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Vera Regitz-Zagrosek *Editor*

Sex and Gender Differences in Pharmacology

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Editor

Sex and Gender Differences in Pharmacology

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Preface

Sex and gender differences in frequent diseases are more widespread than one may assume. In addition, they have significant yet frequently underestimated consequences on the daily practice of medicine, on outcomes and on choice and efficacy of therapies. Using gender-based approaches improves the quality of medical care. Understanding differences in symptoms of myocardial infarction and help-seeking behaviour in women and men leads to a more efficient and faster therapy in both genders. Knowing, for example, that exercise ECG has different sensitivity in subgroups of women and men lowers the threshold to use additional imaging procedures in subgroups of women with suspected cardiac ischemia. The knowledge that a drug has more adverse effects in one gender will affect dosing and choice of therapy. Knowing that some prevention strategies are more voluntarily accepted in women or men will lead to the design of gender-specific campaigns that are more efficient than global approaches.

Efficient pharmacotherapy is affected by pharmacokinetics, resorption, metabolism, distribution and excretion of a drug. These depend on body weight and composition, enzyme activities, organ perfusion and others, all of them differing significantly in women and men. In addition, efficacy and efficiency of drug therapy depend on underlying pathophysiology of a disease. Women and men are clearly similar in most of these parameters (as are mice, pigs and dogs to human) and therefore the majority of drugs that are developed in rodents work in humans of both sexes. Nevertheless, subtle and less subtle differences exist in brain function, in ion channels of heart and kidney, in energy and bone metabolism, in immune responses and in many more. They are due to acute "activational" effects of sex hormones as well as to "organisational" effects that sex hormones exert in utero in the developing foetus, leading to persisting epigenetic marks, and to differences in chromosomal activation between women and men. Disease-associated genes on X and Y chromosomes and sex-specific effects of genetic polymorphisms contribute to sex-specific disease susceptibility.

The more and more we are advancing and refining drug therapy, the more we have to adjust to these subtle differences. Therefore, optimizing drug therapy requires understanding of sex differences in pathophysiology. In addition to sex,

age and related hormonal status play a role. In cycling animals and in cycling women, the phase of the cycle influences a number of physiological parameters outside the reproductive organs.

Gender differences in disease manifestations also determine pharmacotherapy. If the pain threshold is low or a disease is considered life threatening, medications will be started earlier. If a risk factor is underestimated in one gender or gender-specific symptoms are not recognized, treatment may be delayed. Prescription habits depend on gender of patients and physicians and their interaction. Finally the estimated prevalence of a disease in the population influences attitudes in drug development and public health. Gender-based factors also determine the access to health care and the attitudes towards novel drugs.

This book aims at presenting the bases for sex- and gender-sensitive drug development and pharmaceutical therapy. We present sex and gender differences in mechanisms of disease, in pharmacokinetics, in drug development and use and in different therapeutic areas such as cardiology, neuropsychiatry, obesity and diabetes, anaesthetics and pain medication, anti-inflammatory and anti-rheumatic therapy and others. This book provides guidance for the practicing physician, for researchers in drug development, for insurance providers and for health-care authorities. We hope it will stimulate enthusiasm for novel aspects in pharmacotherapy that deserve greatest attention: sex and gender differences.

Berlin, Germany Vera Regitz-Zagrosek

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Part I
Sex Differences in Mechanisms of Disease

Sex and Gender Differences in Clinical Medicine

Vera Regitz-Zagrosek and Ute Seeland

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Abstract Sex and gender differences in frequent diseases are more widespread than one may assume. In addition, they have significant yet frequently underestimated consequences on the daily practice of medicine, on outcomes and effects of therapies. Gender medicine is a novel medical discipline that takes into account the effects of sex and gender on the health of women and men. The major goal is to improve health and health care for both, for women as well as for men. We give in this chapter an overview on sex and gender differences in a number of clinical areas, in cardiovascular diseases, pulmonary diseases, gastroenterology and hepatology, in nephrology, autoimmune diseases, endocrinology, hematology, neurology. We discuss the preferential use of male animals in drug development, the underrepresentation of women in early and cardiovascular clinical trials, sex and gender differences in pharmacology, in pharmacokinetics and pharmacodynamics, in management and drug use. Most guidelines do not include even well-known sex and gender differences. European guidelines for the management of cardiovascular diseases in pregnancy have only recently been published. Personalized medicine cannot replace gender-based medicine. Large databases reveal that gender remains an independent risk factor after ethnicity, age, comorbidities, and scored risk factors have been taken into account. Some genetic variants carry a different risk in women and men. The sociocultural dimension of gender integrating lifestyle, environment, stress, and other variables cannot be replaced by a sum of biological parameters. Because of this prominent role of gender, clinical care algorithms must include gender-based assessment.

Keywords Gender • Sex • Cardiovascular diseases • Clinics • Management • Drugs • Pharmacology

Abbreviations

ECG	Electrocardiogram
CAD	Coronary artery disease
NO	Nitric oxide
HCM	Hypertrophic cardiomyopathy
DCM	Dilated cardiomyopathy
HCV	Hepatitis C virus
HBV	Hepatitis B virus

1 Introduction

The Institute of Medicine in the USA declared repeatedly that being a woman or being a man significantly influences the course of diseases and therefore this fact must be considered in diagnosis and therapy. This statement aimed mainly at the

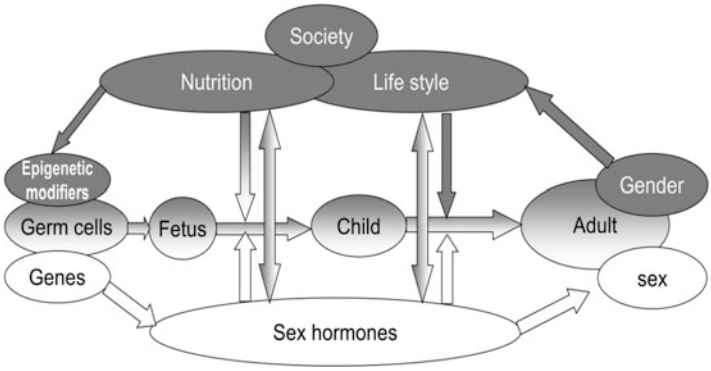

Fig. 1 Complex interdependency of sex and gender in the human (reprinted with permission from sex and gender aspects in clinical diseases, Eds Sabine Oertelt-Prigione and Vera Regitz-Zagrosek, Springer, 2011)

biological differences among female and male animals, or human beings, i.e., sex differences. These are based on differences in sexual hormones, in sex chromosomes and sex-specific gene expression from autosomes. They lead to obvious differences in body composition, in metabolism and physiology and pathophysiology of diseases that are reviewed below. However, gender differences are equally important; gender is the result of a sociocultural process. Gender is associated with behavior, with stress- and lifestyle-associated factors and diseases. It determines access to health care, help-seeking behavior, and individual use of the health care system. For example the use of preventive measures, referral for or acceptance of invasive therapeutic strategies like pacemaker implantation or heart transplantation is largely determined by gender.

In the medical field, sex and gender are closely interrelated. On one hand, sex influences gender roles, i.e., testosterone determines aggressive behavior that may be associated with risk-seeking and negligence of prevention. On the other hand, gender roles, e.g., professional exposition to stress, poor nutrition, environmental toxins or endocrine disrupters may lead to genetic or epigenetic modifications that affect gene expression differently in women and men and thereby modulate the biological phenotype (Fig. 1).

Gender medicine is a novel medical discipline that takes into account the effects of sex and gender on the health of women and men. The major goal is to improve health and health care for both, for women as well as for men. It is based on the hypotheses that both genders benefit from differentiated approaches and better understanding of their specific pathophysiology. In the present chapter, we briefly outline sex and gender differences in clinical manifestations of frequent diseases. A more detailed discussion is found in the novel clinical teaching book "Sex and Gender Aspects in Clinical Medicine" (Oertelt-Prigione and Regitz-Zagrosek 2012).

2 Sex and Gender in Frequent Diseases

A large number of SG differences exist in cardiovascular diseases, pulmonary diseases, gastroenterology and hepatology, in nephrology, autoimmune diseases, endocrinology, hematology, neurology, in pharmacokinetics and pharmacodynamics. The most recent clinical teaching book assembles the relevant literature on sex and gender in these disciplines (Oertelt-Prigione and Regitz-Zagrosek 2012). It is based on a systematic review of the literature that was undertaken in a 2-year-long project by the members of the Institute of Gender in Medicine at Charité Berlin. Sabine Oertelt-Prigione, Vera Regitz-Zagrosek, and coworkers identified more than 10,000 articles dealing with sex and gender differences in clinical medicine and classified them according to major disease entities, epidemiology, pathophysiology, clinical manifestations, outcomes, and management. In each clinical field, about six major diseases were investigated for sex and gender differences (Table 1).

2.1 Cardiovascular Diseases

Cardiovascular diseases are now a major cause of death in women and men in all Western societies (Fig. 2a). Women die more frequently from cardiovascular diseases than men whereas men die more frequently from cancer. In the heart disease and stroke statistics (Rosamond et al. 2008), it is well documented that in the USA 454,613 women and 409,867 men died from cardiovascular diseases in a single year. Among causes of death, heart failure, chronic ischemic heart disease, and myocardial infarction are most prevalent, in Germany as well as in the USA (Fig. 2b).

2.2 Heart Failure

Heart failure is one of the most common cardiac diseases that exhibits significant gender differences. It affects about 10 % of all 70-year olds. Healthy women and men at age 55 have a likelihood of 1:4 or 1:3 to develop heart failure later in life. Heart failure patients have a mean life expectancy below 5 years, the same as patients with malignant disease. Prognosis in heart failure in the Framingham Study improved over the last 50 years and was better in women than in men (Levy et al. 2002). In spite of higher mortality in men, the total number of heart failure deaths is higher in women due to the overall longer lifespan in women. In the Euro Heart Failure Survey systolic heart failure was found predominantly in men, whereas women presented with heart failure with preserved ejection fraction. Major risk factor for heart failure in men is coronary artery disease, whereas in women

Table 1 Publications with sex and gender differences in the most frequent clinical entities

Cardiology	Rheumatology/ immunology	Pneumology	Nephrology	Gastroenterology/ hepatology	Neurology	Endocrinology	Oncology	Hematology
Hypertension (414)	Lupus erythematosus (68)	Asthma (140)	Renal failure (27)	Hepatitis B (22)	Multiple sclerosis (65)	Diabetes mellitus (447)	Skin carcinoma (45)	Anemia (44)
Myocardial infarction (275)	Rheumatoid arthritis (41)	Lung cancer (116)	Diabetic nephropathy (11)	Hepatitis C (26)	Stroke (129)	Obesity (349)	Gastric cancer (25)	Leukemia (49)
Heart failure (153)	Systemic sclerosis (3)	Chronic obstructive pulmonary disease (36)	Glomerulone phritis (9)	Hepatocellular carcinoma (37)	Alzheimer's disease (104)	Osteoporosis (123)	Renal cell carcinoma (17)	Lymphoma (34)
Atrial fibrillation (38)	Fibromyalgia (15)	Pulmonary hypertension (12)	Polycystic kidney disease (12)	Inflammatory bowel disease (13)	Epilepsy (56)	Hypothyroidism (33)	Bladder cancer (22)	Thrombocytopenia (6)
Coronary heart disease (207)	Sjögren's syndrome (8)	Pulmonary embolism (11)	Renal artery stenosis (0)	Colorectal cancer (24)	Parkinson's disease (69)	Hyperthyroidism (16)	Thyroid carcinoma (16)	Purpura (2)
Cardiomyopathy (41)	Ankylosing spondylitis (11)	Sarcoidosis (6)	IgA nephropathy (2)	Autoimmune hepatitis (2)	Muscular dystrophy (11)	Morbus addison/ Cushing's disease (5)	Pancreatic carcinoma (10)	Agranulocytosis (0)

Numbers of relevant publications are in brackets

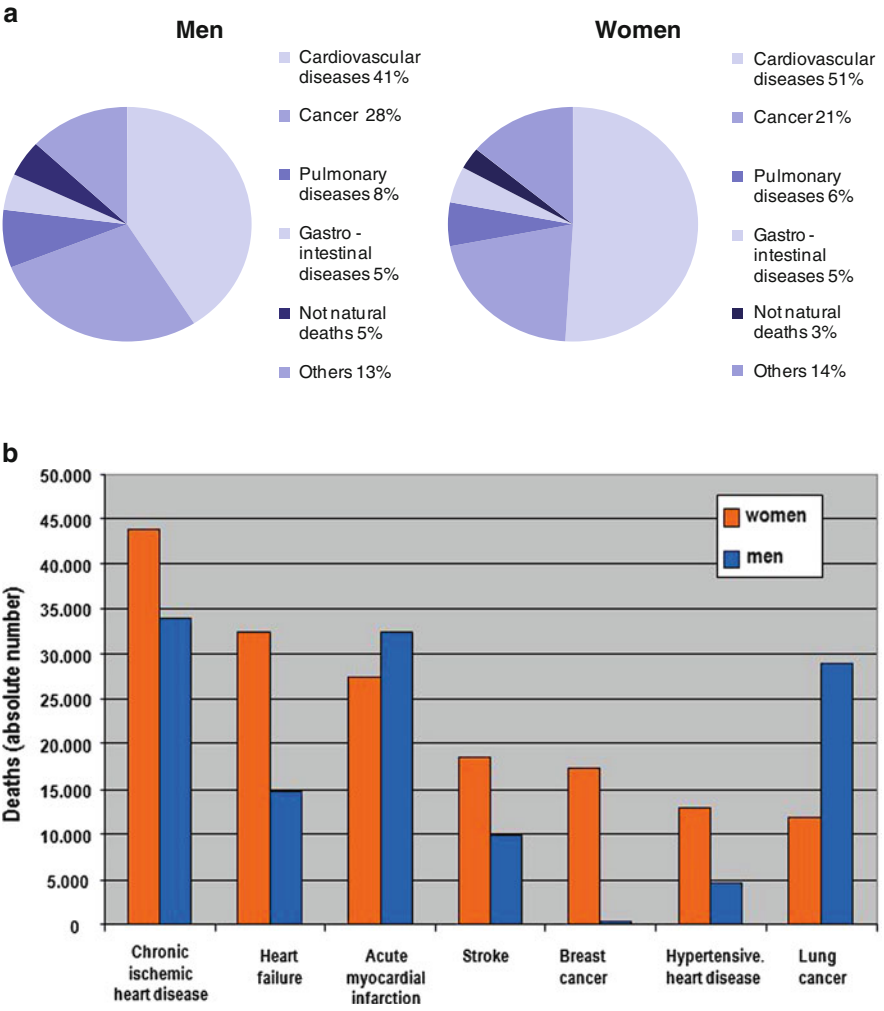

Fig. 2 (**a**) Percentage breakdown of deaths for men and women in Germany (Statistisches Bundesamt, Robert Koch Institut Berlin; 2005). Total may not add to 100 because of rounding. Cardiovascular diseases are the main cause of death for men and women in western industry nations. (**b**) Major causes of death in women and men (*Statistisches Bundesamt, Robert Koch Institut Berlin, 2007*). Chronic ischemic heart diseases are the main cause of death for women. In absolute numbers more men than women die of acute myocardial infarction

hypertension and diabetes are most prevalent risk factors. Sex differences in pathophysiology are not well studied. Gene expression profiling in end-stage failing human hearts reveals less down-regulation of energy metabolism in female hearts and more matrix induction in male hearts. Estrogen receptors are present in the myocardium of both sexes, in nuclear and extranuclear localizations, and change

their localization in heart failure (Mahmoodzadeh et al. 2006). Clinical manifestation in heart failure is quite similar among women and men. Women have a lower prevalence of atrial fibrillation (Benjamin et al. 1994).

Women have more frequently heart failure with normal systolic function whereas men present more frequently with heart failure with reduced ejection fraction. Survival is comparably poor in both forms. A major cause of sex differences is calcium signaling, nitric oxide (NO) synthesis, and profibrotic mechanisms. This depends partially on estrogens and androgens. Nevertheless women do have a better outcome than men (Regitz-Zagrosek et al. 2010a).

2.3 Chronic Coronary Artery Diseases

Sex and gender differences in coronary artery disease are known since long. Women with coronary artery disease (CAD) are about 10 years older than men. Diabetes is a greater risk factor in women than in men. Genetic risk factors for chronic coronary artery disease differ in women and men. There is a locus on the Y-chromosome that determines the risk for coronary artery disease only in men not in women (Charchar et al. 2012).

Pathophysiology between women and men is also different. Men exhibit more main stem stenosis and more triple vessel disease. In contrast, women present more frequently the single vessel disease. They also exhibit more disturbances of the microcirculation and more angina with normal coronary artery disease. This phenomenon has been called syndrome X earlier and is poorly understood in pathophysiology. However, some imaging studies clarified that it is well associated with myocardial ischemia and metabolic markers of disturbed myocardial energy metabolism in a significant percentage of patients (Buchthal et al. 2000). Endothelial dysfunction in the coronaries can be documented by a reduced vasodilating response to intracoronary. If present, this predicts poor outcome (Johnson et al. 2004). The syndrome is characterized by high morbidity, recurrence rate, and treatment costs (Lang et al. 2005).

Women appear to have a greater capability of vascular regeneration. Circulating stem cells increase after estrogen stimulus and stem cell function is increased by estrogen (Zenovich et al. 2008; Nelson et al. 2007).

The exercise ECG is quite frequently misleading in women for not fully understood reasons. However, imaging strategies by echo, szintigraphy or magnetic resonance imaging are of similar high value in both genders. Recommended medical therapy does not differ in both genders; and percutaneous coronary intervention is successful in both. However, it is more often accompanied by bleeding complications in women than in men. Women do have a greater mortality after coronary bypass surgery (Regitz-Zagrosek 2006). The FRISK Study has suggested that women respond less well to aggressive early revascularization in the case of acute angina or acute coronary syndromes. This has however been revised by new studies (Regitz-Zagrosek et al. 2011).

2.4 Myocardial Infarction

Myocardial infarction is considered a disease of men, but it kills almost as many women as men (Rosamond et al. 2008). Women experience the majority of myocardial infarctions about 10 years later than men. Nevertheless, their longer life period leads to the fact that they undergo a very similar absolute number of myocardial infarctions compared with men.

It is now well accepted that the incidence of MI declines worldwide in all parts of the population expect in young women. Nevertheless, young women do have a higher mortality after a first myocardial infarction than age-matched men. This holds also true for increased mortality of younger women after coronary bypass graft surgery. Women and men differ in triggers for myocardial infarction. Psychological stress is more important in women; heavy exercise is more common in men. Women and men also differ in symptoms of myocardial infarction. Women appear to experience a greater variety of symptoms, more so-called atypical syndromes and more vagal activation signs than men (Regitz-Zagrosek 2011). In contrast there is a much higher likelihood for ischemic sudden death in men. Women receive less guideline-based diagnosis and less invasive treatment for MI than men. Social stress is a major determinant of re-infarction after a first MI in women (Regitz-Zagrosek 2006).

2.5 Hypertension

Hypertension is more frequent in young men than in women but increases steeply after the menopause in women. Pulse pressure increases during lifetime in women. Since white coat hypertension is more frequent in women than in men, women may benefit more than men from 24 h ambulatory blood pressure monitoring. Women and men also differ in the pathophysiology of hypertension. Sex differences exist in the activation of the sympathetic nervous system, renin–angiotensin, and endothelium system. Sex differences in vascular activity explain some of the sex differences in age-dependency in hypertension. Androgens increase and castration decreases blood pressure in male animals by renal vascular and other mechanisms (Reckelhoff et al. 1999; Singh et al. 2007). The endothelial pathway and the NO system contribute more to the vasoconstriction in men than in women. In contrast, the role of NO in vascular relaxation is more pronounced in women (Kahonen et al. 1998). The renin–angiotensin system is also sexually dimorphic. It appears that endogenous estrogens may inhibit the hepatic angiotensinogen synthesis. In contrast, testosterone appears to increase renal and hepatic transcription of angiotensinogen. Women have twice the aldosterone response that men have. This phenomenon appears to worsen with age. Production of free-oxygen radicals that correlate with hypertension and atherosclerosis incidence appears reduced in women because of modulation of NADPH oxidases by estrogens (Wang et al. 2006). For yet unknown reasons, hypertension and myocardial hypertrophy are relatively greater risk factors for heart failure in women than in men (for review: Regitz-Zagrosek 2006).

2.6 Cardiomyopathy

Cardiomyopathies occur slightly more frequently in men than in women (ratio 1.5:1 or 2:1).

Among the cardiomyopathies, hypertrophic cardiomyopathy (HCM) is the most frequent in the population with a rate of 1:500 or 1:2,500 per live birth. It is slightly more frequent in men and men have reported to exhibit a more severe phenotype. In dilated cardiomyopathy (DCM), most studies report women:men ratios between 1:2 and 1:3 (Codd et al. 1989). Peripartum CMP is a rare disease, but a leading cause of maternal mortality in Western countries. It occurs obviously only in women, and is considered as an own disease entity. Tako-Tsubo cardiomyopathy affects almost only women.

In pathophysiology, a greater role for autoimmunity in dilated cardiomyopathy has been found in men than in women (Regitz-Zagrosek 2012). In animal models, for genetic cardiomyopathy, female animals in general have a much better survival and develop less hypertrophy and heart failure than male animals. Frequently, the male phenotype can be rescued by estrogen. Sex differences have been reported in a TNF alpha-cardiomyopathy model, in a transgenic mouse model with cardiac overexpression of the beta-adrenergic-receptor and in alcohol-induced cardiomyopathy in mice and specifically in transgenic mice overexpressing alcohol dehydrogenase. Increased sensitivity to the cardiotoxic effects of ethanol in women has been translated to the clinical setting. In contrast, in transgenic mice overexpressing platelet-derived growth factor C, females fare worse than males.

As in heart failure women with cardiomyopathies and systolic failure receive less invasive treatment devices or organ transplant than men. Nevertheless, women with cardiomyopathies and systolic heart failure display better survival than men.

2.7 Atrial Fibrillation

More women than men live with atrial fibrillation because of age dependency and greater longevity in women. Atrial fibrillation increases embolic risk more in women than in men. Women receive less anticoagulation with warfarin; women risk more bleeding complications with different forms of anticoagulation.

2.8 Pulmonary Diseases

Asthma incidence appears increased in young boys compared to girls and this ratio reverses over time as women appear predominantly affected in young adulthood and men at older age (Manfreda et al. 2004; Grohe 2012). Symptoms tend to be more severe in female adolescents. In seniors significant gender differences can

be found in symptom presentation, in the obstructive pattern and in underlying allergic potential. Chronic obstructive pulmonary artery disease is a serious problem in women and men. Due to increasing smoking prevalence in women and a greater sensitivity of women for tobacco toxicity (Dransfield et al. 2006), it will get greater relevance there. Drug treatment and response to drug is also affected by gender. Lung cancer is one of the most frequent solid tumors right now. Its prevalence in women is raising and will reach the prevalence of men due to changes in lifestyle and smoking habits (Manton 2000). It appears that a greater sensitivity to some tobacco toxins and cigarette smoking puts women also at greater risk for lung cancer than men. Pulmonary hypertension is significantly more frequent in women than in men, particularly in young women. Some estrogen dependency has been reported. Nevertheless, the pathophysiology is incompletely understood (Humbert et al. 2010).

2.9 Gastroenterology and Hepatology

There are estimated over 400 million carriers of hepatitis B virus (HBV) worldwide, while chronic hepatitis C virus (HCV) infection has reached pandemic proportions with 170 million individuals estimated to be infected. One of the most severe consequences of chronic viral hepatitis is hepatocellular carcinoma which is nowadays the fifth most common cancer. Women and men differ in the antibody production after infection with hepatitis B or viral clearance after hepatitis C virus infection (Blumberg 1979). The response to therapy after interferon/ribavirin for HCV is sex dependent with more frequent adverse events in women.

Some specific liver diseases, autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis have shown to have significantly higher prevalence in women compared with men, also nonfatty liver disease.

Inflammatory bowel syndromes are much more frequent in women than in men. They exuberate in one third of all pregnancies. Sex-specific therapeutic approaches have been developed (*Voss* et al. *chapter 21*). Colon carcinoma is differently located in women and men. It is more frequently found in the ascending colon in women and more frequently in the descending colon in men (Garcovich and Burroughs 2012).

2.10 Nephrology

Women do have a lower capacity to concentrate urine than men and they also exhibit a lower number of nephrons in their kidney. Women have lower glomerular filtration rates than men, particularly at older age, even if relation to body surface area. In stage 3 chronic kidney disease, more women than men are found (Silbiger and Neugarten 2008). Nevertheless, women experienced more complications of

kidney failure. They exhibit more frequent anemia, more frequent secondary hyperparathyroidism, and associated bone damage. Lupus nephritis is more common in men than in women and displays a more aggressive progression. Glomerular nephritis also appears to have a more severe course in men. Polycystic kidney disease is also more prevalent in men and has a worse outcome in men than in women (Gallieni et al. 2012).

2.11 Autoimmune Diseases

Most autoimmune diseases are more frequent in women than in men (Zandman-Goddard et al. 2012). Ankylosing spondylitis, or Morbus Bechterew, was believed to be significantly more frequent in men in earlier times, but now with advanced diagnostic strategies it becomes clear that the prevalence is similar in women and men (Lee et al. 2008). The disease exhibits sex differences in the genetic background and clinical manifestation in women and men. Women have more frequently a positive family history and may even manifest at younger age. Systemic lupus erythematodes, however, is more frequent in women than in the reproductive age in men (9:1). Its severity is affected by serum estrogen concentration. However, male lupus erythematodes patients are reported to have an increased mortality compared to females. Sjögrens syndrome is also more frequent in women than in men and sex hormone levels have been involved in pathophysiology. Extraglandular manifestation is also more frequent in women than in men, particularly fatigue and arthritis. Fibromyalgia is a hardly understood disease. It is more frequent in women than in men, is associated with psychosocial risk factors like depression, accompanied by altered pain sensitivity. Rheumatoid arthritis has a 3–4:1 distribution in women and men. It is affected by hormone level changes, e.g., during pregnancy. Male patients benefit more from anti-TNF therapies than females with higher remission rates. For yet unknown reasons, in rheumatic diseases there is a significant diagnostic delay in women in comparison to men.

2.12 Endocrinology—Diabetes Mellitus

Gender differences in incidence and prevalence of diabetes mellitus are small and in part controversial (Kautzky-Willer 2012). However, prediabetes is characterized by a higher prevalence of impaired glucose tolerance in women and a greater prevalence of increased fasting glucose levels in men (DECODE 2003). Diabetes carries a greater risk for coronary artery disease in women than in men and is also associated with a greater risk of mortality after a myocardial infarction. In patients with the metabolic syndrome, inflammatory markers have a strong impact on prognosis in women. Thyroid diseases and antithyroid antibodies are more common

in women than in men. However male sex is a risk factor for thyroid cancer. Osteoporosis is more frequent in women than in men. Osteoporosis is frequently underdiagnosed and represents a frequent hidden cause of hip fracture in men.

2.13 Hematology–Oncology

Women are more frequently affected by all forms of anemia; iron deficiency is a frequent cause (Schmetzer and Florcken 2012). Women have more bleeding complications when undergoing invasive therapies (in the coronary arteries) or experience more bleeding upon administration of warfarin. Anti-platelet therapies may act differently in women and men. With chemotherapy, women experience more adverse effects than men.

2.14 Neurology

In younger age (<55 years), fewer women than men have prevalent stroke. In older age (>65 years), 2–3 times as many women have a prevalent stroke than men (Nolte et al. 2012). Stroke mortality may be higher in very old women (>85 years). However, as women on average get older than men, the absolute number of deaths due to stroke is higher in women than in men. Nowadays, strokes are affecting more and more younger women and this may shift the distribution that we know so far. Atrial fibrillation is a greater risk for stroke in women than in men (Dagres et al. 2007; Nolte et al. 2012). Alzheimer's disease is more and more frequent in women and more frequently associated with depression.

3 Gender Differences in Medical Therapy

3.1 Rationale

The effects of pharmacological interventions differ in women and men. Significant differences in pharmacokinetics and pharmacodynamics have been established due to lower body surface in women but also to differences in drug resorption, metabolism by hepatic enzymes, kidney function, and excretion. Many drugs require different doses in women and men for optimal effects. In addition, differences in pharmacodynamics are also evident. Ion channels in heart and kidney, brain function, energy and bone metabolism, immune responses differ between women and men and this may cause sex-specific effects of drugs that are used to modify kidney function or heart rhythm. Major sex and gender differences have been reported for the efficiency and adverse effects of heart failure drugs, like digitalis,

ACE inhibitors, and anti-arrhythmics. Sex and gender differences have also been found in analgesic and neuropsychiatric drugs, in anticancer drugs, cardiovascular drugs, in the effects anti-TNF-alpha and antiviral. Thus, considering sex and gender differences in drug development and testing will increase the likelihood to identify drugs that fit both genders.

3.2 Drug Development and Testing

Drug development is characterized by the fact that most research is done in male animals (Zucker and Beery 2010). However, significant differences exist in the outcomes of male and female mice in models of myocardial infarction, pressure overload and genetic cardiomyopathies (Czubryt et al. 2003; Regitz-Zagrosek 2006; Fliegner et al. 2010) that are often not even considered by the researchers. Most cardiovascular researchers use only male mice (Zucker and Beery 2010). Female mice do have better outcome in most models of pressure overload, in transgenic models for heart failure, and in myocardial infarction whereas males do have a more impressive and severe phenotype. In many cases the male phenotype can be rescued by administration of estrogen. Because of the more severe phenotype, therapeutic effects in males are likely to be greater. In the extreme, a drug may have a major effect in males and may not be effective in females at all. However, even a single gene mutation can vary in its effect in male and female mice and is also dependent on the nutrition, i.e., phytoestrogens supplied in the animal chow (Luczak et al. 2011; Bhupathy et al. 2010).

In addition, adequate animal models for menopause transition are lacking. Mostly, ovarectomy in young female mice is used. This occurs suddenly and affects all ovarian tissues, including testosterone synthesizing stroma cells and not only ovarian follicles are slowly degenerating as it is the case in natural menopause. Therefore a new model has recently been developed.

3.2.1 Clinical Trials in Women

Major efforts have been made to increase the participation of women in all types of clinical trials. However, studies published in 2008 concluded that women were still not included in mixed sex cardiovascular trials in numbers that reflect the disease prevalence among the general population (Kim et al. 2008, 2010). A 2005 study of 300 new drug applications between 1995 and 2000 found that even those drugs that showed substantial differences in how they were absorbed, metabolized and excreted by men and women had no sex specific dosage recommendations on their labels (Anderson 2005). This may be the reason that women are 1.5- to 2-fold more likely to develop an adverse reaction to prescription drugs than men (Zopf et al. 2008). In an assembly of 48 cohort studies for novel drugs in Great Britain, women had a 1.6-fold higher risk for adverse reactions than men (Martin et al. 1998). Even based on the most recent investigations, women are still not

Table 2 Gender differences in management

Women with coronary artery disease I receive less guideline-based diagnosis and less invasive treatment than men	Regitz-Zagrosek (2006)
Women with heart failure receive less guideline-based diagnostic procedures and treatments, less device implantations, and heart transplantations. Nevertheless, women do have a better outcome than men	Regitz-Zagrosek et al. (2010b)
Women at risk for stroke receive less anticoagulation with warfarin, even so women have a greater risk for stroke when atrial fibrillation occurs	Agarwal et al. (2010)
Women obtain dialysis later than men, less kidney transplant, both from living and cadaveric donor	Kausz et al. (2000) and Jindal et al. (2005)
Significant delay in referral of female patients with rheumatoid arthritis to an early arthritis clinic in comparison with male patients is found	Zandman-Goddard et al. (2012)
Osteoporosis and depression are considered female diseases. Both may be underdiagnosed in men	Kautzky-Willer (2012)

adequately represented in clinical trials. They are particularly underrepresented in the area of cardiovascular diseases and in early studies (Melloni et al. 2010); (O'Connor et al. 2010a, b).

3.3 Gender Differences in Management

Studies that analyzed use of drugs in heart failure (Baumhakel et al. 2009) or coronary artery disease (Bischoff et al. 2006; Geller et al. 2007) found undertreatment of women with evidence-based therapies. Moreover, undertreatment of women is most pronounced by male physicians in cardiovascular field (Baumhakel et al. 2009; Journath et al. 2008), for diabetes (Berthold et al. 2008), and in gynecology (Lurie et al. 1993). In Europe, women receive less echocardiography in heart failure, less angiography in coronary artery disease, less percutaneous coronary interventions, and bypass surgery if needed (Daly et al. 2006).

Usually, undertreatment of women is justified by their higher age at manifestation of disease and it is neglected that biological age and remaining life expectancy in old women and 6–8 years younger men are similar. In order to compare persons with the same biological age, women should be compared with 6–8 years younger men. Sex differences in management of frequent extracardiac diseases are manifold. Just a few examples are listed in Table 2.

3.4 Guidelines

These significant differences are related to different efficacy of the drugs but also to differences in administration and use of drugs in the population. First drugs for the

use in only one gender have been marketed and gender-specific recommendations on preferred drug use or dosing are developed, for example in the field of antidepressants or inflammatory bowel disease (*Voss* et al. *chapter 20*). Developing and incorporating these aspects into common management strategies and guidelines will enhance efficiency of pharmaceutical and interventional therapies.

However, so far the established guidelines are not gender sensitive. Even in areas where sex and gender differences are clearly described such as the ischemic heart disease with open coronary arteries, sex differences in symptoms for myocardial infarction or the different sensitivities to some cardiovascular drugs or the different forms of prediabetes in women and men (more elevated fasting glucose in men and more impaired glucose tolerance in women), these are not adequately considered in the guidelines. A positive example the guidelines for management of atrial fibrillation that include female sex as an additional risk factor for stroke (Wann et al. 2011a, b).

3.5 Guidelines for Cardiovascular Diseases in Pregnancy

Guidelines for cardiovascular diseases in pregnancy exist only in the German Cardiac Society (DGK) as well as in the European Cardiac Society (ESC). They have been published by the DGK in 2008 and by the ESC in the form of an expert consensus document in 2003 and as true guidelines in 2011 (Regitz-Zagrosek et al. 2008, 2011). The fact that most recommendations in these guidelines are class C—expert opinions—made clear how big the deficit under standard pregnancy-related diseases still is. There is an immense need of research on pharmacotherapy in pregnancy, on mechanism of pregnancy-related cardiovascular and metabolic and other diseases. This was also recognized by *Nature* in their article "Pregnant women deserve better" (Baylis 2010).

4 Gender and Personalized Medicine

Personalized medicine aims at considering all individual risk factors of a human being—including ethnicity, lifestyle factors, conventional risk factors, personal history, as well as genetic predisposition. The weight that is given to genetic predisposition varies among different protagonists of personalized medicine. However, since only the conventional and laboratory risk factors as well as gene variants can easily be quantitatively assessed or scored, most approaches rely mainly on a combination of these. Some people argue that gender-based medicine will become irrelevant if all individual factors can be taken into account. However large databases reveal that gender remains an independent risk factor after age; comorbidities, scored risk factors, and ethnicity have been taken into account. Some genetic variants carry a different risk in women and men (Siddiqui et al. 2009; Charchar et al. 2012).

The sociocultural dimension of gender integrating lifestyle, environment, stress, and other variables cannot easily be replaced by a sum of biological parameters.

5 Conclusion

Because of the prominent role of gender, it seems mandatory to construct individualized clinical care algorithms based on individual risk profiles on top of gender-based assessment.

Take Home Messages

- Sex and gender differences in frequent diseases—such as cardiovascular diseases, pulmonary diseases, gastroenterology and hepatology, in nephrology, autoimmune diseases, endocrinology, hematology, neurology—are widespread.
- Sex and gender differences have significant consequences on the daily practice of medicine, on outcomes and effects of therapies.
- Gender medicine is a novel medical discipline that takes into account the effects of sex and gender on the health of women and men. The major goal is to improve health and health care for both, for women as well as for men.
- Drug development is still preferentially based on male animals and women are underrepresented in early and cardiovascular clinical trials.
- Management and drug use differs in women and men.
- Most guidelines do not include even well-known sex and gender differences. European guidelines for the management of cardiovascular diseases in pregnancy have only recently been published.
- Personalized medicine cannot replace gender-based medicine. The sociocultural dimension of gender integrating lifestyle, environment, stress, and other variables cannot be replaced by a sum of biological parameters.

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Sex Differences in Animal Models for Cardiovascular Diseases and the Role of Estrogen

S. Mahmoodzadeh, D. Fliegner, and E. Dworatzek

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Abstract Clinical findings show sex differences in the manifestation of a number of cardiovascular diseases (CVD). However, the underlying molecular mechanisms are incompletely understood. Multiple animal models suggest sex differences in the manifestation of CVD, and provide strong experimental evidence that different major pathways are regulated in a sex-specific manner. In most animal studies females display a lower mortality, less severe hypertrophy, and better preserved cardiac function compared with male counterparts. The data support the hypothesis that female sex and/or the sex hormone estrogen (17β-estradiol; E2) may contribute to the sexual dimorphism in the heart and to a better outcome of cardiac diseases in

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females. To improve our understanding of the sex-based molecular and cellular mechanisms of CVD and to develop new therapeutic strategies, the use of appropriate animal models is essential. This review highlights recent findings from animal models relevant for studying the mechanisms of sexual dimorphisms in the healthy and diseased heart, focusing on physiological hypertrophy (exercise), pathological hypertrophy (volume and pressure overload induced hypertrophy), and heart failure (myocardial infarction). Furthermore, the potential effects of E2 in these models will be discussed.

Keywords Animal model • Estrogen • Estrogen receptor • Heart disease • Myocardial hypertrophy • Sex differences

Abbreviations

CVD	Cardiovascular disease
MH	Myocardial hypertrophy
I/R	Ischemia/reperfusion
E2	Estrogen (17β-estradiol)
ER	Estrogen receptor

1 Introduction

Recent epidemiological and clinical data emphasized that sex differences play a major role in the manifestation and outcome of cardiovascular disease (CVD) [for review see Oertelt-Prigione and Regitz-Zagrosek (2012)]. In response to sustained pressure overload, women develop a more concentric hypertrophy with a better preserved left ventricular function than men. In contrast, men tend to develop more eccentric hypertrophy and left ventricular dilatation (Fig. 1) (Carroll et al. 1992; Petrov et al. 2010). Understanding of the mechanisms underlying these sex-based differences in pressure overload is important to develop new approaches for prevention, diagnosis, and treatment. For these purposes, animal models can greatly improve our understanding of the cause and progression of CVD and provide a useful tool to elucidate the mechanisms of sexual dimorphisms in the healthy and diseased heart. Various studies in which animals were subjected to exercise or surgical procedures, treated with drugs or genetically modified, support the existence of sex differences in the cardiovascular system as observed in clinical studies, and showed that females fare better than males (Leinwand 2003; Luczak and Leinwand 2009). Additionally, these animal studies corroborate the hypothesis that the sex hormone estrogen (17β-estradiol; E2) and its respective receptors [estrogen receptors (ER)] protect females and mediate the sex-related differences in the cardiovascular responses to different

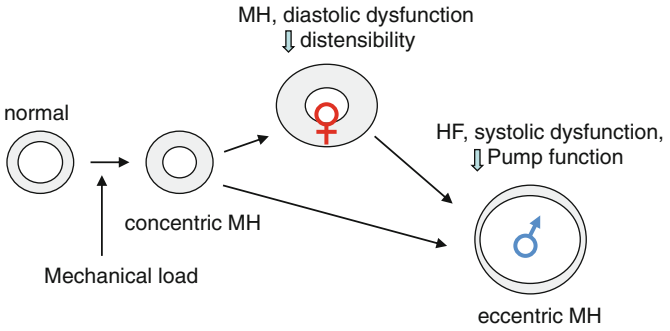

Fig. 1 Schematic illustration of sex differences in the manifestation of heart failure (HF): In response to hypertrophic stimuli, such as pressure overload, hypertension or aortic stenosis, women have a more concentric form of myocardial hypertrophy and better-preserved myocardial function, whereas men exhibit a larger myocyte volume and develop an eccentric form of hypertrophy with a loss of systolic function

physiological and pathological stimuli (Du 2004; Leinwand 2003; Mendelsohn and Karas 2005). In spite of these experimental findings, the molecular mechanisms underlying the sexual dimorphism are very complex and still not clearly elucidated. Learning from the beneficial effects in females could help to build strong foundations for the development of novel sex-sensitive strategies for drug therapies.

E2 mediates its effects predominantly via ER alpha (ERα) and beta (ERβ), which are members of the nuclear receptor superfamily (Mendelsohn and Karas 2005). The ER act as ligand-induced transcription factors and regulate the expression of E2-target genes (genomic effects) (Mendelsohn 2002). They also exert rapid nongenomic effects by interacting with cytosolic proteins and signal transduction pathways (Mendelsohn 2002; Simoncini et al. 2006). The ER are expressed in human and rodent (Fig. 2) hearts in both sexes (Grohe et al. 1997; Nordmeyer et al. 2004), and regulate upon E2 activation the expression of relevant E2-target genes, including connexin 43 (Cx43), alpha-myosin heavy chain (α-MHC), matrix metalloproteinases (MMP), and atrial natriuretic peptide (ANP), which in turn play a role in the pathogenesis of myocardial hypertrophy (Babiker et al. 2004; Grohe et al. 1997; Mahmoodzadeh et al. 2010). They have also been associated with the prevention of apoptosis, regulation of cell–cell interaction, and with the regulation of activity of calcium (Ca^{2+}) channels (Groten et al. 2005; Johnson et al. 1997; Jovanovic et al. 2000; Patten et al. 2004).

Sex differences in cardiac physiology and pathology are multifactorial, and may arise from genetic differences (e.g., differences in X- and Y-chromosome, epigenetic), the action of other sex steroid hormones (e.g., effects of testosterone), or a combination of these factors. The present review focuses on our current understanding of the impact of E2 and its receptors on sex differences in normal cardiac physiology and pathophysiology obtained from animal models.

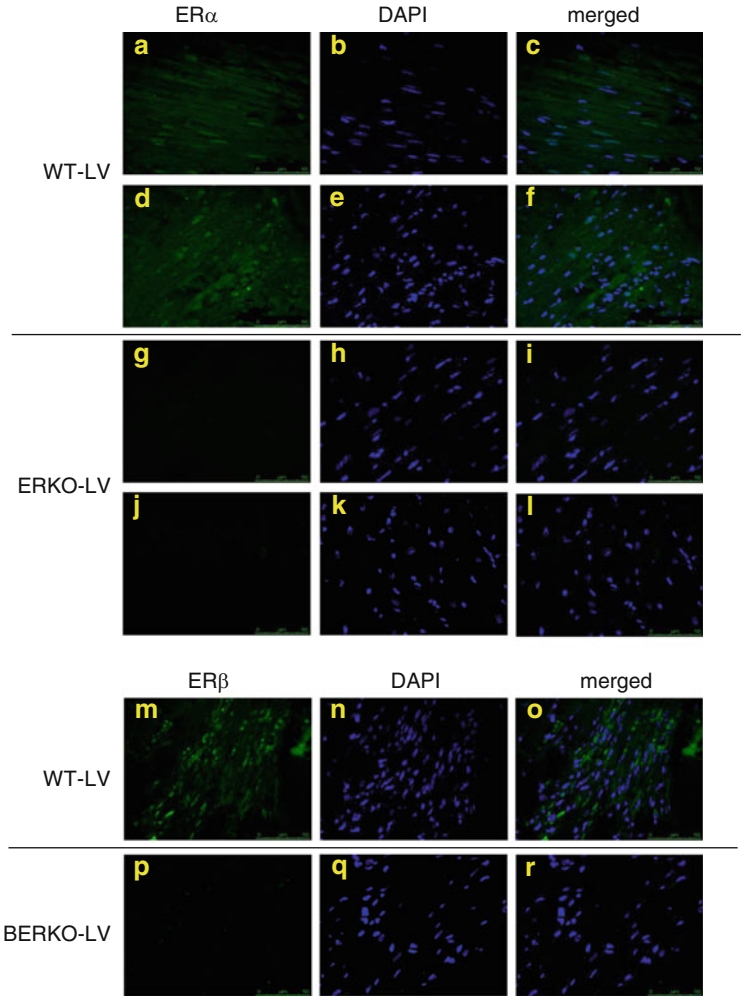

Fig. 2 Representative immunofluorescence (IF) images for localization of ERα and ERβ in LV of WT and estrogen receptor knockout mice. (**a–l**) Detection of ERα in 3 μm paraffin sections of LV from WT and ERKO mice by IF staining and confocal laser-scanning microscopy. IF using two different antibodies against ERα [(**a**): MC-20 (Santa Cruz) and (**d**): G-20 (Santa Cruz)] showed that ERα (*green signal*) is localized in the nuclei of cardiomyocytes and fibroblasts, as well as in the sarcoplasmic reticulum and cytoplasm of cardiomyocytes of WT mice. (**b**) and (**e**) show nuclear staining with DAPI. (**c**) and (**f**) are merged images. (**g–l**): as expected, the same antibodies against ERα showed no signals in the LV section of ERα-deletion mice (ERKO), supporting the specificity of signals for ERα in WT mice. (**m–r**): Detection of ERβ in 3 μm paraffin sections of LV from WT and BERKO mice by IF staining and confocal laser-scanning microscopy. (**m–o**) show the same section of LV of WT stained for ERβ [SP5198P (Acris), *green signal*]; (**m**); DAPI (*blue*) (**n**); and merged images from ERβ and DAPI (**c**). ERβ is mainly localized in the cytoplasm, nuclei and mitochondria of cardiomyocytes. (**p–r**) show the same section of LV of a BERKO mouse stained for ERβ [SP5198P (Acris), *green signal*]. As expected, the signal for ERβ is lacking in the LV section of BERKO mice (**p**), indicating the specificity of the signal for ERβ antibody in the LV of WT mice, DAPI [(**q**), *blue*]; and (**r**) merged images from ERβ and DAPI

2 Sex Differences in Animal Models of Myocardial Hypertrophy

2.1 Sex Differences in Models of Physiological Myocardial Hypertrophy: Exercise

Development of physiological myocardial hypertrophy (MH) is characterized by an increase in cardiac mass and cardiomyocyte dimension. In contrast to the pathological growth of the heart, physiological MH shows preserved or enhanced cardiac function, normal cardiac structure and is reversible (Bernardo et al. 2010). Exercise, pregnancy, and postnatal growth promote the physiological growth of the heart (Hill and Olson 2008). In this review we focus on sex differences in exercise-induced physiological MH, because understanding of the involved cardioprotective signaling pathways and genes is of great importance to treat or prevent heart failure (HF). So far, sex differences in the development of physiological MH induced by exercise have been examined very rarely. But nevertheless in these few studies sex differences have been observed (Table 1). Exercise, independent of voluntary or forced character, induces a sexually dimorphic hypertrophy response of the heart. For example in rats, females subjected to chronic swimming exhibited a marked increase in absolute heart mass associated with increased contractile performance compared with their male counterparts (Mole 1978; Schaible and Scheuer 1979, 1981). In contrast, chronic treadmill running did not increase heart mass in both female and male rats. However, male hearts could show improved performance of the left ventricle (Schaible et al. 1981).

In mouse models, either subjected to voluntary or forced exercise, females exhibited a higher increase in cardiac hypertrophic response compared with male mice (De Bono et al. 2006; Foryst-Ludwig et al. 2011; Konhilas et al. 2004). Additionally, female mice run more on a voluntary wheel than male mice in an age- and strain-independent manner, suggesting that sexual dimorphic cardiac responses are mainly mediated by differences in running distance (De Bono et al. 2006; Konhilas et al. 2004). When the increase in cardiac mass was normalized to running distance, female mice still have an augmented hypertrophic response compared to males, indicating a true sexual dimorphic cardiac response (Konhilas et al. 2004). Whether this differential response to exercise between males and females in response to exercise is due to sex hormones or to genetic properties of the myocardium is still unclear.

2.2 Sex Differences in Models of Pathological Myocardial Hypertrophy: Pressure Overload

Sex differences in the manifestations or significance in MH have been described in human beings. So far, only a few studies focused on sex differences in cardiac hypertrophy in rodents exclusively (Table 1).

Table 1 Sex differences in animal models of physiological and pathological hypertrophy

Physiological/ pathological	Model	Species	Phenotype with sex differences	Pathways involved	Reference(s)
Physiological	Swimming	Rat	Significantly increase in cardiac mass only in females	n.d.	Schaible and Scheuer (1979) and Schaible and Scheuer (1981)
Physiological	Treadmill	Rat	Better contractile function in male hearts	n.d.	Schaible and Scheuer (1981)
Physiological	VCR	Mouse	Significantly higher increase in cardiac mass in females	CaMK, phosphorylation of GSK-3β	Konhilas et al. (2004)
Physiological	VCR	Mouse	Significantly higher increase in cardiac mass in females	n.d.	De Bono et al. (2006)
Physiological	Treadmill	Mouse	Significantly higher increase in cardiac mass in females	Akt pathway	Foryst-Ludwig et al. (2011)
Pathological	I/R	Rat	Postischemic recovery of LV function significantly better, and infarct size significantly smaller in female	Akt- and PKCε pathway	Bae and Zhang (2005)
Pathological	I/R	Mouse	Improved myocardial function in female hearts	PI3K/Akt and pro-apoptotic signaling	Wang et al. (2009)
Pathological	I/R + Isoproterenol (Iso-), or Ca^{2+}-treatment	Mouse	Higher postischemic contractile function and lesser ATP-depletion in female	NO/eNOS signaling	Cross et al. (2002)
Pathological	LAD occlusion/ reperfusion	Rabbit	Significantly less infarct size in female hearts as compared to males	eNOS signaling	Wang et al. (2006a)
Pathological	I/R + Iso-treatment	Mouse	Increased S-nitrosylation of the L-type Ca^{2+} channels, reduced Ca^{2+} entry and reduced heart injury in female hearts	eNOS signaling	Sun et al. (2006)
Pathological	I/R	Rat	Significantly improved postischemic myocardial function in females compared with males	p38-MAPK and pro-inflammatory cytokine	Wang et al. (2005)
Pathological	LAD occlusion	Mouse	Reduced activities of MMP-2 and 9; less accumulation of inflammatory cells, and lower risk of rupture in female hearts	n.d.	Cavasin et al. (2004) and Fang et al. (2007)

Pathological	I/R	Rat, dog	Significantly smaller infarct size, increased activation of sarcKATP- and mitoKATP-channels in female hearts	n.d.	Johnson et al. (2006) and Lee et al. (2000)
Pathological	PO (TAC)	Mouse	Less LVH in females	n.d.	Skavdahl et al. (2005)
Pathological	PO (TAC)	Mouse	Gene cluster response-sex-specific manner to PO	n.d.	Weinberg et al. (2003)
Pathological	PO (TAC)	Mouse	LVH greater in WT males, BERKO mice no SD in LVH, BERKO females highest levels of fibrosis, sex- and genotype-specific gene expression	n.d.	Fliegner et al. (2010)
Pathological	PO (TAC)	Rat	Early transition to HF with cavity dilatation, loss of concentric remodeling elevated wall, and diastolic dysfunction in males	n.d.	Douglas et al. (1998)
Pathological	PO (TAC)	Rat	Males develop diastolic dysfunction, elevated wall stress, and depressed contractile reserve. Females have elevated systolic pressure, no progress into HF	n.d.	Weinberg et al. (1999)
Pathological	PO (SHR)	Rat	Female SHR had normal heart dimensions and function; male SHR LV dysfunction and heart failure	n.d.	Pfeffer et al. (1982)
Pathological	VO (AV Shunt)	Rat	Pro-apoptotic key players such as BAX, caspases 3 and 9 were increased in males and decreased in females	Apoptotic signaling pathway	Dent et al. (2010)
Pathological	VO (AV Fistula)	Rat	Reduced heart failure in females, preserved LV function and chamber size of female hearts	n.d.	Brower et al. (2003) and Gardner et al. (2002)
Pathological	VO (DOCA)	Mouse	Male developed LVH, females maintained their initial physiological adaptive cardiac phenotype	Calcineurin-dependent pathway	Karatas et al. (2008)
Pathological	VO (DOCA)	Mouse	WT males showed a greater degree of LVH than WT females, this effect was diminished in BERKO mice	P38-ERK1/2 pathway	Gurgen et al. (2011)

n.d. not determined

In rodents, the Transverse Aortic Constriction (TAC) has been validated as a reproducible model to study the cardiac response to pressure overload (PO). The TAC model is characterized by a first phase of compensated hypertrophy followed by a transition to HF and mimics human pressure overload-induced heart failure in a number of aspects.

Skavdahl et al. reported that male mice exhibit a significantly higher increase in heart weight-to-body weight ratio than females after TAC. The authors did not specify the form of hypertrophy, i.e., concentric or eccentric (Skavdahl et al. 2005). Other investigators could show in a chronic model of PO that the development of left ventricular hypertrophy (LVH) in WT mice is more pronounced in males than in females. This effect was associated with a greater myocyte hypertrophy and more fibrosis in males (Fliegner et al. 2010; Witt et al. 2008).

PO-induced hypertrophy in male and female Wistar rats led to the observation that males develop a dilated heart accompanied with diastolic dysfunction and elevated wall stress 20 weeks after TAC. In contrast, females develop elevated systolic pressure but they do not progress into HF (Douglas et al. 1998). In a more detailed study with isolated hearts from rats 6 weeks after banding, sex-specific differences in LV contractile reserve and in the genomic response to PO were examined (Weinberg et al. 1999). Higher pressures (contractile force) were observed in females than in males. Despite a similar degree of LVH and systolic wall stress, female hearts had a preserved contractile reserve, whereas male hearts had depressed contractile reserve. The mRNA levels of β-myosin heavy chain (β-MHC) and of the atrial natriuretic factor (ANF) in the ventricular myocardium was greater in male than in female hearts, while the expression of sarcoplasmic reticulum calcium ATPase2A (SERCA2a) was reduced in males and not changed in females (Weinberg et al. 1999).

In spontaneously hypertensive rats (SHR), another model of pressure overload cardiomyopathy, Pfeffer et al. (1982) reported differences in geometric remodeling and an earlier onset of impaired systolic pump performance in male versus female animals. Female SHR (aged 6–18 months) had normal heart dimensions and function whereas male SHR had LV dysfunction and HF by 12 months. When compared with male SHR, female SHR had greater ejection fraction (EF) and cardiac index and smaller end-diastolic and -systolic volumes, despite similar systolic blood pressure values.

A number of animal studies support the anti-hypertrophic effect of estrogens in the myocardium. Long-term E2 treatment of ovariectomized mice after TAC limited the increase in LV mass, ANP, and β-MHC gene expression, while preserving LV chamber size and function (Patten et al. 2008). A different study described similar effects (van Eickels et al. 2001). E2 treatment of ovariectomized female mice caused a reduction of 31 and 26% in PO-induced LVH compared to vehicle-treated animals at 4 and 8 weeks. E2-supplemented animals showed a more pronounced ventricular expression of ANF compared to the vehicle-treated animals. In line with this study, Babiker et al. (2004) demonstrated that E2 exerts profound anti-hypertrophic effects

on ventricular myocytes by transactivation of the ANF gene, and concluded that this mechanism might be responsible for reduction of LVH in female hearts. Another model of PO, the abdominal aortic constriction, performed in ovariectomized female rats exerts also the protective effects of E2 on the development of LVH (Cui et al. 2011). Pathological alterations observed in ovariectomized rats, such as a significant increase of LVH, myocyte diameter and HW/BW ratio, and a decrease of fractional shortening (FS) and EF were largely reversed by administration with E2.

The observed sex differences in animal models support the idea that sex hormones and their sex steroid hormone receptors regulate these cardiovascular responses. Several studies tried to verify the roles of ERα and ERβ in mediating the beneficial effects of E2 using mice with genetic deletion of ERα (ERKO) or ERβ (BERKO). Babiker et al. reported in ovariectomized ERKO mice a significantly reduced ventricular weight comparable to wild type (WT) littermates after addition of E2 (Babiker et al. 2006). This effect was not observed in BERKO mice. Moreover, in BERKO mice, there was a nonsignificant tendency toward hypertrophy when E2 was present, and a tendency toward decreased hypertrophy in the absence of E2. This study supports the hypothesis that E2 has direct, modulating effects on cardiac myocytes and the heart. Similar results were also obtained by the Skavdahl group (2005). ERKO females developed HW/BW nearly identical to that seen in WT littermate females in response to TAC, indicating that ERα is not essential for the attenuation of hypertrophy observed in WT females. In contrast, BERKO females responded to TAC with a significantly greater increase in HW/BW than WT littermate females. These data suggest an important role for ERβ in attenuating the hypertrophic response to PO in females. Another study also supported the involvement of ERβ in the development of cardiac hypertrophy and specified the role of ERβ (Fliegner et al. 2010). The investigators demonstrated that LVH in WT mice is more pronounced in males than in females. This is associated with greater myocyte hypertrophy and more fibrosis in males. The lack of ERβ was leading to an increase of cardiomyocyte hypertrophy and it diminished the observed sex differences in WT mice. It could be assumed that endogenous ERβ acts differently in male and female hearts: while ERβ promotes fibrosis in males, it inhibits fibrosis in female hearts. ERβ limits cardiomyocyte hypertrophy and inhibits apoptosis in both sexes, but with a greater anti-apoptotic effect in male hearts that develop more apoptosis per se. Thus, under PO the loss of ERβ is detrimental for both males and females. These findings indicate that ERβ mediates anti-hypertrophic effects of endogenous E2 in chronic PO. Another study reported demonstrated also that E2 exerts its positive effects via ERβ (Pedram et al. 2008). E2 replacement in female animals inhibited interstitial fibrosis, which was mediated by ERβ.

Taken together, these investigations illustrate the importance of ERβ for the cardiovascular system, in particular for cardiac dimension and its function. ERβ seems to inhibit the development of fibrosis and cardiac growth in both sexes by still unknown mechanism.

2.3 Sex Differences in Models of Pathological Myocardial Hypertrophy: Volume Overload and Mineralcorticoide Excess

Volume overload (VO) leads to eccentric hypertrophy and eventually to HF. There are various models described to achieve VO-induced hypertrophy in an animal model. The most common model is the arteriovenous (AV) *Shunt model*, but also the infrarenal aortoclaval fistula model.

Investigations in rats support the evidence of sex differences during VO. It could be demonstrated that female rats adapt more favorably to VO induced by an infrarenal aortoclaval fistula than male rats do (Gardner et al. 2002). Female hearts developed a concentric hypertrophy with no impairment of the function. This was accompanied by minimal ventricular dilation and no changes in myocardial compliance after 8 weeks of volume-induced dilation. Mortality rate was also higher in males than in females (25 versus 3%), despite a similar degree of VO. The main sex difference in this study was rather the degree of dilation than the degree of hypertrophy. Female hearts develop an appropriate concentric hypertrophy sufficient to maintain a stable compensated state, thus preventing the development of ventricular dilation and HF. In another study, ovariectomy of females abolished these effects and E2 treatment restored the sex-associated patterns of remodeling in this model (Brower et al. 2003).

Furthermore, sex differences in cardiac dysfunction, remodeling, and apoptotic signaling in HF due to VO were identified (Dent et al. 2010). After 4 weeks of AV shunt in rats, both sexes develop cardiac hypertrophy, whereas HF was detected only in males after 16 weeks of AV shunt. Apoptotic key players, such as BAX, caspases 3 and 9 were increased in males and decreased in females at that time point. Phospho Bad was increased and phospho-BCL2 protein was decreased in males. In contrast, females showed an increase only in phopho-BCL2. Ovariectomy abolished this effect and it could be restored by the treatment with E2.

The deoxycorticosterone acetate (DOCA) salt model in rodents serves as a model of secondary hypertension induced by mineralocorticoid excess and volume overload and shows sex differences in blood pressure development. However, independent of blood pressure, a sex-specific dimorphism in cardiac adaptation in response to DOCA-salt was demonstrated in mice (Karatas et al. 2008). Development of LVH in male mice was linked to calcineurin-dependent pathway activation, which increased pro-inflammatory and pro-fibrotic responses. In contrast, female DOCA mice maintained their initial physiological adaptive cardiac phenotype despite mineralocorticoid and salt challenge. In order to investigate the role of ERβ, a follow-up study from the same group was performed (Gurgen et al. 2011). Sex differences, which were observed already earlier in WT mice, were verified also in this study. Furthermore, it could be shown that BERKO mice show another phenotype than WT mice under VO conditions. BERKO females developed the highest HW/TL ratios, exceeding those observed in WT males. Left ventricular wall and septum thicknesses were increased in all of the DOCA animals except for

BERKO females. BERKO female mice instead developed increased left ventricular diameters. In comparison to all other investigated groups, the hypertrophic response in female BERKO mice was accompanied by the highest degree of collagen deposition. Thus, the absence of ERβ in normotensive DOCA-salt mice leads to maladaptive dilative cardiac fibrosis in female mice, implicating a regulatory role of ERβ-related signaling pathways in blood pressure-independent cardiac remodeling processes.

2.4 Sex Differences in Models of Myocardial Injury

Similar to humans (Mehilli et al. 2005), significant sex differences were also found in some animal models of myocardial injury (Table 1). In a model of coronary ligation, female rats showed a concentric form of MH with less cavity dilation and no measurable scar thinning after myocardial infarction (MI), in comparison to male counterparts (Jain et al. 2002). Mice demonstrate a similar sex-dependent response to coronary ligation, such that male mice are more likely to die acutely from cardiac rupture after MI, have significantly worse LV function, greater chamber dilatation and more pronounced cardiomyocyte hypertrophy compared with females (Cavasin et al. 2004; Fang et al. 2007). In a rabbit ischemia/reperfusion (I/R) model, infarct size and apoptotic cell death were significantly attenuated in female rabbits (43.7% and 0.51) compared with males (56.4% and 4.29) (Bouma et al. 2010).

In an in vitro rat model of I/R, Bae and Zhang (2005) observed significantly better postischemic recovery of LV function and smaller infarct size in female (37.1%) than in male (48.3%) hearts. In a similar model, other studies confirmed that isolated perfused female rat hearts have a better recovery and smaller infarct size than male hearts (Brown et al. 2005; Johnson et al. 2006). Mice subjected to I/R demonstrated similar responses, such that female hearts showed improved recovery of contractility ($+dP/dt$) and compliance ($-dP/dt$) and less necrosis in comparison with male hearts (Gabel et al. 2005; Wang et al. 2006c).

All these data provide evidence that female hearts are more resistant to I/R-induced injury and myocardial infarction suggesting that E2 contributes to myocardial salvage after injury in females and to the mechanistic differences between males and females. The cardioprotective effect of E2 in females is supported by the findings that hearts from ovariectomized rodent females exhibits a greater infarct size, reduced functional recovery and myocardial viability in reperfusion, which were reversed by E2 administration (Kolodgie et al. 1997; Liu et al. 2004; Nikolic et al. 2007). Further, a growing body of evidence supports that E2 and/or its receptors are involved in improved myocardial recovery and impairment of myocardial infarct size and cardiomyocyte apoptosis after I/R in different animal models (Booth et al. 2003, 2007; Lee et al. 2004; Nikolic et al. 2007; Patten et al. 2004). For example, experimental administration of E2 reduces the infarct size in rabbit, mice, and rat models (Booth et al. 2003; Hale et al. 1996;

Patten et al. 2004). Administration of the ER antagonist, ICI182 780, dramatically blocked this effect indicating that the E2-induced reduction of infarct size is ER-mediated (Booth et al. 2003; Dubey et al. 2001).

Several studies attempted to determine the role of ERα or ERβ in mediating the beneficial actions of E2 using ERKO or BERKO mice. Under hypercontractile conditions, female BERKO mice exhibit a significantly greater degree of I/R injury than ERKO or WT female mice (Gabel et al. 2005). Babiker et al. (2007) showed that E2 treatment resulted in smaller infarct size in ovariectomized female ERKO mice than in ovariectomized BERKO mice. Pelzer et al. (2005b) showed that deletion of ERβ in ovariectomized female mice subjected to chronic MI increases mortality and aggravates clinical and biochemical markers of HF. These observations support the relevant role for ERβ in mediating an attenuated response in females to MH and HF. In contrast to these studies, other studies reported that the cardioprotective effect of E2 is ERα-mediated. Wang et al. (2006b) showed that female ERKO mice subjected to I/R had a similar recovery of +/− d*P*/d*t* to WT and ERKO males, which was worse than that observed in WT females. In a similar study, it has been demonstrated that the deletion of ERα is associated with more severe cardiac damage following ischemia–reperfusion injury (Zhai et al. 2000). Although these studies utilizing ERKO and/or BERKO mice failed to provide a clear consensus regarding which ER mediates the protection against cardiac injury, nevertheless they suggest that both ER may be involved in cardioprotective function of E2.

To investigate, whether ERα and/or ERβ mediates the beneficial effects of E2, pharmacological studies were also performed using application of ERα- and ERβ-selective agonists (Table 2). Administration of an ERβ agonist, DPN (2,3-bis(4-hydroxyphenyl)-propionitrile), in ovariectomized female mice restored cardioprotection abolished by ovariectomy and resulted in increased functional recovery in postischemic isolated hearts following trauma-hemorrhage (T-H) or isolated perfused hearts (Nikolic et al. 2007; Yu et al. 2006). In a rat model of T-H, Hsieh et al. (2006) reported that E2 as well as DPN, but not PPT [4,4′,4″-(4-propyl-(1H)-pyrazole-1,3,5-triyl) trisphenol; ERα agonist] treatment attenuated the decrease in cardiac mitochondrial ATP and abrogated the T-H-induced lipid accumulation in cardiomyocytes. By contrast to these data reporting the role of ERβ in cardioprotection, there are other studies, which support the cardioprotection effects mediated by activation of ERα. In an in vivo rabbit model of I/R injury, acute treatment with E2 and PPT, but not DPN, resulted in significant reduction of infarct size (Booth et al. 2005). A similar in vivo study with ovariectomized female rats (Jeanes et al. 2008), as well as an in vitro study on isolated perfused ovariectomized female rat hearts (Novotny et al. 2009) showed that acute administration of ERα-agonist significantly reduced the infarct size, neutrophil infiltration, oxidant stress, and necrosis following I/R. In contrast to these studies showing the protective effects by either ERα or ERβ, Vornehm et al. (2009) demonstrated that an acute postischemic treatment with ERα-agonist (PPT) or ERβ agonist (DPN) improves myocardial recovery, indicating both ERα and ERβ are involved in mediating E2-induced rapid cardioprotection following I/R.

Table 2 Cardioprotective effects of ER activation on heart disease

Model	Conditions/treatment	Observed effects	Conclusion from authors	References
Rabbit	Intact, OVX rabbits/LAD occlusion + PPT, DPN, E2, or vehicle	Acute treatment with E2 and PPT, but not DPN significantly decreased infarct size	ERα, but not ERβ, mediates cardioprotective effects of E2	Booth et al. (2005)
Rabbit	OVX rabbits/LAD occlusion + E2, 17α-estradiol or vehicle	E2 led to significantly smaller infarct, rel. normal sarcomere structure and minimal swelling	E2-mediated cardioprotection is mediated by ER	Booth et al. (2003)
Rat	OVX rats/IR + ERα-agonist ERA-45, ERβ antagonist or vehicle	E2 and ERA-45 significantly reduced neutrophil infiltration, oxidant stress and necrosis	ERα mediates the cardioprotective properties of E2	Jeanes et al. (2008)
Mouse	OVX-mice/IR + DPN or vehicle	DPN treatment resulted in better cardiac functional recovery	Chronic treatment of ERβ agonist may confer cardioprotective effects	Nikolic et al. (2007)
Rat	Male rats/T-H + PPT, DPN or vehicle	Only DPN prevented T-H mediated decrease in cardiac output, stroke volume and $\pm$ d*P*/d*t*max	Salutary effects of E2 on cardiac function are mediated via ERβ	Yu et al. (2006)
Rat	OVX-Female SHR or sham + E2, ERα agonist 16α-LE2 or ER antagonists	16α-LE2 or E2 attenuated LVH and increased cardiac output, LV stroke volume and contractility	Activation of ERα affects MH and contractility in OVX SHR	Pelzer et al. (2005a)
Rat	OVX young versus aged female rats/IR + PPT or vehicle	PPT treatment led to reduced infarct size, higher activation of mitochondrial PKCε and pAkt	Acute administration of ERα mediates cardiac protection	Novotny et al. (2009)
Rat	Male rats/T-H + PPT, DPN, E2 or vehicle	Only E2 and DPN normalized cardiac function and mitochondrial gene expression after T-H	ERβ mediates the salutary effects of E2 on cardiac function following T-H	Hsieh et al. (2006)
Rat	Isolated male rat hearts/IR + PPT or DPN	PPT and DPN significantly increased myocardial functional recovery following I/R	Both ERα and ERβ mediate E2-induced rapid cardioprotection following I/R	Vornehm et al. (2009)

Table 3 Sex differences in numerous transgenic mouse models for cardiovascular disease

Transgenic model	Males	Females	References
PLB-KO	DCM at 6 months	Normal EF	Cross et al. (2003)
PLB-OE	Hypertrophy, death at 15 months	No hypertrophy	Dash et al. (2003)
TNFα-OE	HF; increased mortality	Hypertrophy	Kadokami et al. (2000)
PPARα-KO	100 % die early	25 % die early	Djouadi et al. (1998)
HDAC5-OE	100 % die within 7–10 days; mitochondrial dysfunction	Survive during 30 days	Czubryt et al. (2003)
FKPB12-KO	Hypertrophy	No hypertrophy	Xin et al. (2002)

In summary, neither studies using ER deletion mouse models nor studies with ER-agonists did provide a clear answer which ER mediates the effects of E2 in cardiac injury. This discrepancy may be due to the use of different species, different models of injury, different end-points, and different dose and timing of the addition of agonists (Murphy and Steenbergen 2007). However, these studies suggest that the short-term activation of ERα (acute treatment) and long-term activation of ERβ (chronic treatment) are involved in mediating the cardioprotective effects of E2, particularly in females.

3 Sex Differences in Transgenic Mouse Models of Heart Disease

Sex differences in the onset and progression of MH and HF have also been reported in several transgenic mouse models, where the expression of a gene is knocked out or overexpressed (Table 3). Data suggest that male mice are more sensitive than their female counterparts to genetic interventions leading to pathological hypertrophy and HF (Du 2004). Disruption of FKBP12.6 gene, a sarcoplasmic reticulum (SR) protein, which regulates ryanodine Ca^{2+} release channels in cardiomyocytes, results in cardiac hypertrophy in male mice but not in females (Xin et al. 2002). However, female FKBP12.6 knockout mice treated with tamoxifen, an ER antagonist, develop cardiac hypertrophy to a similar level as observed in male mice (Xin et al. 2002). These findings suggest that E2/ER play a protective role in the hypertrophic response of cardiomyocytes to FKBP12.6 deletion. Phospholamban (PLB) is also a SR protein which modifies the activity of the cardiac SR Ca^{2+}-ATPase (SERCA2a) by reducing the affinity for Ca^{2+}. Cross et al. (2003) showed that ablation of PLB exacerbates ischemic injury to a lesser extent in female than male mice. Interestingly, male mice with fourfold overexpression of PLB exhibit ventricular hypertrophy and mortality at 15 months, whereas females do not show these phenotypes at this age (Dash et al. 2003).

Genetic modulation of central molecules in energy metabolism leads also to sex-specific cardiac phenotypes. Peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor implicated in the control of cellular lipid utilization. Disruption of PPARα gene (PPARα-KO) caused massive cardiac lipid accumulation and death in 100% of male, but only in 25% of female PPARα-KO mice. Interestingly, the metabolic phenotype of male PPARα-KO mice was rescued by a pretreatment with E2 (Djouadi et al. 1998). Male doxycycline-regulated transgenic mice that overexpressed a histone deacetylase 5 mutant (HDAC5S/A) specifically in the heart showed also severe cardiac phenotypes (Czubryt et al. 2003). Transgene expression resulted in sudden death in male mice accompanied by loss and morphological changes of cardiac mitochondria and downregulation of mitochondrial key enzymes, such as PGC1α and MEF2A.

Sex differences have also been documented in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine. In this mouse model (TNF1.6), males exhibit HF and increased mortality compared to females (Janczewski et al. 2003; Kadokami et al. 2000). These differences appear to be attributable to the sex-related expression of TNFα within the myocardium. As hearts from E2-deficient rats subjected to I/R have a marked increase in TNFα levels and an E2 replacement reduced TNF levels in LV myocardium and decreased its release after I/R, which was accompanied by improved functional recovery and a decrease in markers of tissue injury and apoptosis (Xu et al. 2006). Furthermore, E2 replacement was associated with increased expression of TNFα-receptor 1. These observations suggest that E2 may have cardioprotective effects, in part, by inhibiting the expression of cardiac TNFα and modulating TNFα receptors expression (Xu et al. 2006).

4 Potential Molecular Mechanisms Involved in the Sex-Based Differences in the Physiology and Pathophysiology of Myocardium: The Role of E2/ER

During recent years, a large number of studies have been performed to assess the molecular and cellular mechanisms associated with sex differences in cardiac physiology and pathology. Different mechanisms and distinct pathways for these sex differences have been proposed. This review focuses on recent findings from animal models relevant for studying the major molecular mechanisms of sexual dimorphisms in the healthy and diseased heart, and highlights the relevant signaling pathways by which E2 and ER affect the cardiac response to mechanical loads.

4.1 Molecular Mechanisms Involved in Physiological Hypertrophy

To identify the mechanisms underlying the sexual dimorphic cardiac response to exercise, Konhilas et al. (2004) examined multiple signaling pathways involved in the development of cardiac hypertrophy in a voluntary exercise mouse model. In trained hearts from females, Ca^{2+}/calmodulin-dependent protein kinase (CaMK) was found to be significantly higher compared with males. Furthermore, females showed a persistent phosphorylation of glycogen synthase kinase 3β (GSK-3β) indicating an inactivation of this anti-hypertrophic factor and stimulation of heart growth (Antos et al. 2002; Haq et al. 2000). Sex differences in the phosphorylation of GSK-3β in trained female hearts could be due to E2 signaling through ER as suggested by studies in the nervous system (Garcia-Segura et al. 2006; Mende et al. 1983; Mendez et al. 2003, 2006). In neuronal cells, E2-induced association of ERα with the insulin-like growth factor-1 receptor (IGF-1R) leads to activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway resulting in phosphorylation and therefore inactivation of GSK-3β (Varea et al. 2010). Data from Foryst-Ludwig et al. (2011) showing increased phosphorylation of Akt only in female hearts and higher increase of LVM compared to males in a forced exercise model emphasize the sex-specific activation of this pathway in physiological myocardial hypertrophy. Studies with transgenic mouse models demonstrated the critical role of the IGF-1R-PI3K-Akt pathway in the physiological cardiac growth (Bernardo et al. 2010). Mice with a cardiac myocyte-specific deletion of the IGF-1R gene attenuated the hypertrophic cardiac response to swim exercise compared to intact mice (Kim et al. 2008). In contrast, transgenic mice overexpressing the IGF-1R in cardiac myocytes displayed cardiac hypertrophy due to increased cardiomyocyte size without necrosis or fibrosis and elevated activation of PI3K and Akt pathway (McMullen et al. 2004). Further studies with mice expressing a cardiac-specific dominant negative form of the catalytic p110α subunit or gene deletion of the regulatory subunit p85 of PI3K, demonstrated that PI3K is also critical for exercise-induced physiological myocardial hypertrophy (Luo et al. 2005; McMullen et al. 2007). Akt, existing in three isoforms (Akt1, Akt2, and Akt3), is a well-known target of PI3K and is also majorly involved in cardiac growth as indicated by studies with Akt transgenic mice (Bernardo et al. 2010). Akt1 gene knockout mice subjected to swim training showed no induction of cardiac hypertrophy, suggesting that Akt1 is required for physiological heart growth (DeBosch et al. 2006). Taken together, it can be assumed that the E2/ER modulation of the IGF-IR-PI3K-Akt signaling pathway in the female heart may be one possible mechanism underlying the sexual dimorphic cardiac response to exercise.

Furthermore, Foryst-Ludwig et al. showed that the hypertrophic response in female mice was associated with increased expression of genes involved in fatty acid uptake and oxidation compared with male mice. They suggest that sex differences in exercise-induced myocardial hypertrophy are also associated with changes in cardiac substrate availability and utilization between males and females (Foryst-Ludwig et al. 2011).

4.2 Molecular Mechanisms Involved in Pathophysiology of MH and HF

The PI3K pathway and its downstream target, Akt, is also one of the best characterized signaling pathways involved in sex-related differences in the development of pathological cardiac hypertrophy. This pathway is involved in a number of physiological responses, including cell survival, gene expression and metabolism (Camper-Kirby et al. 2001). Bae and Zhang showed that there are significant increases in phospho-AKT (p-AKT) and phospho-protein kinase Cε (p-PKCε) levels during reperfusion in female but not in male hearts (Bae and Zhang 2005). They suggested that the increase in p-AKT and p-PKCε levels is likely to play an important role in protecting female hearts and to contribute to the sex-related differences in cardiac susceptibility to I/R injury. As inhibition of PI3K/Akt pathway or PKC before ischemia significantly reduced postischemic recovery and increased infarct size in female rat hearts. In agreement with this study, Bell et al. (2008) also showed that female hearts have increased Akt expression/activity. As a cardioprotective mechanism, Camper-Kirby et al. showed that the localization of phospho-Akt^{473} in myocardial nuclei and Akt kinase activity in nuclear extracts are elevated in female mice hearts versus males (Camper-Kirby et al. 2001). The activation of AKT in a sex-dependent manner may help to explain different susceptibility to cardiovascular disease and support the beneficial role of E2. Another evidence for the beneficial role of E2 in promoting Akt signaling has been shown in cultured rat cardiomyocytes (Camper-Kirby et al. 2001). E2 enhances the accumulation of phospho-Akt^{473} in the nucleus of cultured rat cardiomyocytes, which in turn increases the phosphorylation of forkhead, a pro-apoptotic transcription factor and thus its enhanced translocation from the nucleus to the cytoplasm. Patten et al. (2004) also demonstrated that E2 increases the activation of Akt and reduces the apoptosis in murine cardiomyocytes both in vivo and in vitro. These findings illustrate the importance of the PI3K/Akt signaling pathway in the pro-survival effects of E2 during pathophysiology of the heart, which may partially account for observed sex differences in the myocardial responses to injury. These effects of E2 might be mediated by ER interacting directly with the PI3K. Wang et al. (2009) showed that ERβ, probably through binding to PI3K, increased the PI3K/Akt activation, subsequently decreased caspase-3 and -8, and increased Bcl-2 expression in female hearts. On the other hand, Simoncini et al. (2000) showed that the ligand-activated ERα binds to the p85α regulatory subunit of PI3K in endothelial cells through a nongenomic mechanism by which E2 rapidly activates the ERα-associated PI3K/AKT pathway and endothelial nitric oxide synthase (eNOS).

It has been reported that eNOS plays an important role in the sex-specific cardioprotection. The expression of eNOS is higher in female than in male rodent hearts, and NOS inhibitor (L-NAME) abolishes sex differences in cardiac susceptibility to I/R (Cross et al. 2002; Wang et al. 2006a). One mechanism for the cardioprotective effect in female hearts could be due to the known inhibitory effect of eNOS on Ca^{2+} channel activity and thus reduction of cytosolic Ca^{2+} overload (Cross et al. 2002),

one of the main causes of I/R injury. NOS enhances the S-nitrosylation of the L-type Ca^{2+} channel resulting in a decreased activity of the channel. Sun et al. (2006) showed that the *S*-nitrosylation of the L-type Ca^{2+} channel is increased in female WT hearts following I/R, which led to the reduced Ca^{2+} entry and sarcoplasmic reticulum loading, and thus into the reduced heart injury. Several studies showed that E2 stimulates the expression of eNOS in cardiomyocytes in vivo and in vitro. E2 increases the expression/production of eNOS/NO during I/R resulting in reduction of infarct size and myocardial injury (Node et al. 1997), suggesting that females may be protected at least partly via a NOS-mediated mechanism. In this regard, it has been shown that ovariectomy decreases eNOS level and increases the expression of L-Type Ca^{2+} channel in rat hearts, while a treatment with E2 reversed this effect (Chu et al. 2006; Nuedling et al. 1999). Similarly, E2 treatment of guinea pig cardiac myocytes reduces the Ca^{2+} current and intracellular Ca^{2+} concentration (Jiang et al. 1992). This effect of E2 is apparently mediated by ER, since cardiomyocytes from ERα-deleted mice (ERKO) showed an increased expression and activity of L-type Ca^{2+} Channel (Johnson et al. 1997). Moreover, Lin et al. (2009) showed that chronic E2 treatment and activation of ERβ by DPN treatment lead to increased NOS/NO signaling and cardioprotection against ischemia/reperfusion injury. All these findings suggest that E2/ER may also exert its beneficial effects by acting on Ca^{2+} channel activity in a NOS-dependent manner, thus providing protection against I/R injury.

Sex differences have also been observed in activation of the mitogen-activated protein kinase signaling pathway in pathological hypertrophy. Females are relatively protected against cardiac injury, also possibly due to a less activation of the p38 MAPK signaling pathway (Wang et al. 2005). Because it has been reported that E2 decreases the activation of p38 MAPK (Angele et al. 2003; Wang et al. 2006c), which is ERα-mediated (Wang et al. 2006b), therefore the sex-specific regulation of p38 MAPK might be, in part, responsible for the sexually dimorphic response following cardiac injury.

It has been reported that the activation of p38 and ERK signaling was sex-specifically regulated in DOCA-induced hypertrophy (Gurgen et al. 2011). Male animals showed only minimal p38 MAPK phosphorylation, whereas WT females had strikingly high levels of phosphorylated p38 MAPK. In contrast to all of the groups of male mice with moderate amounts of phosphorylated ERK1/2, WT females showed increased and female DOCA WT mice had very high levels of phosphorylated ERK1/2. Both p38 and ERK1/2 phosphorylation was greatly reduced in female BERKO mice. These findings show explicitly the sex dimorphism in the p38 and ERK1/2 signaling pathway and the involvement of ERβ.

In a model of VO-induced hypertrophy, the arteriovenous (AV) fistula or shunt model could show not only a sex-specific remodeling, with a greater degree of cardiac hypertrophy and larger increase in cardiac output in male than in female animals, but also a sex-specific signaling in the β-adrenoceptor system (b-AR) (Dent et al. 2011). Increases in plasma levels of the catecholamines norepinephrine and epinephrine due to AV shunt were also higher in males than in females. There was no difference in the b1-AR affinity between the sexes observed. But AV shunt induced an increase in b1-AR density higher in female rats than that in males. While

these data demonstrate sex-associated differences in various components of the b-AR system in cardiac hypertrophy due to AV shunt, only higher levels of plasma catecholamines may account for the greater increase in cardiac output and higher degree of cardiac hypertrophy in males.

Not only during VO-induced hypertrophy, also in PO-induced hypertrophy, it was demonstrated that p38 and ERK signaling plays an important role in the development of LVH and being sex-specifically regulated (Cui et al. 2011) The ERK1/2 signaling pathway and also caveolin-3 are important key players in the pathogenesis of hypertension-induced cardiac hypertrophy and it could be shown that E2 attenuates the development of cardiac hypertrophy. In female ovariectomized rats after induction of PO, pathological alterations of cardiac function and dimension were observed. These effects were accompanied with the enhanced expression of ERK1/2 and decreased expression of caveolin-3 in the left ventricle. E2 treatment reversed these alterations. The treatment with E2 restored the levels of caveolin-3 expression and of ERK phosphorylation in these pressure-overloaded rats (Cui et al. 2011). These data indicate that the protective effect of E2 against cardiac hypertrophy induced by PO is mediated by upregulation of caveolin-3 expression and downregulation of ERK1/2 phosphorylation.

Sex differences have been also observed in the cardiac inflammatory response to acute myocardial injury. Female's protection against cardiac injury could be possibly due to a decreased inflammatory cytokine production, e.g., decreased myocardial TNF-α, IL-1, and IL-6 expression (Fang et al. 2007; Wang et al. 2005; Xu et al. 2006). Xu et al. (2006) demonstrated that an increase of TNF-α production after I/R correlated with declined circulating E2 levels in E2-deficient female rats, while E2 replacement reduced TNF-α production and release in LV myocardium after I/R. These data suggest that the sex differences in myocardial inflammation during acute cardiac injury are partly mediated by E2 through regulation of TNF-α levels in the ischemic heart.

Sex differences in cardiac remodeling have also been documented in rodent (Cavasin et al. 2004; Fang et al. 2007). Female hearts show a better cardiac outcome during the development of HF due to a lower rate of cardiac remodeling and thus reduced risk of rupture compared to male hearts. The reduced activities of matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) may represent the molecular mechanism underlying the lower risk of rupture in female hearts (Cavasin et al. 2004; Fang et al. 2007). Additionally, Cavasin et al. (2004) suggested that the mechanisms underlying these differences may be related in part to relative concentrations of collagen type I/III, since E2 treatment prevents increase of collagen type I/III ratio in old female rats (Xu et al. 2003). The effect of E2 in this study is in agreement with the findings from other studies which showed that E2 reduces the expression of collagen I, III and MMP-2 in female rat cardiac fibroblasts (Mahmoodzadeh et al. 2010; Petrov et al. 2010).

It has also been observed that the cardioprotection associated with female sex was accompanied by a greater protein expression of the sarcolemmal and mitochondrial ATP-sensitive potassium (KATP) channels; their blockade during the ischemia increased the degree of injury in the female heart (Johnson et al. 2006;

Lee et al. 2000; Ranki et al. 2001). Thereby, the female sex hormone E2 seems to play an important role. Ranki et al. showed that E2 treatment increases sarcolemmal KATP channel expression and protects cardiac cells from hypoxia re-oxygenation; and that KATP channel antagonist abolished the protection afforded by E2 (Ranki et al. 2002). These data indicate that sarcolemmal and mitochondrial KATP channels may be involved in mechanisms that underlie sex differences in the susceptibility of the heart to I/R injury, which is partly regulated by E2.

5 Conclusions and Clinical Implications

Multiple epidemiological and clinical studies indicate that the predictors and progression to heart diseases are often sex-sensitive. Experimental animal studies have also shown that males and females often differ in their biological responses to cardiac mechanical loads and pharmacological interventions. Although, there are obvious sex differences in the cardiovascular physiology and pathology, most studies fail to include both sexes, and only a limited number of animal researches include female subjects or differentiate between sexes in the data analysis. These might be the reason that nowadays our understanding of molecular and cell-based mechanisms underlying sex-based differences in cardiovascular system is incomplete. Therefore, we do need to take into account sex differences in designing our investigations, and they should also be considered by the selection of optimum diagnostic and therapeutic procedures in clinical practice.

Take Home Messages

- Research on female and male animals is important to understand the mechanisms underlying sex-based differences in the development of cardiac diseases.
- Animal studies allow identifying factors/pathways that confer female's cardioprotection in physiological and pathological hypertrophy, which could help to find appropriate therapeutic targets to inhibit pathological pathways and activate physiological regulators in both sexes.
- Learning from the beneficial effects in females could help to build strong foundations for novel sex-sensitive strategies for drug therapies.
- Female hormone E2 must interfere with a large number of pathways, such as PI3K/AKT, p38MAPK, NO, sarcolemmal and mitochondrial channels and Ca^{2+}-signaling. Identification of these protective pathways could also offer novel therapeutic aspects.

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Sex Differences at Cellular Level: "Cells Have a Sex"

Elisabetta Straface, Lucrezia Gambardella, Marta Brandani, and Walter Malorni

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Abstract Different pathways involved in the complex machinery implicated in determining cell fate have been investigated in the recent years. Different forms of cell death have been described: apart from the "classical" form of death known as necrosis, a well characterized traumatic injury of the cell, several additional forms of cell death have been identified. Among these, apoptosis has been characterized in detail. These studies stem from the implication that the apoptotic process plays

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a key role in a plethora of human pathologies, including cardiovascular diseases. In fact, defects in the mechanisms of cell death, i.e., both an increase or a decrease of apoptosis, have been associated with the pathogenesis of vessel and myocardial diseases. Some new insights also derived from the study of autophagy, a less characterized form of cell damage mainly associated with cell survival strategies but that also leads, as final event, to the death of the cell. Interestingly, very recently, a gender difference has been found in this respect: cells from males and females can behave differently. In fact, they seem to display several different features, including those determining their fate. These gender cytology differences are briefly described here. The study of this gender disparity is of great relevance in cardiovascular disease pathogenesis and pharmacology. The comprehension of the gender-related mechanisms of cell demise can in fact disclose new scenarios in preclinical and clinical management of cardiovascular diseases.

Keywords: Aautophagy • Apoptosis • Cell • Cytopathology • Preclinical studies • Reactive oxygen species • Sex

Abbreviations

Atg	Autophagy-related genes
CVDm	Cardiovascular diseases
E2	Estradiol
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
ER	Estrogen receptor
H_2O_2	Hydrogen peroxides
JNK	Jun N-terminal kinase
$O^{\bullet -}$	Superoxide anion
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
SLE	Systemic lupus erythematosus
TNF	Tumor necrosis factor
VSMC	Vascular smooth muscle cells

1 General Features of the Cells and Gender

As a general rule, different cell types from both males and females show the same main features and/or programs: they can proliferate, undergo differentiation, senescence and, ultimately, death. These main processes are detectable for all the cells from different organs as well as for cells belonging to different histotypes. Vessel cells, neuronal cells, epithelial cells and all the various cell types that form our organs have been studied in the course of the last years in order to precisely assess cell cycle features, cell differentiation pathways and cell death programs but, strikingly, very

Table 1 Cultured cells of widespread use in preclinical studies

Cell "name"	Cell type	Sex	Isolation date
Jurkat	Lymphoid cells	Male	1970
CEM	Lymphoid cells	Female	1964
Hep-2	Epidermoid carcinoma	Male	1952
Hela	Epidermoid carcinoma	Female	1951
U937	Lymphoid cells	Male	1974
NCI-H292	Mucoepidermoid carcinoma	Female	1985
Vero	Kidney (monkey)	Unknown	1962
SH-SY5Y	Neuroblastoma	Female	1970
PC12	Pheochromocytoma (rat)	Male	1976

All these stabilized cell lines derive from tumors

few information has been acquired so far as concerns gender (or sex) of the cells, i.e., in XX and XY cells. Hence, the gender-determinant has very poorly been investigated and this is probably due to difficulties in the establishment of gender-cytology models capable of providing specific information about the role of cell sex in the above issues. In fact, the analysis of cell proliferation and death pathways in XX and XY cells needs valuable model systems that are still lacking or are at the harbor of their potential usefulness. The great majority of the pathways we know derives from the studies on established cell lines, some of them dated more that 50 years ago, generally derived from tumors and considered for their features but irrespective from their origin, i.e., from males or females. Only more recently the use of freshly isolated cells gained the attention of cell pathologists and some of the data obtained on gender cytopathology derived from the studies on these cells, mainly from animals but also, in some instances, from humans. However, we have also to underline that the gender perspective has poorly been considered from a cultural point of view and cells, mainly isolated cells, that provided a mass of data on the genetic and metabolic pathways driving the lifespan of the cells, have often been considered as sexually undetermined objects of study. In Table 1, some cultured cell types that provided information of cell life and death are listed. To note, the gender issue is out of the possibilities of these models. In fact, the high number of subseedings of these cultured cells lead to the establishment of cells with an aberrant chromosomal content, e.g., polyploid cells, with peculiar metabolic pathways and surface molecule expression, high capability to proliferate (a great advantage for cell pathology studies but often giving rise to misleading), and a plethora of altered functions with respect to their original features, including those associated with their "sexual" origin. It was shown that after the first 5–15 subseedings cells can lose at least some of their features such as the expression of key receptors, e.g., hormones and growth factor receptors, and can modify their growth features and metabolic needs. In this complex scenario, however, some research groups took advantage from the study of cell from peripheral blood. Although still at the beginning, generally due to the lack of interest, several lines of evidence have in fact demonstrated that cells freshly isolated from peripheral blood (lymphocytes, granulocytes but, also, platelets and red blood cells) can provide important information not only, as conceivable, for cell pathology per se, but also in terms of gender disparity.

Table 2 The memory of the cells: the birth of "cell sex"

Cell type	Species	No. of in vitro passages (with "memory" of cell sex)	Elective pathology
Fibroblasts	Human, rat, mouse	About 10	Cardiovascular, autoimmune
Vascular smooth muscle cells (VSMC)	Human, rat, mouse	About 10	Cardiovascular, gastroenterology
Resting lymphocytes	Human	–	Immune system and inflammatory diseases
Platelets	Human	–	Hematological, neurodegenerative diseases
Red blood cells	Human	–	Hematological
Freshly isolated cancer cells	Human, mouse	10–15	Experimental chemotherapy
Mouse embryo fibroblasts (MEFs)	Mouse	10–20	Mechanisms of drug toxicity
Keratinocytes	Human	10–12	Dermatology
Neuronal cells	Mouse, rat	–	Neurodegenerative
HUVEC	Human	About 10	Vascular

In this table we list the cell type, the species from which cells have been originated, the putative number of subseedings in which the cells maintain their "sex" (chromosomes, hormone receptors, etc.), and finally the pathologies in which the different cells represent an important model of study

The pathogenetic mechanisms underlying several diseases display significant gender differences also at cellular level, e.g., autoimmune diseases. Some of these are summarized in Table 2 where a list of diseases that shows a different incidence in the two sexes, a different prognosis or a different clinical outcome has been reported. Only in some cases, very few, the possible gender-associated pathogenetic differences have been investigated or, at least, hypothesized. Key issue in this respect is represented by the studies on the implication of cell disturbances in a gender perspective. For example, the studies on the role of hormones in proliferation of some cancers or the induction of cell death in some degenerative diseases, including vascular diseases, could be considered as paradigmatic. In addition, since the alteration of the balance between cell proliferation and cell death is at the basis of the pathogenesis of a plethora of human diseases, some efforts have recently been made in order to acquire some gender-specific information on this important issue, i.e., cell death processes.

2 Apoptosis

Apoptosis, also called type 1 programmed cell death, is an energy-dependent, genetically determined process by which cells self-destruct. The early phases of this process are characterized by cytoplasmic shrinkage, compaction and

aggregation of chromatin that produce the condensed, contracted nuclear and cytoplasmic bodies evident at later stages. Apoptosis can occur after the engagement of tumor necrosis factor (TNF) family receptors (CD95/Fas, TNF-α, TRAIL, etc.) or as a result of the direct targeting of certain drugs (e.g., staurosporine) on the mitochondria, and proceeds through the activation of a complex cascade of specific proteases, caspases, or other lysosomal proteases, such as cathepsins, to the cell demise.

It is well known that disturbances of apoptosis play a key role in the pathogenesis of a variety of diseases including cardiovascular diseases (CVD) (see below). The death receptor Fas is expressed throughout the vessel wall and it is involved in apoptosis of vascular cells. In fact, increasingly Fas–Fas ligand-induced killing has been recognized in the vasculature. Accordingly, many chronic inflammatory diseases, including myocarditis, are attenuated in mice lacking this molecule. Elevated circulating levels of TNF-α, which is the ligand of TNF receptor 1, have been demonstrated in CVD (Dutka et al. 1993; Levine et al. 1990; Pober et al. 2009). Hence, cardiovascular cells are known to express TNF receptor 1 on their cell surface and are sensitive to TNF-α-induced apoptosis.

Reactive oxygen species (ROS, see below) contribute to different phases of the apoptotic pathway, such as the induction of mitochondrial permeability transition and release of mitochondrial death apoptogenic factors, activation of intracellular caspases and DNA damage in all cell types, including cells from the cardiovascular system. ROS-induced oxidative stress plays a role in various CVD such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy, and congestive heart failure. Although the death receptor and mitochondrial pathways are the most studied apoptotic pathways, other less well-defined signaling mechanisms, such as endoplasmic reticulum stress-mediated apoptosis, exist in cardiovascular system (Mizushima and Komatsu 2011).

Apoptotic cell death is observed in a variety of CVD. Cardiomyocyte apoptosis occurs in end-stage heart failure and may contribute to heart failure in a variety of situations (Clarke et al. 2007). In myocardial infarction myocytes initiate apoptosis in response to ischemia, but, owing to the energy demands of the program and the dwindling respiratory capacity of the cells, apoptosis is stalled. Therefore, during prolonged ischemia, myocytes shift from an initial apoptotic to a necrotic fate while, following reperfusion, oxygen is restored allowing apoptosis to complete. The fate of myocyte is decided by the level of adenosine triphosphate (ATP), a factor required for completion of apoptosis in most cells. Thus, in acute infarction both forms of cell death, apoptosis, and necrosis are detectable. Arterial aneurysms are also cited as a classic apoptosis-driven cardiovascular pathology and are typified by vascular smooth muscle cells—poor medial regions that display evidence of degradation and fragmentation of the internal elastic lamina and collagen-rich matrix (Lopez-Candales et al. 1997). Finally, endothelial cell apoptosis may be an important mechanism of vascular injury leading to the disruption of the endothelial barrier with vascular leak, extravasation of plasma proteins, and exposure of the prothrombotic subendothelial matrix (Winn and Harlan 2005; Mallat and Tedgui 2000). Accumulating evidence suggests that endothelial cell apoptosis could play a critical role as an initial pathogenic event in atherosclerosis.

Although many aspects of CVD are similar in women and men, there is a growing body of evidence to support sex dimorphisms in the incidence, presenting symptoms, management, and outcomes of CVD (Pilote et al. 2007; Vitale et al. 2009). The higher incidence of CVD in men than in women of similar age and its increase with the onset of menopausal in women have suggested that gender-related differences in sex steroid hormones play a key role in the development of CVD. However, the great majority of studies failed to demonstrate a definitive correlation between the total levels of circulating sex hormones or their metabolites and the degree of CVD both in men and postmenopausal women. It has been only recently realized that genes show variation in their expression and action in men and women. Sex-specific transcriptional regulation could be due to different growth hormone and sex hormone profiles in the two sexes, which can affect the expression of numerous genes, and these genes in turn affect the transcriptome of many tissues. Originally it was thought that most of the genes on the Y chromosome contribute to male differentiation, but it is now clear that they may also be responsible for differences in blood pressure and stress responses between males and females (Charchar et al. 2004; Ellis et al. 2000). The X chromosome may play an important role in hypertension, cardiovascular malformation, dilated cardiomyopathy, renal disease, and Turner syndrome (Pilote et al. 2007). Sex-specific genetic architecture of human traits contributing to CVD is a result of interplay between sex and autosomal chromosomes. For instance, a polymorphism in the angiotensin II receptor has been associated with hypertension in women but not in men. Moreover, this female-specific association was more pronounced in premenopausal than in postmenopausal women (Jin et al. 2003). Gender differences in lifestyle, which can be important risk factors for CVD, are out of the aims of this article. A good example of gender disparity is the risk of cardiovascular morbidity and mortality in some autoimmune diseases, e.g., by rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (with a gender-dependent incidence characterized by a male/female ratio respectively of 1:3 and 1:9). Cardiovascular events are the first cause of mortality either in SLE or in RA (Salmon and Roman 2008). Compared with expected mortality rates in the normal population, women with SLE and RA have a significantly more compromised life expectancy than men. The high CVD risk has a double origin: an early and progressive atherosclerosis and a prothrombotic propensity. Multiple factors are incriminated, including a higher prevalence of traditional CVD risk factors in SLE and RA population, as well as specific factors such as autoantibodies. In fact, vascular damage and endothelial dysfunction in autoimmune diseases are often associated with the presence of anti-endothelial cell antibodies (Margutti et al. 2007; Praprotnik et al. 2001). These autoantibodies are specific to surface endothelial cell components such as proteins or phospholipids. Different pathophysiological effects have been observed that include direct or indirect cytotoxicity and endothelial cell apoptosis (Tobon et al. 2009). However, the molecular mechanisms of anti-endothelial cell antibodies-mediated damage to host vascular system are scarcely defined. In conclusion, anti-endothelial cell antibodies seem to overcome the effects of sex hormones on endothelial cells, leading to an increased incidence of CVD in women affected by SLE or RA.

Female and male sex hormones directly affect cardiac function, endothelial function, and vascular tone through both genomic and nongenomic effects that are mainly receptor-dependent (Mendelsohn and Karas 2005). Sex steroid hormone receptors do not act alone, but interact with a broad array of coregulatory proteins to alter transcription. However, little is known about the differential expression and function of coregulatory molecules in myocardial and vascular cells. Most research has focused on the effects of estrogens and estrogen receptors (ER) on cardiovascular physiology and disease, whereas androgens and their receptor have received far less attention. Androgens exert their effects either by direct activation of the androgen receptor or trough local aromatization into estradiol. A consistent gender difference in vascular tissue content of ER and androgen receptor does exist (Mendelsohn and Karas 2005). Genetic sex steroid hormone receptor variants influence individual responses to sex hormones and are associated with altered cardiovascular risk in both sexes (Herrington 2003; Shearman et al. 2003), but the physiological consequences of such variants are unexplored.

3 Autophagy

The term autophagy defines a catabolic process regulating the degradation of a cell's own components through the lysosomal machinery (Mizushima and Komatsu 2011). Autophagy is a genetically regulated process that plays an important homeostatic role in cells, preserving the balance between the synthesis, degradation, and subsequent recycling of cellular components. The term "autophagy," derived from the Greek and meaning "eating of self," was first coined by Christian de Duve over 40 years ago, and was largely based on the observed degradation of mitochondria and other intracellular structures within lysosomes (Deter and De Duve 1967). Autophagy is an evolutionarily conserved and strictly regulated lysosomal pathway that degrades cytoplasmic material and organelles. It is activated during stress conditions such as metabolic stress, amino acid starvation, unfolded protein response, or viral infections (Eskelinen 2008; Kundu and Thompson 2008). It has been suggested that autophagy, limiting necrosis and inflammation, inducing cell cycle arrest and preventing genome instability, could prevent tumorigenesis (Karantza-Wadsworth et al. 2007).

Currently, at least 32 different autophagy-related genes (Atg) have been identified by genetic screening in yeast, an important source of information, and, significantly, many of these genes are conserved in slime mold, plants, worms, flies and mammals, emphasizing the importance of the autophagic process in responses to metabolic stress across phylogeny (Nakatogawa et al. 2009). There are three defined types of autophagy: macro-autophagy, micro-autophagy, and chaperone-mediated autophagy, all of which promote proteolytic degradation of cytosolic components at the lysosome.

Macro-autophagy delivers cytoplasmic cargos to the lysosome through the intermediary of a double-membrane-bound vesicle, referred as to autophagosome,

which fuses with the lysosome to form an autolysosome. In micro-autophagy, by contrast, cytosolic components are directly taken up by the lysosome itself through invagination of the lysosomal membrane. Both macro and micro-autophagy are able to engulf large structures through both selective and nonselective mechanisms. In chaperone-mediated autophagy targeted proteins are translocated across the lysosomal membrane in a complex with chaperone proteins (such as Hsc-70) that are recognized by the lysosomal membrane receptor lysosomal-associated membrane protein 2A, resulting in their unfolding and degradation (Saftig et al. 2008).

After induction by a metabolic stress signal, such as nutrient deprivation, the first step in macro-autophagy is the formation of an autophagosome. A flat membrane cistern elongates and wraps itself around a portion of cytoplasm or a specific cargo, forming a double-membrane-bound autophagosome. This membrane cistern is called phagophore, or isolation membrane. The origin of this membrane cistern is still under debate. Probably, it may origin from plasma membrane or endoplasmic reticulum. Autophagosomes next receive lysosomal constituents, such as lysosomal membrane proteins and proton pumps, by fusion with late endosomes or multivesicular bodies. Finally, autophagosomes fuse with lysosomes. The general features and the methodological approaches to analyze autophagy have recently reviewed by Mizushima and Komatsu (2011).

Very important is the role that autophagy pathway plays in several diseases, including CVD. Although altered autophagy has been observed in various heart diseases, including cardiac hypertrophy and heart failure, it remains unclear whether autophagy plays a beneficial or detrimental role in these diseases (Gottlieb and Mentzer 2010; Gurusamy and Das 2009). In the heart, autophagy is important for the turnover of organelles at low basal levels under normal conditions and it is upregulated in response to stresses such as ischemia/reperfusion and in cardiovascular diseases such as heart failure. Tissue-specific deletion of *ATG5* in the heart causes cardiac hypertrophy and contractile dysfunction (Nakai et al. 2007). In addition, increased levels of ubiquitinated proteins and abnormal mitochondria are found, especially after treatment with pressure overload or β-adrenergic stress. This suggests that autophagy is needed in the heart to ensure the availability of sufficient energy substrates and to control cardiomyocyte size and global cardiac structure and function. In fact, autophagy appears to play a protective role in cardiomyocytes: enhancing autophagy by Beclin-1 overexpression reduces Bax activation (a pro-apoptotic molecule) and protects against ischemia/reperfusion injury (Hamacher-Brady et al. 2006). A decrease in autophagy at the hypertrophied state facilitates cardiac hypertrophic response. A further point concerns cardiomyopathy. In patients with terminal heart failure, secondary to ischemic cardiomyopathy or dilated cardiomyopathy, cellular degeneration with granular cytoplasmic ubiquitin inclusion was detected (Knaapen et al. 2001). In human failing hearts with idiopathic dilated cardiomyopathy, the prevalence of autophagic, apoptotic and necrotic cells have also been observed (Kostin et al. 2003). In animal models, dead and dying cardiomyocytes showing characteristics of autophagy have been as well observed (Akazawa et al. 2004). Cardiomyocytes obtained from UM-7.1 hamsters, which is the model of human dilated cardiomyopathy, contain typical

autophagic vacuoles, including degraded mitochondria, glycogen granules and myelin-like figures (Miyata et al. 2006). However, the question remains as to whether autophagy is a sign of failed cardiomyocyte repair or is a suicide pathway for the failing cardiomyocytes.

Several further actors seem to play a role in autophagy pathway in heart disease. For instance, it has been shown that oxidative stress, endoplasmic reticulum stress and changes in the ubiquitin–proteasomal system are intimately involved in the regulation of autophagy (Kim et al. 2007; Kitsis et al. 2007). Conversely, pro-apoptotic factors can be released from the damaged mitochondria, leading to apoptotic cell death (Gustafsson and Gottlieb 2003). In particular, in the absence of autophagy, the accumulation of polyubiquitinated proteins may be responsible for increased endoplasmic reticulum stress, and determine cell death by apoptosis (Nakai et al. 2007). Hence, the balance between autophagy and apoptosis appears as a critical point but, importantly, several signaling pathways that are induced by common cellular stressors regulate either autophagy or apoptosis. For instance, ROS (see below) not only trigger apoptosis but are also essential for autophagy and specifically regulate ATG4 activity (Yamaguchi et al. 2003; Scherz-Shouval et al. 2007). ROS produce damaged proteins and organelles and lipid peroxidation in mitochondria, thereby promoting autophagy (Djavaheri-Mergny et al. 2006; Odashima et al. 2007). Members of the beclin1 and Bcl-2 family could serve as a point of cross-talk between the autophagic and apoptotic pathways. Beclin-1, primarily localized at endoplasmic reticulum, was originally identified as a Bcl-2-interacting protein (Liang et al. 1999). Bcl-2 inhibits Beclin-1-dependent autophagy in mammalian cells (Pattingre et al. 2005). During cardiac reperfusion phase, there is an increased expression of Beclin-1/ATG6 in the heart (Matsui et al. 2007). Recently, inhibition of mTOR by Everolimus has been demonstrated to represent a potential therapeutic strategy to limit infarct size and to attenuate adverse left ventricular remodeling after myocardial infarction (Buss et al. 2009). Altogether these studies indicate that constitutive cardiomyocyte autophagy is required for protein quality control and normal cellular structure and function. Accumulation of abnormal proteins and organelles, especially mitochondria, may directly cause apoptosis and cardiac dysfunction. Autophagy and apoptosis (and their cross-talk) appear thus as two key pathways in the definition of cardiomyocyte survival or death, in turn instructing cardiac integrity and function. However, since autophagic triggering is normally due to metabolic stress and/or nutrient deprivation it is conceivable that ischemic damage could represent a paradigmatic example in vivo of what has been observed in vitro in isolated cells.

Whether autophagy could play a role in gender differences detected in heart disease is still largely unknown. However, several studies evaluated the possible implication of hormones in the modulation of autophagy and some experimental studies at cellular level suggest that further gender-biased specific analyses appear as mandatory. However, it is well known that sex hormones affect body fat distribution and also impact on cardiovascular system. Since obesity represents a high-risk factor for the development of cardiovascular diseases, it has been suggested that adipose tissue, which is distributed in the abdominal viscera, carries

a greater risk for cardiovascular disorders than subcutaneous adipose tissue. In fact, women have more subcutaneous fat, whereas men have more visceral fat. Therefore, obesity-related metabolic disorders are much lower in premenopausal women than in men. In line with this, metabolic signals like leptin and insulin have been suggested to be involved in the food intake, body weight, body fat distribution, and cardiovascular disease. Key areas in the brain, including the hypothalamus, integrates these peripheral adiposity signals to maintain overall adiposity levels, and these brain regions are directly influenced by sex hormones (Nedungadi and Clegg 2009). A last point concerns type 2 diabetes. Type 2 diabetes has to be considered as a gender-associated disease: sex differences play in fact a key role in the onset as well as in the progression of the disease and a higher mortality for cardiovascular diseases is detected in diabetic women with respect to men (Regitz-Zagrosek 2006). Since autophagy is strictly dependent on cell homeostasis in terms of metabolic conditions, the role of autophagic response in patients with type 2 diabetes should be investigated in detail in the near future. Altogether these studies validate the hypothesis of a connection between gender differences autophagy and cardiovascular diseases. However, further research to point out appropriate animal models for gender-biased studies on this issue appears as mandatory.

4 The Role of Reactive Oxygen Species

The intracellular redox state is a key determinant of cell fate. Cells from male and female appear characterized by a huge series of differences in terms of ROS production and oxidative stress susceptibility that can influence their fate. Whether the cells are committed to age, death, or life, depends on small shifts in the particular circumstances or disease condition. ROS are continuously produced within the cell, particularly by the mitochondria and peroxisomes, as a result of mitochondrial electron transfer processes, and activity of several enzymes such as Krebs cycled enzyme complexes, p66Shc, xantine oxidase, lipoxygenases, and cyclooxygenases (Cadenas and Sies 1985). Furthermore, ROS can be generated as a consequence of the intracellular metabolism of foreign compounds, toxins or drugs by cytochrome P450, mono-oxygenases, or because of exposure to environmental factors such as excessive iron salts or UV irradiation. More importantly, inflammation and the products of inflammatory cells, e.g., cytokines, can create a pro-oxidant microenvironment that impacts on cell physiology. The most important targets for these agents (cytokines, ROS, reactive nitrogen species, microenvironmental factors) are cells of vessels. Vascular smooth muscle cells, endothelial cells, and fibroblasts composing vessels are continuously under changes of the environmental milieu and their ability to counteract or adapt to these changes is fundamental for vessel integrity and function. In fact, cells are generally capable of counteracting the production of free radicals through detoxification mechanisms that include enzymatic and nonenzymatic antioxidant defenses. The enzymatic antioxidant system consists of: (1) the family of superoxide dismutase enzymes, which catalyze dismutation of the

superoxide anion ($O^{\bullet -}$) into hydrogen peroxide (H_2O_2); (2) catalase, which removes H_2O_2; and (3) myeloperoxidase, which catalyzes the formation of hypohalous acids from H_2O_2 and chloride (Malorni et al. 2007).

A central role in the regulation of intracellular redox balance is played by reduced glutathione (GSH). It can directly scavenge free radicals or act as a substrate of enzymes, including glutathione peroxidases and glutathione *S*-transferases, involved in the detoxification/reduction of hydrogen peroxide, lipid hydroperoxides and electrophilic compounds.

Depending on their concentrations, ROS can be beneficial or harmful to cells and tissues. It has been recognized that a small amount of ROS can regulate cell proliferation and differentiation, accelerate telomere shortening, cause DNA double-strand breaks, increase beta-galactosidase activity and trigger irreversible arrest of cell growth contributing to a premature senescence of cells (Chen et al. 2004; Straface et al. 2007). Conversely, an excessive ROS production can lead to oxidation of lipids, proteins and DNA, causing damage to the cellular machinery and, as the ultimate consequence, cell death.

In any case, before starting the process of death, cells can degrade oxidized proteins activating the autophagy pathway. As stated above, this process is important for cellular homeostasis, and is considered a survival response to stress and a cytoprotection mechanism. However, excess of autophagy can lead to cell death due to excessive digestion of organelles and essential proteins (Kitsis et al. 2007; Tinari et al. 2008). Furthermore, the generation of ROS or the fluctuation of the cellular redox state leads to the stimulation of various signaling systems, such as p53 and mitogen-activated protein kinases. p53, is considered the "guardian of genome integrity," since it can influence the cell cycle, the DNA repair and, eventually, the apoptotic response after stress insults (Vousden 2000). Normally, the p53 levels are very low in the cells, since it is constantly degraded by the proteasome (Boyd et al. 2000). In situations of cellular stress, p53 degradation is stopped and its levels rapidly rise. In low cellular stress, p53 has an antioxidant role and protects cells from oxidative DNA damage, whereas, in severe cellular stress, high concentrations of p53 promote the expression of genes that contribute to ROS formation and p53-mediated apoptosis (Tomko et al. 2006).

Protein kinases contribute to the regulation of life and death decisions made in response to various stress signals. Therefore, the cell's fate is determined by cross talk between the cellular signaling pathways and the cellular redox state through a strict regulatory mechanism. Phosphorylation of c-Jun N-terminal kinase (JNK) and p38 kinase, increasing the p53 response, may influence the expression of pro- and anti-apoptotic proteins such as Fas ligand and Bcl-2 family proteins, leading to apoptosis. Conversely, phosphorylation of ASK1, mediated by treonin kinase Akt, inhibits the activation of JNK/p38, protecting cells from apoptosis (Matsuzawa and Ichijo 2005). It is absolutely clear that maintaining the redox homeostasis represent a crucial event in the prevention of cell damage.

Gender can also represent a key variable for ROS-mediated cell's fate. It has been demonstrated that a different susceptibility to oxidative stress occurs in cells from males and females. In particular, vascular smooth muscle cells (VSMC) from

male rats undergo oxidative stress-mediated apoptosis in comparison with cells from females that conversely undergo premature senescence (Straface et al. 2009). It has been hypothesized that cells from females are more "plastic" to adapt themselves to changes induced by oxidative stress. Moreover, the antioxidant defenses are apparently different in XX and XY cells and this can lead to, or contribute to, the observed gender disparity (Malorni et al. 2008; Matarrese et al. 2011). For example, the exposure of vascular cells from male and female rats to sub-cytotoxic doses of UVB (200 mJ/cm^2) clearly displayed a gender difference in terms of ROS production. In fact, with respect to cells from female rats, cells from male: (1) produce more ROS and higher levels of 4-HNE (an end-product of membrane lipid peroxidation); (2) undergo alterations of their morphological features with cell shrinking, loss of cell–cell contacts possibly due to oxidative changes, and consequent remodeling of cytoskeleton. This can also result in the loss of cell-basement membrane interaction leading to that form of apoptotic cell death called anoikis. By contrast, cells from female rats respond to oxidative stress with adhesion-associated resistance to apoptosis, the so-called anoikis resistance. This is apparently due to a more adhering phenotype, characterized by a well organized actin microfilament cytoskeleton, to increased phosphorylated focal adhesion kinase, and, more importantly, to a higher propensity to undergo survival by autophagy (Straface et al. 2009). Moreover, some important information as concerns hormone receptor expression and gender-dependent autophagy has also been provided (Straface et al. 2009). It has been demonstrated that estradiol (E2) via estrogen receptors (ERs) regulates the expression of numerous genes involved in the redox control and in E2-induced effects on cardiovascular system (Meyer et al. 2006). ERs mediate diverse and opposite E2 effects. Estrogen receptor alpha (ERα) is considered the master mediator of the E2-induced VSMC growth inhibition; whereas, in acute ischemia/reperfusion injury characterized by oxidative stress, estrogen receptor beta (ERβ) mediates cardioprotection (Wang et al. 2008). In smooth muscle cells it was found that basal cells from female rats express less ERα and ERβ than cells from male rats, whereas the androgen receptor (AR) level did not differ between sexes. More importantly, following induction of a stress (e.g., oxidative stress) the level of both AR and ERs remains unchanged in VSMC from males, whereas in female-derived cells both ERs were found altered: ERβ significantly increased while ERα decreased.

5 Conclusions

Since the study of cellular and molecular mechanisms underlying gender differences in several diseases, including cardiovascular diseases, recently gained the attention of several research groups working on gender disparity at cellular level, the analyses on the possible implication of apoptosis and autophagy as pathogenetic mechanisms and drug targets appear as mandatory and must certainly be improved in the near future. Several works have provided some useful insights

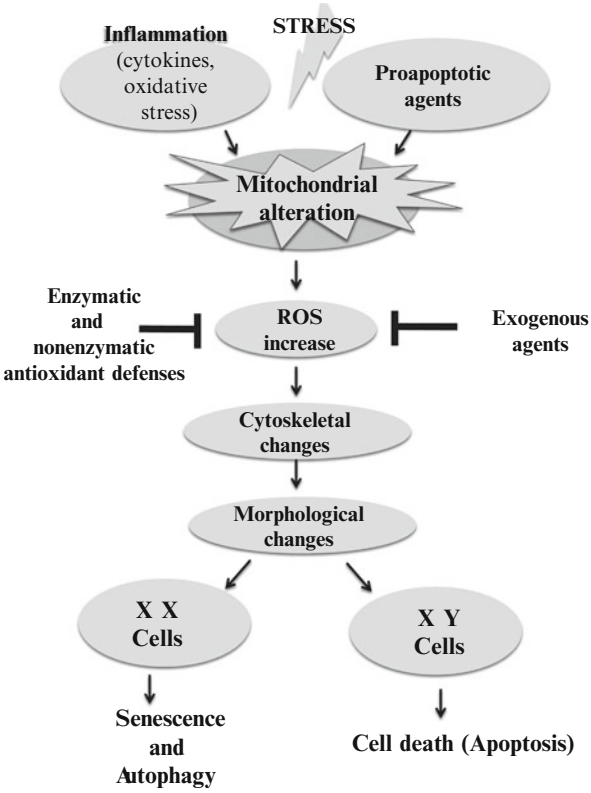

Fig. 1 Stressors and cell fate. This scheme suggests as the exposure to similar stressors, e.g., oxidative stress, cytokines, etc., induces a different fate in XX and XY cells

on this matter (Matarrese et al. 2011; Ortona et al. 2008; Paggi et al. 2010; Pierdominici et al. 2010, 2011; Profumo et al. 2011; Ruggieri et al. 2010) as well as on gender-associated and cell-death-related cellular prognostic biomarkers able to discriminate between males and females (Straface et al. 2010a, b). Furthermore, the advancement of our knowledge on the relationships between apoptosis or autophagy and hormones but, also between "cell sex" and autophagic cytoprotection/apoptotic injury could provide important information to improve the efficacy of specific therapies, including gender-tailored therapies (Lista et al. 2011; Maselli et al. 2009). In fact, the different cell physiology of women relative to men determines a different sensitivity to stressors and predisposition to disease (Fig. 1) resulting in a different response to drug treatments. Thus, the study of cell injury pathways and gender differences appears pivotal in the development of new and more appropriate therapeutic strategies.

Take Home Messages

- The existence of a gender cytology, i.e., dealing with XX and XY cells, has recently been demonstrated.
- A gender disparity has been found either in metabolic pathways or in the cell's defense system, e.g., antioxidant.
- A gender disparity has been demonstrated either in the propensity to apoptosis or in the activation of the autophagic cytoprotection pathway.
- Cells from females, e.g., vessel cells, appear capable of counteracting environmental stress and metabolic disturbances, surviving better than those from males.
- While vessel cells from males show a stereotyped behavior, cells from females are more "plastic" and show a striking adaptive behavior, e.g., a higher resistance to apoptosis and a higher propensity to autophagy.

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What a Difference an X or Y Makes: Sex Chromosomes, Gene Dose, and Epigenetics in Sexual Differentiation

Arthur P. Arnold, Xuqi Chen, and Yuichiro Itoh

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Abstract A modern general theory of sex determination and sexual differentiation identifies the factors that cause sexual bias in gene networks, leading to sex differences in physiology and disease. The primary sex-biasing factors are those encoded on the sex chromosomes that are inherently different in the male and female zygotes. These factors, and downstream factors such as gonadal hormones, act directly on tissues to produce sex differences and antagonize each other to reduce sex differences. Recent studies of mouse models such as the four core genotypes have begun to distinguish between the direct effects of sex chromosome complement (XX vs. XY) and hormonal effects. Several lines of evidence implicate

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epigenetic processes in the control of sex differences, although a great deal of information is needed about sex differences in the epigenome.

Keywords Sex chromosome • Y chromosome • X chromosome • Four core genotypes • Sexome • Gene networks • Hormones

Abbreviations

EAE	Experimental autoimmune encephalomyelitis
FCG	Four core genotypes
Kdm5c	lysine (K)-specific demethylase 5C
Kdm6a	lysine (K)-specific demethylase 6A
Sry	Sex determining region, Y chromosome
Sf1	Steroidogenic factor one, also known as Nr5a1
Trp53	Transformation related protein 53, encodes the p53 protein
XXM	XX gonadal male
XYM	XY gonadal male
XXF	XX gonadal female
XYF	XY gonadal female

1 Introduction: Why Study Sex Differences?

For much of the twentieth century, the study of sex differences focused on large sexual dimorphisms that are functionally related to reproduction. Most investigators in this field attempted to discover the genetic and hormonal factors that cause sex differences in the gonads, external and internal genitalia, and brain. Although the study of sexual differentiation was seen as a subfield of the study of reproduction, these studies served to define basic ideas about the factors that cause sex differences in tissues. Those ideas, and more modern ideas that derive from them, represent a general theory of sexual differentiation, discussed in the next section. In the last 20 years or more, however, it has been realized that many tissues and diseases, not overtly related to reproduction, also differ in males and females (Voskuhl 2011; Ober et al. 2008; Regitz-Zagrosek et al. 2006; Arnold 2010). This means that the best course of treatment of disease might proceed differently in the two sexes. Moreover, one sex may be protected from a disease, or may experience a milder disease course. In some cases, the protection offered by the individual's sex can be greater than that offered by drugs or other therapies. The awareness that sex-biasing factors can protect from disease has drawn attention to the need to identify these factors, with the aim of exploiting this knowledge to develop novel targets of therapy. Thus, in increasing numbers, investigators are interested in animal models and conceptual frameworks for designing investigations that will help identify factors that make the two sexes different.

At the same time, increasing attention is paid to the fact that most experimental subjects in biomedical research have been males, both in clinical and preclinical studies (Beery and Zucker 2011; Taylor et al. 2011). The choice of males is more than just a social bias of the experimenters, who are more often male than female. In animal studies, for example, females have been viewed as more variable than males, because of the changes caused by the estrous cycle. From one perspective, in which physiology is viewed as not likely to be much different between the sexes, it might make sense to study the most experimentally tractable (least variable) sex, with the expectation that the physiology of the male kidney (for example) tells us what we need to know about the physiology of the female kidney. This perspective has two important flaws: the first being that females are not necessarily more variable than males [e.g., see Mogil and Chanda (2005)]; and, even if they are, then the female's physiology is not the same as the male's. Secondly, independent of the issue of variability, numerous aspects of physiology and disease differ in the two sexes. The non-equivalence of the sexes is a strong argument to shift the balance of studies so that females are studied more than in the past, lest the research in physiology and medicine be relevant only to the male half of the human population.

An important point, however, is that more study of females is not enough. There is also a need to compare the sexes directly. The comparison of the sexes can uncover important questions and answers that would not otherwise be investigated. For example, a comparison of the death rates in the two sexes in humans reveals that males die at a faster rate than females, at nearly every life stage beginning before birth (Migeon 2007). Without a direct comparison of the sexes, it would not occur to us to seek to explain what protects females, and/or what makes males vulnerable. If a protective factor can be found, then it might be manipulated to prevent deaths in both the sexes. A second example, of the advantages of direct comparison of the sexes, is that sometimes understanding the physiology of one sex requires the comparison with the other. For example, comparison of the sexes is required to understand the evolution and function of X-inactivation (transcriptional silencing of one X chromosome in XX cells), a process that occurs in nearly every XX female somatic cell, but never in XY male somatic cells. X-inactivation solves problems that arise when the ratio of expression of X to autosomal genes is different in one sex from the other, because such sexual imbalance would mean that the ratio would be non-optimal in at least one of the sexes (Charlesworth 1996). The main effect of X inactivation is to reduce the sexual disparity in X to autosome dose, so that X genes are not inherently expressed higher in females (except for some exceptions discussed below) (Itoh et al. 2007; Arnold et al. 2008). If one tried to study X-inactivation only in females, it would be impossible to understand its function. The conceptual importance of the X to autosome ratio becomes quite relevant when attempting to understand the sex-biased impact of the genes that escape X-inactivation, as discussed below.

2 A General Theory of Sex Determination and Sexual Differentiation

The goal of basic biomedical science is to explain the causal pathways that control physiology and disease. Thus, we envision the function of cells, tissues, and individuals to be controlled by complex intersecting causal pathways, in which specific physical events cause changes in other events. Genes (and their products, RNA, and protein) form networks of interactions as they control and are controlled by each other. The gene networks can be thought to be composed of nodes (gene products) that are connected to limited number of other nodes (van Nas et al. 2009; Arnold et al. 2009; Arnold and Lusis 2012). In this analogy, functional gene networks pulsate with activity, with specific nodes increasing and decreasing in their activity, stimulating and inhibiting each other, creating a dynamic net of interactions that lead to emergent phenotypes (such as heart rate, fat and energy metabolism, etc.). Sex differences in gene networks, and in the phenotypes that they control, are created when the activity of some nodes is greater in one sex than in the other; the sex differences in network functions are caused by sex-specific factors acting in the network. The totality of sex-biased factors in the network comprises the *sexome* (Arnold and Lusis 2012). A major goal is to identify these sex-biasing factors together with their downstream effects on specific parts of gene networks. These factors, and the downstream gene products that they bias sexually, are candidates for manipulation to mimic sex-specific protection from disease.

We can distinguish primary sex-determining factors and secondary factors that are downstream from the primary factors (Arnold 2009b, 2011). The primary factors are encoded by the sex chromosomes, because all sex differences start with the sex chromosomes at some point in life. The sex chromosomes are the only factors that differ in the male and female zygote, and thus they are the factors that give rise to all downstream sex differences thereafter. Four classes of X and Y factors are postulated to comprise the primary sex-determining genes (De Vries et al. 2002; Arnold 2011; see Fig. 1). class I are Y genes, which can only have effects in males. Among the Y genes known to be required to make a complete male are the testis-determining gene *Sry* (Goodfellow and Lovell-Badge 1993), and several Y genes required for spermatogenesis (Burgoyne and Mitchell 2007). Class II are X genes that escape X-inactivation and are expressed from both X chromosomes, resulting in constitutively higher expression in XX cells than XY cells. Because X inactivation appears to vary across tissues and age, the number of such X escapees is likely to depend on species, developmental stage, and tissue, but is greater in humans than in mice (Berletch et al. 2010; Carrel and Willard 2005). Class III are X genes expressed at a higher or lower level in XX than XY cells because of a parental imprint on the gene from the mother or father. Parental imprints on X genes are inherently unequal in the two sexes, because XY cells can only express a maternal imprint on imprinted X genes, whereas XX cells can show the effects of a maternal or paternal X imprint depending on which X chromosome is active in a specific cell. The presence of the paternal imprint in

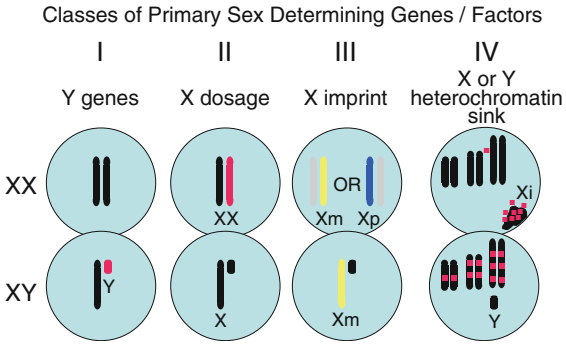

Fig. 1 Four classes of primary sex-determining factors that are encoded by the sex chromosomes. Class I are Y genes found only in males. Class II are X genes that escape inactivation and are inherently expressed higher in females than males. Class III are X genes that are imprinted and have a sex-biasing effect because of expression of the paternal imprint only in XX cells. Class IV are putaitve heterochromatic regions on the sex chromosomes (the X chromosome is illustrated here), which act as sinks to sequester heterochromatizing factors from other chromosomes and alter the epigenetic status of autosomes. Reprinted from Arnold (2011), *Trends in Genetics*, with permission from Elsevier.

about half of the XX cells (when the active X chromosome is from the father) could make XX individuals different from XY. Although some X genes are imprinted (Raefski and O'Neill 2005; Davies et al. 2005; Gregg et al. 2010), and XO mice and humans differ in their cognitive or social behavior depending on the parent of origin of their X chromosome (Davies et al. 2005; Skuse et al. 1997), there are no established cases yet of a sex difference caused by class III genes. Class IV is a newly proposed and speculative class, not of specific genes, but of non-coding regions of the sex chromosomes. These are sex chromosome regions that are heterochromatic in one sex more than the other, and which may alter the availability of heterochromatizing factors that regulate gene expression on all chromosomes. The best evidence for sex-specific heterochromatizing effects is in *Drosophila*, in which the large heterochromatic Y chromosome alters the expression of autosomal genes, not because of any expression of genes from the Y chromosome, but by its effects on the epigenetic status of other chromosomes (Jiang et al. 2010; Lemos et al. 2010). The Y chromosome is also largely heterochromatic but is much smaller in mammals than in *Drosophila*, but it could theoretically have a male-specific effect of this type, although evidence is lacking at present. In addition, however, XX mammalian cells each possess a heterochromatic inactive X chromosome that is absent in XY cells. It is unknown if these chromosomal regions bias expression from the autosomes, but some evidence argues in favor of this idea (Wijchers and Festenstein 2011; Wijchers et al. 2010).

Which of the primary sex-determining factors is most important for causing sex differences in downstream pathways and diseases? The prize would have to go to *Sry*, the class I Y gene that is turned on in the undifferentiated embryonic gonad, and causes differentiation of testes in males, including the activation of genes that

inhibit ovarian differentiation (Koopman 2010). In the absence of *Sry*, autosomal and/or X chromosome genes initiate ovarian differentiation in the XX gonad, activating genes that also block testicular differentiation (Chassot et al. 2008; Dinapoli and Capel 2008). Thus, the molecular switches controlled initially by *Sry* represent the choice between testicular and ovarian development, and therefore set up a lifelong difference in the secretion of gonadal hormones such as testosterone in males vs. estradiol and progesterone in females. These gonadal hormones act on gene networks and are probably the cause of the large majority of known sex differences in function and disease. The molecular effects of gonadal hormones are diverse and beyond the scope of this review.

The effects of the hormones have historically been lumped into two broad classes: activational and organizational. The acute or *activational* effects of gonadal hormones are those that are reversible. In animal models, sex differences that are erased by gonadectomy are attributed to the ongoing activational effects of either testicular or ovarian secretions that were removed by gonadectomy. To do the experiment properly in animals, one has to remove the gonads of both sexes to determine if the sex difference is caused entirely by gonadal secretions. In one study, for example, thousands of genes were found to be expressed consistently at different levels in livers from male or female mice. After removing the gonads, virtually all of these differences disappeared, suggesting the most sex differences in adult mouse liver are caused by activational effects (van Nas et al. 2009). Sometimes, however, sex differences persist when comparing gonadectomized males and females. Males castrated in adulthood, for example, continue to have male genitalia that differ from those of the female, and structural sex differences in the brain. In many cases, these differences are caused by the long-lasting or permanent *organizational* effects of gonadal hormones (Arnold and Gorski 1984). Masculine differentiation of the genitalia and brain is caused largely by the effects of testosterone on the fetus and neonate, which last for the rest of the male's life and differentiate the male from the female. Although the dichotomy between activational and organizational effects has a long history (Phoenix et al. 1959; Arnold and Breedlove 1985; Arnold 2009b), the effects more likely lie along a continuum, with some steroid hormone effects lasting longer than others, after the level of the hormone declines. Exposure of male hamsters to androgens at the time of puberty, like the fetal and neonatal exposure to testosterone, also has long-lasting effects that can be classified as organizational (Schulz et al. 2009).

Although gonadal steroids (downstream of *Sry*'s effect on the gonads) are the dominant factors causing sex differences in physiology and disease, the class I–IV sex chromosome factors also act, via molecular pathways that are not mediated by gonadal hormones, to bias the function of XX and XY cells. These "sex chromosome effects" have been uncovered predominantly in the last 25 years. In a few cases, sex differences have been discovered that occur before the differentiation of gonads, which are therefore not explained by sex differences in gonadal hormones (Renfree and Short 1988; Burgoyne et al. 1995; Bermejo-Alvarez et al. 2011; Dewing et al. 2003). Particularly intriguing is the finding that sex differences in the size and other traits of mammalian embryos exist well before the differentiation

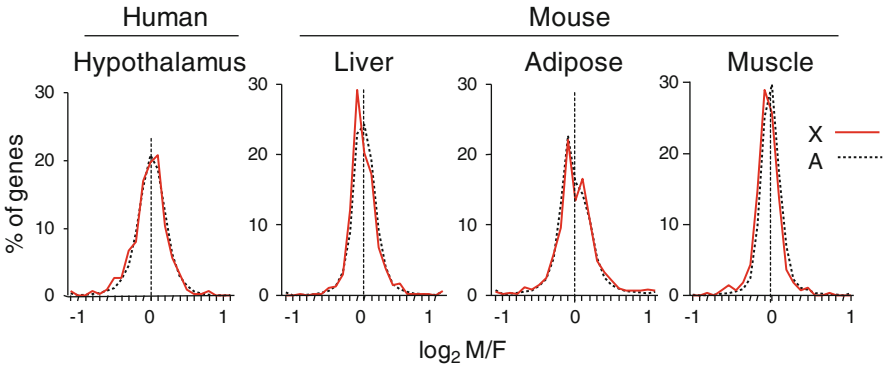

Fig. 2 Sex differences in the mammalian transcriptome. Data from microarray profiling are illustrated. Histograms show the distribution of M–F ratios of expression of all genes measured, including autosomal genes (*black*, *dotted line*) and X chromosome genes (*red*). In each tissue, about the same number of genes are expressed higher in males than in females, and most sex differences are well below twofold. X inactivation is effective in preventing higher expression of most X genes in females. Although the amount of sexual dimorphism (width of the histograms) differs across tissues, the degree of sexual bias in X genes is matched, tissue for tissue, to the sexual bias of autosomal genes, presumably because they interact with each other in gene networks. Reprinted from Itoh et al. (2007)

of the gonads, even before implantation of the embryo. In bovine blastocysts, nearly one-third of all genes measured show sex differences in the level of expression, which probably is the result of the higher expression of X genes in females (Bermejo-Alvarez et al. 2010). The generally higher expression of X genes in females than males is probably due to the incomplete inactivation of one X chromosome in each XX cell of the inner cell mass (the precursor of the embryo proper), so the X genes as a group show higher expression in females (mostly class II effects, Fig. 1). In turn, the sex difference in the expression of X genes causes sex differences in the expression of some autosomal genes. Thus, the mammalian embryo appears to pass through a stage of considerable sexual dimorphism in gene expression, prior to random X-inactivation. It is not known if long-term sex-specific effects might be caused by this sexual inequality. Once X inactivation has occurred, the X genes show about the same amount of sex difference globally as shown by the autosomal genes (Itoh et al. 2007) (Fig. 2).

3 Mouse Models for Uncovering the Origins of Sex Differences

The appreciation of the role of sex chromosome effects has grown steadily in the last 10 years because of the development of mouse models that allow one to separate them from the effects of the gonads (Arnold 2009a). These models are of three types: (1) One approach has been is to manipulate the expression of *Sry*

directly in non-gonadal tissues, to demonstrate or suggest a male-specific effect of this gene that is independent of *Sry*'s differentiating effect in the testes (Dewing et al. 2006; Turner et al. 2011). Similarly, the manipulation of other Y genes in the germ line has demonstrated the importance of several Y genes for specific stages of spermatogenesis (Mazeyrat et al. 2001; Vernet et al. 2011; Burgoyne and Mitchell 2007). The same approach could also work for other class I–IV genes or factors but these experiments have not yet been performed. (2) A second approach is to study mice that lack gonads, to find sex differences that occur in the complete absence of gonadal secretions (but not necessarily in the absence of sex steroid hormones synthesized outside of the gonads). So far, this approach has been reported for mice with a null mutation of *Sf1* (steroid factor 1). *Sf1* is required for gonadal and adrenal differentiation, and thus *Sf1* knockout mice are born without both tissues. The neonates are kept alive by treating them with glucocorticoids to reverse the adrenal insufficiency and then transplanting an adrenal. Such mice show some sex differences in the hypothalamus, which cannot have been caused by gonadal secretions (Majdic and Tobet 2011; Budefeld et al. 2008). (3) The third approach is the use of *four core genotypes* (FCG) mice and related models (Arnold and Chen 2009). In FCG mice, the Y chromosome lacks *Sry* because of a small deletion of a portion of the Y chromosome thought to disrupt only the *Sry* locus (Lovell-Badge and Robertson 1990). This Y chromosome is designated Yminus (Y^-). The loss of *Sry* is complemented by the insertion of an *Sry* transgene onto an autosome (Mahadevaiah et al. 1998). Thus, XY^- mice with the autosomal *Sry* transgene (called XYM here) are functional gonadal males, whereas XY^- mice lacking the *Sry* transgene are gonadal females (called XYF here). Mating XYM to normal XX gonadal females (XXF) gives four core genotypes: XYM, XYF, XXF, XXM (Fig. 3). The four genotypes are a 2×2 comparison that varies sex chromosome complement (XX vs. XY) independent of the presence/absence of *Sry* (Fig. 3). This model is useful for dissecting the differences between XX and XY mice caused by gonadal hormones and sex chromosome complement. For example, when a phenotype is measured in the four groups, and the two groups with *Sry* (gonadal males) are similar to each other but both differ from the two groups without *Sry* (gonadal females), which are also similar to each other, then we conclude that the difference is caused by *Sry*. This *Sry* effect is most likely the result of different effects of gonadal hormones (i.e., different effects of testicular hormones in mice with *Sry* vs. ovarian hormones in mice without *Sry*), but could also be caused by a direct effect of *Sry* on some other tissue. If, on the other hand, the two XX groups are similar to each other, but both are different from the XY groups that are also similar to each other, then we conclude that the sex chromosomes have contributed to the sex difference. In the second example, because the two XX groups are similar to each other, and the two XY groups are similar to each other, there is no apparent effect of gonadal type. Because the FCG model shows effects of gonadal type and also of sex chromosome complement, it offers the possibility of measuring interactions between the two variables, for example when a gonadal effect is different in XX than XY, or when the difference between XX and XY differs in gonadal males and females.

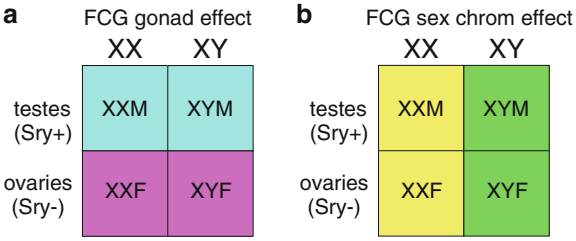

Fig. 3 The four core genotypes model. When XY gonadal males (XYM) are mated to XX gonadal females, the offspring comprise four genotypes: XX and XY gonadal females (XXF, XYF, both without *Sry*), and XX and XY gonadal males (XXM, XYM, both with *Sry*). The four genotypes represent a 2 × 2 comparison of the effects of gonadal sex (comparing mice with testes vs. mice with ovaries) and the effects of sex chromosome complement (XX vs. XY). (**a**) When a sex difference is caused by gonadal hormones, the two groups of mice with testes differ from the two groups with ovaries, irrespective of their sex chromosome complement. (**b**) When the sex difference is caused by sex chromosome complement, then the two groups of XX mice differ from the two XY groups, irrespective of their type of gonad. Not diagramed are cases in which the two factors interact

By the late twentieth century, the dominant theory of sexual differentiation stated that all sex differences in mammals, outside of the gonad, were caused by gonadal secretions (Arnold and Gorski 1984). At the time of the emergence of the FCG model, some evidence argued that sex chromosome complement could also produce sex differences (Arnold 1996; Arnold and Burgoyne 2004). When the FCG model was used to study non-gonadal tissues such as brain and behavior, the results confirmed that several classic sex differences in the brain and behavior were differentiated by organizational effects of gonadal secretions (Fig. 4). For example, male patterns of sexual behavior were found in mice that developed with testes, not mice with ovaries, irrespective of whether they were XX or XY. Thus, the FCG model underscored the dominant effects of gonadal hormones. In addition, study of FCG mice has uncovered a growing number of cases in which XX mice differ from XY mice, irrespective of their gonadal sex. In these studies, the FCG mice have been gonadectomized as adults, and treated with equal amounts of testosterone or with nothing. The point of gonadectomizing the mice is that at the time of testing, the levels of gonadal hormones are equivalent across groups, and group differences in phenotype cannot be attributed to activational effects of hormones. Under these conditions, when effects of *Sry* are found (differences between mice that previously had ovaries vs. testes), they are most likely caused by effects of gonadal secretions that persisted after gonadectomy, for example, organizational effects of gonadal hormones. Because of the novelty of the sex chromosome effects, which were not acknowledged by most twentieth-century theories of sexual differentiation, the published studies using FCG mice have emphasized cases in which XX and XY mice have a different phenotype or different susceptibility to disease.

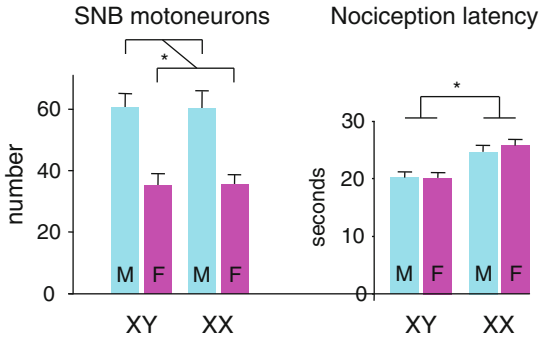

Fig. 4 Representative differences among FCG mice. FCG mice were gonadectomized as adults, and then implanted with equal amounts of testosterone (*left*) or nothing (*right*). On the left the number of neurons in the spinal nucleus of the bulbocavernosus (SNB) is graphed. Gonadal males have more neurons than gonadal females, irrespective of their sex chromosome complement, indicating that this sex difference is dominantly controlled by gonadal hormones. To measure nociception (*right*), the mice were placed on a hot plate and the latency to lick the paws was measured. XX mice responded more slowly than XY mice, irrespective of their previous gonadal status, indicating that the complement of sex chromosomes causes the difference. SNB data from De Vries et al. (2002), and nociception data from Gioiosa et al. (2008a)

4 Examples of Sex Chromosome Effects on Phenotype

Several mouse models of disease show sex differences that are controlled in part by sex chromosome complement. These include models of autoimmune disease, viral infections, neural tube closure defects, and hypertension.

4.1 Autoimmune Disease

In humans, autoimmune diseases, such as multiple sclerosis and systemic lupus erythematosus (SLE), affect females much more than males. A similar predominance of multiple sclerosis in females also occurs in experimental autoimmune encephalomyelitis (EAE), a rodent model of multiple sclerosis. Although androgens are protective in EAE, and therefore account in part for the sex difference (Voskuhl 2011), XX mice are affected by EAE much more than XY mice, irrespective of the type of gonad the mice have (Palaszynski et al. 2005; Smith-Bouvier et al. 2008). Similarly, in a mouse model of SLE, XX mice die sooner and show other signs of greater disease than XY mice. In both models, various immune system markers are influenced by the disease differently in XX vs. XY mice. Differences in sex chromosome number and X chromosome dose have also been suggested to influence the incidence of SLE in humans (Scofield et al. 2008).

4.2 Viral Infection

One study examined sex differences in two types of viral infections in mice, cocksackie virus and influenza A (Robinson et al. 2011a). Both showed sex differences, caused at least in part by gonadal hormones (Robinson et al. 2011b). In addition, however, after gonadectomy XX mice showed greater myocarditis than XY mice in response to cocksackie infection, irrespective of their gonadal type, and the protection in XY mice correlated with increased activation of regulatory T cells and expression of CD4+ forkhead box P3 mRNA.

4.3 Neural Tube Closure Defects

In human populations, anterior neural tube closure defects influence females more than males. Numerous mouse knockouts produce problems of neural tube closure, presumably because this closure is a tightly regulated and complex process involving many gene pathways (Harris and Juriloff 2007). Sex differences are found in some mouse models, including knockout of the tumor suppressor gene *Trp53*. Mice lacking functional *Trp53* at birth are almost all males, because of the pre- or neonatal mortality of females that lack this gene, associated with severe neural tube closure defects. In FCG mice, the sex difference in mortality and neural tube closure is an effect of sex chromosome complement—XX mice are affected more than XY mice irrespective of their gonadal type (Chen et al. 2008). Comparison of mice with two X chromosomes to mice with one X chromosome shows that the sex difference is caused by X chromosome number, not by the presence/absence of Y genes. That finding indicates that class II–IV primary sex-determining factors are implicated in sex differences (Fig. 1).

4.4 Hypertension

In humans, hypertension is more prevalent in males than females. In rodent models, the sex difference is explained in part by the effects of gonadal hormones, but recent evidence suggests that direct effects of sex chromosome genes also play a role. When FCG mice are treated with angiotensin II, either chronically or acutely, the changes in blood pressure and heart rate are different in XX and XY mice irrespective of their type of gonad (Ji et al. 2010; Caeiro et al. 2011). At this point, it is not clear which of the four sex chromosome factors (Fig. 1) are responsible for the sex chromosome effects, but in the FCG model the XX vs. XY difference cannot be attributed to the direct effects of *Sry*. In other studies, however, *Sry* is proposed as a regulator of hypertension because of its effects on the renin–angiotensin system and sympathetic nervous system (Turner et al. 2011; Ely et al. 2010). *Sry* is expressed in

numerous non-gonadal tissues (Turner et al. 2011), and overexpression of *Sry* in adrenal or kidney causes an increase in blood pressure. Molecular studies in vitro indicate that expression of *Sry* leads to changes in promoter activity of tyrosine hydroxylase and genes in the renin–angiotensin related pathways. These intriguing studies suggest a role for male-specific expression of *Sry* as one mechanism leading to sex differences in the regulation of blood pressure, but further studies are needed to downregulate *Sry* expression in vivo to show that *Sry* normally plays this role.

4.5 Behavioral and Brain Phenotypes

FCG and *Sf1* KO mice have also contributed significantly to the conclusion that sex chromosome complement contributes to sex differences in normal physiology and behavior of mice. Many studies have been done on the brain and show that gene expression is different in XX and XY mice irrespective of the gonadal type (or despite the complete absence of gonads). Genes that have been studied include vasopressin in the lateral septum (De Vries et al. 2002; Gatewood et al. 2006), and expression of nitric oxide synthase and calbindin in the hypothalamus, and prodynorphin in the striatum (Abel et al. 2011; Chen et al. 2008; Xu et al. 2005). In addition, neural expression of several class II X genes is higher in XX than XY mice, including two histone demethylases (Xu et al. 2008a, b; Chen et al. 2009). In at least one case, prodynorphin expression in the striatum, subsequent studies showed that the expression is regulated by X genes rather than Y genes (classes II–IV) (Chen et al. 2009). Numerous studies of behavior show differences between XX and XY mice: in the formation of habits related to models of alcohol abuse and drug addiction (Quinn et al. 2007; Barker et al. 2010), in aggressive and parenting behaviors (Gatewood et al. 2006), behavioral measures of nociception (Gioiosa et al. 2008a, b), and investigative and social behaviors (McPhie-Lalmansingh et al. 2008; Cox and Rissman 2011). In studies not using FCG mice, *Sry* has been found to be expressed in the substantia nigra of the midbrain, in neurons that project to the striatum (Dewing et al. 2006). These neurons die in Parkinson's disease, which affects males more than females. When *Sry* expression is experimentally reduced in the brain of adult male mice or rats, the motor behavior of the mice is degraded, and tyrosine hydroxylase is reduced. The deficits are reversed once *Sry* expression is restored. Thus, evidence suggests that there is a direct male-specific effect of *Sry* on the brain that is not mediated by its effects on gonadal differentiation.

5 Practical Approaches to the Study of Sex Differences in Physiology and Disease

As discussed above, the current theory of sexual differentiation suggests that the X and Y chromosomes harbor numerous genes that are the primary factors causing sexual differentiation, because these factors are inherently unequally represented in

XX vs. XY zygotes (Arnold 2011). These primary factors act to cause numerous sex differences in downstream genes and pathways that they regulate. Ultimately, these downstream pathways intersect and interact with each other. In some cases, two sex-biased factors might inhibit each other, which would tend to make the sexes more similar rather than different. The general goal of research on sex differences is to identify the sex-biased factors that explain sex differences in physiology and disease, which involves studying the primary and downstream pathways.

Becker et al. (2005) have suggested a practical experimental approach to finding the sex-biased factors and their downstream products in animal models. Because most sex differences may be caused by activational effects of gonadal hormones, a logical first experiment is to gonadectomize adult animals to determine if the sex difference is abolished. If it is, then one would investigate which gonadal hormones in adulthood were responsible for the sex difference, and investigate downstream pathways modulated by those hormones. If sex differences are still found in gonadectomized animals that have equivalent levels of gonadal hormones, then it is likely that organizational effects of gonadal hormones, or differences in sex chromosome complement, cause the residual sex difference. Organizational effects of gonadal hormones can be tested by manipulating the levels of gonadal hormones at early developmental stages before and/or after birth. In studies of the brain, one would likely start with manipulations of testosterone (or its metabolite estradiol), which is the main hormone causing masculinizing organizational effects in rodent models (McCarthy and Arnold 2011). FCG mice are a good choice for screening for direct effects of sex chromosome complement, except that this model does not test for a direct effect of *Sry* that is independent of its effect on the gonads. If sex chromosome effects are found, then one independently manipulates the number of X and Y chromosomes to determine if the sex chromosome effect is due to X or Y genes (e.g., Chen et al. 2008, 2009) (classes I–IV, Fig. 1). Ultimately, the goal is to find the individual X or Y genes and understand their physiological effects within specific tissues.

6 The Role of Epigenetics in Sexual Differentiation

The recent explosion in the study of epigenetics has several important effects on the study of sex differences. Historically, the genetic control of phenotype has concentrated on variations in phenotype caused by variations in the primary genetic sequence. Variation is also induced by transient and long-lasting epigenetic changes that alter the compaction and loosening of DNA and chromatin, which include processes such as methylation of cytosines in the primary DNA sequence, or changes in the chromatin because of various modifications (acetylation, methylation, ubiquitination, sumoylation, etc.) of specific amino acids in the histone tails comprising the histone octamer around which DNA is wrapped. The epigenetic modifications are fundamental to any biological process, so it is not surprising that they are increasingly studied in the context of sexual differentiation. Particularly

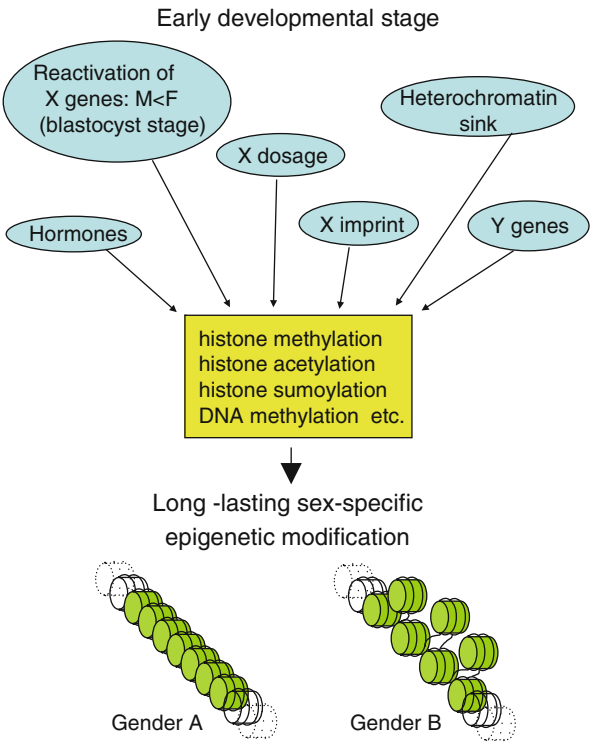

Fig. 5 Summary of possible sex-specific epigenetic modifications that could influence chromatin status and gene expression in a gender-specific manner

intriguing is the finding that epigenetic modifications can last a long time, such that changes early in development can alter the phenotype of the animal much later in life (Zhang and Meaney 2010). Some epigenetic modifications last across generations, and can influence subsequent generations (Guerrero-Bosagna and Skinner 2011; Morgan and Bale 2011). The persistence of epigenetic modifications makes this mechanism an attractive candidate mechanism for explaining some long-lasting effects of gonadal hormones, for example, organizational effects exerted early in development. Accordingly, several groups have begun to analyze epigenetic parameters using research designs that compare the sexes or manipulate hormones at different times of life to determine if steroid hormones have short- or long-lasting influences on the epigenome (McCarthy et al. 2009; Auger and Auger 2011).

Several considerations support the importance of these epigenetic modifications (Fig. 5).

1. The mechanism of action of gonadal hormones involves modification of histones. Sex steroid hormones bind to nuclear receptors (androgen or estrogen receptors, for example), which bind to hormone response elements in the DNA and attract various cofactors that have inherent histone acetyltransferase or methyltransferase activity. The histone-modifying enzymes alter the epigenetic state of gene promoters to which the nuclear receptors bind and change gene

expression (Fu et al. 2004; Leader et al. 2006; Green and Carroll 2007; Auger et al. 2011). Nevertheless, more information is needed to understand where in the genome these changes occur, when in life, and how long they persist.

2. The list of sex chromosome signals that are inherently unequal in most male and female cells (Fig. 1) includes genes that are histone demethylases, *Kdm5c* and *Kdm6a* (Xu et al. 2008a, b). These X-linked genes escape X-inactivation, are expressed widely in many cell types, and are often expressed higher in XX cells than XY cells. They are members of class II of putative sex-biasing factors (Fig. 1). Because of their histone demethylase activity, these genes could have widespread sex-biasing roles in different tissues or stages of development, but the phenotypes influenced by these genes are just beginning to be described. *Kdm5c* is implicated in tumor suppression and mental retardation (Santos-Reboucas et al. 2011; Niu et al. 2011), whereas *Kdm6a* is involved in oncogenesis (Kristensen et al. 2011). However, neither gene is yet to be implicated in the sex bias of an emergent phenotype.
3. In some brain regions, the two sexes differ in patterns of acetylation or methylation of histones by several days before birth, indicating that sex-biased signals have already impacted the brain epigenome by that stage (Tsai et al. 2009; Matsuda et al. 2011). The sex differences are dynamic in this period, with some sex differences appearing and disappearing in the course of a few days (Matsuda et al. 2011). In one study, treatment of female embryos with testosterone masculinized the pattern of acetylation of histone 3 measured at birth, but did not sex-reverse the pattern of methylation of histone 3 (Matsuda et al. 2011). Thus, diverse sex-biased signals, including testosterone secreted by the male, appear to sexually differentiate histone modifications during the perinatal period. Administration of valproic acid, an inhibitor of histone deactylase, at the time of birth, alters acetylation of histone 3 and blocks testosterone-dependent masculine development of the bed nucleus of stria terminalis (Murray et al. 2009, 2011). Knockdown of histone deacetylases in the mating-control regions of the preoptic area in rats inhibited the adult expression of a sexually differentiated behavior, male copulatory behavior (Matsuda et al. 2011).
4. Several studies have examined the patterns of methylation of promoters of interesting relevant genes in several brain regions that are known to be sexually differentiated (masculinized) by testosterone (or estradiol, which is a natural active metabolite of testosterone mediating masculinization of the neonatal male's brain) (McCarthy et al. 2009; Nugent and McCarthy 2011). These studies have found sex differences in the patterns of methylation of cytosines during the critical period for brain masculinization (Edelmann and Auger 2011), and have shown that some but not all of these sex differences can be reversed by treating females with estradiol. That result indicates that neonatal levels of estradiol, which are known to have permanent masculinizing effects, also alter the epigenetic status of some genes. Because some of the methylation patterns are not sex reversed by treating females with estradiol, it is possible that other sex-biasing signals (e.g., sex chromosome complement) may cause the observed sex differences. The sex differences in pattern of methylation are complex in that

they differ according to gene, brain region, age of development, and sometimes according to the lab performing the study. Most of the gene-specific patterns that are masculinized by estradiol are not found to persist into adulthood, so it is not yet clear how the sex-biased alterations of the neonatal epigenome contribute to the long-term development of permanent sex differences in brain function (Schwarz et al. 2010; Nugent et al. 2011). Even when permanent sex differences are found, an important question is whether sex-biased signals such as estradiol cause specific changes in the neonatal epigenome which then change brain function for much of the animal's life, or if the estradiol causes structural and functional differences (e.g., modifying the distribution of cell types in a tissue) which bring with them long-term changes in the epigenome. Many of the sex differences and estradiol-induced changes, in methylation of specific gene promoters, are measured as relatively small differences in methylation. When a specific cytosine is found to be methylated, a few percent more often in one sex than the other, it is not clear how large a functional impact would be expected on gene function. All in all, this field is in its infancy. Only a tiny part of the epigenome has been studied in the context of sex differences, in a few brain regions, so we can expect a great deal of work to clarify these issues in the future (McCarthy et al. 2009; Nugent and McCarthy 2011).

7 Conclusions: Understanding the Sexome

To understand the sexome, the aggregate sex-biasing actions that change cellular systems, the following steps are important: (1) identify the inherent primary genetic sex-biasing factors, encoded by the sex chromosomes, that initiate the process of sexual differentiation. Figure 1 summarizes four possible categories of primary sex-biasing factors, which act in parallel to cause sexual differentiation. (2) Identify the secondary proximate factors, downstream of the action of primary factors, that act on specific molecular networks to cause the networks to function differently in males and females. (3) Identify which molecular network components are sexually dimorphic, and how the influence of sex-biasing factors is propagated throughout the network. Once the sex-biasing process is understood, it may well be possible to find sex-biased factors that protect from disease, and target those factors to develop new therapies

The study of sex differences in reproductive tissues in the last 100 years has given rise to a general theory of sexual differentiation, which provides a conceptual framework for approaching the study of sex differences, as well as experimental strategies and animal models for recognizing and deconstructing the sexome. The vast majority of studies on sexual differentiation of non-gonadal tissues has involved the manipulation of gonadal hormones, which are the most potent proximate factors controlling sexual differentiation. In the past decade, however, animal models have been investigated that allow the study of sex chromosome effects (differential effects of an XX vs. XY genome) independent of the action of gonadal

hormones. The four core genotypes mouse model, for example, produces XX and XY mice, each with testes or ovaries. Under some conditions, XX and XY mice differ from each other, not because of differences in gonadal secretions. Those results indicate that the constitutive sexual bias in X and Y genes contributes to sexual differentiation of the cells. The imbalances of X and Y genes are both important, indicating that multiple primary sex-biasing factors are encoded in the sex chromosomes, and these act in parallel to cause sex differences. The modern theory of sexual differentiation, therefore, envisions multiple sex-biasing signals that act not only in parallel but also interact with each other, such that multiple factors can sum with each other, or counteract each other to buffer and reduce the individual effect of any one sex-biasing factor. Thus, understanding the sexome involves unraveling numerous downstream pathways and figuring out where and how cellular systems are impacted.

Take Home Messages

1. Multiple primary sex-determining genes are found on the sex chromosomes, including *Sry*.
2. The proximate factors causing sex differences in physiology and disease are acute (activational) effects of gonadal hormones, organizational (long-lasting) effects of gonadal hormones, and sex chromosome effects not mediated by gonadal hormones.
3. The four core genotypes mouse model is useful for dissecting the sex-biasing effects of gonadal hormones and sex chromosome complement.
4. Numerous sex differences in physiology and disease can now be traced to the different effect of XX vs. XY sex chromosomes.
5. Numerous hormonal and non-hormonal mechanisms likely alter the epigenome and regulate sex differences via epigenetic mechanisms.

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Part II
Sex Differences in Pharmacokinetics, in Drug Development and Use

Sex Differences in Drug Effects: Interaction with Sex Hormones in Adult Life

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Abstract In recent years, it has become clear that women and men may differ for drug response. Also, there is an increasing recognition on the role of sex hormones on pharmacokinetics and pharmacodynamics as mechanism accounting for sex differences in drug effects.

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In women, the phases of menstrual cycle, of reproductive life and fluctuations in the concentrations of sexual steroids on pharmacokinetics and pharmacodynamics must be considered. Furthermore, the use of oral contraceptives or hormonal replacement therapy, the sex hormone-related changes in total body water or in the amount of fat influence the overall effect of drugs.

On the contrary, the influence of androgens on drug effects is minimal because of the even plasma levels of these hormones in adult males.

Nevertheless, since women have been scarcely included in the early phases of clinical trials, the results obtained in men have been often translated to women and their exact response to drugs is still not well known.

The available evidence suggests that sex hormones influence drug absorption, distribution, metabolism, pharmacodynamics, and adverse effects. For instance, many cardiovascular drugs are metabolized by enzymes of the cytochrome P450 mono-oxygenases system, which is more expressed in females than in males, showing sex differences in drug response.

Upcoming pharmacological research should aim to further clarify the influence of sex hormones on drug effects and, for this purpose, to increase the number of women enrolled in all phases of clinical trials. An evidence-based pharmacotherapy in women is therefore auspicable for women's health.

Keywords Pharmacokinetics • Pharmacodynamics • Sex • Progesterone • Estrogen • Testosterone • Drug adverse effects

Abbreviations

ACE Angiotensin-converting enzyme
CYP Cytochrome P450 mono-oxygenases
RAS Renin-angiotensin system

1 Introduction

In recent years there has been growing interest and increasing recognition of the influence of sex (genetically determined) and gender (related to social factors) in response to drugs in males and females. As acknowledged by several Institutions, Regulatory Agencies and Scientific Societies, "being male or female is an important basic human variable that should be considered when designing and analyzing studies in all areas and at all levels of [. . .] health-related research." (http://www.nap.edu/catalog/10028.html).

A growing wealth of data shows that differences in pharmacokinetics, pharmacodynamics, and physiology exist between women and men and that they contribute to the occurrence of sex–gender differences in drugs response. Beyond differences in gene expression and regulation (Franconi et al. 2007), hormonal

influences play a key role in several aspects of pharmacological response. Sex hormones have direct and indirect effects upon pharmacokinetics and pharmacodynamics. Indeed, in women the phase of the menstrual cycle, the phases of reproductive life (i.e., pregnancy, menopause, etc.), and fluctuations in concentrations of sexual steroids further influence both pharmacokinetics and pharmacodynamics. Furthermore, the different hormonal milieu, the use of oral contraceptives or hormonal replacement therapy, the sex hormone-related changes in total body water or in the amount of fat, all influence the overall effect of drugs (Leinwand 2003; Regitz-Zagrosek 2006). The relationship between sex hormones and drugs is bidirectional, with hormones (both endogenous and exogenous) affecting drug response and, conversely, certain drugs affecting sex hormone levels.

Given the constant plasma levels of androgens in the adult male life the influence of these hormones on drug effects is minimal. Conversely, because of the influence of female sex hormones fluctuations during the menstrual cycle and the adult life, women have been often excluded from the early phases of clinical drug development. Therefore, women have scarcely been included in pharmacological research studies and the results obtained in men have been often translated to women. However, the clinical effect of drugs in women may be different from that expected on the basis of the knowledge gathered in men. These sex differences in the effect of drugs are related to a different genetic susceptibility but greatly to the influence of sex hormones on pharmacokinetics and pharmacodynamics. The possibility of specific different responses to pharmacological therapy in women and in men has progressively emerged and a growing attention has been posed on how sex affects drug pharmacokinetics and pharmacodynamics, with particular regard to the role of sex hormones.

2 Sex Hormones-Related Differences in Pharmacokinetics

Several physiologic differences between men and women may account for variations in pharmacokinetics. Some of these differences may be related to genetic-determined responses (metabolism) to drugs but most are related to the effect of sex hormones on pharmacokinetics. Sex hormones are known to affect dose, plasma levels, interval of administration, excretion with significant clinical effects that are especially relevant for drugs with narrow therapeutic indexes, such as digoxin, warfarin, etc. (Franconi et al. 2011a). These pharmacokinetics differences between men and women are summarized in Table 1.

2.1 Drug Absorption

Numerous differences that may affect drug absorption have been evidenced between men and women in gastrointestinal apparatus and many of them are

Table 1 Sex hormone-related differences in pharmacokinetics

Mechanism	Sex-specific features	Reference
Drug distribution	Effects of estrogens on water and sodium retention[a] and fat amount and distribution	Jochmann et al. (2005)
	Increased volume for lipophilic drugs in women[a]	
	Smaller and fluctuating distribution volume in women[a]	
	Increased volume for hydrophilic drugs in men[a]	
Drug binding	Effects of estrogens on α-acid protein and α-globulins[a]	
	Serum-binding globulins higher in women[a]	
	Effects of testosterone on protein binding[a]	
	α-1 acid glycoprotein higher in men[a]	
Oral absorption	Effects of progesterone and estrogens on intestinal contractility and transit[a]	Schwartz (2003a)
	Longer gastric emptying time in women due to slower motility[a]	Soldin and Mattison (2009)
	Longer gastrointestinal transit time in women[a]	
	Lower gastric secretion in women[a]	
	Higher gastrointestinal pH, related to longer transit time[b]	
Metabolic differences (phase I)	CYP1A2, CYP2E1, CYP2D6 have lower activity in females[b]	Oertelt-Prigione and Regitz-Zagrosek (2009)
CYP	CYP3A4 has higher activity in females[b]	Jochmann et al. (2005)
Metabolic differences (phase II)	Slower metabolism in females[b]	Schwartz (2003a)
Excretion differences	Lower renal clearance in women[b]	Franconi et al. (2011a)
	Lower active secretion in women[b]	
	Slower excretion in women[b]	
	Testosterone-induced increase in muscle metabolism and augmented creatinine clearance in men[a]	
Other hormonal influences	Estrogens influence inflammation, vasodilation, apoptosis, contractility[a]	Oertelt-Prigione and Regitz-Zagrosek (2009)

[a]These differences depend on sex hormones and vary during menstrual cycle and pregnancy
[b]These differences partially depend on sex hormones

influenced by sex hormones (Gandhi et al. 2004; Franconi et al. 2007; Soldin and Mattison 2009). It is well known that progesterone and estrogens inhibit intestinal contractility and transit (Everson 1992), women secrete less gastric acid and tend to have a prolonged gastrointestinal transit time than men (Soldin and Mattison 2009) (Table 1). Gastric and bowel transit time are influenced by the phase of menstrual cycle as progesterone has a relaxing effect on bowel smooth muscle, thereby slowing transit time during the luteal phase. The differences in basal gastric pH may influence the rate of dissolution of drugs that have pH-dependent solubility in acidic environment while the differences in transiting may influence drug

absorption. Gastric empting is lower in women than in men leading to prolonged gastric retention and delayed drug absorption from the small intestine. These differences have been associated with unexpected variations between sexes, particularly regarding modified-release dosage forms. The prolonged gastrointestinal transit time observed in women could require a longer wait after eating before drug taking, such as anticoagulants that must be ingested on an empty stomach. The sex hormone-induced differences in drug absorption are not limited to gastrointestinal tract but involve also the transdermal or subcutaneous administration (Franconi et al. 2011a), in relation to the different subcutaneous lipid amount related to sex hormones. Despite correction for height, body weight, body surface area, and body composition, sex-dependent differences in biotransformation have been observed for a few specific drugs such as nicotine, aspirin (acetylsalicylic acid), and heparin (Soldin and Mattison 2009). However, these differences are dependent upon genetic traits and are not greatly influenced by sex hormones.

2.2 Drug Distribution

The effect of sex hormones in determining the sexual dimorphism in body composition and body weight, in the amount of intra and extracellular water as well as in the extent of plasma protein binding of the drug may account for different drug distribution. Specifically, sex hormone-dependent physiological differences that may affect drug kinetics include the effect on body mass index and body fat deposition, on absolute and relative water compartment and on plasma proteins. The effects of sex hormones lead to a greater muscle mass and a larger intravascular volume in men and in postmenopausal women.

Drug distribution is also affected directly by sex hormones. Indeed, the percentage of tissue-water fluctuates in women throughout the menstrual cycle, as high estradiol concentrations are associated with sodium and water retention. Testosterone has less effect on tissue-water content. For these reasons, the same dose of a hydrophilic medication will have a higher serum concentration in a woman than in a man due to the smaller amount of water in which the medication will disperse. Similarly, the same dose of a lipophilic medication will have a lower serum concentration in a woman compared to a man of the same weight because there is a relatively larger lipophilic compartment in which the medication resides. Furthermore, the distribution of a hydrophilic drug will be different in the same woman in the different phases of the cycle because of the water-retentive effect of estrogens and the antimineralocorticoid and diuretic effect of progesterone.

In plasma, albumin, α-acid protein, and α-globulins are the most relevant proteins for drug binding and therefore drug distribution. The two proteins are influenced by estrogens whereas albumin, the major plasma protein involved in reversible drug binding, is not consistently affected by sex hormones (Soldin and Mattison 2009). Estrogens also increase the levels of the serum-binding globulins, such as

sex hormone-binding globulin, thyroxine-binding globulin, corticosteroid-binding (Wiegratz et al. 2003), thus altering the free (active) fraction of medications.

In men, testosterone affects protein binding of drugs and their distribution and this effect may also be affected by pharmacological use of testosterone. As mentioned, sex hormones may also influence drug distribution indirectly through their effect on muscle and fat mass. Differences in adipose tissue are present even after correction for body mass index and depend in both sexes on the level of sex hormones. As aforementioned, testosterone is known to increase muscle mass and to decrease body fat. However, in the case of exogenous testosterone its effects depend on the type of androgen and on the degree of methylation of androgens into estrogens. Estrogen deficiency is associated with an increase in visceral fat and a decrease in muscle mass, while estrogen replacement therapy reduces the menopausal-related increase in body fat. As already mentioned, the larger proportions of body fat in women, and especially in pregnant women, may increase the body burden of lipid-soluble, slowly metabolized toxicants.

2.3 Drug Metabolism

Drug metabolism is influenced by sex (Franconi et al. 2011a). The sex differences in drug metabolism, however, are more dependent upon gene expression than on the effect of sex hormones. Both phase I (oxidation, reduction, hydroxylation, etc.) and II (glucuronidation, sulfation, acetylation, methylation, etc.) are sexual dimorphic (Table 1). In general, lipophilic compounds have a tendency to pass through biological membranes and/or to be stored, and are often susceptible to phase I types of metabolism (Custodio et al. 2008).

2.3.1 Phase I

Cytochrome P450 mono-oxygenases (CYP) are involved in phase I catalyzing the metabolism of various endogenous and exogenous substrates. Many cardiovascular drugs are metabolized by enzymes of the CYP system, showing sex differences partly related to the effect of sex hormones. Sex differences have been found with regard to CYP1A2, CYP2D6, CYP2E1, and CYP3A4 and are particularly relevant in cardiovascular medication (Table 2).

Whereas men seem to have higher activities of the CYP450 isoenzymes, such as CYP1A2, CYP2D6, and possibly CYP2E1, women appear to have a higher clearance of CYP3A4 substrates (Zhu et al. 2003; Schwartz 2007).

These sex differences are of particular relevance as CYP3A4 is involved in the metabolism of nearly 50 % of all drugs and is more expressed in females than in males. In general, it appears that women metabolize medications processed by the 3A4 system 20–40 % faster than men (Krecic-Shepard et al. 2000). CYP3A4 is involved in the metabolism of steroid sex hormones and therefore its activity is

Table 2 Sex hormone-related differences in pharmacodynamics of cardiovascular drugs in women and men

Class	Sex-specific features	Side effect	Comments	Reference
Beta-blockers	Greater reduction in blood pressure in women	Greater incidence of adverse event in women because of the higher plasma levels	Sex hormones modulation of β-adrenergic receptors in heart and vessels. Hormone supplementation with estrogens and progestins can prevent β1-receptors upregulation	Luzier et al. (1999) Gilmore et al. (1992) Thurmann et al. (2006)
ACE-inhibitors	Similar effectiveness in women and men	Adverse drug reactions in the form of cough occur twice as frequently in women	Estrogens and testosterone increase angiotensinogen	Shekelle et al. (2003)
			Estrogens reduce ACE and renin activity	Jochmann et al. (2005)
			Testosterone increase renin activity	
Antiplatelet therapy	Less clear benefit in primary prevention of myocardial infarction in women	–	Testosterone increases the aspirin-induced inhibition, estradiol has no effect	Jochmann et al. (2005)
			Pharmacokinetics differences (i.e., bioavailability) disappear in women taking hormonal contraception	Spranger et al. (1989)
Antiarrhythmics	–	Greater incidence of Torsade de Pointes	Direct role of sex steroids on QT length	Schwartz (2003a)
		More tachycardia in women		

influenced by the phases of ovarian cycle, the phase of reproductive life, and by the use of estrogens for contraception or hormonal replacement therapy. Therefore, the effect of drugs such as diltiazem, nifedipine, amiodarone, amlodipine, atorvastatin, gemfibrozil, lovastatin, and simvastatin can be greatly influenced by sex hormones.

In contrast, the CYP1A2 enzyme, which metabolizes clopidogrel and propranolol, is more active in males, resulting in higher levels of these medications in females (Jochmann et al. 2005).

Also CYP2D6 is more expressed in males, who show an increased medication clearance compared to females, especially in the case of extensive metabolizer (Schwartz 2007). This enzyme metabolizes a large number of antiarrhythmics (encainide, flecainide, mexiletine, propafenone) and betablockers (Oertelt-Prigione and Regitz-Zagrosek 2009), and may in part explain why the anti-arrhythmic effect and ADE frequency with these drugs are more marked in women than in men (Franconi et al. 2007).

Few data exist on sex differences on metabolism of CYP2C9 (metabolizer of ibuprofen, irbesartan, losartan, etc.) and CYP2C19 substrates and suggest a slower clearance in women compared to men (Jochmann et al. 2005).

As for CYP2C19, a study (Hagg et al. 2001) failed to find any gender difference in its activity but revealed that the association of estrogen with progestin reduced the activity of CYP2C19, an effect that seems to be related to the ethinylestradiol component. Thus, CYP2C19, which metabolizes many drugs including clopidogrol, warfarin, lansoprazole, omeprazole, irbesartan, and in part propranolol, does not show relevant sex-specific differences but may be inhibited by the association of estrogen with progestin (Zingone et al. 2009).

With the exception of pravastatin, rosuvastatin (both without significant CYP metabolization), and fluvastatin (predominantly CYP2C9 metabolization), all statins are primarily subject to hepatic metabolism via CYP3A4 (Jochmann et al. 2005), and therefore influenced by estrogens.

2.3.2 Phase II

As for phase II metabolism (glucuronidation, sulfation, acetylation, methylation, glutathione conjugation), genetic polymorphisms and racial differences have received extensive investigations, although some data on sex differences exist. Drugs that are metabolized only by conjugation are, in general, cleared faster by men than by women (Schwartz 2003a). Examples of cardiovascular medications undergoing glucuronidation include propranolol and labetalol. Glucuronidation of propranolol is faster in men compared with women and propranolol concentrations were higher in postmyocardial infarction women patients receiving the same doses of propranolol as men (Schwartz 2003b). Clearance of labetalol (cleared by combined processes) was also higher in male hypertensive patients compared with female patients (Johnson et al. 2000). These effects, however, are little influenced by sex hormones.

2.4 Drug Elimination

Men and women also differ in the elimination of drugs. Women have a lower creatinine clearance, while in men, testosterone-induced increase in muscle metabolism is associated with augmented creatinine clearance (Schwartz 2003a). The lower renal clearance observed in women compared with men further decreases with age. It is important to underline that significant decreases in renal function are often present in older women despite normal serum creatinine, thus increasing the risk of increased toxicity in elderly women (Singh et al. 2000), above all for those drugs with a narrow therapeutic to toxic ratios, such as digoxin (Rathore et al. 2002).

The differences in drug excretion are only in part affected by sex hormones.

3 Sex Hormones-Related Differences in Pharmacodynamics

Despite several data are available on pharmacokinetic differences between males and females, few studies are available on pharmacodynamic sex differences. Women respond to cardiovascular medication differently than men (Jochmann et al. 2005). Several cardiovascular targets show sex-specific pathways such as myocardial calcium handling, mitochondrial activity, β-adrenergic receptors density (Ostadal et al. 2009), reactive oxygen species production and antioxidant defenses (Malorni et al. 2007), renin-angiotensin-aldosterone system (Pretorius et al. 2005; De Silva et al. 2009), and sympathetic system (Hinojosa-Laborde et al. 1999).

However, these pharmacodynamic differences are only in a small part dependent upon sexual hormones (Arias-Loza et al. 2007; Malorni et al. 2008).

Sex differences in pharmacodynamic responses generally include increased adverse cardiovascular drug effects in women compared with men (Torsade de Pointes arrhythmias, increased risk of hemorrhagic consequences of anticoagulation or thrombolytic therapy, electrolyte abnormalities with diuretics, myopathy with HMG Co-A reductase inhibitors, cough with angiotensin-converting enzyme (ACE) inhibitors, and increased incidence of thrombosis). This may be related in part also to differences in pharmacokinetics leading to greater plasma levels of cardiovascular drugs in women (i.e., digoxin, beta-blockers) when dosage is not adequately adjusted. Indeed, it is commonly believed that, as the most prominent factor in adapting medication dosages between the sexes is to tailor for body size, it is sufficient to normalize the dose for body weight or surface to obtain similar clinical effect in women. However, due to the numerous differences mentioned above, it is clear that adjustment for body size does not automatically optimize the therapeutic effect, as commonly believed, since there are differences in drug metabolism that remain also after these corrections.

Few data are available on the interactions between sex hormones and specific cardiovascular drugs. While the effect of sex hormones are clear for drugs with

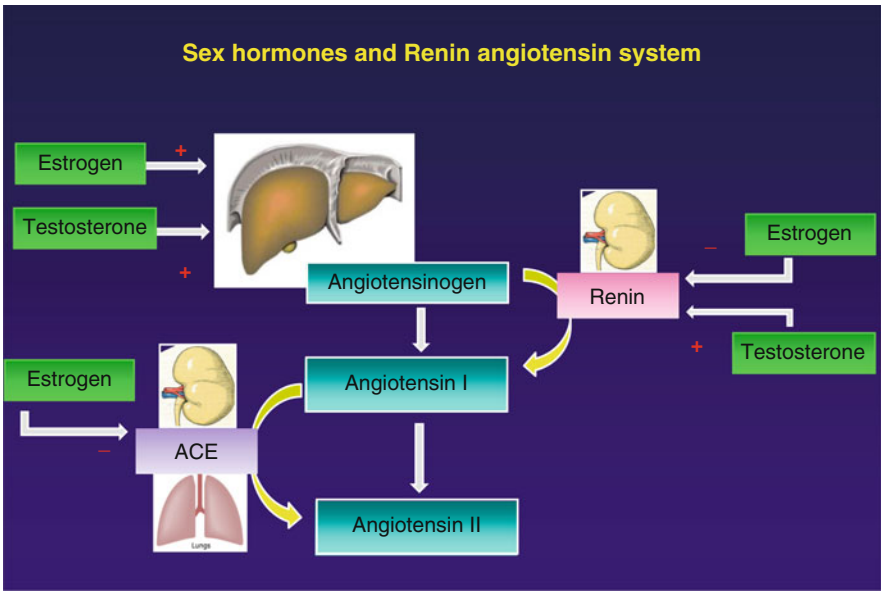

Fig. 1 Sex hormones and renin angiotensin system

narrow therapeutic indices, for most of cardiovascular drugs there is no clear evidence of drug interaction with sex hormones. However, evidence exists for the interaction between sex hormones and drugs acting on renin-angiotensin system (RAS). Indeed, endogenous sex hormones interact with the RAS (Fig. 1) that plays a significant role in the regulation of cardiovascular system and disease. Endogenous and exogenous estrogens increase angiotensin II plasma levels and decrease ACE, renin activity, and the expression of the angiotensin II Type-1 receptor (Fischer et al. 2002). Indeed, premenopausal women have slightly higher angiotensinogen levels than postmenopausal women, and both estrogen replacement therapy or contraceptive medication increase angiotensinogen in the circulation. However, transdermal estrogen replacement therapy, avoiding the first pass effect, is associated with mild, if any, stimulation of angiotensinogen production (Fischer et al. 2002). The cardioprotective effects of endogenous estrogens may result in part from inhibition of the RAS. It has not been established, whether these hormonal influences on the RAS modulate effectiveness of therapy with ACE-inhibitors or with drugs acting on the RAS. Androgens have been shown to upregulate the RAS, increasing angiotensinogen messenger RNA and plasma renin activity, and appear to produce an overall vasopressor effect (Fischer et al. 2002).

The bioavailability of acetylsalicylic acid is greater in women than in men, due to a more rapid adsorption and hydrolyzation in females than in males, a larger distribution volume or a slower clearance. However, acetylsalicylic acid has also hormonal influence because the difference in bioavailability disappears in women

under hormonal contraception, and at mid-cycle absorption is slowest (Franconi et al. 2011b). A part of these pharmacokinetic differences, gender differences in the effect of aspirin was found as acetylsalicylic acid inhibits spontaneous aggregation only in men (Franconi et al. 2011b), supporting the more common aspirin resistance among women than among men. Indeed, testosterone enhances the aspirin-induced inhibition, while estradiol has no detectable impact (Spranger et al. 1989).

As women have increased platelet function compared with men (Qayyum et al. 2008), it seems that women have a major benefit from glycoprotein IIb/IIIa inhibition (abciximab, eptifibatide, tirofiban). However, women have an increased risk of major bleeding complications (Boersma et al. 2002).

4 Sex Hormones and Adverse Drug Effects

Women have a 50–70 % greater risk of suffering from adverse drug reactions than men, and 60 % of patients admitted to hospital with an adverse drug effects are female (Pirmohamed et al. 2004; Patel et al. 2007).

Although the underlying reasons are not perfectly clear, hormonal factors, differences in pharmacokinetics and pharmacodynamics, and polytherapy may have a role. The sex-related differences in pharmacokinetics cause women to be more frequently overdosed than men. In addition, pharmacodynamics may play a role in causing females to be more sensitive to drugs than males.

Drug interactions that have the potential (inhibitors of cytochrome P450 isoenzymes) to result in high drug concentrations can be another predisposing factor to develop these adverse drug effects. Women have also a greater susceptibility to some adverse drug effects such as in the case of long QT syndrome (Kurokawa et al. 2009). Indeed, severe cardiac arrhythmia and Torsade de Pointes occur approximately twice as often in women as they do in men. Estrogens are known to have electrophysiological effects because of a direct effect on cardiac myocyte electrophysiology (Rosano et al. 2000).

Acute estrogen administration has been shown to stabilize atrial electrical activity. Estrogens may alter cardiac repolarization because of this similarity to digoxin and may influence repolarization interval.

In addition to antiarrhythmics, several other classes, such as antibiotics, gastrointestinal drugs, antipsychotics, and antihistaminics (Anthony 2005) are potentially associated with prolongation of the QT interval and with the risk of Torsade de Pointes tachycardia in women (Schwartz 2003a).

Explanations for this phenomenon include the longer corrected QT interval in women, which persists also after correction for heart rate, hormonal influences, dosage not adjusted for body weight (Schwartz 2003a). The role of sex steroids on QT length is supported by several data. QT interval before sexual maturity is of equal length in both sexes, shortens after puberty in men and gradually increases

until approximately 60 years of age, when it approaches that in women (Kurokawa et al. 2009). The repolarization duration is shorter in the luteal phase than in the follicular phase by about 10 ms (Kurokawa et al. 2009), hormonal replacement therapy with estrogen alone in postmenopausal women causes a slight but significant QT interval prolongation, while the combination of estrogen and progestin consistently shortens it (Kurokawa et al. 2009). Studies on the occurrence of arrhythmia in menstruating women have also shown that arrhythmia has a cyclical pattern with a greater incidence at ovulation and during the follicular phase and that this cyclical variation is related to the plasma levels of estradiol and progesterone (Rosano et al. 1996). Experimental investigations suggest that sex hormones may alter either Ca^{2+} currents, K^{+} currents, or both and that actions on these ionic currents may account for the gender differences in cardiac repolarization.

5 Clinical Implications

Although several pharmacokinetic and pharmacodynamic differences related to sex are now well known, the appropriate dosage and dosing schedule and, above all, the clinical effects and the differences in clinical outcomes are still not recognized for many drugs routinely used in clinical practice in women.

It is now recognized that sex hormones influence drug effects, throughout pharmacokinetic and pharmacodynamic influence. Menstrual cycle, pregnancy, and menopause can be associated with changes in the pharmacokinetics of drugs, mostly as a result of changes in sex steroid concentrations and alterations in total body water (e.g., expansion of total body water, increase of renal plasma flow, and glomerular filtration during pregnancy) or body fat composition and distribution. In addition, interactions with exogenous hormone therapy such as oral contraception and hormone replacement therapy must be taken into account. For instance, estrogens and progestins interact with a number of cardiovascular drugs, possibly by inhibiting CYP enzymes or increasing drug glucuronidation (Schwartz 2003b; Jochmann et al. 2005). Since the metabolism of adipose tissue differs from that of muscle tissue, some of the differences between men and women are attributed to body composition metabolism of adipose tissue (Soldin and Mattison 2009). A lower basal metabolic rate per unit of body surface area reflects the lower lean body mass in women due to a smaller skeletal muscle component (Soldin and Mattison 2009).

However, because of the lack of pharmacological clinical trials dedicated to women, their exact response to drugs is not well known.

It is therefore of pivotal importance taking into account the influence of sex hormones in pharmacokinetics and pharmacodynamics, drug efficacy, and adverse events. However, due to the lack of solid data obtained from clinical studies, evidence-based pharmacotherapy in women is still lacking.

6 Conclusions

The physiological differences between men and women and, particularly, the different role of sex hormones, strongly suggest that drug responses are influenced by sex. Hormonal effects on drug therapy are significant, as sex hormones influence drug absorption, distribution, metabolism, pharmacodynamics, and adverse effects.

Further studies are required to better clarify the influence of sex hormones on drug effects and to investigate the importance of the balance between sex hormones in both sexes.

Take Home Messages

- Menstrual cycle, pregnancy, and menopause, as well as oral contraception and hormone replacement therapy, can be associated with changes in pharmacokinetics.
- Estrogens and progestins interact with many cardiovascular drugs, possibly by inhibiting CYP enzymes or increasing drug glucuronidation.
- Sex differences in pharmacodynamic responses generally include increased adverse cardiovascular drug effects in women compared with men.
- Sex hormones affect drug dosage, plasma levels, interval of administration and excretion, with clinical effects that are especially relevant for drugs with narrow therapeutic indexes.
- The appropriate dosage and the differences in clinical outcomes are still not recognized for many drugs routinely used in clinical practice in women.
- Due to the lack of solid data obtained from clinical studies, evidence-based pharmacotherapy in women is still lacking and further studies are needed.

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Sex and Gender in Adverse Drug Events, Addiction, and Placebo

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Abstract Sex–gender-based differences in response to pharmaceutical treatments are still under evaluation but evidence already exists regarding the impact of sex–gender-related differences on drug safety profile, drug abuse/addiction, and placebo effects. For a number of drugs it is well recognized that a sex–gender dimorphic profile in terms of drug adverse effects exists and appears to be more frequent and severe in women than in men. However, it is not well known whether this is due to pharmacodynamic or pharmacokinetic differences. Indeed the optimization of therapy requires that attention is paid to single sex–gender. Numerous pharmacokinetic, pharmacodynamic, and sociocultural differences between women and men in drug abuse have been described. Here we focus on sex–gender differences in alcoholism and nicotine addiction. The relevance of sex and gender differences in addiction appear to be relevant. Specific programs aimed to address addicted women's specific needs (child care, pregnancy, housing, and violence and others) are recommended. Finally, this article discusses the possible effect of sex–gender on placebo response in the light of the more significant recent literature evidencing that studies are urgently required in order to better understand the role of sex–gender on placebo mechanism and its impact on randomized clinical trials outcomes.

Keywords Adverse reactions • Aging • Sociocultural • Addiction • Stress • Pregnancy • Placebo • Pain

Abbreviations

SGD	Sex–gender differences
ADE	Adverse drug events
RCT	Randomized controlled trials
ADH	Alcohol dehydrogenase
OC	Oral contraceptives
HRT	Hormone replacement therapy

1 Introduction

Although the terms sex and gender are commonly used interchangeably, for the purpose of this review the term sex–gender differences (SGD) will be used to describe any difference observed between men and women as it may impact several aspects of experimental medicine and clinical therapeutics. SGD will reflect both physiological distinctions between each sex, as well as environment influences dictated by differences in diet, life style, and exposure to environmental pollutants (tobacco, alcohol). Where appropriate, differences due to cultural and behavioral responses are highlighted and contrasted against those due to hormonal and anatomical differences (Kim and Nafziger 2000).

Drug's pharmacokinetics and pharmacodynamics are sex–gender dependent. Importantly, the impact of SGD on response is not limited to drugs but is extended

Table 1 SGD in response to drugs and xenobiotics modified by Gochfeld (2007)

Exposure[a]	Xenobiotics kinetic	Xenobiotics dynamic	Modifiers
Occupation	Body size and composition, blood and organ volumes	Membrane	Hormonal environment including the use of OC and HRT
Time-activity budget	Absorption	Receptors and channels	Reproductive status
Locations	Transport and distribution	Interactions with macromolecules	Nutritional factors
Diet	Protein binding	Target organ response	Smoking
Critical periods (pregnancy, lactation)	Metabolism: phases I and II		Alcohol
	Excretion/sequestration		
	Solubility		
	Tissue affinity		
	Mobilization and blood levels		

[a]In many societies, exposure opportunity differs between sexes and involves when, and how people come in contact with a xenobiotics in air, water, soil or dust, food, or in the home, community, or workplace environment

to all xenobiotics such as substances of abuse (Anthony and Berg 2002a, b; Benowitz 2010; Ceylan-Isik et al. 2010; Fattore et al. 2008; Gochfeld 2007) (Table 1).

During the drug development process, SGD which are species dependent are well evidenced, particularly in terms of biodisposition. Yet these differences have not received adequate attention in human pharmacology (Franconi et al. 2007, 2011a, b; Regitz-Zagrosek 2006; Schwartz 2007; Soldin and Mattison 2009). Historically, females have not been prospectively included in early phase studies, and when incorporated, the experimental design does not formally address potential SGD in exposure parameters and response. The lack of systematic evaluation of the influence of sex–gender during the drug development process, including differential sensitivity or exposure, may result in post-hoc and post-marketing safety surveillance data suggesting a higher rate and severity of adverse drug events (ADE) in women (Krahenbuhl-Melcher et al. 2007; Patel et al. 2007; Pirmohamed et al. 2004).

This article dedicates also attention to placebo response and risk of addiction from a sex–gender perspective. Placebo is commonly present within randomized controlled trials (RCT), and an examination of SGD in placebo response leads to significant clinical implications (Barsky et al. 2001, 2002; Beecher 1955; Kaptchuk 1998; Koshi and Short 2007).

2 Adverse Drug Events

Besides therapeutic effectiveness, drug tolerability is a key issue especially for treatments that must be chronically taken or that involve drugs with narrow therapeutic indexes, somewhat arbitrarily defined as a difference of less than or equal to threefold between a dosage level producing unacceptable toxicity and the

dosage producing acceptable efficacy (Levy 1998). Numerous pharmacokinetic and pharmacodynamic SGD are described and they are largely reviewed (Donovan 2005; Franconi et al. 2007, 2011a, b; Gandhi et al. 2004; Kashuba and Nafziger 1998; Regitz-Zagrosek 2006; Schwartz 2007; Soldin and Mattison 2009; Zopf et al. 2008). However, the studied SGD have not been translated into a change in therapeutic protocols. Consequently, the evidence from studies in men is commonly extrapolated to women, ignoring variables such as age, hormonal status, and other logical and culturally dictated differences between men and women. Inevitably, as simple extrapolation, this may lead to an inappropriateness of care, and to an increase in the incidence of ADE in women compared to men (Franconi et al. 2011a, b; Rademaker 2001). Also the severity of ADE can be more pronounced in females (Krahenbuhl-Melcher et al. 2007; Rademaker 2001; Schwartz 2007; Zopf et al. 2008): in fact, 60 % of the patients admitted to hospital for ADE are reported to be females (Patel et al. 2007; Pirmohamed et al. 2004; Sikdar et al. 2010). Dose-related ADE are the dominant type in female subjects, particularly those referable to the cardiovascular system (Krahenbuhl-Melcher et al. 2007; Rademaker 2001; Zopf et al. 2008).

Multiple factors can be involved in the higher rate of ADE in women and they are summarized below.

2.1 Drug Consumption and Polytherapy

Women consume more prescriptional and nonprescriptional drugs than men (Franconi et al. 2007). Since polytherapy is higher in women than in men, ADE from drug interactions can occur more frequently (Gurwitz 2005; Office of Strategic Planning Health Care Financing Administration 1998).

2.2 Aging

Importantly, nearly three out of five people aged 65 years or older are females (Office of Strategic Planning Health Care Financing Administration 1998); therefore, those factors impacting drugs safety and tolerability in elderly people, not surprisingly affect females more than males. Aging modifies psychological and cognitive characteristics of patients, drugs' pharmacokinetics and pharmacodynamics, and increases drug consume (Gurwitz 2005). Women show differential hemodynamic responses contingent upon the age, with younger patients paradoxically showing more liability in terms of orthostatic changes, while older female patients are generally indistinguishable for males. Likewise, aging-related modifications in gastric emptying time, splanchnic blood flow, fat/muscle ratio, and renal clearance mechanisms can be more accentuated in females (Cheng et al. 2011), leading to a decreased tolerability profile respect to males.

2.3 Underrepresentation of Women in Clinical Studies

Historically, women are underrepresented in clinical studies, predominantly in early phase development (phases 1, 2) where disease-related differences in demographics do not dictate selection based on sex–gender (Pinnow et al. 2009). Regulatory authorities have noted that exclusion of females particularly from early investigation of novel chemical and biological entities have historically influenced past researches of diseases and development of novel therapies. Regarding phase 3 (pivotal trials, i.e., a study which will form the basis for therapeutic decisions in a clinical setting), the percentage of women included now coincides with the prevalence of the diseases in many areas, with few salient exceptions such as cardiovascular diseases (Yang et al. 2009). Given that SGD may exist in respect to the tolerability to the study drug, underrepresentation of female patients would not allow to detect sex–gender-related differences in the incidence of ADE, in the absence of adequate stratification by sex and of adequate sample size for detecting the significance of such differences.

2.4 Pharmacokinetics and Pharmacodynamics

SGD in pharmacokinetic and pharmacodynamic have been extensively reviewed (Donovan 2005; Franconi et al. 2007, 2011a, b; Gandhi et al. 2004; Kashuba and Nafziger 1998; Regitz-Zagrosek 2006; Schwartz 2007; Soldin et al. 2011; Soldin and Mattison 2009). Men and women may have different pharmacological targets (Komukai et al. 2010; Niesters et al. 2010; Regitz-Zagrosek 2006). As an example, the difference in cardiac repolarization, which becomes longer in women after puberty (Kurokawa et al. 2009) explains the higher susceptibility of female respect to males to the iatrogenic long QT syndrome, a syndrome induced by more than 80 drugs and that can also be fatal (Drici and Clement 2001; Kurokawa et al. 2009). More information on this syndrome is available on http://www.torsades.org (Table 2).

There are many biological SGD that can impact pharmacokinetic parameters (Franconi et al. 2007, 2011a, b). Briefly, women and men differ in body size and composition, metabolism, elimination, oral absorption, which, as previously indicated, can also be compounded by age. The recommended dose of a drug, if calculated for 70 kg healthy young males, may result in higher plasma level or area under the concentration time curve in women. Generally speaking, in women hydrophilic drugs have smaller distribution volume (the theoretical volume in which the total amount of drug would need to be uniformly distributed to produce the desired blood concentration of a drug), whereas lipophilic drugs have higher volume of distribution.

Renal clearance may be different in men and women (~10 % less in females) and this will materially impact the elimination of those products subject to elimination by renal clearance mechanisms (Franconi et al. 2007). Although some dosing

Table 2 Some SGD in ADE rate with the exception of iatrogenic QT

Drugs	ADE type	F/M	Reference
Antineoplastic–immunosopressive drugs[a]	Agranulocytosis	+F	Rodenburg et al. (2011)
	Nausea/vomiting		
	Pneumonia		
	Fever		
5-fluorouracil	Hematological toxicity	+F	Chansky et al. (2005)
	Mucositis		
Anticoagulants and Salicylates[a]	Hemorrhage nonspecified	+F	Rodenburg et al. (2011)
	Hematuria	+M	
	Intracranial bleeding	+M	
	Hemoptysis	+M	
Procainamide[b]	Lupus	+F	Borchers et al. (2007)
Hydralazine[c]	Lupus	+F	Borchers et al. (2007)
Quinidine[d]	Lupus	+F	Borchers et al. (2007)
Amiodarone	Bradyarrhythmia	+F	Batcher et al. (2007)
	Hypotiroidism	+F	Borchers et al. (2007)
	Lupus[e]	+F	Essebag et al. (2007)
Thiazidic diuretics	Hypokalaemia	+F	Toner and Ramsay (1984)
	Hyponatremia	+F	Liamis et al. (2008)
Antiepileptic drugs	Rash	+F	Alvestad et al. (2007)
Valproate	Body weight gain and carbohydrate craving	+F	El-Khatib et al. (2007)
Tacrolimus[e]	Alopecia	+F	Tricot et al. (2005)
Infliximab	Immunomediated liver dysfunction	+F	Mancini et al. (2010)

[a]Risk of hospitalization
[b]Elevated risk
[c]Moderate risk
[d]Low risk
[e]T in f male kidney–pancreas transplant recipients

regimens are corrected for estimated renal function, this does not routinely occur in both clinical trials and routine clinical care, resulting in increased toxicity, especially in elderly women who present other changes in their physiology which render them at greater risk. Many of pharmacokinetic parameters are sensible to sexual hormones, which vary during menstrual cycle and pregnancy (Franconi et al. 2011a, b; Rivero and Curtis 2009).

2.5 *Females' Vulnerability Respect to Specific Classes of Drugs*

Both frequency and character of ADE in women may depend on the drug type (Rodenburg et al. 2011). For example, iatrogenic osteoporosis and fractures can be induced by corticosteroids, thiazolidinedione, and aromatase inhibitors.

Anti-androgen therapy, high doses of thyroxin, calcineurin inhibitors, antiretroviral drugs, selective inhibitors of serotonin reuptake, anticonvulsants, loop diuretics, heparin, oral anticoagulants, and proton pump inhibitors could be of special relevance in postmenopausal women, which are more prone to develop osteoporosis (Mazziotti et al. 2010).

The risk of ADE is also associated with depression (Onder et al. 2003), whose incidence is higher in females than in males (Zender and Olshansky 2009). The onset of ADE is also linked to hepatic and renal functions, and heart failure (Franconi et al. 2011a, b; Soldin et al. 2011).

2.6 Sociocultural Factors

Other variables such as living accommodations, marital status, education level, cognitive impairment, and alcohol and tobacco habits might be associated with incidence of ADE (Okuno et al. 2001; Onder et al. 2002). However, an Italian paper indicates that socioeconomic factors are not significantly associated with hospital admissions for ADE, whereas they are linked with gender and age, polytherapy, nutritional state, and renal condition at least in elderly (Caamano et al. 2005).

ADE could be also related to the role and social function of a woman. In many societies, women are the primary caregivers: this can have a detrimental impact on their health and productivity as their range of responsibilities commonly includes an extended family. For example, elderly Alzheimer's caregivers have an accelerated decline in antibody response to pneumococcal and influenza vaccine following immunization than age-matched (Glaser et al. 2000; Kiecolt-Glaser et al. 1996; Vedhara et al. 1999).

Men and women could present differences in the onset and description of symptoms, which could influence reporting of ADE (Barsky et al. 2001). Finally, doctors can have a different therapeutic interaction between men and women, based upon cultural determinants.

3 Conclusions

Data on the impact of SGD on ADE are mostly obtained by post-hoc analysis and/or metanalysis, therefore, conclusions are limited. Design of clinical studies does not include specific sex–gender related hypotheses and does not consider the influence of hormonal variation on pharmacokinetic and pharmacodynamic, nor evaluates the placebo response in both sexes (see below).

In a different approach, the identification of the possible associations between ADE and social factors, which in turn can be related to SGD, could give to health administrations the necessary information to identify the subjects with a higher level of risk for any given level of exposure and to facilitate the design of programs to reduce the economic and health consequences of ADE.

Take Home Message
Clinical trials need to be designed and sampled in order to early detect sex/gender-related differences in drug safety. This can help to reduce the incidence of ADE in female subjects.

4 Addiction

Cannabis derivates, opiates, cocaine, amphetamine, methamphetamine and "ecstasy," tranquillizer, nicotine and alcohol are the most abused substances (Benowitz 2010; Ceylan-Isik et al. 2010; Greenfield et al. 2010; World Health Organization 2002), being smoking and alcoholism the main causes of preventable death in developed countries (Greenfield et al. 2010).

Drug addiction is reported to be more frequent among men than women (Substance Abuse and Mental Health Services Administration 2005). However, data on pain killer and tranquilizers abuse recognize some discrepancies across the different studies, some of them indicating that use of these drugs is approximately twice in women than men (Grella 2008), while other studies report equivalent rates between sexes or higher rates among men (Greenfield et al. 2010).

Global studies indicate that due to changing socioeconomic factors, women have become increasingly more at risk for drug abuse (Substance Abuse and Mental Health Services Administration 2005). Even if most drug abuse investigations historically have been shaped by demographic characteristic of male addicts, recently SGD have been identified in biological, psychological, and social aspects of addiction (Becker and Hu 2008; Broome et al. 2010; Fattore et al. 2008; Fox and Sinha 2009; Greenfield et al. 2010; Hudson and Stamp 2011; Unger et al. 2010).

4.1 Impact of Genes and Hormones

Genes may play a role in SGD as this aspect is perceived in use and abuse of substances (Becker and Hu 2008), but how genetics, sex, and/or hormones interact with environment is not clarified and still matter of investigation, as well as, SGD differences in the biology of motivational function, which deregulating the patterns of intake, is the hallmark of addiction (Becker and Hu 2008).

Estrogens induce functional changes in dopamine terminals and in GABAergic neurons (Ceylan-Isik et al. 2010; Lajtha and Sershen 2010) being this last also influenced by progesterone-derived molecule (Quinones-Jenab and Jenab 2010). Indeed, the use of progesterone as a therapeutic agent has been proposed especially in nicotine and cocaine female users (Lynch and Sofuoglu 2010; Quinones-Jenab and Jenab 2010).

Circulating sexual hormones partially account for the SGD in nicotine addiction. Cessation, withdrawal, and craving vary during the menstrual cycle, with the luteal phase characterized by a higher craving and more severe withdrawal symptoms. Consequentially, women who attempt to quit during the first days of their menstrual

cycle seem more likely to succeed than women who attempt to quit in the second half of the cycle (Greenfield et al. 2010). Circulating hormones influence also women response to stimulants (cocaine, amphetamine, methamphetamine), and this response is accentuated in the follicular phase of the menstrual cycle (Fox and Sinha 2009; Greenfield et al. 2010). However, the profile of the influence of menstrual cycle on endocrine disruption and stimulant drug use is still scarcely studied (Fox and Sinha 2009).

4.2 Stress Reactivity

The stress-system function is controlled by complex interactions between the hypothalamic-pituitary-adrenal axis and the hypothalamic–pituitary–gonadic axis and may be altered following drug abuse in a sex–gender specific way (Fox and Sinha 2009). In summary, it is believed that substance-abusing women may show increased emotional sensitivity to stress-system changes compared to men.

4.3 Pharmacokinetics

SGD were detected in pharmacokinetics of xenobiotics. Body size and body composition are the basis of the lower distribution volume of ethanol in women respect to men (Benowitz 2010). The lower first passage of ethanol could be ascribed to a lower alcohol metabolism due to lower level of an isoform of alcohol dehydrogenase (ADH) in women gastric mucosa (Benowitz 2010; Ceylan-Isik et al. 2010). Importantly, with aging, ADH declines in men but increases in women (Ceylan-Isik et al. 2010), indicating that SGD are also age dependent.

In contrast, nicotine is more quickly metabolized in women than men (Benowitz 2010), as women express higher activity of CYP2A6 (Ceylan-Isik et al. 2010). People with genetic polymorphism, which induces low activity of this enzyme, smoke fewer cigarette/day and are able to maintain abstinence better in comparison with people with higher activity of enzyme and this could explain the major difficulty of women in sustaining complete abstinence from smoking (Benowitz 2010). The enzyme activity is increased by oral contraceptives (OC) and pregnancy, suggesting the influence of sexual hormones in nicotine metabolism (Benowitz 2010). Other substances of abuse such as cocaine do not present any significant SGD in pharmacokinetics (Evans and Foltin 2010).

4.4 Psychological, Cultural, and Socioeconomic Factors

Biological factors are linked to socioeconomic, psychological, and cultural factors and these last may have a different impact in men and women on the risk of addiction (Greenfield et al. 2010). In alcohol abuse, women may show different

causes and family influences when compared with men (Curran et al. 1999). Socioeconomic status is a prominent predictor of alcohol dependency among men, while family history of alcohol disorders is a stronger predictor among women (Greenfield et al. 2010). Alcohol-addicted women are significantly more likely to have co-occurring psychiatric disorders and are more likely to die from suicide, alcohol-related accidents, circulatory disorders, and liver cirrhosis than male alcoholics (Smith and Weisner 2000).

Women and men also differ on patterns of cigarette smoking and assumption of alcohol (Greenfield et al. 2010). At least for smoking, nonbiological factors seem to be more important for women; consequentially nonpharmacological treatment may be more effective in women than men (Greenfield et al. 2010). Nicotine replacement therapy does not seem to work in females as it does in males, but data on this point are not conclusive (Greenfield et al. 2010). Whereas non-nicotine medications are equally effective in men and women, in contrast, bupropion is more active in women (Greenfield et al. 2010). Thus, the interplay of pharmacology, with biological and cultural SGD yields a complex therapeutic challenge. As an example, an obstacle to quit smoking for women is represented by the prospect of weight gain following smoking discontinuation, which also leads to relapse more often than in men (Greenfield et al. 2010). Indeed, some data suggest that weight gain is higher in women than men during the "*quit process*" (National Institute on Drug Abuse 1998).

4.5 Pregnancy

Pregnancy and lactation are pertinent drug abuse-related concerns for women and their offspring. Prenatal exposure to drugs of abuse is the single largest preventable cause of developmental alterations and compromise many children today (Malanga and Kosofsky 2003). The teratogenic effect of ethanol is extensively described (The Committee on Substance Abuse and the Committee on Children with Disabilities of the American Academy of Pediatrics 2000), whereas nicotine, cocaine, and opioids have a variety of growth and neurological deficits (Addis et al. 2001; Greenfield et al. 2010; Kandel et al. 1994; Varvarigou et al. 2009).

Importantly, preclinical studies indicate that exposure in uterus to substances of abuse may have divergent effects in male and female offspring (Malanga and Kosofsky 2003). However, there are only few clinical observational studies regarding the SGD as a consequence of exposure in uterus. For example, smoking in pregnancy delays fetal growth mainly in boys (Greenfield et al. 2010; Varvarigou et al. 2009), whereas daughters of women who smoke during pregnancy are more likely to smoke as adults in comparison with sons (Kandel et al. 1994). These data seem to suggest that smoking modifies the development trajectory in a sex–gender specific way and emphasizes the importance of epigenetic.

5 Conclusions

Most treatment models of addiction have been designed for men and are based predominantly on male norms (Greenfield et al. 2010). Gender-specific interventions are beginning to emerge in response to mixed-gender programs, which often fail to address addicted women's specific needs (child care, pregnancy, housing, and violence and others). Notably, some findings suggest that both sexes show better relapse outcomes if they receive tailored care to their particular challenges (e.g., psychosocial issues, provision of child care) (Broome et al. 2010).

Take Home Message
Sex and gender differences in addiction appear to be relevant. Specific programs aimed to address addicted women's specific needs (child care, pregnancy, housing, and violence and others) are recommended.

6 Placebo

Definition of "placebo" and "placebo effect" is far from easy and this reflects the double role that placebo can have in clinical trials (an artifact) and in clinical practice (therapeutically valuable response) (Ernst 2007).

The magnitude of the placebo response is highly variable both within and across therapeutic areas and general statements regarding the impact of SGD on that response are difficult to confirm. However, since a SGD in the placebo response would have a paramount consequence in the evaluation of results from RCT, we attempt to evaluate if placebo effects are influenced by sex–gender determinant, with a focus on the placebo effect in pain/analgesia.

Extensive reviews on different aspects of placebo response have been published, including historical overviews which antedate the initiation of controlled clinical trials (Benedetti 2009; Benedetti et al. 2007; Colloca and Benedetti 2005; Goffaux et al. 2010; Klosterhalfen and Enck 2008; Price et al. 2008). These aspects are illustrated in the following paragraphs.

6.1 Cognitive Psychological Processes

Expectancy (conscious process) and conditioning theories (essentially unconscious process) have been used to explain the placebo effect (Howland 2008) and conditioning (Ernst 2007; Stewart-Williams and Podd 2004). It is a matter of debate if expectancy and conditioning can be distinguished from each other (Kirsch 2004). Pavlovian conditioning may explain placebo effects seen in animals (Jaeger et al. 2005) being also relevant in humans. For example, in humans, both decrements in peripheral leukocyte counts induced by cyclophosphamide and insulin response can

be conditioned (Giang et al. 1996; Stockhorst et al. 2004). There are other examples both in animals and humans on the salutary effect of exposure to conditioned stimuli previously paired with therapeutic agents (Ader and Cohen 1982; Ader et al. 2010; Exton et al. 1998; Goebel et al. 2002; Gorczynski 1990; Jones et al. 2008; Klosterhalfen and Klosterhalfen 1983; Olness and Ader 1992).

No research has been focused on the influence of sex–gender on expectancy and conditioning in placebo setting, thus it is not known whether expectancy and conditioning are sex–gender regulated processes.

SGD may be found under special conditions (Aslaksen et al. 2011; Frank et al. 1999; Jensen and Karoly 1991). As an example, the induction of placebo responses by verbal information may be larger in males compared to females in a setting of placebo analgesia (Aslaksen et al. 2011). In benzodiazepine withdrawal syndrome, where patients, but not controls, expect a reduction of their symptoms after the injection of the drug/placebo, it has been shown that female patients have higher placebo response respect to males (Saxon et al. 2001). Instead, in an experimental setting with placebo analgesia during ischemic pain, males respond to the manipulation of expectancies through pain information, while women do not (Flaten et al. 2006). Worth mentioning that a not recent positron emission tomography study had evidenced that females when exposed to placebo show significantly greater activation of the contralateral prefrontal cortex when compared to the males (Paulson et al. 1998).

6.2 Pain Reporting/Verbalization

Traditional gender roles influence the verbalization of pain (Kallai et al. 2004; Sanford et al. 2002). In fact, in western society, the stereotypical male role characterizes men as stoic and intending to impress women with their ability to withstand pain, while the corresponding female role expects women to exhibit increased sensitivity in order to evoke a protective response in men (Levine and De Simone 1991). Different studies report instead that during painful stimulation, pain reporting is influenced by SGD in emotional activation (Frot et al. 2004).

6.3 Medical–Patient Relationship

Psychosocial context of clinical setting may have an important role in placebo response (Miller et al. 2009). An interaction between experimenter's gender and subject's gender for pain tolerance has been detected (Kallai et al. 2004). Men show higher placebo analgesia after heat stimulus when a male acted as experimenter (Aslaksen and Flaten 2008). In a model where pain was induced by the submaximum tourniquet technique, expectations and pain information were reported to decrease pain only in males being the experimenters only women (Flaten et al. 2006).

Importantly, SGD are also noted in an acupuncture trial with male and female acupuncturists, with females inducing greater trust than male experimenters (White et al. 2003). However, the behavior rather than the gender of the experimenter could be the relevant variable (Aslaksen and Flaten 2008), even if an electrophysiological investigation evidenced the biological basis for different empathic pain response between sexes (Han et al. 2008).

6.4 Biological Process

The biological mechanisms underlying the placebo response are very complex and have been extensively reviewed (Benedetti 2009; Goffaux et al. 2010; Howland 2008). In particular, the administration of opioid antagonists can inhibit the analgesic effects of placebo indicating that placebo response utilizes opioid system (Sauro and Greenberg 2005). Dopamine activation is also salient for placebo response (Scott et al. 2007). Two imaging studies demonstrate that the blinded administration of intravenous glucose induces dopamine release and increases the binding potential in the striatum of men but not of women (Haltia et al. 2007, 2008). Importantly, SGD have been described in opioid and dopaminergic systems, offering a background explanation of SGD in placebo effects (Niesters et al. 2010).

6.5 SGD in Placebo Analgesia

Although, numerous SGD have been documented in pain both in experimental and clinical setting (Paller et al. 2009), SGD in placebo-analgesia have rarely been reported (Greenspan et al. 2007). There are, however, a few findings of stronger placebo analgesic responses in males (Berkley et al. 2006; Fillingim et al. 2009). Others failed to find SGD in placebo response in the post-third molar extraction (Averbuch and Katzper 2001), in transcutaneous electrical stimulation setting (Robinson et al. 1998), and pressure algometer test (Olofsen et al. 2005). A systemic review of literature shows that placebo response is not sex related in fibromyalgia and painful peripheral diabetic neuropathy (Hauser et al. 2011).

6.6 Clinical Trials Design

Placebo response is influenced by the study design and placebo sequence (Batterman 1965; Moertel et al. 1976; Sunshine et al. 1964). Relapse is delayed among patients who are given placebos upon withdrawal of active medication (Greenberg and Roth 1966). This could be of importance in double-blind crossover

studies in which issues of carryover and sequence effects can jeopardize the underpinnings of biostatistical analyses (Ader 1989; Suchman and Ader 1992).

7 Conclusions

Considering the high interindividual variability of placebo responses (Beecher 1955; Wager et al. 2011), which is due to factors such as optimism, expectation, behavioral activation (Schweinhardt et al. 2009), desire for relief, opiate sensitivity (Zubieta et al. 2006), anticipatory activity (Wager et al. 2011), striatal "reward" responses (Scott et al. 2008), human conditions that are difficult to eliminate. Thus it is difficult to measure and to uniformly described placebo response (Clegg et al. 2006). Indeed, response to placebo probably varies during the life of the single individual considering that people with pain, depression, anxiety seem to be more "placebo–nocebo prone" than others (Clegg et al. 2006).

Trial methodologists routinely demand and researchers routinely attempt to include placebo groups in every major clinical trial, and this occurs without knowing the influence of sex–gender on the placebo mechanism.

At this stage, results about sex–gender effect on placebo clinical practice are too preliminary in order to reach to any conclusion. However, in view of the fact that many social, psychological, and biological factors could have a role (Charron et al. 2006; Kaptchuk et al. 2006; van Leeuwen et al. 2006), a sex and gender effect is conceivable. In this contest, clinical research in all therapeutic areas should appropriately take sex–gender into consideration when tailoring treatment to provide optimal effect for each individual.

Take Home Message

Studies are needed in order to better understand the role of sex–gender on placebo mechanism and its impact on RCT outcomes.

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Considerations of Sex and Gender Differences in Preclinical and Clinical Trials

Limor Raz and Virginia M. Miller

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Abstract Women continue to be underrepresented in clinical trials, particularly in Phases I and II of experimental drug studies in spite of legislative guidelines in the USA, Canada, the European Union, Australia, and Japan requiring the inclusion of

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women in clinical trials. As such, women remain a vulnerable population subject to the adverse effects of pharmacological therapies. Thus, women experience higher rates of adverse drug reactions than do men and for women of reproductive age or who may be pregnant, therapeutic options may be limited. This chapter provides a brief history of inclusion of sex and gender as variables in clinical trials, summarizes governmental legislation for consideration of sex and gender in clinical trials and provides specific examples of drugs which have been withdrawn from the market because of side effects in women. Additional information related to sex and gender in preclinical testing, trial design, challenges to recruitment of women for clinical trials and statistical methods for analysis of data also is considered.

Keywords Animal studies • Cultured cells • Definitions • Legislation • Phase I • Phase II • Phase III • Statistical analysis

1 Prologue

Women have received clinical treatments based on evidence and recommendations from clinical studies conducted mainly on men, in spite of documented differences in metabolism, distribution of fat and physiological mechanisms between males and females. As a result, adverse consequences and disparities in medical outcomes for women have served as a fulcrum for women's health reform. Concerns regarding the safety and efficacy of drugs in women have generated guidelines and recommendations worldwide for the inclusion of women in clinical trials (Fleisch et al. 2005; Foulkes 2011). Additionally, women are more likely to live longer than men, thus rendering females more susceptible to chronic illnesses and increased drug interactions. As the field of women's health progresses, the involvement of women in the latter phases of clinical trials has increased, yet there are unmet challenges in experimental design and statistical analyses. The majority of studies do not consider sex and gender differences, fail to report negative findings and may be underpowered to identify whether or not sex and gender differences exist. This chapter highlights the pertinent issues facing researchers and clinical investigators in the field of women's health, while suggesting potential solutions which bring sex and gender to a focus in preclinical and clinical trials with the goal of improving women's health and reducing sex and gender disparities in health outcomes.

2 Introduction

Basic science and medical literature abound in evidence of physiological and pathophysiological differences between males (men) and females (women) affecting development, progression, presentation, and symptomatology of disease beyond those defined by reproduction. As identified in other chapters of this

book, differences in metabolism and liver function affect absorption and disposition of pharmacological agents thus affecting treatment efficacy (Freire et al. 2011; Waxman and Holloway 2009). Therefore, it is only logical that in development and testing of novel diagnostics and therapeutics, sex and gender should be considered as important biological variables. This chapter will define terminology used to describe clinical trials in therapeutic development, briefly review the legislative history affecting representation of sex and gender differences in clinical trials, discuss study design including issues related to preclinical studies with specific examples of drugs where gender influenced outcomes, challenges with recruitment and retention of women and men into clinical trials, and finally, statistical analysis of data by sex and gender.

3 Terminology

Sex and gender. The terms sex and gender are often used interchangeably in basic science and clinical literature. According to the 2001 definitions of the Institute of Medicine (Wizemann and Pardue 2001b), sex is a biological construct dictated by the presence of the sex chromosomes and functional reproductive organs; gender is a spectrum of maleness or femaleness determined by socio-environmental factors. In clinical trials, participants are categorized based on sex or gender often without clear differentiation between the terms or without the understanding that environmental influences which affect the phenotypic expression of a male or female genotype is influenced not only by endogenous sex steroid hormones but also by environmental exposures to chemicals/toxins and behaviors influenced by culture, societal norms, diet, geographical location and lifestyle affecting attitudes toward and access to healthcare. In this chapter, the term sex will be used when discussing study design related to preclinical studies using isolated cells and tissues or experimental animals. The terms male and female will be used to distinguish gender in reference to design of clinical trials.

Classification of clinical trials. In order to assure the efficacy and safety of therapeutic products, strict guidelines must be followed which include development and testing in cell and tissue systems or experimental animals before tests are initiated in humans. The foundation for the regulation of clinical trial processes began in the USA in 1938 with the Federal Food, Drug and Cosmetic Act which was developed in response to deaths resulting from the use of a product containing sulfonamide dissolved in diethylene glycol (Ashton 2000). Until this time, there were no legislative directives requiring testing of drugs in animals prior to use in humans. The Federal Food, Drug and Cosmetic Act set the requirement for preclinical testing. The classification for phases of drug testing is outlined here.

Preclinical studies. As indicated above, the requirement for preclinical testing began in the USA in 1938 to provide insight into what might be the expected and unexpected actions (i.e., toxicity, teratogenicity, and cancer) of the compound when used in humans (Collins et al. 1990). The 1938 Act modified the Pure Food and

Drug Act of 1906 which required products containing alcohol, cocaine, heroin, morphine, and cannabis to be labeled with contents and dosage. Preclinical testing can involve studies conducted on cultured cells, tissues, and experimental animals. These studies help to ensure the biological mechanisms of the compound and physiological consequences of its action. On average, only one compound in a thousand will make it to being tested on humans in clinical trials. Preclinical drug studies and trials last approximately 5 years, following which the researcher(s) or company must apply for approval from the regulatory agency to continue to move the experimental drug to testing in humans. The cost to bring a new drug to market is estimated to vary from around $0.8–$1.0 billion US dollars, depending on the therapy or the developing firm (Adams and Brantner 2006; DiMasi et al. 2003).

Phase I. The objective of Phase I clinical trials is to develop the safety profile for a particular drug by determining the absorbance, distribution, metabolism, and excretion of the compound in humans as well as the duration and effects of the drug. This phase of testing may take 1–3 years (Umscheid et al. 2011). The test population often consists of healthy volunteers, although terminally ill patients may be included in some circumstances. The cohort of subjects will typically consist of 100 individuals. Once a drug enters Phase I of clinical trials, it has a 13–20% chance of receiving approval for use in humans (DiMasi et al. 2003; Rosenberger and Haines 2002).

Phase II. Once the safety of the product has been established, the drug moves to Phase II clinical trials which enroll 100–300 patient volunteers, who suffer from the condition or disease targeted by the drug. These small and well-controlled experiments continue to assess the drug's safety, side effects and to optimize the dose. Statistical endpoints for the drug are established here, which represent the targeted favorable study outcomes. Phase II lasts approximately 2 years and approximately 69% of drugs tested have a chance of being approved for use in humans (Sharma et al. 2011).

Phase III. The goal of Phase III trials is to identify the effectiveness of the test drug in treating the particular disease under study, as established by statistical endpoints determined in Phase II trials. Phase III trials serve as the "gold standard" of treatment efficacy, continuing to build the drug's safety profile, monitoring long-term side effects as well as adverse reactions. The study design will include defined inclusion and exclusion criteria for participation and consist of a double-blinded treatment assignment, with a sample size of over 1,000 subjects possibly from more than one geographical location. Phase III trials may last between 3 and 4 years. If the drug is effective, the trial is deemed successful and the drug then has a 70% chance of being released to the market (Scher et al. 2011).

Phase IV. Upon the release of the drug to the market, a company must continue to evaluate the drug's safety profile by conducting additional large trials, observational studies, or physician reports of adverse events. Additional medical conditions for which the drug may be applicable and appropriate can be investigated in this phase. Once a drug reaches Phase IV, it has the highest probability (over 70%) of being approved for prescribed use (Ashton 2000; Umscheid et al. 2011).

4 Historical Perspective

Around the globe, failure to understand and study female biology in medicine has resulted in higher mortality and co-morbidities in women (Berlin and Ellenberg 2009; Kim and Menon 2009). Historically, clinical trials worldwide excluded women of childbearing age from participation in Phases I and II trials because of concerns regarding potential genetic and developmental abnormalities to the fetus. The following section highlights, in chronological order, the legislation history of policy changes which impacted the inclusion of women into clinical research.

During World War II, the Nazi regime performed brutal experiments on humans, with studies uniquely targeted toward the gender of the victims. In concentration camps, both Jewish and non-Jewish women were subjected to sterilization experimentation and abortions directed by Dr. Josef Mengele (Georges and Benedict 2006). Newly arriving pregnant women were immediately sent to special barracks, where premature birth was induced in order to preserve the "Aryan" Nazi race, while women who resisted were sent to the gas chamber for extermination. These horrific "war crimes against humanity" became known to the world community in 1945 during the Nuremburg Trials, which persecuted ten Nazi leaders for "encouraging and compelling abortions" (Caplan 2005; Weindling 2001). These atrocities led to the development of the Nuremberg Code, a set of research ethical principles for human experimentation established at the end of the Second World War. The Nuremberg Code includes such basic humanitarian principles as informed consent, the absence of coercion, properly designed scientific experimentation, and beneficence toward experiment participants (Ghooi 2011).

Following the Nuremburg Trials, two other influential doctrines emerged, providing ethical guidelines for the participation of patients and improved subject design: the 1948 Declaration of Geneva and the 1964 Declaration of Helsinki, both developed by the World Medical Association (WMA). The Declaration of Geneva is an agreement of physicians' ethical duty which binds the physician with the words, "The health of my patient will be my first consideration," and is based on the International Code of Medical Ethics which declares that, "A physician shall act in the patient's best interest when providing medical care" (Frewer 2010; Pillay 2008). Along the same lines, the Declaration of Helsinki was developed, based on the ten principles stated in the Nuremberg Code integrated with the stipulations of the Declaration of Geneva. Uniquely, the Declaration of Helsinki defines the fundamental guidelines of human experimentation and is widely regarded as the milestone document of human research ethics (Puri et al. 2009). Prior to the 1947 Nuremberg Code, there was no generally accepted code of ethical conduct governing human research. The Nuremberg Code and the related Declaration of Helsinki serve as the basis for the Code of Federal Regulations issued by the United States Department of Health and Human Services governing federally funded research in the USA and other countries around the world (Macrae 2007). Links to specific policies for some countries in the international community are found in Table 1; key features of these policies are summarized below.

Table 1 Policies on the inclusion of women in clinical trials

Country	Organization name	Links to legislation/guideline websites
USA	National Institute of Health (NIH)	http://grants.nih.gov/grants/funding/women_min/guidelines_amended_10_2001.htm
Canada	Canadian Institute on Health Research	http://publications.gc.ca/collections/collection_2008/cihr-irsc/MR21-103-2003E.pdf
Europe	European Commission	ftp://ftp.cordis.europa.eu/pub/science-society/docs/gendervademecum.pdf
Japan	Ministry of Health Labour and Welfare	http://www.nihs.go.jp/mhlw/yakuji/yakuji-e_20110502-02.pdf
Australia	Australian Institute of Health and Welfare	http://www.mja.com.au/public/issues/188_11_020608/bal10871_fm.html

4.1 United States Policy on Inclusion of Women in Clinical Trials

In the USA, the Food and Drug Administration (FDA) prior to 1993 enacted to exclude women from Phases I and II of all clinical trials based on the complexities of female hormones and the possibility of pregnancy by female participants (Parekh et al. 2011; Reichmann 2011). The rationale for this exclusion was twofold: (1) There was and remains a desire to protect unborn children; (2) The differences in disease between the sexes was attributed to differences in the reproductive systems. Similarly, there was a growing body of evidence indicating that European women were underrepresented in clinical trials resulting from the increased number of birth defects in the 1950s in European women taking the sedative Thalidomide (Kuehn 2010; Ruiz Cantero and Angeles Pardo 2006) (refer to Sect. 4.3), thus harming their healthcare and leaving women further behind their male counterparts in terms of disease and quality of life.

Gradual changes in global healthcare policy began around the late 1980s, early 1990s, with the recognition by the scientific and medical communities of disparities between the sexes in disease courses and drug interactions resulting from differing risk factors due to roles given to men and women by society, innate genetic and biological factors, hormonal levels and metabolic considerations which cumulatively influence disease outcomes (Fleisch et al. 2005; Merkatz et al. 1993). The realization of the need for reform and inclusion of women in medical trials resulted in the National Institutes of Health Revitalization Act of 1993, legislating consideration of biological sex in design of clinical trials and the inclusion of women in clinical research including Phase I and Phase II trials (Geller et al. 2006, 2011). The legislation required clinical studies funded by the National Institute of Health (NIH) to include men, women and ethnic minorities in studied populations, thus ensuring the right of every citizen to participate in clinical research and to gain better healthcare. The General Accounting Office monitors the enrollment and study of sex and gender differences. It is due to this agency that U.S. investigators may not obtain NIH funding if their plans of including and analyzing data on women are not detailed in the submitted NIH grant applications (Curfman et al. 2005).

4.2 Canadian Policy on Inclusion of Women in Clinical Trials

The inclusion of women in clinical trials in Canada began in 1997 following the establishment of guidelines published by Health Canada. These guidelines called for the inclusion of women of childbearing age as well as postmenopausal women in all stages of research, particularly from the earliest stages of drug development. The new guidelines called for the analysis of the results by sex so that any male–female differences may be identified. Yet unlike the NIH policy of the USA, the guidelines only sought to "encourage" the participation of women in clinical trials and did not make it an absolute requirement. The document states "patients of both sexes ... be included in the same trials in numbers adequate to allow detection of clinically significant sex-related differences in drug response." However, the guidelines did specify the importance of reporting gender differences in women using oral contraceptives or "estrogen replacement therapy" but remained unenforced in Canadian clinical studies (Broyles et al. 2005). Thus, although not mandatory, in Canada, there is a governmental commitment to the application of gender-based analyses to policy and program development (Enserink 2005; Lippman 1995).

4.3 European Policy on Inclusion of Women in Clinical Trials

European agencies such as the International Conference on Harmonization (ICH) and European Medicines Agency (EMEA) began enforcing stricter guidelines for clinical trials as a consequence of birth defects in children of women taking Thalidomide during pregnancy in the 1950s (Ruiz Cantero and Angeles Pardo 2006). This incident resulted in clinical trials conducted in Europe including predominantly male participants until the 1990s when the ICH began addressing sex and gender disparities in development of new regulatory standards and governing rules of present-day clinical trials. There are several ICH guidelines (Karbwang and Torres 2011), which specifically address inclusion of sex and gender in design of clinical trials:

1. Guideline E8 requires that Phase I pharmacokinetic information be obtained in women
2. Guideline E4 requires that dose–response data must be obtained according to gender
3. Guideline E18 (general considerations for clinical trials) requires a study population representative of the target patient population
4. Guidelines E3 and M4E call for the characterization of the patient population and that data analyses be performed with respect to gender

Similar to trends reported by the United States' FDA, a EMEA survey covering 2000 to 2003 and reviewing over 240 pivotal clinical trials performed in Europe

reported that, although women achieved equal representation as compared to men in late Phase II and Phase III clinical trials, women were underrepresented in the earlier development-phase studies. Additionally, the EMEA survey found women to be underrepresented in certain therapeutic categories (i.e., hypertension) and overrepresented in others (i.e., allergy). One major limitation of the findings by the EMEA of representation of women in clinical trials was that the survey covered only drug trials conducted by industry (Harris and Douglas 2000; Meinert et al. 2000).

In 2004, the *European Clinical Trials Directive 2001/20/EC* implemented a legislative framework for clinical research in Europe with the ultimate goal to make Europe as competitive in clinical research as the rest of the world through the enhanced protection of research subjects. Yet astonishingly, there was no mention of the word "women" throughout the document. Albeit the absence of specific directives to include women in clinical trials guidelines in Europe, female representation was higher in 2005 in international trials (32.7%) as compared to clinical studies conducted in the USA (26.7%) (Melloni et al. 2010). However, guidance and regulation of ethics committee members and the participation of women in clinical trials needs to be strengthened, while greater attention is needed in gender equity and justification for the exclusion of women from particular clinical studies (Druml et al. 2009).

4.4 Australia Policy on Inclusion of Women in Clinical Trials

Research on humans in Australia is governed by the *National statement on ethical conduct of research involving humans*. The 2007 National Statement contains a revised section on the inclusion of men and women in research. A survey of the human research ethics committee (HREC) was conducted in 2008 with the objective of exploring the role played by the HREC with regard to inclusion of men and women in Australian clinical research (Ballantyne and Rogers 2008). Interviews with 25 HREC chairs were conducted. Surprisingly, most of the chairs did not believe that sex discrimination was a significant problem and were unaware that sex equity was not being met. This discrepancy between the sentiments of the HREC chairs and the reality of clinical research has delayed the progress and advancement of clinical trials in Australia (Loblay 2008; Rogers and Ballantyne 2008).

4.5 Japanese Policy on Inclusion of Women in Clinical Trials

In addition to the USA and Europe, Japan is also an essential part of the ICH, and as such, must adhere to industry and pharmaceutical guidelines specified by the ICH committee regarding the inclusion of women in clinical trials. However, the ICH has accepted that Japan has different safety standards than other foreign countries,

and thus, the regulatory guidelines set forth regarding the inclusion of women of childbearing age have not been clearly defined. Studies report 58% representation of women in clinical trials out of the total study population. Yet as compared to the rest of the world, Japanese Phase I clinical trials are composed of only 4% of women, while in foreign trials, that percentage has been reported as significantly higher (31%), which may be explained by the implementation of stricter research guidelines in Japan (O'Neill 1995; Smith 1996).

4.6 International Policy on Inclusion of Women in Clinical Trials

The ICH has addressed the issue of inclusion of women in research (Karbwang and Torres 2011). Guidelines from this conference refer to the need to study pediatric and geriatric populations due to age being a confounding factor influencing medication effects. Thus, more research on drug development is needed on these two vulnerable populations. However, there are no specific guidelines addressing women, despite the established requirements that the study population represent the target population.

Taken altogether, these legislative directives and guidelines identify a significant problem, whereby women are underrepresented in early drug development. Furthermore, although there have been major efforts to increase the number of women participating in clinical trials, these policies and actions do not always result in the desired quantitative objective with an average participation of only 37% in trials conducted in a variety of disciplines from 1995 to 2010 (Fig. 1) (Foulkes 2011). Improvements in these deficiencies must be made in order to improve understanding of the underlying biological mechanisms directing sex and gender differences in absorbance, metabolism, elimination and dose-dependent safety and efficacy of drugs.

5 Study Design Considerations

Sex-specific approaches are required to increase the foundation of basic knowledge upon which to discover novel therapies and to build translational approaches to medical care of humans (Arain et al. 2009; Bairey Merz et al. 2010; Ginsburg and Willard 2009; Hamburg and Collins 2010).

5.1 Preclinical Studies

Preclinical studies often are not designed to account for specific variables such as sex, hormonal status or reproductive history that may be representative of the human population affected by a condition or disease or of the patient population

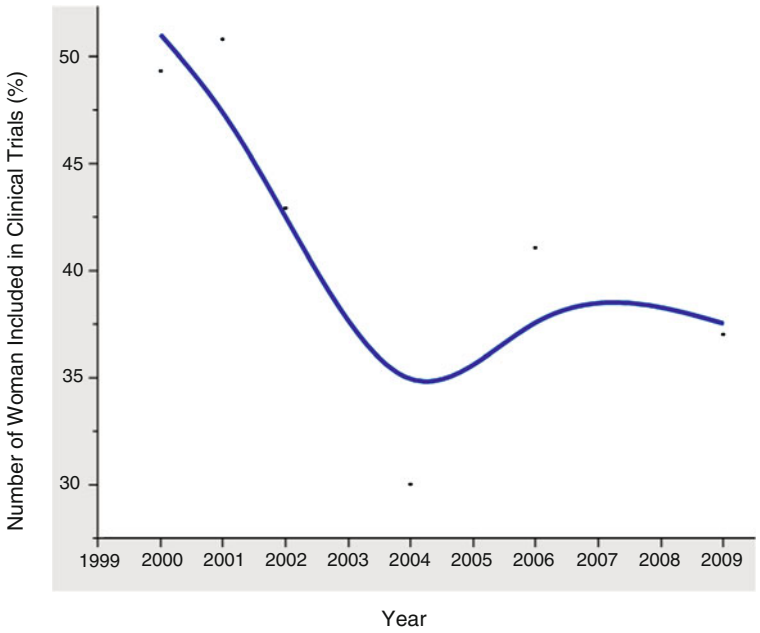

Fig. 1 Inclusion of women in clinical trials conducted in the USA and Canada from 2000 to 2009. Percentage of women included in clinical trials as derived from meta-analyses of clinical trials conducted in the USA and Canada. Data are derived from references list here (Fleisch et al. 2005; Geller et al. 2011; Melloni et al. 2010; Yang et al. 2009). The percentage of women participating in clinical research has declined since the year 2000 in spite of legislative directives for their inclusion

most likely to be in need of or who use a specific intervention. Several excellent reviews are available that describe strategies and methods for investigation of sex differences for studies of brain and behavior (Becker et al. 2005; Gillies and McArthur 2010), neuroprotection after stroke (Fisher et al. 2009), pain and analgesia (Greenspan et al. 2007), and cardiovascular disease (Miller et al. 2011). Although preclinical studies for discovery and testing of putative new therapeutics or biomarkers are not directed to specifically investigate "sex differences," investigators should be aware that sex-specific factors may affect their results, interpretations, and conclusions in all phases of drug discovery.

5.1.1 Cultured Cells and Progenitor (Pluripotent Stem Cells)

Every cell has a sex. Although most signaling pathways are present in cells/tissues derived from female and male animals, they may be differentially expressed. That is, transcription of genes, expression of RNA and translation of the signal to protein (i.e., enzymes and receptors) which affect expression of a given signaling pathway will be influenced by the sex of the cell and the hormonal environments from which

the cells are derived. These influences include the possibility that a single exposure to a sex steroid hormone may influence cellular metabolism through multiple passages of the cells in culture (Antoniucci et al. 2001; Avner and Heard 2001; Csaba et al. 1990; Hager et al. 2008; Isensee and Ruiz Noppinger 2007; Isensee et al. 2008; Ivanova and Beyer 2003; McEwen 2001; Ober et al. 2008; Ostrer 1999; Pierce et al. 2009; Rzewuska-Lech et al. 2005; van Nas et al. 2010; Wang et al. 2006; Waxman et al. 1985; Yang et al. 2006; Yao et al. 2010). However, the sex and hormonal or reproductive status of the cell/tissue donor or experimental animals is not consistently reported (Beery and Zucker 2011; Taylor et al. 2011; Zucker and Beery 2010). The following points should be considered when using cultured cells including isolated progenitor cells to test potential therapeutic agents:

- Is expression of the target receptor, pathway, enzyme, etc., affected by the sex or hormonal status of the donor animal [i.e., see Yang et al. (2006)]? That is, would gene expression/receptor or pathways signaling differ depending on whether cells were derived from a pre- or postpubescent animal or one that was reproductively senescent (aged or gonadectomized before or after sexual maturity) or null- or multiparous?
- Is expression of the target receptor, pathway, enzyme, etc., affected by hormones in the culture media? How does the phenotype or differentiation potential change with passage of cells exposed to hormones in culture media using fetal bovine serum (Csaba et al. 1990; Waxman et al. 1985)?
- Is the choice of cell/tissue donor appropriate for the mechanism of interest related to human disease in regard to sex, age, and hormonal status (i.e., are human umbilical vein endothelial cells representative of or appropriate for the study of mechanisms of insulin resistance and endothelial dysfunction in human adult type II diabetes)? Are the numbers of the cell type being isolated (e.g., bone marrow, blood, heart, or vasculature) or their viability affected by the sex or hormonal status of the donor animal?
- Is the number or potency of the pluripotent stem cells affected by gonadectomy either before or after sexual maturity, age or disease status?
- For investigation of cell-based therapies and given potential sex differences in cell number, phenotype, and potency, should sex-mismatched allogeneic cells be considered as a therapeutic option? If so, is the donor appropriate for the human disease of interest in regard to sex, age, and hormonal status?

5.1.2 Experimental Animals

The choice of an experimental animal in which to develop diagnostic tests or therapies should depend upon the degree to which the phenotype/pathology of interest represents disease expression in humans (Miller et al. 2011). Unfortunately, selection of male animals is often the “default” choice because of perceived additional costs introduced by including female animals or because of variability associated with fluctuations in female hormonal cycles (Beery and Zucker 2011;

Zucker and Beery 2010). This bias in use of male animals persists even with documented sex and gender differences in disease prevalence and outcomes in humans, for example, in efficacy of treatment of hypertension, all-cause mortality for cardiovascular disease, diabetes, multiple sclerosis, stroke, cognitive decline, osteoporosis, pulmonary disease, influenza, etc. (Aguero-Torres et al. 1998; Gall et al. 2010; Klein et al. 2010; Miller et al. 2010; Mosca et al. 2011; Ober et al. 2008; Regitz-Zagrosek and Lehmkuhl 2005; Roger et al. 2012; Wizemann and Pardue 2001a; Zakeri et al. 2011).

Other considerations for the selection of the appropriate experimental model include cost, size, and housing requirements. However, variables which often are not considered but could impact outcomes include diurnal activity cycle, diet requirements (soy or plant-based influencing consumption of phytoestrogen) (Luczak et al. 2011), lifespan, environmental components of disease, or modulation of drug metabolism (Dodds and Abelseth 1980) such as stress imposed by disruption of sleep/wake cycles, patterns of social interaction (or degree of social isolation), or handling which may influence other parameters (e.g., corticosteroids and catecholamines) that interact with the test drug (Kaplan and Manuck 1999; Kaplan et al. 1985; Miller et al. 2011).

5.2 Clinical Implications

Consideration of choice of sex and hormonal status of the preclinical experimental subject has practical consequences. For example, of ten prescription drugs withdrawn from the USA market between 1997 and 2001, four which were developed and prescribed to treat diseases in both men and women and fell into the general categories of antihistamine, cardiovascular and gastrointestinal therapies, posed greater risk for Torsades de Pointes in women compared to men (Heinrich 2001). Although adverse reactions resulting in withdrawals of drugs from the market may occur because a particular drug was used incorrectly by physicians or patients or used for purposes other than for that which it was developed (off-label use) (Heinrich 2001), the potential sex differences in physiology and pharmacology affecting drug reaction and efficacy cannot be ignored. For example, Torsades de Pointes is a type of ventricular tachycardia which has higher incidence in women than in men. It results from ionic imbalances that affect electrical conduction in cardiac myocytes. Preclinical strategies and tests usually are chosen because they have high sensitivity and high specificity and have some ability to screen high numbers of compounds. There are several animal and tissue tests available for identifying effects of new compounds on cardiac ion channels (Arrigoni and Crivori 2007; Joshi et al. 2004; Liu et al. 1999). However, it is unclear whether selection variables such as sex or hormonal status of the cell, tissue, or animal are considered (Yang and Clancy 2010). Given that two-thirds of cases of Torsades de Pointes occur in women using drugs not intended for anti-arrhythmic therapy suggests that

blockade of potassium channels may be an off-target effect of many compounds and that consideration of sex and hormonal status in the testing process may identify these effects early in the drug development process (Bednar et al. 2002).

Attention to sex and hormonal status in preclinical studies may also benefit development of new therapeutic approaches for the treatment of diabetes and heart failure with preserved ejection fraction (HFpEF). Although diabetic men have realized a 50% relative reduction in cardiovascular mortality (from 26.4 to 12.8 annual deaths/1,000 persons), there has been no reduction in mortality for diabetic women (Gregg et al. 2007). This gender mortality gap is projected to intensify with our aging population and epidemics of obesity and diabetes (Roger et al. 2012). Rezulin (troglitazone) prescribed to treat diabetes caused more liver failure in women than men and was withdrawn from the market (Heinrich 2001). Rosiglitazone used to treat type II diabetes also was suspended by the EMEA and restricted by the United States Federal Drug Administration because of increased cardiovascular risk (Hancox 2011). Rosiglitazone acts through peroxisome proliferator-activated receptor γ (PPAR γ), a receptor which is also modulated by metabolites of estrogen (Barchiesi et al. 2010). Rosiglitazone may also inhibit estrogen synthesis in ovarian tissue which could indirectly as well as directly affect bone resorption (Harslof et al. 2011; Seto-Young et al. 2011). Since development of type II diabetes and associate cardiovascular risk differs in men and women, preclinical testing in obese, female animals of various hormonal status may have provided additional insight into drug efficacy prior to testing and use in humans (Ajayi et al. 2003; Bai et al. 2010; Jiang et al. 1999).

Incidence of stroke also differs between men and women (Beal 2010; Siegel et al. 2010) and understanding factors that account for these differences could lead to sex-specific approaches in humans. For example, activation of poly-ADP-ribose polymerase (PARP1) protects against ischemic damage in brains of male animals (Hagberg et al. 2004), but paradoxically, enhances ischemic injury in the brains of female animals (McCullough et al. 2005; Yuan et al. 2009). For these drugs, female sex and hormonal status impacted efficacy of the therapy. While basic research into cellular mechanisms of sex differences may not always precede initiation of a clinical trial for a drug (Miller et al. 2009), the question can be raised regarding whether or not preclinical testing of drugs in female animals would alter the course of the design and outcomes of a clinical trial.

HFpEF, unlike type II diabetes, is a complex syndrome whose pathophysiology remains unclear and therapeutic strategies undefined (Zakeri et al. 2011). In addition, the unexplained female predominance, HFpEF has received little attention in experimental work including efforts to understand potential differences in the underlying mechanisms of hypertension between males and females (Bhatia et al. 2006).

The absence of data from female animals and/or cells is striking in many fields of basic science which impact preclinical testing (Beery and Zucker 2011; Taylor et al. 2011; Zucker and Beery 2010). Thus, the opportunity is available to not only explore the physiological basis of diseases that present with a female predominance, but to also explore interactions of drugs with other confounders for disease

treatment such as hypertension and heart failure in the presence of obesity and diabetes. Viewing preclinical study design through a gender lens might hasten translation of potential treatments with better efficacy and fewer adverse effects in both women and men from the preclinical status to the clinical arena.

6 Limitations of the Currents Status of Clinical Research in Women

Concerns about epidemiological concepts such as external validity and generalizing use of research drugs have been raised in clinical trials (Hammad et al. 2011). If women are not studied appropriately during the development of the drug in early Phases I and II of clinical trials, then how can the safety and efficacy of a particular compound be assessed with confidence for the general population? Alternatively, grouping all women to a similar outcome through sex-specific trials assumes a certain homogeneity which may not exist and may be influenced by other physiological consequences such as the number of pregnancies, pregnancy-associated diseases, ethnicity and cultural attitudes/customs which affect disease progression, attitudes and use of treatments and access to care. Recruitment of women into clinical trials may be affected by their geographical location (urban or rural), access to transportation, child- or elder care, education and socio-economic status and other cultural issues. Inability to recruit women into a trial may limit statistical power even if the original trial was designed to include women in proportion to disease presentation. Another limitation of current clinical trial design is the insufficient attention to polypharmacy, or multiple drug interactions in an individual (Col 2005; Drici and Clement 2001). Women have a longer lifespan than men and are faced with a higher prevalence of chronic disease. Therefore, it becomes essential to at least consider monitoring of cumulative effects of several compounds in a human research study.

While some regulations regarding drug development and testing were driven by adverse teratological outcomes in babies of pregnant women, ethical guidelines developed by the Council for International Organizations of Medical Sciences stipulate that pregnant women are eligible to participate in biomedical research (Baylis 2010) (Table 2 for specific references). However, concerns regarding adverse effects on the developing fetus remain and pregnant women, thus, may decline participation in clinical trials.

When pregnant women require medication, often physicians prescribe drugs off label or make dosing recommendations which are not evidence-based or standardized for physiological changes associated with pregnancy, such as increased plasma volume, fat disposition and fluctuations in hormones. Open databases or retrospective analysis of data collected from pregnant women taking an unproven drug while pregnant may help to provide more evidence base for decision making in the future (Baylis and Kaposky 2010).

Table 2 Guidelines for participation of pregnant women in clinical trials and prescribing practices

Country	Organization name	Guidelines for participation of pregnant women in clinical trials and prescribing practices
USA	Food and Drug Administration (FDA)	http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073246.pdf
Canada	Health Canada	Baylis and Kaposky (2010)
Europe	European Medicines Agency (EMEA)	http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500059887.pdf http://www.tga.gov.au/pdf/euguide/phvwp31366605en.pdf
Japan	Ministry of Health Labour and Welfare	http://www.nihs.go.jp/mhlw/yakuji/yakuji-e_20110502-02.pdf
Australia	Australian Drug Evaluation Committee (ADEC)	http://www.huidziekten.nl/richtlijnen/medpreg.pdf

Women participating in clinical trials may be required to use contraception but it should be considered that some agents might interfere with actions of hormonal-based contraception, and thus, increase the risk of pregnancy or have other adverse drug–drug interactions. Use of barrier types of contraception may be recommended for women and men enrolled in early stages of drug development and may be recommended to continue in men through a spermatogenic cycle (ICH M3 stipulation) until genetic and reproductive testing has assured safety of the agent.

6.1 Data Analysis and Statistical Considerations

Legislation requiring or recommending inclusion of women in clinical trials does not guarantee compliance (Fig. 1). However, it is merely not enough to only include women in clinical trials but it is also necessary to ensure that results are presented in a statistically meaningful way, allowing for the correct analyses and interpretation of data in a scientifically correct manner. Designing studies with adequate sample size to detect sex and gender differences in early stages of clinical trials is essential in order to improve therapeutic options for women. In studies enrolling both males and females, analysis of data by sex and gender as a biological variable and not just as a covariate will provide better information about outcomes if the results are reported as such. It should be clearly stated in the paper whether or not the study was underpowered to detect sex and gender differences. If a gender-specific analysis was performed, both null and positive findings should be reported. Moreover, since only a *fourth* of all clinical research studies conducted in the USA and Europe provide a sex-based analysis of the results (Melloni et al. 2010), effects of therapeutics in women remain unclear and the ineffective trial design, confounded by a low number of female subject participants, creates a significant health hazard for women (Fleisch et al. 2005; Tsang et al. 2011). With the development of the

discipline of health outcomes research, it is essential that sex and gender be considered in all phases of drug development as a requirement for scientific excellence (Nieuwenhoven and Klinge 2010) and for improved treatments to assure better health outcomes for both men and women (Gochfeld 2010).

7 Conclusions and Clinical Implications

Application of strategies to account for sex differences are not required by regulatory agencies in the design of preclinical studies. However, investigators should no longer assume that pharmacological modulation of specific mechanistic pathways are similar in cells derived from males and females or in experimental animals of all ages, both sexes, different hormonal status, or reproductive history. Accounting for sex and hormonal status in the design and interpretation of basic research of anatomical structure, physiological and pathophysiological function and disease processes in design of preclinical and clinical trials is essential to facilitate development of novel and innovative therapeutic approaches, to reduce adverse drug reactions when these therapies are tested and used in humans and is equivalent with scientific excellence (Kim et al. 2010). Evaluating design of clinical trials through a gender lens should provide a mechanism to reduce costs of drug/test development and improve outcomes by reducing side effects, and in the long term, reduce the number of drugs withdrawn from the market place.

Take Home Messages

- Preclinical studies in screening and testing potential new therapeutic agents should be conducted in cells/tissue or animals of both sexes.
- Women should be included in all phases of clinical trials and in sufficient numbers to assess sex and gender differences. Although various governmental and scientific guidelines encourage or legislate inclusion of women into clinical trials, such inclusions often fail to be realized.
- Reporting results from clinical trials should include sex and gender differences in the analysis with reporting of null findings if no differences are detected.
- Clinical trial structure should be re-evaluated in order to provide more evidence-based safety and efficacy data for drug use in pregnant women.
- Single sex trials should be constructed and evaluated to account for potential effects of reproductive status, race, ethnicity, and socioeconomic class.

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"Gender-Specific Drug Prescription in Germany" Results from Prescriptions Analyses

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Abstract There are still considerable differences in the medication supply for men and women. While the prescription volumes for both men and women have, for some time, been similar or have even risen for the men, there are still characteristic differences between the sexes when it comes to the prescription of certain indication groups. Women are still prescribed clearly more drugs in the field of psychotropic medication, especially antidepressants, hypnotic drugs, and tranquilisers. As the American Beers criteria for quite some time now and, more recently, the German PRISCUS list have shown, the effects of such drugs are potentially dangerous, particularly for older women. The known adverse effects are, apart from dependence problems, restricted cognitive capacities, insecure or instable walking, and badly healing wounds from falling accidents that are followed by patient's nursing care dependency. In secondary prophylaxis after acute myocardial infarction, the characteristic prescription features of the various medicinal products that are used for both men and women (such as platelet aggregation inhibitors, beta-receptor blockers, ACE-inhibitors, statins) have become similar; women's still higher mortality risk

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appears to go back to the fact that too much time is spent before proper hospital treatment commences. In general, more attention should be paid to the right medication, the right length of treatment, and the right dosage of the medication prescribed to women; the evidence concerning women's supply of medicinal products should also be improved.

Keywords Acute myocardial infarction • Antidepressants • Medication prescriptions for women • Psychotropic drugs • Secondary data analysis • Secondary prophylaxis • Tranquilisers

Abbreviation

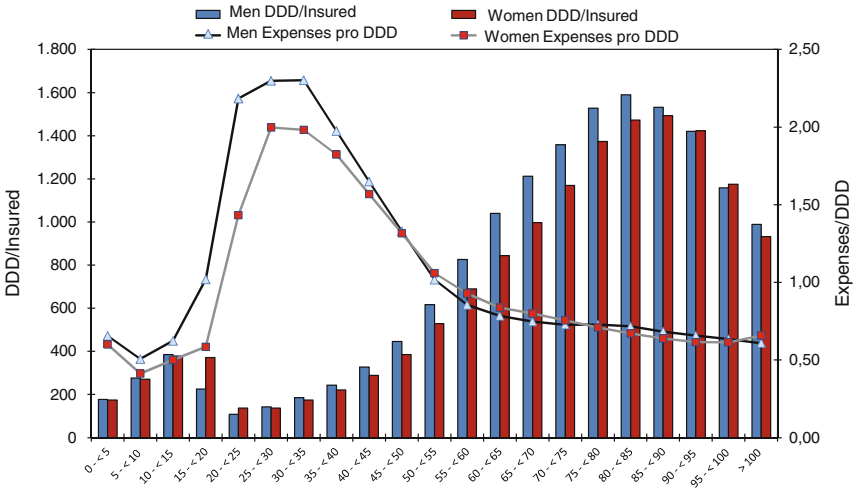
DDD	Defined daily doses
SSRI	Selective serotonin reuptake inhibitor
NSMRI	Non-selective monoamine reuptake inhibitor
ATC Code	Anatomical therapeutic chemical classification system (WHO)
ICD-10	International statistical classification of diseases and related health problems (WHO)
Rx	Prescription only drug

1 Introduction

In the past, women have benefited from the gain in lifetime more than men. While in Germany in the years from 1900 to 2010 the men's average life expectancy at birth has increased from 44.8 to 77.5 years, women's life expectancy has increased from 48.3 to 82.6. In the higher age groups, the number of women outweighs the number of men (Statistisches Bundesamt 2012). However, the additional years are, in most cases, not devoid of illness. The consumption of medicinal products actually depends on both age and sex. Data from the statutory health insurance fund show that men are given more, and mostly also more expensive, medicines. That the expenses decrease with age has to do with those chronic diseases (hypertension, cardiac insufficiency, or diabetes) that occur more often in elderly people and that are treated with less expensive generic drugs.

In this chapter, we analyse secondary data from the GEK, a statutory health insurance, and after the fusion with the BARMER Ersatzkasse in 2010, from the BARMER GEK, one of Germany's largest statutory health insurances with about nine million insured persons. Graph 1 shows the distribution situation for persons insured with BARMER GEK.

When comparing the figures with earlier data, it is surprising to see that the number of drugs prescribed to women is basically not higher than that for men. It is also obvious that older men get prescribed more defined daily doses (DDD) than women. Only 10 years ago, the relation was clearly to the women's disadvantage:



Graph 1 The 2010 BARMER GEK per-capita prescriptions and expenditure per DDD in Euro according to age and sex (Glaeske and Schicktanz 2011)

With an average of 441 daily doses, they were given 50% more DDD than men who got 295 daily doses. The changes that have occurred since then are due to the fact that since 1 January 2004 adults non-prescription drugs are no longer reimbursed by the statutory health insurance. This regulation affected a great number of drugs that are more frequently prescribed to women such as medicines for the treatment of venous diseases, or herbal remedies for menstrual cycle, or menopause complaints. Furthermore, the desired decrease of hormone therapy prescribed during menopause to treat menopause complaints also affected the prescribed volumes: In former years, these preparations were prescribed on a continuous basis to 30–40% of women older than 45 years—also to prevent osteoporosis.

However, sex- and gender-specific differences are still conspicuous with respect to certain medication groups. Women are, in general, more frequently than men given sexual hormones, osteoporosis preparations, thyroid therapeutics, and minerals. The most conspicuous differences still occur in the field of psychotropic drugs: In 2010, with an average of 33.4 DDD women received 56% more psychotropic drugs than the men who were prescribed an average of 21.0 DDD. Apart from this, there are signs that the prescription volumes vary for the chronically ill according to the sex, and that men, for instance, more frequently get antithrombotic drugs (+33%) or lipid-lowering drugs (+21%) (Coca and Nink 2011, p. 954). However, these data have to be interpreted with certain reservations since such general overviews of medication consumption do not take the diagnoses into consideration. Regarding indication groups, analyses of health insurance funds data from earlier years showed clear differences in the volumes prescribed for men and for women—these differences still exist today (Glaeske and Janhsen 2006).

With regard to prescribed packages, women, when compared with men, receive per capita.

- 5.4 times more migraine medicines
- 2.9–3.5 times more thyroid preparations
- 1.9–2.9 times more neuroleptic drugs and antidepressants
- 1.6 times more sleeping pills

On the other hand, men get, when compared with women,

- 1.8 times more lipid-lowering agents
- 1.5–1.8 times more types of insulin or oral anti-diabetic agents
- 1.1–1.5 times more beta-blockers or calcium antagonists
- 1.8 times more anti-platelet aggregation drugs

Part of these differences will be analysed more carefully below by looking at current evaluations.

This distribution shows that women are rather given medication that has psychological effects while men tend to receive medication for the treatment of physical disorders, primarily of the cardiovascular system. For psychostimulants, that is, stimulating psychotropic drugs, and for certain neuroleptic drugs, the per-capita prescription percentage is for men far higher than for women. Here, role stereotypes appear to influence the prescription pattern—it is rather the women who are associated with diseases caused psychologically or by emotional strain while the men are associated with somatically influenced illnesses.

Due to this variance among the prescriptions of psychotropic drugs, different hypotheses are being discussed which describe the mechanisms of prescribing psychotropic drugs to women and what kind of effects they have on patients or on society (Glaeske 2002, p. 527, following Franke et al. 1998):

According to the "Women-are-expressive"-hypothesis, women's higher sensitivity and emotionality with respect to illness, together with a lower threshold to look for help, let them visit physicians more frequently. In the case of certain strain symptoms, the physicians know of no way to deal with them other than through prescribing psychotropic drugs.

Following the *substitution hypothesis*, women's coping strategies differ from those of the men: women tend to swallow down stress, anxiety, and everyday problems by taking medication while men are inclined to use alcohol.

The *convergence hypothesis* claims that medication consumption decreases while the use of alcohol in the context of profession or career increases (in a "male-dominated" culture).

The prescribing physicians, much like their patients, work according to similar, socially based patterns of behaviour and interpretation, or are at least familiar with them. They are, furthermore, influenced by their professional teachers' (prejudices) and opinions. Therefore, physicians are required to discipline themselves and keep their prescription behaviour as far as possible free of role stereotypes.

With regard to the prescription of psychotropic drugs, addictive benzodiazepine tranquilisers that were formerly often prescribed to older women have, for some years now, been prescribed less frequently and have been replaced by antidepressants. By comparison, prescriptions of sleeping pills which contain benzodiazepine or similar

substances (such as Z-drugs, for instance, zolpidem or zopiclone), however, have not gone down; their use has remained relatively stable over the last years (Hoffmann et al. 2006, 2009, 2010).

2 Age- and Sex-Specific Medication Consumption

2.1 Costs and Quantities

An overview of the medication prescriptions of one of the largest statutory health insurances in Germany shows the distribution patterns for both insured men and insured women. According to this overview, in 2010 per 100 men 790 medication prescriptions were issued while the figures for women were much higher: per 100 women it was 953 prescriptions (+20.6%). The costs were less different: per 100 insured men they came up to 41,100 Euro; per 100 insured women they were 45,000 Euro (+9.5%). These figures indicate that women are, when compared to the men, obviously "provided for" at lower costs—this corresponds with the data represented in Graph 1. The BARMER GEK medication expenses in 2010 amounted to a total of 3.9 billion Euro; about 80.3 million medication packages were prescribed for about 9.1 million insured persons (see Table 1). For men, a prescription caused an average expenditure of 52 Euro while it was 47 Euro for women; the average total price per package was 49 Euro (Glaeske and Schicktanz 2011).

That there are age- and sex-specific differences in the percentage of insured persons who are given medication by physicians is quite remarkable. While for both men and women up to the age of 10 years, the figures are almost the same, and while in very few cases only young persons are prescribed medication more frequently, the distribution pattern clearly changes for people over 10 and up to the age of 60 years (Table 2).

It is obvious that women of almost all age groups make use of the medication supply system to an extent that is if not markedly then at least slightly higher than that of the men. This is also connected with the frequency of contact within the system (see Table 3): About one-third of the entire population of the patients of a smaller statutory health insurance who in 2005 received a prescription consulted one physician only; another 30% consulted two physicians; another 15% three physicians. 15% visited four or five physicians, and the remaining 5% went to see a markedly higher number of doctors. Women are clearly "leading" when it comes to contacting more than just one or two physicians (Glaeske and Janhsen 2006).

This is most probably one of the reasons that the number of prescribed medicinal products is higher for women than for men—after all, in Germany nearly each and every contact with a physician ends with the prescription of a drug.

Table 1 General key figures of the BARMER GEK medication data from 2009 to 2010 (Glaeske and Schicktanz 2011)

	2009	2010
Number of insured		
Total	8,793,714	9,074,128
Men	3,647,502 (41.5%)	3,799,130 (41.9%)
Women	5,146,212 (58.5%)	5,274,998 (58.1%)
Average age	44.6 years	44.6 years
Men	41.5 years	41.4 years
Women	46.9 years	46.9 years
Number of prescriptions (packages)		
Total	77,015,678.72	80,279,593.78 (+4.24%)
Men	28,575,088.10 (37.1%)	30,021,217.07 (37.4%)
Women	48,440,590.62 (62.9%)	50,258,376.71 (62.6%)
Total medication expenses	3,684,153,549.87 €	3,933,602,649.13 € (+6.77%)
Men	1,447,226,748.22 € (39.3%)	1,560,699,786.42 € (39.7%)
Women	2,236,926,801.65 € (60.7%)	2,372,902,862 71 € (60.3%)
Prescriptions (packages) per 100 insured	875.80	884 71 (+1.02%)
Men	783.42	790.21
Women	941.29	952.77
Expenses per 100 insured	41,895.31 €	43,349.65 € (+3.47%)
Men	39,677.20 €	41,080.45 € (+3.54%)
Women	43,467.44 €	44,983.96 € (+3.49%)

2.2 Medication Supply Quality

Apart from paying attention to the quantity of prescription medication, it is important, particularly in the case of elderly patients, to know about the problematic use of so-called potentially inappropriate medication. In this context, "inappropriate" means that the selected drug, its dosage, or the therapy duration are, in general, not recommended since the potential risks outweigh the potential benefits and since there have to be safer alternative drugs or the therapy is considered as not sufficiently effective.

In the context of inappropriate medication use among elderly patients, the Beers criteria, established by Mark Beers and his team of scientists, have become particularly significant (Fick et al. 2003). The majority of these drugs (such as, for instance, flurazepam, amitriptyline, promethazine, or diazepam) affect the nervous system. Unlike younger persons, elderly people tend to react with undesired effects to the use of psychotropic drugs in general and of benzodiazepines in particular (including sleeping pills or tranquilisers such as flurazepam and diazepam) (Madhusoodanan and Bogunovic 2004; Mort and Aparasu 2002). In 1989, Ray and his research group did a case–control study and for the first time reported of an

Table 2 Insured persons according to age and sex, and percentage of claims of medication-based therapy in 2010 (Glaeske and Schicktanz 2011)

Age/sex	Number of insured 2010	Insured with medication
0 to under 10		
Total	701,675	605,560 (86.30%)
Men	362,458	313,855 (86.59%)
Women	339,217	292,376 (86.19%)
10 to under 20		
Total	871,649	604,894 (69.40%)
Men	456,193	288,709 (63.29%)
Women	415,456	316,376 (76.15%)
20 to under 30		
Total	1,123,808	673,148 (59.90%)
Men	531,416	265,181 (49.90%)
Women	592,392	407,969 (68.87%)
30 to under 40		
Total	981,552	628,987 (64.08%)
Men	419,501	232,022 (55.31%)
Women	562,051	396,970 (70.63%)
40 to under 50		
Total	1,396,065	965,776 (69.18%)
Men	547,296	339,901 (62.11%)
Women	848,769	625,880 (73.74%)
50 to under 60		
Total	1,336,244	1,053,543 (78.84%)
Men	516,974	379,537 (73.42%)
Women	819,270	674,006 (82.27%)
60 to under 70		
Total	1,146,927	1,000,066 (87.27%)
Men	436,665	370,996 (84.96%)
Women	709,262	629,071 (88.69%)
70 to under 80		
Total	983,685	914,864 (93.00%)
Men	370,828	342,483 (92.36%)
Women	612,857	572,381 (93.40%)
80 to under 90		
Total	452,911	431,798 (95.34%)
Men	141,548	134,518 (95.03%)
Women	311,363	297,280 (95.48%)
Over 90		
Total	80,612	76,722 (95.17%)
Men	16,251	15,345 (94.42%)
Women	64,361	61,377 (95.36%)
Total	9,074,128	6,955,358 (76.65%)
Men	3,799,130	2,682,547 (70.61%)
Women	5,274,998	4,273,686 (81.02%)

Table 3 Patients' contact with prescriptions—not only medication—2005 contacts with different physicians (Glaeske and Janhsen 2006)

Number of physicians	Number of insured persons (% of total)	Men (% of total)	Women (% of total)
	1,135,949 (100%)	619,684 (100%)	516,265 (100%)
1	460,109 (40.6%)	284,240 (45.9%)	175,869 (34.1%)
2	329,189 (28.9%)	180,140 (29.1%)	149,049 (28.9%)
3	181,697 (16.1%)	88,271 (14.2%)	93,416 (18.1%)
4	89,395 (8.0%)	38,825 (6.3%)	50,570 (9.8%)
5	41,279 (3.7%)	16,401 (2.6%)	24,878 (4.8%)
>6	27,876 (2.6%)	11,807 (1.9%)	23,087 (4.3%)

increased risk of hip fractures in the elderly in the context of benzodiazepines use (Ray et al. 1989). Since then further studies have been published showing the same results for short-acting or for all benzodiazepines (Herings et al. 1995; Hoffmann and Glaeske 2006). Two overview studies suggest that for both falls (Leipzig et al. 1999) and hip fractures (Cumming and Le Couteur 2003) the half-life time of benzodiazepines does not seem to be the decisive factor. Instead, more recent studies clearly show that especially at the beginning of a treatment phase the increased risk of fracture is due to unsteady gait, restricted attention, and limited walking stability (Hoffmann and Glaeske 2006; Wagner et al. 2004).

Since the Beers criteria were designed for American medication supplies, numerous medicines of the Beers list are not available in Germany or have hardly any market relevance. This is why the PRISCUS list was established for Germany. It mentions 83 generally prescribed substances which potentially have problematic effects for elderly people; these substances primarily include medication which affects the nervous system and whose prescription may mean a considerable hazard potential for elderly persons (Holt et al. 2010).

It is, therefore, most important to study the above-mentioned therapy problems with the relevant age groups. Since pharmaceutical companies normally have only little interest both in drug utilization studies regarding the tolerability of medicines in polypharmaceutic use or in direct comparison with approved drugs are normally of little interest to pharmaceutical companies. Therefore, the investigation of these essential issues has to be publicly financed by, for instance, in the tolerance of certain medicines as compared with each other and in polypharmacy, the investigation of these essential issues has to be publicly financed by, for instance, sickness funds membership fees or in the context of research programmes. What is at stake here is the therapy safety of elderly people, an issue that in most of the cases cannot be deduced from licensing trials because elderly people are, even for medication that is primarily used with them, only seldom included in clinical studies. It is, therefore, high time to examine medicines and their effects on patient populations that are treated primarily with them, also after these medicines have been given market authorisation. Since January 2007, a European regulation has been in force which demands that medication for children must be examined in studies that fulfil

all criteria required for a pharmaceutical trial when the drugs are classified as necessary in paediatrics.

The much larger scale issue of adequate medication supply for elderly people is still in need of such an obligatory regulation, although it has been known for some time now that the older body reacts to medication differently from the younger body: kidneys and liver function only to a limited extent, the immune system is out of balance, and the muscle mass has decreased. Thus, older people take longer to decompose the chemical substances of medicinal products. Patients over 65, who on average take 5 times more drugs than younger persons, pose a particular problem. They are also more vulnerable to adverse reactions. The PRISCUS list includes 83 substances which should possibly be avoided in old age. Still, about every fourth patient is given one of these potentially dangerous drugs. And it is the women who are most affected by this. They are given 5–7% more drugs that are on the PRISCUS list (WIdO 2012). Psychogenic substances such as painkillers and antidepressants as well as medication for the treatment of cardiovascular diseases are among the 20 most frequently prescribed PRISCUS substances. In 2011, for instance, 20 million DDD of Amitriptyline were prescribed. This substance belongs to the group of antidepressants which are particularly often represented in the PRISCUS list. Amitriptyline is prescribed to women 3 times more frequently than to men. The PRISCUS substance Etoricoxib was particularly frequently prescribed (13.4 million DDD). This anti-rheumatic drug is also more frequently prescribed to women—their intake of Etoricoxib is more than twice the amount of that of the men.

It is quite interesting to note that men physicians prescribe PRISCUS substances more frequently than women physicians do. So far, nothing has been found to really explain this difference (WIdO 2012).

Besides psychotropic medication, there are other groups of medicines that show marked sex-specific distribution differences (our own evaluations). Table 4 represents examples which orientate themselves on a normal distribution of persons insured with BARMER GEK (41.9% men, 58.1% women):

This overview already shows the differences in the distribution of medication groups—for equal distribution a ratio of 4.2–5.8 would have to be expected: Again, the impression is reinforced that women are clearly more frequently prescribed psychotropic medication (such as antidepressants, tranquilisers, hypnotics, SSRI) while medication with precise indications clearly show fewer gender-based differences and are often more frequently prescribed to men. The relatively high portion of non-beta receptor blockers for women could be explained by migraine prophylaxis.

2.3 Special Evaluations: Antidepressants and Benzodiazepines

With regard to the prescription frequency of thrombocyte aggregation inhibitors which are typically used after myocardial infarction or stroke to prevent thrombi from building up again, the difference is particularly marked (the following

Table 4 2010 BARMER GEK indication groups with conspicuous sex-specific prescription volumes

Indication group (ATC-Code)	Total prescriptions	Total costs in Euro	Prescriptions for men	Costs for men in Euro	Prescriptions for women	Costs for women in Euro
Beta-blockers (C07A)	4,432,538	70,324,419.41	1,659,986 37.4 %	26,705,578.07	2,772,552 62.6 %	43,618,841.34
ACE-inhibitors (C09AA)	2,873,191	40,581,755.74	1,273,844 44.3 %	18,106,638.74	1,599,347 55.7 %	22,475,117.00
Anti-depressants, tri- and tetracycl. (N06AA, N06AX03, N06AX11)	1,305,605	29,617,368.73	324,548 24 9 %	7,886,223.53	981.057 75.1 %	21,731,145.20
Tranquilisers (N05B)	773,126	10,975,322.73	209,751 27.1 %	3,074,464.25	563,375 72.9 %	7,900,858.48
CSE-inhibitors (C10AA)	2,000,321	53,991,615.13	919,733 46.0 %	25,669,384.16	1,080,588 54.0 %	28,322,230.97
Ca-antagonists (C08)	2,204,853	37,294,283.84	877,686 39.8 %	14,939,577.01	1,327,167 60.2 %	22,354,706.83
Insulin (A10A)	1,229,548	133,201,505.45	629,324 51.2 %	69,451,563.81	600,224 48.8 %	63,749,941.64
Thrombocyte aggregation inhibitors (B01AC)	984,662	52,048,638.08	521,111 52.9 %	29,149,130.63	463,551 47.1 %	22,899,507.45
Sulfonyl-urea (A10BB)	378,027	7,861,429.28	186,834 49.4 %	3,936,083.49	191,193 50.6 %	3,925,345.79
Hypnotics (N05C)	900,067	14,483,490.32	245,490 27.3 %	4,020,334.66	654,577 72.7 %	10,463,155.66
Serotonin uptake inhibitors (N06AB)	745,306	34,689,760.99	193,469 26.0 %	9,305,357.78	551,837 74.0 %	25,384,403.21

Table 5 DDD classes for SSRIs (such as, for instance, citalopram, fluoxetine, sertraline, etc.)

DDD classes	Men	In (%)	Women	In (%)
≤30	873	2.69	1,499	4.61
31–60	1,522	4.68	2,513	7.73
61–90	247	0.76	429	1.32
91–120	1,991	6.12	3,288	10.11
121–150	658	2.02	1,194	3.67
>150	6,824	20.99	11,473	35.29

Table 6 DDD classes for citalopram

DDD classes	Men	In (%)	Women	In (%)
≤30	607	3.46	1,017	5.80
31–60	1,007	5.74	1,632	9.30
61–90	146	0.83	252	1.44
91–120	1,165	6.64	1,840	10.49
121–150	389	2.22	695	3.96
>150	3,447	19.65	5 342	30.46

Table 7 DDD classes for NSMRI (such as, for instance, amitriptyline, doxepin, trimipramine, etc.)

DDD classes	Men	In (%)	Women	In (%)
≤30	6,478	12.81	11,814	23.37
31–60	3,471	6.86	6,152	12.17
61–90	2,085	4.12	3,713	7.34
91–120	1,401	2.77	2,582	5.11
121–150	1,287	2.55	2,246	4.44
>150	3,619	7.16	5,714	11.30

Table 8 DDD classes for benzodiazepines as muscle relaxants (M03BX07) (such as, for instance, tetrazepam)

DDD classes	Men	In (%)	Women	In (%)	Total
Up to 30	15,760	43.44	18,444	50.84	34,204
31–60	504	1.39	672	1.85	1,176
61–90	113	0.31	181	0.50	294
91–180	169	0.47	239	0.66	408
>180	83	0.23	112	0.31	195
Total	16,629	45.84	19,648	54.16	36,277

Table 9 DDD classes for anxiolytics (N05BA), particularly benzodiazepines

DDD classes	Men	In (%)	Women	In (%)	Total
Up to 30	8,163	25.45	13,558	42.27	21,721
31–60	1,662	5.18	2,417	7.54	4,079
61–90	616	1.92	980	3.06	1,596
91–180	999	3.11	1,416	4.41	2,415
>180	1,014	3.16	1,251	3.90	2,265
Total	12,454	38.83	19,622	61.17	32,076

Table 10 DDD classes for tranquilisers (N05CD), particularly benzodiazepines

DDD classes	Men	In (%)	Women	In (%)	Total
Up to 30	977	19.32	1,279	25.30	2,256
31–60	424	8.39	528	10.44	952
61–90	151	2.99	184	3.64	335
91–180	236	4.67	350	6.92	586
>180	396	7.83	531	10.50	927
Total	2,184	43.20	2,872	56.80	5,056

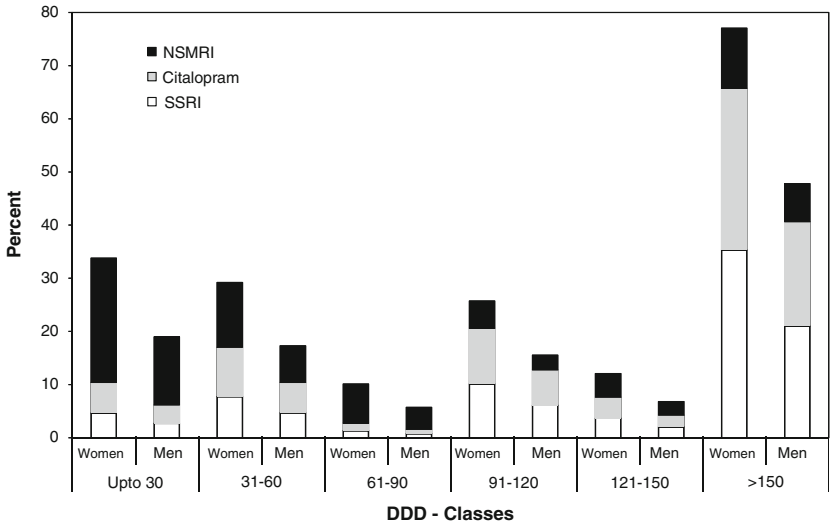
Table 11 DDD classes for Z-drugs (N05CF) (particularly zolpidem and zopiclone)

DDD classes	Men	In (%)	Women	In (%)	Total
Up to 30	3,583	23.04	5,001	32.16	8,584
31–60	1,068	6.87	1,478	9.50	2,546
61–90	369	2.37	456	2.93	825
91–180	699	4.50	1 032	6.64	1,731
>180	742	4.77	1,122	7.22	1,864
Total	6,461	41.55	9,089	58.45	15,550

Table 12 Insured persons with acute myocardial infarction

			Prevalence	
Sex	Age	Insured	Patients	Percent
Men				
	30–39	120,964	37	0.03
	40–49	189,240	255	0.13
	50–59	141,047	428	0.30
	60–69	96,419	463	0.48
	70–79	54,724	403	0.74
	80+	14,692	183	1.25
Total		617,086	1,769	0.29
Women				
	30–39	112,480	6	0.01
	40–49	166,729	47	0.03
	50–59	113,994	94	0.08
	60–69	67,575	96	0.14
	70–79	39,657	149	0.38
	80+	16,267	138	0.85
Total		516,702	530	0.10

Tables 5, 6, 7, 8, 9, 10, 11, and 12 are based on our own analyses of GEK or BARMER GEK data). Although the prevalence of problems after myocardial infarction is indeed for men markedly higher (32.7–16.7%) than for women, problems after stroke are more frequent with the latter. Therefore, the distribution pattern would have to be investigated more carefully to exclude women's under-supply of the medicines mentioned earlier. This also holds for CSE inhibitors which



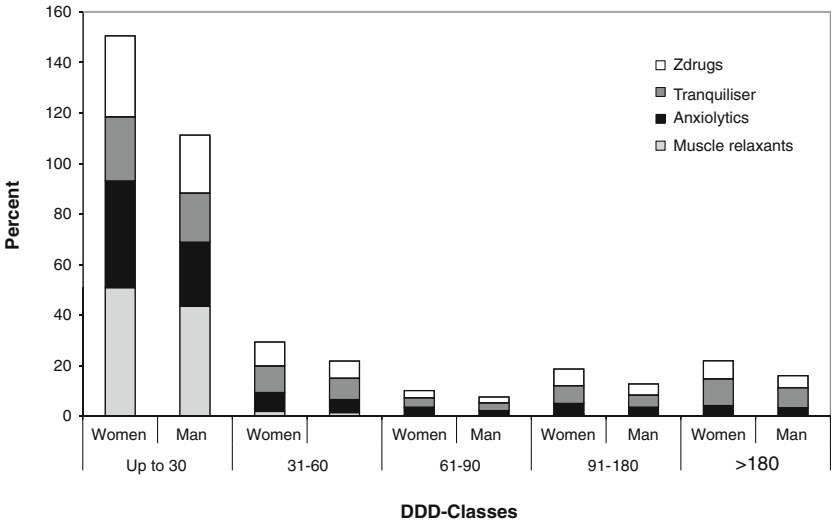
Graph 2 Prescription characteristics of antidepressants (SSRIs, citalopram, NSRMIs)

are prescribed in the case of elevated cholesterol levels to prevent vessel damages, and after myocardial infarction and stroke to prevent another myocardial infarction.

The first example will analyse prescription differences in serotonin reuptake inhibitors (SSRIs). The figures clearly show that more women are prescribed these medicines, particularly over a lengthy period of time. Of those insured that in 2010 were prescribed 150 DDDs and more, 21% were men and 53.3% were women (see Graph 2). The particular example of the most frequently prescribed SSRI citalopram shows comparable sex-specific prescription and distribution patterns (see Tables 5 and 6).

For non-selective monoamine reuptake inhibitors (NSMRIs), sex-specific distribution is also obvious (see Graph 2); the prescription duration characteristics, however, vary over a prescription period of 1 year—these medicines, unlike SSRIs, are prescribed less frequently over a lengthy period of time, but even here women are clearly "leading" in terms of the number of prescriptions.

The analyses of medication with substances from the group of benzodiazepines or benzodiazepine antagonists (Z-drugs such as zolpidem or zopiclone) show similar patterns; this also holds for benzodiazepine derivatives that are applied to relax muscles. Insured persons who have been prescribed benzodiazepine at least once are included in the evaluations according to indication groups and DDD categories. All substances show relatively higher prescription figures for women than for men. In the case of benzodiazepines and similar substances, the danger of becoming addicted is high and starts after 2–3 months (that is after 60–90 DDD). It is especially the tranquilisers that show a high percentage of patients who are treated with such drugs on a continuous basis—to prevent withdrawal symptoms that would occur when the drug is no longer taken. The evaluations show that it is



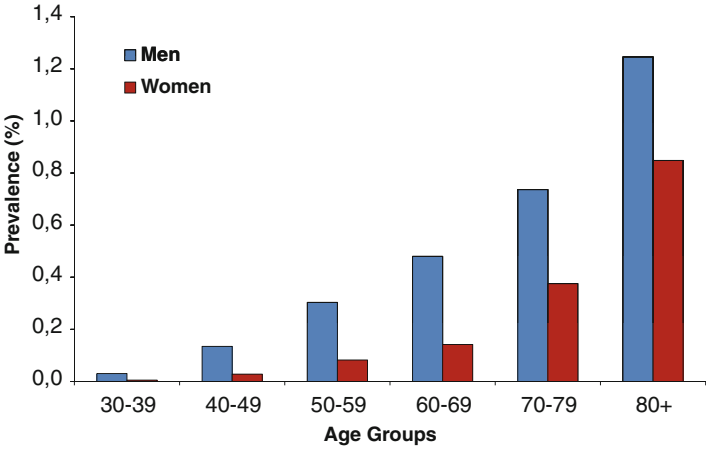
Graph 3 Prescription characteristics for benzodiazepines

mostly the women who suffer from undesired medication effects. In the meantime it has been found out that sleeping pills of the Z-drugs type (see Graph 3 and Table 11) are also prescribed on a long-term basis (more than 90 DDDs), which may also imply that addiction already exists. The progress characteristics of this medication are similar to those of tranquilisers (see Graph 3 and Table 10); the level of long-term volumes is, however, lower than with tranquilisers. From all this it may be concluded that medication addiction is "female" (Glaeske 2011).

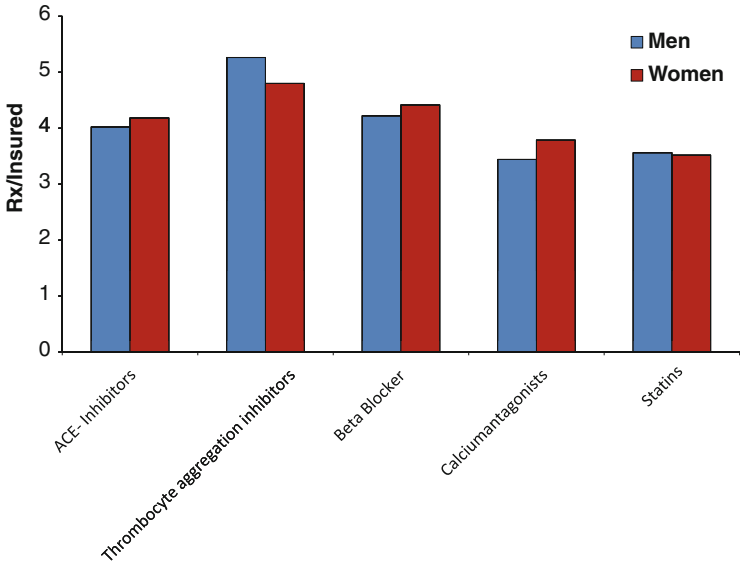
2.4 Diagnosis-Based Sex-Specific Prescriptions

It has often been speculated that medication for secondary prophylaxis, for instance, following myocardial infarction is prescribed differently to men and to women. To examine the validity of this speculation, an analysis of data from a statutory health insurance with 1.7 million insured persons (GEK) used the medication data from 2008 to 2009 as well as the stationary data from 2008. In a second step, insured persons with acute myocardial infarction diagnosis were identified (ICD-10 from I21.0 to I22.9) and then examined with regard to their medication prescriptions until the end of 2009. Only insured patients older than 30 years of age were included in the analysis. Altogether, the following 12-month prevalence could be observed (Graph 4):

As for medication prescriptions that are typically recommended after myocardial infarction ("ABC-therapy", such as, for instance, ASS, beta-blockers, CSE-inhibitors, especially statins), the data used for analysis suggest that women do not, like in former years, appear to be treated significantly different from the men (see, for instance,



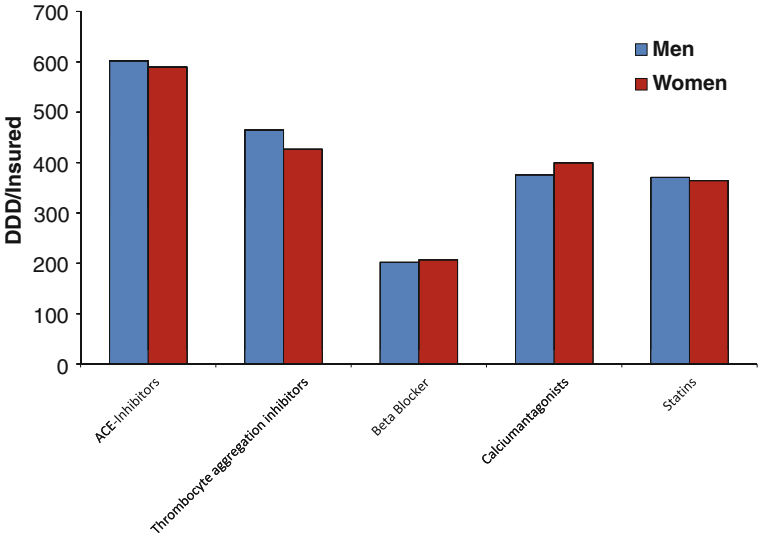
Graph 4 Prevalence of acute myocardial infarction



Graph 5 Prescription frequency of medication used as secondary prophylaxis after myocardial infarction

Hippisley-Cox et al. 2001; Theres et al. 2004). However, whether this is also reflected positively in the outcome should be investigated.

The average follow-up observation period for outpatient medical care after hospitalisation due to myocardial infarction was 12 months. During this time, the average prescription volume of 3.8–5.2 prescriptions (see Graph 5) meant about 200 DDD for beta-receptor blockers; about 400 DDD for thrombocyte aggregation inhibitors, calcium antagonists, and statins; and about 600 DDD for ACE inhibitors



Graph 6 Prescribed volumes (according to DDD) of medication used as secondary prophylaxis after myocardial infarction

(see Graph 6). Prescriptions before and after the follow-up observation period were not taken into consideration.

To reduce the risk of another myocardial infarction, medication-based follow-up treatment is most important—beside changes in the life style. According to what is currently known about this issue and according to the evidence available, simultaneous and long-term treatment with medicines from the a.m. four substances—thrombocyte aggregation inhibitors for "thinning" blood; beta-receptor blockers for reducing blood pressure; ACE inhibitors for enhancing heart performance; and lipid reducers, particularly statins, for normalising the metabolism—promise the best results. These medicines are also represented among the prescriptions for patients with myocardial infarction, yet their DDDs volumes vary. Particularly, the beta-blockers (preferably propranolol, timolol, acebutolol, and metoprolol-succinate) are not enough for the average observation period: about 360 DDDs are expected, only 200 DDDs are prescribed.

However, it can also be observed that women are supplied with medication to an extent that is similar to that of the men, with only slight differences (men, for instance, are on the average prescribed more thrombocyte aggregation inhibitors). In this context, however, it is important to point out that there are considerable differences between men and women with regard to the pharmacokinetics of beta-receptor blockers. After a dose of 100 mg Metoprolol, women show a maximum concentration of Metoprolol in the plasma that is about 40% higher than in men (Thürmann et al. 2006); see Chap. 5 of Thürmann in this book. The same must be said about other non-selective beta-receptor blockers such as Propranolol (Walle et al. 1985). For women this means increased reduction of heart rate and

blood pressure as well as more frequently occurring, serious adverse medication reactions.

Therefore, the dosages must be adapted to the patient's sex. According to the results of a meta-analysis of the effectiveness of Metoprolol administered after myocardial infarction, no differences between men and women could be observed with regard to mortality (Olsson et al. 1992). The actual results of prescription analyses at least suggest that men and women are given quite similar treatment after a diagnosed myocardial infarction; they also suggest that in secondary prophylaxis sex-specific disadvantages as described in several studies (see, for instance, Hippisley-Cox et al. 2001) need no longer be expected.

Women's higher risk of dying in hospital after acute myocardial infarction appears to rather be connected with the fact that they in comparison to men's rather diffuse and atypical complaints [see Seeland and Regitz-Zagrosek (2012)] are not quickly enough treated in hospital: Women's complaints are still obviously falsely interpreted, with the result that adequate hospital treatment starts too late (Löwel et al. 2002). This underscores the urgent necessity of considering sex-specific diagnosis aspects when diagnosing acute infarction. According to the formerly mentioned substitution hypothesis, women tend to not connect the symptoms of exhaustion and dizziness to myocardial infarction but look for normal medicinal help instead of emergency medical aid.

3 Conclusion and Clinical Implications

The evaluations of health insurance funds data still present examples of gender differences in the prescription characteristics. Conspicuous differences are observed particularly in the field of antidepressants and hypnotics—in these fields women are prescribed 2–3 times more medicines than men. This is why especially older women are particularly vulnerable to being prescribed potentially inappropriate medication, medication that, according to the PRISCUS list, is connected with certain potential hazards (such as reduced cognitive capacities, insecure gait, falls followed by difficult-to-heal fractures) (Wang et al. 2001) caused by adverse reactions such as fatigue, e.g. this kind of prescription pattern should urgently be replaced by improved safety and tolerance of women's medication supply. The often quoted differences in medication-based therapy for men and women, particularly in secondary prophylaxis after myocardial infarction seem to have become less relevant. Anyhow, the analyses presented here show that men and women are after such an event treated with comparable quantities of the same medicines: thrombocyte aggregation inhibitors, ACE inhibitors, beta-receptor blockers, and cholesterol-lowering drugs (especially statins). There are, however, considerable differences with regard to dying in hospital. This may have to do with the fact that women suffer from these unspecific complaints longer than men before going to hospital, which diminishes the hospital treatment prognosis. This, however, implies that especially the time between the diagnosis of acute myocardial infarction and the commencement of hospital treatment is of

particular significance for the survival differences in men and women, and that further medication-based treatment to reduce the risk of another infarction shows hardly any sex-specific differences: When women have survived a myocardial infarction and are, after the hospital stay, given evidence-based and medication-based treatment, hardly any sex- or gender-specific differences can be observed with regard to infarction-based mortality. This good message is still contrasted by the higher risk of women with acute myocardial infarction to die even before secondary prophylaxis begins.

Take Home Messages

- The greatest gender-based difference in the supply of medicinal products can still be observed in the field of psychotropic medication—women are 2–3 times more frequently than men prescribed antidepressants, tranquilisers, or hypnotics.
- Many of these medicines are listed in the PRISCUS list (a German Beers list) and contain potentially inappropriate substances for the elderly. Women are prescribed clearly more of these inappropriate medicines.
- Women are definitely more frequently than men jeopardised by potentially inappropriate medication; therefore, when prescribing medication to women, the selection of medicines, the dosage, the prescription-based therapy duration, and the sex-based differences of pharmacokinetics (for instance, of Metoprolol) should be paid attention to.
- Not only medication therapy but also diagnostics and acute intervention must follow the available evidence. Hospital lethality is for women with acute myocardial infarction still higher than for men because too much time passes before they get proper hospital treatment.

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Gender and Polypharmacotherapy in the Elderly: A Clinical Challenge

Christina Hofer-Dückelmann

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Abstract Polypharmacotherapy is a major concern in the elderly and especially in older women after the age of 80. It results from the intake of prescription and non-prescription drugs, being often a problem of evidence-based therapy. Besides the fact that women live longer than men and outnumber them, reasons for polypharmacy in women are diverse and include a different attitude towards intake of drugs between men and women, the propensity of women to rather see a physician and talk about their problems, the load of family responsibility as women are the main caregivers within a family, the influence of physician sex on patient care, the level of education, social deprivation and self-rated health. Women are more often prescribed potentially inappropriate medication and more often become victims of adverse drug reactions. This is not only due to the number and quality of drugs prescribed but also to differences in pharmacokinetics and -dynamics which make them more vulnerable to drug exposure. Thus, inappropriate

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prescribing contributes to hospitalization, poor quality of life, costs, compliance issues and poor outcomes. More preclinical and clinical studies with elderly patients and especially elderly women are needed to study the underlying mechanisms of the pharmacologic differences and obtain more insight into the difference in risk between men and women. Attention to prescribing of medications, consistent review of medication lists, and reevaluation of indications and outcomes of prescribing are essential to ensure that drugs are used appropriately in elderly women, polypharmacy is minimized and safety for patients is maximized.

1 Introduction

1.1 Definition and Incidence of Polypharmacy

The term "polypharmacy" or "polypharmacotherapy" ranges from the use of a large number of medications to the use of potentially inappropriate medications (PIMs) or medication duplication (Maggiore et al. 2010). It can refer to perceptions of prescribers or consumers and may or may not include non-prescription medication like over-the-counter remedies or herbal/supplementary agent use (Maggiore et al. 2010; Junius-Walker et al. 2006). Thus, the definition of polypharmacy is vague. European studies defined polypharmacy according to the number of medications taken, whereas the studies conducted in the USA defined polypharmacy according to whether a medication was clinically indicated (Fulton and Allen 2005). Clients may have several diagnoses and comorbidities, necessitating the use of multiple medications; therefore, a definition of polypharmacy based upon the number of medications may be inappropriate (Fulton and Allen 2005; Viktil et al. 2007). There is no consensus or commonly used cut-off point referring to the number of drugs anyway (Hajjar et al. 2007). One of the most commonly used definitions is the concurrent use of five or more drugs (Haider et al. 2009). In the Kuopio 75+ Study, "polypharmacy" was defined as the use of six to nine drugs whereas the concomitant use of ten or more drugs was referred to as "excessive polypharmacy" (Jyrkka et al. 2009). Whichever level is the basis of the definition, polypharmacy stands out as a marked risk factor for developing drug related problems (Viktil et al. 2007).

Polypharmacy is most common in the elderly but is also widespread in the general population (Haider et al. 2007). Its prevalence in elderly people is reported to be 5–78% (Fulton and Allen 2005). In the USA, among adults 65 years of age or older, 58% take five to nine medications and 18% take ten or more (Patterns of Medication Use in the United States 2006). In Germany, people over 60 years of age currently represent about 27% of the population, but consume 66% of prescribed medications (Thürmann et al. 2012) (Fig. 1).

Prescribing for elderly persons with several chronic diseases is a balance between limiting the number of drugs and using all drugs that may be beneficial (Colley and

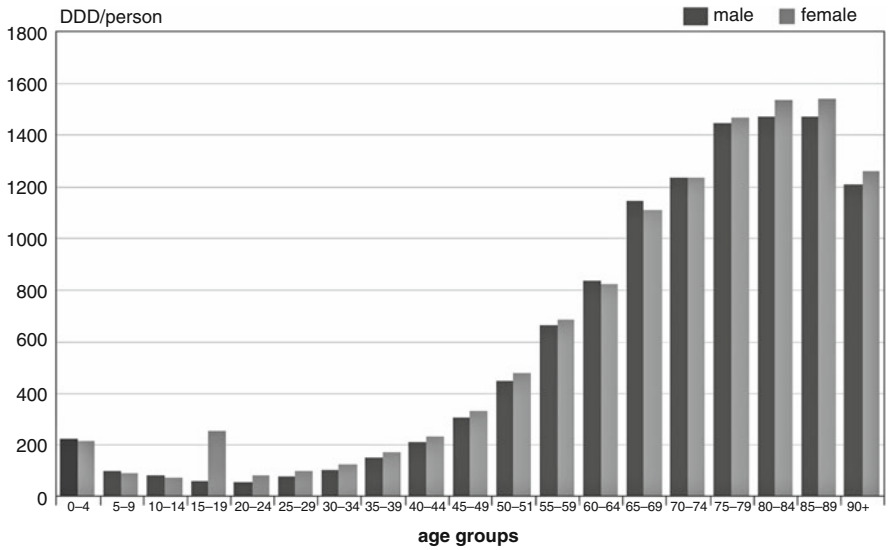

Fig. 1 Prescription of DDD per person for men and women according to age groups in the German population in 2010 (Thürmann et al. 2012)

Lucas 1993; Rochon and Gurwitz 1999). According to current guidelines, a patient with diabetes, hypertension, heart failure and atrial fibrillation requires a minimum of six different drugs. Redefining thresholds (e.g., for blood pressure and cholesterol) and marketing of new drugs (most of them without adequate testing in the older population) also promote polypharmacy, thus making polypharmacy a problem of evidence-based medicine and medical progress (Schuler et al. 2008).

Besides multiple diseases and advanced age, additional patient-related risk factors for polypharmacy were low level of self-rated health, living in an institution, number of visits to a primary care provider per year and *being female* (Chen et al. 2001; Fulton and Allen 2005; Jyrkka et al. 2009). On average, each German person of the 17 million people aged 65 years or more was prescribed 1,303 defined daily doses (DDD)/year in 2010, but women used 3% more than men (1,320 versus 1,281 DDD) (Thürmann et al. 2012).

2 Polypharmacy and Women

2.1 International Studies Confirm that Women Consume more Medication than Men

In Sweden, women ≥65 years treated in primary care had more non-fatal diagnoses and used an average of 4.8 prescription medications whereas men consumed an average of 3.8 (Jörgensen et al. 2001). Linjakumpu et al. (2002) also found that in

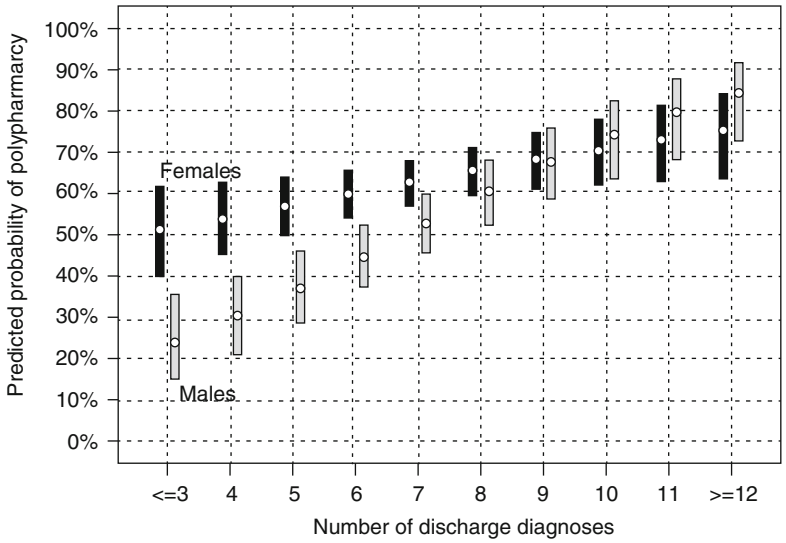

Fig. 2 Predicted probability for polypharmacy by gender and the number of diagnoses (Schuler et al. 2008)

Finland community-dwelling women aged 64 years or over used more prescription medications than men, with 81% of women using medications compared to 74% of men in 1990. Until 1998, drug consumption even increased with 93% of women using medications compared to 82% of men. These changes were most prominent among persons aged 85 years or over.

Among the ambulatory adult population of the USA, the rate of prescription medication was similar in men and women aged 65 years or older but differed in men and women younger than 65 years of age where women used much more drugs than men. Between 45 and 64 years of age, 90% of females used at least one prescription drug, 44% used five to ten, and 14% took at least ten different prescription drugs per week versus 83, 30, and 6%, respectively, in men (Patterns of Medication Use in the United States, 2006).

In an Austrian study, women with few diagnoses had a significantly higher risk for polypharmacy than men with comparable morbidity, although in the more severely ill patients (number of discharge diagnoses >9 or Charlson comorbidity score >5), drug prescription rates were similar between the sexes (Fig. 2) (Schuler et al. 2008). In an assessment of drug therapy in the UK, women used more drugs than men; however, the prevalence of polypharmacy, defined as concomitant use of ≥5 drugs, was similar in both genders (Kennerfalk et al. 2002).

2.2 *Is there any Reason Why Women are Rather Exposed to Polypharmacy?*

Women constitute the majority of old people because women life longer than men. Currently, women outnumber men by about 70 million among those aged 60 years or over worldwide. Among those aged 80 years or over, women are nearly twice as numerous as men, and among centenarians women are between 4 and 5 times as numerous as men (United Nations 2009). In Germany, women >65 years represent 23% of the population, whereas the rate of men is 18% (Thürmann et al. 2012).

Furthermore, consulting rates differ between men and women. Between the ages of 15 and 64 years, consulting rates for British women exceeded that for men in general practice (Martin et al. 1998). German women also attended primary care providers more often than men (68 versus 53%) (Zok 2006). Since consulting a physician is a risk factor for polypharmacy, this can be another reason why women are more endangered.

Non-prescription drugs are often used simultaneously with prescription drugs. According to an evaluation in Germany, six non-prescription drugs are consumed on average in addition to eight prescribed drugs per year (Zok 2006). Women use self-medication more frequently than men (58 versus 41%), the rate increasing with income, education and being (or feeling) sick (Zok 2006). Therefore, there must be a factor beyond age, polymorbidity and consulting rates. It seems that women have a different attitude towards drug intake and that their expectations concerning drug therapy are more positive. This is a field that requires further research.

A prediction model for polypharmacy also concluded that educational level accounted for the most variation in polypharmacy in individuals aged 65 and older. Women, on average, consumed more prescription medications than men, though gender differences were non-significant. Older, more educated women may be most likely to engage in polypharmaceutical consumption, suggesting a greater likelihood of an adverse drug event (Perry and Turner 2001). The so-called gender-paradox describes a discrepancy between morbidity and mortality among women: Although women's self-rated health is generally poorer than that of men, they have more preventive check-ups and visits to doctors and use more medication, they still live longer than men (Schwabe and Paffrath 2008).

Thus, the scientific literature, the public press, and numerous authors recognize the existence of differences between the sexes and the greater numbers of women than men at older ages, but with the exception of sex-specific conditions or diseases like osteoporosis or depression, the approach to medication therapy in the elderly has not traditionally included sex-specific considerations (Schwartz 2007). Contributing factors may be: (a) the misconception that as sex hormones diminish at older ages, pharmacokinetic or pharmacodynamic differences between the sexes are less at older ages, and (b) the lack of significant numbers of older women enrolled in premarketing pharmacokinetic or pharmacodynamic studies or in randomized clinical trials of common disorders in older populations (Schwartz 2007).

3 Consequences of Polypharmacy

Polypharmacy is a potential risk factor for medication problems. The most common results of polypharmacy are inappropriate medication, increased adverse drug reactions (ADRs), drug–drug interactions, medication errors such as administration of an inappropriate dose of medicine or giving the wrong drug, hospitalization, poor quality of life, and higher costs (Haider et al. 2007; Jyrkka et al. 2009; Hajjar et al. 2007; Shi et al. 2008). Furthermore, the more drugs an elderly person uses, the greater the likelihood of lower adherence to the medication regimen and poorer health outcomes (Barat et al. 2001; Sorensen et al. 2005). Polypharmacy has also been reported to increase the risk of falls (Baranzini et al. 2009; Sommeregger et al. 2010). However, there is evidence that fall risk is associated with the use of polypharmacy regimens that include at least one established fall risk-increasing drug such as psychotropic medication, antiarrhythmics or antiparkinson drugs rather than with polypharmacy per se (Baranzini et al. 2009).

4 Use of Potentially Inappropriate Medication

For the last decades, panels of experts in geriatric medicine and pharmacology developed lists of drugs that are considered as potentially inappropriate in the elderly because of their risk of causing an adverse effect outweighing the potential benefit. The first publications of drugs "to avoid in the elderly" were led by Marc Beers and colleagues (Beers 1997).

A number of studies of community-dwelling elders have found that older women were more likely than men to receive drugs "to avoid in the elderly" (Bierman et al. 2007; Zhan et al. 2001; Goulding 2004; Howard et al. 2004; Schuler et al. 2008; Johnell et al. 2009; Thürmann et al. 2012).

Zhan et al. (2001) stratified a subset of 33 drugs from the Beers drug list into three categories: (1) drugs that should always be avoided, (2) drugs that are rarely appropriate, and (3) drugs that have some indications but are often misused. An analysis of rates of potentially inappropriate medication (PIM) use among persons aged >65 enrolled in US managed care plans in 2000–2001 indicated that 6% of elderly females used at least 1 of 11 drugs classified as "should always be avoided," compared with 4% of men. For medications classified as "rarely appropriate," 16% of women received these drugs compared with 11% of men. Medications with "some indications but which are often misused" were used by 19% of women and 14% of men (Gurwitz 2005).

In a population of veterans aged >65 years, again women were more likely than men to have received PIMs in all three categories: always avoid, rarely appropriate, and some indications before and after adjustment for demographic characteristics, number of unique medications and characteristics of care (Bierman et al. 2007). There were higher rates of use of analgesic, psychotropic, and anticholinergic

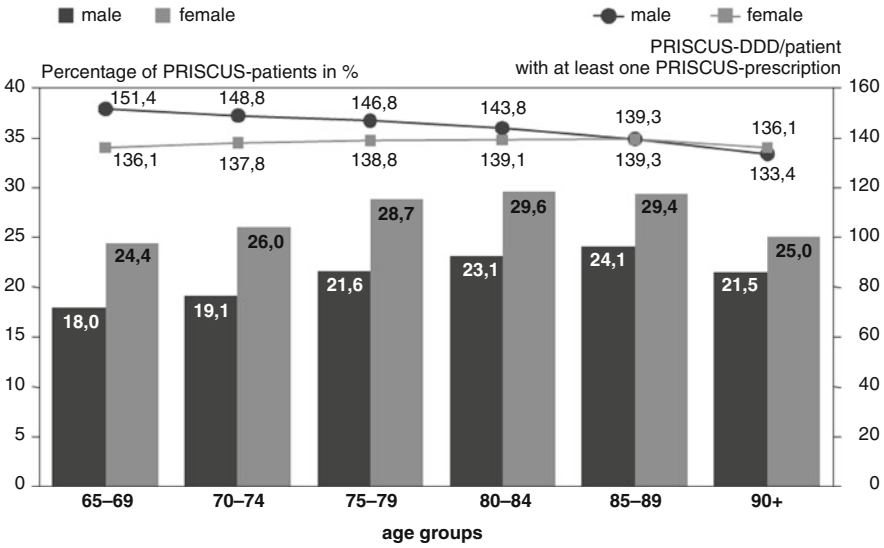

Fig. 3 Percentage of men and women with potentially inappropriate medication according to the PRISCUS list and prescribed DDD for men and women according to age in 2010 (Thürmann et al. 2012)

medications that should be avoided among women compared with men. For both men and women, those using more medications were more likely to have been prescribed an inappropriate medication (Bierman et al. 2007).

In Germany, the PRISCUS list defines "PIMs for the elderly" (Holt et al. 2010). According to this list, 5% of prescribed medications to elderly patients were considered as "PIM". Women were prescribed 5.7% and men 4.4%, respectively. Interestingly, the ratio of PIM keeps increasing from 65 years of age in men and women and has its peak in women of 90 years of age (Thürmann et al. 2012). However, men are prescribed a higher amount of DDD of PIM medications per prescription (148 versus 138 DDD) as they use bigger packages or more potent drugs (Fig. 3). Men and women consume different kinds of PIMs: Whereas men are prescribed 4% PIMs for cardiovascular therapy and 35% psychiatric drugs, in women psychiatric drugs represent 49% and cardiovascular drugs 25%. In an Austrian study, women were also found to have a much higher rate of inadequate drugs than men (38 versus 18%) (Schuler et al. 2008).

5 Which Medication and Why?

Numerous studies have documented the greater use of psychotropic drugs by women relative to men (Simoni-Wastila 1998; Goulding 2004; Thürmann et al. 2012). As the prevalence of mental illness, patient's and physician's age, self-rated

psychological well-being and physician specialty are associated with psychotropic drug prescription in men and women, reasons for women's greater use are not readily apparent. Simoni-Wastila (1998) found that the probability of receiving any psychotropic drug is 55% greater in office visits by women than men. Gender differences in coping with and expressing distress, willingness to seek medical care, perceptions of illness and physician prescribing bias might be some explanations (Lagro-Janssen 2008; Cafferata and Meyers 1990). Family responsibilities, but also marital separation, divorce, or widowhood and the presence of family stressors such as an ill spouse are correlated with the likelihood of obtaining a psychotropic drug prescription (Cafferata and Meyers 1990). However, women do not receive more of all types of psychotropics; rather, general practice office visits (in contrast to psychiatric visits) by women are more likely than men's visits to result in an anxiolytic or antidepressant prescription (Simoni-Wastila 1998).

Among ambulatory care visits with pain relievers prescribed, women's visits were more likely than men's to involve inappropriate pain relievers (Goulding 2004).

Some authors believe that women are more likely than men to report pain or depressive symptoms and therefore are more likely than men to be diagnosed and then treated for these conditions. Physicians are used to prescribing the older and inappropriate drugs in antidepressants, antianxiety agents, sedatives/hypnotics and pain relievers and may not be aware of their risks. Furthermore, older drugs tend to be less expensive and cost may play a role, thus leading to the high percentage of PIM in elderly women (Goulding 2004). However, if providers are more likely to attribute somatic complaints in women to mental health conditions, then sex bias in clinical decision making may contribute to these differences, and this would need to be addressed (Bierman et al. 2007; Lagro-Janssen 2008). Indeed, some studies suggest that the interaction between physician and patient gender may influence the amounts and types of treatments provided by physicians (Lagro-Janssen 2008). The fact that sedative and antidepressant drugs are prescribed twice as often in women suggests that polypharmacy in elderly women might also be a consequence of social deprivation (Bierman et al. 2007). Because of higher survivorship and lower propensities to remarry, older women are more likely than older men to live alone. Globally, an estimated 19% of women aged 60 years or over live alone, whereas just 8% of men in that age group do so (United Nations 2009).

In a study of very old people ($\geq$85 years), significant gender disparities in the prescription of several other drugs, such as diuretics, nitroglycerin and oral antihyperglycemic drugs, were observed. In most cases, women were prescribed more drugs than men in general. There was a strong association between female sex and the prescriptions of thiazide diuretics, potassium-sparing diuretics and diuretics as a whole. On the other hand, men more often had undergone coronary artery surgery. These disparities could only in part be explained by differences in diagnoses and symptoms (Brännström et al. 2011).

In an Austrian inpatient study, again some drugs were significantly more often prescribed for women: diuretics, betablockers, antidepressant and antipsychotic, benzodiazepines and levothyroxine, whereas allopurinol was more common in men (Schuler et al. 2008).

6 Women and Adverse Drug Reactions

Women are perceived to be more prone to drug-related problems than men, especially at an age of ≥65 years (Martin et al. 1998; Drici and Clement 2001; Zopf et al. 2008; Krähenbühl-Melcher et al. 2007; Hofer-Dueckelmann et al. 2011; Rodenburg et al. 2010). In a study evaluating polypharmacy and inappropriate prescribing in elderly internal-medicine patients (≥75 years) in Austria, 18% of elderly patients suffered from an adverse drug event in which women were much more frequently affected. Indeed, female gender was an independent predictor of adverse drug events (Schuler et al. 2008). Polypharmacy is another risk factor for ADRs and thus has been shown to predict hospital readmission related to ADRs. ADRs are often aggravated by another drug with the same side effects due to pharmacodynamic interactions (Jyrkka et al. 2009). Van der Hooft studied ADR-related hospitalizations in the Netherlands in 2001. The proportion of females with ADR-related hospitalizations varied between the different age categories, increasing with increasing age from 50.5% in the age group 65–79 years to 66.6% in the highest age group (80 years and older) (van der Hooft et al. 2006).

A 1.5- to 1.7-fold higher ADR rate for the female gender cannot be explained by age or number of prescriptions only (Rodenburg et al. 2010; Zopf et al. 2008). There seems to exist an increased vulnerability to drug toxicity with advancing age and female sex (Martin et al. 1998).

Such a propensity may result from gender-associated differences in drug exposure as well as from possible differences in the way the adverse event is perceived (Drici and Clement 2001; Zopf et al. 2008). A pharmacological explanation for the increase in ADR rates in females may be differences in pharmacokinetics and—dynamics such as lower body size and weight in females with consequent changes in apparent volume of distribution (Martin et al. 1998). Other possible explanations are differences in body composition like muscle mass, organ blood flow and organ function, activity of receptors or physiological (menopause, pregnancy, menstruation) aspects (Beierle et al. 1999).

Indeed, sex differences in the incidence of ADRs and pharmacatoxicity have been reported for several classes of drugs and specifically for several cardiovascular preparations. Given the high incidence of cardiovascular conditions and thus the broad use of these drugs in the general population, these reports have to be considered of relevance to public health (Oertelt-Prigione and Regitz-Zagrosek 2009). For example, two-thirds of the cases of drug-induced tachycardia occur in women. Therefore, this ADR represents a perfect example of gender differences impairing women's health. Estrogens facilitate bradycardia-induced prolongation of the QT interval and the emergence of arrhythmia with antipsychotics, antihistamines, antiarrhythmics or antibiotic treatment whereas androgens shorten the QT interval and blunt the QT response to drugs (Drici and Clement 2001; Zopf et al. 2008).

Earlier studies suggest that women present more commonly with gastrointestinal and cutaneous allergic drug reactions (Martin et al. 1998).

Table 1 Organ systems involved in ADEs (Hofer-Dueckelmann et al. 2011)

Involved organ system	*n*	*m* (%)	*f* (%)
Electrolytes	91	31.00	42.00
Hyponatremia	44	14.00	22.00
Hypokalemia	21	8.00	9.00
Hyperkalemia	10	3	5
Others	16	7.00	7.00
Coagulation system	70	28	30
Hemorrhage/bleeding	19	9.00	7.00
Over-anticoagulation	51	19.00	23.00
Renal/genitourinary	41	21.00	14.00
Creatinine	26	13	9
Acute renal failure	15	8.00	5.00
Cardiac arrhythmia	39	21.00	13.00

In an own study analyzing community acquired ADRs identified on admission, more women than men encountered an ADR (10 versus 6%, $p < 0.005$). Analyzed separately by age groups, this gender difference became significant at an age of $\geq$81 years, when patients are often living alone in deprived circumstances with nobody looking after them regularly. Women were more often admitted because of an ADR and had a significantly higher percentage of life-threatening and fatal ADRs than men (23 versus 12%, $p = 0.031$). Hyponatremia due to diuretics or psychotropic drugs occurred significantly more often in women, whereas renal failure and cardiac arrhythmia due to ADRs were more commonly found in men (Table 1). Coagulation problems were seen more often in women. Since many of the causative drugs are prescribed very often like ACE-inhibitors, diuretics or vitamin K antagonists, regular monitoring of laboratory values might have prevented harm to patients (Hofer-Dueckelmann et al. 2011). Thus, vigilance to the higher vulnerability in the elderly and especially elderly women has to be improved.

Dose-related ADRs (52%) were the dominant type in female subjects (Martin et al. 1998; Zopf et al. 2008). Patients weighing 50 kg or less ($n = 155$) received milligram-per-kilogram doses of three study drugs that were 31–46% higher than the group mean and 70–88% higher than patients weighing more than 90 kg (Campion et al. 1987). In another study, again patients with polypharmacy and low body weight had a significantly higher risk for wrong dosage (31 versus 13%, $p < 0.0005$, OR 3.05) (Schuler et al. 2008). Intravascular volumes, organ volumes, and muscle volumes are usually smaller in older individuals than younger individuals, and the smallest volumes of distribution encountered in clinical practice will usually be seen in older Caucasian or Asian women. The impact of a reduced volume of drug distribution is most evident when a loading or intravenous bolus dose of a medication is given, and for those drugs that have a narrow toxic to therapeutic ratio. Weight adjustment for loading doses of digoxin, lidocaine and other type I antiarrhythmic drugs, type III antiarrhythmic drugs, aminoglycoside antibiotics, chemotherapy regimens, and for unfractionated heparin is routinely

recommended. The difference in body weight is thought by some to be the most important gender difference that affects drug concentrations (Schwartz 2007).

As cytochrome P 450 activity varies between men and women, it is recommended to reduce the doses of CYP 450 2D6 substrates like carvedilol, metoprolol or paroxetin by approximately 20% in older patients and by another 10–20% in women compared with men as women are more susceptible to ADRs. Renal clearance will be lower in women than in men, and lowest in older women. Thus, routine estimation of glomerular filtration or creatinine clearance is recommended to guide dosing of renally cleared medications to reduce adverse events in both the acute and chronic care setting (Schwartz 2007). Because of slower clearance of cardiotonic glycosides in women, drug effects may be greater if doses have not been adjusted (Rodenburg et al. 2010).

7 Women and Clinical Trials

Many of the evidence-based therapies include drugs that have not been tested in the older population or in women at all since they are underrepresented in clinical trials (Lee et al. 2001; Gurwitz 2005). Although the authorities emphasized the importance of including more women in clinical trials as early as 1986, women are still underrepresented in clinical research nowadays (Kim et al. 2010).

A study examining trial enrollment of women and elderly persons in published randomized controlled trials of acute coronary syndromes during 1966–2000 found that both women and elderly persons remained highly underrepresented in the published literature, much of the sex disparity being a byproduct of underenrollment of elderly persons (Lee et al. 2001). The universal extension of trial results from a younger, mostly male population to women and elderly patients of both sexes may be inappropriate. In a post hoc subgroup analysis of the Digitalis Investigation Group Trial assessing sex-based differences in the effects of digoxin therapy, digoxin was associated with an increased risk of death from any cause among women, but not men, with heart failure and depressed left ventricular systolic dysfunction (Rathore et al. 2002). Drug-related risks are not always apparent until a drug is used in large numbers of elderly patients, many of whom are older, more likely to be female, and have more comorbidities than the participants in clinical trials (Gurwitz 2005). On the other hand, the paucity of information regarding the safety and efficacy of therapies in women and elderly persons may lead some physicians to withhold evidence-based drugs and interventions in these subgroups and rather prescribe old and PIM, although some drugs might be safe if used in an appropriate way (Lee et al. 2001). Concern about the burden of trial participation can make it more difficult to recruit a diverse population to participate in clinical trials, however, it is ethically clear that investigators have a duty to make special efforts to recruit a population of subjects that adequately reflects the population who will be exposed to the therapy that is the subject of the trial (Morse et al. 2004). Phase IV trials and registries are required to

detect safety problems in this vulnerable subpopulation to alter these patterns of exclusion from clinical trials to ensure evidence-based care to all patients.

8 Conclusion

Polypharmacy is a complex and worrying phenomenon that leads to medical problems especially in the elderly. Female gender appears to be a potential risk factor for polypharmacy. Older women are more likely to be prescribed PIM and to suffer from ADRs as they have an increased vulnerability to drug toxicity. To ensure evidence-based care to all patients, efforts are necessary to increase the representation of elderly female and male participants in preclinical and clinical trials and registries and to address drug safety issues more rigorously.

Take Home Messages

- Polypharmacy is common in the elderly, female gender being a risk factor for it.
- Polypharmacy in elderly women results in higher rates of PIM and ADRs.
- Reasons for ADRs are higher vulnerability due to differences in pharmacology in elderly women.
- More research is needed in elderly women to ensure safe medication therapy independent of age and gender.

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Role of Physician Gender in Drug Therapy

I. Gouni-Berthold and H.K. Berthold

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Abstract There is evidence that female patients receive less intensified drug therapy in many medical conditions than male patients. However, there are only limited data regarding the influence of physician gender on drug therapy. It has been

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shown, for example, that female physicians tend to adhere more closely to guideline-recommended pharmacotherapy compared to their male counterparts. In some medical conditions where drug therapy is only one among various components of a complex interplay of therapeutic regimes (e.g., diabetes, cardiovascular diseases, depression, pain management), female physicians seem to achieve better overall intermediate outcomes and some studies suggest that "better" drug therapy is provided by female compared to male physicians. The reasons for the overall better outcomes may be superior communication skills of female physicians, participatory decision making, and consequently improved drug adherence in addition to or in combination with more effective non-pharmacologic treatment results. It is impossible to distinguish between the individual contributions of drug- and nondrug-related influence on such improved outcomes and thus to determine whether they are due to unconfounded physician gender effects on drug therapy. There is until now in no area of medicine evidence to suggest that a patient will consistently receive higher quality of drug therapy by switching to a physician of a specific gender.

Keywords Physician gender • Gender dyads • Drug therapy • Patient–physician communication • Cardiovascular disease • Diabetes mellitus • Chronic heart failure • Pain management

Abbreviations

ACE	Angiotensin converting enzyme
ARB	Angiotensin receptor blocker
BMD	Bone mineral density
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
LDL	Low-density lipoprotein
PCP	Primary care physician
SBP	Systolic blood pressure

1 Introduction

There is a large body of evidence suggesting that in many areas of medical conditions, patient gender is associated with the quality of care provided. For example, in cardiometabolic diseases, the vast majority of findings suggest that female patients receive less intensified care than male patients. This could be demonstrated in the treatment of angina pectoris (Crilly et al. 2007; Daly et al. 2006), acute coronary syndromes (Blomkalns et al. 2005), acute myocardial infarction (Chandra et al. 1998; Peterson et al. 2008), cardiovascular secondary prevention (Cho et al. 2008), and in type 2 diabetes (Gouni-Berthold et al. 2008; Wexler et al. 2005). There are also examples, however, where the opposite could be

demonstrated. Namely, a multilevel analysis of adherence to osteoporosis practice guidelines showed that male patients had an odds ratio of 0.17 (95% CI 0.12–0.23) of undergoing bone mineral density scans or of receiving an osteoporosis medication compared to female patients (Solomon et al. 2004). These examples suggest that some diseases may have gender-specific connotations, i.e., heart disease may be more associated with male patients and osteoporosis more with female patients. This may lead to differential awareness of the diagnostic and therapeutic measures that are necessary for adequate quality of care.

The question whether physician gender also plays a role in the quality of care provided has been discussed controversially for quite some time (Beran et al. 2007; Cassard et al. 1997; Henderson and Weisman 2001; Kreuter et al. 1995; Osborn et al. 1991; Schmittdiel et al. 2000; Streja and Rabkin 1999). It has been shown that physician level characteristics such as race, specialty training, and degree of surgical experience may potentially influence quality of care (Birkmeyer et al. 2003; Cooper-Patrick et al. 1999; Greenfield et al. 2002). There is evidence from some studies that physician gender also influences patient care. Specifically, some findings have suggested that female physicians provide more comprehensive treatment than their male counterparts, for both male and female patients (Cassard et al. 1997; Ewing et al. 1999; Franks and Clancy 1993; Henderson and Weisman 2001; Kreuter et al. 1995; Lurie et al. 1997; Osborn et al. 1991), while other studies found no difference (Schmittdiel et al. 2000; Streja and Rabkin 1999). A Canadian study of general practitioners (GPs) identified that female GPs were more likely to value psychosocial factors in patient care (Maheux et al. 1990). In an Australian study of 113,000 general practice encounters, female GPs were more likely to manage psychosocial problems (Britt et al. 1996). In a Californian study, the gender of the GP was shown to influence the communication pattern and to contribute to the female GPs being viewed as achieving greater patient satisfaction (Bertakis et al. 1995). In addition to these aspects of medical care, there is evidence that physician gender may also affect the way drugs are prescribed. One study found that patients with HIV treated by male physicians received protease inhibitor (PI) therapy earlier than patients treated by female physicians (Beran et al. 2007). Interestingly, gender concordance (patient and clinician are of the same gender) was not a significant predictor of time until PI initiation. This shows that there are no simple associations in the complex interplay of patient/physician gender, drug prescription, and communication patterns.

Are there true differences in the way male and female physicians use drugs? What are the proposed explanations for these potential differences? The purpose of this article is to critically examine the existing evidence regarding the role of physician gender in drug therapy of various diseases, with the main focus on drug therapy of complex (mostly chronic) diseases, such as cardiometabolic diseases, because cardiovascular disease (CVD) is the leading cause of death for both men and women in industrialized countries (Maas et al. 2011) and its incidence will be steadily increasing in the future (World Health Organization 2008).

2 Examples of Physician Gender Influence on Medical Therapy

2.1 Hypertension and Cardiovascular Risk Factors

Hypertension is a well-established cardiovascular risk factor (Kannel 1996). The treatment goals for hypertensive patients are systolic blood pressure (SBP)/diastolic blood pressure (DBP) <140/90 mmHg or <130/80 mmHg for patients with diabetes mellitus, renal disease, or at very high cardiovascular risk (Chobanian et al. 2003; Mancia et al. 2007; Whitworth 2003). However, there is a big gap between guideline recommendations and everyday clinical practice (Primatesta and Poulter 2006; Weinehall et al. 2002; Westheim et al. 2001). Various factors have been implicated in explaining this observation, such as the educational level of the patient, varying adherence to antihypertensive medication, decision making of the providers, and the way in which patients interact with physicians regarding the treatment decisions made (Roumie et al. 2006). Journath et al. (2008a) using a database from a national survey (Journath et al. 2008b) studied the association between physicians' gender and blood pressure, lipid control, and cardiovascular risk factors of men and women treated for hypertension. The national survey used was the "Hyper-Q" survey which was carried out in primary health care centers in Sweden from September 2002 to September 2005. The inclusion criteria for the patients were a diagnosis of hypertension and the primary care physicians (PCPs) came from across Sweden. The PCPs registered on a web-based form connected to a central database. In order to minimize selection bias, each PCP contributed data from at least ten patients' medical records. In all, 6,537 patients (48% men) with a mean age of 66.1 years (SD 12.1) were recruited by 264 PCPs. Patients were included consecutively in the same order as they visited the health care center. The mean age of the PCPs (187 men and 77 women) was 51.8 years (SD 6.1). There was no age difference between the male and female physicians. Hypertensive women more often reached target systolic/diastolic blood pressure levels (<140/90 mmHg) when treated by female PCPs than when they were treated by male PCPs (32 vs. 24%, $P < 0.001$). This difference remained when comparing female and male physicians' nondiabetic female patients. Both male and female patients had better control of total cholesterol and LDL cholesterol when treated by female PCPs than when treated by male PCPs, respectively (total cholesterol <193 mg/dl [5 mmol/l]: women 30 vs. 24%, $P < 0.001$; men 42 vs. 34%, $P < 0.001$; LDL cholesterol <116 mg/dl [3 mmol/l]: women 39 vs. 33%, $P < 0.01$; men 41 vs. 35%, $P < 0.05$). The results of this study suggest that female physicians seem to more often reach the treatment goal for blood pressure in female patients and cholesterol levels in all patients than did male physicians (Table 1).

A higher percentage of men 56–70 years of age treated by male PCPs had microalbuminuria compared with men in the same age group treated by female PCPs ($P < 0.01$). Moreover, the men in this age category (56–70 years of age) who were treated by female PCPs had a higher prevalence of smoking than did those treated by male PCPs ($P < 0.01$). Hypertensive men and women with female PCPs

Table 1 Percentage of hypertensive patients (~24% with diabetes) not achieving treatment target values or not receiving drug treatment, stratified by primary care physician gender in a Swedish population [after Journath et al. (2008a)]

	Male patients			Female patients		
Parameter	Male physician	Female physician	*P*-value	Male physician	Female physician	*P*-value
Total cholesterol ≥195 mg/dl [5 mmol/l]	66	58	<0.001	76	70	<0.001
LDL cholesterol ≥115 mg/dl [3 mmol/l]	65	59	<0.05	67	61	<0.01
Systolic blood pressure ≥140 mmHg	68	67	n.s.	75	66	<0.001
Diastolic blood pressure ≥90 mmHg	28	31	n.s.	24	20	<0.01
Receiving ACE inhibitors	25.7	30.8	<0.01	16.4	20.2	<0.01
Receiving ARBs	36.2	32.4	<0.05	33.5	33.8	n.s.
Receiving lipid-lowering drugs	33.0	38.5	<0.01	27.5	32.5	<0.01

LDL low-density lipoprotein, *ACE* angiotensin converting enzyme, *ARB* angiotensin-receptor blocker

received more often angiotensin-converting enzyme inhibitors (ACE inhibitors) and lipid-lowering medication than did patients of male PCPs ($P < 0.01$ for both). Treated hypertensive men with male PCPs were more often treated with angiotensin II receptor blockers (ARBs) compared with treated male hypertensive patients of female PCPs ($P < 0.05$). Male PCPs prescribed ARBs more often in total and to hypertensive women than did their female colleagues ($P < 0.01$ for both). On average, both men and women were prescribed a mean of 2.0 drugs by male PCPs and 1.9 drugs by female PCPs.

In the female patients treated by female physicians, there was an additional 3.2 mmHg reduction in SBP. Could this difference be clinically relevant? The answer is most likely affirmative, since a 2 mmHg lowering of SBP has been shown to result in reduction of mortality rates of 4–7% for coronary heart disease (CHD) and 6–10% for stroke (Lewington et al. 2002; Stamler et al. 1989). In this regard, it has also been shown that even a 1 mmHg decrease in SBP can decrease the covariate-adjusted CHD mortality by around 2% (Gerber et al. 2005). Moreover, a recent meta-analysis in 73,913 patients with diabetes (Reboldi et al. 2011) showed that for each 5 mmHg reduction in SBP there is a 13% decrease in the risk of stroke (95% CI 5–20, $P = 0.002$) and an 11.5% decrease (95% CI 5–17) for each 2 mmHg reduction in DBP. Moreover, a 10% decrease in blood pressure and total cholesterol has been shown to reduce CVD by 10% (Emberson et al. 2004).

It is of interest to point out, however, that irrespective of the gender of the treating physician, only one-third of the patients reached target blood pressure levels.

Journath et al. (2010) examined the association between physicians' gender with risk factor control in patients with hypertension and dyslipidemia, receiving both antihypertensive and lipid-lowering treatment. Dyslipidemia and hypertension are not only two major risk factors for cardiovascular disease (CVD) but often occur concomitantly (Neaton and Wentworth 1992). A 10% decrease in blood pressure

and total cholesterol has been shown to reduce CVD by 10% (Emberson et al. 2004). The study was a cross-sectional study of 4,319 patients (53% men) on lipid-lowering and antihypertensive treatment from two national Swedish surveys, the HyperQ, which has already been previously discussed, and the EKO study (Journath et al. 2010), which was also carried out in primary health care centers in Sweden from August 2003 to June 2006, with inclusion criteria for the patients being a diagnosis of treated dyslipidemia and hypertension. The PCPs came from all over Sweden. Male physicians ($N = 244$) included 1,643 men and 1,311 women in the study, and female physicians ($N = 103$) included 605 men and 648 women. All data were collected consecutively from medical records. Female patients were older, had a higher SBP, pulse pressure, total cholesterol and LDL-C, SBP $\geq$140 mmHg, and had more often isolated systolic hypertension compared with male patients. Men compared with women had more often diabetes, higher cardiovascular risk, as calculated using the European risk evaluation algorithm SCORE (Conroy et al. 2003), and achieved treatment goals more often for blood pressure in nondiabetic patients and total cholesterol in both nondiabetic and diabetic patients. The female diabetes patients treated by female PCPs more often achieved treatment goals for blood pressure (<130/80 mmHg). Male patients with diabetes treated by female PCPs were more often well controlled (well controlled defined as SBP $\leq$140 mmHg and total cholesterol $\leq$190 mg/dl [5 mmol/l]) (Journath et al. 2010).

Tabenkin et al. (2010) evaluated CVD risk factor management in patients cared for by 39 male and 16 female PCPs in 30 practices in southeastern New England. All adult patients of these practices ($N = 51{,}078$) were sent a letter by their physician inviting them to participate in the project. Of those, 5,218 participated in a telephone interview where they responded to a questionnaire eliciting information about factors such as age, race/ethnicity, marital and smoking status, and education and income levels. Eventually data were analyzed from 4,195 patients (40% men, 60% women). Information on the physicians was obtained by completing a questionnaire and data from the state medical licensing board. The authors found that in multilevel adjusted analyses, styles of CVD risk factor management differed by the gender of the physician, with female physicians providing more diet and weight loss counseling for hypertension (OR 2.22; 95% CI 1.12–4.0) and obesity (OR 2.03; 95% CI 1.30–3.18) and more physical activity counseling for obesity (OR 2.03; 95% CI 1.30–3.18) and diabetes (OR 6.55; 95% CI 2.01–21.3). Diabetes management differed by the gender of the patient, with less women receiving glucose-lowering medications (OR 0.49; 95% CI 0.25–0.94), ACE inhibitor therapy (OR 0.39; 95% CI 0.22–0.72), and aspirin prophylaxis (OR 0.30; 95% CI 0.15–0.58). However, patients of female physicians were not more likely to reach guideline-recommended target values in LDL cholesterol, blood pressure, or HbA1c of <100 mg/dl [2.6 mmol/l], <130/85 mmHg, and <7%, respectively [Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III] 2001; Standards of medical care in diabetes – 2006 2006; Chobanian et al. 2003), compared to the patients of male physicians.

Regarding the question whether physician gender affects the type of antihypertensive drugs prescribed, the results have been contradictory. Sequeira et al. (2003) found that there are physician gender-based differences in the prescription of antihypertensive drugs, with female physicians prescribing more ACE inhibitors, methyldopa, and calcium channel blockers while male physicians prescribing more beta-blockers and diuretics. Burge et al. (2001), however, in a retrospective, population-based study of 1,466 physicians in Canada did not find any evidence of physician gender affecting the prescribing patterns regarding antihypertensive medications.

2.2 *Heart Failure*

The prevalence of chronic heart failure (CHF) is rising, especially in the aging population of Western industrialized countries (Remme and Swedberg 2001). The outcomes of patients with CHF have been improving over the past 20 years due, at least partially, to the increased use of evidence-based drug therapy management with ACE inhibitors, ARBs, and beta-blockers (Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). The CONSENSUS Trial Study Group 1987; Heidenreich et al. 1997; Pfeffer et al. 1992; Werner et al. 2010). Baumhäkel et al. (2009) examined the influence of physician gender on guideline-recommended treatment of CHF. Consecutive patients with heart failure ($N = 1{,}857$; 52.5% men) were evaluated between March and November 2006 in centers in eastern Germany regarding co-morbidities, New York Heart Association classification, current medical treatment, and dosage of ACE inhibitors, ARBs, and beta-blockers. The patients were treated by 829 physicians (65.3% general practitioners, 27.6% internists, and 7.1% cardiologists). There was no gender-related difference in the specialization of physicians (63.2% male vs. 68.0% female general practitioners; 29.0% male vs. 25.7% female internists; 7.8% male vs. 6.2% female cardiologists, all *n.s.*). Duration since medical board examination was comparable for both genders (male 23.9 $\pm$ 9.1 years and female 23.5 $\pm$ 8.8 years, *n.s.*). Baseline characteristics of patients and physicians were comparable for males and females. Treatment with an ACE inhibitor or ARB was documented in 80.4% of the patients (72.5% ACE inhibitors, 4.3% ARB, 3.6% ACE inhibitor and ARB), whereas treatment with beta-blockers was documented in 69.9% of the patients. Female patients were less frequently treated with ACE inhibitors, ARBs, or beta-blockers ($P = 0.021$) and recommended doses tended to be higher in male patients ($P = 0.058$). There was no difference in use or dosage of the aforementioned medication in patients treated by a male or female physician (*n.s.*). Male patients tended to receive more beta-blockers ($P = 0.075$) with significantly higher doses ($P = 0.021$), compared with female patients. There was no difference in dosing between the genders of treating physicians. However, use of beta-blockers tended to be higher in patients treated by a female physician ($P = 0.054$). Achieved doses were lower in female compared with male patients. Guideline-recommended drug

Table 2 Independent parameters influencing the prescription of beta-blockers in patients with chronic heart failure [after Baumhakel et al. (2009)], with permission from Oxford University Press

Parameter	Estimate	95% confidence interval	*P*-value
Ejection fraction	0.006	0.003–0.009	<0.001
Use of ACE inhibitor or ARB	0.124	0.034–0.214	0.007
Physician gender	−0.31	−0.521 to −0.1	0.004
Patient gender	−0.22	−0.426 to 0.013	0.037
Physician gender/patient gender interaction	0.166	0.645–1.538	0.016
COPD	0.132	0.024–0.24	0.017
Patient age	0.001	−0.004 to 0.003	0.93
Physician specialty	0.003	−0.042 to 0.048	0.89
Duration since medical board examination	0.002	−0.002 to 0.005	0.39
Hypertension	0.033	−0.058 to 0.124	0.48
Coronary heart disease	−0.048	−0.119 to 0.24	0.20
NYHA classification	0.015	−0.024 to 0.054	0.45

ACE angiotensin converting enzyme, *ARB* angiotensin-receptor blocker, *COPD* chronic obstructive pulmonary disease, *NYHA* New York Heart Association

use and achieved target doses tended to be higher in patients treated by female physicians. There was no difference in the treatment for male or female patients by female physicians. Male physicians, however, used significantly less medications ($P < 0.05$ for both ARBs or ACE inhibitors and beta-blockers), and lower doses ($P < 0.05$ for doses of both ARBs or ACE inhibitors and beta-blockers), in female patients compared to male patients.

In multivariate analysis, adjusted for use of ACE inhibitors or ARBs, age of patients, ejection fraction, NYHA classification, hypertension, CHD, chronic obstructive pulmonary disease, and also possible physicians' confounders such as physician specialization and time since medical board examination, physician gender, patient gender, and the interaction of physician and patient gender were independent predictors for use of beta-blockers (Table 2). Use of ACE inhibitors or ARBs was not dependent on physician gender in multivariate analysis (−0.174 to 0.225, $P = 0.80$). The authors suggest as possible explanations for their findings that (1) female physicians are more likely to work fewer hours than male physicians (Linzer et al. 2002; Uhlenberg and Cooney 1990; Weizblit et al. 2009) and part-time workers have been shown to have higher quality performance than physicians working longer hours (Parkerton et al. 2003), (2) female physicians emphasize patients' needs and opinion more than male physicians, and (3) they encourage questions and cooperation from their patients more effectively than male physicians. It can be safely assumed, however, that there are more at present unknown explanations for the observed effects.

2.3 Diabetes Mellitus

In 2008, our group investigated the influence of physician gender on quality of care in patients with type 2 diabetes in Germany (Berthold et al. 2008). We performed

a cross-sectional study in 51,053 outpatients (48.6% male), treated by 3,096 office-based physicians (74.0% general practitioners, 21.8% internists, and 4.2% diabetologists). We used the data of the DUTY registry (*D*iabetes mellitus needs *u*nrestricted evaluation of patient data *t*o *y*ield treatment progress). In short, between February 2002 and November 2003, a total of 6,700 office-based physicians were approached to participate in the study. Physicians in mid-sized and large practices (general practitioners, internists, and diabetologists) qualified for participation if they treated patients with diabetes. Each physician was asked to recruit 20 consecutive patients with diabetes under his treatment, type 1 and type 2 (yielding a total of 134,000 expected patients). Reports of 59,075 patients from 3,213 physicians were received. Of these patients, 89.8% had type 2 diabetes, 5.7% type 1, and in 4.5% the type of diabetes was not identified. For this study, we considered only patients with type 2 diabetes. Moreover, only datasets where physician and patient gender could be identified were considered. Thus, data of 51,053 patients were analyzed, obtained from 3,096 physicians, 2,053 male (66.3%) and 1,043 female (33.7%). Main outcome measures were (1) diabetes processes of care, including receiving eye and foot examinations, urine albumin testing, and lipid and HbA1c testing, (2) intermediate outcomes including HbA1c, LDL cholesterol, and blood pressure levels, and (3) glycemia, lipid, and blood pressure management. Quality of care measures were based on current ADA guidelines (Standards of medical care in diabetes – 2006 2006). Hierarchical regression models were used to avoid case-mix bias and to correct for physician-level clustering. Adjusted odds ratios were calculated controlling for age, gender, disease duration, and presence of atherosclerotic disease. Male physicians treated 67.2% of the total patients and female physicians 32.8%. Whilst male physicians treated similar proportions of men and women (50.6% male vs. 49.4% female), female physicians had significantly more female than male patients (44.6% male vs. 55.4% female). Patients of female physicians were more often women, more obese, older, and had more often atherosclerotic disease (34% in the total cohort). The patients of female physicians more often reached target values in glycemic control, LDL cholesterol, and blood pressure. They tended to receive more structured diabetes education and were more likely to receive antihypertensive drug therapy in general and ACE inhibitors in particular.

Figure 1 summarizes some of the findings and shows the results of the unadjusted and fully adjusted models. Even though the observed differences were highly significant, some of the actual sizes of the differences were small and therefore could be considered of limited clinical relevance. However, small increases in multiple risk factors could add up to a sizeable combined effect. In this context, it has been shown that individual therapy with antihypertensive agents, lipid-lowering drugs, and aspirin each reduces the risk of cardiovascular events by 25%, and the results appear to be additive (Stratton et al. 2006; Yusuf 2002). Moreover, data from the UKPDS study have shown that the use of statins and antihypertensive drugs seem to have the largest effect in reducing cardiovascular risk, with hypoglycemic agents and aspirin being the next most important interventions (Gaede and Pedersen 2004). Regarding HbA1c, it has been shown that in patients with diabetes, the

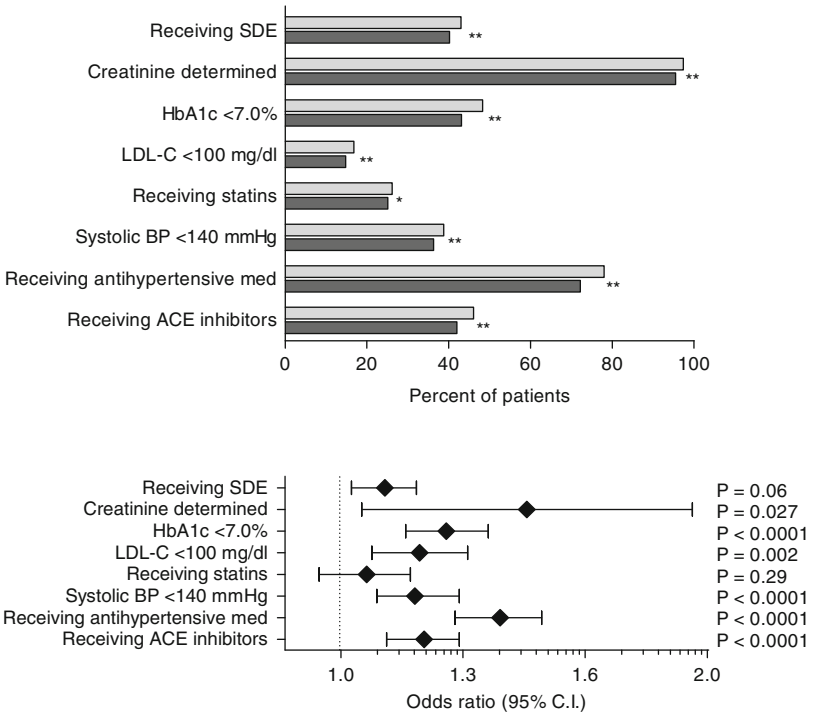

Fig. 1 Process and intermediate outcomes of 51,053 patients with type 2 diabetes mellitus treated by 3,096 physicians in Germany [after Berthold et al. (2008)]. *Upper panel*: Percent of patients receiving measure or achieving target. *Lighter shading* indicates female physicians and *darker shading* male physicians. $^{**}P < 0.0001$, $^{*}P < 0.01$ (unadjusted *P*-values from logistic regression analysis). *Lower panel*: Fully adjusted multilevel regression model. Adjustments were made for patient age, patient gender, diabetes duration, presence of atherosclerotic disease, and physician-level clustering. The odds ratios indicate the association of physician gender and listed parameters with the male physician gender as referent group. Odds ratios >1 indicate a higher likelihood of patients to achieve the respective target when treated by female physicians, and vice versa. *SDE* structured diabetes education

relationship between HbA1c levels and CHD increases throughout the range of HbA1c values and that the risk ratio of CHD per 1% point increase in HbA1c level is 1.14 (95% CI 1.07–1.21) (Selvin et al. 2004, 2005). Furthermore, it has been shown that even very small shifts of HbA1c levels (in the range of 0.1–0.2% points) can dramatically affect the future incidence of cardiovascular disease (Khaw et al. 2004). The same is true for LDL cholesterol where a 1 mg/dl [0.026 mmol/l] change decreases the risk ratio of CHD by ~1% (Grundy et al. 2004). The positive effects of systolic blood pressure reduction (even that of 1 mmHg) have been discussed previously in the "Hypertension" section. Moreover, the fact that patients of female physicians achieved more often target values in glycemic, lipid, and blood pressure control seems clinically highly relevant since a recent analysis has demonstrated that in the general US population (amongst them many patients with diabetes) half

of the decrease in deaths from CHD may be attributable to reductions in major risk factors (Ford et al. 2007).

Schmittdiel et al. (2009) examined, among other parameters, the association between physician gender and cardiovascular risk factor control and treatment in patients with diabetes mellitus. Patients with type 2 diabetes ($N = 157{,}456$; mean age 61 years; 52% male) were selected from the Kaiser Permanente (KP) diabetes registry. KP is an integrated health care delivery system providing comprehensive medical care to ~3.2 million members in Northern California. Selected patients had a PCP within KP and they were assessed for uncontrolled levels of cardiovascular risk factors, namely a HbA1c value of $\geq$8%, LDL cholesterol $\geq$100 mg/dl [2.6 mmol/l], and SBP $\geq$130 mmHg in 2005. Medication adherence and appropriate CVD treatment intensification were assessed using pharmacy data. Probit models assessed the adjusted marginal effects of PCP gender on control, adherence, and treatment intensification. Intensification was defined as any one of the following three occurrences: (1) an increase in the number of drug classes, (2) an increase in the daily dosage of at least one ongoing drug class, or (3) switch to a medication in a different drug class.

Of the 1,750 PCPs in the study, 43% were female. Male patients were more likely to have a male PCP (73%), while female patients were equally likely to have a male or female PCP. Female PCPs were more likely than their male counterparts to intensify therapy for hyperlipidemia and hypertension. Moreover, female patients of female PCPs had the highest adjusted rates of HbA1c control of the four patient–physician gender dyads (70% vs. 66–68%, $P < 0.01$). These findings were not altered when the authors, to verify the robustness of the results, reanalyzed the data using different risk factor cutoff points (HbA1c <7%, LDL cholesterol <130 mg/dl [3.4 mmol/l], SBP <140 mmHg).

2.4 Pain Management

In a study examining the decisions about pain management, 111 PCPs (61 male, 50 female) in the Northeast United States were asked to treat three hypothetical patients with pain (kidney stone, back pain) or a control condition (sinusitis) (Weisse et al. 2001). Symptom presentation and severity were held constant, but patient gender and race were varied. No overall differences with respect to patient gender or race were found in the decisions to treat or in the maximum prescribed doses. However, for renal colic, male physicians prescribed higher doses of hydrocodone to white vs. black patients (426 vs. 238 mg, $P = 0.003$). This pattern was repeated for persistent kidney stone pain. For persistent back pain, male physicians prescribed higher doses of hydrocodone to males vs. females (406 vs. 201 mg), but female physicians prescribed higher doses to females (327 vs. 163 mg, $P = 0.03$). These results suggest that when treating pain, male and female physicians may react differently to gender and/or racial issues. The reasons for these differences can only be speculative, such as physicians sympathizing with

patients of the same gender or race (in the case of male physicians) or with patients with disadvantaged groups (in the case of female physicians).

A subsequent study from the same authors further investigated the question in a sample of 712 practicing physicians (414 male, 272 female) (Weisse et al. 2003). Medical vignettes were used to vary patient gender and race experimentally while holding symptom presentation constant. Treatment decisions were assessed by calculating the maximum prescribed doses of the narcotic analgesic hydrocodone prescribed for initial pain treatment and for follow-up care. No overall differences by patient gender or race were found in decisions to treat or in maximum prescribed doses. However, for persistent back pain, female physicians prescribed lower doses of hydrocodone, especially to male patients. Physician gender did not predict pain treatment for renal colic in this study. It remains unclear why physician characteristics might influence treatment approaches for one pain-associated condition but not for another.

In a prospective, multicenter, observational study, Safdar et al. (2009) investigated pain management in 842 consecutive patients (56% women) presenting with complaints of moderate to severe pain at 16 US and 3 Canadian hospitals. The authors found that female physicians were more likely to administer analgesics than male physicians (66 vs. 57%, $P = 0.009$). With regard to opioid administration, female physicians were more likely to prescribe opioids to females ($P = 0.006$) while male physicians were more likely to prescribe them to males ($P = 0.05$). This study suggests that physician gender, as opposed to patient gender, may influence pain management. The reasons for this can only be speculative. For example, compassion and communication skills, two characteristics attributed predominantly to female physicians (Cooper-Patrick et al. 1999; Roter and Hall 2004), are considered key to treating pain. Furthermore, whether female physicians are better listeners when evaluating pain and whether they are more aware of the subjective nature of pain complaints remain unclear.

Morphine is a preferred narcotic agent over meperidine (=pethidine) since the latter has a metabolite with a long half-life and is known to have central nervous system adverse effects, especially in elderly patients and patients with renal insufficiency (Forman 1996; Golembiewski 2002). Nonetheless, meperidine is still frequently prescribed despite the proven superiority of morphine. A study investigated determinants of meperidine prescription in hospitalized patients and found, among other parameters, that female physicians were more likely to order meperidine than morphine compared to male physicians (Panda et al. 2004). The reasons for this finding, which barely reached statistical significance, are not apparent.

2.5 Depression

Psychiatric disorders and especially depression seem to be suitable medical areas for investigating the influence of physician gender on care since there is a complex interplay of diagnostic measures, facilitating open exchange with the patient,

listening and speaking, behavioral therapy and psychotherapy, and last but not least prescribing drugs. Female physicians seem to enter into more active physician–patient partnership behaviors (Roter et al. 2002; Skelton and Hobbs 1999) and are more likely to treat those with psychological problems (Bensing et al. 1993). In a study from Australia investigating the management of a first episode of depression by GPs, female GPs are seen as distinctly more caring than male physicians (Parker and Hyett 2009). Moreover, the study investigated specifically whether male GPs are more likely to prescribe or suggest medications as a first-line option for depression and, in turn, less likely to offer or suggest psychological strategies. Male GPs were more likely to prescribe medication, more likely to prescribe medication alone, more likely to seek less information than expected by the patient, and less likely to refer to a specialist (Parker and Hyett 2009). Of those patients prescribed medication, the percentage of those expecting more information to be obtained prior to prescribing was 68% in male and 50% in female physicians ($P = 0.003$). There were distinct physician/patient gender interactions in as far as female patients were more likely to rate female rather than male GPs as caring, and less likely to receive medication from GPs of either gender. The impact of such differential level of care is clearly not trivial, since female patients were slightly less likely to return to a male GP—and particularly less likely if the GP favored medication as the only option (Parker and Hyett 2009).

There are not many studies investigating the psychiatric field. Regarding the association between physician gender and prescription of psychotropic drugs, the results are conflicting. Male physicians prescribed amitriptyline less frequently to males than females, while female physicians prescribed it in equal rates to male and female patients in a Canadian study (Rosser 1981). A Swedish study found that female physicians are less inclined to prescribe benzodiazepines than male physicians (Jarbrink et al. 1999). However, in a Swiss study there was no significant difference between male and female physicians' rates of prescribing benzodiazepines (Morabia et al. 1992). Guldbrandsen et al. (1998), on the other hand, concluded that female physicians prescribe a psychotropic drug three times as frequently as male physicians when influenced by a patient's social problems.

2.6 *Other Medical Conditions and Facts*

In infectious diseases in pediatrics, there was no association found between physician gender and prescription pattern of *antibiotics* (high vs. low prescribers) for colds in children (Mainous et al. 1998).

Male physicians have been shown to perform more *injections* compared to their female counterparts in a study involving 644 clinics in Korea (Hwang et al. 2007).

In Italy, female GPs have been found to be more likely to write *prescriptions* and to have higher average *drug expenditure* than male GPs (Orzella et al. 2010).

Moreover, female physicians have been shown to treat patients with *osteoporosis* according to recommended guidelines more often compared to male physicians

(Kirigaya et al. 2011; Solomon et al. 2004). In a structured review of the literature on predictors of screening and treatment of osteoporosis, it was found that male physicians were less likely to test patients using bone mineral density (BMD) scans (Morris et al. 2004b). The authors had examined 28 studies of which 17 analyzed patient and/or physician correlates of therapy, but only 11 studies had conducted multivariable analyses. Most of the studies combined any prescription or nonprescription medications for osteoporosis as evidence of treatment. Although male patient gender was consistently associated with an increased risk of not receiving osteoporosis treatment, there was no clear indication that male physician gender had an influence on osteoporosis treatment due to a lack of the respective primary data. However, male physician gender was associated with an increased risk of not obtaining BMD tests. Moreover, in several studies, the proportion of patients receiving treatment was higher among patients with lower BMD, suggesting that physicians respond to BMD test results. The authors concluded that quality improvement efforts need to be focused mainly on male patients, male physicians, and generalist physicians (Morris et al. 2004b).

Regarding treatment practices in *obesity*, female physicians were 1.80 times (95% CI 1.08–2.99, $P = 0.024$) more likely than male physicians to recommend one or more supervised diet programs (Warner et al. 2008). Interestingly, physicians who were themselves overweight or obese were 2.97 times (95% CI 1.03–8.52, $P = 0.043$) more likely to recommend nonprescription diet aids, which shows that gender is not the only confounder that has to be considered when investigating physician characteristics.

2.7 *Overall Care*

There is evidence from large-scale epidemiologic studies that female physicians deliver an overall better care than their male counterparts, which obviously comprises drug therapy. Reid et al. (2010) examined in a large sample of Massachusetts physicians the relationship between physician characteristics and performance on a broad range of quality measures. The authors calculated overall performance scores on 124 quality measures from RAND's quality assessment (QA) tools for each of 10,408 Massachusetts physicians using claims generated by 1.13 million adult patients. The claims-based QA tools measures included 124 indicators of quality of care for 22 acute and chronic conditions, as well as preventive care. Specifically, the conditions examined were asthma, atrial fibrillation, benign prostatic hyperplasia, breast cancer, cerebrovascular disease, COPD, colorectal cancer, CHF, CHD, depression, diabetes mellitus, headache, hip fracture, hyperlipidemia, hypertension, hysterectomy, low back pain, pneumonia, prenatal and preventive care, senile cataract, sexually transmitted diseases, and urinary tract infections. Each physician's composite performance score was created by dividing the number of instances in which recommended care was delivered by the number of instances in which patients were eligible for such care and were assigned to that

physician (overall score method) (Reeves et al. 2007). The patients were continuously enrolled in 1 of 4 Massachusetts commercial health plans from 2004 to 2005. Physician characteristics were obtained from the Massachusetts Board of Registration in Medicine. Associations between physician characteristics and overall performance scores were assessed using multivariate linear regression. The mean overall performance score was 62.5% (5th to 95th percentile range, 48.2–74.9%). Three physician characteristics were independently associated with significantly higher overall performance: female gender (1.6% points higher than male gender; $P < 0.001$), board certification (3.3% points higher than noncertified; $P < 0.001$), and graduation from a domestic medical school (1.0% points higher than international; $P < 0.001$). Moreover, using separate regression models for male- and female-specific measures, the authors found that female physicians had higher performance scores than male physicians both on female-specific (4.4% points higher; $P < 0.001$) and male-specific measures (5.2% points higher; $P = 0.22$). The latter difference was, however, not statistically significant. It has to be pointed out, however, that the results of this study considered overall performance scores with a large number of sub-items and did not explicitly specify aspects of drug therapy, although it is safe to assume that quality of drug treatment is part of the overall performance.

2.8 Dealing with Public and Industrial Drug Information

In many health systems, various efforts are taken to promote the rational use of drugs based on evidence-based principles of drug therapy on all levels of the health care system. This is mainly and best achieved by objective and independent education and information about drugs. However, the pharmaceutical industry sponsors and thus influences drug information to a large extent. There has been much debate on how the pharmaceutical industry influences physicians, assessment agencies, and other stakeholders in the health care systems (Avorn 2007; Lexchin et al. 2003). In 2005, the House of Commons has published its report about the influence of the pharmaceutical industry on all areas of health systems and has stated its concern regarding the biased influence of the pharmaceutical industry which may act against public interest (House of Commons Health Committee 2005). Physicians mostly perceive collaboration with the industry as appropriate (Ross et al. 2009). It has been shown though that there are differences between physician characteristics (specialty, level of training, etc.) and their attitudes towards the pharmaceutical industry (Korenstein et al. 2010). It is an interesting question whether in the approach towards the pharmaceutical industry physician gender may also play a role.

A study from Sweden investigated GPs from primary health care centers and asked their opinion on public- and industry-provided drug information (Skoglund et al. 2011). Interestingly, male physicians were found to be more oriented towards industry-provided drug information compared to female physicians, the latter

valuing information from public authorities to a much greater extent than their male counterparts. Older physicians, the ones with longer work experience and the ones working in the private sector, were also more positive towards industry-provided information. The study was, however, small and its results need to be confirmed by others.

3 Possible Mechanisms and Explanations for Physician Gender Differences

If the described differences in drug therapy between male and female physicians are valid, they could reflect various processes. It could be a "men from Mars, women from Venus" scenario, in which the female doctors wish to spend more time in discussion versus male physicians "cutting to the chase" and making rapid technical decisions, with the female physicians viewed as more caring in consequence (Parker and Hyett 2009). Before we discuss if and how the process of prescribing drugs can be evaluated separately from the whole medical treatment process, the question arises what the proposed explanations for the potential differences may be?

3.1 Differences in Communication Style

One factor may be the physicians' communication skills which have been linked to a variety of positive outcomes, such as patient satisfaction, levels of adherence to treatment, and better health status in general. Within this context, gender has stimulated a great deal of interest as a possible source of variation in the interpersonal aspects of medical practice (Roter et al. 2002). It has been suggested, for example, that female physicians may use different communication styles (e.g., display more partnership and psychosocial orientation, positivity, empathy, focus on feelings, good listening, encouragement, and expressions of respect or praise) that contribute to improved quality of care (Cooper-Patrick et al. 1999; Hall et al. 1994; Mechanic et al. 2001; Roter et al. 1991, 2002).

It has also been shown that female physicians use participatory decision making more often (Cooper-Patrick et al. 1999) and this practice has been found to improve, for example, diabetes outcomes (Kaplan et al. 1989). Another suggestion was that female physicians may focus more on preventive therapies (Bertakis et al. 1995; Boerma and Brink-Muinen 2000; Brody et al. 2000; Ewing et al. 1999; Fang et al. 2004; Frank and Harvey 1996; Gardner et al. 2008; Hall et al. 1990; Lurie et al. 1993, 1997; Maheux et al. 1990; Markova et al. 2011; McKinlay et al. 2002; Morris et al. 2004a, b), while male physicians seem to spend more time with history taking, physical examination, and discussing treatment (Tabenkin et al. 2010). Some (Franks and Bertakis 2003; Mechanic et al. 2001; Meeuwesen et al. 1991;

Roter et al. 2002) but not all (Bertakis et al. 1995; Bertakis et al. 2003) studies have shown that female physicians spend more time with patients than male physicians. A systematic review concluded that medical visits of female physicians last on average 10% longer than the ones of male physicians (Roter and Hall 2004) and that patients of female physicians spoke more overall and seem to talk differently to female physicians, disclosing more biomedical and psychosocial information (Hall and Roter 2002), all factors that may improve quality of care. Moreover, female physicians are more likely to listen to patients without interruptions (Rhoades et al. 2001). In addition, the patients' social problems have a significant influence on management in general practice (Gulbrandsen et al. 1998). It has been shown that the doctor's age, gender, and time spent in their present practice were the only variables related to doctor or practice (as opposed to patient-related variables) that correlated with the influence of social problems on management (Gulbrandsen et al. 1998). In all, female physicians are more likely than their male counterparts to offer a patient-centered communication (Levinson and Lurie 2004; Roter et al. 2002) and the latter has been shown to improve outcomes of care (Greenfield et al. 1985; Kaplan et al. 1989; Mead and Bower 2002; Stewart et al. 2000; Stewart 1995), Therefore, physician gender may be associated with the quality of care provided.

Crude associations of physician gender and isolated quality parameters of medical care (such as prescribing a specific drug more or less often) are certainly an oversimplification of what is of real interest for patient care. Even if differences exist in the frequency a specific drug is being prescribed, the question remains open if less or more of this drug is better for the patient. Intermediate or endpoint outcomes should be available. If pharmacologic and non-pharmacologic therapies are combined and endpoints were available, even then the question whether less or more drug prescription is better would remain open. For example, if hemoglobin-A1c reduction is the outcome of interest and one physician group attains the goal by better implementing the outcome by dietary measures and another one by drug therapy, it would be difficult to conclusively decide which of the two approaches is the best.

In addition, studies investigating physician level characteristics (such as gender) should adjust for other physician-level characteristics such as years of experience, performance quality measures, board certification, age, geographic location, malpractice claims and disciplinary actions, hospital or practice workplace, and many more parameters. In other words, useful studies must apply complex multivariable statistics, thus avoiding potentially confounded results regarding the effects of physician gender on (drug) treatment.

3.2 Influence of Gender Dyads

A gender dyad is defined as one out of four specific gender constellations, e.g., female patient/female physician. In some areas such as gynecology, there are only two dyads possible. It is obvious that in certain areas of medicine the specific gender

dyad is of crucial importance (Bensing et al. 1993; Hall et al. 1994). Studies investigating physician–patient communication are crucial in understanding how patients participate in treatment decisions and how they self-manage their conditions. Although the influence of gender on this communication is relatively well studied, the impact of gender dyads on physician–patient communication is not well characterized. A systematic review (Sandhu et al. 2009) concluded that, even in the presence of a small evidence base, some differences were evident in communication style, nonverbal communication, exhibition of power and status, and length of communication. The authors believe that the conditions with more effective communication improve consultation outcomes and their findings show that these conditions are more likely to exist in some dyads than in others (Sandhu et al. 2009).

A study investigating gender dyads in a nationally representative sample from the US National Ambulatory Medical Care Survey examining encounters of 41,292 adult patients found that physician–patient gender concordance is a key determinant of the pattern of these encounters (Franks and Bertakis 2003). One of the most interesting findings was that female physicians had significantly longer visits with their female patients than any other physician–patient dyad. The second long visits occurred between male physicians and their male patients. But, when pelvic and breast examinations were excluded, there was no overall physician gender effect on visit length (Franks and Bertakis 2003). It would therefore appear that the visit length is associated with the performance of gender-specific (breast and pelvic) physical examinations. Female patients may preferentially see female physicians and these patients may make their selection based on a preference for a physician of the same gender to perform their breast and pelvic examinations. The data suggest the assumption that a similar selection process may occur when complex pharmacotherapies such as in diabetes or pain management have to be performed, but there is no proof of such a theory. Another explanation may be that as gender is a substantial component of social status, same-gender physician–patient dyads may be closer in social status (greater status congruence) than opposite-gender dyads (Waller 1988) and therefore facilitate effective consultation in pharmacotherapy. A study investigating the aspects of drug therapy in patients with diabetes in the Kaiser Permanente Northern California Program ($N = 157{,}457$ patients) found only week associations of physician–patient gender concordance in the control of cardiovascular risk factors, but the female patients of female physicians, for example, had the highest adjusted rates of HbA1c control among the four gender dyads (Schmittdiel et al. 2009). Similar results had been shown before in a study from Germany (Berthold et al. 2008).

4 Conclusions and Clinical Implications

Altogether, the evidence available addressing the question whether physician gender plays a role in the drug therapy provided is relatively small. Even though the question can therefore not be answered conclusively, the existing data do not

suggest a major difference in the patterns of medication prescription between male and female physicians. If they exist, they are small in magnitude and their clinical relevance is uncertain. There is until now in no area of medicine evidence to suggest that a patient will consistently receive higher quality of drug therapy by switching to a physician of a specific gender. However, there is evidence strongly supporting the notion that female physicians may provide an overall more comprehensive care for patients in many medical conditions. This apparently contradictory finding may be at least partially explained by the fact that patient-centered communication between physicians and patients, a characteristic of female physicians (Levinson and Lurie 2004), can enhance outcomes of care, such as patient adherence to treatment and outcomes in chronic disease (Greenfield et al. 1985; Parker and Hyett 2009; Stewart 1995). In turn, since in the long-term treatment of complex chronic diseases drug therapy is just one component of the treatment plan, better drug therapy may be part of the overall better care, but it is methodologically difficult to investigate its influence independent of the influence of non-pharmacologic measures.

While the reasons for the possible differences in overall care are still unclear, it has been postulated, and seems in our opinion rather plausible, that the practice and communication styles of female physicians, such as spending more time with a patient, hearing and listening more effectively, and including more preventive measures, may result in more efficient clinical encounters (Britt et al. 2005; Phillips and Austin 2009). A warm interpersonal relationship between patients and physician has been shown to independently add a substantial therapeutic benefit to a therapeutic regimen (Kaptchuk et al. 2008). Not surprisingly, it has been suggested that even placebo effects may be more likely seen in patients of female physicians (Enck et al. 2005), a potential explanation being that the benefit provided by placebo "drugs" is derived from the positive effects of the clinical encounter (Brody and Miller 2011; Colloca et al. 2004). The benefit of good communication on patient care and outcomes is unequivocal (Levinson et al. 2010) and steps to improve patient–physician communication should be identified and implemented (Levinson and Pizzo 2011; Olson and Windish 2010).

Further research with a primary focus on the effects of physician gender effects on clinical outcomes is needed. Since randomized blinded studies do not seem feasible, observational studies should consider all reasonable efforts to adjust for as many potential confounders as possible. If in fact different therapeutic outcomes based on the gender of the treating physician could be established, the responsible behaviors should be identified and the relevant skills taught to all physicians, irrespective of their gender (Arouni and Rich 2003).

Take Home Messages

- Women receive lesser quality drug therapy in many medical areas but the influence of physician gender has not been well investigated.
- Female physicians provide better overall therapy and better drug therapy in some medical areas.

- Better communication styles of female physicians may contribute to better drug therapy and better overall outcomes.
- The current literature is limited by inconsistent inclusion of common covariates and a lack of multivariable analyses and thus physician gender as an independent parameter influencing drug therapy is difficult to establish. Especially, the influence of gender dyads on quality of drug therapy has been investigated only in a limited numbers of studies.
- It is methodologically difficult to separate the influence of the quality of pharmacologic therapy from non-pharmacologic therapies in the overall quality of care.
- There is until now in no area of medicine evidence to suggest that a patient will consistently receive higher quality of drug therapy by switching to a physician of a specific gender.

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Part III
Sex Differences in Different Therapeutic Areas

Sex and Gender Differences in Cardiovascular Drug Therapy

Ute Seeland and Vera Regitz-Zagrosek

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Abstract This chapter outlines sex differences in pharmacokinetics and pharmacodynamics of the most frequently used drugs in cardiovascular diseases, e.g., coronary artery disease, hypertension, heart failure. Retrospective analysis of

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previously published drug trials revealed marked sex differences in efficacy and adverse effects in a number of cardiovascular drugs. This includes a higher mortality among women taking digoxin for heart failure, more torsade de pointes arrhythmia in QT prolonging drugs and more cough with ACE inhibitors. Trends towards a greater benefit for women and/or female animals have been observed in some studies for endothelin receptor antagonists, the calcium channel blocker amlodipine, the ACE-inhibitor ramipril and the aldosterone antagonist eplerenone. However, reproduction of these results in independent studies and solid statistical evidence is still lacking. Some drugs require a particularly careful dose adaptation in women: the beta-blocker metoprolol, the calcium channel blocker verapamil, loop-, and thiazide diuretics. In conclusion, sex differences in pharmacokinetics and pharmacodynamics have to be taken into account for cardiovascular drug therapy in women.

Keywords ACE inhibitors • Angiotensin receptor blockers • Beta-blockers • Calcium channel blockers • Endothelin receptor antagonists • Diuretics • Sex differences

Abbreviations

AT1-R	Angiotensin II type 1 receptor
AT2-R	Angiotensin II type 2 receptor
ACE2	Angiotensin converting enzyme-2
Ang II	Angiotensin II

1 Introduction

Gender differences have been observed in the clinical effects of a number of major cardiovascular drugs and drug families. In general, women are at greater risk than men of experiencing an adverse reaction to medication. In an analysis of 48 cohort studies in Great Britain, Martin and colleagues found a 1.5- to 1.7-fold higher risk for adverse events in women compared to age-matched men (Martin et al. 1998). In the cardiovascular area, more adverse effects in women are consistently reported for QT prolonging drugs, for ACE inhibitors and thrombolytics and sex differences in efficacy have been described for others, as digoxin. Parts of the differences may be related to pharmacokinetics. Women and men differ in body composition, weight, mechanisms of absorption and drug distribution, metabolic enzymes and routes of excretion. In addition, true sex differences in pharmacodynamics are also well described. Ion channel composition of different organs, kidney and heart, differs in women and men. Another part of the difference may be due to the fact that drug development is mainly done in young male animals of a well-selected genetic background. Old males are neglected, as well as pre- and postmenopausal

females. Male and female rodents are usually kept on a diet rich in phytoestrogens that may interfere with drug effects and they are kept in a pathogen free environment that may preclude the detection of interactions of drug effects with an activated immune system. These reductionist approaches impair the development of optimal drugs for subgroups, for elderly women and men, and the successful translation of new drugs to the clinics. Phase 1 and 2 studies also include mainly young men, preventing systematic development of adequate doses for women and the elderly [see Raz and Miller (2012)]. These shortcomes in drug development may contribute to the observed sex and gender differences in drug effects, in addition to the biological differences in pharmacokinetics and pharmacodynamics that are reviewed below.

2 Mechanisms of Sex/Gender Differences in Cardiovascular Drug Response

2.1 Role of Genetic Polymorphisms

Genetic polymorphisms may modify drug response. This has been best documented for some anticancer drugs (Deenen et al. 2011). In the cardiovascular field, genetic polymorphisms have been associated with the response to ACE inhibitors, to the metabolism of beta-blockers and calcium-channel blockers ("poor metabolizers"). Some autosomal gene polymorphisms are associated with cardiovascular phenotypes in a sex-specific manner and the resulting sex differences in phenotypes may lead to sex differences in drug responses. Such genes are related to the renin–angiotensin system (see below), lipid metabolism, homocysteine and folate metabolism and to the phenotypes of blood pressure, longevity, ischemic heart disease, cardiovascular mortality and depression (van Suylen et al. 1999; Linnebank et al. 2005; Kokubo et al. 2005).

Genes that are relevant in the cardiovascular system and that are located on the X-chromosome are also candidates for causing sex/gender differences. Mutations in these genes or functionally relevant polymorphisms might be better compensated in women, who have two copies of the gene, than in men. In addition about 15 % of the X-chromosomal genes are assumed to escape X-inactivation, which could result in higher gene doses in women (Carrel and Willard 2005). Genes for angiotensin converting enzyme-2 (ACE2) (Tipnis et al. 2000) and angiotensin II receptor (AT2R) are located on the X-chromosome. The AT2R has been shown to modulate left-ventricular hypertrophy in women with hypertrophic cardiomyopathy independently of the circulating RAS, but not in men. In women left ventricular mass decreased with the number of C alleles of the AT2R gene A/C(3123) polymorphism suggesting an antihypertrophic effect of AT2 in women (Deinum et al. 2001).

In an impressive number of recently reviewed transgenic animal models, a more severe cardiovascular phenotype developed in male than in female animals, and the

progression of heart failure and death from heart failure occurred earlier in the male animals (Leinwand 2003; Du 2004). Reasons for these sex differences are multiple but also dependent on food intake. In particular, phytoestrogens seem to be relevant (Luczak et al. 2011; Bhupathy et al. 2010) and can modify cardiovascular phenotypes and drug responses in a sex-specific manner. Frequently, the more severe cardiovascular phenotype in male animals or in ovariectomized females can be rescued by the administration of estrogens (Xin et al. 2002). Genes involved in these pathways are also candidates for causing sex/gender differences.

2.2 Role of Sex Hormones

Estrogens interact with a large number of cardiovascular drugs [see Spoletini et al. (2012)]. Variation of estrogens and other hormones during the menstrual cycle may modify the response to other drugs. Among others, estrogens interfere with angiotensinogen synthesis in the liver and angiotensin I receptor (AT1R) expression in the myocardium. Furthermore, endogenous estrogens may increase the expression of the angiotensin II receptor (AT2R) in the myocardium (Regitz-Zagrosek et al. 2004).

Progesterones expressed in the cardiovascular system, in the myocardium and great vessels, interact partially synergistically/partially antagonistically with estrogens (Edwards 2005). The effect of progesterone on the action of estrogens in the development of atherosclerosis was studied in ovariectomized rabbits treated with estradiol, progesterone or combined sex hormones. Progesterone was dose dependently able to inhibit the beneficial effects of estrogens in experimental atherosclerosis, suggesting that progesterone exerts a direct inhibitory effect on the athero-protective action of estrogens (Hanke et al. 1996). Furthermore, treatment with progesterone significantly ($P < 0.05$) reduced myocardial infarct area, lipid peroxidation level, activity of myeloperoxidase and inhibited serum CK activity and the incidences of ventricular tachycardia in myocardial ischemia/reperfusion (I/R) injured female rats. These cardioprotective effects have not been observed in ischemic male rats. The protective effect could be mediated by the interaction with endogenous estrogens.

Testosterone is a precursor for estrogen biosynthesis. Therefore, its effects can be mediated by testosterone or directly by estrogens. Sometimes these possibilities are difficult to differentiate. Testosterone affects the cardiovascular system through both genomic and non-genomic mechanisms. In human observational studies, men with lower testosterone levels tended to have a higher incidence of cardiovascular diseases. Supplementation of testosterone has been used for improving performance in patients with heart failure and reducing exertional angina threshold. However, results were not convincing. Adverse effects occur mostly with supraphysiological doses, e.g., in athletes abusing androgens (Kaushik et al. 2010).

Sex differences in drug metabolism are due in part to the female-predominant expression of CYP3A4, the most important P450 catalyst of drug metabolism in

human liver. The sexually dimorphic expression of P450s and other liver-expressed genes is regulated by the temporal pattern of plasma growth hormone (GH) release by the pituitary gland, which shows significant sex differences. These differences are most pronounced in rats and mice, where plasma GH profiles are highly pulsatile (intermittent) in male animals versus more frequent (nearly continuous) in female animals (Waxman and Holloway 2009).

Little information is available regarding the effects of the human menstrual cycle or the rat estrous cycle on expression and activity of the CYP-dependent enzyme system. Very recently, Lee et al. determined the expression and activity of CYP-dependent drug-metabolizing enzymes in the liver and ovary during the rat estrous cycle. The results indicated that hepatic and ovarian expression and activity of CYP isoforms, cytochrome b5, and NADPH-dependent CYP reductase were not different between diestrus and proestrus, although serum estradiol concentrations were markedly increased in the proestrus phase. This suggests that the CYP 450-dependent system is not sensitive to changes in the estrous cycle (Lee et al. 2012).

3 Sex/Gender Differences in Cardiovascular Drug Families

3.1 Digoxin

In 1997, the Digitalis Investigation Group (DIG) reported the positive results of a randomized trial evaluating the efficiency of digoxin therapy for patients with heart failure, however, without running a sex-specific analysis (DIG 1997). Thereafter, guidelines strongly endorsed the use of digoxin for these patients. However, in a post hoc subgroup analysis, digoxin was associated with a significantly higher risk of death among women taking digoxin compared with those taking placebo, an effect that was not observed in men (Rathore et al. 2002). Dose-related effects, as well as an interaction with hormone replacement therapy, were discussed as potential explanations for this unanticipated result. Higher serum digoxin concentrations were associated with increased crude all-cause mortality in men (Rathore et al. 2003). In women, a similar trend was observed, but did not reach significance because the number of women participating in the study was too small. Subsequently, higher drug serum levels due to reduced distribution volume and lower drug elimination due to reduced glomerular filtration rate (GFR) (Yukawa et al. 1997) leading to drug concentrations in the upper normal range were held responsible for the unfavorable survival effects reported in women.

In the absence of definitive evidence, digoxin plasma concentration should be below 0.8 ng per ml (Rathore et al. 2003). These data reinforce the possibilities of gender-related effects and therefore underscore the need to perform gender-specific analysis and to include sufficient numbers of women in trials (Regitz-Zagrosek 2006).

3.2 Endothelin Receptor Antagonists

3.2.1 Sex Differences in the Endothelin System

Endothelin is synthesized in the endothelium, the heart, brain, lung, kidney, and some circulating cells. Since 1988 endothelin-1 (ET-1) is known as an endothelial cell-derived contracting factor. ET-1 is one of the most potent vasoconstrictors and became a target for cardiovascular research with the aim to develop a new drug for the treatment of hypertension in humans. Injection of ET-1 in rats (Mortensen and Fink 1990) as well as in the model of human forearm blood flow (Haynes et al. 1995) showed vasoconstrictor effects with increase in blood pressure and total peripheral resistance in healthy volunteers.

Three endothelin isoforms, such as ET-1, ET-2, and ET-3, are expressed in humans. Endothelial ET-1 acts in an autocrine/paracrine manner to produce its physiological effects on vessels (Iglarz and Clozel 2010). Endothelin concentrations acting on the underlying VSMCs may be several orders of magnitude higher than it is in plasma (Benigni and Remuzzi 1999). Signaling pathways activated by ET-1 and sensitizing the contractile proteins of vascular smooth muscle cells to calcium during contraction are under further investigation (Wirth et al. 2008; Rautureau and Schiffrin 2012). ET-1 is cleared from the circulation by endothelial ETB receptors (Kelland et al. 2010). ET is hydrophilic and unable to cross the plasma membrane, therefore it must bind to specific cell surface receptors.

The type A (ETA) receptor has a higher affinity for ET-1 and ET-2 and less for ET-3. Type B (ETB) receptor affinity is not different to the isoforms. ETA receptors are expressed in vascular smooth muscle cells (VSMCs) and coupled to trimeric G proteins (Galpha q/11 or G alpha 12/13) to induce vasoconstriction and cell proliferation. ETB receptors are found on endothelial cells. The ETA receptor is the predominant receptor subtype in the human coronary vasculature (Saetrum Opgaard et al. 1994). The ability to test the specific actions of ETA and ETB is limited because selective agonists are only available for ETB, although antagonists have been developed for both receptors (Pollock 2010).

Sex differences have been shown concerning the function of human ETB receptors. Forearm skin blood flow was measured in male and female volunteers after perfusion of an ETB receptor antagonist and sodium nitroprusside. In men, ETB receptors mediated tonic vasoconstriction but in women tonic vasodilatation. Thus, the contribution of ETB receptors to resting cutaneous vascular tone differs between men and women (Kellogg et al. 2001). Stauffer et al. assessed forearm blood flow in response to intra-arterial infusions of endothelin-1 (ET-1), or a selective ETA or ETB receptor antagonist by venous occlusion plethysmography in 21 women and 25 men. Middle-aged and older men were under greater ETA receptor-mediated vasoconstrictor tone than age-matched women. There was no difference in the vasoconstrictor response to ET-1 between the sexes (Stauffer et al. 2010). Sex difference in vasoconstrictor tone could be a mechanism contributing to the higher prevalence of cardiovascular diseases in middle-aged and older men than

women. Furthermore, the contractile response to ET-1 appears to be modulated by the relative density and distribution of ETA and ETB receptors. Analysis of binding data with endothelium-intact saphenous vein samples obtained from patients undergoing coronary artery bypass graft surgery showed lower endothelin binding capacities of ETA and ETB receptors in women compared to men. In addition, ET-1 induced contractions were twofold higher in men than in women (Ergul et al. 1998). These results indicate sex differences in the ratio and density of ET receptors, which might be an important factor in the regulation of the contractile response of vessels and explain greater vasoconstrictor tone in men.

Further evidence suggests that more differences between the sexes in the endothelin system are modulated by sex hormones: Ovarian hormones reduce ET-1 production and effects. 17ß-estradiol attenuates ET-1 induced coronary artery constriction both in vivo (Lamping and Nuno 1996) and in vitro (Sudhir et al. 1997). Plasma ET-1 levels are higher in men than in age-matched women and testosterone administration in transsexual female patients increases plasma ET-1 levels (Polderman et al. 1993). Estrogen replacement therapy and pregnancy, where estrogens levels are high, decrease ET-1 plasma levels (Best et al. 1998).

Genetic hypertensive animal models treated with ETA- or ETA/ETB receptor antagonists and normalization of blood pressure might suggest that ET participates in the pathophysiology of hypertension. However, blood pressure of ETA knockout mice is high and overexpression of ET-1 or human preproET-1 (transgenic mouse models) is associated with normal blood pressure. In animal models, ET-1 is only responsible for the hypertrophic arterial remodeling when animals are exposed to high salt intake like Dahl salt-sensitive rats and deoxycorticosterone salt-treated rats and mice (Schiffrin 2005). ETB receptor-deficient rats (spotting-lethal rats) are marked by severe salt-sensitive hypertension (Gariepy et al. 2000).

The impact of gene polymorphisms on blood pressure and on the change in blood pressure in humans during antihypertensive treatment has been studied by Hallberg et al. (2004). Preproendothelin-1 mRNA is expressed by human cardiomyocytes and interstitial cells that synthesize and secrete mature ET-1 (Plumpton et al. 1996). ETA and ETB receptors are present in the human myocardium (Molenaar et al. 1993). A G5665T gene polymorphism of preproendothelin-1 has been shown to be associated with higher blood pressure in overweight patients. Patients carrying the T-allele have higher blood pressure than those with the G/G genotype (Iglarz et al. 2002; Tiret et al. 1999). The study of Hallberg et al. suggests a sex-specific relationship between the G5665T preproET-1 polymorphism and the degree of reduction of systolic blood pressure during antihypertensive treatment with the AT1-receptor antagonist irbesartan and the ß1-blocker atenolol. The authors determined the preproET-1 genotype in 102 patients with essential hypertension and LV hypertrophy, randomized to treatment with either irbesartan or atenolol. Carriers of the T-allele responded on average with a more than twofold greater reduction of systolic blood pressure than those with the G/G genotype. This genetic difference was only seen in men. In women no statistically significant differences in blood pressure change have been observed between G/G genotypes and carriers of the T-allele.

3.2.2 Endothelin Receptor (ET) A and ETB Antagonists

The first dual ETA- and ETB receptor antagonist, bosentan, could decrease blood pressure in patients with essential hypertension. However, bosentan as well as other ET receptor antagonists was not successful for the treatment of hypertension or heart failure. The Research on Endothelin Antagonism in Chronic Heart Failure (REACH-1) trial (Lechat et al. 1998) and the Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure (ENABLE) study (Coletta et al. 2002) were terminated prematurely because of early worsening of heart failure, an increase in concentrations of liver enzymes and lack of improvement in clinical outcomes. However, endothelin receptor antagonists were useful treatment of portopulmonary hypertension (Kahler et al. 2011). In addition, bosentan, sitaxsentan, atrasentan, and clazosentan are indicated for the management of pulmonary artery hypertension, a condition that predominantly affects women. Clazosentan has been developed initially for treatment of subarachnoid hemorrhage. After infusion of clazosentan plasma levels have been 18 % higher in females compared with males, mainly attributable to a difference in clearance (van Giersbergen et al. 2007).

3.3 Beta-Blockers

Beta-blockers are cornerstones in the treatment of heart failure. Women have so far been a minority in clinical trials testing beta-blockers for this indication, representing 20–30 % in the first major trials (Jochmann et al. 2005). Two major trials, the MERIT-HF (metoprolol CR/XL) study and the COPERNICUS trial, failed to find a beneficial effect on mortality in women (MERIT-HF 1999; Packer et al. 1996). In a detailed gender-specific analysis for the CIBIS II study, women profited significantly from treatment with bisoprolol (Simon et al. 2001; CIBIS-II 1999), which had a greater unadjusted effect on all-cause mortality in women than in men. Pooling of mortality results from MERIT-HF, CIBIS II, and COPERNICUS showed survival benefits in both women and men (Ghali et al. 2002). The lack of evidence in some large beta-blocker studies is therefore probably due to the under-representation of women in the trials.

Sex-specific differences in the pharmacokinetics of beta-blockers lead to greater drug exposure in women and frequent reports of drug toxicity in females (Luzier et al. 1999). Drug-metabolizing hepatic enzymes like cytochromes and P-glycoproteins are differently expressed in women and men. CYP2D6 is particularly relevant in the metabolism of beta blockers. Metoprolol and propranolol are primarily metabolized by CYP2D6 which has a lower activity in women (Labbe et al. 2000; Tanaka and Hisawa 1999). In addition, women display lower distribution volumes for metoprolol, leading to increased plasma concentrations in women compared to men. Propranolol reaches plasma levels that are up to 80 % higher in

women compared to men. Moreover, oral contraceptives can interact with metoprolol and further increase its plasma levels (Kendall et al. 1982). Accordingly, women suffer from adverse effects when beta blocker dosage is not adequately adjusted (Walle et al. 1994).

Evidence exists that deficiency of sex hormones like estrogens up-regulates myocardial ß1-receptors. Estrogen supplementation can prevent this. Consistently endogenous estrogens reduce cardiac sympathetic response to catecholamines.

Metoprolol is administered as a racemic mixture *S*-metoprolol, that possesses the predominant ß1 blocking actions of this drug. Women have a greater exposure corresponding to a lower clearance of metoprolol enantiomers compared to men. This resulted in a greater reduction in exercise heart rate in women (Luzier et al. 1999). Approximately 85 % of an administered dose of metoprolol is metabolized into three major metabolites: α-hydroxymetoprolol (10 % of the administered dose), *O*-desmethylmetoprolol, and deaminated metoprolol (Lennard 1985). The α-hydroxylation pathway is exclusively mediated by CYP2D6 (Lennard et al. 1983). Administration of diphenhydramine, a prototype classic antihistamine, results in a greater inhibition of clearance of CYP2D6 substrates, like metoprolol, with a resulting higher risk of pronounced pharmacological and adverse effects in women compared to men (Sharma et al. 2010).

Sex differences in the systemic response to adrenoreceptor antagonists during sympathetic activation have been described in a forearm plethysmography study. Enhanced β(2)-adrenergic stimulation in women leads to attenuation of noradrenaline-mediated vasoconstriction. Isometric forearm contraction increased heart rate and mean arterial pressure in both sexes. The ß-blocking agent esmolol attenuated the rise in mean arterial pressure in men but not in women. This study supports findings of sex differences in adrenergic responsiveness and suggests that it is systemically relevant (Coulson and Cockcroft 2011).

Since the pharmacological actions of ß-blockers are also mediated by blocking effects on Ca(2+)-channels, Na(+)-channels and various native cardiac K(+) channels, sex differences in the response to ß-blocking agents could be due to sex differences in ion channel compositions of the myocardium.

3.4 Calcium-Channel Blockers

Two main types of calcium-channel blockers are the “non-dihydropyridine-type” like verapamil and the “dihydropyridine-type” like amlodipine and nifedipine. Verapamil is a calcium ion influx inhibitor with antiarrhythmic, antianginal and antihypertensive properties (Gupta et al. 1995). This is a drug with a high first-pass metabolism including the intestinal and hepatic drug metabolizing enzymes cytochrome P450, especially CYP3A4. Verapamil is also known as a substrate for the human MDR1 (multidrug-resistance gene 1) gene product P-glycoprotein (P-gp). Women have only one-third to one-half of the hepatic P-gp level of men resulting in increased intrahepatocellular substrate (verapamil) availability and increased

hepatic CYP3A4 metabolism (Meibohm et al. 2002). The active metabolite is norverapamil. After administration of a single 80-mg oral dose verapamil to healthy volunteers the area under the blood concentration–time curve (AUC) for norverapamil to that for verapamil was significantly higher in women than in men (Dadashzadeh et al. 2006). The authors conclude that norverapamil production is a sex-dependent process that is carried out more extensively in women than in men because of a higher activity of CYP3A4 or lower activity of P-gp (Ueno and Sato 2012). Further pharmacokinetic parameters differed by sex like the significantly shorter verapamil half-life (t1/2) and mean residence time in women than men. These data support the finding of faster elimination of oral verapamil in healthy women. Higher activity of CYP3A4 in women compared with men has been reported for different drugs (Wolbold et al. 2003). Cytochrome P450 isoforms in humans show moderate differences in activity for CYP2E1 and CYP1A2 (higher in men than women) but a female predominant expression and activity of the most clinically relevant human isoform CYP3A. Pharmacokinetic studies investigating the influence of sex steroid hormone levels on CYP3A4 activity are mainly done with rodents or in vitro, e.g., progesterone has been shown to increase CYP3A4 activity (Kharasch et al. 1997). These results could be very interesting for explanation of sex differences in drug metabolism but it should be considered that rodent CYP isoforms are different from human isoforms.

Results of previous studies concerning sex differences of verapamil treatment were inconclusive. This could be explained with the complexity of pharmacokinetics because of individual variations, alternative pathways in the metabolism of CYP3A4 substrates, route of drug administration, and differences in competition for transport mechanisms (Ueno and Sato 2012). One point extensively discussed concerning sex-related differences in verapamil metabolism has been the oral versus intravenous route of drug administration. Krecic-Shepard et al. reported 2000 that verapamil oral clearance was faster in men compared with women administered as either a sustained release or a regular release formulation (Krecic-Shepard et al. 2000a, b). Previously, this group and others reported of faster clearance of intravenously administered racemic verapamil, R-verapamil and S-verapamil, in women (Krecic-Shepard et al. 1999; Dilger et al. 1999). As mentioned earlier, Dadashzadeh et al. (2006) demonstrated a faster elimination rate of verapamil in female than men following single oral dose of 80 mg verapamil. It is possible that the sex differences observed could be due to differences in intestinal P-gp activity, which has not been investigated in human beings. Sex differences in CYP3A or P-gp in the gut counterbalance the sex-related effects in the liver. Sex-related dietary or hormonal differences could also play a role (Rosenberg 1991; Lown et al. 1997). Continued attention should be drawn to the role of intestinal factors determining in vivo pharmacokinetics after oral drug dosing. Cummins et al. (2002) determine that drugs that are substrates for both CYP3A4 and P-gp typically had higher clearance values in women, whereas drugs that were metabolized by CYP3A4 but not transported by P-gp did not exhibit sex-related differences. It is obvious that the data obtained from healthy subjects and the findings of sex-related differences in drug metabolism need to be confirmed in patient groups.

It is not clear whether the pharmacokinetic differences among calcium channel blockers have relevant clinical impact. The major hypertension trials with calcium channel blockers like The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), Invention as a Goal in Hypertension Treatment (INSIGHT), Systolic Hypertension in Europe (Syst-Eur) trial, the Swedish Trial in Old Patients with Hypertension-2 (STOP-Hypertension-2) study, and Nordic Diltiazem (NORDIL) study have revealed no evidence for gender differences in outcomes. On the other hand, the data of the Amlodipine Cardiovascular Community Trail (ACCT) trial showed that therapy with amlodipine resulted in more pronounced blood pressure reduction in women than in men. However, this effect depended on whether women used hormone replacement therapy. The Hypertension Optimal Treatment (HOT) study demonstrated a trend toward a decreased rate of myocardial infarction in women with low diastolic blood pressure treated with felodipine.

Amlodipine, a dihydropyridine-type, long-acting calcium channel antagonist, has been extensively assessed in several studies. In contrast to verapamil it has low rates of first-pass metabolism, high bioavailability, is metabolized by several CYP pathways and is not considered to be a P-gp substrate. Kloner et al. (1996) compared amlodipine effects in men and women with mild to moderate hypertension in a prospective study. More women responded with decreased diastolic blood pressure to amlodipine therapy than men. The authors also reported that women had a higher incidence of edema, although this was combined with a greater therapeutic response. Considering the older population, faster clearance of oral administered amlodipine in women compared to men was observed (Kang et al. 2006). These data are similar to that for many other substrates of CYP3A4 due to a greater decrease in CYP3A4 in older men compared with women of the same high age (Greenblatt et al. 2004). The sex differences seem, however, to be small and further evidence is needed to support clinical relevance.

3.5 Angiotensin-Converting Enzyme Inhibitors

Angiotensin-converting enzyme inhibitors are part of evidence-based therapy of heart failure and hypertension (BP). In several multicentre studies, e.g., CONSENSUS I, SAVE, and SOLVD, ACE inhibitors led to much smaller mortality reductions in women compared with men. However, these studies were not powered to detect gender differences and only small percentages of women were included (Regitz-Zagrosek 2006). A meta-analysis including 7,105 heart-failure patients claimed that the effects were comparable in women and men, but detailed data by gender were not included (Garg and Yusuf 1995). Later trials, such as AIRE and HOPE, showed a significant benefit of ACE inhibition in women, especially with regard to the secondary prevention of cardiovascular events in high-risk patients (Regitz-Zagrosek 2006). However, the "Second Australian National Blood Pressure Study" (ACE inhibitors versus diuretics) demonstrated a significant

reduction in cardiovascular events in men, but not in women, despite similar reductions in blood pressure in both sexes (Wing et al. 2003). A recently published study has demonstrated that the use of ACE inhibitors is associated with a significant decrease in overall mortality and cardiovascular events in patients with diastolic heart failure (Wu et al. 2010). Despite 32–42 % women have been included in the study groups detailed data by gender are missing. Pharmacokinetics of captopril and lisinopril exhibited no significant sex-specific differences in healthy volunteers (Massana et al. 1997; Saenz-Campos et al. 1996). However, higher plasma levels of ramipril occurred in women than in men, due to women's lower body weight when taking the same dose of 5 mg (Vree et al. 2003).

Adverse effects of ACE inhibitors, especially a typical dry cough are more frequent in women than in men (Mackay et al. 1999; Gibson 1989; Os et al. 1992). Cough occurs within hours of the first dose or weeks or months after the initiation of the treatment. ACE-inhibitor-induced cough has been related not only to the sex of the patient, but also to tobacco habits, ethnicity, and comorbidities and seems to be dose independent. The role of angiotensin-converting enzyme in the metabolism of kinins, mainly bradykinin, has been proposed as a pathogenic mechanism (Kawakami et al. 1998). Bradykinin has been shown to induce the production of proinflammatory metabolites, such as prostaglandins and nitric oxide, which could promote cough (Trifilieff et al. 1993). Very recently, Mas et al. identified genetic polymorphisms in bradykinin receptors (BDKRB2) and ABO genes, related to ACE levels, being associated with ACE-inhibitor-induced cough. The effect of polymorphisms in ABO is sex specific. These results provide ABO as a good candidate gene for pharmacogenetic studies of ACE-inhibitor-related cough (Mas et al. 2011).

Estrogens elevate angiotensin II (Ang II) plasma levels and reduce via negative feedback ACE and renin activity (Fig. 1). Expression of the angiotensin II type 1 receptor (AT1-receptor) is decreased (Fischer et al. 2002; Harrison-Bernard et al. 2003). Premenopausal women may benefit from an estrogen-induced inhibition of the renin–angiotensin system (RAS). Whether this contributes to the relative protection of premenopausal women from cardiovascular events remains to be determined.

The RAS is a key regulator of blood pressure. Endogenous RAS activity differs between men and women (Zapater et al. 2004). Animal models are potentially useful to examine the mechanisms leading to differential blood pressure responses in males and females. Male Sprague–Dawley rats (Tatchum-Talom et al. 2005) and also male mice (Xue et al. 2005) have a greater pressure increase after chronic infusion with Ang II when compared to female rodents. After gonadectomy this response was abrogated in mice (Xue et al. 2005).

Treatment of SD rats with the ACE inhibitor enalapril reduced blood pressure to a greater extent in female than in male rats (Sartori-Valinotti et al. 2008). Venegas-Pont et al. tested whether female C57BL/6J mice also exhibit a greater blood pressure response to ACE inhibition during chronic Ang II. Male and female

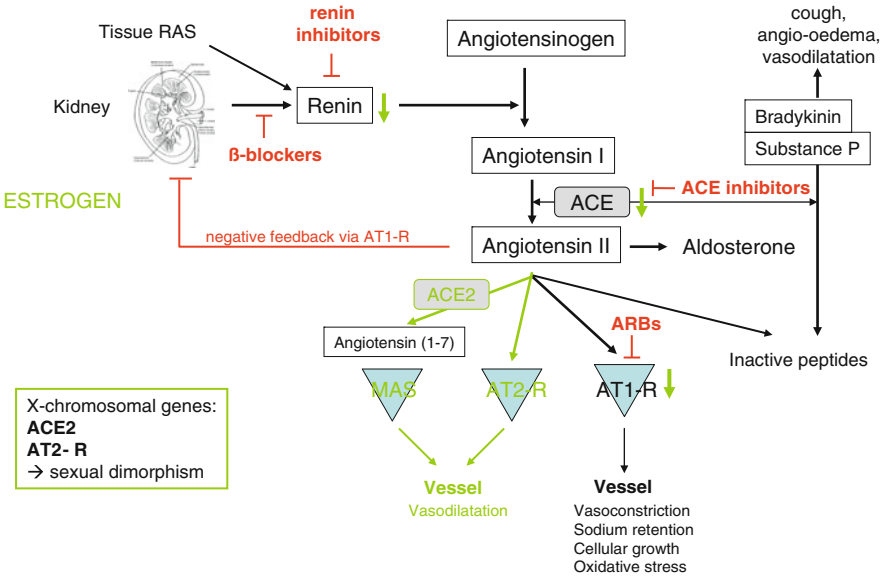

Fig. 1 Estrogens, sex, and RAS—schematic representation of estrogen (E2) effects and sexual dimorphisms (*green*) and effects of interfering drugs (*red*) on the renin–angiotensin system (RAS). Hepatic angiotensinogen synthesis can be regulated by E2. The major peptide angiotensin (Ang) II exerts its main actions by binding to the angiotensin 1-receptor (AT1-R) which is reportedly down-regulated by E2 in some cardiovascular tissues. Some Ang II effects may result from binding to the receptor AT2. Ang II can be further cleaved by angiotensin-converting enzyme 2 (ACE2) into angiotensin-(1–7) which exerts mainly vasodilating effects through its receptor MAS. ACE2- and the AT2-R genes are located at X chromosome and may therefore exhibit higher or more stable expression in women. Polymorphisms of RAS genes are candidates for sexual dimorphic effects of RAS activation. *Dashed lines* refer to experimental data that need further confirmation. *AT1-R* angiotensin II type 1 receptor, *AT2-R* angiotensin II type 2 receptor, *ACE2* angiotensin converting enzyme-2, *ARBs* angiotensin receptor blockers, *MAS* mas receptor

mice, treated with enalapril, were assigned to groups receiving either Ang II or saline for 2 weeks. Blood pressure was higher in male mice than female mice treated with enalapril and Ang II and blood pressure was not different between mice treated with enalapril alone (Venegas-Pont et al. 2010). These experiments show that males exhibit higher blood pressure than females in response to Ang II even during blockade of the endogenous ACE (Venegas-Pont et al. 2010). The mechanisms responsible for the dimorphic blood pressure response are not clear. Another model of Ang II-induced hypertension in rats during ACE inhibition showed that the sex differences were modulated by salt intake. Female rats fed a low-salt diet during ACE inhibition exhibited a greater blood pressure response to Ang II than males. When the same rats were fed a high-salt diet during ACE inhibition, the blood pressure response to Ang II was greater in male rats compared with females (Guo et al. 2008). In contrast to the study in rats, male mice fed a normal-salt diet and treated with enalapril exhibited a greater blood pressure

response to chronic Ang II when compared to female mice (Venegas-Pont et al. 2010). These data suggest that there are sex-specific and species differences in the mechanisms of the blood pressure response to Ang II with or without ACE inhibition. Because endogenous production of Ang II may not be the responsible factor for the dimorphic blood pressure effects in mice, scientists discuss about a differential expression or regulation of Ang II type 1 (AT1) receptors.

3.6 Angiotensin II Receptor Blockers

Major studies have investigated the effects of AT1 receptor antagonists. Losartan Intervention for Endpoint Reduction in Hypertension (LIFE), Evaluation of Losartan in the Elderly (ELITE II), and Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan (OPTIMAAL) tested losartan for the treatment of hypertension, in the elderly and after myocardial infarction. Valsartan Antihypertensive Long-Term Use Evaluation (VALUE) and Valsartan Heart Failure Trial (Val-HeFT) investigated valsartan for hypertension and heart failure, Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) has been conducted with candesartan and patients with heart failure and I-PRESERVE (Irbesartan in heart failure) analyzed irbesartan in heart failure, for example. These studies found no gender-specific differences and showed the same safety profile in both sexes. It should be considered that these studies included fewer women than men with the exception of LIFE (54 % women). In addition, propensity-score matching was preferentially used for statistical analysis in these trials. Propensity-score matching adjusts for differences in all covariates as well as for sex and will only lead to the detection of sex differences if this is specifically the aim of the investigation. To detect sex differences, prespecified subgroup analyses for sex differences in treatment groups would have been more useful. Ofili et al. (2008) published a post hoc analysis of the Irbesartan/Hydrochlorothiazide Blood Pressure Reductions in Diverse Patient Populations (INCLUSIVE) trial. Treatment with irbesartan/hydrochlorothiazide (HCTZ) combination therapy was associated with significant reductions in both systolic and diastolic blood pressure values in women, including "difficult-to-treat" female subgroups, such as the elderly, African Americans, and those with type II diabetes mellitus (Ofili et al. 2008). Systolic and diastolic blood pressure reduction in women was similar to those obtained in the male population of this trial (Saunders et al. 2008) arguing against major sex differences in Irbesartan effects.

Although clinical data do not show any major sex differences concerning the effects of angiotensin receptor blockers, experimental data suggest sex-specific differences in response to renin–angiotensin system activation. It should be noted that results of experimental data could be species dependent and not transferrable to human conditions in all cases. Ang II acts via two main subtypes of receptors, the

angiotensin II type 1 receptor (AT1-R) responsible for vasoconstriction, sodium reabsorption and cell proliferation and the angiotensin II type 2 receptor (AT2-R) characterized by vasodilatation and antiproliferation in humans (Berry et al. 2001; You et al. 2005) (Fig. 1). AT1-R isoforms as well as AT2-R expression differ in rat and human. Sampson et al. hypothesized that chronic administration of Ang II may affect arterial pressure in females differently from males. The group examined the effect of AT2-R blockade on the hemodynamic response to chronic low-dose Ang II treatment in male and female rats (Sampson et al. 2008). The findings suggest an increase in the vasodilatory effects of the RAS in female compared with male rats (Sampson et al. 2008). The hypotensive response to Ang II in female rats agrees with previous data suggesting that the AT2R mediates vasodilatation in both in vitro and in vivo studies (You et al. 2005). Furthermore, basal left ventricular expression and renal AT2R mRNA expression are markedly greater in females as compared with males (Sampson et al. 2008). The AT2R gene is located on the X chromosome. It is speculated that the transcription or expression of this gene would have a greater impact in females compared with males, given that they have two copies of the gene (Lazard et al. 1994). One alternative reason for the shift in balance to vasodilatation in females could be the effects of sex hormones. Sex steroids play an important role in the modulation of ATR function. Estrogens decrease AT1R expression, ovariectomy increases AT1R expression (Nickenig et al. 1998; Zheng et al. 2006) and estrogens upregulate AT2R expression (Armando et al. 2002).

Another finding contributing to the hypothesis that female sex shifts the balance of the RAS toward vasodilatation is the upregulation of the renal ACE2 mRNA. This effect occurs in both sexes but to a greater extent in female rats (Sampson et al. 2008). ACE2 cleaves Ang II into angiotensin-(1–7), which exerts its effects through its Mas receptor. The actions mediated by the Mas receptor and the AT2-R oppose the actions of the AT1-R in vessels. The Mas receptor contributes to vasodilatation and vascular protection, is known for antifibrotic and antiproliferative effects, and reduces proinflammatory cytokines in animal studies (Clarke and Turner 2012).

The complex role of the kidney in arterial pressure regulation and the response to Ang II and salt intake deserve further attention. The mechanisms responsible for postmenopausal hypertension in women and the greater salt sensitivity of blood pressure in men than in premenopausal women are not completely understood. In Dahl salt-sensitive rats, high-salt diet increases blood pressure more in males than in females. In this model, the systemic RAS is suppressed in response to high salt in male rats and intrarenal angiotensinogen expression is increased. Testosterone replacement in castrated rats increased blood pressure and renal angiotensinogen. Thus, testosterone may contribute to the development of hypertension in male Dahl salt-sensitive rats on high-salt diet through activation of the intrarenal RAS (Yanes et al. 2009). In addition, the increase of vasodilatory components of the RAS (AT2-R and ACE2) in female rats may also contribute to the sex differences observed in response to RAS activation. The enhancement of the vasodilator pathway of the RAS may be one mechanism for the relative cardiovascular protection in females.

3.7 Renin Inhibitors

Renin inhibitors block the renin–angiotensin system at its origin, by the inhibition of renin. Renin cleaves angiotensinogen to Ang I and ACE converts Ang I into Ang II. ACE inhibitors activate renal renin secretion since they interrupt the normal feedback suppression of renin secretion by circulating Ang II levels. The sequence of renin differs between species. Preclinical studies must be done in primates or in rat models transgenic for human renin and angiotensinogen.

Aliskiren is the first non-peptide active renin inhibitor with a sufficient oral bioavailability, specificity and efficacy. Aliskiren monotherapy (150 and 300 mg) provided equally effective, dose-dependent blood pressure lowering in women and men with mild-to-moderate hypertension. A pooled analysis of eight studies with aliskiren in hypertensive women demonstrated blood pressure lowering also in the elderly, obese or those with metabolic syndrome (Gradman et al. 2010).

A pooled analysis of 17 clinical studies demonstrated small effects of sex on the pharmacokinetics of aliskiren in healthy volunteers. The area under the aliskiren plasma concentration–time curve (AUC) was 22 % and the peak aliskiren plasma concentration (C_{max}) was 24 % lower in men than in women (Jarugula et al. 2010). The authors conclude that gender and differences in body weight are unlikely to have a clinical impact on the efficacy of aliskiren because the higher body weight of men is associated with the reduction in systemic exposure. Furthermore, analysis of clinical studies in patients with hypertension demonstrated no significant differences in the effect of aliskiren to inhibit plasma renin activity and blood pressure effects (Jarugula et al. 2010). The incidence of cough was low (<2 %) in both women and men, although cough was more frequent in women than in men at 300 mg aliskiren. Thus, there is no evidence for significant gender differences in aliskiren effects.

3.8 Aldosterone Receptor Antagonists

Aldosterone receptor antagonists are used as novel therapeutic elements in addition to ß-blockers and ACE inhibitors or angiotensin II receptor blockers (ARBs) for the management of severe systolic heart failure. Eplerenone is similar to the diuretic spironolactone though it is more specific for the mineralocorticoid receptor with minimal effects at other steroid receptors, thereby minimizing many of the hormonal adverse effects. Eplerenone reduces mortality and improves post-myocardial infarction (MI) remodeling in humans (Pitt et al. 2003). Whether these effects are modulated by gender is unclear. The major clinical trial of eplerenone in patients with acute MI and left ventricular dysfunction EPHESUS showed a trend towards a greater benefit for women, treated with eplerenone, at 30 days all-cause mortality analysis compared with men ($p = 0.089$). These results were not verified at 16 months (Pitt et al. 2003). The RALES trial published some years before

investigated the effect of spironolactone on symptomatic heart failure patients without any difference in a treatment benefit between men and women (Pitt et al. 1999). However, just 30 % of the patients enrolled have been women and the trial was not powered to detect gender differences.

Because aldosterone and estrogen signaling pathways interact, mediated through a common pathway involving protein kinase C (PKC-α) (Harvey et al. 2001), the question still remains if aldosterone blockade may depend on sex. Male and female infarcted rats receiving eplerenone or placebo have been investigated for left ventricular remodeling and gene expression. Eplerenone attenuated left ventricular chamber enlargement more effectively in female than in male rats and improved ejection fraction in females. Furthermore, eplerenone also reduced infarct size and cardiac fibrosis in females but not in males (Kanashiro-Takeuchi et al. 2009). Eplerenone preferentially restored altered gene expression to normal in post-MI female rats. Microarrays revealed that in females 19 % of downregulated genes and 44 % of upregulated genes post-MI were restored to normal by eplerenone. In contrast, eplerenone only restored 4 % of overexpressed genes in males. These alterations occurred among others in the renin–angiotensin- and fibrosis-inducing pathways (Kanashiro-Takeuchi et al. 2009).

Eplerenone is primarily metabolized by the cytochrome P450 enzyme CYP3A4. Sex differences in the pharmacokinetics of eplerenone may arise from extensive metabolism in male rats.

3.9 Diuretics

From the database of the German Network of Regional Pharmacovigilance Centres (NRPZ) it is known that women experience more frequently adverse drug reactions associated with diuretics. Data have been collected in urban hospitals between 2000 and 2006. Diuretics caused serious adverse drug reactions in 375 patients, of which 258 occurred in women ($p < 0.001$). The authors considered the fact that physicians prefer to prescribe diuretics to hypertensive women. However, prescription habits in this study could not explain the observed gender difference in the rate of adverse drug reactions (Werner et al. 2008) and additional mechanisms may be involved. Animal studies support the notion that sex-related differences of adverse and therapeutic effects of diuretics may exist. Female rats displayed a significant lower renal clearance of furosemide (loop diuretic), potentially due to the fact that furosemide is a substrate for the renal organic anion transport system (Cerrutti et al. 2002). Furthermore, furosemide as well as torasemide induced diuresis, natriuresis, and kaliuresis more effectively in female rats than in males (Brandoni et al. 2004). Adverse effects like hyponatraemia and hypokalaemia occur more frequently in women than in men taking diuretics, and both of these electrolyte disturbances have the potential to cause severe arrhythmia. This might suggest the possibility that women will experience more arrhythmia than men especially with more long QT-associated rhythm disturbances. However, this suspicion could not be confirmed

in clinical trials. Nevertheless, this fact should be considered when prescribing diuretics for women with a related risk profile.

Werner et al. identified sex as a potential determinant of torasemide pharmacokinetics and examined the impact of genetic polymorphisms of CYP2C9 and of liver-specific organic anion transporting peptide 1B1 (SLCO1B1) in a patient population with hypertension or heart failure. Torasemide, a loop diuretic, is frequently used for the treatment of these patients. Torasemide is cleared from the circulation mainly by hepatic metabolism, partly through the genetically polymorphic cytochrome P450 enzyme CYP2C9, and also by excretion into the urine. The study demonstrated a significant impact of sex and confirms the impact of the SLCO1B1 SNP on the steady-state pharmacokinetics of torasemide. These observations may in part explain the unbalanced distribution of torasemide adverse drug reactions among males and females (Werner et al. 2010).

For thiazide diuretics few sex differences are published. The density of the thiazide receptor was twofold higher in female Sprague–Dawley rats than in males. Ovariectomy decreased thiazide receptor by more than 20 %. In females the excretion of sodium, chloride and ammonium caused by bendroflumethiazide was greater than in male rats. It can be concluded that the renal excretion of electrolytes is, in part, controlled by sex and sex hormones via their regulation of the renal density of the thiazide diuretic receptor (Chen et al. 1994). Single nucleotide polymorphisms in genes encoding or influencing renal sodium transport systems were investigated in patients with hypertension treated with hydrochlorothiazide. However, most polymorphisms investigated were not associated with significant variation in blood pressure response (Turner et al. 2005).

4 Clinical Implications

Women are at greater risk than men of experiencing an adverse reaction to most cardiovascular drugs. Genetic mechanisms like polymorphisms modifying drug response to ACE inhibitors, beta-blockers, and calcium-channel blockers interfere with the effect of sex hormones and menstrual cycle, age, comorbidities, comedication, and self-medication.

Therefore, a complete drug history should be obtained when treating women. Since women often have lower body weight and/or kidney function compared with men, adequate dosing should be discussed when starting novel drug therapy in women. Creatinine clearance should be calculated in all women with borderline kidney function and drug doses should be adapted to kidney function. This may be particular relevant for digoxin, beta-blockers, diuretics and thrombolytics [see Rauch (2012)]. Doctors should be suspicious for adverse effects of beta-blockers or ACE inhibitors (dry cough more frequent in women than in men). In addition, potential interaction of drugs with endogenous hormones or therapeutically supplied hormones should be checked. It should be considered that there are differences in the adequate dose of drugs between pre- and postmenopausal

women, depending on hormonal status, intestinal uptake, hepatic metabolism and renal function. Polypharmacy in women is more frequently associated with arrhythmias than in men because QT time prolongation is known as an adverse effect of multiple drugs like antibiotics, antidepressants and cardiovascular drugs. EKG control should be performed readily if women are treated with such drugs. Administration of the aldosterone receptor antagonist eplerenone might have advantages for women with heart failure and post-myocardial infarction but there is still no firm evidence available. Adverse effects of diuretics may be particularly relevant for women. In summary, drug treatment of cardiovascular syndromes should be done in consideration of gender-related effects.

Take Home Messages

- Sex-related disparities in pharmacokinetics are common and some but not all of them will lead to clinically relevant differences in adverse effects and efficacy.
- Treatment with digoxin should lead to plasma levels below 0.8 ng per ml for both sexes. Impairment of renal function should be specifically considered in women treated with digitalis.
- Sex differences in the pharmacokinetics of beta-blockers lead to greater drug exposure and more adverse effects in women. Beta-blockers lead to similar survival benefits in heart failure in women and men.
- Sex differences in pharmacokinetics and effects of calcium channel blockers are small. In the elderly, clearance of oral administered amlodipine was faster in women.
- Adverse effects of ACE inhibitors, especially a typical dry cough are more frequent in women than in men.
- The major clinical trial of eplerenone in patients with acute myocardial infarction and left ventricular dysfunction EPHESUS showed a trend towards a greater benefit for women, for 30 days all-cause mortality. Animal studies support sex differences in eplerenone effects.
- Adverse effects like hyponatraemia and hypokalaemia occur more frequently in women than in men taking diuretics, and both of these electrolyte disturbances have the potential to cause severe arrhythmia.

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Sex and Gender Aspects in Antiarrhythmic Therapy

Junko Kurokawa, Masami Kodama, Tetsushi Furukawa, and Colleen E. Clancy

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Abstract Although cardiac arrhythmia had long been considered a predominantly male syndrome, it is now clear that arrhythmia is also a primary cause of mortality in women. Notably, the manifestation of specific arrhythmia syndromes appears to be gender specific. In particular, female sex is an independent risk factor for development of torsade de pointes (TdP) arrhythmias not only in congenital long QT syndromes but also in acquired long QT syndromes which occur as adverse effects of existing drugs. Males, on the other hand, are more likely to develop Brugada syndrome. Recent clinical and experimental studies suggest that these differences may stem from intrinsic sex differences in cardiac tissue. These include fundamental electrical differences resulting from variable ion channel expression and diverse sex hormonal regulation via long-term genomic and acute nongenomic pathways, and sex differences in drug responses and metabolisms. Undoubtedly, determining the effect of gender on cardiac function will be difficult and require

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sophisticated methodologies. However, gender differences underlying predilection to distinct arrhythmia syndromes must be revealed so that new therapeutic strategies that take gender into account can be applied to at-risk patients.

Abbreviations

TdP	Torsade de pointe(s)
ECG	Electrocardiograms
QT_C	Rate-corrected QT
ER	Estrogen receptor
MAPK	Mitogen-activated protein kinase
eNOS	Endothelial nitric oxide synthase
PI3K	Phosphoinositide 3-kinase
I_{Ks}	The slow delayed rectifier K^+ current
$I_{Ca,L}$	L-type Ca^{2+} current
hERG	Human ether-a-go-go-related gene
I_{Kr}	The rapid delayed rectifier current
E2	17β-estradiol
WPW	Wolff–Parkinson–White

1 Introduction

Sex differences in electrocardiograms (ECG) were reported for the first time in 1920 (Bazett 1920). In general, women have faster resting heart rates (Liu et al. 1989) and longer rate-corrected QT intervals in comparison with men (Merri et al. 1989). Moreover, it is becoming clear that sex differences in cardiac electrophysiology play a role in the prevalence of clinical arrhythmias. The mounting evidence implicating sex differences in the manifestation of clinical arrhythmias suggests that gender must be taken into account when developing and prescribing antiarrhythmic therapy.

While the gender differences in susceptibility to distinct arrhythmias are increasingly recognized, gender-specific therapies for arrhythmias remain to be established (Kohli and Gulati 2010). In order to develop appropriate gender-specific treatment modalities for cardiac arrhythmias, the mechanisms of sex differences must be determined. Two likely possible contributors to sex differences exist—transient hormonal regulation by the sex steroids estrogen, progesterone and testosterone, and fundamental differences in cardiac tissue stemming from genetic differences in males and females. Because the development of antiarrhythmic therapies requires understanding of the physiological differences and the molecular mechanisms behind them, this chapter reviews known biological sex differences in cardiac electrophysiology, such as heart rate and ECG characteristics, and the current

knowledge on the molecular mechanisms underlying these differences. We also summarize gender differences in several types of cardiac arrhythmias and attempt to discuss future development of gender-specific antiarrhythmic therapy including cardiac safety testing of new drugs.

1.1 Sex Differences in Cardiac Electrophysiology

1.1.1 Heart Rate

In young adults, women have higher heart rates than do men, corresponding to shorter sinus cycle length (Burke et al. 1996; Storstein et al. 1991). However, the mechanisms underlying differences in heart rate between males and females are still not clear. The sex difference in heart rate remains after double autonomic excision, indicating an involvement of intrinsic differences of heart rates sinus node rather than autonomic tone (Burke et al. 1996). However, a multivariate analysis in the same study suggests that the exercise capacity, which presumably alters autonomic tone, has a more significant impact on sex differences in heart rate compared with intrinsic differences in sinus node function (Burke et al. 1996). Heart rate in females fluctuates during the menstrual cycle and it seems to be faster during the ovulatory or luteal phases, which are higher estrogen states (Burke et al. 1996; James et al. 2007; Moran et al. 2000), suggesting that there is also an influence of the ovarian steroids on heart rates. It should be noted that other contrasting reports conclude that there is no change in the heart rate during the menstrual cycle in women and suggest contribution of neurohormonal regulation (Hirshoren et al. 2002; James et al. 2007) (Table 1).

1.1.2 QT Interval and Ventricular Repolarization

Shown are voltage space–time plots for the 50th paced beat at a cycle length of 1,000 ms in simulated tissue strands. (1) Phases of the menstrual cycle are shown as follows: (a) early follicular phase, (b) late follicular phase, (c) luteal phase. Simulated action potential duration (APD) in the presence of two physiological concentrations (d and e) of testosterone. (2) The computed virtual electrograms show the effect of fluctuating hormone levels during the menstrual cycle and two physiological concentration of testosterone on QT intervals (lower panels). The vertical bar graph shows the QT intervals under different circumstances (top panel) (Fig. 1).

It has been long known that women have a longer rate-corrected QT (QT_C) interval than do men by 2–6 % (Bazett 1920; Bidoggia et al. 2000; Lepeschkin 1956). The gender difference was first observed by Bazzet almost a century ago (Bazett 1920), and since confirmed by many researchers (Bidoggia et al. 2000; James et al. 2007; Lepeschkin 1956; Rautaharju et al. 1992). The evident gender differences in ECG are thought to reflect biological sex differences in the process of

Table 1 Sex differences in cardiac electrophysiology

Cardiac electrophysiology	Sex differences	Reference(s)
Resting heart rates	F > M	Liu et al. (1989)
Sinus cycle length	F < M	Burke et al. (1996) and Storstein et al. (1991)
Rate-corrected QT (QT_C) interval	F > M	Bazett (1920), Bidoggia et al. (2000), Lepeschkin (1956), and Merri et al. (1989)
JT interval	F > M	Yang and Clancy (2010)
QT dispersion (QT_d)	F < M	Kassotis et al. (2000), Mayuga et al. (2010), Nakagawa et al. (2003), Taneja et al. (2001), and Tran et al. (2001)
Variability of T-wave peak to T-wave end (T_{p-e}) interval	F < M	Kassotis et al. (2000), Mayuga et al. (2010), Nakagawa et al. (2003), Taneja et al. (2001), and Tran et al. (2001)
Vectorial deviation between the R and T waves	F < M	Cheng (2006)
Atrial effective refractory periods (ERP)	F < M	Tse et al. (2001)
P-wave interval	F < M	Dhala et al. (2002), Liu et al. (1989), and Storstein et al. (1991)
PQ or PR interval	F < M	Dhala et al. (2002), Liu et al. (1989), and Storstein et al. (1991)
AH interval	F < M	Liu et al. (1989)
HV interval	F < M	Liu et al. (1989)

ventricular repolarization, which determines the duration of the ventricular action potential. The duration of the ventricular action potential corresponds to QT intervals on the ECG. At the cellular level, the ventricular action potential is characterized by a long-lasting "plateau" period in which balance is maintained between small inward and outward currents. Minute perturbations to this balance can have severe functional consequences in the cardiac repolarization process, where the delay results in prolongation of QT intervals or JT intervals. Since excessive prolongation of APD can induce early after depolarization, which may act as arrhythmia triggers, sex differences in QT_C intervals may profoundly impact susceptibility to arrhythmia, especially for torsades de pointes (TdPs). Because the sex difference in QT_C intervals persists in the presence of autonomic regulation or autonomic blockade (Burke et al. 1997; Teplitz et al. 2005; Nakagawa et al. 2005), an involvement of intrinsic sex differences is a likely contributor to timing of ventricular repolarization.

When effects of sex differences on the process of ventricular repolarization are examined more closely, it is evident that men have a greater QT dispersion and T-wave peak to T-wave end corresponding to variability of the ventricular recovery time and transmural dispersion in repolarization, respectively (Kassotis et al. 2000; Mayuga et al. 2010; Nakagawa et al. 2003; Taneja et al. 2001; Tran et al. 2001). Global heterogeneity in ventricular repolarization measured with the vectorial deviation between the R and T waves is greater in men than in women. It is possible that the sex differences associate with diverse arrhythmic risks (Cheng 2006).

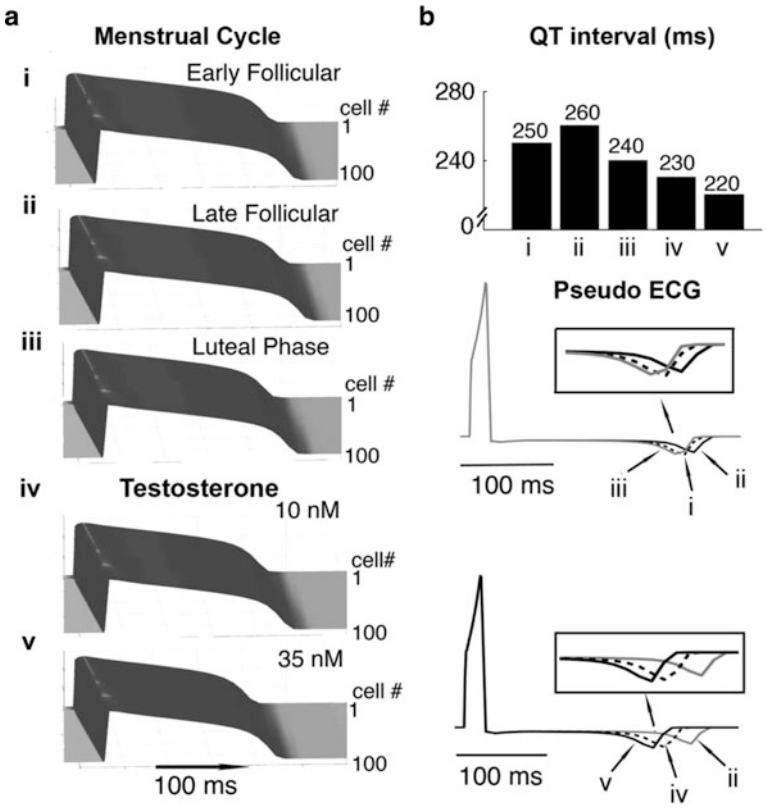

Fig. 1 Simulated combined effects of female hormones during the menstrual cycle and male hormones on cardiac action potentials [from Yang et al. (2010)]

Enhanced variability of QT dispersion and T-wave peak to T-wave end interval in males may provide a substrate for reentrant arrhythmias which can associate with the risks of sudden cardiac death in men (Kannel and Schatzkin 1985; Nakagawa et al. 2003), while prolonged QT or JT intervals in females may associate risks of triggered activity which predispose to TdP (Yang and Clancy 2010).

The sex disparity of ventricular repolarization heterogeneity or dispersion (QT dispersion and T-wave peak to T-wave end) and duration (QT and JT) are evident in the manifestation of electrical pattern on the body surface. Men have greater QT dispersion throughout life from adolescence until senescence than do women (Kassotis et al. 2000), indicating no overt age dependence, while QT intervals show a clear age dependence as discussed in the next paragraph. The circadian variation in QT dispersion is found primarily in men (Hansen et al. 2007), where the sex difference in QT dispersion is the greatest in early morning around at 6 A.M. (Mayuga et al. 2010; Smetana et al. 2002), while the sex difference in QT_C is constant throughout the day (Smetana et al. 2002).

Regarding the influence of aging on the sex difference in QT intervals, the QT interval is similar in young children before puberty (Rautaharju et al. 1992). Differences in QT intervals in males and females then appear from the time of puberty (Rautaharju et al. 1992; Stramba-Badiale et al. 1995), so sex steroid hormone effects on cardiac repolarization have been implicated. At the time of puberty, QT_C values in females remain unchanged, while those in males decrease by 20 ms, resulting in the sex differences in QT_C intervals between men and women until around the age of 50 (Rautaharju et al. 1992). The QT_C interval in men then gradually increases until the age of ~60 years, when it approaches that in women (Merri et al. 1989; Rautaharju et al. 1992; Stramba-Badiale et al. 1995). The international long QT syndrome (LQTS) registry 1998 reported that females had higher risk of a first cardiac event between 15 and 40 years (Locati et al. 1998). Moreover, clinical findings observed that more than 68 % of drug-induced TdP occur in women (Drici et al. 1998; Lehmann et al. 1996; Makkar et al. 1993). As sex hormone levels fluctuate during this period, the influence of sex hormones to differences in QT_C intervals between men and women has been documented (Merri et al. 1989; Rautaharju et al. 1992; Stramba-Badiale et al. 1995). Bidoggia et al. (2000), in fact, showed that women with virilization exhibit accelerated repolarization compared to intact women and castrated men. Consistent with the effects of testosterone on human ventricular repolarization, androgen therapy has also reported to decrease QT dispersion in men with congestive heart failure (Malkin et al. 2003). In animal models, Pham et al. (2001) showed in male rabbits that higher serum testosterone level, but not the estradiol level, associates with lower APD and less incidence of drug-induced arrhythmias. The same group also showed in female rabbits that testosterone diminishes drug-induced arrhythmias (Pham et al. 2002b). Taken together, these data suggest that testosterone is an important regulator of sex differences in ventricular repolarization and propensity to arrhythmias.

Female hormones are also involved in gender differences, both in QT_C intervals and in the susceptibility to TdP, although the situation is more complex than that for androgens. In females, there are dynamic fluctuations in QT interval and TdP risk during the menstrual cycle and pregnancy, which may correlate with changes in serum levels of ovarian steroids (Nakagawa et al. 2006). Whether normal hormonal fluctuations are sufficient to account for variability in QT during the menstrual cycle in not yet clear. Neither are the effects of physiological concentrations of hormones on arrhythmia susceptibility well understood. Some studies do report that dynamic fluctuations in QT intervals during the menstrual cycle are related to changes in susceptibility to TdP risk (Nakagawa et al. 2006; Rodriguez et al. 2001). Other data have not found marked fluctuation in QT interval during specific phases of the menstrual cycle (Burke et al. 1997; Hulot et al. 2003; Rodriguez et al. 2001). Burke et al. (1997) found that the corrected QT (QT_C) interval does not significantly change through menstrual cycle in premenopausal women; however, QT_C is reduced in the luteal phase after autonomic blockade. A study of drug-induced QT prolongation during the menstrual cycle observed that QT_C did not vary during

the menstrual cycle, but QT_C shortening was more pronounced in the luteal phase with ibutilide application (Rodriguez et al. 2001).

Nonetheless, both clinical and experimental data suggest that women have both longer QT intervals than men and are more likely to develop long-QT-dependent arrhythmias and TdP arrhythmias (Lehmann et al. 1996; Rodriguez et al. 2001). Women are especially susceptible to increased arrhythmia risk in response to QT-prolongation drugs (Gowda et al. 2004; Lehmann et al. 1996; Regitz-Zagrosek 2006; Rodriguez et al. 2001). One possibility is that an analysis of pooled QT intervals may not be sensitive enough to observe significant individual differences in QT intervals as they are fluctuating throughout the menstrual cycle, since biological variability between patients may be larger than fluctuations in individual patients. A computer simulation study investigated the acute effects of sex steroid hormones on cardiac cell and tissue dynamics and on fluctuations of QT interval (Yang et al. 2010). Because estrogen and progesterone dominate in the different phases of menstrual cycle, simulations show that during the late follicular phase (prior to ovulation) of the menstrual cycle, QT interval is longer than in the luteal phase when progesterone is increased, which is consistent with the clinical observation by Nakagawa et al. (2006). Notably, the fluctuations in QT interval during the menstrual cycle predicted by the computer model were within a relatively narrow range of 20 ms, which approximates the clinically assessed standard deviation in pooled QT intervals for a patient population assessed at each phase of the menstrual cycle (Burke et al. 1997).

Several studies have evaluated the potential impact of hormone replacement therapy on QT_C intervals in postmenopausal women (Abi-Gerges et al. 2004; Kadish et al. 2004) as estrogen hormone replacement therapy prolongs QT intervals and increases arrhythmia risk (Carnethon et al. 2003; Haseroth et al. 2000; Kadish et al. 2004). Although conflicting findings exist regarding hormone replacement therapy, these clinical findings imply that the dynamic changes in levels of female hormones have cyclical effects on QT_C intervals (Fig. 2).

1.1.3 Other Cardiac Electrophysiological Parameters

In contrast to longer QT_C interval in women, P-wave interval and PQ or PR interval are shorter in women (Dhala et al. 2002; Liu et al. 1989; Storstein et al. 1991), suggesting possible sex differences in atrial/AV nodal electrophysiology. Although there are conflicting reports regarding sex difference in atrial effective refractory periods, at least a study reported that premenopausal women have shorter atrial effective refractory periods compared with postmenopausal women or age-matched men, suggesting that the effect of the menopause on the sex differences in atrial effective refractory periods exists and is mediated by ovarian steroids (Tse et al. 2001). The intervals between the atria and His bundle (AH interval) and His-ventricle (HV interval) were also significantly longer in men than women (Liu et al. 1989). ST elevation is commonly seen in young, healthy men (Bidoggia et al.

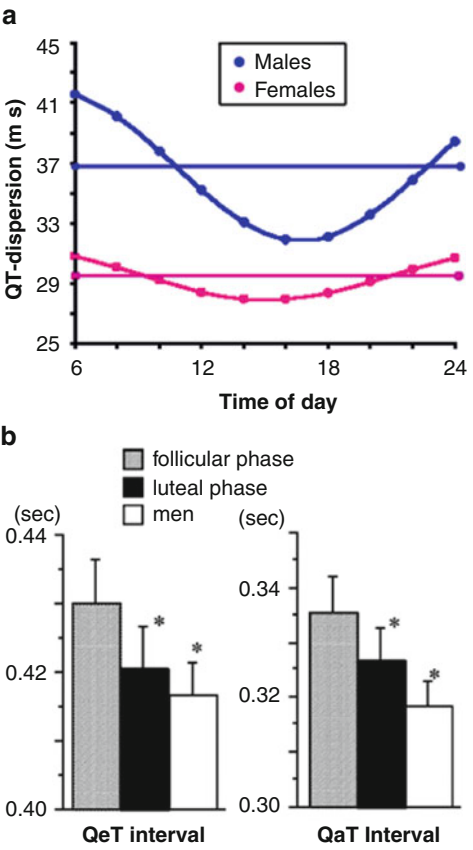

Fig. 2 Gender-specific cycle-dependent ventricular repolarization parameters; (**a**) circadian-dependent QT dispersion [adapted from Mayuga et al. (2010)]. (**b**) Menstrual cycle-dependent QT interval [adapted from Nakagawa et al. (2006)]

2000). The age and gender difference are not apparently due to differences in autonomic tone (Endres et al. 2006).

1.2 Sex Differences at Cellular and Molecular Level

Studies have shown both gender-specific effects of sex steroid hormones that modulate gene expression and acutely modulate ion channels and genomic differences in ion channel expression that underlie heterogeneous electrophysiological substrates (Verkerk et al. 2005).

It is known that one way sex steroid hormones cause functional physiological changes is via transcriptional regulation. Sex hormones may bind to sex hormone receptors and then translocate into the nucleus. In the nucleus, a ligand-bound sex hormone receptor acts as a transcription factor by binding to the promoter region of genes containing a hormone responsive element, leading to regulation of gene expression. For example, in the heart, lipocalin-type prostaglandin D synthase has

been found to be transcriptionally upregulated by estradiol and estrogen receptor (ER) (Otsuki et al. 2003). This genomic action requires several hours before the effects can be observed. In addition to the genomic pathway, sex steroid hormones may induce a rapid activation of mitogen-activated protein kinase (MAPK) leading to transcription factor activation (Behl and Holsboer 1999; Improta-Brears et al. 1999) as well as activation of membrane bound endothelial nitric oxide synthase (eNOS) (Furukawa and Kurokawa 2007; Mendelsohn and Karas 2005).

Cardiac ion channels are targets of modifications that permit cardiac function, drive or suppress disease states, and induce or respond to processes like aging (Catterall and Yu 2006; Furukawa and Kurokawa 2007; Kaab and Schulze-Bahr 2005; Nerbonne and Kass 2005). An important acute regulatory mechanism is via sex steroid hormones that activate cell signaling cascades and modulation of cardiac ion channel targets.

Testosterone acutely modulates a primary repolarizing potassium current in the heart, I_{Ks}, and the major depolarizing plateau current, L-type Ca^{2+} current ($I_{Ca,L}$), via a pathway involving phosphoinositide 3-kinase (PI3K)/Akt–dependent eNOS activation (Asada et al. 2009; Bai et al. 2005; Nakamura et al. 2007). Testosterone-induced phosphorylation of the Ser/Thr kinase Akt and eNOS leads to eNOS activation and production of nitric oxide (Bai et al. 2005). NO leads to *S*-nitrosylation of cysteine residues on the channel underlying the slow delayed rectifier K^+ current (I_{Ks}) (Asada et al. 2009). $I_{Ca,L}$ is conversely suppressed by NO via a cGMP-dependent pathway. Regulation of I_{Ks} and $I_{Ca,L}$ by testosterone is dose dependent (Bai et al. 2005) and leads to shortening of APD (Bai et al. 2005) and QT intervals (Liu et al. 2003; Malkin et al. 2003; Pham et al. 2001). In adult men, the serum testosterone level is reported to be 10–35 nM (Dorgan et al. 2002); however, circulating levels of testosterone begin to decline in men as young as 40 (Allan and McLachlan 2004). QT intervals are shorter in adult men than in adult women until around the age of 50 (Rautaharju et al. 1992), suggesting a likely role for circulating testosterone.

In females, progesterone fluctuates through the menstrual cycle. The reported serum progesterone level is 2.5 nM in the follicular phase and 40.6 nM in the luteal phase (Janse de Jonge et al. 2001). It was recently shown by Nakamura et al. that progesterone, like testosterone, increases I_{Ks} current through the NO production pathways and prevents cAMP enhancement of $I_{Ca,L}$ (Nakamura et al. 2007).

The apparent result of acute effects of progesterone and testosterone is to shorten APD and diminish incidence of arrhythmias (Bai et al. 2005; Nakamura et al. 2007; Pham et al. 2001, 2002b). Recently, experiments have suggested protective effects of testosterone against arrhythmia. In vivo experiments show that orchiectomized male rabbits treated with dihydrotestosterone had shorter QT interval and APD_{90} (APD at 90 % repolarization) compared to non-dihydrotestosterone-treated rabbits (Liu et al. 2003; Pham et al. 2001). Also, experiments in testosterone-treated female animals have shown that dihydrotestosterone reduces drug-induced arrhythmia by dofetilide (Pham et al. 2002b).

The acute effects of estradiol result in suppression of human ether-a-go-go-related gene (hERG) underlying the rapid delayed rectifier current (I_{Kr}) by directly

binding to the channel, altering channel kinetics, and reducing current (Kurokawa et al. 2008). Kurokawa and co-workers showed that 17β-estradiol (E2) accelerates the channel rate of closure (deactivation) and lessens repolarizing current (Kurokawa et al. 2008). They also showed that in the presence of E2, hERG is more sensitive to block by drugs. The group proposed that aromatic centroid of E2 may be responsible for increasing the sensitivity of hERG block by E4031 via interaction with the aromatic side chain of Phe^{656} and aromatic rings of the hERG blocker. Because (1) the concentration of E2 is not constant through the menstrual cycle, but rather fluctuates from the peak follicular phase serum E2 level of 1–0.7 nM in the luteal phase, and (2) E2 has dramatic effects on sensitivity to hERG block within this range, it stands to reason that susceptibility to drug-induced arrhythmia by hERG block may vary through the menstrual cycle.

1.3 Sex Differences in Risks of Arrhythmia

Risk factors for arrhythmias are dependent on the underlying arrhythmia initiation mechanism or manifestation. Although, to date, gender-specific guidelines for administration of antiarrhythmic therapy are not definitively established, increasing evidence suggests that risk stratification and treatment will include a gender component in the near future. This section reviews major types of cardiac arrhythmias or effectors, which show clear gender predominance. A summary is presented in Table 2.

1.3.1 Drug-Induced Long QT Syndrome

It is now apparent that the life-threatening ventricular tachyarrhythmia termed *torsades de pointes* (TdP) can be induced by many commonly used drugs which delay cardiac repolarization (Clancy et al. 2003; Drici et al. 1998; Hashimoto 2007; Hreiche et al. 2008; James et al. 2007; Lehmann et al. 1996; Makkar et al. 1993; Tamargo 2000), and such induction is the most common reason for withdrawal of medications from the market (Abriel et al. 2004; Fermini and Fossa 2003). Drug-induced arrhythmia, one of acquired long QT syndrome, is associated with prolonged QT_C intervals on ECG, resulting from delay of cardiac repolarization caused mostly by inhibition of the promiscuous drug target—the hERG channel, which conducts I_{Kr}. The incidence of drug-induced arrhythmia is also affected by other risk factors such as gender (Drici et al. 1998; Lehmann et al. 1996; Makkar et al. 1993) and/or sympathetic nervous system activity (Kurokawa 2007; Marx et al. 2002). Because drug-induced QT prolongation is such a major concern in drug manufacturing, pharmaceutical companies and basic researchers are striving to improve ways to predict the risk of novel agents as early as possible (Fermini and Fossa 2003; Satoh et al. 2000; Takahara et al. 2007). Thus, unraveling the molecular basis of effects of

Table 2 Sex differences in risks of arrhythmia

Types of cardiac events	Gender predominance	Effectors	Reference(s)
Torsades de pointes (TdP)	F > M	Drug-induced/ congenital long QT syndrome (LQTS)	Drici et al. (1998), Ebert et al. (1998), Liu et al. (1998a, b), Locati et al. (1998), Makkar et al. (1993), and Marx et al. (2002)
Arousal-induced arrhythmias	F > M	Autonomic control of cardiac activity, QT interval	Kim et al. (2010)
Supra-ventricular tachyarrhythmia (SVT)	F > M	PR/AH intervals, AV effective refractory period	Deneke et al. (2009), Jackman et al. (1992), Liu et al. (2001a, b), and Rodriguez et al. (1992)
Manifest Wolff–Parkinson–White (WPW) syndrome	F < M	AH interval, HV interval	Liu et al. (2001a)
Atrial fibrillation (AF)	F < M	Size of left atrium	(Benjamin et al. (1994) and Friberg et al. (2003)
Brugada syndrome	F < M	Ion channel gene SCN5A, testosterone	Antzelevitch (2006), Escarcega et al. (2009), Shimizu et al. (2007), and Wilde et al. (2002)

such risk factors may be beneficial for avoiding lethal side effects of unintended hERG blockade.

Female sex is an independent risk factor for development of TdP in the case of acquired (drug-induced) long QT syndrome. Thus, women are more prone to develop TdP than men in response to QT-prolonging drugs, with 65–75 % of cases of drug-induced TdP occurring in women (Drici et al. 1998; Ebert et al. 1998; Lehmann et al. 1996; Liu et al. 1998a, b; Locati et al. 1998; Makkar et al. 1993). Although the mechanisms underlying the gender differences in development of TdP have not yet been clarified, the higher susceptibility of females to drug-induced TdP has been thought to be associated with the longer baseline QT_C intervals in women by about 20 ms in comparison with those in men (Bazett 1920). Since QT dispersion is lower in women, it has been suggested that the fundamentally longer QT intervals in females is what predisposes to increased TdP risk (Kassotis et al. 2000). Although direct evidence from humans is still missing, it is likely that the gender differences in susceptibility to TdP also depend on age, which may correlate with changes in serum levels of sex hormones (Rodriguez et al. 2001). The age-dependent changes in QT_C intervals in men imply the involvement of testosterone in these gender differences.

1.3.2 Congenital Long QT Syndrome

The congenital (familial) form of long QT syndrome is a rare disease associated with recurrent syncope and a propensity to TdP and sudden death. The disease phenotype is caused by inherited defects in the cardiac repolarization process and genetic variants of the disorder have been labeled according to the chronology of linkage of specific genes. The functional characterization of mutant protein in long QT syndrome has provided plentiful information about the contribution of specific cardiac ion channels to cardiac repolarization. As of today, at least 13 genes harboring mutations have been associated with long QT syndrome and named in chronological order of discovery (LQT1-13). A large majority of long QT syndrome patients carry mutations in first three genes, LQT1, LQT2, and LQT3. LQT1 and LQT2 mutations are loss of function and located in genes of cardiac delayed rectifier K^+ channels: *KCNQ1* which encodes the α-subunit of the I_{Ks} channel and *hERG* which encodes the α-subunit of the I_{Kr} channel, respectively. LQT3 mutations are gain of function and localized to the gene of the cardiac Na^+ channel *SCN5A*, which encodes α-subunit of the I_{Na} channel. In affected long QT syndrome patients, risk factors to develop arrhythmias are gene specific, which has to be taken into account for diagnosis and medication. Usually, differences in risk factors are revealed once the genetic lesion is unequivocally attributed to LQT1 or LQT2 arrhythmias patients with K^+ channel mutations and LQT3 patients with Na^+ channel mutations.

As in drug-induced long QT syndrome, there are sex differences in the prevalence of the congenital forms of long QT syndrome (Locati et al. 1998). It has been reported that there is a female predominance in symptomatic long QT syndrome patients with the congenital form, although the inheritance pattern is autosomal (Moss et al. 1991). Close examination reveals that in LQT1 and LQT2, women are at greater risk than men of TdP arrhythmias (Locati et al. 1998). Notably, even in the congenital forms, there are no sex differences in baseline QT_C intervals in boys and girls younger than 15 years (Locati et al. 1998). However, after 16 years old—at puberty, QT_C intervals in female patients are longer than those in male patients. The first cardiac events in males occur prior to puberty, while first events in female occur uniformly throughout life (Locati et al. 1998). An age dependence of sex differences is also evident in LQT1 patients (Locati et al. 1998) (Fig. 3).

In pregnancy TdP risk associated with LQT2 is low and suddenly increased postpartum (Seth et al. 2007). On the other hand, sex differences in QT_C intervals and risks of cardiac events among LQT3 patients were not obvious in several studies (Lehmann et al. 1997; Zareba et al. 2003). Sex-specific phenotypes have been reported in KCNJ2 R67W mutation associated with Andersen's syndrome/LQT7 (Andelfinger et al. 2002) and KCNE1 D85N polymorphism associated with QT prolongation (Lahtinen et al. 2011).

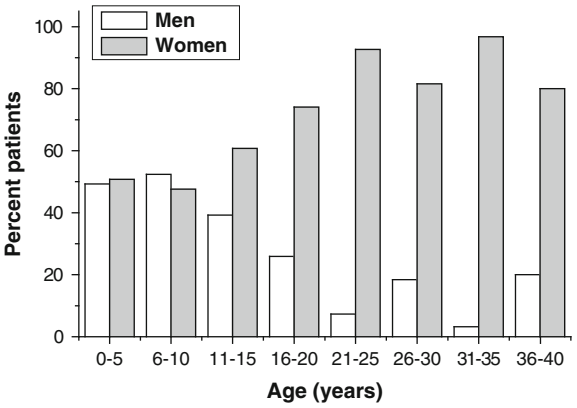

Fig. 3 Percent distribution of probands by sex and age at baseline ECG. Horizontal axis is age by 5-year increments. The male:female ratio is almost 1:1 until 15 years old, whereas females became predominant afterward [adapted from Locati et al. (1998)]

Gender and the Risk for Arousal-Triggered Cardiac Events in LQT2 Patients

Profound differences in the manifestation of arousal-induced arrhythmias in males and females suggest differences in autonomic control of cardiac activity between males and females. Although the rate of arousal-triggered events was similar in males and females during childhood, after the onset of adolescence the rate of arousal-triggered events was starkly higher in women than in men (Kim et al. 2010). At 40 years of age the rate of cardiac events triggered by acute arousal (usually a sudden loud auditory stimulus) was more than fourfold higher for women than men. In older age groups, women had an even greater ninefold increase in the risk for arousal-triggered cardiac events compared with men. During sleep, parasympathetic tone is elevated and sympathetic tone is depressed. Topaz et al. (1988) proposed that auditory stimuli trigger an efferent pathway channeled to the heart via the stellate ganglia and cardiac sympathetic nerves. Sudden auditory stimuli abruptly activate the sympathetic nerves in the setting of high vagal tone. Usually an increase in sympathetic discharge occurs concomitantly with vagal withdrawal, such as during exercise, and leads to a rapid shortening QT interval. However, an abrupt sympathetic surge without vagal withdrawal leaves the QT interval almost unchanged. A recent study showed a sluggish adaptation of the QT interval to an increase in heart rate in the arousal setting (Nakagawa et al. 2009). As a result, heart rate corrected QT interval was markedly prolonged after sudden arousal from sleep compared with daytime awake status. This finding led to the hypothesis that a mismatch of the heart rate and the repolarization time may generate electrical instability and subsequent arrhythmias that appear to be especially potent in females.

1.3.3 Supra-Ventricular Tachyarrhythmia

Supra-ventricular tachyarrhythmia (tachycardia), SVT, is broadly defined as a narrow tachycardia that requires atrial tissue or the AV node, and/or the accessory

pathway as an integral part of the arrhythmia substrate. The majority of clinically important supra-ventricular tachyarrhythmia is caused by the presence of an accessory electrical connection between the atrium and ventricle (i.e., the bundle of Kent) or within the AV node itself, resulting from reentry. Understanding the mechanism of reentry is critical to understanding the targets of therapeutic strategies for supra-ventricular tachyarrhythmia and the risk factors including sex differences.

AV nodal reentrant tachyarrhythmia (AVNRT), the most common atrial reentrant tachycardia, has a 2:1 female–male predominance (Jackman et al. 1992; Rodriguez et al. 1992). This female predominance has been explained to be due to a shorter PR/AH intervals (conduction time) and a shorter AV effective refractory period in women (Deneke et al. 2009; Liu et al. 2001a, b). Although the underlying mechanisms are still largely unknown, changes in plasma levels of ovarian hormones may be of importance in determining episodes of arrhythmia in supra-ventricular tachyarrhythmia patients. Reflecting the cyclical changes in supra-ventricular tachyarrhythmia risks during the menstrual cycle, plasma progesterone has a positive correlation with episode number and duration of supra-ventricular tachyarrhythmia, while plasma estrogen has a negative correlation with them (Rosano et al. 1996). On the other hand, a male predominance has been reported in patients with manifest Wolff–Parkinson–White (WPW) syndrome, but not in those with concealed WPW syndrome, which has been suggested to be due to a longer AV conduction in men (Liu et al. 2001a).

1.3.4 Atrial Fibrillation

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice and has multifactorial nature of disease. To date, therapy for AF is multidimensional spanning pharmacological therapy to invasive electrophysiological intervention. The principal goal is relief of symptoms and prevention of stroke. The pharmacological therapy includes antiarrhythmic support such as control of heart rate or rhythm, and anticoagulation. Although benefits of anticoagulation and/or catheter-based ablation are promising, there are still debates regarding strategies of control of the ventricular rate (rate control) or maintenance of sinus rhythm (rhythm control) to improve quality of life (Connolly 2010; De Caterina and Hylek 2011; Nattel 2011; Savelieva et al. 2011; Sellers and Newby 2011; Wakili et al. 2010).

Both the Framingham and the Copenhagen City Heart Studies show that men have greater risk of developing AF than women after adjusting for age and other risk factors (Benjamin et al. 1994; Friberg et al. 2003). One explanation is the larger size of left atrium in men, which allows circuit pathways for reentry. AF leads to shortening of the arterial effective refractory periods, resulting in an enhanced susceptibility of further AF episodes (AF beget AF). Actually, the shortening of the effective refractory periods in response to a brief episode of AF was attenuated in young women, suggesting involvement of ovarian steroids (Tada et al. 2001).

1.3.5 Brugada Syndrome

Brugada syndrome is an inherited ventricular tachycardia syndrome with variable electrocardiographic features characteristic of right bundle-branch block, persistent ST-segment elevation in the precordial leads at rest (from V1 to V3), and sudden cardiac death (Antzelevitch 2006; Escarcega et al. 2009). Sudden death in Brugada patients tends to occur during the early morning hours (Wilde et al. 2002). Currently, mutations that cause Brugada syndrome have been identified in five genes encoding cardiac ion channels or channel modulators. The most frequent mutation is found in *SCN5A* which encodes for the α-subunit of the cardiac Na^+ channel (Antzelevitch 2006; Escarcega et al. 2009; Wilde et al. 2002). Most *SCN5A* mutations associated with Brugada syndrome (only about 20 % of all cases) lead to a "loss-of-function" phenotype, reducing the Na^+ influx during the early phases of the action potential. Two specific types of ST-segment elevation, coved (type 1) and saddleback (type 2), are observed in the Brugada syndrome, the former of which is reported to relate to a higher incidence of ventricular fibrillation and sudden cardiac death.

Brugada syndrome has a clear male predominance in phenotype and symptoms (Benito et al. 2008; Kamakura et al. 2009; Shimizu et al. 2007; Wilde et al. 2002). The prevalence of the disease is one of the important causes of sudden cardiac death of middle-aged males, particularly in Asian countries (Antzelevitch 2006). Since all reported *SCN5A* mutations show autosomal-dominant transmission, males and females are expected to inherit the defective gene equally. An involvement of transient outward K^+ current (I_{to} current) in the male predominance has been pointed out (Di Diego et al. 2002). Varying levels of transient outward (I_{to}) current between endocardium and epicardium are presumed to underlie the observed difference in the "notch" or phase 2 of action potential. Reduced Na^+ current is then anticipated to cause premature repolarization in epicardial cells with large transient outward (I_{to}) currents but not in endocardial cells. This repolarization dispersion is the hallmark of phase 2 reentry, which is the presumed underlying mechanism in ventricular tachyarrhythmia development in Brugada syndrome. Males exhibit larger transient outward (I_{to}) currents dispersion, which may predispose them to arrhythmias compared to female. Although the mechanism for the male predominance is not fully understood, it is believed that testosterone is also involved (Shimizu et al. 2007). A study suggests that higher testosterone level associated with lower visceral fat may have a significant role in the male predominance in Brugada syndrome (Shimizu et al. 2007).

1.4 Pharmacokinetics of Antiarrhythmic Drugs

There are several sex differences in pharmacokinetics that can control plasma concentrations of drugs. Pharmacokinetic parameters include interference of drugs with absorption, distribution, metabolism, and excretion. Drug metabolism

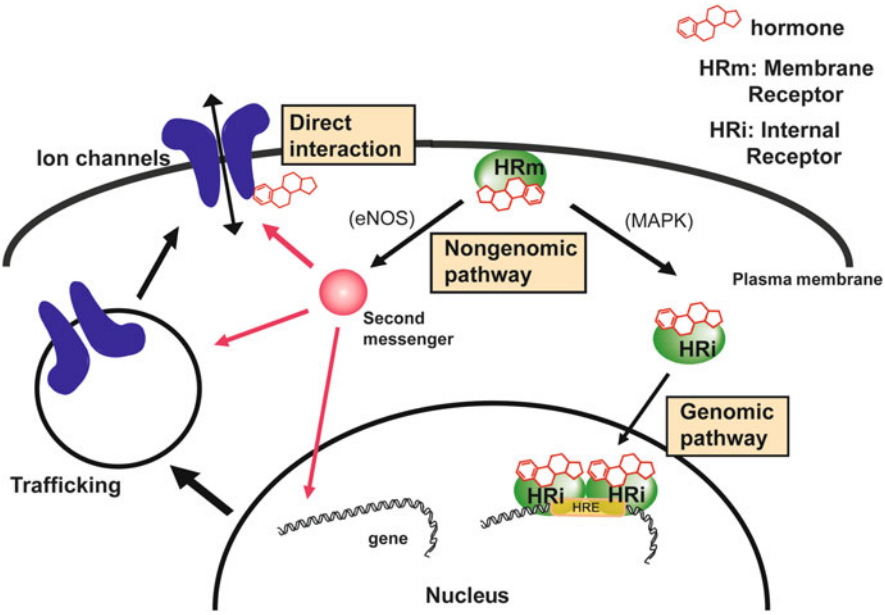

Fig. 4 Genomic and nongenomic signaling pathway of sex steroid hormones regulates cardiac ion channels [adapted from Kurokawa and Abriel (2009)]

in women may be affected by sex-specific factors such as menopause, pregnancy, and menstruation. Cytochrome P-450 (CYP) enzymes are central to metabolism of drugs in humans. CYP3A4 is the most abundant P450 in the human liver and is known to metabolize more than 50 % of drugs (Anzenbacher and Anzenbacherova 2001). It has been reported CYP3A4 exhibits higher activity in women than in men (Tanaka 1999), while metabolism by CYP2C9, CYP2C19, and N-acetyltransferase do not show clear sex differences (Schwartz 2003). Higher plasma concentration of cisapride, a QT prolonging drug, can be seen upon decrease of metabolism with CYP3A4, suggesting an involvement of pharmacokinetics in susceptibility of drug-induced arrhythmias (Wysowski et al. 2001).

1.5 Possible Mechanisms for Sex Differences

Sex steroid hormones bind to cytosolic sex hormone receptors. Ligand-bound sex hormone receptors translocate into nucleus, dimerizes, and binds to the genes containing hormone responsive element in the promoter region, leading to transactivation or transrepression (genomic pathway). In addition to the classical genomic pathway, sex steroid hormones exhibit rapid actions via an activation of MAPK or eNOS in a membrane-limited manner (nongenomic pathway) (Fig. 4).

Table 3 Sex differences in ion channel subunit expression from nondiseased ventricles

	Female		Male			
Ion channels	Epi	Endo	Epi	Endo	Species	Reference
I_{to} (Kv1.4) (protein level)	1.2	2.5	1	4.5	Human	Gaborit et al. (2010)
I_{Ks} (MinK) (protein level)	0.61	0.64	1	1.3	Human	Gaborit et al. (2010)
I_{Kr} (HERG) (protein level)	0.8	0.8	1	1	Human	Gaborit et al. (2010)
I_{K1} (Kir2.3) (mRNA)	0.24	0.6	1	1	Human	Gaborit et al. (2010)
Na^+/K^+-ATPase a1 (mRNA)	2.47	3	1	1.3	Human	Gaborit et al. (2010)
Ca^{2+}-ATPase 4 (mRNA)	1.8	1.8	1	1.13	Human	Gaborit et al. (2010)
PLB (mRNA)	0.8	0.74	1	1	Human	Gaborit et al. (2010)
Cx43	0.44	0.52	1	1	Human	Gaborit et al. (2010)
Cx43 (protein level)	1.5		1		Mouse	Rosenkranz-Weiss et al. (1994)
PLB_{S16P} (protein level)	0.5		1		Human	Dash et al. (2001)

One possible mechanism underlying sex differences in the manifestation of Brugada Syndrome is that high estrogen levels during the late follicular phase of the menstrual cycle and the interplay between larger net inward currents and low progesterone levels in females set the stage for action potential prolongation and arrhythmogenic afterdepolarizations that trigger ventricular arrhythmias.

1.5.1 Gene Expression of Cardiac Ion Channels

Recent clinical and experimental studies suggest that some gender differences may stem from fundamental intrinsic differences in cardiac tissue (Di Diego et al. 2002; Fish and Antzelevitch 2003; Hara et al. 1998; Pham et al. 2002a; Pham and Rosen 2002; Xiao et al. 2006; Korte et al. 2005). These include intrinsic electrical differences resulting from variable ion channel expression (see Table 3). Measured differences in ion channel expression profiles between human males and females have also been published and will surely alter human action potential waveforms (Table 3) (Gaborit et al. 2010).

1.5.2 Hormonal Regulation

Clinical Studies

Female gender is an independent risk factor for development of TdP in both congenital and acquired long QT syndrome (described in Sect. 1.3.1). The differences in QT_C interval and TdP incidence are not different between females and males from the birth to the onset of puberty, but emerge upon onset of puberty. These clinical data indicate protective effects of testosterone against long QT syndrome. Indeed, the testosterone patch has been considered for development by pharmaceutical companies to prevent and reverse QT prolongation in females, although, the potential for multiple other adverse reactions arising from testosterone applications in females has hindered progress.

In females, QT_C interval and the propensity for drug-induced QT_C prolongation show cyclical changes during the menstrual cycle: QT_C interval is shorter in the luteal phase compared to the follicular phase and drug-induced QT_C prolongation is less prevalent in the luteal phase compared to the follicular phase. In pregnant women, the incidence of TdP dramatically increases following delivery in LQT patients. For postmenopausal females, hormone replacement therapy with estrogen alone prolongs QT_C interval, while hormone replacement therapy with estrogen and progestin shortens QT_C interval. These clinical findings imply protective effects of progesterone against QT prolongation. It is, therefore, interesting to examine if progesterone has nongenomic effects similar to testosterone.

The testosterone effect to shorten QT intervals in men, presumably by increasing repolarizing current, may contribute to the Brugada Syndrome linked pathological early repolarization (described earlier) that is observed more frequently in men. Di Diego and colleagues (Di Diego et al. 2002) also suggested that a more prominent transient outward (I_{to}) currents in males lead to the male predominance of Brugada phenotype. Indeed, in male RV-epicardium, higher level expression of the transient outward (I_{to}) K^+ channel β-subunit KChIP2 has been observed and may favor early repolarization in Brugada patients (Gaborit et al. 2010). In a recent ion-channel expression-pattern study, Gaborit et al. (2010), found lower lever expression of repolarizing ion-channel subunits including KCHIP2, HERG, Kir2.3, Kir6.2, and SUR2 in females that may protect females against the Brugada phenotype (but also make them susceptible to long QT as discussed earlier).

1.5.3 Chronic Effects of Sex Steroid Hormones

Steroid hormone receptors have been traditionally been thought to act via the regulation of transcriptional processes, involving nuclear translocation and binding to specific response elements, and ultimately leading to regulation of gene expression (McKenna and O'Malley 2002). Binding of sex hormones to their receptors releases the receptor from an inhibitory complex with heat shock proteins, leading to homo-dimerization and translocation of the receptor complex into the nucleus. The sex hormone receptors then bind to a short palindromic sequence called the hormone responsive element, located in the promoter region of target genes. Sex hormone receptors-ligand complex recruits a co-regulator complex to the promoter in tissue-, cell-, and promoter-specific manner, resulting in transactivation or transrepression (McDonnell and Norris 2002; Rosenfeld et al. 2001). This classical pathway involving nuclear actions is referred to "genomic," "nuclear," or "transcriptional" action of sex hormones. Since genomic action requires the process of transcription and translation, it takes several hours to days for functional manifestations to occur.

1.5.4 Acute Effects of Sex Steroid Hormones

Recently, the observation of effects elicited by steroid hormones which are too rapid to be mediated by activation of RNA and protein synthesis has prompted the search

for alternative signaling mechanisms (Levin 2002), conceptually defined by Selye (1942). It has been reported that rapid actions of estrogen afford beneficial effects against heart failure and corresponding myocardial remodeling (Farhat et al. 1993; McHugh et al. 1995; Node et al. 1997). In terms of cardiac arrhythmias, involvements of the nongenomic sex steroid hormones in acute effect on cardiac ion channels have been implicated. There are several reports showing that both estrogen and androgen rapidly inhibit various cardiac ion currents including $I_{Ca,L}$ (Berger et al. 1997; Gupte et al. 2002; Nakajima et al. 1999), T-type Ca^{2+} currents ($I_{Ca,T}$) (Nakajima et al. 1999), I_{Ks} and I_{Kr} (Nakajima et al. 1999; Tanabe et al. 1999), transient outward current (I_{to}) (Berger et al. 1997), and inwardly rectifying K^+ current (I_{K1}) (Berger et al. 1997; Carnes and Dech 2002) in isolated single rat ventricular myocytes, and guinea pig ventricular and atrial myocytes. However, estrogen and androgen require more than several μM concentrations at which steroids exhibit nonspecific binding to various molecules. Therefore, physiological relevance of these inhibitory effects of sex hormones on cardiac ion currents has been unclear.

In 2005, our group demonstrated that sex steroid hormones rapidly regulate cardiac ion currents (Bai et al. 2005). In guinea pig hearts, physiological concentrations of testosterone (nM order) shortened QT_C intervals and action potential duration within 5 min (Bai et al. 2005). Testosterone enhanced I_{Ks} with an EC_{50} of 1.1 nM in guinea pig ventricular myocytes in an androgen receptor-dependent manner through a PI3K-Akt-NOS3 pathway. The nongenomic action of testosterone on cardiac ion channels is mediated by NO production. The same study (Bai et al. 2005) also showed that E_2 also enhances I_{Ks} and shortens action potential duration; the EC_{50} (~30 nM) for I_{Ks} enhancement was slightly higher than the physiological range of E_2 (0.1–10 nM).

In contrast to estrogen, progesterone exerts a nongenomic action within the physiological range that manifests functionally with changes to cellular electrophysiology that are similar to those observed with testosterone (Nakamura et al. 2007). The acute effect of progesterone on $I_{Ca,L}$ was shown to be cAMP dependent, while the effect on I_{Ks} was cAMP independent (Nakamura et al. 2007), and rather explained by distinct mechanisms via NO regulation. NO effects I_{Ks} by two distinct mechanisms. NO binds to the heme iron of soluble guanylate cyclase, and activated soluble guanylate cyclase converts GTP to cGMP (Murad 1986, 2006). NO also modifies proteins by *S*-nitrosylation; the coupling of an NO moiety to a reactive crystal thiol to form an *S*-nitrosothiol (Hess et al. 2005; Jaffrey et al. 2001; Saraiva and Hare 2006). I_{Ks} enhancement by testosterone was cGMP independent but may nonetheless be due to *S*-nitrosylation, while an soluble guanylate cyclase-dependent pathway plays a role in $I_{Ca,L}$ inhibition (Asada et al. 2009; Bai et al. 2005; Nakamura et al. 2007).

2 Conclusions

It is becoming increasingly clear that gender differences need to be considered in diagnosis and treatment of cardiac arrhythmias. This is because recent studies have begun to show that the mechanisms of arrhythmia initiation, sustenance, and

termination appear to be gender specific. Clinical and experimental studies suggest that these differences may stem from fundamental gender differences in cardiac tissue. These include intrinsic electrical differences resulting from variable ion channel expression and diverse sex hormonal regulation via long-term genomic and acute nongenomic pathways. Methodologies derived from cutting edge technologies must be developed to improve understanding of gender effects on cardiac function that may lead to development of new therapeutic strategies that take the sex of patients into account.

Take Home Messages

- Cardiac arrhythmia is a primary cause of mortality both in women and men. Sex differences in cardiac electrophysiology play a key role in the prevalence of clinical arrhythmias.
- Enhanced variability of QT dispersion and T-wave peak to T-wave end interval in males may provide a substrate for reentrant arrhythmias, while prolonged QT or JT intervals in females may associate risks of triggered activity which predispose to TdP.
- Sex differences in cardiac electrophysiology stem from fundamental intrinsic differences in cardiac tissue and hormonal regulations which is either chronic or acute.

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Sex and Gender Aspects in Anesthetics and Pain Medication

Ilaria Campesi, Marco Fois, and Flavia Franconi

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Abstract The influence of sex and gender on anesthesia and analgesic therapy remains poorly understood, nevertheless the numerous physiological and pharmacological differences present between men and women. Although in anesthesiology sex–gender aspects have attracted little attention, it has been reported that women have a greater sensitivity to the non-depolarizing neuroblocking agents, whereas males are more sensitive than females to propofol. It has been suggested that men

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wake slower than women after general anesthesia and have less postoperative nausea and vomiting. Sexual hormones seem to be of importance in the onset of differences. Nevertheless, in the last years, sex–gender influences on pain and analgesia have become a hot topic and data regarding sex–gender differences in response to pharmacologic and non-pharmacologic pain treatments are still scanty, inconsistent, and non-univocal. In particular, females seem to be more sensitive than males to opioid receptor agonists. Women may experience respiratory depression and other adverse effects more easily if they are given the same doses as males. Evidently, there is an obvious need for more research, which should include psychological and social factors in experimental preclinical and clinical paradigms in view of their importance on pain mechanism, in order to individualize analgesia to optimize pain relief.

Keywords Anesthetics • analgesic drugs • gender

Abbreviations

SGD	Sex–gender differences
ADE	Drug adverse effects
BIS	Bispectral index scores

1 Introduction

Women have historically been neglected or excluded from many drug development studies because of the hormonal variability and concerns for potential teratogenicity of drugs (Anderson 2005; Franconi et al. 2007, 2011; Schwartz 2007; Soldin et al. 2011). The exclusion of women from clinical studies and female animals from preclinical studies (Beery and Zucker 2010) may have caused the loss of opportunities to identify specific sex–gender differences (SGD) in drug pharmacokinetics and pharmacodynamics. Consequently, guidelines for prevention, diagnostic testing, and medical and surgical treatments for diseases are based on studies conducted predominantly on middle-aged men and data mainly obtained in men have been transferred to women (Franconi et al. 2007, 2011; Schwartz 2007; Soldin et al. 2011). Now we know that men and women present numerous physiological differences; consequentially they could have a different response to drugs (Anderson 2005; Franconi et al. 2007, 2011; Marino et al. 2011; Soldin et al. 2011).

Indeed, growing evidence suggest that patient sex–gender is an independent factor influencing drug responses in terms of efficacy and safety (Anderson 2005; Franconi et al. 2007, 2011; Soldin et al. 2011). In this chapter, we focus our attention on the influence of sex–gender on anesthetic and analgesic drugs.

2 What Determines SGD in Pharmacokinetics?

The physiological differences between men and women which impact pharmacokinetic parameters have been extensively described in many recent reviews (Anderson 2005; Franconi et al. 2007, 2011; Marino et al. 2011; Soldin et al. 2011). Indeed in humans, it is estimated that there is a 40% difference in pharmacokinetics between men and women (Anderson 2005). Shortly, women are smaller and have more fat and lower muscle when compared with men. Women have a lower (~15–20%) total body water than men. In addition, extracellular fluids and plasma sodium vary during the menstrual cycle, being more elevated during mid-follicular and ovulatory stages than during the luteal phase and with the use of oral contraceptives due to the effect of estrogens and progesterone on renal sodium reabsorption (Olson et al. 1996; Stachenfeld et al. 1999; Stachenfeld and Taylor 2004; Campesi et al. 2012). Generally, water-soluble drugs, such as muscle relaxants, have a lower distribution volume in women (Pleym et al. 2003; Buchanan et al. 2009). On the contrary, lipid-soluble drugs (benzodiazepines, opioids, propofol, eltanolone) generally have a larger volume of distribution in women than in men (Pleym et al. 2003; Buchanan et al. 2009). These SGD in distribution volumes are especially relevant when the drugs are administered in fixed dosages (mg) instead of considering body weight or body surface (mg/kg or mg/m^2) as frequently noted with the premedication agents and postoperative analgesics (Booij 2008).

SGD have been described at gastrointestinal levels (Anderson 2005; Franconi et al. 2007, 2011; Schwartz 2007; Soldin et al. 2011). For example, women have a higher bioavailability of oral midazolam than men (Pleym et al. 2003). SGD also exist in cardiac output, liver perfusion, and glomerular filtration rate. Many of these factors have been recently discussed (Anderson 2005; Franconi et al. 2007, 2011; Schwartz 2007; Soldin et al. 2011). In addition, there are differences in metabolism and transport proteins (Franconi et al. 2007, 2011; Schwartz 2007; Soldin et al. 2011). Indeed, much of the SGD could be due to differential expression of drug metabolism genes between men and women. SGD are described in both phase 1 (Table 1), mediated by cytochrome P450 enzyme, and phase 2 enzymes which catalyze glucuronidation, sulfation, acetylation, or methylation either within or outside the liver. They have been already described in many reviews (Anderson 2005; Schwartz 2007; Franconi et al. 2007, 2011; Soldin et al. 2011). In particular, the activity of plasma cholinesterase, an enzyme that metabolizes some neuroblocking agents (Table 2), is higher in men older than 10 years in comparison with women of the same age (Lepage et al. 1985). Notably, in the postmenopausal women, the plasma choline esterase activity reaches the level observed in men, whereas pregnancy and the use of estrogen-containing oral contraceptives lead to a reversible decrease in plasma cholinesterase activity (Lepage et al. 1985) indicating the importance of sexual hormones.

Several anatomic and physiologic SGD have been described in pulmonary system (Harms 2006). Additionally, estrogens and progesterone can influence ventilation, substrate metabolism, thermoregulation, and pulmonary function; pulmonary parameters can vary during menstrual cycle and pregnancy (Schoene et al. 1981; Bayliss and Millhorn 1992). Differences involve smaller airway diameter, vital capacity and

Table 1 Metabolic pathway of drug used in anesthesia

	General anesthetics	Analgesics	Local anesthetics	Benzodiazepines
CYP1A2 (more expressed in female)			Ropicavaine	
CYP2B6	Propofol[a], ketamine			
CYP2C9	Propofol[a], ketamine			
CYP2C19	Hexobarbital			Diazepam
CYP2D6 (more activity in male)		Codeine, tramadol, oxycodone, hydrocodone		
CYP2E1 (more activity in male)	Halothane, enflurane, isoflurane, sevoflurane, ketamine			
CYP3A4 + (more expressed in female)				Fentanyl, alfentanil, sufentanil, methadone, buprenorphine, lidocaine, ropicavaine, buvicavaine, mizandol, diazepam
Glucuronidation + (more expressed in male)	Propofol	Paracetamol		

Data are obtained from Scandlyn et al. (2008) and Restrepo et al. (2009)
[a]Propofol is an inhibitor of CYP3A4, CYP1A2, CYP2E1 (Janicki et al. 1992; Lejus et al. 2002; McKillop et al. 1998)

maximal expiratory flow rates, and diffusion surface. These differences may have an effect on the integrated ventilatory response, respiratory muscle work, and in pulmonary gas exchange and thus could be important for volatile anesthetics.

3 Preanesthetic and General Anesthetic Drugs

3.1 Preanesthetic Agents

The use of preanesthetic drugs is aimed to reduce anxiety and stress without producing large drowsiness, provides amnesia for the preoperative period, maintains cooperation before the loss of consciousness, and, if necessary, should relieve pain

Table 2 Metabolic pathways of neuromuscular blocking agents and pharmacokinetic SGD

	Pharmacological properties	Metabolism and elimination	Sex and gender differences
Succinycholine	Depolarizing	Plasma choline esterase	
d-tubocurarine	Competitive antagonist		
Atracurium[a]	Competitive antagonist	Plasma choline esterase, Hofmann degradation	No univocal results
Doxacurium	Competitive antagonist	Is not metabolized and that the major elimination pathway is renal and bile excretion	
Mivacurium	Competitive antagonist	Plasma choline esterase	
Pancuronium	Competitive antagonist	Mainly renal elimination	Lower Vd in women, females require less drugs
Pipecuronium	Competitive antagonist	Renal and liver elimination	Lower Vd in women
Rocuronium	Competitive antagonist	Mainly hepatic elimination	Lower Vd in women, females require less drugs
Vecuronium[b]	Competitive antagonist	Mainly hepatic elimination and biliary excretion	Lower Vd in women, females require less drugs

[a]Neill et al. (1983) and Pleym et al. (2003)
[b]Xue et al. (1998, 1999)

(Brunton et al. 2011). In addition, it should reduce the need of inhalation agents reducing their drug adverse effects (ADE) (Brunton et al. 2011). The achievement of previous goals requires a cocktail with two and three drugs and they include benzodiazepines, barbiturates, antihistamines, phenothiazines, butyrophenones, opioids, antiemetics, and anticholinergics. Many of them have been treated in other chapters; therefore, we examine only neuromuscular blocking agents.

3.2 Neuromuscular Blocking Agents

Neuromuscular blocking agents are used to relax skeletal muscle through nicotinic cholinergic receptors at neuromuscular junctions (Brunton et al. 2011). They are distinguished on the basis of their ability to induce depolarization of the motor end plate (Table 2). Generally, women are more sensitive to non-depolarizing muscle relaxants than men (Xue et al. 1997; Pleym et al. 2003).

The muscle relaxants also have a longer duration of effect in women because, as discussed above, these hydrophilic drugs have a smaller volume of distribution in women than in men. This leads to higher plasma concentrations and thus more pronounced effects. Indeed, it has been reported that women require ~30% less rocuronium than men to achieve the same degree of neuromuscular block

(Pleym et al. 2003; Adamus et al. 2008), suggesting that females are more sensitive than males to this drug. This has also been demonstrated for atracurium (Xue et al. 1999) and vecuronium (Xue et al. 1997, 1998, 1999). On the contrary, no SGD in the onset time or clinical duration of cisatracurium are observed (Adamus et al. 2008).

3.3 SGD in ADE Induced with Neuromuscular Blocking Agents

Adverse allergic reactions in anesthesia, which are more frequently due to neuromuscular blocking drugs, have a higher incidence in women (70% in women versus 30% in men) (Light et al. 2006). The same authors evidence that men are more likely to suffer ADE to atracurium, whereas women experienced more reactions to succinylcholine (Light et al. 2006). Women, for unknown reasons, also report more pain than men on injection of rocuronium (Mencke et al. 2008), indicating the importance of the single medication.

3.4 General Anesthetics

The state of general anesthesia, a drug-induced absence of all perceptions, can be achieved with a variety of drugs either alone or more often in combination. It is relevant to recall that the first SGD observed in pharmacology regards barbiturates (Holck et al. 1937). In fact, after administration of barbiturates, female rats have a longer sleeping time than male rats. General anesthetic agents are classified according to the route of administration as intravenous (barbiturate, propofol, etomidate) and inhalatory (enflurane, isoflurane, desflurane, sevoflurane, nitrous oxide) drugs.

Importantly, neurosteroides such as a progesterone derivate, pregnenolone, have anesthetic properties (Merryman et al. 1954). Increased production of progesterone during the luteal phase of the menstrual cycle (Erden et al. 2005) and pregnancy (Gin and Chan 1994; Chan et al. 1996) can decrease anesthetic drug requirements.

However, it is still unclear if sex–gender significantly modifies the sensitivity to general anesthesia (Eger et al. 2003; Wadhwa et al. 2003). It is now emerging that the postoperative outcomes and, in particular, speed of recovery from general anesthesia could be sexually dimorphic [(Pleym et al. 2003; Buchanan et al. 2009) and quoted literature]. In particular, many authors observed a faster emergence time from anesthesia in women than men (Gan et al. 1999; Hoymork et al. 2000, 2003; Myles et al. 2001; Hoymork and Raeder 2005; Bajaj et al. 2007), although some authors suggest that SGD are clinically not relevant (Moller and Glass 2003). It has been also described that women have higher bispectral index scores (BIS) at similar concentrations of anesthesia. Notably, plasma progesterone concentrations in women negatively correlated with the time to eye opening (Buchanan et al. 2009, 2011).

3.5 SGD with Propofol

Propofol is a non-barbiturate intravenous anesthetic agent that became widely adopted for the induction and the maintenance of anesthesia as well as for sedation during regional anesthesia or intensive care (Brunton et al. 2011). Individual variation in the pharmacokinetics and dynamics of intravenous anesthetics is considerable even if the dose is adjusted to weight; therefore, it is difficult to individuate SGD. In addition, various studies have not included sex–gender determinant; thus no conclusive data are available because the influence of sex–gender on pharmacokinetic derives from post hoc analyses. Nevertheless, Shafer et al. (1988) show that women have higher clearance and higher distribution volume compared with men. In line with these data, it has been found that males have higher plasma level of propofol than females, despite a similar dosing (Schnider et al. 1998; Ward et al. 2002; Hoymork and Raeder 2005) suggesting that propofol kinetics is sexually dimorphic as the clearance is indirectly affected by the relatively smaller lean body mass in women. In contrast, others (Schuttler and Ihmsen 2000) do not find any significant SGD in propofol kinetics. The young and old females (>65 years) seem to need 10% higher infusion rates than males (Vuyk et al. 2001; Hoymork and Raeder 2005) to have the same blood propofol concentrations. It has also been shown that women require more propofol to achieve the same depth of anesthesia, monitored by BIS or Narcotrend (Kraus et al. 2003; Kreuer et al. 2003). Notably, women have a faster emergence time from anesthesia than men (Gan et al. 1999; Myles et al. 2001; Hoymork et al. 2000, 2003; Hoymork and Raeder 2005; Bajaj et al. 2007).

The described pharmacokinetic differences partially explain the SGD, but some data suggest the presence of pharmacodynamic SGD because, at least in a cohort of patients, plasma progesterone concentrations in women are negatively related with the time to eye opening (Buchanan et al. 2009).

3.6 SGD in ADE Induced by General Anesthetics

Several reports reveal that women are more exposed to ADE than men (Franconi et al. 2007, 2011). General anesthetic use also leads more frequently to ADE in women than men with the exception of malignant hyperthermia. This rare ADE triggered by agents such as desflurane, sevoflurane, and isoflurane shows a higher prevalence in males than females (Brady et al. 2009). Whereas to be a women, it is the most important patient-related risk factor for the occurrence of postoperative nausea and vomiting at least in the postpubertal age group (Gan 2006). Importantly, a number of operations frequently performed in women (laparoscopic surgery, breast surgery, and major gynecological surgery) carry to an increased risk for nausea and vomiting. Women, in fact, require more treatment for nausea and vomiting (Buchanan et al. 2011).

Volatile anesthetics may prolong the QTc interval (Owczuk et al. 2005) and this may result in severe cardiac arrhythmias. It is well known that to be a woman is a risk factor for drug-induced long QT syndrome with two-thirds of all cases of drug-induced torsades de pointes occurring in females (Drici and Clement 2001). It has also been described that both intravenous and volatile anesthetics may cause greater decrease in blood pressure, with reflex increase in heart rate in women than in men (Daelim and Hi-Lim 2004). Conversely, sympathetic activation from laryngoscopy and tracheal intubation may lead to greater increases in blood pressure in men than in women (Daelim and Hi-Lim 2004).

Finally, the duration of recovery room is longer in women and the 40-item quality of recovery for the first 3 days is lower in women than in men (Buchanan et al. 2011).

3.7 Local Anesthetics

Information on the effect of sex–gender on local anesthetic is very scanty and does not permit any conclusion (Betts et al. 1995; Robinson et al. 1998; Pleym et al. 2003; Li et al. 2010), and a recent editorial declares "sex-based differences in the efficacy of local anesthetics have not been demonstrated to date" (Benhamou 2011). Nevertheless, it has been reported that lidocaine could have a larger distribution volume in women than in men (Pleym et al. 2003) and ADE with local anesthetic seems to be more frequent in females than in males (Nazir and Holdcroft 2009).

Take Home Message
Our knowledge of SGD in anesthesiology is scant and needs additional research. However, anesthesiologists should pay attention to this topic to improve the practice of anesthesia because SGD in pharmacokinetics and pharmacodynamics of anesthetic drugs seem to occur and they seem to be of relevance for propofol and non-depolarizing muscle relaxants. SGD with volatile anesthetics are less known and they regard the drug safety (Tanifuji et al. 1988; Zhou et al. 1995; Gin and Chan 1994; Chan and Gin 1995; Eger et al. 2003; Pleym et al. 2003; Booij 2008; Zacny and Jun 2010; Brunton et al. 2011).

4 Analgesic Drugs

4.1 Opioid Drugs

A number of evidence suggests that the response to pain is a sexually dimorphic process (Toomey 2008). Pain is a complex phenomenon regulated by a variety of psychological, cellular, and hormonal modulations including sexual ones and there are evidences that sex–gender could be a factor that could modify the response to analgesic drugs (Pleym et al. 2003; Niesters et al. 2010). Recently, it has become apparent that sex–gender is important for pharmacological activity of opioid drugs (Rasakham and Liu-Chen 2011). SGD are reported for alfentanil, which seems to

have lower plasma level in women (Pleym et al. 2003), and buprenorphine, a μ-receptor partial agonist (Brunton et al. 2011), and its metabolites, that have higher area under the plasma concentration curves and maximum plasma concentrations in women than in men (Moody et al. 2011). The authors attribute these differences to the impact of body composition.

SGD also exist in morphine-induced analgesia in both experimental pain studies and clinical studies, with greater morphine efficacy in women (Niesters et al. 2010). Males, need higher morphine doses than females to achieve equivalent pain relief (Niesters et al. 2010). However, using cold pressure test and mechanical pressure test, it has been evidenced that morphine induces similar analgesic effects in men and women (Comer et al. 2010), indicating that more studies are necessary to clarify the presence of SGD in therapy with opioids.

The data on non-morphine μ and mixed μ/κ-opioids are less convincing and require further studies. Indeed, the κ-opioid ligands (pentazocine, nalbuphine) seem to produce greater analgesia in women than in men (Gear et al. 1999; Pleym et al. 2003; Rasakham and Liu-Chen 2011). In conclusion, women may be more vulnerable to opioid therapy than men.

SGD have also been seen in patient-controlled epidural analgesia (opioid plus local anesthetics) after major surgery. Women, in fact, show lower total epidural analgesia consumption compared to men being also influenced by body mass index and vomiting (Schnabel et al. 2012). Indeed, women have a greater motor block than men, which with vomiting could explain the lower consumption of epidural analgesia of women in comparison with men (Schnabel et al. 2012).

4.2 Psychosocial Factors

Finally, we remind that psychosocial factors profoundly affect the experience of pain and coping with it (Darnall and Stacey 2012). For example, women with solicitous spouses are more likely to use greater amounts of opioids; the same is not true for men with solicitous spouses (Darnall and Stacey 2012). The same authors, however, evidence that men report significantly greater positive mood effects, whereas women report more negative mood effects compared to men confirming the importance of psychosocial factors in analgesic therapy. A very recent meta-analysis evidences that subjects who considered themselves more masculine are less sensitive to pain, indicating that gender stereotypes specifically affect pain response (Alabas et al. 2012).

4.3 SGD in ADE Induced by Opioid Analgesics

Notably, women have more ADE with opioid administration. In particular, women report increased nausea and vomiting and higher ratings of “sluggish feeling” and “dry mouth” than men (Zacny 2001; Zun et al. 2002; Cepeda et al. 2003; Fillingim

et al. 2005; Bijur et al. 2008). Additionally, females experience respiratory depression more frequently than males [(Franconi et al. 2007) and quoted literature]. Both men and women are at risk of opioid-induced endocrinopathy (Vuong et al. 2010), but women have specific features. Fertile women have amenorrhea and infertility and postmenopausal women have a reduced level of sex steroids (Daniell 2008). The previous hormonal imbalance is linked with more pain intensity and secondary complications (Daniell 2008). Please recall that opioid use in pregnancy can induce fetal malformations (Broussard et al. 2011).

4.4 SGD with Nonsteroidal Anti-Inflammatory

Sex–gender-specific analgesic responses to nonsteroidal anti-inflammatory drugs have been scantily explored. Some authors (Walker and Carmody 1998; Butcher and Carmody 2012) indicate ibuprofen as a compound more active in men than in women. This is not a general finding for the majority of this kind of agents (Pleym et al. 2003). Pharmacokinetic differences have been described for paracetamol and aspirin, whereas the ibuprofen pharmacokinetic is similar in men and women (Pleym et al. 2003).

Take Home Message

A deeper understanding of the underlying events in analgesia in men and in women may be essential to gain insights into pain therapy. Additionally, the effect of sex–gender on pain therapy needs more research focused either on the biological aspect of pain or on the psychosocial aspects.

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Psychopharmacological Treatment of Mood and Anxiety Disorders During Pregnancy

Stephanie Krüger

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Abstract Many women with psychiatric disorders want to become mothers and only a minority seek advise prior to becoming pregnant. In those women, in whom pregnancy can be planned, the decision, if a medication is required for stabilisation and which one to choose if this is the case, is easier to make than in women in whom pregnancy occurs unplanned. The physician has to weigh the risk that a relapse of the psychiatric disorder during pregnancy poses to the foetus against the reproductive risk of psychotropic drugs. This presentation is intended to assist in understanding the general principles of pharmacotherapy during pregnancy as well as the morphological, perinatal and neurobehavioural toxicity of antidepressants, antipsychotics, benzodiazepines and mood stabilisers.

Keywords Antidepressants • Antipsychotics • Benzodiazepines and mood stabilisers • Pregnancy

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1 Introduction

Many women with psychiatric disorders want to become mothers, despite the well-known risk of illness recurrence during and after pregnancy and despite the reproductive risks of psychotropic medications.

The situation is even more complicated by the pregnancy-related hormonal changes which may produce unaccustomed mood swings and physical discomfort that may exacerbate existing psychiatric disorders or generate new ones (Steiner et al. 2000).

The prevalence of psychiatric disorders in pregnancy is high: up to 10% of women develop a depressive episode, 5% of women with unipolar depression relapse, 23% of women with bipolar disorder relapse, especially when not receiving mood stabilising medication and up to 15% of women experience a clinically relevant anxiety disorder (Viguera et al. 2011). In the postpartum, the risk of relapse is 3.5 times higher than during pregnancy (Viguera et al. 2011).

Thus, physicians will eventually have to advise women on whether they should take medications in order to prevent psychiatric disorders and if yes, which ones are safe for the unborn. Naturally, there are many insecurities on both sides—women's and physicians', on which is the right decision and whether any one decision is ever risk-free.

This chapter will summarise clinical aspects of pharmacological treatment of mood and anxiety disorders during pregnancy. The objectives are:

1. To present principles of psychopharmacological treatment during pregnancy.
2. To discuss the risk–benefit assessment for psychotropic drugs against the background of their reproductive toxicity.

2 General Principles of Psychopharmacology During Pregnancy

It is very important to listen to the women's needs and to educate them about the risks and benefits of treatments for their psychiatric condition.

Ideally, the woman comes for advice prior to becoming pregnant as it is incomparably more difficult to adjust medication once pregnancy has occurred.

Unfortunately, many pregnancies in women with psychiatric disorders occur unplanned and it is often too late to adjust medication in order to avoid potential damage to the foetus.

Most clinicians would agree that ideally, psychiatric symptoms during pregnancy should be treated with non-pharmacological interventions. However, many women are already receiving medication for an existing disorder that would most certainly get worse if the medication was discontinued. Furthermore, the high rate of de novo occurrence of severe psychiatric disorders during pregnancy often calls for rapid, fast acting pharmacological interventions and thus the decision to either stop existing or not initiate pharmacotherapy must be carefully reviewed.

There are several clinical situations in which the risks associated with failing to treat psychiatric conditions are greater than the risks associated with drug therapy.

These include suicidality, failure to eat or drink, mania with disinhibited behaviour, psychotic symptoms which cause the expecting mother to harm herself or the unborn, obsessive-compulsive disorder with obsessions about killing the unborn or inability to attend prenatal care appointments because of depression or severe anxiety. Also, concomitant alcohol and drug use as well as excessive smoking during pregnancy pose a significant risk to the unborn (Sawada Feldman et al. 1962).

Untreated psychiatric conditions may have severe impact on the foetus: developmental delays, small for date babies, stillbirths and poor neonatal adaptation have all been associated with untreated maternal illness.

In addition, violent behaviour, foetal abuse, neonaticide and attempts at premature self-delivery have been reported in mothers whose psychiatric illness during pregnancy was either not diagnosed or not treated adequately (Kauppi et al. 2008, 2010; Barr and Beck 2008).

Thus, the development of an appropriate treatment strategy during gestation is mandatory. The following factors should be considered here (Steiner et al. 2000):

1. The symptom profile and severity of the current psychiatric condition
2. Prior psychiatric history with a focus on episodes associated with the reproductive cycle (PMDS, previous ante- or postpartum episodes)
3. History of illness with a focus on number and severity of recurrences and length of the symptom-free interval
4. Prior treatment and response to psychotropic drugs including current medication
5. History of psychosocial problems
6. Presence of familial conflicts, e.g., parental deprivation, abuse, potential conflicts about being pregnant
7. Stability about coping with the stresses associated with pregnancy
8. Stability about dealing with the physical changes associated with pregnancy
9. Social support systems
10. Medical history, especially previous miscarriages and abortions
11. Known congenital disorders in the family or own handicapped/challenged child
12. Family history of psychiatric disorders

Benefits gained from psychopharmacological treatment must be weighed against the potential reproductive risk to the foetus. No drug has been approved by the EMEA or the FDA for the use during pregnancy; thus no decision can ever be risk-free. All drugs cross the placenta mostly by diffusion. There are several factors associated with how much medication is transferred to the foetus, including drug characteristics, the mother's weight and the maturation of the placenta.

The foetus can metabolise medications to a certain degree via its own hepatic enzyme system, which appears about 8 weeks of gestation. However, this system plateaus mid pregnancy and is not able to perform complex metabolic tasks such as glucuronisation. This is possible 6 weeks after delivery and therefore drugs which are mainly metabolised by glucuronisation will lead to major perinatal complications in the child (Soyka et al. 2006).

There are three types of reproductive toxicity caused by psychotropic drugs, or drugs, alcohol and nicotine in general.

1. Teratogenicity means malformation of limbs or organs. The incidence of major birth defects is 2.6–4% in Europe and the USA and the cause of the majority of these defects is not known (AMA 1983). The genetic susceptibility of each organ/limb to develop malformations varies broadly. Each drug has its specific target organ.
   The problem with reporting drug-induced teratogenic effects is that there is a bias against publication of no or insignificant differences between comparison groups, which may lead to exaggerated estimates of teratogenic risks.
   In addition, for ethical reasons, no double-blind, placebo-controlled trials can be performed to assess the teratogenic risk of psychotropic drugs; therefore, information comes from small prospective studies, spontaneous reports and for some drugs from registries, which may be national or international.
2. Perinatal or neonatal toxicity means side effects, withdrawal symptoms or signs of drug dependence in the child. These usually appear as poor neonatal adaptation that often requires intervention (Cupit and Rotmensch 1985).
3. Behavioral teratogenicity describes postbirth delays or other abnormalities in the behaviour or in the intellectual, emotional and motor development of the child after in utero exposure to psychotropic drugs. Behavioural teratogenicity can occur throughout pregnancy because the functional development of the brain is extremely sensitive to teratogens. It is difficult to associate developmental delays or behavioural abnormalities in the child with drug exposure as a causal connection between the two cannot be proven. Behavioural abnormalities, for example, may also occur because of the mother's mental disorder and a pathological interaction between her and her baby. This may also be due to a genetic disposition for mental disorders in the child itself (Cupit and Rotmensch 1985).

The pregnant woman herself is at increased risk from pharmacological treatment as she may be more vulnerable to side effects. The physiological changes occurring during pregnancy, for example, progesterone-induced delayed gastric emptying, decreased protein-binding capacity, increased renal elimination, increased uterine blood flow and increased glomerular filtration can alter medication metabolism and lead to increased or decreased serum concentrations (Livezey and Reyburn 1992; Wadelius et al. 1997).

It is mandatory to inform the female patient about the risks and benefits of the possible treatment options. Careful documentation of the decision-making process and of the information given to the patient is important. In cases, where birth defects occur, these must be reported. If the physician is convinced that either because of the severity of the mental disorder or because of the type, dose and combination of pharmacotherapy, or because of both, a pregnancy cannot be recommended or is explicitly advised against, the woman should be informed about this and this proceeding should be documented.

It is important to stress the fact that it is impossible to come up with a complete risk–benefit profile. As pointed out before, no decision can ever be risk-free and the woman should be aware of this.

Ideally, the woman comes for advise prior to becoming pregnant, so that the necessary steps can be taken in advance.

3 Reproductive Toxicity of Psychotropic Medication During Pregnancy

3.1 Antidepressants

3.1.1 Tricyclic Antidepressants

General Information

Tricyclic Antidepressants (TCAs) are metabolised via hepatic mechanisms. Hepatic metabolism, however, is increased during pregnancy and thus, progressively increasing doses of TCAs are required. There are studies showing that during the last trimester TCAs need to be dosed up to twice the non-pregnant dose in order to achieve sustained remission from depression (Dalmizrak et al. 2011).

Teratogenic Toxicity

To date, there are no studies showing an increased risk for morphological teratogenicity of TCAs, although some reports found dysmelia associated with amitriptyline (Pariante et al. 2011; Misri and Sivertz 1991).

Perinatal Toxicity

TCAs have been associated with severe neonatal adaptation problems, since the hepatic system of the newborn is not capable of metabolising these drugs. Symptoms include icterus, hepatitis, seizures, cyanosis, tachypnoea, tachycardia, irritability, tremor, urinary and bowel retention and sweating. Because of these symptoms, TCAs are not recommended during pregnancy (Wen and Walker 2004).

Behavioural Toxicity

Several studies have been performed to evaluate behavioural toxicity in children exposed to TCAs during pregnancy. They found normal motor skills and behaviour up to 3 years after birth. Nulman et al. performed a study comparing TCA-exposed children with a control group. The groups were tested at birth and again at 16 and 86 months. There were no differences between the groups with respect to motor and

emotional development as well as global intelligence (Misri and Sivertz 1991; Nulman et al. 1997).

3.1.2 Selective Serotonin Reuptake Inhibitors

Teratogenicity

Until 2009, selective serotonin reuptake inhibitors (SSRIs), in general, were considered safe during pregnancy with respect to causing major malformations (Einarson and Einarson 2005). However, when analysing the different substances subsumed under this group, it became clear that a general statement on the safety of these drugs cannot be made.

A recent study of teratogenicity data from 151,800 births in Denmark reported that 4.9% of children born to mothers who took SSRIs early in pregnancy had congenital malformations, compared with 3.4% of children born to mothers who did not take SSRIs during pregnancy, yielding a 1.34 increased relative risk (Pearlstein 2008 Wogelius et al. 2006). The common malformation types were cardiovascular (29%), muscle and bone (31%) and digestive organ (14%). As in many studies, this study did not control for underlying maternal psychiatric disease. Two recently published large case–control studies have reported a small increased risk of omphalocele, craniosynostosis and anencephaly with early pregnancy use of SSRIs as a group (Alwan et al. 2007) as well as an association of sertraline with omphalocele and of paroxetine with right ventricular outflow tract obstruction defects (Louik et al. 2007). However, the absolute risks were low. Other studies have reported a lack of elevated congenital malformation rates with SSRIs (Hendrick et al. 2003; McElhatton et al. 1996; Sivojelezova et al. 2005; Ericson et al. 1999).

Three recently published meta-analyses on paroxetine exposure in pregnancy and cardiovascular malformations have not been consistent (Hendrick et al. 2003; Wen et al. 2006; Malm et al. 2005): in the meta-analysis published by Bar-Oz et al. (2007), first trimester paroxetine exposure was associated with a significant increase in the risk of cardiac anomalies.

In this study, significantly more women receiving paroxetine used the medication for anxiety or panic disorders compared to women using other SSRIs. Detection bias was suggested as a contributing factor to the observed risk of cardiovascular malformations with paroxetine. In the meta-analysis published by O'Brien Bar-Oz et al. (2008), no increased risk of congenital malformations was associated with paroxetine. Cardiac malformation rates were similar and within population norms. In the meta-analysis published by Wurst et al. (2010), there was an increased risk for combined cardiac defects with first trimester paroxetine use.

The definition of cardiovascular malformations varied among studies, some including small septal defects, while others excluded them. The inconsistency across these studies may be explained by differences in study design, by confounding factors, for example, maternal underlying psychiatric disorder, coadministered

medications, lifestyle factors (smoking, drinking), maternal BMI and diabetes, or they may be spurious. Overall, there are over 33,000 reported pregnancy outcomes after prenatal exposure to various SSRIs.

In a recently published review by Diav-Citrin and Ornoy (2012), the overall rate of major congenital anomalies and of cardiovascular anomalies in the published prospective studies after prenatal exposure to SSRIs was calculated as (Pastuszak et al. 1993; Chambers et al. 1996; McElhatton et al. 1996; Goldstein et al. 1997; Wilton et al. 1998; Kulin et al. 1998a, b; Sivojelezova et al. 2005; Einarson et al. 2008; Diav-Citrin et al. 2008; Oberlander et al. 2008) 3.8% (189/4,920) and 0.9% (53/6,094), respectively, both well within their baseline risk in the general population.

The authors concluded that "the majority of the prospective studies (on SSRIs) have not shown an increase in the overall risk of major malformations. The studies which have suggested that SSRIs may be associated with a small increased risk for malformations were particularly with paroxetine."

Preterm Delivery

SSRIs have been associated with the risk of preterm delivery.

However, study results are inconsistent: in a Finnish study, there was no increase in the rate of preterm delivery (Malm et al. 2005). In other studies, the risk of both low birth weight and preterm delivery was increased in infants who were born to mothers who had received SSRI therapy (Simon et al. 2002; Wen et al. 2006; Reis and Källén 2010). The rate of preterm delivery was calculated to be twice as high in women exposed to SSRIs (14%) compared to non-exposed women (SSRIs). In most of these studies, neither the gestational age at which SSRIs were first taken, nor the underlying psychiatric disorder were controlled for and are thus a confounder.

In summary, associations were found in some studies between the use of SSRIs during pregnancy and risk of preterm delivery. Most of these studies are potentially confounded. If an SSRI is selected for depression treatment during pregnancy, it should either be citalopram/escitalopram or sertraline.

Perinatal Toxicity

SSRIs readily cross the placenta and perinatal symptoms have been described in up to 30% of neonates exposed to SSRIs late in pregnancy (Levinson-Castiel et al. 2006, Rampono et al. 2009). They occur primarily following prenatal exposure to fluoxetine, but also to paroxetine and sometimes other SSRIs (Zajecka et al. 1997; Stahl et al. 1997; Stiskal et al. 2001; Costei et al. 2002; Trenque et al. 2002; Källén 2004; Sanz et al. 2005, pp. 47–52). However, with the exception of fluoxetine, they tend to be mild and are self-limited. Perinatal symptoms comprise poor neonatal adaptation, irritability and tremor. In rare cases, seizures have been observed.

3.1.3 Persistent Pulmonary Hypertension

One specific issue related to SSRI exposure is the occurrence of persistent pulmonary hypertension (PPH) of the newborn (Chambers et al. 2006; Källén and Otterblad-Olausson 2008), which has been calculated to be <1%. In the study which used data from the Swedish Medical Birth Register (Källén and Otterblad-Olausson 2008), the 11 infants whose mothers reported the use of SSRI in pregnancy and had PPHN survived the neonatal period. Other studies, however, did not find an association between SSRI exposure and PPH. These studies were possibly underpowered. Mode of delivery may also be associated with PPH, as one study reported caesarean delivery prior to the onset of labour to lead to PPH. None of the women included in this study had used SSRIs in the second half of pregnancy (Wilson et al. 2011).

In conclusion, an absolute risk of <1% for PPHN in infants exposed to SSRIs cannot be excluded, although studies are not consistent.

Neurobehavioural Toxicity

None of the reports investigating potential neurobehavioural sequelae of SSRI treatment in pregnancy to date have controlled for maternal depression. As pointed out by motherly depression or in fact any psychiatric disorder in the mother will inevitably impact on the child's development and may impair its emotional, cognitive and maybe even motor capacities. Pawluski (2011) has reviewed preclinical and clinical data on this topic and concludes that untreated depression and a dysregulated stress hormone system will negatively influence hippocampal function and may cause delayed cognitive development in the offspring, whereas treatment with SSRIs may reverse this procedure. Regardless, no conclusions can be drawn from this as most data come from animal studies.

3.1.4 Other Antidepressants

Teratogenic Toxicity

There is a paucity of data with respect to the reproductive toxicity of SNRI, mirtazapine, bupropion, agomelatine and (ir)reversible MAO inhibitors (for review, see Patil et al. 2011).

About 1,500 pregnancies have been documented on bupropion, only one was associated with an increased risk of major malformations (Patil et al. 2011). The authors found an increased risk of left outflow heart defects in newborns whose mothers had taken bupropion during the first trimester; however, they had not controlled for smoking and psychiatric disorders in their study design. Thus, these results need to be interpreted with caution.

About 170 cases of venlafaxine exposure have been documented, none of which was associated with an increased risk of major malformations (Patil et al. 2011).

For the other antidepressant drugs, data are even more scanty and no reliable information can be given on their reproductive safety.

Perinatal Toxicity

Placental transfer of dual acting antidepressants is significant, whereas bupropion does not cross the placenta equally readily (Rampono et al. 2009). Several studies albeit with small numbers of subjects have confirmed perinatal complications after intrauterine exposure to both bupropion and venlafaxine: poor neonatal adaptation including respiratory stress, feeding difficulties and tachypnoea have been observed in up to 30% of the cases and seizures have been noted in one case after venlafaxine and another case of bupropion exposure (Patil et al. 2011). No data are available for the MAOIs, mirtazapine, agomelatine and duloxetine.

Neurobehavioural Toxicity

There is one study in which an increased risk of ADHD was observed in children whose mothers had taken bupropion during pregnancy. However, maternal psychiatric illness and possible genetic aspects of the ADHD were not accounted for in this study. Until further studies have confirmed these findings, no final conclusions can be drawn (Patil et al. 2011; Pawluski 2011).

In summary, none of these antidepressants can be recommended for first-line depression treatment during pregnancy. If citalopram or sertraline is not sufficient in treating depressive symptoms, bupropion and venlafaxine can be considered with the necessary caution.

3.1.5 Antipsychotics and Pregnancy

The majority of antipsychotics has not been well researched with respect to their reproductive toxicity (Gentile 2010). Regardless, these substances are widely used across almost all psychiatric disorders and are often prescribed in women of childbearing age.

3.1.6 Butyrophenones and "Typical" Antipsychotics

Teratogenic Toxicity

Reviews of studies involving haloperidol have not suggested increased rates of congenital malformations (Gentile 2010; Seay and Field 1968; Godet and Marie-Cardine 1991; Van Waes and Van de Velde 1969; Diav-Citrin et al. 2005), although case reports of limb defects have led to the suggestion of increased monitoring of the pregnancy during the first trimester.

The findings with phenothiazines have been mixed (Rawlings et al. 1963; Milkovich and van den Berg 1976; Rumeau-Roquette et al. 1975; Slone et al. 1977a, b). Phenothiazines are hardly used anymore in psychiatry; nevertheless, the studies concerning their reproductive safety are reported here. A meta-analysis of first-trimester phenothiazine exposure reported a small increase in the relative risk of malformations (2.4%) relative to the 2.0% risk in the general population.

Perinatal Toxicity

The use of traditional antipsychotics has been associated with extrapyramidal motor symptoms in the newborn, particularly neonatal dyskinesias, tremor, akathisia-like symptoms, hypertonicity, difficulty with oral feeding, apathy and cholestatic jaundice (Sexson and Barak 1989; Mohan et al. 2000; O'Collins and Comer 2003). Phenothiazines are associated with cholestatic jaundice.

Neurobehavioural Toxicity

No adverse effects on behavioural and cognitive functioning in children with in utero typical antipsychotic exposure have been identified, but the data are scanty (Slone et al. 1977a, b).

3.1.7 Atypical Antipsychotics

The "atypical" antipsychotics (clozapine, olanzapine, risperidone, quetiapine, aripiprazole, ziprasidone, asenapine) are heterogenous drugs with equally heterogenous side-effect profiles. They are widely used in affective disorders and schizophrenia; however, metabolic issues such as weight gain and the induction of diabetes mellitus should be considered when prescribing them in women of childbearing age.

Teratogenic Toxicity

Clozapine has not been associated with an increased risk of major malformations.

However, it may cause gestational diabetes in women taking this medication (Stoner et al. 1997; Di Michele et al. 1996; Yogev et al. 2002; Klys et al. 2007; Barnas et al. 1994; Gupta and Grover 2004; Tényi and Trixler 1998; Duran et al. 2008).

With olanzapine, sporadic cases of foetal major malformations, onset or worsening of gestational diabetes have been reported (Biswas et al. 2001; Goldstein et al. 2000; McKenna et al. 2005; Arora and Praharaj 2006; Yeshayahu 2007; Vemuri and Rasgon 2007).

A number of case reports have described positive outcomes in infants exposed to quetiapine. No definitive conclusions can be drawn from this though, because in some cases, the pregnant women had been treated with a combination of psychotropic medications (Tényi et al. 2002; Pace and D'Agostino 2003; Taylor et al. 2003; Gentile 2006; Cabuk et al. 2007). Manufacturer's updated information reassessing spontaneous reports on the reproductive toxicity of quetiapine reveals that no recurrent pattern of anomalies was recorded. Of note is though that in 295 reported cases the outcome was not known. The authors also identified prospectively 36 women treated with quetiapine during early pregnancy: quetiapine was not associated with an increased risk of malformations (Twaites et al. 2007).

Recently, Coppola et al. (2007) published a comprehensive review comprising all prospective and retrospective reports of pregnancies exposed to risperidone received by the Benefit Risk Management, which is a division of Johnson and Johnson Pharmaceutical Research and Development, LLC: 201 unpublished cases of risperidone-exposed pregnancies were identified. A number of cases of birth defects have been reported, but most reports were confounded by the concomitant use of other psychotropic medications. The authors concluded that an increased risk of teratogenicity could not be identified in risperidone-exposed foetuses. However, such results did not derive from true incidence rates but from percentages of voluntarily reported prospective cases or retrospectively identified cases where the subsequent outcome was known or reported. This, however, is not specific for risperidone, but applies to almost all reports on the reproductive toxicity of antipsychotics.

There are six case reports regarding foetal aripiprazole exposure during pregnancy.

In two of these cases, the drug was given after week 20 of gestation. No malformations were documented (Mendhekar et al. 2006a,b; Mervak et al. 2008; Watanabe et al. 2011; Lutz et al. 2010; Gentile et al. 2011).

Ziprasidone caused cardiac malformations in animals. There is one case report that ziprasidone may be associated with cleft palate (Peitl et al. 2010), however, more human data are not available to date.

There is no information on the reproductive risk of asenapine.

Perinatal Toxicity

Clozapine can lead to shoulder dystocia in the newborn, and, in addition, the newborn very often is overweight due to maternal gestational diabetes caused by this drug. Furthermore, newborns exposed to clozapine in the third trimester may experience neonatal adaptation problems including seizures. Infants exposed in utero to clozapine should have a complete blood count obtained to rule out agranulocytosis. Clozapine overdose may cause lethal poisoning of the foetus (Waldman and Safferman 1993; Mendhekar et al. 2003; Karakula et al. 2004; Stoner et al. 1997; Yogev et al. 2002; Klys et al. 2007).

With respect to olanzapine: Newport et al. (2008) investigated the placental passage (defined as the ratio between umbilical cord and maternal plasma concentrations) of different antipsychotic agents. Olanzapine showed the highest amount of placental passage of all antipsychotics causing perinatal complications such as poor neonatal adaptation and metabolic issues requiring admission to neonatal intensive care units.

In the same study, quetiapine showed the lowest amount of placental passage when compared with both typical and specific atypical antipsychotics (risperidone and olanzapine) (Newport et al. 2008). Moreover, drug maternal serum levels did not show relevant changes during pregnancy (Klier et al. 2007). Perinatal complications are thus expected to be low.

In humans, the amount of placental passage of risperidone was quite high, causing perinatal complications. These comprise extrapyramidal motor symptoms and akathisia as well as neonatal adaptation difficulties (Newport et al. 2008).

Aripiprazole is associated with small for date babies and poor neonatal adaptation in dosages up to 10 mg/day (Watanabe et al. 2011; Nguyen et al. 2011; Lutz et al. 2010).

No information is available on the perinatal toxicity of ziprasidone and asenapine.

Neurobehavioural Toxicity

Studies are needed on the long-term effects on child development of atypical antipsychotic use during pregnancy. There is some evidence that clozapine and olanzapine may lead to metabolic issues later in life in children who were exposed to these drugs during pregnancy; however, the results of this study need to be replicated (. . .).

In conclusion, information on the reproductive risk of most atypical antipsychotics is scarce. Typical and atypical antipsychotics are associated with an increased risk of perinatal complications. Clozapine and olanzapine increase the risk of gestational metabolic complications and babies being large for gestational age and with mean birth weight significantly heavier as compared with babies exposed to other antipsychotics. The FDA has rated most antipsychotics pregnancy category C, which means that there is evidence of human foetal risk, but the benefits from use in pregnant women may be acceptable despite the risk. This means that they can be used if the drugs are needed in a life-threatening situation or for a serious disease for which other, possibly safer drugs cannot be used or are ineffective. Hence, first-generation antispychotics should not be the first choice in pregnant women despite their relatively low risk for malformations. When pregnancy occurs during antipsychotic treatment, the current therapy should be continued under close prenatal monitoring.

3.2 Mood Stabilisers

3.2.1 Lithium

Teratogenic Toxicity

Reports from an International Register of Lithium Babies established in the 1970s, based on a voluntary physician reporting system, described an excess of cardiovascular malformations, particularly Ebstein's anomaly (Nora et al. 1974; Weinstein 1976; Grover et al. 2006). The risk for this malformation in infants with first trimester lithium exposure was initially proposed to be 400 times higher than that in the general population. Later, based on a pooled analysis of the data, Cohen et al. (1994) estimated the risk for Ebstein's anomaly following first trimester exposure to be between 1/1,000 (0.1%) and 1/2,000 (0.05%) births. Although the relative risk of Ebstein's anomaly in lithium-exposed infants is 10–20 times higher than in the general population, the absolute risk is small (0.05–0.1%), and lithium thus is considered relatively safe in pregnancy in women who require mood stabilizing medication. (Cohen et al. 1994). Other congenital abnormalities reported include large for gestational age infants (Jacobson et al. 1992), anencephaly (Grover and Gupta 2005) and oromandibular-limb hypogenesis (Tekin and Ellison 2000).

Perinatal Toxicity

Lithium is a drug with considerable perinatal toxicity. The "floppy infant syndrome" includes poor neonatal adaptation as shown in low APGAR scores, muscular hypotonia and cyanosis. Furthermore, neonatal hypothyroidism, nephrogenic diabetes insipidus and polyhydramnios have also been described (Yonkers et al. 1998; Ananth 1976). Considering the risk this conveys to the newborn, it is recommended to discontinue lithium about 2 weeks prior to delivery (Jacobson et al. 1992; Llewellyn et al. 1998). The last trimester is the period of greatest possible stability in women with psychiatric disorders, so a temporary discontinuation of the drug can be tolerated. It is mandatory, however, to reinstitute lithium during the postpartum period.

Neurobehavioral Toxicity

There are two previous reports with very small sample sizes that found no neurobehavioural sequelae in lithium-exposed children aged between 3 and 5 years (Schou 1976a, b; Jacobson et al. 1992).

In a recent observational retrospective cohort study (van der Lugt et al. 2011), 15 children who were exposed to lithium in utero were investigated at 3–15 years of age. One child had signs of a minor neurological dysfunction, but without further clinical implications. The results of the cognitive tests were within normal limits, although most children had lower scores on the performance IQ subtest. Growth, behaviour and general development were within the normal range.

These reports do not permit a final conclusion on the developmental toxicity of lithium.

3.3 *Anticonvulsants and Pregnancy*

3.3.1 Anticonvulsants

The majority of studies on the teratogenic risk of anticonvulsants come from epilepsy. However, many of the described teratogenic effects of anticonvulsants are clearly related to the compounds themselves and not to the underlying epilepsy and thus the findings are applicable to treatment of women with mood disorders. Generalisability is more complicated in issues related to neurobehavioural sequelae of anticonvulsants as here differentiation as to the role of medication vs. the role of the mother's underlying illness in the child's neurodevelopmental delay is not easy to make.

3.3.2 Valproic Acid

Teratogenic Risk

A consistent finding that has emerged in multiple pregnancy registries and prospective pregnancy studies indicates that valproic acid (VPA) causes a significant dose-dependent increased risk to the foetus. Meador et al. (2008) published a meta-analysis that revealed that VPA is associated with a 10.7% (95% CI: 8.16–13.29) risk of malformations, especially spina bifida but also dysmelia and facial dysplasia.

Pregnancy registries demonstrate an increased risk of malformations associated with VPA exposure which is higher than that of the other anticonvulsants. All national and international registries on the use of antiepileptic drugs in pregnancy reported similar results: for example, in a recent publication by Jentink et al. (2010a, b) from the EUROCAT Antiepileptic Study Working Group exposure to VPA monotherapy was recorded for a total of 180 cases. The data set included 98,075 live births, stillbirths or terminations with malformations among 3.8 million births in 14 European countries from 1995 through 2005. As compared with no use of an antiepileptic drug during the first trimester, use of VPA monotherapy was associated with significantly increased risks for 6 of the 14 malformations under consideration; the adjusted odds ratios were as follows: spina bifida, 12.7 (95% confidence interval [CI], 7.7–20.7); atrial septal defect, 2.5 (95% CI, 1.4–4.4); cleft palate, 5.2 (95% CI, 2.8–9.9); hypospadias, 4.8 (95% CI, 2.9–8.1); polydactyly, 2.2 (95% CI, 1.0–4.5); and craniosynostosis, 6.8 (95% CI, 1.8–18.8). Results for exposure to VPA were similar to results for exposure to other antiepileptic drugs.

There seems to be a clear dose dependency: dosages above 1,000 mg/day are associated with the highest risk of malformations. Several large pregnancy

registries, including the North American Pregnancy Registry (Wyszynski et al. 2005) (10.7 vs. 2.9% other AED monotherapies), the Australian Pregnancy Registry (Vajda and Eadie 2005) (17.1 vs. 2.4% other Anticonvulsants), the Swedish Medical Birth Registry (Wide et al. 2004) (9.7 vs. 4.0% carbamazepine), the Finnish National Medical Birth Registry (Artama et al. 2005) (10.7 vs. 3.5% carbamazepine), the United Kingdom Pregnancy Registry (Morro et al. 2006) (6.2 vs. 2.2% carbamazepine) and the International Lamotrigine Pregnancy Registry (Cunnington et al. 2005) (12.5% polytherapy with valproate vs. 2.7% polytherapy without valproate) have confirmed this finding. Based on the clear evidence it is safe to say that VPA should not be used as a first-line prophylactic medication in women with mood disorders in their childbearing years, and if there are no other options, the dose should be limited.

Perinatal Toxicity

Near-term application of VPA, doses >1,000 mg/day or non-sustained release formulations, is associated with neonatal complications like heart rate decelerations, liver toxicity, hypoglycaemia, reductions in neonatal fibrinogen levels and withdrawal symptoms of irritability, jitteriness, feeding difficulties and muscular weakness (Harden et al. 2009a, b, c). However, generally, VPA is compatible with nursing thus reducing these difficulties.

3.3.3 Carbamazepine

Teratogenic Toxicity

Originally, carbamazepine (CBZ) use during pregnancy was thought to be as teratogenic as VPA exposure (Grover et al. 2006). However, recently published data from pregnancy registries have failed to find an increased risk of malformations due to CBZ exposure (Jentink et al. 2010a, b).

For example, Meador et al. reported a malformation rate of 4.6% (95% CI: 3.48–5.76) in a meta-analysis including 4,411 woman taking CBZ throughout their pregnancies. When compared with other antiepileptic drugs, the teratogenic potential of CBZ was significantly lower. With respect to type of malformation, two studies (reviewed in Jentink et al. 2010a, b) have found in utero CBZ exposure to be associated with an increased risk of orofacial clefts and of spina bifida.

Perinatal Toxicity

Carbamazepine is associated with transient hepatic toxicity in newborns exposed to the drug during pregnancy (Frey et al. 1990; Merlob et al. 1992). Of note is that if carbamazepine is used during pregnancy it can cause foetal vitamin K deficiency.

Adequate levels of vitamin K are necessary for normal mid-facial growth and for the functioning of clotting factors and carbamazepine exposure in utero could increase the risk of neonatal bleeding and mid-facial abnormalities. Twenty milligrams per day of oral vitamin K are required in the last month of pregnancy in women taking carbamazepine (Harden et al. 2009b).

3.3.4 Lamotrigine

Originally, lamotrigine (LTG) was thought to be less teratogenic than the other anticonvulsants. However, even smaller sample sizes than that of the other anticonvulsants and uncontrolled clinical trials do not permit recommendations to the safety of this drug.

This is especially true, because recently, data from three registries found that prenatal LTG exposure may increase the risk of craniofacial defects. Cunnington et al. (2011) showed that LTG exposure was associated with a higher prevalence of orofacial clefts. Similar findings were reported by the European Registry for Anticonvulsants in Pregnancy. Morrow et al. (2009), however, observed only a single case of isolated cleft palate in 1,151 LTG monotherapy-exposed pregnancies reported in a UK pregnancy registry. Because of the limited information, it is not yet possible to conclude that LTG exposure is associated with an increased risk for oral clefts. Regardless, women of childbearing age who are treated with LTG should be informed about this potential risk.

Perinatal Toxicity

During pregnancy, LTG clearance has been shown to progressively increase to a peak of greater than 300% at 32 weeks of gestational age compared with that of baseline, prepregnancy levels (e.g., Madadi and Ito 2010). Whether this corresponds with a worsening of psychiatric symptoms during the seventh month of gestation is not entirely clear, but the patient should be monitored closely. After 32 weeks, clearance values steadily decrease and reach prepregnancy levels as rapidly as 1 month after birth. As a result, maternal LTG-induced toxicity is prevalent in the first several weeks postpartum, probably because LTG doses that were increased during pregnancy are not tapered accordingly. Newborns may thus suffer from neonatal adaptation problems including apnoea, decreased muscle tone and sedation (e.g., Harden et al. 2009c).

3.3.5 Pregabaline

None of the pregnancy registries have released data on the reproductive toxicity of pregabaline. This is due to the fact that only a few cases of monotherapy with pregabaline in pregnancy have been reported so far.

3.3.6 Neurobehavioural Toxicity of Anticonvulsants

Accumulating evidence suggests that in utero exposure to anticonvulsants confers a risk of cognitive and behavioural problems: these include low mental development mean scores and significantly lower IQ scores. One study investigated verbal fluency/flexibility and originality in 54 children (mean age: 4.2 years) exposed to CBZ, LTG and VPA. The results revealed that fluency and originality were lower in the VPA group than the LTG and CBZ groups (Meador et al. 2011; Bromley et al. 2009; Shallcross et al. 2011).

While evidence is still scarce, several studies suggest that anticonvulsant exposure results in altered cognitive function even later in development. Kantola-Sorsa et al. (2007) reported that children exposed to AED monotherapy performed poorer on attentional tasks, while children exposed to AED polytherapy performed poorer on auditory attention, sentence repetition and the fine motor task. While too small to allow drug comparisons, a follow-up study of older AED-exposed children (aged 10–20 years) revealed that, with the exception of CBZ, all anticonvulsants had a negative impact on intellectual functioning (Titze et al. 2008). It should be noted, however, that the majority of these studies did not control for maternal illness.

3.3.7 Prophylactic Folate Treatment

It is recommended that high-risk women take 4 mg/day of folic acid to prevent having a child with spina bifida (Van Allen et al. 1993). Additionally, it must be noted that folic acid must be taken prior to becoming pregnant in order to protect against NTDs. At the least, it must be present at day 25 of the pregnancy.

Unfortunately, there is evidence questioning the prophylactic properties of folate in women treated with anticonvulsants, mainly VPA. For example, the North American Pregnancy Registry recently observed that 6.7% of the registry-enrolled infants had a major congenital malformation; maternal periconceptional use of folic acid was not associated with a statistically significant reduction in the risk of having an infant who had a major malformation, including spina bifida (Craig et al. 1999). The problem with this report is that dosage of folic acid was not considered and that as pointed out already, higher dosages of folate supplementation may be needed to prevent neural tube defects in women who are taking anticonvulsants. There are some reports that high dosages of magnesium may be protective against malformations; however, these reports are inconclusive and it has yet to be established whether these findings will lead to clinically relevant recommendations.

In summary, it seems clear that a periconceptional dose of folic acid of 0.4 mg daily is not beneficial in women using antiepileptic drugs. Whether the recommended 4 mg/day dose is more beneficial in protecting against neural tube defects is not supported by data.

3.4 *Benzodiazepines*

3.4.1 Teratogenic Toxicity

Benzodiazepines with a long half-life such as diazepam have been associated with causing orofacial clefts (Safra and Oakley 1975, 1976a, b). These studies, however, had several methodological problems: the information was collected retrospectively and the authors did not control for multiple drug use and for dosage taken. In addition, there is evidence that many women used benzodiazepines without being monitored by their physician and without reporting their use, which raises the question whether the information gathered in these reports is accurate (Leppée et al. 2010).

More recent studies have not found any particular association between orofacial clefts (Marinucci et al. 2011; Rosenberg et al. 1983) or other congenital malformations (Gidai et al. 2008a, b, c). Gidai et al. (2008a, b, c) documented 112 live-born pregnancies with a history of prenatal exposure to doses of diazepam up to 30 mg/day without a higher risk of developing malformations, including orofacial clefts. Wikner et al. (2007) found a higher incidence of pylorostenosis or alimentary tract atresia in newborns exposed to benzodiazepines in the first trimester. However, no correlation between malformation type and the drug's half-life was detected.

3.4.2 Perinatal Toxicity

Late third trimester use and exposure during labour seems to be associated with much greater risks to the foetus/neonate. Many infants, especially those who were exposed to benzodiazepines with a long half-life, exhibited either the floppy infant syndrome or marked neonatal withdrawal symptoms (Rochow et al. 2008; Weinstock et al. 2001; Laegreid et al. 1992). There has been no significant increase in the incidence of neonatal jaundice and kernicterus, though. Symptoms vary from mild sedation, hypotonia and reluctance to suck to apnoea, cyanosis and impaired metabolic responses. These symptoms have been reported to persist for periods from hours to months after birth (Laegreid et al. 1992). This correlates well with the pharmacokinetic and placental transfer of the benzodiazepines and their disposition in the neonate (Hudak and Tan 2012).

3.4.3 Neurobehavioural Toxicity

The prolonged use of benzodiazepines throughout pregnancy raised the concern about neurobehavioural toxicity. There is hardly any information on this topic, though. In ~550 children who were followed up to 4 years of age, no increase on neurobehavioural development and IQ was noted (McElhatton 1994). Although

some of the data indicate that a small number of children were slower to develop during the first year, they seemed to catch up growth and most had developed normally by 4 years of age. Where developmental deficits persisted, it was not possible to prove a cause–effect relationship with benzodiazepine exposure. These children were often from families where maternal illness was not controlled properly or where environmental and social factors negatively influenced the postnatal health and development of the child (McElhatton 1994).

4 Conclusions and Take Home Messages

Assisting the pregnant woman with a psychiatric illness in deciding how to proceed with medication during pregnancy and the postpartum is an important and difficult task.

Treatment choices are affected by many factors including severity and course of illness, perception of the woman on teratogenicity of medications and her likelihood to terminate the pregnancy for fear of these potential risks. Therefore, making the woman an informed participant in the decision-making process is of paramount importance. Ideally, but not always possible, is including the partner in the educational process.

The physician should inform the woman about risks and benefits of the various treatment options and not conceal the risks of the underlying psychiatric illness for the unborn/newborn child.

Once a treatment regimen has been decided upon, it needs to be reassessed throughout pregnancy as the physiological and psychological changes occurring during gestation require repeated modifications of the chosen medication.

Pharmacological treatment during pregnancy must be integrated with psychosocial interventions to ensure optimal outcome that ideally lasts throughout the postpartum period as well.

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Obesity and Diabetes

Alexandra Kautzky-Willer and Rosa Lemmens-Gruber

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Abbreviations

5-HT	5-Hydroxytryptamine
AMPK	5′ Adenosine monophosphate-activated kinase
BMI	Body mass index
CB1	Cannabinoid-1
DPP-4	Dipeptidyl peptidase 4
DPP-4i	Dipeptidyl peptidase 4 inhibitors
EMA	European Medicines Agency
FDA	Food and Drug Administration
GLP-1	Glucagon-like peptide 1

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GLP-1R Glucagon-like peptide 1 receptor
IGF Insulin-like growth factor
IGT Impaired glucose tolerance
PCOS Polycystic ovary syndrome
SU Sulfonylureas

1 Obesity

Obesity and type 2 diabetes are chronic lifestyle diseases which are dramatically increasing worldwide leading to high costs and an enormous burden on the public health care system. Both biological and psychosocial factors, environment and lifestyle are involved in the pathogenesis of these metabolic disorders and thus important sex- and gender-based differences can be found. These differences have also important implications for lifestyle intervention (nutrition and exercise) and drug therapy.

The dramatic increase in the prevalence of overweight and obesity, affecting more than 1.1 billion individuals worldwide, has resulted in a major burden on healthcare resources in developed countries (Field et al. 2009; Hainer 2011). Excess fat mass increases the risk of mortality overall and increases individually the risk of chronic diseases like type 2 diabetes, hypertension, cardiovascular disease, stroke, osteoarthritis, obstructive sleep apnoea and certain cancers with sex-specific differences (Kautzky-Willer 2011; Kautzky-Willer and Handisurya 2009). Although obesity is classified by the World Health Organisation as one of the principal causes of preventable chronic diseases, prevention strategies and treatment options are limited up to now. Ideally treatment of obesity comprehends an ongoing lifelong program including lifestyle (diet, exercise) and behavioural modifications and if necessary drug therapy. Adjunctive pharmacological therapy is currently recommended for subjects with body mass index (BMI) $\geq$ 30 kg/m^2 or those with BMI $>$ 27 kg/m^2 and associated co-morbidities (Snow et al. 2005).

1.1 Drug Therapy for Obesity

Anti-obesity drugs can act through different mechanisms: via suppression of food intake in the central nervous system or by decreasing gut absorption of food and/or by increasing energy expenditure, thermogenesis or oxidation of nutrients (Hainer 2011). Drugs reducing food intake act primarily on neurotransmitters of the central nervous system and include noradrenergic drugs, serotonergic drugs, serotonergic and adrenergic drugs, drugs binding to the cannabinoid receptors and others. The efficacy of anti-obesity drugs should be validated by reduction of fat stores (Table 1), in particular visceral adipose tissue, maintenance of weight loss, reduction of obesity-related health risks and mortality and improvement of quality of life (Hainer 2011). Treatment should be effective and safe and individually tailored considering age, sex and presence of co-morbidities including careful assessment of the risk/

Table 1 Sex/gender differences in existing drugs for the therapy of obesity

Drug/substance	Rimonabant	Sibutramine	Orlistat
Class	Cannabinoid-1 receptor blocker	Serotonin and norepinephrine reuptake inhibitor	Reversible gastrointestinal lipase inhibitor
Sex-specific features	No sex analyses conducted in humans	No information is provided regarding potential sex differences in cardiovascular events in humans	Women report more weight-related psychosocial problems. Men were at 40% greater risk of progression to diabetes
	Animal studies suggest that cannabinoid system may be differentially sensitive in its modulation of appetitive behaviour in males vs. females	In animal studies sex differences in the protection of fluidity of plasma membranes via antioxidant effect were described	Clozapine- or olanzapine-treated overweight/obese patients with schizophrenia showed that men (but not women) experienced weight loss of −2.4 kg, and women showed significant decrease of fasting glucose and triglycerides
		Acceleration of the sperm transit time and decrease in the sperm reserve in the epididymal cauda observed in rats	
		Gender-specific sensitivity to different doses observed in rats	In women with PCOS Orlistat combined with diet improves metabolic and hormonal parameters and the atherogenic profile, decreases the LH: FSH ratio and androgen levels. It reduces total testosterone, serum cholesterol and triglycerides and induces ovulation in obese PCOS patients
Comments	Withdrawn from European market; not approved elsewhere	Withdrawn from market	
References	Ward and Walker (2009)	Bellentani et al. (2011), Guzman et al. (2009), and LeBlanc and Thibault (2003)	Karlsson et al. (2003), Tchoukhine et al. (2011), Diamanti-Kandarakis et al. (2007), Ghandi et al. (2011), Metwally et al. (2009), and Sönmez et al. (2005)

benefit profile. The serotonergic drugs fenfluramine and dexfenfluramine, which caused the release of serotonin to suppress appetite and reduced food intake, were withdrawn from market in 1997 because of heart valve damage. Common reported side effects were palpitations, agitation and nervousness, headache and insomnia (Li and Cheung 2011). However, a preliminary report in a small group of women without any history of cardiac diseases ($N = 24$) receiving fenfluramine–-phentermine revealed unusual valvular morphology resembling ergotamine-induced heart valve disease and newly documented pulmonary arterial hypertension (Connolly et al. 1997). More recently, two other widely used anti-obesity drugs, rimonabant and sibutramine, were withdrawn from the market due to serious side effects. On the other hand, some anti-obesity drugs with potential cardio-excitatory and psycho-stimulatory effects, such as phentermine, ephedrine and caffeine mixtures are still available for short-term use (<3 months) in some countries. Ephedrine and caffeine were shown to increase energy expenditure and thermogenesis.

1.1.1 Rimonabant

Rimonabant, a selective cannabinoid-1 (CB1) receptor blocker, was the first member of a new class of drugs targeting the endocannabinoid system. CB1 receptors are distributed abundantly in the central nervous system but were also found throughout the gastrointestinal tract, in adipose tissue and the cardiovascular system (Sam et al. 2011). Leptin, which controls energy balance, plays an important role in the regulation of hypothalamic endocannabinoids (Di Marzo et al. 2001; Ravinet Trillou et al. 2004). Blockade of CB1 receptors results in an increase of leptin receptors expressed on the membrane of orexigenic neuropeptide Y neurons. Moreover, 5′ adenosine monophosphate-activated kinase (AMPK), which increases food intake (Kim et al. 2004; Minokoshi et al. 2004; Xue and Kahn 2006), is regulated by both leptin and endocannabinoids. Thus, leptin- and rimonabant-caused inhibition of AMPK may result in the observed synergistic effects on food intake (Boustany-Kari et al. 2011). In addition, rimonabant increases the expression of adiponectin by adipocytes (Bensaid et al. 2003; Despres et al. 2005), and this is supposed to be one reason for the improved insulin sensitivity with rimonabant observed in vivo (Ülgen et al. 2011). Inhibition of peripheral CB1 receptors in adipocytes directly promotes transdifferentiation of white adipocytes into a mitochondria-rich, thermogenic brown fat phenotype, resulting in enhanced thermogenesis and insulin sensitivity (Perwitz et al. 2010). Furthermore, retinol binding protein 4 (RBP4), an adipocyte-secreted hormone proposed to link obesity with insulin resistance, is positively associated with markers of inflammation in obese and pro-atherogenic condition. Rimonabant may improve vascular function via modulating retinol binding protein 4 along with proinflammatory cytokines (Mohapatra et al. 2011). In accordance, recent studies have demonstrated that CB1 receptor blockade ameliorated inflammation, endothelial and/or cardiac dysfunction, and cell death in models of nephropathy, atherosclerosis and

cardiomyopathy. Activation of CB1 receptors may play an important role in the pathogenesis of diabetic retinopathy by facilitating mitogen-activated protein kinase activation, oxidative stress and inflammatory signalling (El-Remessy et al. 2011). Therefore, CB1 receptor inhibition may be beneficial in the treatment of this devastating complication of diabetes.

Rimonabant showed reduction of body weight and improved cardiometabolic risk factors in obese patients in several large randomised double-blind placebo-controlled trials (Curioni and Andre 2006). Rimonabant 20 mg per day produced a 4.9 kg greater reduction of body weight vs. placebo in trials with 1-year results. The modest weight loss of ~5% was associated with significantly more adverse effects, especially of nervous system, psychiatric or gastrointestinal origin. A meta-analysis showed that the number needed to harm was 25 individuals for adverse events and 59 for more serious adverse events (Christensen et al. 2007). Patients given rimonabant were 2.5 times more likely to discontinue the treatment because of depressive mood disorders than were those given placebo. This problem could be solved by development of new drugs, because recent observations indicate that peripherally restricted CB1 receptor antagonists retain efficacy in reducing weight and improving metabolic abnormalities in mouse models of obesity without causing behavioural effects predictive of neuropsychiatric side effects in humans (Kunos and Tam 2011).

Although no explicit sex analysis was conducted in these trials most events probably occurred in women because the majority of participants [52–81% in the rimonabant in obesity (RIO) studies] were female. This is not surprising because women in general suffer more from overweight and therefore more often seek medical help and are more willing to take drugs or undergo surgery for reduction of body weight. Depression and mental illness were an exclusion criteria for study participants of the RIO programme. However, depression is often a co-morbidity of overweight/obesity and in particular a common disease in women supporting the hypothesis that severe obesity causes or aggravates depression (Dixon et al. 2003). Use of 20 mg rimonabant was associated with psychiatric side effects in 26% of the participants compared to 14% in those on placebo (Samat et al. 2008). The probability of incidence of suicide was almost doubled in all available studies (including smoking cessation trials) examined by the Food and Drug Administration (FDA) for 20 mg rimonabant versus placebo (OR 1.9), although only two deaths occurred from suicide in the entire database (U.S. Food and Drug Administration Endocrinologic and Metabolic Drugs Advisory Committee 2007). The drug was never approved in the US for treatment of obesity and marketing approval has been removed by the European Medicines Agency (EMA) following the postmarketing surveillance studies.

Women have a doubled life-time risk of major depression compared to males and in obesity this risk is further increased in both men and women. Severely obese subjects, especially younger women with poor body image, are at particular high risk for depression, but they also show sustained improvement with weight loss (Dixon et al. 2003). BMI is strongly associated with the presence of common mental disorders, and this association varies with gender and age (McCrea et al. 2011). In young women the probability of having a disorder increased as BMI

increased, whereas in young men depressive disorders were higher for both underweight and obese men. Age and gender differences must be taken into account when investigating the link between obesity and common mental disorders and efficacy and safety of anti-obesity drugs. In particular, novel drugs affecting the endocannabinoid system should be studied in a sex-specific analysis as growing literature from animal studies suggests that the cannabinoid system may be differentially sensitive in its modulation of appetitive behaviour in males vs. females (Ward and Walker 2009). Moreover, recent genomic studies provide evidence that variants of the CB1 receptor gene (CNR1) alone or in combination with the gene of the serotonin transporter (SLC6A4) contribute to the development of anxiety and/or depression, suggesting that high-risk individuals for rimonabant-associated psychiatric side effects could be identified through genetic testing (Lazary et al. 2011).

1.1.2 Sibutramine

Sibutramine is a serotonergic and adrenergic drug that inhibits the reuptake of serotonin and norepinephrine. 5-Hydroxytryptamine (5-HT) reuptake inhibition is necessary but is not sufficient for sibutramine's efficacy in humans, supporting preclinical data suggesting that the hypophagic effect requires the co-inhibition of both serotonin and norepinephrine transporters (Talbot et al. 2010). The two 5-HT receptors most critically involved in the control of feeding behaviour and body weight homeostasis are the 5-HT_{1B} and 5-HT_{2C} receptors in the central nervous system (Bello and Liang 2011; Dalton et al. 2006; Garfield and Heisler 2009). Serotonin activates the precursor pro-opiomelanocortin (POMC) to the anorectic peptide α-melanocyte stimulating hormone (α-MSH) and inhibits orexigenic neuropeptide Y/agouti-related peptide (NPY/AGRP) to reduce food intake. Findings with 5-HT_{2C} receptor knockout mice indicated that these animals had elevated fasted blood glucose and insulin levels and impaired glucose tolerance compared with wild types (Nonogaki et al. 1998). Furthermore, it was demonstrated that 5-HT_{2C} receptor agonists significantly improve glucose tolerance and hyperinsulinemia in murine models of obesity and type 2 diabetes via an melanocortin-4 (MC4) receptor-dependent mechanism (Zhou et al. 2007). Also the UCP2 gene may contribute to weight loss and fat change in response to sibutramine therapy (Hsiao et al. 2010).

Sibutramine suppresses appetite, causes satiety and stimulates thermogenesis mainly through its active metabolites, *N*-desmethyl and *N*-bisdesmethyl sibutramine (Nisoli and Carruba 2000). It is metabolised by the enzymes Cytochrome P450 2B6 (CYP2B6) and Cytochrome P450 2C19 (CYP2C19). Clopidogrel as an inhibitor of CYP2B6 and CYP2C19 significantly increased the half-life and area under the plasma concentration–time curve of sibutramine and decreased the clearance of sibutramine. Thus, clopidogrel may increase the adverse effects of sibutramine (Bae et al. 2011).

A meta-analysis of 11 orlistat trials and 5 sibutramine trials lasting at least 1 year showed that patients given orlistat lost 2.7 kg and those given sibutramine 4.3 kg

more weight than did those taking placebo (Padwal et al. 2003). The most frequent side effects in users of sibutramine were headache, insomnia and constipation, but sibutramine was also found to increase heart rate and cause a mean of 2 mmHg increase in both systolic and diastolic blood pressure at a dose of 10–15 mg per day in particular in young and more obese patients (Nisoli and Carruba 2000). Preliminary reports of the 5-year Sibutramine Cardiovascular OUTcomes (SCOUT) trial led to the recommendation to suspend the use of sibutramine by the EMA and FDA 2010 because this drug may pose unnecessary cardiovascular risks to patients. The effects of sibutramine on the autonomic nervous system are complex as the drug might have opposing effects on peripheral and central sympathetic activity (de Simone et al. 2005). The SCOUTtrial which included more than 10,000 overweight/obese subjects (42% women) with cardiovascular disease, hypertension or type 2 diabetes showed an increased risk of serious non-fatal cardiovascular events such as myocardial infarction and stroke in sibutramine vs. placebo after 3.4 years (James et al. 2010). The rates of non-fatal myocardial infarction and non-fatal stroke were 4.1 and 2.6% in the sibutramine group and 3.2 and 1.9% in the placebo group. All analyses were adjusted for sex, and no information is provided regarding potential sex differences in cardiovascular events. However, in animal studies sex differences were described in the protection of fluidity of plasma membranes via the antioxidant effect of sibutramine stimulated by changes in endogenously produced tryptophan levels in rat brains (Guzman et al. 2009).

Sibutramine provoked acceleration of the sperm transit time and decrease in the sperm reserves in the epididymal cauda. This alteration is probably related to the sympathomimetic effect of this drug, as shown by the in vitro assays. In humans, use of this drug may present a threat for male fertility (Bellentani et al. 2011).

Male and female rats' food intake differed according to sibutramine doses. For example at 2 h post-administration, the lower doses (2.5 and 5 mg/kg) had a stronger effect on the female rats' carbohydrate-rich diet intake than in male rats' intake indicating that female rats could be more sensitive to lower doses of sibutramine shortly after administration. In contrast, sibutramine at a dose of 10 mg/kg was shown to consistently decrease carbohydrate and fat intake regardless of sex and diet. All three doses of sibutramine resulted in significant weight loss in the male rats the day following the gavage day, but the weight loss was not always significant for the female rats. Therefore, the possible gender-specific sensitivity to different doses of sibutramine should be addressed in further studies (LeBlanc and Thibault 2003).

Presence of hypothalamic neuropeptides, their metabolites and the interaction with their receptors should also be taken into account in future studies as neurotransmitters play an active role in control of hunger.

1.1.3 Orlistat

Orlistat is a reversible gastrointestinal lipase inhibitor which inactivates hydrolysation of dietary fat and reduces fat absorption by ~30%, thereby decreasing energy intake of patients (Li and Cheung 2009). The non-absorbed unbroken

triglycerides as well as orlistat are eliminated in the faeces. Therefore, orlistat has no systemic action. It is approved for long-term weight management (Ioannides-Demos et al. 2006). The most common side effects include diarrhoea, flatulence, bloating, abdominal pain and dyspepsia (Snow et al. 2005). Long-term decrease in fat absorption can result in deficiency of fat-soluble vitamins A, D, E and K. Its role in adolescent overweight management is limited by the FDA to those aged 12–18 years old and having BMI more than two units above the 95th percentile for age and gender. The use of orlistat for 1 year appeared to be safe in adolescents and was associated with a modest but significant decrease of weight vs. placebo (Godoy-Matos et al. 2009). Its use in paediatric patients is limited by the side effects fatty stools and possible malabsorption of fat soluble vitamins. Orlistat may also be considered as adjunctive therapy in patients with binge-eating disorders but there are only small trials giving orlistat in combination with or without cognitive behavioural therapy mostly for short periods and predominantly in women (Golay et al. 2005). The XENDOS study (Torgerson et al. 2004) was performed over a period of 4 years and included 3,305 obese patients (55% women) with normal (21%) and impaired glucose tolerance (IGT, 79%) in Sweden. This RCT aimed to investigate the effectiveness of orlistat plus lifestyle changes (diet, exercise) compared to lifestyle modification alone for prevention of type 2 diabetes. Eight per cent of the participants given orlistat and 4% of those given placebo withdrew from the study because of adverse events. Overall orlistat therapy was associated with modest reduction of weight and improvement of cardiometabolic parameters but relative risk reduction of 37% for diabetes (diabetes incidence 6.2% in orlistat-treated vs. 9% in placebo-treated subjects). The number needed to treat (NNT) in IGT for orlistat plus lifestyle was 10/4 years to avoid one case of diabetes. The only documented sex differences were that women reported more weight-related psychosocial problems (Karlsson et al. 2003) and that men were at 40% greater risk of progression to diabetes. Another study showed that orlistat therapy following a very-low-energy diet in patients with metabolic syndrome or type 2 diabetes was associated with better maintenance of weight reduction (−2.4 kg) und lower diabetes incidence over 3 years (Richelsen et al. 2007). Studies in patients with type 2 diabetes also showed a benefit in patients given orlistat in comparison to placebo—in some even without differences in weight loss—with reduced postprandial free fatty acids probably related to effects on triglyceride absorption and postprandial lipemia, improved insulin sensitivity, lower inflammatory and enhanced anti-inflammatory adipocytokines and improved glycaemic control overall (Mancini and Halpern 2008). Of note it was also shown that orlistat can enhance the incretin levels of glucagon-like peptide 1 (GLP-1), which can improve postprandial insulin secretory response and thus postprandial hyperglycaemia and can decrease food intake (Damci et al. 2004). In obese geriatric women, after 6 months of treatment with orlistat and hypocaloric diet, weight loss improved ventricular diastolic function and decreased left ventricular mass. It also contributed to partial improvement in right ventricular systolic function (Varli et al. 2010). Furthermore, an acceptable pharmacoeconomic profile was calculated for orlistat with an estimated average incremental cost–utility ratio of 75.3 € × 1,000/quality-adjusted life year (Iannazzo et al. 2008). A study exploring long-term effects of orlistat in

adult clozapine- or olanzapine-treated overweight/obese patients with schizophrenia showed that men (but not women) experienced weight loss of −2.4 kg (Tchoukhine et al. 2011) similar to that of non-psychiatric population in big trials, but women showed significant decrease of fasting glucose and triglycerides in the long term. In women, however, changes in body weight appeared not to have been related to orlistat treatment but to dietary and behavioural factors. Orlistat combined with diet was also shown to improve metabolic and hormonal parameters and the atherogenic profile and to be associated with a decrease of androgen levels in women with polycystic ovary syndrome (PCOS) independently of BMI changes (Diamanti-Kandarakis et al. 2007). Comparison of the effects of metformin or orlistat on hormone, lipid profile and ovulation status in obese women with PCOS revealed a significant reduction in total testosterone, serum cholesterol and triglycerides. The ovulation rate was higher in metformin-treated patients although the difference to orlistat was not statistically significant (Ghandi et al. 2011). Women in the orlistat group had a 15% ovulation rate which is much lower than in women with significant weight loss by lifestyle and diet modification for 6 months with an ovulation rate of 60–70% (Clark et al. 1995, 1998). These results confirm a previous study on obese anovulatory women with or without PCOS (Metwally et al. 2009), which showed that metformin as well as orlistat can induce ovulation in these patients with slightly higher ovulation and pregnancy rates in the metformin group. Due to the small sample size in this study an independent analysis of PCOS versus non-PCOS obese patients was not permitted. Similar, with acarbose the luteinising hormone:follicle-stimulating hormone (LH:FSH) ratio and total testosterone concentrations decreased and ovulation rates increased in PCOS patients, however, again the rates were lower than with metformin (Sönmez et al. 2005).

1.2 Conclusion and Future Perspectives

The need and market of weight loss drugs is enormous. There is urgent demand for new safe, effective and well-tolerated anti-obesity drugs. It should be considered that one in four patients using anti-obesity drugs has at least on one occasion used a psychotropic drug such as anxiolytics/sedatives or antidepressants concomitantly (Amundsen et al. 2010). This use is extensive in obese patients, especially among women and therefore clinicians should be informed of drug interactions between psychotropic drugs and anti-obesity drugs. The risks of centrally acting weight loss drugs are now recognised and long-term data on cardiovascular events and potential psychiatric effects should be mandatory for approval of new drugs. Of note several new anti-obesity drugs, such as phentermine/topiramate or buproprion/naltrexone were not approved by the FDA due to safety concerns. However, recently published data from the CONQUER trial (56-week phase 3 trial, low dose, controlled-release, phentermine plus topiramate combination plus lifestyle interventions in overweight/obese adults with or without co-morbidities; 70% women) are promising with sustained reduction of weight and improvement of blood pressure and

metabolic parameters (Gadde et al. 2011). Potential sex differences regarding efficacy, safety and outcomes should be reported in all ongoing and future anti-obesity trials as sex and gender differences are obvious in obese subjects with obesity-related co-morbidities but completely neglected in most reports.

2 Diabetes

Various forms of diabetes with different aetiology and pathophysiology can be distinguished: the most important forms are immune-mediated type 1 diabetes, going along with destruction of beta cells and absolute insulin deficiency, and more heterogeneous type 2 diabetes (~90%), representing a disease caused by two prominent defects, insulin resistance and a (relative) defect in insulin secretion, caused by overweight in the majority of cases. For the immune-mediated disease type 1 diabetes with the characteristic feature of absolute insulin deficiency, insulin replacement therapy is absolutely mandatory. These patients need special care and education programmes improving self-management skills. They should be treated by continuous insulin therapy (pumps) or basal–bolus-insulin regimes with multiple daily insulin injections. Puberty might accelerate onset of type 1 diabetes in genetically susceptible females mediated by oestrogen (Gillespie et al. 2005). In general, oestrogen appears to increase insulin sensitivity while higher testosterone levels associate with an increased risk of type 2 diabetes in females; on the other hand, low testosterone levels relate to diabetes risk in males. Sex differences in the insulin sensitivity of adipose tissue, especially the intraabdominal depot, relate to differences in physiological levels of sex steroids and their receptors and the release of adipokines. Females display better whole-body insulin sensitivity despite higher fat content than males, who also more often feature fatty liver disease potentially contributing to their higher progression of the disease.

Diabetes is a chronic progressive disease which needs complex medical management, with varying intensity at different stages, with a focus on management of hyperglycaemia but also targeting associated features such as dyslipidaemia, hypertension, hypercoagulability and inflammation. The goal is to reduce diabetic microangiopathy (retinopathy and nephropathy), macroangiopathy (cardiovascular complications) and neuropathy and to reduce increased mortality. Diabetes management should include a lifestyle intervention program to increase activity levels and to promote weight control (Nathan et al. 2009). There is currently a selection of pharmaceutical treatment options (Fig. 1) with similar absolute reductions in HbA1c (Bolen et al. 2007; Schernthaner et al. 2010) and no clear benefit in one sex. Lowering HbA1c to below or around 7% has been shown to reduce microvascular and in the long term cardiovascular complications if reached early in the course of the disease without hypoglycaemic events. However, the choice of glycaemic goals and drugs used to achieve these treatment goals must be individualised for each patient balancing the potential benefits (efficacy to lower HbA1c, long-term outcomes and adherence, tolerability, non-glycaemic effects,

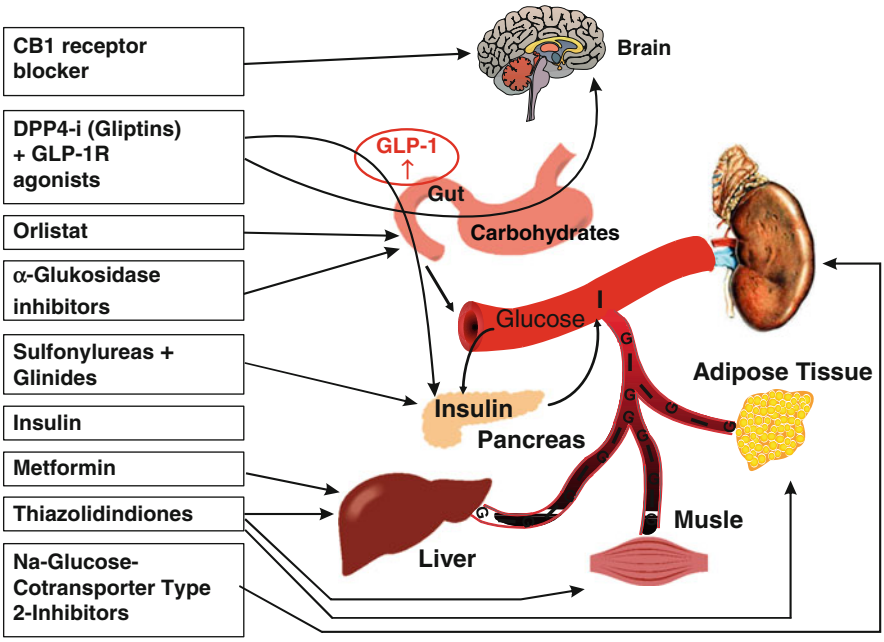

Fig. 1 Main effects of pharmacological anti-hyperglycaemic agents. Metformin and thiazolidinediones (insulin-sensitisers) are both insulin-sparing drugs that enhance insulin action in peripheral tissues by up-regulating glucose transporters and limiting hepatic glucose output. Metformin primarily decreases hepatic glucose production and gluconeogenesis, while glitazones primarily decrease lipolysis and increase glucose uptake. Sulfonylureas and non-sulfonylurea secretagogues (meglitinides) are insulin-providing drugs that stimulate insulin secretion from the beta-cells. Alpha-glucosidase inhibitors interfere with breakdown of complex carbohydrates and delay intestinal glucose absorption. GLP-1R agonists (subcutaneous administration) and the DPP-4 inhibitors (DPP-4i) belong to the incretin-based therapies, which target the incretin system enhancing the effects of GLP-1. GLP-1 increases postprandial insulin secretion and reduces glucagon release but also has multiple extrapancreatic effects including central effects (reduction of appetite, increase of satiety) and delay of gastric emptying. Sodium/glucose co-transporter 2 (SGLT-2) inhibitors are a novel class of drugs in development with a new insulin independent mechanism of action complementary to those of available anti-hyperglycaemic agents. SGLT-2 is a low-affinity high-capacity glucose transporter located in the proximal tubule which is responsible for 90% of renal glucose reabsorption; therefore the drugs act by increasing renal excretion of glucose. The reversible gastrointestinal lipase inhibitor orlistat limits the absorption of fats from the human diet, thereby reducing caloric intake. Various anti-obesity drugs, some potentially exerting additional anti-hyperglycaemic effects, can act via suppression of food intake in the central nervous system acting primarily on neurotransmitters of the central nervous system (noradrenergic drugs, serotonergic drugs, serotonergic and adrenergic drugs, drugs binding to the cannabinoid receptors (CB), etc.)

weight reduction, ease of use) and risks (hypoglycaemia, weight gain, cardiovascular events, specific side effects, long-term safety, drug interactions). In addition, sex and age are important determinants (fertility, menstrual cycle, pregnancy, lactation,

Table 2 Personalised treatment: clinical characteristics of patients which should be considered in drug therapy in metabolic diseases

A	*A*ge
B	*B*ody weight, *B*MI and *B*iomarkers
C	*C*o-morbidity
D	*D*iabetes duration
E	*E*thnicity
F	*F*emale sex
G	*G*enes (biological aspects) and *G*ender (psychosocial aspects)
H	*H*ormones (including sex hormones)

menopause, sexuality, erectile dysfunction, paediatric patients, geriatric co-morbidities) (Table 2).

2.1 Oral Anti-hyperglycaemic Drugs

2.1.1 Metformin

Metformin is the most widely prescribed and available biguanide and can also be used in paediatric patients (10–16 years). It decreases hepatic glucose production and gluconeogenesis, increases peripheral insulin sensitivity in liver and muscle, lowers fasting glucose and reduces HbA1c by ~1–1.5%. Metformin exerts membrane-related events, including suppression of mitochondrial respiratory chain, increased insulin receptor tyrosine kinase activity, stimulation of translocation of GLUT4 transporters to the plasma membrane and activation of the enzyme AMPK (Musi et al. 2002); in addition, metformin was shown to up-regulate adiponectin gene expression (Zulian et al. 2011).

Metformin treatment in overweight patients reduced the rate of all micro- and macrovascular complications (Schernthaner et al. 2010; UKPDS 1998) and a significant benefit in all-cause mortality was found compared to sulfonylurea or insulin (Holman et al. 2008). Metformin has the advantage to be weight neutral and not to induce hypoglycaemia. Initial metformin monotherapy is therefore recommended in both sexes if no contraindication is present (Nathan et al. 2009); in particular, caution is necessary in patients with renal insufficiency [contraindication: estimated glomerular filtration rate (eGFR) < 60 ml/min; elevated serum creatinine levels ($\geq$136 mmol/l in men and $\geq$124 mmol/l in women)], although metformin was recently shown to be safe and effective unless the eGFR falls to $<$30 ml/min (Shaw et al. 2007). Metformin may be used in patients with stable congestive heart failure if renal function is normal. Metformin use is associated with more gastrointestinal problems than other oral diabetes agents (nausea, vomiting, bloating, diarrhoea, abdominal pain and loss of appetite) (Bolen et al. 2007). A serious, potentially fatal but very rare side effect is lactic acidosis (less than one out of every 100,000 patients) (Salpeter et al. 2002) and was reported to be

no more common in metformin recipients without co-morbid conditions than in recipients of other oral anti-hyperglycaemic drugs (Bolen et al. 2007). Among the oral anti-diabetic agents, metformin and acarbose are classified as category B (no evidence of risk in humans) and all others as category C. Analysis of inquiries made to regional poisons unit involving overdoses with metformin, sulfonylureas and insulin indicated that most inquiries ($N = 262$) concerned sulfonylureas (48% females), followed by metformin ($N = 172$; 61% women) and insulin ($N = 191$; 54% women) (von Mach et al. 2006). Aetiology mostly revealed self-poisoning (60% for oral anti-diabetic agents, 85% for insulin). Overdose with biguanides led to most deaths, while insulin overdose caused highest number of major and serious symptoms.

Metformin was also successfully used as pharmacological intervention to prevent diabetes in subjects at increased risk with a reduction of 31% in IGT conversion to type 2 diabetes (Knowler et al. 2002). An American Diabetes Association (ADA) Consensus conference recently proposed metformin therapy in addition to exercise and diet in high-risk individuals (HbA1c $> 6\%$, BMI > 30 kg/m^2, age < 60 years) with prediabetes (Nathan et al. 2007).

Subgroup analyses suggested that in the prevention of progression to overt diabetes metformin might be more effective in young obese men and acarbose in older non-obese women. A recently published post hoc analysis studying the effect of age and gender on weight loss in the Diabetes Prevention Program (DPP) showed diminished weight loss among black women in comparison with other race–gender groups in a lifestyle intervention but not metformin (West et al. 2008); within metformin treatment, all race–gender groups including black women lost similar amounts of weight.

Another subgroup analysis showed that women with a history of gestational diabetes (GDM) had a 71% higher diabetes incidence rate than other women at risk but experienced greater benefit from metformin therapy than those with no gestational diabetes during pregnancy (Ratner et al. 2008). Another specific group of women who may benefit from metformin therapy are those with PCOS. Approximately two-thirds of women with PCOS will not ovulate on a regular basis and may need treatment for ovulation induction. A change in endometrial gene and protein expression after in vitro stimulation with metformin was demonstrated, including a diminished decidualisation process and changes in genes relevant to implantation (Germeyer et al. 2011). Metformin concomitantly restored menstrual cycles and increased conception rates in women with PCOS (Pasquali and Gambineri 2006). Notably, PCOS patients with a positive response to metformin treatment had significantly lower pretreatment sex-hormone binding globulin (SHBG) levels (Wassell et al. 2011). A systematic review and meta-analysis provides evidence of metformin-induced changes in circulating androgens and SHBG levels in women but the quality of evidence is not high. However, there are no data from randomised controlled trials regarding these effects in postmenopausal women or healthy premenopausal women (Barba et al. 2009).

Prescribing of metformin increased in particular amongst girls aged 16–18 in the last decade (Hsia et al. 2011) with the main indication PCOS. However, at present

metformin is not licensed for PCOS and obesity treatment in adults or children. As there is a steady increase in prescriptions of metformin in young people, further studies are required to investigate the efficacy and safety, in particular, in girls. The use of metformin throughout gestation in women with PCOS is controversial: some studies proposed that the number of first trimester spontaneous abortions and the development of gestational diabetes may be reduced by continued treatment, other studies found no evidence and raised concern about metformin transfer through the placenta; recently changes of pharmacokinetic parameters were observed in non-diabetic pregnant women and the umbilical/maternal metformin plasma concentrations ratios were found to be around 0.7 requiring metformin dosage adjustment (de Oliveira Baraldi et al. 2011). However, women with gestational diabetes randomly assigned to metformin or insulin therapy showed that metformin was as safe and efficient as insulin regarding maternal and foetal/neonatal outcome but women clearly preferred the oral anti-diabetic drug (Rowan et al. 2008). Data from pregnant women with type 2 diabetes are reassuring that metformin is not teratogenic and do not indicate any harm of its use in early gestation and throughout pregnancy but interpretation of data of unplanned pregnancies is often difficult because glycaemic control is always an important confounder. Therefore, use of metformin may be an option in pregnancies complicated by type 2 diabetes (National Collaborating Centre for Women's and Children's Health 2008), but women should be informed of the evidence regarding its associated risks and benefits to enable an informed choice over its use (Simmons 2010). In addition, metformin may be also beneficial in oligo-terato-asthenozoospermic patients with metabolic syndrome, as the use of metformin was associated with a statistically significant reduction in insulin resistance and SHBG levels, a statistically significant increase in serum androgen levels and a consequent improvement in semen characteristics (Morgante et al. 2011).

Interesting studies on sex differences in mice showed that metformin treatment may increase survival and life span in particular in the females with most benefit when started early in life (Anisimov et al. 2010, 2011). Chronic treatment of inbred 129/Sv mice with metformin decreased life span of male mice, but increased that of female mice independently of body weight changes. Further metformin failed to influence spontaneous tumour incidence in the males but decreased by 3.5 times the incidence of malignant tumours in female mice. Metformin treatment increased the number of chromosome aberrations in male mice. It can be hypothesized that metformin could be geroprotective and anti-carcinogen in certain circumstances. Via activation of AMPK metformin can inhibit down-stream mammalian effector target of rapamycin (mTOR) and thus cancer cell growth. The authors also showed that metformin increased lifespan in female SHR mice and postponed tumours when started at young age; in addition, treatment improved reproductive function independent of age. Metformin slowed down disturbances in the oestrous function of the mice.

Epidemiologic studies in humans also reported that metformin is associated with a lower risk for cancer while exogenous insulin was associated with an increased risk, although these associations are complex and may be confounded by biological,

diabetes/obesity-related changes. In particular, insulin therapy has been related to increased risk of breast cancer, which could be explained by activation of insulin-like growth factor (IGF) signalling pathways and increased signalling through the oestrogen receptor. On the other hand, women with diabetes and breast cancer who were taking metformin therapy experienced better chemotherapy response rates, possibly due to reduction of growth factor signalling and induction of cell cycle arrest. Metformin decreased breast cancer risk in diabetic women (Martin-Castillo et al. 2010). There are data from various countries suggesting that metformin also protects from colorectal, pancreatic and hepatocellular cancer (Currie et al. 2009). Taiwanese patients on metformin featured a decrease of total, colorectal and hepatocellular cancer incidence to near or below non-diabetic levels but with varying degrees depending on gender and cancer type (Lee et al. 2011). Risk reduction was higher in women for colorectal cancer and in men for hepatocellular cancer. Adjustment for other oral anti-diabetic drugs and co-morbidity made the benefit of metformin even more evident. There was a significant gender interaction with metformin in colorectal cancer which favoured women who even had less cancer risk than the non-diabetic population. The authors speculate that metformin might act differentially and favourably on oestrogen-deficient elderly women. The dosage for significant decrease in cancer was $\leq$500 mg/day.

Recent studies provide evidence that the use of metformin may be associated with reduced incidence and improved prognosis of certain cancers. The ability of metformin to suppress the oncogene, metastasis promoter and breast cancer stem cell marker CD24 may open a novel molecular avenue in the therapeutic management of highly metastatic subgroups of triple-negative (basal-like) breast cancers naturally enriched with CD44posCD24pos tumour-initiating cell populations (Vazquez-Martin et al. 2011). Metformin induces apoptosis in human ovarian adenocarcinoma cell lines OVCAR-3 and OVCAR-4 in an AMPK-independent manner and provokes a cell cycle arrest in the S and G2/M phase. Moreover, metformin can induce apoptosis in these cells by activating caspases 3/7, down-regulating Bcl-2 and Bcl-xL expression and up-regulating Bax and Bad expression. The metformin-induced apoptosis is enhanced by cisplatin (Yasmeen et al. 2011). In addition, metformin activates p53 and it induces tumour cell apoptosis through the extracellular signal-regulated kinases (ERK) pathway (Malki and Youssef 2011). Metformin's molecular functioning to prevent invasive breast cancer can be explained in terms of its ability to efficiently up-regulate the tumour-suppressive microRNAs let-7a and miRNA-96 and to inhibit the oncogenic miRNA-181a, thus epigenetically preserving the differentiated phenotype of mammary epithelium while preventing epithelial-to-mesenchymal transition-related cancer-initiating cell self-renewal (Oliveras-Ferraros et al. 2011). A long-term treatment with progestin commonly results in progestin resistance in endometrial cancer. Metformin down-regulates the expression of glyoxalase I (GloI), a mediator of chemotherapy resistance in endometrial carcinoma. It reverses progestin resistance, enhances progestin-induced cell proliferation inhibition and induces apoptosis in progestin-resistant Ishikawa cells. In addition, medroxyprogesterone acetate-induced mTOR phosphorylation is blocked by metformin (Zhang et al. 2011). However, despite

these promising in vitro data, recent findings suggest that metformin use during adjuvant chemotherapy does not significantly impact survival outcomes in diabetic patients with triple receptor-negative breast cancer (Bayraktar et al. 2011).

2.1.2 Alpha-Glucosidase Inhibitors

Alpha-glucosidase inhibitors reduce the rate of digestion of polysaccharides in the proximal intestine, lower postprandial glycaemia, are weight neutral, do not cause hypoglycaemia and have a very good safety profile (Nathan et al. 2009). However, their use is associated with gastrointestinal side effects leading to poor adherence and their efficacy to improve glycaemic control is rather modest. In the STOP-NIDDM trial, the drop-out rate was 75% in men and 25% in women (Chiasson et al. 2002). Furthermore, acarbose therapy may be associated with less cardiovascular complications (Hanefeld et al. 2004). This property might be due to the fact that, besides its effects on fasting plasma insulin and postprandial insulin, acarbose decreases important markers for inflammation such as soluble adhesion molecules, interleukin-6 and high-sensitivity C reactive protein (Derosa et al. 2011). Furthermore, in type 2 diabetic patients plasma adiponectin is significantly decreased, and it is reported that hypoadiponectinemia is associated with endothelial dysfunction and platelet activation. The increase of adiponectin by acarbose may have an antiplatelet effect via promotion of nitric oxide (NO) production. In addition, acarbose significantly decreases plasma platelet-derived microparticles and selectins which, together with adiponectin, play an important role in the development of atherosclerosis in diabetes (Shimazu et al. 2009).

Acarbose and voglibose were also shown to be able to decrease the risk to develop diabetes in IGT subjects (Chiasson et al. 2002; Kawamori et al. 2009). This may be ascribed to inhibition of carbohydrate absorption, but also to augmentation of incretin hormone release and effects on gut microbiota flora (Defronzo and Abdul-Ghani 2011). Subgroup analyses from the STOP NIDDM trial (acarbose) suggested that acarbose may be more effective in the prevention of progression to overt diabetes in older non-obese women. This observation may be related to the fact that women more often feature isolated impaired glucose tolerance and to the assumption that higher postprandial glucose levels may be more often the dominant metabolic defect in women compared to men. A delay of gastrointestinal glucose absorption in women compared to men resulting in higher 2 h glucose levels was also seen in healthy subjects (Anderwald et al. 2011). Therefore, it can be hypothesized that drugs acting primarily on reduction of postprandial glucose levels may be of greater benefit in women and those acting primarily on insulin resistance may be of greater benefit in males at least in the early stage of the disease. Males usually are more insulin resistant than women (Kautzky-Willer and Handisurya 2009). However, with the progression of disease combination therapies will be necessary in both sexes.

2.1.3 Glitazones

Both, pioglitazone and rosiglitazone, increase the risk of heart failure with high strength of evidence (SOE), and also oedema (high SOE) and fractures in women (moderate SOE) (Habib et al. 2010; Jonas et al. 2011) (see also Benz et al. 2012).

The thiazolidinedione ring in the glitazone molecules was shown to contribute to hepatic injury. A thiazolidinedione ring-containing compound (DCPT) produced liver damage in rats of both sexes. However, male rats were more sensitive to DCPT as shown by increased serum alanine aminotransferase levels and altered hepatic morphology 24 h postdosing (Patel et al. 2008).

Rosiglitazone induced regrowth of fat in female but not male MORE-PGKO (Global Mox2-Cre-Ppar-gamma-knockout) mice. Insulin sensitivity increased but was not associated with the typical changes in adipokines or lipids. However, increases in alternatively activated macrophage markers, which have been previously associated with metabolic improvement, were observed in the regrown fat independent of peroxisome proliferator-activated receptor-gamma (PPAR-γ). In contrast, rosiglitazone improved glucose homeostasis in male mice with similar PPAR-γ deficiency by increasing insulin production with an apparent expansion of pancreatic islets. The insulin-sensitising effect of rosiglitazone is dependent on PPAR-γ in this male lipodystrophic model (Duan et al. 2010).

2.1.4 Sulfonylureas

Sulfonylureas (SU) are widely used effective anti-diabetic drugs, which are often given in combination with metformin. SU bind to an ATP-dependent K^+ (K_{ATP}) channel on the cell membrane of pancreatic beta cells and increase insulin release. Drug therapy is usually associated with significant increase of weight and moderate risk of hypoglycaemia although both risks may vary between different agents with different pharmacokinetics (Schernthaner et al. 2004, 2010). The therapeutic efficacy of oral hypoglycaemic drugs varies between individuals, and pharmacogenetic factors contribute to this variability (Seeringer et al. 2010). In the UKPDS, intensive therapy initiated with SU (chlorpropamide or glibenclamide) or insulin showed only a reduction in microvascular complications (UKPDS 1998), but after 10 years of observational follow-up a significant reduction in myocardial infarction and death was described (Holman et al. 2008). In observational cohort studies of patients newly treated with oral hypoglycaemic agents, those treated with SU only were at higher risk of adverse cardiovascular outcomes than those treated with metformin alone (Evans et al. 2006). In addition, patients given combinations of SU and metformin were found to have a higher risk of cardiovascular events and mortality although data are controversial (Rao et al. 2008). The proportion of women included in these clinical trials was adequate. Sex was used for adjustment of data or as covariate.

In a bioequivalence assessment of glimepiride and pioglitazone in a fixed dose combination formulation, gender analysis revealed that women had higher (16 and 30%) exposure than men for glimepiride and pioglitazone (Karim et al. 2007). However, due to considerable overlapping of the concentration curves the authors concluded that gender-dependent dosing is unnecessary.

There is only one SU which may be considered for use in pregnancy. Glibenclamide (glyburide) was a clinically effective alternative to insulin therapy in women with gestational diabetes (Langer et al. 2000). The cord-serum insulin concentrations were similar in the two groups, and glyburide was not detected in the cord serum of any infant in the glyburide group. However, long-term follow-up of children is missing and oral anti-diabetic drugs are still not used in clinical practice in many countries when the gold standard pharmaceutical therapy during pregnancy, insulin, is easily available.

SUs may also exert other effects than lowering glucose. In in vitro experiments glibenclamide inhibited the vasorelaxation to levosimendan in the arteries from males but not in those of females (Akar et al. 2007). In a model of stroke-prone spontaneously hypertensive rats glibenclamide increased blood pressure, plasma insulin and ratios of heart weight to body weight in young female rats but not in male rats (Peuler et al. 1993). In one study in humans increasing doses of SU were associated with increasing levels of NT-proBNP with no sex differences in any age category (Tildesley et al. 2007).

2.1.5 Incretin (Gut-Derived Hormone)-Based Therapies

The incretin GLP-1 is a peptide produced by the L cells of the small intestine which is released in response to nutrient ingestion and potentiates glucose-stimulated beta cell insulin secretion in a glucose-dependent manner (Cernea and Raz 2011). Naturally occurring GLP-1 and glucose-dependent insulinotropic peptide (GIP) are rapidly degraded by dipeptidyl peptidase 4 (DPP-4), which is expressed in many tissues including endothelial and immune cells. When vildagliptin was administered to normal, wild-type, mice and to mice with a β-cell targeted dominant-negative mutant hepatocyte nuclear factor-1α (HNF-1α), these mice had a defective islet response to glucose. After 8 weeks, vildagliptin augmented the insulin response after gastric glucose by fivefold in male mice and 30-fold in female mice. Furthermore, glucose-stimulated insulin secretion from isolated islets was markedly enhanced by 9 weeks treatment with vildagliptin. In contrast, in transgenic mice, the severely suppressed insulin response was only marginally improved by vildagliptin in males, and not affected at all in females. Thus, it is concluded that DPP-4 inhibition improves islet function and increases beta cell secretory responses on a long-term basis and that this is dependent on intact expression of HNF-1α (Ahrén et al. 2005).

Because the biological activities of the large number of chemokines, adipokines, neuropeptides and incretins are modified by DPP-4-mediated cleavages, extrapancreatic effects are likely and neuroprotective and beneficial cardiovascular

effects (mainly animal studies) were proposed (Cernea and Raz 2011). Two drug classes have been developed to target the incretin system enhancing the effects of GLP-1 for treatment of type 2 diabetes, GLP-1 receptor (GLP-1R) agonists, requiring subcutaneous administration, and the DPP-4 inhibitors (DPP-4i), delivered as oral tablets. Fixed combinations of DPP-4i and metformin may have synergistic effects further enhancing GLP-1 action and convenience of use and adherence.

There are several DPP-4i on the market (sitagliptin, vildagliptin, saxagliptin) with limited postmarket data, while others recently got approval (linagliptin) or are still waiting for approval (alogliptin). To date there are two licensed GLP-1R agonists available, exenatide (exendin-4 mimetic with 53% sequence identity to native GLP-1) and liraglutide (GLP-1 analogue with 97% sequence identity to native GLP-1). Recently, the first once-weekly prolonged release of exendin GLP-1R agonist received approval by EMA. DPP-4i are weight neutral and GLP-1R agonists even enhance weight loss; both classes do not cause hypoglycaemia, but a possible link to pancreatitis was described (Schernthaner et al. 2010). GLP-1R agonists may be associated with gastrointestinal side effects (particularly nausea), but were also shown to exert a beta cell protective effect, whereas with DPP-4i infections appeared more frequent (Cernea and Raz 2011). All-cause infections increased significantly after sitagliptin treatment but did not reach statistical significance following vildagliptin therapy (Richter et al. 2008).

Information on sex/gender-differences of incretin-based therapies are scarce, but in general it can be assumed that the drug profiles with weight loss, low risk of hypoglycaemia and reducing postprandial glucose peaks may be particularly attractive for women who in general have more psychological problems associated with even mild weight gain (Table 3).

The various DPP-4i differ in metabolism and elimination. Vildagliptin and saxagliptin are structurally similar (cyanopyrrolidine based), while sitagliptin (beta amino acid derivate) and linagliptin (xanthine derivate) have different structures (Tiwari 2009). Linagliptin is excreted primarily in the bile and appears to be safe in patients with renal impairment without dose adjustments. Sitagliptin and saxagliptin are partially metabolised via the cytochrome P450 3A4 (CYP3A4) pathway, while vildagliptin is not metabolised by cytochrome P450 (CYP450) and does not inhibit any of the important CYP450 enzymes. Gliptins do not act as inhibitor or inducer of the cytochrome (CYP) system. Therefore, drug interactions (e.g. statins) or interference with sexual hormones is not likely in vildagliptin-treated patients (Halimi et al. 2010) but may be of some clinical relevance in patients treated with other DPP-4i. This is in contrast to what has been reported with other glucose-lowering agents such as sulfonylureas, glinides and glitazones (Scheen 2010). Sitagliptin is primarily excreted by renal elimination as unchanged drug (Migoya et al. 2009); moderate hepatic insufficiency had no clinically meaningful effect on the pharmacokinetics of sitagliptin in men or women. Saxagliptin has the advantage that no dose adjustment is necessary for patients with mild renal impairment or any degree of hepatic impairment. For patients with severe renal impairment, 50% of the usual dose of saxagliptin is recommended (Boulton et al. 2011). A systematic assessment of cardiovascular outcomes in the saxagliptin

Table 3 Sex/gender differences in existing drugs for the therapy of diabetes

Drug/substance	Metformin	Acarbose	Glimepiride	Glibenclamide
Class	Biguanide	Alpha-glucosidase inhibitors	Sulfonylurea	Sulfonylurea
Sex-specific features	Prevention of progression to overt diabetes more effective in young obese men Beneficial in oligo-terato-asthenozoospermic patients (reduction in insulin resistance and SHBG levels, increase in serum androgen levels, improvement in semen characteristics) Decreases risk for hepatocellular cancer in men Restores menstrual cycle and increases conception rates in women with PCOS. Changes in circulating androgens and SHBG levels in women described Decreases breast cancer risk and risk for colorectal cancer in women. Improves chemotherapy response rates in diabetic breast cancer patients Increased survival and life span, decreased incidence of malignant tumours and slowed down disturbances in the oestrous function in female animals; Decreased life span and increased number of chromosome aberrations in male animals	Prevention of progression to overt diabetes more effective in old non-obese women	In a bioequivalence assessment of glimepiride and piogolitazone in a fixed dose combination formulation revealed that women had higher exposure for both than men	In vitro inhibition of vasorelaxation to levosimendan in male arteries. In female rats increase of blood pressure, plasma insulin and heart weight:body weight ratio

Comments	Greater therapy benefit for women with a history of GDM compared to those women without history of GDM Not licensed for PCOS and obesity treatment; use throughout gestation is controversial			Only sulfonylurea considered for use in pregnancy
References	Anisimov et al. (2010, 2011), Barba et al. (2009), Currie et al. (2009), Lee et al. (2011), Morgante et al. (2011), Pasquali and Gambineri (2006), Ratner et al. (2008), and West et al. (2008)	West et al. (2008)	Karim et al. (2007)	Akar et al. (2007), Langer et al. (2000), and Peuler et al. (1993)

Drug/substance	Exenatide	Vildagliptin	Saxagliptin	I-Glargine
Class	GLP-1R agonists	DPP-4 inhibitor	DPP-4 inhibitor	Long-acting insulin analogue
Sex-specific features	Comparable outcomes in men and women	Not metabolised by CYP 450 No sex differences in pharmacokinetics and dynamics Augments insulin response to glucose stronger in female mice	Men experience a greater reduction of cardiovascular events in comparison to control group than females	Women may have an increased risk of breast cancer (inconclusive data/ observational studies)
Comments				
References	Buysschaert et al. (2010)	Ahrén et al. (2005), He et al. (2008), and Halimi et al. (2010)	Frederich et al. (2010)	Kurtzhals et al. (2000), Teng et al. (2011), and Jovanovic (2009)

development showed that overall as well as in subgroups with higher baseline cardiovascular risk the use of saxagliptin was not associated with increased cardiovascular risk (Frederich et al. 2010). Men on saxagliptin experienced a greater reduction of cardiovascular events in comparison to the control group than females (7.7 vs. 22.5 events per 1,000 patient-years in males and 4.9 vs. 6.0 in women) (Frederich et al. 2010).

A recently published study showed effects of age, gender or BMI on pharmacokinetics and pharmacodynamics of the DPP-4i vildagliptin in healthy subjects (He et al. 2008). Peak concentration and exposure of vildagliptin were somewhat higher in elderly than young subjects. However, no clinically relevant changes were observed between young and elderly, male and female, lean or obese subjects. The results suggest that no dose modification is necessary for vildagliptin based on age, gender or BMI of a subject.

Sex differences were studied in diabetic men and women matched for age and BMI given add on therapy of vildagliptin to metformin. Both sexes had an improvement in weight, HbA1c and liver and heart lipid content as well as of the cardiac ejection fraction within 6 months of therapy (Kosi et al. 2011).

Studies directly comparing GLP-1R agonists with DPP-4i in parallel group or head-to-head crossover design showed a more robust glucose-lowering effect by GLP-1R agonists (Cernea and Raz 2011; Pratley et al. 2010). In addition, GLP-1R agonists significantly reduced postprandial glucagon and gastric emptying. For exenatide a study showed that use in routine practice was associated with comparable outcomes in men and women (Buysschaert et al. 2010). GLP-1R agonists may also be of benefit if used in the prediabetic state or in obese subjects (Defronzo and Abdul-Ghani 2011). Seventy-seven per cent of the subjects using exenatide along with lifestyle intervention for 24 weeks reverted to normal glucose tolerance compared to 56% on placebo (Rosenstock et al. 2010). Long-acting forms given once weekly could be promising agents to prevent diabetes and reduce obesity in the future.

2.2 Insulin

Insulin is the only treatment option for type 1 diabetes and also often has to be initiated in type 2 diabetes in combination with oral anti-diabetic drugs or alone in the course of the disease; furthermore, insulin is still the gold standard pharmacological therapy in diabetic pregnancy. Insulin has the greatest glucose-lowering efficacy, potentially unlimited with up-titration. However, the concerns of starting insulin in type 2 diabetes in early stages of the disease are weight gain, moderate to high risk of hypoglycaemia, requirement of frequent blood glucose monitoring and a potential link to cancer risk. Weight gain appears to be proportional to the correction of glycaemia (Nathan et al. 2009). Insulin caused twice the absolute number of severe hypoglycaemic episodes than non-insulin anti-hyperglycaemic agents (Gross et al. 2011). In addition, there is no evidence that insulin therapy as such reduces risk of macrovascular complications. The diabetes and

insulin–glucose infusion in acute myocardial infarction (DIGAMI-2) study in type 2 diabetes showed no difference in total mortality and even a trend towards more cardiovascular events in patients receiving acute or chronic insulin therapy; a post hoc analysis further revealed that although total mortality did not differ between genders, the combined cardiovascular end-point of death, re-infarction or stroke was more common in women, but this difference disappeared after age adjustment (Venskutonyte et al. 2010). The use of long-acting (I-Detemir or I-Glargine) and very rapid acting insulin analogues (I-Aspart, I-Lispro or I-Glulisine) may be associated with lower risk of hypoglycaemia than the older human insulin formulations (Horvath et al. 2007). Insulin analogues lead to greater flexibility and convenience of dosing and, thus, greater patient satisfaction and improved quality of life. However, there still may be some safety concerns. An increased risk of breast cancer was reported in women treated with I-Glargine in several observational studies. In fact, I-Glargine has the highest affinity to IGF-1 receptors (six- to eightfold increased IGF-1 receptor affinity and mitogenic potency compared with human insulin) (Kurtzhals et al. 2000). Further, Glargine promoted the proliferation of breast adenocarcinoma cells in vitro, probably by preventing apoptosis (Teng et al. 2011). Recent reports including analysis in more than 4,500 Glargine users outlined that the risk of breast cancer in women with type 2 diabetes is not increased during the first 5 years of insulin Glargine use (Suissa et al. 2011). However, longer term use may increase this risk, particularly in women with long-standing use of insulin before starting insulin Glargine. Using data from dispensing records from community pharmacies individually linked to medical records of 2.5 million individuals in the Netherlands, the lowest risk of cancer in general (hazard ratio 0.75) was observed in subjects treated with I-Glargine but also lower risk was described in those treated with other I-analogues (hazard ratio 0.85) in comparison to human insulin (Ruiter et al. 2012). However, an increased risk in I-Glargine treated women was found for breast cancer, although a dose–response relationship could not be identified.

Unfortunately reports on sex- or gender-differences regarding insulin therapy are scarce. In a post hoc analysis it was recently shown that the insulin dose and the rate of hypoglycaemia were different between men and women with type 2 diabetes at the end of an insulin intervention trial (Jovanovic 2009); women had higher insulin dose and more hypoglycaemic events; thus, it was demanded that sex differences should be explored and considered more intensively in future clinical trial design and analysis. Premenopausal women with normal menstrual cycles have varying plasma levels of oestrogens and gestagens which differently affect insulin sensitivity and glucose metabolism. Female diabetic patients on insulin therapy, in particular type 1 diabetic women with absolute insulin deficiency, may recognise that in the second half of their menstrual cycle when progestagen levels increase they have a higher demand of insulin in order to maintain normoglycaemia.

In pregnancy all human insulins can be used and the short-acting insulin analogues I-Aspart and I-Lispro are also recommended (de Valk and Visser 2011). The latter were shown to be safe for mother and child and may help to reduce postprandial glucose peaks and risk of hypoglycaemia during pregnancy. There is also growing evidence that the long-acting insulin analogues I-Detemir and I-Glargine are not associated with increased perinatal complications but long-term follow-up data of the children are missing.

2.3 Conclusion and Future Perspectives

Diabetes is a progressive life-lasting disease requiring increasingly more complex pharmacological treatment over time. Glycaemic control remains a main goal, but multifactorial intervention is necessary to prevent cardiovascular complications in diabetic patients in particular in women. Diabetes increases the cardiovascular risk of women two- to threefold more than in diabetic males in comparison to non-diabetic subjects of the same sex and diabetes-related mortality is even higher in diabetic women than men. Therefore, therapy should not only target blood glucose levels but also treat hypertension and hyperlipidaemia as recommended in specific guidelines. Multiple complex treatment often includes three or more anti-diabetic agents (>60% in the ACCORD trial in intensively treated patients) (Gerstein et al. 2008) which is a challenge for both patients and physicians. Analysis of anti-hyperglycaemic therapy in type 2 diabetic patients treated at a university clinic showed no sex differences in the prescription of specific drugs or their combinations (Kautzky-Willer and Handisurya 2009). However, women more often were treated with diet alone. Goal and medication should be based on individual factors, needs and concerns (Table 2). Treatment personalisation including fixed-dose combination therapies may increase adherence to multitherapy and improve glycaemic control (Thayer et al. 2010).

Take Home Messages

- Biological and psychosocial factors, culture and environment are involved in the pathogenesis and progression of the metabolic disorders obesity and type 2 diabetes with implications of sex- and gender-differences for lifestyle intervention and drug therapy.
- Obese women have more pronounced subcutaneous or peripheral fat mass and males more pronounced visceral obesity and fatty liver disease.
- Orlistat has modest effect on weight reduction in both sexes and some beneficial metabolic effects in several subgroups, like women with PCOS or men with schizophrenia.
- Women report more weight-related psychosocial problems and seek more often medical help for treatment of obesity including bariatric surgery. Obese or diabetic women suffer more from depressive symptoms, stigmatisation and physical limitations.

- The use of psychotropic drugs is extensive in obese patients, especially among women and therefore clinicians should be informed of drug interactions between psychotropic drugs and anti-obesity drugs.
- Men appear to have more pronounced insulin resistance and a higher risk of progression to diabetes.
- Prevalence of prediabetes shows sexual dimorphism with a higher rate of impaired glucose tolerance in women and a higher rate of impaired fasting glucose in men; this may be attributed to prolonged gut absorption and lower body height of women.
- Diabetic women are characterised by poorer control of modifiable cardiovascular risk factors, especially by increased LDL cholesterol and higher systolic blood pressure levels, which further increase after menopause.
- Diabetes is associated with higher cardiovascular mortality in women.
- Metformin is the first choice medication for patients with type 2 diabetes independent of sex although it may be particularly effective in younger obese men, women with a history of gestational diabetes and women with PCOS. In addition, anti-cancer effects with sex-specific aspects may be considered.
- Drugs primarily acting on insulin release and reducing postprandial glucose peaks without concomitant increase of risk of hypoglycaemia or weight gain may be of even greater benefit in women than men as women often suffer more from postprandial hyperglycaemia at least in the early phase of the disease and seem to be particularly vulnerable to hypoglycaemia and weight gain.

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Adrenal Disorders

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Abbreviations

11b-HSD2	11β-hydroxysteroid-dehydrogenase
ACTH	Adrenocorticotropin

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BCRP1	Breast cancer-resistance protein
BETA	Betamethasone
CAH	Congenital adrenal hyperplasia
CBG	Corticosterone-binding globulin
DEX	Dexamethasone
DHEA	Dehydroepiandrosterone
GR	Glucocorticoid receptor
MR	Mineralocorticoid receptor
sGC	Synthetic glucocorticoid

1 Primary Adrenal Failure

The most frequent cause nowadays is autoimmune adrenalitis, which can be confirmed by the presence of 21-hydroxylase-antibodies (Oelkers 1996). When suspected on clinical grounds, adrenal insufficiency has to be confirmed by inappropriately low serum cortisol with elevated adrenocorticotropin (ACTH) (in case of primary adrenal insufficiency). A morning plasma cortisol $\leq$3 μg/dl is indicative for adrenal failure, whereas a cortisol $\geq$19 μg/dl rules out the disorder (Grinspoon and Biller 1994) if corticosterone-binding globulin (CBG) is normal. All other patients need dynamic testing (short ACTH stimulation test). A low ACTH in the presence of low cortisol points to secondary adrenal failure, in which case ACTH stimulation might be less reliable, especially when of recent onset.

1.1 Therapy of Adrenal Failure

1.1.1 Hydrocortisone

Hydrocortisone is resorbed completely and metabolic clearance is primarily by the liver (Charmandari et al. 2001). Clearance is higher during puberty compared to pre- and post-pubertal stage, and the half-life of free cortisol appears significantly shorter in females compared with males, although the half-life of total cortisol does not change (Charmandari et al. 2001). Weight, height and body surface area were major variables affecting hydrocortisone kinetics, and dose also made a significant contribution, while age and gender had no significant effect (Mah et al. 2004). A new sustained release form (Chronocort®) may improve treatment in both adrenal insufficiency and congenital adrenal failure by allowing more physiological diurnal hormone profiles (Verma et al. 2010).

Side Effects

Patients with primary adrenal insufficiency receive more glucocorticoids than the endogenous production. The study of Gow et al. (2011) on hair specimen cortisol content showed comparable cortisol content in healthy and cortisol-substituted females with adrenal insufficiency, whereas in men substituted individuals had significantly higher cortisol content. Although the authors not specifically draw any conclusions from that finding their results could point at under-treatment of women or over-treatment of men with adrenal insufficiency. This is in line with a cross-sectional study of two cohorts of Addison patients, which finds more reduced bone mineral density (femoral neck, Z-scores) in men than women, as compared to a reference population (Lovas et al. 2009).

1.1.2 Dehydroepiandrosterone (Neary and Nieman 2010)

At birth dehydroepiandrosterone (DHEA) levels are low, around age of 6–10 years begin to increase, peak around age 24 and decline thereafter. DHEA does not have a specific target but can be transformed to androgens and oestrogens and may have immune-modulatory and neuropsychological properties. Some postulate that DHEA insufficiency explains the impaired quality of life (QOL) in primary adrenal failure, and a meta-analysis of randomised trials showed a small benefit, particularly in women, in doses of 25–50 mg daily (Alkatib et al. 2009). Healthy men have plenty of testicular androgens which explains why DHEA is less important in men. When offered to women target concentrations (24 h after last dose) should follow age and gender reference concentrations. Therapy is associated with common androgenic side effects (Traish et al. 2011) and is hampered by the poor quality of available over-the-counter (OTC) DHEA preparations (Parasrampuria et al. 1998).

1.1.3 Fludrocortisone

Fludrocortisone is a synthetic mineralocorticoid (9a-Fluorocortisol) with high oral bioavailability. Since it is not inactivated by 11β-hydroxysteroid-dehydrogenase (11β-HSD2) type 2 its potent (>100-fold compared to hydrocortisone, see Table 1) action on mineralocorticoid receptors (MR) in the kidney is very strong although it binds to the receptor with similar binding affinity as hydrocortisone. No gender differences in pharmacokinetics have been published. For the use in pregnancy adequate, well-controlled human studies are lacking, and limited data from animal studies have shown risk to the foetus. Food and Drug Administration (FDA) has categorised the substance in pregnancy risk category: C (chance of foetal harm if the drug is given during pregnancy, but the potential benefits may outweigh the potential risk) (National Center for Biotechnology Information. PubChem Compound Database CID 31378) Table 2.

Table 1 Comparison of anti-inflammatory, mineralocorticoid, growth retarding and androgen-suppressing effects of various steroid preparations [according to Claahsen-van der Grinten et al. (2011)]

Steroid	Anti-inflammatory glucocorticoid effect	Growth retarding, glucocorticoid effect, androgen-suppressing effect	Salt retaining, mineralocorticoid effect
Hydrocortisone	1	1	1
Prednisolone	4	5–15	0.8
Prednisone	3.5–4		0.8
Dexamethasone	30	70–100	0
Fludrocortisone	15		200

1.2 Primary Adrenal Failure in Pregnancy

Adrenal failure is rare in pregnancy (Lekarev and New 2011). Diagnosis is suggested by a low early morning cortisol <3 μg/dl in the non-stressed state and in the setting of typical clinical symptoms confirms the diagnosis. In the second and third trimester cortisol rises to levels two- to threefold above those in the non-pregnant state, therefore a baseline level of <30 μg/dl warrants further evaluation. ACTH-stimulated normal cortisol values have been established for each trimester.

Progesterone continually increases throughout pregnancy and antagonises the mineralocorticoid effects of aldosterone by competing with aldosterone for binding to the MR in the kidney. Pregnancy is a state of relative aldosterone resistance associated with physiological secondary aldosteronism (Corry and Tuck 1995) and remarkable potassium retention, possibly independent of a direct anti-mineralocorticoid effect (Elabida et al. 2011).

1.2.1 Therapy in Pregnancy

Hydrocortisone, which does not cross the placenta, is the glucocorticoid treatment of choice, and fludrocortisone is used as mineralocorticoid replacement in patients with primary disease (Arlt 2008). CBG normally increases gradually during pregnancy and, during the third trimester of pregnancy, free cortisol rises. Therefore, it may be necessary to increase hydrocortisone replacement during this period.

Peripartal hydrocortisone replacement follows recommendations for major surgery: 100 mg/24 h starting with the onset of labour (25 mg i.v. every 6 h, 100 mg i.v. at time of delivery, followed by rapid tapering after delivery within 3 days). Saline hydration is started with the start of labours.

About 10 % of the maternal serum concentration applied as the non-fluorinated glucocorticoids prednisolone, methylprednisolone or prednisone arrive in the foetus (with prednisone being activated to prednisolone in the liver, prednisolone is then inactivated by 11β-HSD2 in the placenta) (Brown et al. 1996). This enzyme is also

Table 2 Sex/gender differences in existing drugs for the therapy of adrenal disorders

Drug	Hydrocortisone	DHEA	Fludrocortisone	Dexamethasone	Betamethasone	Prednisone	Prednisolone	Methylprednisolone
Class	Steroid hormone	Steroid hormone	Synthetic mineralcorticoid	Synthetic glucocorticoid	Synthetic glucocorticoid	Glucocorticoid prodrug	Synthetic glucocorticoid	Synthetic glucocorticoid
Sex-specific features	Half-life shorter in females Sex-specific side effects: under-treatment of women or over-treatment of men, and more reduced bone mineral density in men Impairs fear conditions in men and improves fear conditions in women	Small benefit for women, in doses of 25–50 mg/day For men less important	No gender differences in pharmacokinetics published	Adult men do not need therapy Irregular menstruation and acne reverse within 3 months of treatment in women, hirsutism within 30 months In animals exposed offspring show lower male to female sex ratio and produces sex-selective alterations in activity, learning, and memory	In animals exposed female offspring show reduced fecundity Affects hippocampal MR protein expression stronger in male offspring			
Comments	CAH treatment of choice for pregnant women Does not cross placenta		FDA pregnancy risk category C	Should be avoided in sexually active women not on contraception, because it crosses the placental barrier Has to be avoided during pregnancy, except prenatal therapy is indicated	Has to be avoided during pregnancy	FDA pregnancy risk category B Between pregnancy weeks 8 and 11 doses should not exceed 10 mg/day	FDA pregnancy risk category B Between pregnancy weeks 8 and 11 doses should not exceed 10 mg/day	
References	Charmandari et al. (2001), Gow et al. (2011), Lovas et al. (2009), Merz et al. (2010), Hui and Bianchi (2011), Lekarev and New (2011), Arlt (2008)	Alkatib et al. (2009)		Merke (2008), New (2006), Dunn et al. (2010), Kreider et al. (2005), Weinstock (2011)	Dunn et al. (2010), Owen and Matthews (2003)			

responsible for the much lower foetal glucocorticoid concentrations compared to concentrations in the maternal circulation (Beitins et al. 1973). The placental barrier, however, is incomplete, and high maternal concentrations elevate foetal glucocorticoid levels (Benediktsson et al. 1997). It has been recommended to limit the dose of prednisolone and prednisone to a maximum dose of 15 mg a day in the first trimester due to a slightly elevated risk of cleft lip and cleft palate (Carmichael et al. 2007), although such doses are hardly ever used in adrenal failure and congenital adrenal hyperplasia (CAH). Foetal glucocorticoid exposure may predispose to adult affective disorders (Wyrwoll and Holmes 2011), and use of prednisone during pregnancy has been reported to be associated with an increase in still-birth, foetal distress, placental insufficiency (Warrell and Taylor 1968) and low birth weight neonates (Reinisch et al. 1978). Interestingly, females seem to be less sensitive to glucocorticoid exposure via increased placental inactivation by 11β-HSD2 (Clifton and Murphy 2004; Liu et al. 2001), which thus does not apply to dexamethasone (DEX) and betamethasone (BETA).

The use of prednisolone and prednisone in pharmacological doses in pregnancy has been classified (U.S. Food and Drug Administration 2007) as risk factor category B.

Mineralocorticoids may also require adjustment, due to effect of progesterone (see above). The need should be judged based on sitting and erect blood pressure and serum sodium and potassium levels. In essence, in case of postural hypotension or hyperkalaemia fludrocortisone dose is increased, in case of hypertension or hypokalaemia it is decreased. If plasma renin activity (PRA) [or plasma renin concentration (PRC)] is used to monitor adequacy of fludrocortisone dose, pregnancy-specific reference ranges (which are higher) have to be observed, although there is no published evidence.

1.2.2 Breastfeeding

Only small amounts of prednisone and prednisolone appear in breast milk. Of 10 mg of prednisone and prednisolone applied orally, peak milk concentrations after 2 h were 26.7 and 1.6 ng/ml, respectively (Katz and Duncan 1975), as breastfeeding in doses up to 20 mg (prednisone) or 50 mg (prednisolone) should pose no adverse effect in the breastfed infant; with high doses avoiding breastfeeding for 4 h after dose limits the amount taken up by the child (Makol et al. 2011).

2 Congenital Adrenal Hyperplasia

2.1 Definition and Pathophysiology

The term describes a family of autosomal recessive disorders of steroidogenesis secondary to a deficiency in any of the five enzymes necessary for the synthesis of cortisol (Vos and Bruinse 2010). Cortisol production is reduced, via negative

feedback ACTH is increased, and hormones (immediately) before the enzymatic block may be overproduced and are shunted into the androgen pathway, yet cortisol may be underproduced.

The most common form (>90 % of all patients with CAH) is caused by a deficiency of 21-hydroxylase (caused by severe mutations in the CYP21A2 gene). In the most severe form, the classical salt wasting form, the residual enzymatic activity is less than 1 % leading to cortisol deficiency as well as decreased (in 75 %) aldosterone synthesis. Female patients are virilised prenatally due to adrenal androgen excess because the androgen pathway is not affected by the enzyme deficiency and neonates of both sexes suffer from life-threatening adrenal crisis (Trapp and Oberfield 2011; Vos and Bruinse 2010). In classic simple virilising CAH, the residual enzyme activity is 1–2 %, generally enough for sufficient aldosterone production to prevent salt wasting. Cortisol synthesis is impaired and affected females present with genital ambiguity due to prenatal virilisation. In the case of the non-classic form, 21-hydroxylase activity is reduced to 20–50 % of normal, cortisol and aldosterone production are normal, and there is mild androgen excess, but females are not virilised at birth (Merke 2008; Trapp and Oberfield 2011; Vos and Bruinse 2010). Females may be asymptomatic or symptoms arise in childhood/adolescence with extreme variability: premature pubarche, cystic acne, hirsutism and menstrual disorders, often polycystic ovary syndrome. Some patients present first in adulthood with fertility problems (Claahsen-van der Grinten et al. 2011).

2.2 Epidemiology

The disorder is uncommon, with an incidence of classic CAH of 1:10,000–20,000 births and an estimated carrier frequency of 1:50–60. Salt-loosing CAH accounts for two-thirds, simple virilising CAH for one-third of classic CAH (Merke and Bornstein 2005). Non-classic CAH is much more frequent, published numbers vary between 1:27 (Ashkenazi Jews), 1:50 (Slavs), 1:100 (Austrians) and 1:300 (Italians) (Baumgartner-Parzer et al. 2005; New 2006).

2.3 Treatment of CAH

2.3.1 Glucocorticoid Therapy

The aim of treatment in classic CAH is to suppress androgen production while preserving function of the hypothalamic–pituitary–adrenal (HPA) axis. Children are typically treated with hydrocortisone because of the short half-life in order to avoid iatrogenic Cushing syndrome and optimise childhood growth and weight gain (Speiser et al. 2010). After achieving adult height, patients are often switched to longer-acting glucocorticoid preparations that can be given once or twice daily, such as DEX 250–500 mg at night (Merke 2008).

If glucocorticoid therapy is necessary in children with non-classic CAH, the same principles apply. Adult men do not need therapy, and adult females can often be treated with an oral contraceptive and antiandrogen (Merke 2008). If treated with DEX, a small study suggested that irregular menstruation and acne are reversed within 3 months of treatment with DEX (0.25 mg at the hour of sleep each day), whereas hirsutism may be reversed within 30 months (New 2006).

Relative potency is expressed as factor compared to hydrocortisone.

In general, DEX should be avoided in sexually active CAH females not on oral contraceptives because it is not inactivated by placental 11β-HSD2, thus crosses the placental barrier and leads to suppression of the foetal adrenal gland. DEX is only used in pregnant CAH females when prenatal therapy is indicated (see section on prenatal treatment).

2.3.2 Mineralocorticoid Therapy in CAH

In classical salt-wasting CAH fludrocortisone is given to normalise electrolytes and renin (in addition to salt supplements in infants). It is also recommended in simple-virilising CAH because it allows a lower dose of glucocorticoids to be prescribed (Merke 2008; Vos and Bruinse 2010).

2.3.3 Prenatal Glucocorticoid Therapy

In the more severe classical form, excess of prenatal androgen causes external genital masculinisation and ambiguity in newborn females and progressive postnatal virilisation in males. In pregnancies at risk for a child affected with virilising adrenal hyperplasia suppression of foetal androgen production may decrease female genital ambiguity in females. This has been achieved by administering oral DEX to the mother (David and Forest 1984). As compared with hydrocortisone, DEX has no salt-retaining activity and crosses the placenta because it is not metabolised significantly by placental 11β-HSD2 (White et al. 1997). The typical dose is 20 mg/kg/day based on pre-pregnancy weight to a maximum of 1.5 mg daily in three divided doses have been shown to normalise androgen precursor levels (17-Hydroxy-Progesterone) in affected foetuses (Speiser et al. 2010; Vos and Bruinse 2010). Approximately 70 % of prenatally treated females are born with normal or only slightly virilised genitalia.

Prenatal diagnosis and treatment are usually offered because of a previously affected child, since the overall prevalence is very low (Speiser et al. 2010; Vos and Bruinse 2010). The critical time for development of the external genitalia (weeks 7–12) is before foetal sex can be determined by chorionic villus sampling. Prenatal treatment of CAH to prevent virilisation (in the case of a female foetus) must thus start before invasive tests can tell whether the foetus is affected and whether it is male (in which case treatment is stopped) or female (Hui and Bianchi 2011). Foetal sex determination using foetal Y-chromosomal DNA in maternal plasma is a

non-invasive method of prenatal diagnosis that allows women carrying male foetuses to avoid prenatal DEX exposure (Avent and Chitty 2006) and reduces the number of those unnecessarily exposed.

If prenatal treatment is undertaken, it should be considered experimental and "pursued through protocols approved by Institutional Review Boards at centres capable of collecting outcomes data on a sufficiently large number of patients" and should commence before 8 weeks' gestation (6–7th week postmenstrual) (Speiser et al. 2010). Prenatal treatment prevents virilisation in approximately 80–85 % of female foetuses (Speiser et al. 2010). Treatment failures are usually attributed to poor compliance, cessation in later pregnancy, or late onset of treatment (Vos and Bruinse 2010). The authors of the guideline explain that they place a higher value on preventing unnecessary prenatal exposure of mother and foetuses to DEX and avoiding potential harms associated with this exposure, and a relatively lower value on minimising the emotional toll of ambiguous genitalia on parents and patients. The significant maternal risks of long-term DEX therapy include hypertension, abnormal glucose tolerance, infection, and (potentially) osteoporosis or cataracts. Foetal risks include growth restriction, possible disruption of the HPA axis with long-term behavioural changes, and neurodevelopmental changes (Speiser et al. 2010).

Postnatal genitoplasty and hormone therapy remain alternatives to prenatal steroid use to treat female virilisation, but this does not address the postulated psychological effects of androgen exposure in affected females. There are similar concerns about the potential negative metabolic, cognitive, and behavioural effects of long-term prenatal steroid treatment on the growing foetus and the child. A large European follow-up study of children prenatally exposed to DEX was reassuring, with no reported differences in psychopathology, behavioural problems, or adaptive functioning, as compared to non-exposed children (Hirvikoski et al. 2008). A US-based comprehensive follow-up study investigating potential long-term adverse side effects of prenatal DEX treatment in children with 21-hydroxylase deficiency and their mothers is ongoing (Office of Rare Diseases (ORD) 2008). Despite decades of medical experience with prenatal DEX, CAH remains a controversial topic and debate continues online (http://www.fetaldex.org).

2.3.4 Long-Term Effects of Intrauterine Exposure to Fluorinated Corticosteroids (Ostensen et al. 2008)

Several animal studies and a few human studies have raised concern about possible negative long-term effects of BETA and DEX given to accelerate foetal lung maturation. It was suspected that antenatal exposure to BETA might result in insulin resistance in adult offspring, but a 30-year follow-up of a randomised controlled trial found no clinical effect on cardiovascular risk factors such as body size, blood lipids, blood pressure, prevalence of diabetes or history of cardiovascular disease (Dalziel et al. 2005).

In 16 anti-Ro/SSA-positive children exposed in utero to very high dosages of DEX (mean total dose 186.6 mg) no negative effects on the neuropsychological development were found (Brucato et al. 2006). This dose is much higher than that used to enhance foetal lung maturation (usually 48 mg, 24 mg with BETA), and are not comparable with the DEX therapy starting early in pregnancy. The findings of Brucato et al. seem reassuring considering that doses of fluorinated steroids were large compared to those used with CAH children.

2.3.5 Treatment of CAH in Pregnancy

The treatment of choice for pregnant women affected with CAH is hydrocortisone, and fludrocortisone is added for those with the salt-wasting form of the disease (Hui and Bianchi 2011; Lekarev and New 2011).

If the foetus is at risk for classical CAH, DEX treatment can be used prenatally to prevent masculinisation of the genitalia in a female infant (q.v.). Because DEX crosses the placenta, it should not be used to treat pregnant women with CAH if the foetus is not at risk for the disease.

2.3.6 Mineralocorticoid Therapy of CAH in Pregnancy

The mineralocorticoid dose has to be increased continuously in pregnant women with adrenal failure (primary adrenal failure or CAH) under tight observation of blood pressure and serum potassium. Offspring of these mothers have to be checked for signs and symptoms of adrenal insufficiency (National Center for Biotechnology Information. PubChem Compound Database CID 31378).

2.3.7 Mineralocorticoid Therapy of CAH During Breastfeeding

It is unknown whether effective doses of fludrocortisone are taken up by the infant with breast milk. Small amounts of glucocorticoids appear in breast milk; this may also apply for fludrocortisone.

2.3.8 Adverse Effect of Mineralocorticoid Therapy (Claahsen-van der Grinten et al. 2011)

Overtreatment with fludrocortisone should be avoided to prevent the effects of iatrogenic hyperaldosteronism (fluid retention and hypertension). Regular monitoring of blood pressure, plasma renin, serum sodium, and potassium concentrations is required. The hypokalemic effect of β_2 agonists and theophylline could be potentiated by fludrocortisone, therefore caution must be taken when using these drugs in asthma (Padidela and Hindmarsh 2010). The small glucocorticoid effect of

fludrocortisone may be relevant, especially in children (0.1 mg fludrocortisone is about equivalent to 1.5 mg hydrocortisone). On the other hand, as the mineralocorticoid potency of 20 mg hydrocortisone is equivalent to 50 μg of fludrocortisone, if high doses of hydrocortisone are given, e.g. for surgery extra mineralocorticoid treatment usually is not necessary (Padidela and Hindmarsh 2010).

3 Other Sex- and Gender-Dimorphic Effects of Glucocorticoids in Animal and Human Studies

3.1 Pregnancy-Related Animal Models: Effects on Development of Offspring

Repeated antenatal synthetic glucocorticoid (sGC) exposure has sex-specific effects on HPA development in the foetal and adult guinea pig. Glucocorticoid receptors (GR) and MR exhibit sex differences in their temporal and spatial expression during foetal and early postnatal life. Maternally administered BETA reduces foetal plasma ACTH and cortisol concentrations. BETA significantly affected hippocampal MR protein expression, and this effect was greatest in males. BETA was unable to autoregulate GR protein during foetal life, indicating that regulation of brain corticosteroid receptors is fundamentally different in foetal compared with adult life. The sex differences in the pattern of GR and MR expression during development may indicate different windows of vulnerability to prenatal glucocorticoid exposure in foetal life (Owen and Matthews 2003).

Administration of glucocorticoids to sheep early in pregnancy results in offspring with hypertension in adulthood. Hypertension in offspring exposed prenatally to cortisol is associated with increased total peripheral resistance, mimicking observations in human patients with chronic hypertension. The increased vascular resistance appears to be dependent, at least in part, on an increased effect of sympathetic vasomotor drive (Moritz et al. 2009).

A rat model, designed to determine if foetal DEX exposure is associated with decreased insulin sensitivity in adulthood, manifested as decreased binding of p85 to phosphorylated insulin receptor substrate-1 (IRS-1) within the phosphatidylinositol 3-kinase (PI 3-kinase) pathway of insulin signalling, showed decreased insulin signalling at the level of p85 binding to IRS-1. This effect was not enhanced by administration of insulin prior to sacrifice, nor was a sex-dependent effect noted (O'Brien et al. 2008).

Prenatal exposure to sGCs reduces the incidence of respiratory distress syndrome in babies at preterm delivery. Therefore, administration of multiple courses of sGCs became common practice. Animal and human studies have demonstrated that multiple courses of sGCs can have long-term effects. While the majority of animal studies have been undertaken in male offspring, it is emerging that there are profound sex differences in the consequences of prenatal sGC exposure. When

pregnant guinea pigs were administered BETA, DEX or vehicle, the BETA-exposed females exhibited increased GR mRNA expression in the hippocampus and MC2R (melanocortin 2 receptor) mRNA in the adrenal cortex, and the DEX-exposed animals exhibited higher hippocampal GR and MR mRNA levels. BETA-exposed females showed reduced fecundity. In DEX-exposed females there was a lower male to female sex ratio. The study demonstrated that there are long-term effects of prenatal sGC exposure on HPA axis activity and regulation in adult female guinea pigs. The outcomes are different depending on whether BETA or DEX was the sGC administered during pregnancy. This highlights the importance of long-term follow-up in children who were treated with either sGC (Dunn et al. 2010).

There is emerging evidence of subsequent neurobehavioural abnormalities. In rats, gestational treatment with DEX produces sex-selective alterations in activity, learning, and memory. Locomotor activity in females was reduced to the lower level typical of males, and habituation of activity similarly was impaired in females. Male rats in the DEX group showed a partially feminised pattern of habituation (Kreider et al. 2005). Restraint stress in late pregnancy of rats impaired cognitive development and dysregulated progestogen formation in the brain (Paris and Frye 2011). Learning deficits are more prevalent in males and anxious behaviour in females. Cognitive deficits and anxiety are linked to a sex-dependent reduction in neurogenesis and in measures of dendritic morphology in the prefrontal cortex and hippocampal formation. Corticosterone administration to the dam to mimic levels induced by stress reinstates only the anxiety, indicating that it arises from foetal exposure to corticosterone from the maternal circulation. Learning deficits in males may result from a combination of a reduction in testosterone and in aromatase activity, together with the action of other adrenal hormones. Sex differences in the behaviour and brain morphology of rats stressed prenatally were found during the equivalent period of neuronal development in humans. In humans, recent prospective studies have shown that gestational stress is more likely to cause cognitive and emotional problems in the offspring if it occurs during weeks 12–20 of pregnancy (Weinstock 2011).

This accumulating evidence from animal studies has raised concerns regarding the long-term consequences of prenatal treatment. Hirvikoski et al. (2008) reported that direct neuropsychological assessment of children exposed to DEX and controls show normal full-scale IQ, learning, and long-term memory. However, the children exposed to DEX during the first trimester had an impaired verbal working memory which was significantly associated with low self-perceived scholastic competence. In addition, the children showed increased self-rated social anxiety. The results indicate less masculine and more neutral behaviour in short-term DEX-exposed boys (Lajic et al. 2011). These findings indicate that long-term follow-ups of this group of patients are of extreme importance and that future DEX treatment of CAH may be questioned. However, additional studies on larger cohorts in order to draw more decisive conclusions about the safety of the treatment are encouraged.

Inhaled corticosteroids (ICS) are currently advised for the control of asthma during pregnancy, despite the lack of evidence regarding potential systemic effects on maternal, placental, and foetal systems. Foetal growth inhibition is a known

sequelae of in utero glucocorticoid exposure and has long-term consequences for adult health. Sex-specific foetal growth patterns are observed in pregnancies with maternal asthma and may be due to differential sensitivity of the placenta to glucocorticoids. It is currently unknown whether expression of the placental GR becomes altered with asthma or the use of ICS. The sex-specific associations between cortisol and birth weight observed in pregnancies with asthma are not due to altered GR expression; however, they may be due to differential glucocorticoid sensitivity via preferential transcription of GR isoforms or post-translational modifications (Hodyl et al. 2010). Foetal adrenal function appeared unaffected by ICS in pregnancies of both males and females. This provides clinically important information suggesting that ICS do not exert effects on glucocorticoid-regulated pathways in the foetus, and therefore are unlikely to contribute to adverse effects on foetal growth and development in human (Hodyl et al. 2011).

3.2 Effects on Expression of Genes and Proteins

Breast cancer-resistance protein (BCRP1), encoded by Abcg2 mRNA, limits the penetration of a spectrum of compounds into the brain. The foetal brain is a primary target for many BCRP1 substrates. sGCs increase Abcg2/BCRP1 expression and function in vitro in endothelial cells derived from brain microvessels. A regulatory role of glucocorticoids on Abcg2/BCRP1 in the foetal brain is of importance given that approximately 10 % of pregnant women are treated with sGCs for threatened preterm labour. Abcg2 mRNA expression significantly decreases with advancing gestation, while BCRP1-mediated neuroprotection increases. Furthermore, there is a dose-, sex-, and age-dependent effect of DEX on Abcg2 mRNA in the foetal brain in vivo, indicating a complex regulatory role of glucocorticoid during development (Petropoulos et al. 2011).

The constitutive expression level of CYP2B mRNA in the kidney of adult C57BL/6NCrj mice is higher in female than in male mice. In the liver more CYP2B9 is expressed in the females, and more CYP2B10 is expressed in the males. After treatment with DEX, induction of CYP2B10 mRNA and protein in the kidneys was far greater in male than in female mice. These observations suggest that there are multiple regulatory pathways in the expression of Cyp2b genes, one of which used by DEX is mediated via the GR, and sex hormones may play a role in the regulation of the sex-dependent expression of Cyp2b genes in the mouse (Jarukamjorn et al. 2001).

To determine whether glucocorticoid-inducible expression of hepatic hydroxysteroid sulfotransferase is conserved in the mouse, the effects of DEX on hydroxysteroid sulfotransferase (mSULT2A) gene expression were investigated in primary cultured hepatocytes prepared from C57BL/6J mice. In female mouse hepatocytes, DEX increased the amounts of mSULT2A mRNA relative to control. By contrast, mSULT2A mRNA levels were undetectable in male mouse hepatocytes. Female-predominant mSULT2A mRNA expression was also observed

in liver samples from C57BL/6J mice and three other mouse strains. Treatment of primary cultured female mouse hepatocytes with dihydrotestosterone in the presence of DEX suppressed mSULT2 expression. Transfection of primary cultured male or female mouse hepatocytes with a rat SULT2-40/41 reporter construct revealed that hepatocytes of both sexes have sufficient machinery to achieve DEX-inducible SULT2 transcription (Wu et al. 2001).

Na(+)/H(+) exchanger (NHE) activity regulates intracellular pH in the placental syncytiotrophoblast. In other tissues aldosterone and cortisol have been shown to up-regulate NHE activity via an acute, non-genomic effect. Syncytiotrophoblast NHE activity is increased acutely by aldosterone and, when 11β-HSD2 is blocked, by cortisol. These non-genomic effects are only evident in placentas from female babies and are mediated by classical MR and/or GR (Speake et al. 2010).

In the rat pituitary, the level of functional corticosteroid receptors is subject to a 20 % down-regulation by circulating levels of oestrogen. This raises the possibility that the lower number of receptors in females may act to reduce their sensitivity to the negative feedback effects of glucocorticoids at the level of the pituitary (Turner 1990).

3.3 Effects on Neural Correlates of Emotional Learning

A human functional magnetic resonance imaging (fMRI) study observed acute effects of the stress hormone cortisol on prefrontal structures. Men showed evidence for impaired fear conditioning after cortisol treatment, while the opposite pattern was found for women. In contingency-unaware participants cortisol has in some brain regions sex-specific effects on neural correlates of emotional learning. These effects might translate into a different vulnerability of the two sexes for anxiety disorders (Merz et al. 2010).

3.4 Anti-inflammatory Effects

Males and females show differences in the prevalence of many major diseases that have important inflammatory components to their aetiology. These gender-specific diseases, which include autoimmune diseases, hepatocellular carcinoma, diabetes, and osteoporosis, are largely considered to reflect the actions of sex hormones on the susceptibility to inflammatory stimuli. Glucocorticoids are the primary physiological anti-inflammatory hormones in mammals, and synthetic derivatives of these hormones are prescribed as anti-inflammatory agents, irrespective of patient sex. Sexually dimorphic actions of glucocorticoid regulation of gene expression may contribute to the dimorphic basis of inflammatory disease by evaluating the rat liver, a classic glucocorticoid-responsive organ. Glucocorticoid administration expanded the set of hepatic sexually dimorphic genes. Eight distinct patterns of

glucocorticoid-regulated gene expression were identified, which included sex-specific genes. Experiments also defined specific genes with altered expression in response to glucocorticoid treatment in both sexes, but in opposite directions. Pathway analysis identified sex-specific glucocorticoid-regulated gene expression in several canonical pathways involved in susceptibility to and progression of diseases with gender differences in prevalence. Moreover, a comparison of the number of genes involved in inflammatory disorders between sexes revealed 84 additional glucocorticoid-responsive genes in the male, suggesting that the anti-inflammatory actions of glucocorticoids are more effective in males. These gender-specific actions of glucocorticoids in liver were substantiated in vivo with a sepsis model of systemic inflammation (Duma et al. 2010). The data confirm with the finding in patients suffering from inflammatory bowel disease as men showed a significantly higher remission rate than women. Although it has also to be mentioned that women received significantly less immunosuppressive medication compared to men (Blumenstein et al. 2011).

Take Home Messages

- Hydrocortisone clearance is higher during puberty compared to pre- and post-pubertal stage, and the half-life of free cortisol appears significantly shorter in females compared with males, although the half-life of total cortisol does not change between sexes.
- *Substitution therapy in adrenal insufficiency during pregnancy*:
  - Therapy should never be stopped.
  - Between weeks 8 and 11 prednisone and prednisolone doses should not exceed 10 mg/day.
  - Dexamethasone and betamethasone have to be avoided.

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Thyroid Disorders

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Abstract All forms of thyroid diseases are much more frequently observed in women than men, although the reasons are still not completely elucidated.

Hyperthyroidism is defined by elevated circulating free thyroid hormones. The prevalence is about 2 % in women and 0.2 % in men. The most frequent causes are various forms of thyroid autonomy in elderly women and Graves' disease, which occurs mostly in younger women.

Hypothyroidism is defined by a lack of thyroid hormones. It is a common endocrine disorder caused by autoimmune thyroiditis (Hashimoto thyroiditis), iodine deficiency or following surgery or radioiodine therapy. Thyroxine requirements depend on fat-free mass and are, therefore, somewhat higher in males who are more often undersubstituted. In pregnancy lower TSH-reference ranges have to be considered and thyroid function should be monitored throughout pregnancy to avoid harm to the foetus caused by maternal thyroid dysfunctions. If overtreated women more often feature fractures, whereas males more often develop atrial fibrillation.

Keyword Hyperthyroidism • Graves disease • Hypothyroidism • Pregnancy • Iodine • Thyreostatic drugs • Thyroxine therapy

Abbreviations

CBZ	Carbimazole
PTU	Propylthiouracil
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine-binding globulin
TMZ	Thiamazole
TSH	Thyroid-stimulating hormone
TSH-R-ab	Thyroid-stimulating hormone-receptor antibody

1 Hyperthyroidism

1.1 Causes of Hyperthyroidism

Hyperthyroidism is defined by elevated circulating free thyroid hormones. The prevalence is about 1.3%, with a male/female ratio of 1 to 5–7 (2% in women, and 0.2% in men) (Cooper 2003; Dasgupta and Savage 2005; Fumarola et al. 2010), even in childhood (Rivkees and Dinauer 2007), and in its most severe form, hyperthyroid storm, where the peak incidence is shifted to the 8th decade (Karger and Fuhrer 2008). This is in line with the increasing prevalence with age (4–5% in elderly women) (Hollowell et al. 2002).

The most frequent causes are various forms of thyroid autonomy and Graves' disease. The ratio between Graves' disease and toxic-nodular goiter depends on the regional iodine supply (Laurberg et al. 2006). Graves' disease occurs mostly in younger women, autonomy more frequently in elderly women.

1.1.1 Graves' Disease

Graves' disease is responsible for 60–80% of all cases of hyperthyroidism and is significantly more frequent in women (about 0.5 cases per 1,000 women per year, gender ratio 1:5 to 1:10) (Weetman 2000). It occurs more frequently in areas with good iodine supply and usually manifests in the 4th decade (Holm et al. 2005; Streetman and Khanderia 2003).

Pathogenesis. Autoreactive T and B cells that infiltrate the thyroid gland (and the eye socket) produce cytokines and finally thyroid-stimulating hormone (TSH)-receptor antibodies (TSH-R-ab). Requirements are endogenous factors such as female gender, a genetic predisposition (sisters and daughters of Graves' patients have a 5–8% disease risk, monozygotic twins having a concordance rate of 20%), indicating that additional environmental factors such as infections, stress, oral iodine intake and smoking (dose-dependently) trigger the disease in susceptible individuals (Cooper 2003; Holm et al. 2005).

Therapy. Thionamides are usually offered as first-line therapy in Central Europe. Due to the lower liver toxicity thiamazole (TMZ) is usually preferred (Cooper and Rivkees 2009). The suboptimal remission rate with thionamide therapy (Allahabadia et al. 2000; Vaidya et al. 2008) led about 20% of endocrinologists in the UK and 70% in the US to offer radioiodine therapy as primary treatment (Wartofsky et al. 1991). In women desiring pregnancy radioiodine is not first choice, since TSH-R-ab tend to persist longer after radioiodine therapy (compared with medical therapy and surgery) and women thus incur a greater risk of foetal/neonatal hyperthyroidism (Laurberg et al. 2008).

1.2 Thyrostatic Drugs

1.2.1 Mechanism of Action and Pharmacokinetic Properties

Thyrostatic drugs dose-dependently inhibit the action of the haeme-enzyme thyroid peroxidase fixed on the luminal side in the cell membrane of the thyroid follicle. By serving as an alternative substrate for the iodinating intermediate, thionamides compete with thyroglobulin-linked tyrosine residues and thus inhibit the formation of thyroid hormones (Cooper 2005). Thionamides (sulphur-containing thyrostatic drugs) have the greatest importance. Starting from one goitrogen (phenylthiourea) all the substances now in use were found: propylthiouracil (PTU), carbimazole (CBZ) and TMZ (Brent and Koenig 2011). CBZ, a prodrug of TMZ, is rapidly converted into TMZ by cleaving of the ethoxycarbonyl residue (Cooper 2003).

Based on longer half-life, better potency and more favourable side effect profile, TMZ is considered as drug of choice (Cooper and Rivkees 2009).

Thionamides are used as short-term therapy before surgery or radioiodine therapy, or long-term as primary treatment to achieve remission in Graves' disease. A comparison of four randomized prospective studies showed that therapies shorter than 12 months led to more frequent relapses, while treatment longer than 18 months had no additional benefit on relapse rate (Cooper 2003, 2005).

1.2.2 Side Effects

Side effects of thionamides are dose-dependent (Cooper 2003).

Agranulocytosis. The frequency of agranulocytosis is estimated as 0.3% of all treatments (Tajiri and Noguchi 2004; Watanabe et al. 2011). Most cases (85%) occur within the first 100 days after start of therapy (Bahn et al. 2009; Cooper 2005). The disorder may be life-threatening, and is therefore the most serious side effect of thionamide treatment (Cooper 2003). Agranulocytosis differs from transient mild neutropenia (granulocyte count below 1,500/μl), which may accompany Graves' hyperthyroidism (Cooper 2005). A rare variant is agranulocytosis ending up in pancytopenia with bone-marrow morphology similar to aplastic anaemia, which nevertheless accounted for 10% of cases of agranulocytosis in a large retrospective analysis, possibly indicating that thionamides damage stem cells (Watanabe et al. 2011). Data spontaneously reported to the UK-wide pharmacovigilance agency point to a higher risk of PTU (Pearce 2004).

The risk depends on TMZ dose. Low dose therapy (15mg or lower) is associated with up to 10 times lower incidence rates of agranulocytosis compared to the formerly used high dose therapies (Takata et al. 2009; Tsuboi et al. 2007). Since agranulocytosis is an autoimmune-mediated disease it can occur at any dose ("idiosyncratic"), mostly in the *first 2–3 months of therapy* (Takata et al. 2009), presumably more frequent in *women* (Andres and Maloisel 2008; Cooper et al. 1983; van der Klauw et al. 1998) at least over 15 and under 65 years (Ibanez et al. 2005), *over 40* years 6.4-fold more frequent than under 40 years of age (Andres and Maloisel 2008; Cooper et al. 1983).

The reason why women are affected more frequently could be explained by pharmacokinetic differences between men and women (Table 1), immunologic (T-cell function) and hormonal factors as well as differences in co-medications.

Heretics think that the higher prevalence could be due to bias because of the longer life expectancy of women and thus longer exposition etc. (Andres et al. 2002). However, the data of Ibanez et al. on community-acquired drug-induced agranulocytosis do not support that assumption (Ibanez et al. 2005).

Neutropenia may precede agranulocytosis. This led to the recommendation to obtain a neutrophil count before starting thionamide therapy (Bahn et al. 2009; Cooper 2005). This recommendation might bar people with "ethnic neutropenia", like persons of African origin, Sephardic Jews and others from getting thionamide therapy (Tesfa et al. 2009). The control of neutrophil counts during TMZ therapy is under debate because of the sudden onset and lack of cost effectiveness (Cooper

Table 1 Pharmacokinetic differences between men and women

Lower fat-free body mass
Lower hepatic clearance
Different activity of cytochrome P450 enzymes
CYP3A4 activity↑, CYP2D6, CYP2C19 and CYP1A2↓
Differences in conjugation, absorption, protein binding and renal elimination

Adapted from Rademaker (2001)

2005; Vanderpump et al. 1996); however, it was shown that mandatory neutrophil count programmes save lives (Tajiri and Noguchi 2004; Tesfa et al. 2009). Improvement of management (including G-CSF-therapy) led to a drop in mortality to 5% or less (Andres and Maloisel 2008; Watanabe et al. 2011). Most patients recover within 2–3 weeks. Owing to risk of cross-reactivity after agranulocytosis with either PTU or TMZ/CBZ both drugs are contraindicated (Bahn et al. 2009).

1.2.3 Thionamide Therapy and Remission Rate of Graves' Disease

The remission rate of Graves' disease under thyrostatic therapy is 50% or less, and significantly higher in women as compared to men (40 vs. 19.6%) (Allahabadia et al. 2000; Vaidya et al. 2008). Unfavourable prognostic factors are familiar predisposition for autoimmune diseases of the thyroid gland, younger age, male gender, smoking, stressful life events, problems with coping, severe hyperthyroidism (in initial lab), high triiodothyronine/thyroxine (T3/T4) ratio, a long time delay from the onset of symptoms to start of therapy, large goiter, presence of ophthalmopathy or dermatopathy, presence of nodules, high TSH-R-ab titre at onset or end of thionamide therapy as well as high intrathyroidal blood flow at the end of therapy (Allahabadia et al. 2000; Hegedüs 2009).

Childhood. Graves' disease rarely occurs in childhood, the maximum incidence is reached between 10 and 15 years, and girls are affected more frequently (Kaguelidou et al. 2008). Although symptoms are similar to those in adult age, diagnosis frequently is late. In contrast to adult age only about 30% of children achieve remission with thionamides (Kaguelidou et al. 2008). It is unclear whether girls have a lower relapse risk (Kaguelidou et al. 2009).

2 Graves' Disease in Pregnancy and Breastfeeding

Hyperthyroidism occurs in 0.1–0.2% of all pregnancies. Untreated hyperthyroidism increases the risk of stillbirth, premature birth, low birth weight and increased neonatal mortality and morbidity. The mother is at an increased risk of gestational hypertension, preeclampsia, thyrotoxic crisis or heart failure (Stagnaro-Green et al. 2011).

The elevated concentration of progesterone and the formation of regulatory T cells with Th2 status serve to protect the foetus and reduce the activity of many

autoimmune diseases in pregnancy (Quintero et al. 2011). During the first trimester, women may experience an exacerbation of their symptoms, but in the course of the pregnancy significant reduction in disease activity takes place (most women can reduce thionamide doses in the course of the second and third trimesters, some may even discontinue therapy!) (Stagnaro-Green et al. 2011; Weetman 2010). If high TSH-R-ab titres persist foetal/neonatal hyperthyroidism can ensue (Abalovich et al. 2007; Laurberg et al. 2008; Stagnaro-Green et al. 2011; Weetman 2010). The titres of antibodies decrease with the progression of the pregnancy and should be low by the end of the second trimester. In patients with active Graves' disease, recent radioiodine-therapy or surgery TSH-R-ab titres should be measured by 24–26 week gestation. In case the titre is elevated (over three times the upper limit of normal) foetal and neonatal thyroid gland functions have to be monitored. Signs of potential foetal hyperthyroidism in ultrasound are foetal tachycardia, intrauterine growth restriction, advanced bone age, formation of goiter or signs of congestive heart failure and foetal hydrops (Laurberg et al. 2008; Stagnaro-Green et al. 2011).

Postpartum worsening ("rebound", "flare") of a pre-existing autoimmune thyroiditis (postpartum thyroiditis) may be associated with the return to the Th1 environment. Therefore, current guidelines recommend follow-up visits 6 weeks postpartum (Stagnaro-Green et al. 2011). In rare cases (intolerance or requirement of very high thionamide doses) surgery is recommended in the second trimester.

2.1 Thionamide Therapy and Pregnancy

Traditionally PTU was used for the therapy of hyperthyroidism in pregnancy because of the higher risk for embryonal malformations and more teratogenic TMZ effect (Abalovich et al. 2007). Inborn anomalies such as aplasia cutis as well as the TMZ-embryopathy syndrome (choanal- or esophageal atresia as well as facial anomalies) more frequently occur with TMZ (Clementi et al. 2010). Because of the significant risks of liver failure (Cooper and Rivkees 2009), this recommendation, however, was changed in favour of TMZ in the second and third trimesters (Bowman et al. 2011; Stagnaro-Green et al. 2011). Thus, the American Thyroid Association guidelines recommend a switch from PTU to TMZ after the first trimester (Bahn et al. 2009; Stagnaro-Green et al. 2011).

The dose depends on the severity of hyperthyroidism, a typical starting dose being 5–15 mg TMZ per day, or 50–300 mg PTU, respectively, in divided doses, equivalent doses for TMZ compared to PTU are 1:10–15 (Stagnaro-Green et al. 2011). All thionamides cross the placenta; therefore, to avoid adverse consequences for the foetus, the lowest possible dose should be used with free T4 levels at the upper limit of normal or slightly elevated. Potential problems of overtreatment are foetal goiter or hypothyroidism (Ochoa-Maya et al. 1999; Stagnaro-Green et al. 2011). Serum T3 determination is no longer recommended in the management of Graves' hyperthyroidism (exception: isolated T3-hyperthyroidism in autonomy), TSH remains suppressed with this approach in many women (Momotani et al. 1997;

Stagnaro-Green 2011; Stagnaro-Green et al. 2011). Placental PTU extraction with active transport to the foetus was suspected earlier (Mortimer et al. 1997). Gardner et al. (1986) showed that the concentrations of PTU were higher in umbilical cord blood than in the mother's serum. Therefore, the risk of hypothyroidism in the foetus with all potential consequences may be more likely (Momotani et al. 1997; Mortimer et al. 1997). The transplacental passage of PTU and CBZ/TMZ does not differ (although earlier studies had suggested a lower placental transfer to the foetus of PTU) (Clark et al. 2006; Mortimer et al. 1997).

In a very recent case–control study the newborns of 2,830 hyperthyroid women who took higher doses (>150 mg/day) of PTU during pregnancy showed lower birth weights, compared to babies of women who had taken TMZ/CBZ (Chen et al. 2011).

Breastfeeding. PTU and TMZ can be detected in breast milk (Low et al. 1979). Women who were on thionamide therapy for many years were advised against breastfeeding (Clementi et al. 1999). However, only small amounts of PTU get in the milk, corresponding to a milk–plasma ratio of about 0.1 (Low et al. 1979). Even high doses such as 750 mg/day showed no impact on the thyroid function of breastfed children (Momotani et al. 2000). TMZ has a milk–plasma ratio of about 1. Breastfed children of mothers on daily TMZ showed normal thyroid functions (Azizi 1996) and physical and intellectual development (Azizi et al. 2003).

Moderate doses (TMZ less than 20–30 mg/day, PTU less than 300 mg/day) taken in divided doses and always after breastfeeding appear safe, but testing thyroid function in breastfed offspring is still recommended (Stagnaro-Green et al. 2011).

Pregnancy and thyroid surgery. Recently Kuy et al. (2009) showed the significantly elevated risks of thyroid surgery in pregnancy. Surgery should therefore be performed only when the advantages of therapy outweigh the higher risks. If necessary, the second trimester is the optimal time (Owen et al. 2010; Stagnaro-Green et al. 2011).

2.2 Lithium

Lithium is clinically used as lithium carbonate for the treatment of bipolar disorders. Women on lithium therapy develop goitres and hypothyroidism more than three times as much as men (41 vs. 13%), even if thyroid-autoantibodies are negative (van Melick et al. 2010). In a dose of 600–1,000 mg/day, similar to iodine, it blocks the release of hormones and can be given in iodine allergy. Therefore, lithium is useful for the therapy of (thionamide)-resistant hyperthyroidism. Presumably lithium increases the retention of radioactive iodine in the thyroid gland (Lazarus 2009). Via reduced hormone release it can be given to lower the risk of hyperthyroidism with radioiodine-therapy when the cardiovascular risk is high (Saleem et al. 2011). A controlled study with 900-mg lithium before radioiodine therapy, however, was negative (Bal et al. 2002). Lithium is filtered freely as free ion, reabsorbed in the proximal tubules, and excreted by the kidneys proportional to

the glomerular filtration rate. Via its effect on glomerular filtration rate gender affects pharmacokinetics (Chiu et al. 2007). In the last months of pregnancy the clearance of lithium rises by about 30–50%. Lithium appears in equal concentration in breast milk and serum (Grandjean and Aubry 2009).

2.3 *Inorganic Iodine*

Iodine is an essential component of thyroid hormones. Gestation is accompanied by increased iodine requirements. During pregnancy and lactation women have an increased iodine demand (Glinoer 1997). Iodine deficiency may impair neurogenesis, migration and myelination (Zimmermann 2009). The most dangerous consequence of an inadequate iodine supply is the critical shortage of the developing brain with thyroid hormones, foremost the delicate development of myelin sheaths in the foetal- and early neonatal period (Barradas et al. 2001). World Health Organization (WHO) in 2004 raised the recommendations for the iodine intake in pregnancy from 200 to 250 μg/day (de Benoist et al. 2004). National Health and Nutrition Examination Survey (NHANES) data from 2007 to 2008 show that (unchanged to the previous survey) iodine supply in the US is worse in women than in men (Caldwell et al. 2011). This imbalance is observed worldwide, starts in childhood and gets worse in adolescence, which is mirrored in a higher prevalence of goiter (Cobra et al. 1997). A high percentage of pregnant women even in the USA is slightly undersupplied with iodine, 15% have severe iodine deficiency (Caldwell et al. 2011). Similar data were collected by our group in the Vienna area on pregnant women, generally considered an area of iodine sufficiency. A general supplementation with 150 μg iodine per day was already recommended in 2006 by the American Thyroid Association in order to improve iodine supply of pregnant and breastfeeding women (Becker et al. 2006). Ideally this substitution has to start already months before pregnancy (at least in areas with mild iodine deficiency) to avoid the small rise which occurs when iodine is started during pregnancy (Moleti et al. 2011). In many western countries a significant part of iodine ingestion in women happens accidentally (via milk and dairy products which contain iodophores used as disinfectants) (Zimmermann 2011). Therefore, worldwide efforts for sufficient iodine supply have to be intensified especially in women of childbearing age.

2.3.1 Treatment of Hyperthyroidism with Iodine

Before thyroid surgery iodine blocks the release of thyroid hormones immediately and reduces blood flow in the thyroid gland although the exact mechanism is unclear. Side effects are mostly mild and comprise rashes, nausea, vomiting, diarrhoea, dysuria and, very rarely, acute renal failure and thrombocytopenia (Pearce and Braverman 2004). Gender-specific differences concerning dose or side effects are not known.

2.4 Radioiodine Therapy in Hyperthyroidism

Radioiodine therapy with the beta emitter ^{131}I is used for the treatment of hyperthyroidism in Graves' disease as well as in cases of thyroid autonomy. Similar to surgery it is an ablative method which can lead to hypothyroidism (Ross 2011). This risk increases with time (55.8% after 1 year, 86.1% after 10 years) and, in case of Graves' disease, is the treatment goal (Ahmad et al. 2002). "Risk factors" for the development of hypothyroidism are (apart from Graves' disease) female gender, radioiodine dose, no pre-treatment with thionamides, no palpable goiter, less severe hyperthyroidism as well as positive autoantibodies. In the opposite sense women have higher success rates as compared to men (Boelaert et al. 2009), and the risk of relapse is higher after pre-treatment with thionamides (Bonnema et al. 2004).

The use of radioiodine imaging and/or uptake determination or therapeutic dosing is contraindicated during pregnancy (Cooper et al. 2009; Stagnaro-Green et al. 2011). The foetus is exposed to radiation from ^{131}I circulating in the mother's blood, as well as from the transit of ^{131}I in the urinary and gastro-intestinal tract, which is ~0.5–1.0 rad (5–10 mGy) per mCi administered (Abalovich et al. 2007; Hyer et al. 2011).

Prior to radioiodine application all women have to be asked for the possibility of pregnancy and before therapy a negative result of a pregnancy test "should" be on hand (Abalovich et al. 2007; Cooper et al. 2009) which has to be performed within 72 h or less before ^{131}I treatment (Hyer et al. 2011).

3 Hypothyroidism

3.1 Therapy of Hypothyroidism

Substitution is carried out with synthetic L-T4. Typical doses are roughly 100–125 μg/day for women, about 125–150 μg/day for men.

3.1.1 Pharmacology and Metabolism

In young euthyroid individuals T4 has a half-life of about 7 days, thus a "steady state" is reached only after 4–6 weeks (Singer et al. 1995). Interestingly, there is a lack of data on T4 pharmacokinetics (Soldin et al. 2010). In older individuals half-life is longer (about 9 days in the 7th decade) (Gregerman et al. 1962). In hypothyroid patients T4-metabolism is slowed and a steady state is reached later after start of therapy or dose changes, for which reason longer control intervals can be chosen initially. The contrary applies to T4-metabolism in hyperthyroidism, thus shorter control intervals should be chosen initially. Counter-intuitively, in pregnancy T4 elimination rates seem to be slower than in the non-pregnant state, despite the increase in cardiac output and the increased clearance of nearly all drugs in

pregnancy (Soldin et al. 2010). About 80% of T4 is absorbed within the first 3 h after oral ingestion (Benvenga et al. 1995). The amount absorbed depends on the galenics as well as simultaneously ingested food, drugs or minerals.

3.1.2 Thyroxine Requirement

Thyroxine is a narrow therapeutic index drug, precise dosing is critical (Soldin et al. 2010). In case of (functionally or post-ablation) absence of a thyroid gland T4 requirement mounts to about 1.6 (−1.8) μg/kg body weight per day (Roberts and Ladenson 2004). Typical doses are 100–125 μg/day for women and 125–150 μg/day for men. The required dosages for TSH suppression with differentiated thyroid carcinoma are about 2.0–2.5 μg/kg (Burmeister et al. 1992; Mandel et al. 1993).

The individual T4 requirement is variable. It depends on body weight (Burmeister et al. 1992; Fish et al. 1987; Jonklaas 2010; Mandel et al. 1993), mainly on the fat-free body mass (Cunningham and Barzel 1984; Santini et al. 2005), especially in men and elderly women (Cunningham and Barzel 1984), age (Cunningham and Barzel 1984), body surface (Hennessey et al. 1986) and last but not least, pregnancy status. Santini et al. postulated in their study that age- and gender-dependent differences could be explained by differences in the proportion of fat-free body mass, which could not be corroborated in other studies (Mistry et al. 2011).

In women on oral oestrogen therapy (or pregnant) thyroxine-binding globulin (TBG) is elevated up to 50%. Thereby the T4-demand becomes higher, or pre-existing subclinical hypothyroidism turns into overt thyroid failure (Arafah 2001). Twelve weeks after start of oral oestrogens (or oestrogen-containing oral contraceptives) women on T4-therapy should have TSH checked and the dosage adapted if necessary (Arafah 2001). Transdermal oestrogens, due to the lack of a first-pass effect on the liver, hardly have any effect on TBG concentrations and thus the T4 requirement (Steingold et al. 1991). Even when Tamoxifen and Droloxifen, selective oestrogen-receptor modulators (SERM), increase TBG in postmenopausal women, the impact on T4 requirement, however, is a controversial matter (Mamby et al. 1995; Marqusee et al. 2000). Androgens, via its TBG-lowering effect, lower T4 requirements. Some women on T4 substitution therapy who received androgens for breast cancer developed hyperthyroidism (Arafah 1994). In a study with 58,567 hypothyroid patients on T4 substitution men were much more often poorly substituted (TSH $>$ 4 mU/l, odds ratio 2.86) (Okosieme et al. 2011). This is corroborated by recent data of the Scottish Tayside Study (Flynn et al. 2010), as well as indirectly by the Cardiovascular Health Study (3,678 individuals over 64), which more frequently showed a lower TSH in women (Somwaru et al. 2009).

3.1.3 Side effects of Thyroxine Therapy

Side effects of T4 therapy are increased fracture risk, especially in the elderly. In a recent study on individuals over 70 on current T4 therapy fractures were more

frequent in both sexes, although in women fractures (esp. hip fractures) generally occurred more often compared to individuals who had not taken T4 tablets for more than 6 months. In this very large (213,511 individuals on T4 therapy) populations-based cohort study (Turner et al. 2011) even moderate doses (44–93 μg/day) increased the fracture risk with an odds ratio of 2.62 compared to doses lower than 44 μg/day. Although fracture risk was higher in women on T4 therapy, the risk of increase with higher T4 doses was much stronger in men compared to women, possibly related to differences in T4-pharmacokinetics (steeper dose–effect curve in men compared to women) (Flynn et al. 2010; Somwaru et al. 2009; Turner et al. 2011). If the last T4-intake dated back more than 6 months hip fractures were already significantly less frequent. The strength of this study is its size, which allowed the detection of dose dependency, as well as the exclusion of patients with previous or present hyperthyroidism, thyroid cancer, on haemodialysis or in palliative settings. In the USA over 20% of elderly women take T4-tablets (Canaris et al. 2000). The elevated fracture rate even at rather low doses could be a consequence of the higher TSH-reference range in older age (Surks and Hollowell 2007), TSH within the "usual" reference range already meaning over-substitution. In addition, the required T4 doses are lower in the elderly. Although optimum T4 doses for adults are 1.6–1.8 μg/kg/day (Roberts and Ladenson 2004), elderly frequently need much lower doses. In the elderly (age $>$ 65 years) a dose of 0.5 μg/kg/day is recommended (Roberts and Ladenson 2004; Sawin et al. 1983).

In line with the increased risk of atrial fibrillation in (subclinical) endogenous hyperthyroidism even when TSH is in the low normal ("pre-subclinical") range (Auer et al. 2001; Kirchhof et al. 2012), risk is also elevated in exogenous hyperthyroidism (Batrinos 2006). Atrial fibrillation affects men more frequently than women, and hyperthyroid men have a higher risk (odds ratio 1.8) for atrial fibrillation than women (Frost et al. 2004). It can be assumed that the risk for atrial fibrillation is increased (more in men than in women) in individuals either over-treated or under suppressive doses of T4 for thyroid carcinoma.

In atrial fibrillation, on the other hand, the risk of ischaemic stroke is higher in women (relative risk 1.6–2.0) than in men (Camm et al. 2010; Fang et al. 2007), possibly even when on anticoagulation therapy (Poli et al. 2009). Gender has therefore been incorporated as "clinically relevant non-major risk factor" in the current CHA_2DS_2-VASc-Score for the calculation of the stroke rate as base for whether or not to anticoagulate (Camm et al. 2010). Stroke risk seems to be higher when atrial fibrillation is caused by hyperthyroidism (Siu et al. 2009). In this context the current guidelines advise anticoagulation "in the presence of risk factors for stroke" as class I recommendation (Camm et al. 2010). Due to the increased clearance of vitamin K-dependent coagulation factors, the sensitivity of thyrotoxic patients to the anticoagulant effect of warfarin is increased and may require lower doses (Kellett et al. 1986; Shenfield 1981). This could increase the bleeding risk especially in women (Poli et al. 2009).

3.2 Hypothyroidism and Pregnancy

For diagnosis and therapy targets lower TSH-reference ranges have to be considered. In the second- and third trimester free T4 concentrations are lower compared to non-pregnant women (Stagnaro-Green et al. 2011).

An initial, retrospective study (Haddow et al. 1999) showed an IQ decline of seven points in children of mothers who were (subclinically) hypothyroid during pregnancy (compared to children of euthyroid mothers). Despite all weaknesses this study pointed to the importance of treatment of hypothyroidism in pregnancy, the optimal adjustment of preexisting hypothyroidism, as well as screening for thyroid disorders in pregnant women and in women seeking pregnancy.

Autoimmune thyroiditis in pregnancy increases the risk for miscarriage and premature birth and 50% of the women concerned experience some thyroid dysfunction in the context of postpartum thyroiditis (PPT) (Glinoer et al. 1994). The risk for the development of hypothyroidism during pregnancy is high (Lazarus et al. 2002). About 2–3% of all "healthy" women in reproductive age have an elevated TSH (2–2.5% subclinical hypothyroidism, 0.3–0.5% overt hypothyroidism). In pregnancy overt hypothyroidism is defined as TSH $>$ 10 mU/l and or TSH $>$ 2.5 mU/l plus low free T4 (Stagnaro-Green et al. 2011). Due to the elevated risks for pregnancy complications and impairment of neurointellectual development, the requirement of treatment of overt hypothyroidism is clear-cut (Stagnaro-Green et al. 2011). Even in subclinical hypothyroidism a (dose dependent with TSH) risk for pregnancy complications exists. A correlation with intelligence impairment, however, has never been shown definitely (the respective "Controlled Antenatal Thyroid Study" is ongoing). A recent prospective randomized treatment study on 21.846 pregnant women found no benefit of treatment on IQ scores of the children at 3 years of age (Lazarus et al. 2012). The scientific evidence for a benefit of T4 substitution in subclinical hypothyroidism without elevated thyroid peroxidase (TPO) antibodies thus is unclear. If antibodies are concomitantly elevated, therapy is recommended, the target of therapy being normalization of TSH into the trimester-specific reference range (Stagnaro-Green et al. 2011). As much as 50–85% of all women with treated hypothyroidism need higher T4-dosages already in the 4th–6th week of pregnancy, often before pregnancy is formally confirmed. Therefore, it has been proposed to take two extra daily doses of T4 per week, if a pregnancy test is positive or the period is missing, corresponding to an increase of T4 by 29% per week (Yassa et al. 2010). If pregnancy is planned TSH should be optimized already preconceptionally (to $<$ 1.5 or presumably even better to $<$ 1.2 mU/l) to avoid mild hypothyroidism early in pregnancy (Abalovich et al. 2010). There are factors other than preconceptional TSH concentrations which have an effect on size and pace of the rise of T4 requirement. For example, the (individual) changes of the maternal oestrogen concentrations correlate with the change of the T4 requirement (Alexander et al. 2004). To avoid a rise of TSH, thyroid function should be tested every 4 weeks until the 20th pregnancy week, when T4 demand does not increase any further (Stagnaro-Green et al. 2011; Yassa et al. 2010) (Tables 2 and 3).

Table 2 Sex/gender differences in existing drugs for the therapy of hypothyroidism

Drug	Synthetic L-thyroxine (T4)	Selenium	Triiodothyronine (T3)
Class			
Sex-specific features	In women on oral oestrogen therapy (or pregnant) adaptation of dosage is necessary because of elevated TBG Women receiving androgens for breast cancer can develop hyperthyroidism Fracture risk higher in women, but increases with higher doses much stronger in men	Women are better supplied with selenium than men Selenium supply in women correlates negatively with thyroid gland volume and presence of multiple thyroid nodules	In men higher scores of depression are related to lower total T3 concentration In animal studies T3 affects gender-specific behaviour and is a mediator of sexual dimorphism in the effect of ageing on the functional decline of BAT
Comments			
References	Arafah (2001), Arafah (1994), and Turner et al. (2011)	Xun et al. (2011) and Rasmussen et al. (2011)	Bunevicius et al. (2006), Lifschytz et al. (2006), and Valle et al. (2008)

Table 3 Sex/gender differences in existing drugs for the therapy of hyperthyroidism

Drug	Propylthiouracil	Carbimazole	Thiamazole	Iodine
Class	Thionamide	Thionamide	Thionamide	
Sex-specific features			The side effect agranulocytosis is more frequent in women	Gender-specific differences not known
Comments				Radioiodine therapy is contraindicated during pregnancy
References			Andres and Maloisel (2008), Cooper et al. (1983), and Van der Klauw et al. (1998)	Cooper et al. (2009) and Stagnaro-Green et al. (2011)

3.3 Selenium and Thyroid

Selenium is an essential trace element which is important for the activation of T4 to T3 (part of the deiodinase enzymes), possibly involved in the developments of nodules and carcinomas as well as development of autoimmune diseases (via detoxification of reactive oxygen radicals by selenium-containing oxidases and

effects on the immune system) (Hoffmann and Berry 2008; Schomburg 2011). It seems that women are better supplied with selenium than men (Xun et al. 2011). This, however, is a mixed blessing since in case of excellent selenium supply metabolic syndrome and diabetes occur more frequently (see below). At least in women the selenium supply correlates negatively with the volume of the thyroid gland, as well as with the presence of multiple thyroid nodules (Rasmussen et al. 2011). Derumeaux et al. (2003) found a negative effect of worse selenium supply in women, but not men, which explains contradictory results of some selenium studies, the mechanism, however, is unclear (Rasmussen et al. 2011). Similarly, Nacamulli et al. (2010) reported in a therapy study a possible protection from thyroid autoimmunity (decline of TPO- and Thyroglobulin [Tg]-autoantibody titres) via sufficient selenium supply. Marcocci and colleagues (2011) described a favourable effect of low-dose selenium in mild endocrine ophthalmopathy. Another study described a protective effect of selenium on the appearance of PPT (Negro et al. 2007). In the NHANES III study men had a higher diabetes risk when their serum selenium concentrations were in the highest tertile (Bleys et al. 2007), whereas another cross-sectional study found an inverse relation between selenium concentration in the toenails and diabetes risk in elderly men (Rajpathak et al. 2005). A French longitudinal study showed a protective effect of a higher selenium concentration on the incidence of diabetes in men, but not women (Akbaraly et al. 2010). These apparently contradicting results could be due to the selenium status of the respective cohorts (lower in Europe, f.i. the French studies of Akbaraly et al., Marcocci et al., and Negro et al.). The results could be explained by a U-shaped relation between selenium supply and diabetes risk. The possibly elevated risk of type 2 diabetes both in epidemiologic as well as therapeutic studies is a strong argument against a general selenium supplementation in autoimmune diseases without knowledge of the individual selenium status (Stranges et al. 2007).

4 Other Thyroid Hormone Therapy-Related Sex-and Gender-Differences: Animal and Human Studies

4.1 Antidepressive Effects

Thyroid hormones, particularly T3, have long been used for the treatment of depression, most frequently to enhance the therapeutic activity of other antidepressants. Blockade of the antidepressant-like effects of T3 by the thyroid hormone receptor α (TRα) antagonist, dronedarone, suggests that these effects are TRα-mediated (Lifschytz et al. 2011). The mechanism underlying the antidepressant effect of T3 may involve a reduction in 5-HT1A and 5-HT1B receptor transcription rates (Lifschytz et al. 2010). Of note, attenuation of 5-HT1A receptor agonist-induced hypothermia occurred only in males, which shows the need to evaluate a possible gender disparity in the role of presynaptic 5-HT1A receptors in

T3 antidepressant mechanisms (Lifschytz et al. 2011). Evidence for sex-dependent effects of T3 and fluoxetine on behaviour of male and female rats has been demonstrated also in the forced swim test, which is a test used for screening antidepressant-like activity of compounds in animals. While fluoxetine reduced immobility and increased active behaviours in male rats, it had no effects in female rats. However, the effects of fluoxetine in male rats were not potentiated by T3. In contrast, in female rats T3 decreased immobility and increased swimming with some latency. These results provided some support from an animal model for the efficacy of T3 as antidepressant therapy in female patients, but did not provide support for the augmentation and acceleration effects seen clinically when T3 is used in conjunction with established antidepressants such as fluoxetine (Lifschytz et al. 2006). Recently, however, it has been shown that the enhancement of antidepressant action of fluoxetine by T3 may be related to its effect of increasing hippocampal neurogenesis (Eitan et al. 2010).

Patients with coronary artery disease (CAD) and depressive symptoms had a higher prevalence of cardiac failure, higher N-amino terminal fragment of pro-B-type natriuretic peptide (NT-pro BNP) concentrations, and lower free T3 concentrations than patients with CAD but without depressive symptoms. In men, higher scores of depression were related to lower total T3 concentration and to higher NT-pro BNP concentration. These findings suggest that symptoms of depression in patients with CAD are associated with changes in thyroid axis function and with cardiac impairment, especially in men (Bunevicius et al. 2006).

4.2 Energy, Steroid and Bile Acid Metabolism

Sex and thyroid hormones are among the factors modulating energy metabolism through regulation of mitochondrial oxidative capacity. Brown adipose tissue (BAT) in old female rats has been shown to maintain the ability to produce heat better than males when exposed to cold. Sex steroid hormones were well correlated with BAT parameters when both genders were considered; however, T3 was the hormone with the strongest positive correlations in female rats. Thus, it is suggested that T3 may be a potential mediator of the sexual dimorphism in the effect of ageing on the functional decline of BAT (Valle et al. 2008).

Hormone [adrenocorticotropin (ACTH), endorphin, progesterone, T3] content in immune cells of rats is gender-dependent with higher levels in females, independent of sexual maturation (Csaba and Pallinger 2009). In rat liver, the sexually differentiated microsomal enzyme steroid 5 alpha-reductase, which catalyses the NADPH-dependent conversion of testosterone to 5 alpha-dihydrotestosterone, is expressed at a tenfold higher level in adult female as compared to adult male rats. The pituitary regulation of this enzyme and its mRNA was studied in untreated and hypophysectomized rats and in rats rendered hypothyroid by treatment with methimazole. It was demonstrated that thyroid hormones act at a pretranslational level to modulate the expression of some growth hormone-stimulated hepatic

mRNAs and that both T4 and growth hormone can independently contribute to the sex-dependent expression of hepatic enzymes of steroid metabolism (Ram and Waxman 1990).

Thyroid hormones exert significant changes in the metabolism of bile acids. Previous studies have shown that the activity of the human cholesterol 7 alpha-hydroxylase (cyp7a) gene promoter is repressed by T3 in cultured cells. However, in humans, the effect of thyroid hormone on cyp7a, the rate-controlling enzyme in the classical bile acid biosynthetic pathway, is difficult to study directly in vivo. It was hypothesized that T3 would negatively regulate human CYP7A1 gene expression in vivo. Therefore, this hypothesis was tested by inducing hypo- and hyperthyroidism in transgenic mice expressing the human CYP7A1 gene. Hypothyroidism did not affect the abundance of human cyp7a mRNA in transgenic mice. In hyperthyroid male mice, human cyp7a mRNA abundance was decreased, whereas no significant change in cyp7a mRNA abundance was observed in hyperthyroid female mice. Gender differences in the amount of cholesterol and bile acids in gallbladder bile were also observed. These data indicate that thyroid hormone can repress the human CYP7A1 gene in transgenic mice, and this effect is dependent on gender in this in vivo model (Drover and Agellon 2004). Finally, the conversion of cholesterol to bile acids and subsequent faecal excretion is an important mechanism for the removal of cholesterol from the body. Thyroid hormone is known to play a role in the regulation of bile acid synthesis, but this is unlikely to be a primary mechanism for lipid changes in patients with hypothyroidism.

The thyroid hormone receptor β (TRβ) is predominant in liver and mainly responsible for effects on cholesterol and lipoprotein metabolism, whereas TRα is most important in fat, muscle and heart. Thyroid hormone analogues (thyromimetics, tiromes) have been developed that activate TRβ. They are selectively taken up and/or activated by the liver. Such compounds stimulate hepatic LDL receptors, cholesterol elimination as bile acids and cholesterol, and presumably promote reverse cholesterol transport. In animals, they retard atherosclerosis progression. In humans, eprotirome exerts favourable lipid-modulating effects while lacking thyroid hormone-related side-effects and maintaining normal hypothalamic-pituitary–thyroid feedback. When added to statins, it reduces LDL and non-HDL cholesterol, apolipoprotein B and triglycerides as well as lipoprotein (a) (Angelin and Rudling 2010). However, clinical trials to date have not consistently shown a beneficial effect of levothyroxine treatment on serum lipid levels in subclinically hypothyroid patients (Pearce 2011).

4.3 Cardiovascular Effects

Oxidative stress (OS) has been documented in hypothyroidism, and OS has been reported to be more prevalent in male hypothyroid patients. Considering the negative influence of OS on health, extra attention should be paid to male hypothyroid patients in spite of the low prevalence of this disease in men (Nanda et al. 2008); in accordance with the report of Imaizumi et al. (2004) male survivors of the

Nagasaki atomic bombing with subclinical hypothyroidism had lower survival than their female counterparts. A recent metaanalysis, however, reported no differences in cardiovascular risk between men and women (Rodondi et al. 2010).

The literature on the effect of excess thyroid hormone on ventricular repolarization is controversial. To study whether free T4 and TSH are associated with QTc prolongation a cohort study with exclusion of participants with hypothyroidism, use of antithyroid drugs, thyroid hormones or digoxin, left ventricular hypertrophy, and left and right bundle branch block was conducted. High levels of free T4 were associated with substantial QTc prolongation in men of up to 10 ms. Free T4 was associated with a significantly increased risk of borderline and prolonged QTc values with its risk of sudden cardiac death (van Noord et al. 2008). Interestingly, long QT syndrome together with bradycardia, flat T waves and central low voltage are—when occurring together—highly predictive of hypothyroidism (Klein 2005).

Take Home Messages

- *Risk factors for agranulocytosis*
  - Advanced age
  - Female
  - Higher TMZ dose
  - Therapy with PTU
- *Thyroxine requirement is different in men and women*
  - About 100–125 μg vs. 125–150 μg/day
  - Dependent on fat free mass
  - Pregnancy status, estrogen intake
  - Men more often undersubstituted
  - If overtreated presumably more fractures in women, but less atrial fibrillation
  - Treatment of subclinical hypothyroidism is recommended in pregnancy, evidence of benefit poor
- *Selenium and thyroid*
  - Women possibly better supplied
  - Effect of selenium deficiency in women possibly more unfavorable

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Sex-Specific Differences in Type 2 Diabetes Mellitus and Dyslipidemia Therapy: PPAR Agonists

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Abstract The influence of sex on the development of obesity, Type 2 Diabetes Mellitus (T2DM), and dyslipidemia is well documented, although the molecular mechanism underlying those differences reminds elusive. Ligands of peroxisome proliferator-activated receptors (PPARs) are used as oral antidiabetics (PPARgamma agonists: thiazolidinediones, TZDs), or for the treatment of dyslipidemia and cardiovascular diseases, due to their lipid-lowering properties (PPARalpha agonists: fibrates), as PPARs control transcription of a set of genes

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involved in the regulation of lipid and carbohydrate metabolism. Given a high prevalence of those metabolic disorders, and thus a broad use of PPAR agonists, the present review will discuss distinct aspects of sex-specific differences in antiobesity treatment using those groups of PPAR ligands.

Keywords PPARgamma • PPARalpha • Nuclear receptor cross talk • Type 2 diabetes mellitus • Hypertriglyceridemia • Hypercholesterolemia • TZDs • Fibrates

Abbreviations

BMI	Body mass index
CAD	Coronary artery disease
ER	Estrogen receptor
NHR	Nuclear hormone receptor
PPAR	Peroxisome proliferator-activated receptor
RXR	Retinoid acid receptor
T2DM	Type 2 diabetes mellitus
TZD	Thiazolidinediones

1 Sex-Specific Differences in Obesity and Metabolic Disorders

It is well known that glucose and lipid metabolism, and as a consequence whole body composition, differ between sexes (Dayspring and Pokrywka 2010; Hsieh et al. 2007). Women exhibit a greater accumulation of adipose tissue, predominantly in the gluteal–femoral region of the body, whereas men show increased accumulation of abdominal fat, which has been associated since years with a higher risk for cardiovascular morbidity/mortality, development of T2DM, and other metabolic complications (Kissebah et al. 1982; Ross et al. 2002; Yusuf et al. 2005). This sex-specific pattern changes after menopause when a redistribution of fat from subcutaneous to visceral depots occurs. Differences between the basal and stimulated lipolysis of different fat depots as well as differences between males and females have been identified in different studies, but results remain controversial (Blaak 2001).

There are consistent results in regard to lipolysis and fat oxidation during endurance training. When compared with men women have better ultra-endurance capacity and oxidize more fat (Carter et al. 2001; Maher et al. 2009). Interestingly, different studies showed in the muscle of women when compared to male tissue a higher mRNA expression of genes involved in lipid metabolism, like fatty acid transporters, enzymes of ß-oxidation, and others, providing an explanation for the higher lipolytic activity in women during exercise (Maher et al. 2010). Further examples for higher expressed genes are hormone sensitive lipase (HSL) (Roepstorff et al. 2006), lipoprotein lipase (LPL), cytosolic fatty acid binding

protein (FABPc), sterol regulatory binding protein-1c (SREBP-1c) (Tarnopolsky 2008), and Fatty Acid Translocase/CD36 (Kiens et al. 2004).

Although the prevalence of diabetes seems to be comparable in women and men, a study performed by Vaccaro and colleagues (2008) on T2DM-patients in Italy showed a significantly higher average body mass index (BMI) in diabetic women, when compared to men. Also glycated hemoglobin (HbA1c) and plasma LDL-cholesterol were increased, reflecting a more adverse cardiovascular risk factors profile with a pronounced negative effect on the development of cardiovascular diseases in diabetic women compared to men.

Also other studies indicated an increased cardiovascular risk in women with T2DM, when compared to diabetic men (Rivellese et al. 2010) but the cause of those sex-specific differences is not completely understood, and could be explained—according to the work from Rivellese and colleagues—by biological and behavioral differences, such as heavier burden of cardiovascular risk factors, functional and structural differences of the cardiovascular system, and discrepancy in medical treatment and treatment response.

The common problem with the identification of sex-specific differences is connected with the fact that women are underrepresented in most of the clinical studies. For years it has been a common standard (in human and animal studies) to include only males due to unwanted effects of sex hormones. But the role of sex hormones is, as investigated in the last years, tremendous and not negligible. Even if women are increasingly involved in clinical trials nowadays, the study design and randomization of the groups often unable the analysis of sex-specific information, such as sex-related differences in pharmacokinetics and pharmacodynamics, as well as sex-specific side effect profiles (Gandhi et al. 2004). Only few studies subdivide groups for specific analysis of sex interactions. In addition, women who are included in the studies are mainly postmenopausal. In summary, although sex-specific aspects of obesity are well known, sex-specific strategies for the treatment of metabolic disorders and diabetes are not available at the moment.

2 Treatment of Metabolic Disorders and PPARs

The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptors (NHR) superfamily, consisting of several transcription factors and transcriptional regulators, such as thyroid receptors, estrogen receptors (ERs), retinoid acid receptors (RXRs), and others (Ferre 2004; Lonard and O'Malley 2006). PPARs control transcription of a set of genes involved in the regulation of carbohydrate and lipid metabolism, vascular endothelial functions, and pancreatic beta-cell functions (Tobin and Freedman 2006). Up to now three diverse PPARs have been identified: subtype PPARalpha, gamma, and delta (Reddy 2004).

PPARalpha is highly expressed in the liver and skeletal muscle and modulates intracellular lipid metabolism by transcriptional regulation of genes involved in fatty acid uptake/oxidation and triglyceride catabolism (Claudel et al. 2005).

PPARalpha ligands comprise of endogenous and pharmacological compounds (fibrates) for the treatment of dyslipidemia and cardiovascular diseases, due to their lipid-lowering properties (Forman et al. 1997).

PPARgamma is a key regulator of glucose and lipid metabolism by modulating energy homeostasis in adipose tissue, skeletal muscle, and liver (Brun et al. 1996; Kliewer et al. 1994; Picard and Auwerx 2002; Semple et al. 2006). The native and modified polyunsaturated fatty acids and prostanoids were recognized as potential endogenous ligands for this subtype of PPARs (Forman et al. 1995). Glitazones or thiazolidinediones (TZDs) are high-affinity PPARgamma agonists and are used for the treatment of T2DM as potent insulin sensitizers.

PPARdelta is ubiquitously expressed, and was shown to be involved in overall energy regulation and fatty acid oxidation, especially in the skeletal muscle. Activation of PPARdelta was also linked with an increase of plasma high-density lipoprotein cholesterol (HDL-C) in diabetic db/db mice and obese rhesus monkeys (Oliver et al. 2001). Data published by Wang and colleagues has demonstrated that overexpression of PPARdelta in white adipose tissue protects against diet-induced obesity in mice, and that treatment with a PPARdelta agonist reduces weight gain without effects on food intake in high-fat diet fed mice (Wang et al. 2003). Although the potential physiological properties of the putative PPARdelta activators are highly attractive, selective PPARdelta agonists for clinical use are still to be developed.

2.1 Mechanism of PPARs Activation

The PPARs share a common structure, with N-terminal transactivation domain, conserved DNA binding domain consisting of two Zn-fingers, ligand binding domain, and C-terminal second transactivation domain mediating ligand-dependent transactivation (Glass 2006). PPAR activity is regulated by selective PPAR ligands/agonists in a similar manner comparable to most of the NHR. In the unstimulated state, PPAR forms a heterodimeric receptor complex together with RXR. In the basic state this hetero-complex is associated with a multiprotein corepressor network. One of the accepted models of PPARs activation implies that the PPAR/RXR complex remains in the cytoplasm und upon ligand-specific activation translocates to the nucleus. The second model indicates that PPAR/RXR complex is—irrespective of the ligand binding status—bound to the target promoters in repressed state (Glass 2006), and this repression is induced through nuclear receptor corepressors such as SMRT (silencing mediator for retinoic acid receptor and thyroid hormone receptor), N-COR (nuclear receptor repressor) (Jepsen et al. 2000), or RIP140 (NRIP1, Nuclear receptor interacting protein 1) (Cavailles et al. 1995). PPAR target promoters are defined through the presence of so-called PPAR-specific binding sites (PPAR response elements/PPREs) (Desvergne and Wahli 1999) (Fig. 1).

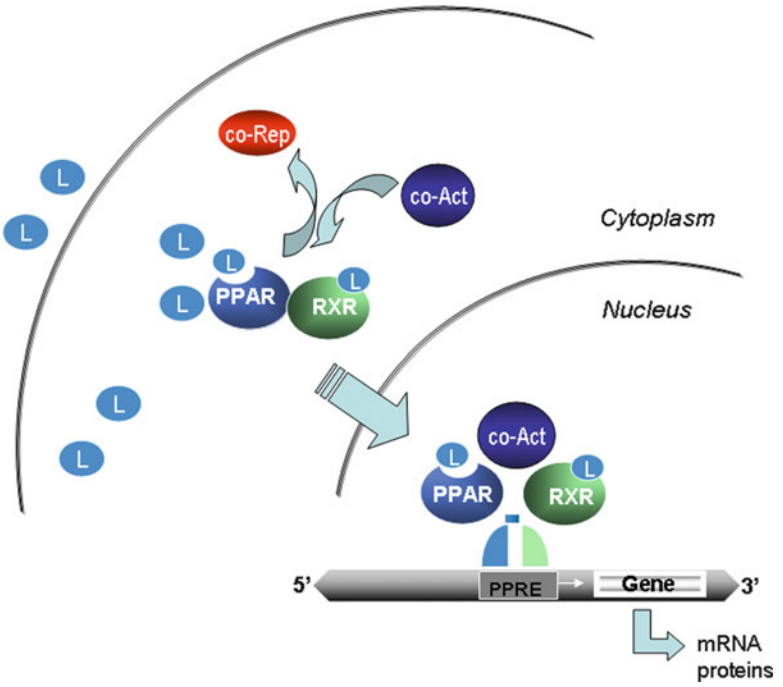

Fig. 1 Mechanism of PPARs activation. Ligand (L) binding within the PPAR ligand binding domain causes a conformational transformation leading to the exchange of corepressor (Co-Rep) for coactivator proteins (CO-Act). This induces the translocation of the complex to the nucleus and progression of the PPAR/RXR heterodimer binding to the target promoters, followed by the initiation of transcription. PPRE, PPAR response element

Agonist binding within the PPAR ligand binding domain causes a conformational transformation leading to the exchange of corepressor for coactivator proteins. This induces the progression of the PPAR/RXR heterodimer binding to the PPREs, followed by the initiation of transcription. By this manner agonist binding within the ligand-binding domain causes a switch from gene repression to activation. Numerous coactivators have been reported including the p160/SRC (steroid receptor co-activator) family of coactivators including steroid receptor coactivator 1 (SRC1), transcriptional intermediary factor 2 (TIF2), and CBP cAMP-response-element-binding protein CREB-binding protein/p300 (Guan et al. 2005; McKenna and O'Malley 2002). There are also several specific PPARgamma coactivators such as PGC1alpha or PGC1 beta both present in the white or brown adipose tissue. Whereas PGC1alpha stimulates adaptive thermogenesis and mitochondrial biogenesis, the PGC1beta regulates mitochondrial function. Both coactivators were also reported to regulate the activity of others NHRs, including estrogen-related receptor alpha (Kamei et al. 2003).

The coregulator network of PPARs appeared to play a crucial role in the modulation of metabolism and metabolic adaptations by these receptors. PPAR

activity is controlled not only by variations in the corepressor and coactivator complexes, but also by variations of PPAR interactions with other NHR, discussed below, and eventually the differences within PPRE sequences.

3 PPARgamma and Sex-Specific Differences in Treatment of Metabolic Disorders

PPARgamma is recognized as a key regulator of glucose and lipid metabolism, and synthetic ligands activating selectively PPARgamma–TZDs such as pioglitazone (Actos: Takeda Pharmaceuticals) and rosiglitazone (Avandia: Glaxo-Smith-Kline) are common oral antidiabetic drugs used for the treatment of T2DM as insulin sensitizers since 1999. TZDs were demonstrated to induce adipogenesis and adipose tissue remodeling, followed by an improvement of glucose tolerance and insulin sensitivity by increasing the expression of GLUT4 transporter in skeletal muscle and adipose tissue, and by elevating plasma levels of adipose tissue derived adipocytokines such as adiponectin, leptin, and resistin (Yki-Jarvinen 2004). PPARgamma was also recently identified as a key modulator of inflammatory responses mainly due to the PPARgamma-mediated inhibitory actions on NFkappaB and AP1 signaling. Importantly, in comparison to other oral antidiabetic drugs, TZDs were shown to improve lipid metabolism (Deeg and Tan 2008). Disruption of the PPARgamma gene in macrophages leads to insulin resistance and glucose intolerance in mice (Straus and Glass 2007).

Several independent meta-analysis have recently suggested that rosiglitazone increases cardiovascular risk and induces manifestation of severe cardiovascular adverse events, such as myocardial infarction and coronary heart diseases in diabetic patients (Home et al. 2009; Lincoff et al. 2007; Mannucci et al. 2010; Singh et al. 2007) reviewed by Schernthaner and Chilton (2010). Under this concern in September 2010 rosiglitazone was withdrawn from the European market.

3.1 Sex-Specific Differences in T2DM and Coronary Artery Disease Patients

Numerous clinical studies as well as large meta-analysis of published randomized clinical trials on factors associated with TZDs therapy of T2DM indicate no significant sex-specific differences regarding antidiabetic effects such as HBA1c reduction, postprandial plasma glucose lowering effects (Phatak and Yin 2006), or cardiovascular risk (Lincoff et al. 2007; Nissen and Wolski 2007; Schernthaner and Chilton 2010). The absence of significant sex-specific differences for metabolic glitazone actions was also documented in the DREAM (Diabetes Reduction

Assessment with Ramipril and Rosiglitazone Medication) trial for rosiglitazone (Gerstein et al. 2006). The only published large prospective clinical endpoint trial with glitazones, the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events) did not report a statistical analysis for the interaction between sex and treatment on outcome (Dormandy et al. 2005).

Most of the studies described earlier include diabetic patients of both sexes, and due to a randomization of the groups and statistical power a potential sex effect is abrogated. Only few studies subdivide groups for specific analysis of sex interactions. Diabetic women were reported to show increased incidence of hypoglycemia under rosiglitazone or pioglitazone treatment, when compared to men, which could be explained by higher sensitivity of the women for the TZDs treatment (Patel et al. 1999; Vlckova et al. 2010).

Recent data published by Amoruso and colleagues (2009) indicate a significant difference in PPARgamma expression level measured in monocytes and macrophages isolated from patients suffering from coronary artery disease (CAD). PPARgamma-dependent gene expression was also strongly upregulated in monocytes/macrophages from CAD patients in comparison to healthy controls. The study also indicated that PPARgamma expression was even more elevated in female CAD patients in comparison to male patients. Accordingly, female CAD patients showed low plasma TNFalpha expression, which was linked to known anti-inflammatory properties of those nuclear receptors. The authors concluded that administration of TZDs to patients with a high protein level of PPARgamma might result in enhanced clinical effects.

3.2 Sex-Specific Differences and TZD-Specific Side Effects

Several lines of evidence indicate that there are important sex differences regarding TZDs side effects. Recent data designate that long-term use of TZDs in diabetic patients leads to the development of bone loss, fractures, and development of secondary osteoporosis, mostly due to an unbalanced bone remodeling induced by PPARgamma activation in mesenchymal and hematopoietic cells, reviewed by Lecka-Czernik (2010). Treatment with TZDs is linked with an increased differentiation and recruitment of osteoclasts and suppression of bone formation by osteoblasts, at least in animal models (Lazarenko et al. 2007; Wan et al. 2007). Interestingly, several independent clinical studies as well as meta analysis from ten different randomized controlled clinical trials based on long-term treatment of T2DM with TZDs indicate an increased risk of fractures exclusively in diabetic women, but not in men (Aubert et al. 2010; Kahn et al. 2010; Loke et al. 2009). Moreover, risk of fractures under TZD treatment is additionally increased in postmenopausal diabetic women, which is indicative for an estrogen-PPARgamma interaction (Bilik et al. 2010; Habib et al. 2010). Importantly, when compared parallel, both rosiglitazone and pioglitazone increase the fracture risk in a similar manner (Aubert et al. 2010).

3.3 Pharmacokinetic of TZDs

Sex differences in the pharmacokinetics (bioavailability, distribution, metabolism, and elimination) and pharmacodynamics are well documented [reviewed by Gandhi et al. (2004)]. According to Gandhi and colleagues, most differences are based on body weight and BMI, which are lower in women; body fat mass, which is higher in women; plasma volume, which is higher in men; expression pattern and activity of hepatic CYP-enzymes (CYP3A4 is higher expressed in women's liver, and different renal clearance, which strongly depends on the given drug.

Different sex-specific metabolic actions of TZDs described earlier could be explained, at least in part, by differences in pharmacokinetics of those antidiabetic agents. After single oral administration of pioglitazone in rats, plasma levels of pioglitazone and its active metabolites were significantly higher in female animals when compared to male rats, and accordingly the elimination rate was significantly increased in male animals (Fujita et al. 2003). Also studies performed by Beconi et al. (2003) on dual PPARgamma/PPARalpha agonist indicated a principal sex difference in the pharmacokinetic of TZDs, as plasma level of the P1 (substituted 2,4-thiazolidinedione) was significantly higher in female rats, dogs, and monkeys in comparison to male littermates. In addition, studies from Fujita and colleagues demonstrated that plasma clearance of the active metabolite was significantly higher in male rats, when compared to female littermates.

3.4 Sex-Specific Differences and Polymorphism of PPARgamma

Numerous recently published clinical studies indicate that PPARgamma polymorphisms could be considered as an important factor determining TZD-based therapy. There are two well-characterized human PPARgamma isoforms: PPARgamma 1 and 2, both generated by alternative promoters. Polymorphism of human PPARgamma2 gene, coding for PPARgamma with predominant expression in adipose tissue and monocytes/macrophages, seems to have serious metabolic consequences. Although the etiology of obesity is still not completely understood, there are some experimental data indicating its heritable origin (Hofbauer 2002). It is well known that the Pro12Ala polymorphism, resulting from a single nucleotide exchange at the position 34, and consequently a switch from proline to alanine in the mature PPARgamma2 (Yen et al. 1997), is linked with an elevated risk of obesity-related metabolic disorders (Beamer et al. 1998; Vaccaro et al. 2000). Moreover, Pro12Ala substitution was linked with lower PPAR activity and reduced adipogenic response to TZDs in vitro (Masugi et al. 2000), as well as lower BMI and improved insulin sensitivity in the individuals caring this allele (Deeb et al. 1998). Studies performed by Mattevi and colleagues (2007) on Brazilian and European obese individuals suggested that the Pro12Ala variant of PPARgamma2

(variant allele frequency of 0.09) was not associated with a change of total plasma cholesterol, LDL- and HDL-cholesterol and triglycerides, characteristic for obesity and the obesity-related phenotype. On the other hand, the BMI was significantly increased in male individuals carrying the Pro12Ala variant of PPARgamma in comparison to wild-type genotype. As no effects of this polymorphism were observed in women, the authors concluded that this genotype/phenotype interaction is regulated in a sex-specific manner. In consonance, studies published by Ben Ali and colleagues (2009) conducted in the Tunisian population of obese and metabolically healthy individuals showed that the Pro12Ala variant of PPARgamma2 is linked with increased obesity in men. In addition, only obese male patients with Pro12Ala genotype showed a significantly higher BMI and elevated plasma leptin levels, when compared to patients with wild-type genotype of PPARgamma2. Those metabolic parameters were not differently regulated in female obese participants, independent of the genotype. Similar results indicating sex-specific differences of obese patients and children with the Pro12Ala variant of PPARgamma2 were recently published by others (Dedoussis et al. 2007, 2009; Morini et al. 2008).

Results published by Rebalta et al. (2005) indicate that postprandial triglyceride plasma levels, on average lower in women, are significantly elevated in female individuals carrying the Pro12Ala variant, and that additional genotypic variations in other obesity-related genes such as ApoE and FABP-2 resulted in additively increased triglyceride values up to the plasma level normally measured in men.

In contrary, studies done on long-lived subjects with a lower BMI and improved insulin sensitivity indicated that the Pro12Ala genotype was associated with a lower BMI in men, but not in women (Barbieri et al. 2004).

In summary, one may conclude that the Pro12Ala polymorphism in the PPARgamma gene plays a more dominant role in males than in females.

3.5 PPARgamma and Estrogens

Sex-specific effects of genes involved in the regulation of lipid glucose metabolism—such as PPARgamma—could be primarily attributed to hormonal differences, like plasma level of estrogens, and ER signaling. The PPARgamma/RXR complex was demonstrated to suppress ER-induced target gene expression through competitive binding to an ERE site in the vitellogenin A2 promoter (Keller et al. 1995). Also Wang and colleagues (2002) demonstrated that ERs are able to inhibit ligand-induced activation of PPARgamma in breast cancer cell lines. Our group has recently shown that ERbeta is a negative regulator of ligand-induced PPARgamma activity in vitro, in the adipocytic cell line 3T3-L1, and in the diet-induced obesity mouse model in vivo (Foryst-Ludwig et al. 2008). Using high-fat diet fed ERbeta-deficient mice we could demonstrate that ERbeta negatively regulates insulin and glucose metabolism, which results from an impairment of regular adipose tissue function. These effects were directed by a negative cross talk

between ERbeta and PPARgamma. Similar observations had been described earlier (Wang and Kilgore 2002). Loss of ER resulted in enhanced body weight gain and increased white adipose tissue in high-fat diet animals in comparison to control littermates. Absence of ERbeta prevented hepatic/muscular triglyceride overload, preserved insulin sensitivity in liver and skeletal muscle, and improved whole body insulin sensitivity and glucose tolerance in these mice. The observed metabolic phenotype was linked with increased endogenous PPARgamma activity, measured in gonadal fat from high-fat diet fed ERbeta-deficient mice. The application of PPARgamma-directed antisense oligonucleotides in ERbeta-deficient mice reversed their metabolic phenotype. Also in vitro analysis using luciferase reporter assay specific for PPARgamma, differentiation assays and ChiP experiments done in the nuclear fractions of adipose tissue isolated from high-fat diet fed ERbeta-deficient and wild-type mice indicated a mutual cross talk between PPARgamma and ERs. Repression of PPARgamma activity through ERbeta was reversed by titration of the p160 coactivators, SRC1 and TIF2, suggesting that the suppressive action of ERbeta is a result of p160 coactivators interaction with ERbeta thereby preventing the binding of PPARgamma to the same coactivators.

Recent work published by Yepuru and colleagues (2010) underlined the PPARgamma–ERbeta interactions in vivo using selective ERbeta ligands in a diet-induced obesity mouse model. In addition, the authors used an ovariectomy-induced obesity model, where ovariectomized or sham-operated female mice were treated with vehicle or an ERbeta-specific agonist, and afterwards metabolically characterized. The results indicated that ERbeta agonists reduced body weight gain, diminished fat mass, and increased lean mass of the mice, when compared to control animals. In addition, selective ERbeta agonist used in those models led to a significant reduction of plasma cholesterol, leptin, and glucose levels. The authors concluded that the observed antiobesity effects of ERbeta selective ligands are mediated by a PGC1alpha-dependent inhibitory effect of this ER on PPARgamma activity. Similar interactions were described previously for ER interactions with the thyroid receptor (Lopez et al. 1999).

Wang and collaborators demonstrated that troglitazone—a withdrawn antidiabetic drug belonging to the group of TZDs—not only binds and activates PPARgamma, but also exerts its anticancer properties directly by inhibiting the activity of estrogen-related receptors EERalpha and beta (Wang et al. 2010). ERRalpha and beta are known regulators of mitochondrial biogenesis and energy homeostasis together with PGC1alpha and beta. Along this line, rosiglitazone was recently demonstrated to induce bone loss by stimulating bone resorption and inhibiting bone formation, mediated by inhibitory effects of PGC1beta on osteoclast differentiation (Wei et al. 2010). Moreover, the authors demonstrated that under rosiglitazone stimulation, PPARgamma increased EERalpha expression in osteoclasts, which together with PGC1beta-mediated increased mitochondrial biogenesis in osteoclasts, which in turn lead to escalation of bone loss.

In summary, the metabolic interactions between PPARgamma, estrogens, ERs, or ER-related cofactors could explain, at least in part, some sex-specific differences in the TZD-based treatment.

4 PPARalpha and Sex-Specific Differences in the Treatment of Metabolic Disorders

PPARalpha is predominantly expressed in tissues that metabolize high amounts of fatty acids like liver, kidney, heart, and muscle indicating its regulatory function on lipid metabolism (Auboeuf et al. 1997; Mandard et al. 2004). PPARalpha can be activated by both endogenous [e.g., long-chain polyunsaturated fatty acids, eicosanoids, prostaglandins (Forman et al. 1997; Keller et al. 1993; Krey et al. 1997)] and synthetic ligands. Synthetic ligands are the fibrates and PPARalpha is thereby used as pharmacological target to exert a positive effect on lipid metabolism by reducing triglycerides and raising HDL-cholesterol (Pejic and Lee 2006; Singh et al. 2010). The mechanism for these effects is based on the regulation of target genes after ligand binding and thereby exerting a positive effect on fatty acid uptake, intracellular lipid metabolism, lipoprotein, and triglyceride plasma concentrations (Berger and Moller 2002; Fruchart 2001).

For example, carnitine palmitoyltransferase 1, an enzyme responsible for the transport of fatty acids across the mitochondrial membrane is upregulated under PPARalpha activation (Mandard et al. 2004). Likewise, the major enzymes of the ß-oxidation pathway are higher expressed under activated PPARalpha. In summary the upregulation of these enzymes causes a reduction of intracellular fatty acid concentrations, decreased plasma triglycerides and VLDL levels, and increased HDL-cholesterol concentration—which all exert positive impacts on the development of cardiovascular diseases (Lefebvre et al. 2006). The phenotype of the PPARalpha null mice, with lowered levels of several fatty acid metabolizing enzymes, which causes abnormalities in triglyceride and cholesterol metabolism and a development of late onset obesity, serves as a proof of concept (Mandard et al. 2004).

As mentioned earlier, different studies showed that muscle of women express a higher level of mRNA/proteins of genes involved in lipid metabolism compared to men, which could explain the higher lipolysis in women during exercise (Costet et al. 1998; Lee et al. 1995). In consonance with these data also the mRNA expression of PPARalpha in whole muscle homogenate in the follicular phase of the menstrual cycle is higher in women than in men (Maher et al. 2010). Animal studies revealed compared to clinical studies even stronger but contrary sex-specific differences in the expression of PPARalpha and its regulated genes. These sex-specific differences are mediated by a higher PPARalpha expression in the liver of male rodents when compared to females (Tarnopolsky 2008). Gonadectomy in male mice decreased hepatic PPARalpha levels in the liver to the level of female rats implicating a cross talk between sexual hormones and PPARs (Ciana et al. 2007; Jalouli et al. 2003; Sundseth and Waxman 1992) which will be discussed later. It appears that sex-specific differences exist regarding the expression level of PPARalpha; these differences are regulated in a tissue- and species-specific manner.

4.1 Sex-Specific Differences in Fibrate Treatment

Fibrates (e.g., Gemfibrozil, Bezafibrate, Etofibrate, Fenofibrate) are used as lipid-lowering drugs in humans, while in rodents these substances induce increased hepatic peroxisome number, hepatomegaly, and carcinogenesis in higher doses (Jalouli et al. 2003). The clinical indications for a fibrate treatment are hypertriglyceridemia and hypercholesterolemia (Kitson et al. 2010).

4.2 Clinical Studies

The absence of an adequate female sample size in clinical studies or the randomization of groups is a major problem in the identification of sex-specific differences in drug treatment. A meta-analysis of efficacy and safety of HDL-C-increasing compounds by Birjmohun and colleagues indicated the sex imbalance in the clinical trials: in the analysed studies patricipated only 373 women in comparison to 13945 men. (Staels et al. 1998).

Examples for clinical studies on fibrate treatment:

Study		Year	Analysis for sex-specific differences
CDP (Birjmohun et al. 2005)	Coronary Drug Project	1975	Men only
HHS (Carlson and Rossner 1975)	Helsinki Heart Study	1990	Men only
LOCAT (Manttari et al. 1990)	Lopid Coronary Angiography Trial	1997	Men only
VA-HIT (Syvanne et al. 1997)	The Veterans Administration-HDL intervention Trial	1999	Men only
DAIS (Rubins et al. 1999)	Diabetes Artherosclerosis Intervention Study	2003	Women are underrepresented, no analysis of sex-specific differences
FIELD (Vakkilainen et al. 2003)	Fenofibrate Intervention and Event Lowering in Diabetes	2005	Sex-specific analysis, no significant difference
ACCORD (Keech et al. 2005)	Action to Control Cardiovascular Risk in Diabetes	2010	Prespecified subgroups sex-specific difference identified

The newest study listed above (ACCORD study) investigated the potential superior effect of a combination therapy (statins + fibrates) in high-risk T2DM patients versus monotherapy with statins regarding the rate of cardiovascular events. In this study, sex showed a significant interaction with treatment on outcome, while men seemed to profit from the combination therapy, women showed increased adverse events and harmful reactions (Ginsberg et al. 2010). However, this result is in contrast with the outcome of the FIELD study, where no

significant interaction effect was seen between treatment and sex (Ginsberg et al. 2010). In both studies, fenofibrate was used as a PPARalpha ligand. Since, in FIELD patients in the intervention group received fenofibrate monotherapy without statin treatment, and in ACCORD a combination therapy (statin + fibrate) was applied, it is likely that sex differences may occur from adverse effects of combining these two lipid-lowering strategies in women. The exact reason for these sexual dimorphisms, however, is still unclear.

A study by Koskinen and colleagues focused on sex-specific differences in response to gemfibrozil treatment. They showed that serum triglyceride levels in postmenopausal women not taking hormone replacement therapy decreased stronger than in men. Furthermore, HDL_3 (HDL subfraction 3) concentration increased significantly more in postmenopausal women than in middle-aged men. Whether this effect is also observed in premenopausal women with a higher endogenous estrogen level is not discussed in the study (Keech et al. 2005). Nerbrand and colleagues showed that postmenopausal women do not exhibit any additional effect of a combination therapy with estrogens and fibrates. Both drugs show in a single therapy a positive effect on lipid profile, but no additional benefit was reported concerning the serum lipids or lipoprotein profile when they are used contemporaneously (Koskinen et al. 1992).

In summary, these studies provide evidence that a negative cross talk between PPARalpha and estrogens may be possible.

4.3 Animal Studies

The reaction of humans and rodents to fibrates differs as fibrates induce hepatic peroxisome proliferation, hepatomegaly, and hepatocarcinogenesis in rodents, and these toxic effects are absent in humans. Therefore, species-specific functional and ligand-binding properties of PPARalpha have to be carefully evaluated (Nerbrand et al. 2002) and an extrapolation of data from rodents to humans is critical. Several explanations have been provided for these differential effects, such as a tenfold lower PPARalpha DNA binding activity in humans than in mouse hepatic lysate, in relation with lower amounts of the receptor (Lefebvre et al. 2006). In an experimental setting with mice fed a fenofibrate-supplemented high-fat diet, Yoon and colleagues observed in males a reduction of all effects caused by the high-fat diet (body weight, visceral white adipose tissue mass, serum triglycerides, cholesterol levels). In contrary, female mice did not show any decrease in body weight, white adipose tissue mass, or cholesterol levels. They only reduced triglycerides but to a smaller extent compared to male mice. In consonance, the PPARalpha target genes encoding for enzymes for fatty acid ß-oxidation increased much higher in males compared to females. These data propose a sex-specific difference in the control of obesity mediated by PPARalpha ligands, and further suggesting a cross talk between fenofibrate and estrogens (Costet et al. 1998; Palmer et al. 1998). Several groups showed in male rodents that fibrates act as regulators of energy homeostasis

by decreasing body weight in a high-fat diet model, without changing food intake (Yoon et al. 2002). The authors postulate that the increase of energy expenditure is mediated by an upregulation of UCP gene expression. This hypothesis was proven at least in male mice after fenofibrate treatment or fish oil supplementation (Chaput et al. 2000; Guerre-Millo et al. 2000). Mancini and colleagues showed a similar body weight reducing effect in a rat model with male Wistar rats. Fenofibrates were able to prevent excessive weight gain and to mobilize fat from adipose tissue. They could not observe any change in UCP-2 or UCP-3 expression in the liver, nor in the muscle, and concluded that fenofibrate acts as a "weight-stabilizer" through enhancement of lipid catabolism in the rat liver (Tsuboyama-Kasaoka et al. 1999).

In summary, animal studies discussed here demonstrated a consistent result: male rodents are more responsive to fibrates and show a higher hepatic expression of PPARalpha compared to females (Mancini et al. 2001).

4.4 PPARalpha and Estrogens/ERs

Estrogens have an important role in the regulation of lipid metabolism and lipoprotein levels. For example, female rodents undergoing ovariectomy develop obesity. This phenotype is rescued by hormone replacement therapy implicating the role of estrogens in lipid metabolism (Jalouli et al. 2003). In consistence with this animal model, the menopause has a pronounced effect on the circulating levels of lipids and lipoproteins (Rogers et al. 2009) with raising LDL and decreasing HDL concentrations. The role of estrogens and ERs in PPARalpha signaling is controversially described and varies from induction to suppression. The results presented here arise from animal studies.

Djouadi et al. revealed a sex-specific difference in PPARalpha null mice. When treated with an inhibitor for carnitine palmitoyltransferase 1, which blocks the fatty acid flux to the mitochondria, only PPARalpha null male mice suffered from massive hepatic and cardiac lipid accumulation and severe hypoglycemia followed by death. A weaker reaction and almost no life-threatening effects were observed in female mice. A pretreatment with estradiol rescued the male mice, underlining the pivotal role of estrogen in cardiac and hepatic lipid metabolism. Although the cross talk between estradiol and PPARalpha was not discussed in this study, the authors already implicated the existence of an estradiol-triggered fatty acid utilizing pathway similar to the effect of PPARalpha activation (de Aloysio et al. 1999). Costet and colleagues performed further investigations on PPARalpha deficient mice reporting that PPARalpha knock out mice show a monogenic, late onset, spontaneous obesity with a stable caloric intake and remarkable sexual dimorphism, as only males developed a considerable hepatic enlargement. However, PPARalpha deficient females exhibit a markedly higher level of plasma triglycerides and adipose tissue storage when compared to males. Interestingly, the late onset of obesity was not due to hyperphagia (Djouadi et al. 1998). Campbell and colleagues presented data that demonstrate a significant regulation of the genes regulating lipid oxidation

in the skeletal muscle and PPARalpha by the ovarian hormones. The expression of PPARalpha in the muscle of female rats was regulated by the presence of estradiol as ovariectomy of rats caused a decrease in PPARalpha mRNA and protein content, which was abolished after 17ß-estradiol treatment. This indicates that the presence of ovarian hormones regulates the expression of PPARalpha in the muscle and thereby regulates the expression of lipid metabolizing enzymes (Costet et al. 1998).

In summary, these studies show that male rodents are more sensitive to a loss of PPARalpha and exhibit a stronger response to PPARalpha ligands including an augmented expression of PPARalpha target genes. In contrast, females appear to be able to compensate for the absence of PPARalpha involving an estrogen-dependent lipid oxidative pathway currently unknown. These results cannot be extrapolated to humans, as the hepatic liver expression is lower and sex-specific differences were not observed to date in such a dimension.

A negative cross talk of estrogens and PPARalpha ligands is described in a study by Jeong and Yoon. The induction of enzymes in the liver by fibrates was decreased when combined with estradiol treatment, in ovariectomized mice. Thereby the estrogens decreased the ability of these drugs to reduce body weight and adiposity in female mice, indicating that the combination of those substances with a positive effect on lipid metabolism and body weight had no additional effect and impaired the action of PPARalpha agonists alone (Campbell et al. 2003).

In summary, PPARalpha and ERs share common structures and a negative cross talk, e.g., involving cofactor competition might exist, however, additional molecular studies are required.

4.5 PPARalpha and Anti-inflammatory Effects

Fibrates showed in different clinical studies a positive effect on the progression of artherosclerotic lesions (Yoon et al. 2002). This effect is certainly mediated by a positive regulation of lipoprotein metabolism but additionally PPARalpha activation exerts an anti-inflammatory response. The first hint of a relation of PPARalpha to inflammation revealed a study in PPARalpha-deficient mice that showed a prolonged response to inflammatory stimuli (Frick et al. 1997; Ruotolo et al. 1998). Afterwards different studies showed that PPARalpha activation reduced or inhibited the expression of various inflammatory genes and showed anti-inflammatory activity in different cell types like monocytes/macrophages, endothelial cells, smooth muscle cells, and T-lymphocytes (Devchand et al. 1996). Different mechanisms and pathways how fibrates act on the immune system are discussed, and data point towards an anti-inflammatory effect by the inhibition of the NFkappaB- and AP1-pathway (Delerive et al. 1999). There are known sex-specific differences in immunological responses, as women are more susceptible than men to develop autoimmune diseases. Sex hormones are believed to account at least partially for these differences (De Bosscher et al. 2006). In several studies estrogens were associated with different markers of systemic inflammation, whereas other studies describe estrogens

as an anti-inflammatory hormone and enhancer of immune response (Fairweather et al. 2008). Crisafulli et al. showed that the anti-inflammatory effects of estradiol were reduced in PPARalpha knock out mice compared to wild-type animals, suggesting that the presence of PPARalpha is required for the anti-inflammatory action of estrogens. However, the molecular mechanism remains unclear (Cutolo et al. 2010). Dunn et al. identified in an animal study PPARalpha as a gene in CD4 + T-cells that is sensitive to androgen levels, as its expression is downregulated after castration of male mice. The elevated PPARalpha expression in male T-cells was associated with a decreased binding of transcription factors involved in inflammatory responses like NFkappaB. This study provides an idea why males show different immune responses or susceptibility to autoimmune diseases compared to female mice (Crisafulli et al. 2009). Further studies have to investigate whether PPARalpha also exhibits a sexual dimorphic pattern of expression in human immune cells.

5 Clinical Implications

As described in the present review, regarding the clinical actions of PPARgamma agonists no significant sex difference could be detected for their clinical efficacy whereas certain side effects such as osteoporosis are more present in women. The cardiovascular protective action of PPARalpha ligands seems to be stronger in men than in female. Future studies are required to explore the underlying mechanisms of these sexual dimorphic responses to PPAR agonists.

6 Conclusion and Perspectives

The accumulating knowledge on PPARs biology, based on several recently published studies, has allowed a better understanding of a regulatory PPAR-transcriptional network. PPAR activity results not only in the regulation of PPAR-target promoters but also influences—due to the activation and binding of the certain sets of corepressor or coactivator proteins—activity of other NHRs. Importantly, such a cross talk has significant physiological consequences, as underlined by several studies discussed earlier. In particular, metabolic interactions between PPARgamma and estrogens or ERs could explain, at least in part, the sex-specific differences in the treatment of metabolic disorders with PPARs agonists. While sex-specific differences in obesity are well documented, sex-specific approaches for the treatment of metabolic disorders and diabetes are still to be established.

In order to develop more efficient and safer PPAR-ligands for clinical practice, a better understanding of distinct cross talks within a NHR-cofactor network will be absolutely required.

Take Home Messages.

- Ligands of PPARs are used as oral antidiabetics (PPARgamma) or for the treatment of dyslipidemia (PPARalpha).
- Although sex-specific aspects of obesity are well known, sex-specific strategies for the treatment of metabolic disorders and diabetes are not available at the moment.
- Most of the clinical studies on TZD-based therapy of T2DM indicate no significant sex-specific differences regarding antidiabetic effects. Those studies include diabetic patients of both sexes, and due to a randomization of the groups and statistical power a potential sex effect is abrogated.
- There are important sex differences regarding TZDs side effects. Long-term use of TZDs in diabetic patients leads to the development of severe bone loss, fractures, and secondary osteoporosis. Those side effects were reported predominantly by female patients.
- Most clinical studies focussing on fibrate treatment do not analyze for sex-specific differences, only in few studies a difference in the reaction to a combination treatment (fibrates + statins) could be observed.
- Male rodents are more responsive to fibrates and show a higher hepatic expression of PPARalpha compared to females. These data cannot be extrapolated to humans.
- Metabolic interactions between PPARgamma or PPARalpha and estrogens, ERs, or ER-related cofactors could explain, at least in part, some sex-specific differences in PPARs-based treatment.

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Sex Differences in the Drug Therapy for Oncologic Diseases

Oliver Schmetzer and Anne Flörcken

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Abstract There are clear gender-dependent differences in response rates and the probability of side effects in patients treated with chemotherapy. Sex-biased expression levels of metabolic enzymes and transporters in liver and kidney leading to different pharmacokinetics have been described for most common anti-cancer drugs. In women, half-life is often longer, which is associated with improved survival, but also increased toxicity.

Some chemotherapy protocols lead to a better response rate in women without increasing toxicity (e.g., cisplatin and irinotecan), while others only increase toxicity, but do not improve response rates in women (e.g., 5-fluorouracil). The increased toxicity often correlates with different pharmacokinetics, but women also show a higher sensitivity to some agents with shorter half-life (e.g., steroids). Organ-specific toxicities like cardiac toxicity after doxorubicin treatment or neurotoxicity associated with ifosfamide are more severe in women due to gender-specific changes in gene expression. Novel therapies like tyrosine kinase inhibitors or monoclonal antibodies show very complex, but clinical significant differences depending on gender. Antibodies often have a longer half-life in women, which is associated with an improved response to therapy.

Side effects appear to be highly dependent on different tissue properties, as women have a higher incidence of oral mucositis, but lower rates of gut toxicity. Nausea and vomiting is a greater problem in females during therapy due to the lower activity of anti-emetic drugs. Nausea and vomiting pose a bigger challenge in female patients, as anti-emetic drugs seem to be less effective.

Keywords drug • metabolism • half-life • cytostatic therapy • gender

Abbreviations

5-FU	5-Fluorouracil
GH	Growth hormone
STAT	Signal transducers and activators of transcription
EGF	Epidermal growth factor
CYP	Cytochrome P450
GST	Glutathione *S*-transferase

1 Introduction

Gender- and sex-related adaptations in dosing of cytostatic drugs are easier to perform than interindividual dose adjustments. Women are more vulnerable to most cytostatic drugs and have different metabolic activities. Therefore, the common clinical practice of reducing the dose after a first cycle with high side effects is also effective in women. In contrast, the start of the therapy with a non-sex-adapted dosage regime has tremendous influence on severe adverse effects and also on outcome. Differences in side effects, mortality, and response to treatment are more severe during the first cycle. Therefore sex-adapted therapies need to be developed.

For most gender-associated differences in metabolism of drugs, different enzyme expression levels and/or enzyme activities regulated mainly indirectly by sex hormones have been identified. In addition, the sensitivity of tissues and tumors (e.g., DNA-repair capacity or muscle/fat ratio) plays a role in treatment efficacy. The sensitivity to a drug changes during the menstrual cycle, which partly explains the contradicting data, e.g., in pre- and postmenopausal women. Novel studies, which include sex-dependent analyses, have shown that there are at least 40% difference in pharmacokinetics between men and women for most drugs (Anderson 2005) (Fig. 1).

One explanation for sex-related gene expression patterns might be the genomic localization of these genes. It has been shown that a higher number of differentially expressed genes can be found on the X chromosome (Saifi and Chandra 1999). The concentration of genes on the sex chromosomes renders them susceptible to various epigenetic changes (e.g., different methylation, X inactivation).

The different metabolism of drugs and other xenobiotics is likely caused by the need for different metabolism of steroid hormones, which is performed by the same enzymes (Waxman and Holloway 2009). The importance of the resulting kinetic differences is often outweighed by non-kinetic factors of variability of response to cytotoxic agents.

2 Sex and Gender Differences in Bioavailability, Enzyme Expression, Tissue Distribution, and Activity

2.1 Control of Enzyme Expression

To understand the differing drug pharmacokinetics in men and women, an extensive knowledge in expression control of metabolizing enzymes and its dependence on various hormones is required. In the last decade multiple data have evolved describing in great detail how the differing expression is regulated. Central for the control of the gender dimorphic expression in the liver is the transcription factor signal transducers and activators of transcription 5b (STAT5b) (Clodfelter et al.

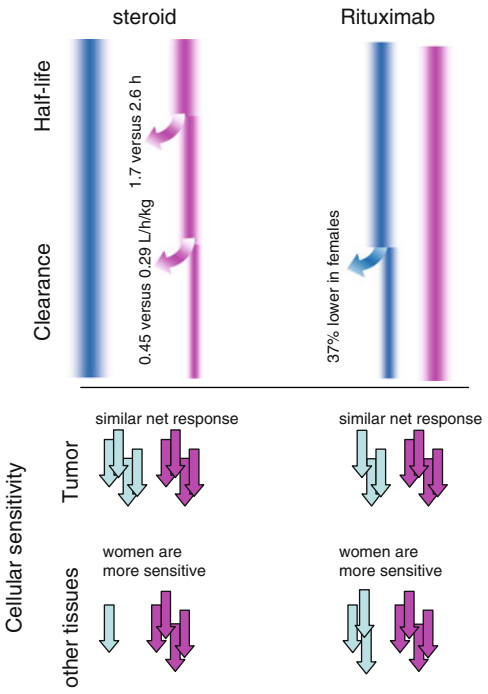

Fig. 1 Sex and gender influence in oncologic drug therapy. Substances can have different half-lives and clearance rates in females and males. However, the clinical response can be independent of pharmacokinetics due to different drug sensibility of tissues and tumors

2006). In mice 4% of the genome is controlled in a gender-dependent fashion, 90% of these 4% in male mice is induced by STAT5b. In female mice about 60% of the gender-dependent genes are suppressed by STAT5b, demonstrating its central relevance for gender- and sex-based differences in gene expression. The activity of STAT5b is mainly regulated by *growth hormone (GH)*, but also by various interleukins and epidermal growth factor (EGF) (Herrington and Carter-Su 2001). The secretion of GH by the pituitary gland is under the control of gonadal steroids (Jansson et al. 1985; Veldhuis et al. 2001) and blocked by somatostatin, which is down regulated in a feedback regulatory mechanism by GH (Bennett et al. 2005). Especially, neonatal exposure to testosterone has been shown to regulate GH secretion (Jansson et al. 1985; Waxman and Celenza 2003). In human men and in rodents a pulsative pattern of GH secretion can be found, while in females a continuously lower plasma level can be seen (Veldhuis 1998; Veldhuis and Bowers 2003). These secretion patterns are controlled by gonadal hormones and control the gene expression in the liver (Fig. 2).

In addition to Stat5b, HNF4alpha (NR2A1) is required for full male-specific liver gene expression (Lamba et al. 2003). Conversion of heterochromatin to euchromatin by HNF3beta (FOXA2) is an additional regulatory mechanism to especially activate female genes (Wiwi et al. 2004).

Estrogen has been shown to control expression of non-GH-responsive genes (Stahlberg et al. 2005). This is another mechanism how female gene expression pattern in the liver in about 30% of female-enriched genes can be achieved.

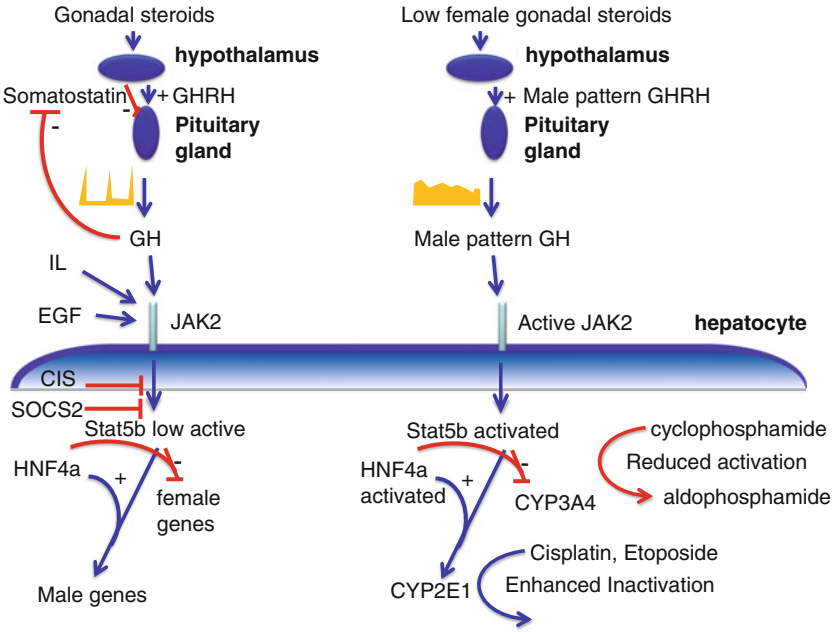

Fig. 2 Major pathways involved in control of drug metabolism. The expression of dimorphic gender-specific enzymes is controlled by the release pattern of growth hormone (GH). Peaks and low stable concentration lead to female pattern (*left*) while high stable plasma concentration leads to male pattern (*right*)

GH-regulated genes cannot only be found in the liver, but also in the heart and kidneys (Flores-Morales et al. 2001). However, in the kidney, additional gene expression regulated directly by *androgens* has been demonstrated (Tullis et al. 2003). Androgens can increase the function of AU-rich RNA-binding proteins which stabilize labile mRNAs and in an opposite fashion decrease the function of proteins which destabilize labile mRNAs (Sheflin et al. 2001). The function of these translational control proteins can be mediated via redirection from nucleus and cytosol to polyribosomes enabling a very rapid, gene expression independent response to androgens. Major target proteins of these androgen-mediated mRNA-stabilization are EGF, EGF receptor, and the androgen receptor.

2.2 *Tissue Distribution*

Surprisingly, most of the differently expressed genes are involved in metabolism, especially in steroid and foreign substance metabolic pathways. Therefore, these genes are predominantly expressed in liver, kidney, and gut, while some different genes can be identified in heart tissue, but not, e.g., in the hypothalamus (Rinn et al. 2004). For example, the P450 monooxygenase is higher expressed in the male jejunum (Sundseth and Waxman 1992).

2.3 Activity

Gender-associated differences are not only based on gene expression, but can also be due to post-translational control of enzyme activity. Estrogen containing contraceptives can induce cytochromes, e.g., CYP2A6 and increase their function (Higashi et al. 2007a, b).

3 General Sex and Gender Differences in Drug Uptake, Metabolism, Excretion, and Ethnic Effects

3.1 Bioavailability

In addition to differing first-pass effects, oral bioavailability is often reduced in women: kinetics of absorption can be different. Estrogen slows down gastrointestinal mobility and gastric emptying (Waxman and Holloway 2009). Women have a reduced drug uptake of oral applied drugs due to a reduced absorption in the gut. One study identified the reduced expression of P-glycoprotein (MDR1A gene, a major transporter of cytotoxic agents) in women as a possible explanation. Importantly this might also influence drug resistance during treatment (Davis 2005).

3.2 Differences in Liver Metabolism

Liver microsomes are specialized enzyme-rich compartments, which play an important role in drug metabolism. Due to the many isoforms of the detoxifying enzymes, a detailed analysis of expression and activity has been difficult. Recently, dominating enzymes in men and women have been identified (Huang et al. 2011) (Table 1).

Besides GH effects mediated via STAT5, several other expressed genes regulate proteins in the liver. The KRAB zinc finger repressors are also controlled by GH and repress several male-specific genes (Waxman and Celenza 2003). This effect remains active in females throughout life, but the repression is switched off in males after puberty by a pulsative pattern of GH secretion.

Beside clear differences in cytochrome P450 expression and activity, other metabolic processes are clearly differing according to gender. Glucuronidation is higher in males and can lead to an increased clearance and decreased bioavailability of drugs (e.g., acetaminophen or aspirin) (Miners et al. 1983; Franconi et al. 2007). Redox-systems also differ in men and women. The expression of Glutathione *S*-transferase A (GST A) is elevated in female livers; therefore, detoxification or drug inactivation might be increased with some agents (Mulder et al. 1999).

Table 1 Gender-specific enzyme expression and activity differences

	Expression	Activity	Id level		References
Alcohol dehydrogenase 1	Higher in female		Protein	Inactivation of cyclophosphamide (aldophosphamide to alcophosphamide)	Huang et al. (2011)
Aldehyde dehydrogenase	Higher in male		Protein	Inactivation of cyclophosphamide (aldophosphamide to carboxy-phosphamide)	Huang et al. (2011)
ATP-binding cassette sub-family D member 3	Higher in female		Protein	Drug transporter	Huang et al. (2011)
Carboxylesterase 3	Higher in male		Protein	Activation of irinotecan and capecitabine	Huang et al. (2011)
Cytochrome P450 1A2		Lower clearance in women, mediated by caffeine	Function		Schwatz (2007)
Cytochrome P450 2B6	Lower in females		Protein	Major: activation of cyclophosphamide, thiotepa, procarbazine Minor: ifosphamide	Lamba et al. (2003)
Cytochrome P450 2D6		Higher in male	Function		Schwatz (2007)
Cytochrome P450 2E1	Higher in male	Higher in male	Protein, function	Activation of dacarbazine, Inactivation of cisplatin and etoposide	Huang et al. (2011) and Franconi et al. (2007)
Cytochrome P450 3A4	Higher in females	30 % higher in female	Protein	Minor: Activation of cyclophosphamide, thiotepa, geftinib Major: ifosphamide and inactivatuon of vinca-alkaloids, docetaxel, etoposide, irinotecan, taxol, teniposide, paclitaxel, busulfan, cisplatin, doxorubicine,	Huang et al. (2011) and Waxman and Holloway 2009

(continued)

Table 1 (continued)

	Expression	Activity	Id level		References
				topotecan, mitoxantrone, thioTEPA, imatinib, gefinitib	
Dimethylaniline monooxygenase [*N*-oxide-forming] 3	Higher in male		Protein	Oxidation of tamoxifen	Huang et al. (2011)
Fatty acid synthase	Higher in female		Protein	Resistance to various cytotoxic agents	Huang et al. (2011)
Glutathione S-transferase alpha-1, kappa 1, Mu1 and Mu2	All higher in male		Protein	Activation of cyclophosphamide (phosphoramide mustard); Inactivation of busulfan and chlorambucil	Huang et al. (2011)
Liver carboxylesterase 4 and B-1	All higher in male		Protein	Activation of Iriniotecan?	Huang et al. (2011)

Most data is derived from rodents

3.3 Kidney

Several genes are differentially expressed in the kidney (Rinn et al. 2004). Predominantly, there are three kinds of gender-associated regulated genes: hormone receptors (e.g., prolactin receptor), metabolic enzymes (cytochromes, transferases, etc.), and transporter channels. The channels are mainly involved in amino acid and organic anion transport. mOATL-6 is increasingly expressed in female kidneys, while Slc21a1 and Slc7a12 are found increased in the male kidney. The expression of the same enzymes can be regulated by GH in the liver, but by testosterone in the kidney, e.g., for CYP4A2 (Sundseth and Waxman 1992). This can be explained by the low level of androgen receptors in the liver, as steroid metabolizing enzymes are present in high concentration and therefore sex hormones will have a very short half-life in liver cells.

Redox-capabilities again also differ in the kidney. Renal GST activity is higher in women (Temellini et al. 1995).

3.4 Excretion

In general, the same drugs which are metabolized by CYP3A4 are excreted into the bile by P-glycoprotein. Due to its 2.4-fold reduced expression in females, drug excretion is reduced and half-life prolonged (Schuetz et al. 1995; Benet et al. 2004).

Routinely used equations to calculate the glomerular filtration rate and dose (e.g., of carboplatin) underestimate the dose needed for women by more than 10% and overestimate the dose for male patients even when "area under the curve" values are used (Dooley et al. 2002).

3.5 Ethnic Effects

Only very few studies have analyzed ethnic differences important for the treatment of cancer patients. However, most studies have shown differences in pharmacokinetics and impressive differences concerning hematological toxicity, which are related to ethnics and gender (Sekine et al. 2008). A 3.6- to 5-fold higher CYP2B6 function has been identified in women of Hispanic origin (Lamba et al. 2003). Ethnic differences important in treatment with antibodies and biologicals have also been identified between patients of Caucasian (American) and Asian (Japanese) origin.

3.6 *Relevant Gender-Associated Pharmacokinetic Differences: Cytostatic Agents*

For most oncologic drugs, dramatic sex differences in plasma concentrations have been clearly demonstrated. However, interindividual differences are often more remarkable, as every cancer patient's tumor reacts with a different dose–response relationship and many polymorphisms exist in important drug metabolizing enzymes. Even more complicated are intra-individual differences, like previous treatments, cancer stage, grade and progression, and hormonal changes. In these limits of variability, it has still been demonstrated that women benefit from different doses. Therefore, it might be helpful to use different dosing schedules adjusted to gender and age. This has already been implemented for a few drugs, where doses are reduced in older patients to minimize side effects (e.g., vinca alkaloids) (Tables 1 and 2).

Steroids like cortisol are more rapidly metabolized in women due to 25–200% higher expression and much higher activity of CYP3A4 (Shimada et al. 1994; Gleiter and Gundert-Remy 1996; Inagaki et al. 2002; Wolbold et al. 2003).

5-FU has a significantly slower clearance rate in women (179 vs. 155 l/h/m^2; $p = 0{,}013$) (Milano et al. 1992). *Folates* have also a 2.5-fold higher clearance in men (Mader et al. 1995).

Anthracyclin clearance, as shown for Doxorubicin and Epirubicin, is significantly higher in men (Lee et al. 1980; Rodvold et al. 1988; Wade et al. 1992; Morgan and Bray 1994; Dobbs et al. 1995; Hempel et al. 2002). Importantly, cardiac toxicity might be increased in women due to a decreased expression of *p*-glycoprotein, which leads to accumulation of anthracyclins in the heart muscle (van Asperen et al. 1999a, b).

Gemcitabine has a 25% decreased clearance rate and a 20% decreased distribution volume in women (Halm et al. 2000).

Docetaxel is also metabolized via CYP3A4 and a clear gender bias in clearance rate has been demonstrated (Marre et al. 1996; Puisset et al. 2007). Another explanation is the high binding of docetaxel to serum alpha1-acid glycoprotein (Urien et al. 1996). This protein has a significant higher serum concentration in males (0.814 vs. 0.739 g/l, $p < 0.01$), which increased with age only in females, but not in males (Blain et al. 1985). Therefore, with standard dosing, the concentration of free drug might be much higher in young- and middle-aged women.

The sex-related pharmacokinetic differences of *paclitaxel* treatment have been analyzed in great detail. Women reach saturation of the peripheral compartment at lower plasma levels (0.83 vs. 1.74 µmol/l), have lower plasma maximal elimination capacities, a lower maximal transport rate to the first peripheral compartment (both are 20% lower than in men), but a longer time to saturation of drug elimination (1 vs. 0.5 h) (Joerger et al. 2006). The maximum plasma concentration is higher in females, even after a body-surface-adjusted dose. This is likely due to different muscle/fat ratios explaining the different saturation concentrations and due to different transport processes (e.g., due to differing *p*-glycoprotein activity) explaining the longer maximal transport rate in females. However, the elimination

Table 2 Examples of gender differences in the clearance and half-life of drugs used in oncology

Drug	Gender differences in clearance	Gender differences in half-life	Comment	Source
Methylprednisolone	Women (luteal phase) exhibited a greater methylprednisolone clearance (0.45 vs. 0.29 l/h/kg)	Women (luteal phase) have a shorter elimination half-life (1.7 vs. 2.6 h)	Although women are more sensitive to methylprednisolone as measured by cortisol suppression, they eliminate the drug more quickly, generally producing a similar net response. Higher rate of second. DM in women	Lew KH, Ludwig EA, Milad MA, Donovan K, Middleton E, Ferry JJ, Jusko WJ (1993) Gender-based effects on methylprednisolone pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 54(4): 402–414 Marosi (2006) Wien Med Wochenschau
MTX	Reduced clearance in women			Godfrey et al. (1998) Br J Clin Pharm Wall et al. (2000) Leukemia
Docetaxel			Nausea, headache, skin local toxicity, and rash/itch were seen more frequently in females (62 TCF-treated subjects) than in males (159 TCF-treated subjects)	European Medicines Agency (EMA): http://www.ema.europa.eu
Paclitaxel	Drug elimination was 20 % higher in male patients compared with female patients			Joerger M, Huitema ADR, van den Bongard DH, Schellens JH, Beijnen JH (2006) Quantitative effect of gender, age, liver function, and body size on the population pharmacokinetics of Paclitaxel in patients with solid tumors. Clin Cancer Res12: 2150

(continued)

Table 2 (continued)

Drug	Gender differences in clearance	Gender differences in half-life	Comment	Source
Fluorouracil	Clearance higher in male (179 vs. 155 l/h/m^2)		10 % increased survival in women; greater hematological and GI-toxicity in women	Davis WM (1998) Impact of gender on drug responses. Drug Topics, October 5 1998 Marosi (2006) Wien Med Wochenschau Wang and Huang (2007) Curr Drug Discov Technol Mader (2006) Wien Med Wochenschau
Capecitabine and main metabolites of capecitabine (5′DFUR, 5-FU, FBAL)	Clearance of capecitabine in women is less than that in men		The AUC and C_{max} of FBAL are approximately 10 and 20 %, respectively, higher in women than in men	Midgley R, Kerr DJ (2009) Capecitabine: have we got the dose right? Nat Clini Pract Oncol 6(1): 17–24 European Medicines Agency (EMA): http://www.ema.europa.eu
Gemcitabin	Clearance in men is higher (92.2 vs. 69.4 l/h/m^2 at age 29; 75.7 vs. 57.0 l/h/m^2 at age 45; 55.1 vs. 41.5 l/h/m^2 at 65; 40.7 vs. 30.7 l/h/m^2 at 79)	Half-life in women is higher (42 vs. 49 min at 29; 48 vs. 57 min at 45; 61 vs. 73 min at 65; 79 vs. 94 min at 79)		FDA: http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/020509s033lbl.pdf Wang and Huang (2007) Curr Drug Discov Technol
Topotecan	38 % higher clearance after oral administration in males (237 ± 105 l/h) vs. females (163 ± 62.5 l/h)			Loos WJ, Gelderblom HJ, Verweij J, Brouwer E, de Jonge MJ, Sparreboom A (2000) Gender-dependent pharmacokinetics of topotecan in adult patients. Anticancer Drugs 11(9): 673–680 Wang and Huang (2007) Curr Drug Discov Technol

Etoposide	Reduced hepatic clearance in women	Cell lines derived from females were more sensitive	Huang RS, Kistner EO, Bleibel WK, et al (2007) Effect of population and gender on chemotherapeutic agent-induced cytotoxicity. Mol Cancer Ther 6: 31–36 Wang and Huang (2007) Curr Drug Discov Technol
Doxorubicin and epirubicin	Doxo: men had a significantly higher clearance women (median values, 59 and 27 l/h/m^2, respectively; $P = 0.002$) Epi: similar data found by Wade et al.		Dobbs NA, Twelves CJ, Gillies H, James CA, Harper PG, Rubens RD (1995) Gender affects doxorubicin pharmacokinetics in patients with normal liver biochemistry. Cancer Chemother Pharmacol 36(6): 473–476. 10.1007/BF00685796 Marosi (2006) Wien Med Wochenschau Wang and Huang (2007) , Curr Drug Discov Technol
Temozolomide		Higher rates of Grade 4 neutropenia: 12 vs. 5 %, and thrombocytopenia: 9 vs. 3 %, in women vs. men in the first cycle of therapy	European Medicines Agency (EMA): http://www.ema.europa.eu

(continued)

Table 2 (continued)

Drug	Gender differences in clearance	Gender differences in half-life	Comment	Source
Melphalan (24 h infusion)	Clearance in men is higher (14.3 ± 4.5 l/h/m^2 vs. 12.3 ± 4.5 l/h/m^2)			Mougenot P, Pinguet F, Fabbro M, Culine S, Poujol S, Astre C, Bressolle F (2004) Population pharmacokinetics of melphalan, infused over a 24-hour period, in patients with advanced malignancies. Cancer Chemother Pharmacol 53(6): 503–512. 10.1007/s00280-003-0761-2
Carmustine			Female gender is an independent risk factor and predictor for oral mucositis	Vokurka et al. 13:554-558, (2005 Vokurka S, Bystrická E, Koza V, Scudlová J, Pavlicová V, Valentová D, Visokaiová M, Misaniová L (2006) Higher incidence of chemotherapy induced oral mucositis in females: a supplement of multivariate analysis to a randomized multicentre study. Support Care Cancer 14(9): 974–976
Nelarabine			ara-GTP AUC0-t and C_{max} were 4.2 and 2.1 times higher ($p = 0.020$ and $p = 0.074$), respectively, in adult females than in adult males (AUC0-t 3,752 vs. 896 µmol h/l, respectively, and C_{max} 158 and 74.2 µmol/l, respectively)	European Medicines Agency (EMA): http://www.ema.europa.eu

Carboplatin/cisplatin			Cell lines derived from females were less sensitive to platinating agents than cell lines derived from males higher rate of nausea/vomiting in females	Huang RS, Kistner EO, Bleibel WK, et al (2007) Effect of population and gender on chemotherapeutic agent-induced cytotoxicity. Mol Cancer Ther 6: 31–36 Wang and Huang (2007) Curr Drug Discov Technol
Bevacizumab	Clearance 17 % higher in men (0.220 vs. 0.188 (l/day)	Longer half-life in men (20 days vs. 18 days)		European Medicines Agency (EMA): http://www.ema.europa.eu Final Multivariate Model for PFS in Study AVF2107g
Cetuximab	Female patients had a 25 % lower intrinsic clearance than male patients			European Medicines Agency (EMA): http://www.ema.europa.eu
Rituximab	Female patients have a 37 % lower clearance of Rituximab			
Ofatumumab	14–25 % lower clearance and volume of distribution in female patients compared to male patients			

Adapted from Oliver Schmetzer and Anne Flörcken: Sex/gender differences in hematology in sex and gender aspects in clinical medicine

rate is also slower, likely due to reduced enzyme activities in women (e.g., CYP3A4). In contrast, only a 5% reduction in drug elimination can be found for every 10-year increase in age. Therefore, the elimination of paclitaxel in a 30-year old woman is comparable to a 70-year old man. These pharmacokinetic differences lead to an increased time above the toxicological threshold concentration in women and might explain the increased toxicity compared to male patients.

The metabolism of *vinorelbine* depends on CYP3A4 activity in liver microsomes and is increased in females (Kajita et al. 2000; Puisset et al. 2005). In contrast to other drugs, which are metabolized via CYP3A4, the vinca alkaloids do not depend on *p*-glycoprotein-mediated transport; therefore, excretion is not decreased in female patients (van Asperen et al. 1999a, b; Davis 2005).

Treatment protocols consisting of several agents often show gender-associated differences due to many different pharmacokinetic properties of the drugs.

3.7 *Non-Pharmacokinetic-Based Differing Actions of Cytostatic Agents in Females*

DNA-repair capacity in lymphocytes of cancer patients is lower in females (Wei et al. 2000). Moreover, it is possible that tumor-DNA-repair is also reduced in women which could explain the better response to chemotherapy.

One explanation for higher tumor resistance to 5-FU in females might be the effect of estrogen on the 5-FU-target-enzyme: thymidylate synthase. This enzyme is under control of the estrogen receptor in female colon carcinoma cells which down regulates thymidylate synthase, therefore possibly rendering the cells more resistant to 5-FU based therapy (Horie et al. 1995; Nussler et al. 2008). In contrast, other studies suggested that female colon carcinomas are more sensitive to 5-FU, because of a lower expression of the rate-limiting catabolic enzyme for 5-FU: dihydropyrimidine dehydrogenase (Yamashita et al. 2002).

4 Relation of Pharmacokinetics on Response to Therapy

In hematological diseases increased bone marrow toxicity is more common in women. However, this increased rate of side effects is associated with an improved treatment. In Hodgkin Lymphoma the higher rate of severe neutropenia in females was correlated with an improved treatment response (Klimm et al. 2005). The sex-specific improved survival in female Hodgkin Lymphoma patients is therefore likely based on different drug metabolism during treatment.

Worse outcome could only be demonstrated in young male adults with Hodgkin Lymphoma, Ewing sarcoma, and osteosarcoma, but not in patients with acute lymphoblastic leukemia or rhabdomyosarcoma. Predominantly, the excess mortality occurred in 15–30-year-old male patients. Young female adults had similar response

rates as children of either sex, indicating that young male patients are generally under-dosed with current treatment protocols. This can be explained by changes in liver enzymes during puberty due to gonadal steroids, which have not been taken into account in many modern clinical trials. The study showed that survival and response rate have not changed in young male adults over two decades.

Different response rates and higher side effects in female patients treated with *5-FU* have first been noted in patients with colorectal cancer (Jansman et al. 2000). However, a pooled analysis did not demonstrate any gender effect in *adjuvant 5-FU treatment* in 3,302 patients with colorectal cancer stages II and III (Gill et al. 2004).

Temozolomide prolonged survival in women with glioblastoma multiforme (Stupp et al. 2005).

The median progression free survival was also longer in women treated with *carboplatin and paclitaxel* for lung cancer (5.3 vs. 4.4 months, $p = 0.0081$) (Yamamoto et al. 2008). Better response to chemotherapy in female patients with lung cancer has also been shown in other studies (O'Connell et al. 1986; Shinkai et al. 1992; Wakelee et al. 2006). Interestingly, in some studies the response to chemotherapy is improved in women but side effects are comparable between the sexes (2-year OS 50.3 vs. 28.2%) (Han et al. 2006). While response rate in male patients was better with an optimized chemotherapy application sequence (*cisplatin followed by irinotecan*), there was no different response rate found in females.

4.1 Sex Differences in Toxicity: Clinics and Pathophysiology

The sex differences in metabolic activity generally lead to a higher possibility of adverse effects in women during therapy with cytostatic agents (Mader 2006). Especially in high dose therapies, e.g. given for stem cell transplantation, the mortality in females is higher than in men (Socie et al. 2001). In young adults (15–30 years) treated with high-dose chemotherapy, male patients experienced lower toxicity (Khamly et al. 2009). Especially, the rates of oral mucositis are higher in females treated with high-dose chemotherapy (BEAM or HD-L-PAM) followed by autologous stem-cell transplantation (86 vs. 60%, $p = 0.0016$) (Vokurka et al. 2006).

4.1.1 Mucosa-Associated Effects

In contrast to the mouth mucosa, the gut mucosa in men appears to be more sensitive to cytostatic agents and women therefore have lower gastrointestinal side effects during treatment. While gastric absorption is minimal, most xenobiotics are resorbed in the duodenum and jejunum. Mucosa cells are protected against toxic compounds, the redox system being the most important system involved. Glutathione is used as redox-buffer to inactivate reactive oxygen species and electrophiles. It has been shown that the cytosolic glutathione *S*-transferase is significantly lower expressed and active in the male gut (Singhal et al. 1992; Hoensch et al. 2002). Therefore, the

male mucosa is less protected against oral toxins. Sex is an independent variable of glutathione *S*-transferase function, but as fruits and vegetables increase the expression of the enzyme, there might also be a life-style-associated phenomenon. However, in postmenopausal women this difference to men is less pronounced. Cytostatic drugs additionally reduce the expression of the enzyme (Hoensch et al. 2002). In contrast, cortisol derivates enhance anti-oxidative capability by increasing GST P. Studies in rodents suggest that the increased expression in women is at least in part induced by hormones (thyroxin, pituitary growth hormone, and testosterone) likely due to increased gene expression levels (Howie et al. 1990; Srivastava and Waxman 1993; Hayes and Pulford 1995). In humans, insulin has been shown to increase GST P-level (Hoensch et al. 2002). Interestingly, these increased anti-oxidant activities can already be found in neonatal tissue and might protect selected female tissues from oxidative stress in order to improve genetic stability (Lavoie and Chessex 1997). Another important detoxification system is the P450 monooxygenase which is higher expressed in male jejunum (Sundseth and Waxman 1992).

4.2 Sex-Specific Adverse Effects of Some Drugs

Higher neurotoxicity in females treated with *ifosfamide* might be induced by CYP3A4 mediated more rapid increased hepatic microsomal *N*-dechloroethylation (Schmidt et al. 2001).

In colorectal cancer, women have been shown to experience a higher degree of toxicity than men (Gusella et al. 2006; Ramani et al. 2006; Kaminski et al. 2007). 5-FU has been shown to lead to increased non-hematological grade 3 and 4 [hand–foot syndrome, (1998)] and hematological [neutropenia, infection, (Stein et al. 1995)] side effects in women. However, enzyme activity of dihydropyrimidine dehydrogenase could not be correlated directly to the amount of side effects, even if it is linked to clearance rates. In contrast, newer studies have demonstrated mainly increased hematological side effects (Chansky et al. 2005; Farker et al. 2006). Severe toxicity and mortality are also higher in women (Tsalic et al. 2003).

Surprisingly, side effects due to *steroid* treatment are more often found in women than in men, even when steroids in women have a much shorter half-life (Marosi 2006). Rates of secondary diabetes mellitus are increased.

4.3 Sex-Specific Side Effects of Some Regimes

Women treated with *carboplatin and paclitaxel* for lung cancer had a higher rate of severe (grade 3–4) leukopenia ($p < 0.001$) and a lower median leukocyte nadir ($p < 0.001$) (Wakelee et al. 2006; Yamamoto et al. 2008). Another study showed also increased hematological toxicity in females treated with cyclophosphamide, vincristine, doxorubicin, etoposide, and cisplatin (Singh et al. 2005). Nausea and

vomiting have been reported to be more severe in women receiving chemotherapy for lung cancer (Gralla et al. 1999).

4.4 Sex-Specific Side Effects of Some Localized Therapies

Localized therapies can have tremendous sex-dependent differences in side effects. This has been demonstrated for hyperthermic intraperitoneal *mitomycin C* after cytoreductive surgery. Women had a dramatic increased incidence of neutropenia (57.6 vs. 21.3%, $p < 0.0001$) in one study and increased rates of urinary tract infection ($p < 0.01$) (Lambert et al. 2009). However, no increase in mortality or hospitalization could be found.

In treatment of young adults for Hodgkin lymphoma, Ewing sarcoma, and osteosarcoma, women had a higher rate a neutropenic fever (2.5 episodes vs. 1.7 in male patients), a lower hemoglobin level (12.6 vs. 14.4 g/dl; $p < 0.0001$), but in contrast also a significant lower need for blood transfusions (every 3.3 cycles vs. 1.9 cycles in men, $p = 0.027$) (Khamly et al. 2009). Less toxicity in males with Ewing sarcoma was also reported in the EURO-Ewing 99 study (Juergens et al. 2006).

5 Targeted Therapy

Non-small cell lung cancer and its treatment have been thoroughly analyzed for gender-associated differences in clinical characteristics and treatment outcomes in clinical oncology.

Multiple studies have demonstrated gender-based differences in clinical factors such as disparities in histological subtypes, age, and smoking habits.

Women with non-small cell lung cancer tend to be of younger age, are more likely to be never-smokers or current non-smokers, and show higher incidences of adenocarcinoma.

Interestingly, these clinical features have been shown to have impact on treatment outcomes, in a number of clinical trials an improved overall survival of women could be observed. This could be seen in surgical interventions as well as chemotherapy [reviewed in Harichand-Herdt and Ramalingam (2009)].

As EGF receptor is frequently overexpressed in non-small lung cancer, tyrosinekinase inhibitors targeting the EGF receptor such as gefitinib and erlotinib represent modern therapeutic options. Clinical trials have shown a favorable efficacy profile in women concerning response rate and overall survival (Fukuoka et al. 2003; Kris et al. 2003; Perez-Soler et al. 2004; Ciardiello 2008; Yamamoto et al. 2008). However, this has been partly attributed to disparities in clinical factors such as age, smoking habits, and histological subtypes. Furthermore, women tend to show a higher prevalence of EGF receptor mutations which are associated with an improved

response rate to EGF receptor inhibitors (Paez et al. 2004; Harichand-Herdt and Ramalingam 2009).

Additionally, there is emerging evidence that hormone substitution in postmenopausal women using combined estrogen and progestin leads to a higher incidence of deaths from lung cancer (Heiss et al. 2008). Further analyses on estrogen receptor expression are warranted to fully understand any significant association.

5.1 Tyrosine-Kinase Inhibitors

Imatinib is a potent selective inhibitor of the protein kinase Bcr-abl, platelet-derived growth factors, and c-KIT. It is approved for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. Pharmacokinetic studies have shown no gender differences (Peng et al. 2005). Toxicity and quality of life improvement related to imatinib treatment appear not to be gender-associated (Aziz et al. 2011). In contrast, there seem to be gender-related differences in clinical outcome after imatinib therapy: women seem to have a superior outcome or at least a comparable outcome when presenting with less favorable prognostic factors (Lee et al. 2009; Bjorkholm et al. 2011).

The multi-tyrosinekinase inhibitor *sorafenib* is an effective and approved targeted therapy in metastatic hepatocellular carcinoma and metastatic renal cell cancer. Effectiveness in several other cancer types, e.g., sarcoma and leukemia is still under investigation. In a phase II study in sarcoma, female gender was associated with increased toxicity, another analysis showed menstruation alterations in females treated with sorafenib (Maki et al. 2008; Zhang et al. 2009). However, large phase III trials which led to approval of the drug did not confirm increased toxicity in females (Escudier et al. 2007; Llovet et al. 2008). In a smaller cohort of hepatocellular carcinoma patients treated with sorafenib, female sex was an independent prognostic factor for prolonged total time to progression (Tortora et al. 2011).

Interestingly, the second-generation tyrosinekinase inhibitor *axitinib*, which is expected to be approved for treatment of metastatic renal cell cancer in 2012, showed significant gender differences in progression-free survival (Rini et al. 2011).

In other tyrosinekinase inhibitors such as *sunitinib* or *pazopanib*, no gender differences in efficacy could be observed (Motzer et al. 2007, 2009; Sternberg et al. 2010). However, a different study documented female gender to be related with significantly higher toxicity (van der Veldt et al. 2008).

5.2 mTOR-Inhibitors

Proliferation-signal inhibitors or mammalian target of rapamycin (mTOR) inhibitors are effective immunosuppressive agents (Webster et al. 2006). Further

development has led to therapeutic use in renal cell cancer and mantle cell lymphoma.

It has been shown that these agents impair male gonadal function with low testosterone levels and increased luteinizing hormone (LH). Additionally, spermatogenesis seems to be disrupted and follicle-stimulating hormone (FSH) levels are elevated [reviewed in Huyghe et al. (2007)].

5.3 Monoclonal Antibodies

5.3.1 Models in Antibody Metabolism

Models of Antibody Catabolism

One explanation for the prolonged half-life of exogenous therapeutic antibodies in females could be an inhibition of antibody production and/or uptake of antibodies for degradation by estrogen alpha receptor stimulation Table 3. FcRn is a Fc-receptor essential for the antenatal transfer from mother to the embryo (Mould and Sweeney 2007). IgG-molecules are transendosomaly transported and protected from degradation. In adults, the receptor is mainly expressed in endothelial cells, in low density on monocytes and in high density on some macrophages. Increased function and/or expression of FcRn can therefore increase recycling of the antibody in females and prolong half-life which is termed IgG-protection (Leveque et al. 2005; Mould and Sweeney 2007). Mice deficient of the receptor have a remarkably lower half-life of IgG (Junghans and Anderson 1996). In humans it has been demonstrated that murine, e.g., chimeric antibodies have a shorter half-life, maybe due to their reduced binding to FcRn and consecutive lower rate of recycling (Ober et al. 2001). In contrast, several studies demonstrated a higher clearance of rhTNF-Fc in females which is incongruent with this model (Nestorov et al. 2004; Fang et al. 2010). In addition to clearance mediated by the rediculoendothelial system, several unexpected factors seem to play a major role in pharmacokinetics of monoclonal antibodies: The antigen mass (tumor mass and antigen expression) clearly has a tremendous effect on pharmacokinetics and response to treatment as has been shown for rituximab (Cartron et al. 2011). IgG1 subtypes are mainly cleared by the endothelial system and not via the liver (Brahmer et al. 2011). In addition, clearance is 20% increased in patients with low albumin (<29 g/l) (Lu et al. 2008). While some human/full-humanized IgG1-antibodies (adalimumab, alemtuzumab, efalizumab, trastuzumab) show non-linear pharmacokinetics, others (bevacizumab, daclizumab, omalizumab) show clear linear pharmacokinetic behavior (Mould and Sweeney 2007). The only difference between these mAbs is the antigen-binding-side (CDRs), the residual Fab, and the complete Fc-parts are identical. The different behavior clearly demonstrates that characteristics of the antigen-binding and not Fc-type are important in these differences in metabolism. Therefore, more studies are needed to clarify this issue.

Table 3 Examples of gender differences in the clearance of antibodies used in oncology

Biological	Target	Gender differences	Fc-part	
Bevacizumab	Anti-VEGF	Clearance 17 % higher in men (0.220 vs. 0.188 (l/day)	HuIgG1	European Medicines Agency (EMA): http://www.ema.europa.eu Final Multivariate Model for PFS in Study AVF2107g
Cetuximab	Anti-EGFR	Female patients had a 25 % lower intrinsic clearance than male patients	HuIgG1	European Medicines Agency (EMA): http://www.ema.europa.eu
Denosumab	Anti-RANKL	No difference	huIgG2	European Medicines Agency (EMA)
Ofatumumab	Anti-CD20	14–25 % lower clearance and volume of distribution in female patients compared to male patients	HuIgG1	Nightingale, Ann Pharmacother 2011
Panitumumab	Anti-EGF	No difference	huIgG2	Ma j clin pharmacol 2009
Rituximab	Anti-CD20	Female patients have a 37 % lower clearance of rituximab	Murine	European Medicines Agency (EMA): http://www.ema.europa.eu

Independent of the Fc-part the half-life is associated with the sex of the patient

Findings for Well-Characterized Antibodies

Rituximab was the first monoclonal antibody for the treatment of cancer approved by the US Food and Drug Administration. It is directed against the CD20 antigen on B-cells and is widely used in the treatment of B cell lymphomas and leukemia.

Its clearance is about 30–37% higher in male individuals (Ng et al. 2005) and males show lower trough serum levels during treatment (Pfreundschuh et al. 2009). While male gender is a known adverse prognostic factor in Hodgkin's lymphoma and in some non-Hodgkin's lymphoma studies in the prerituximab era of lymphoma therapies, it could be shown that women tend to respond better to Rituximab-containing immunochemotherapy (Riihijarvi et al. 2011). This has been attributed to the different pharmacokinetics between men and women. Additionally, possible gender-associated genetic polymorphisms which lead to higher resistance have been postulated (Ternant et al. 2009; Cho et al. 2010). Based on these observations, the German lymphoma group is currently recruiting patients to a clinical trial in which men receive higher dosages of rituximab to compensate for lower serum levels (DENSE-R-UP-Trial, DSHNHL 2004-1).

The monoclonal EGF receptor antibody *cetuximab* has been approved for therapy in colorectal carcinoma and head and neck cancer. It shows a 25% higher clearance rate in male patients (http://www.ema.europa.eu). Male gender was also associated with a higher rate of hypersensitivity reactions during infusion (Hansen et al. 2011).

Another therapeutic option in non-small cell lung cancer is the blockade of vascular endothelial growth factor. *Bevacizumab* is a monoclonal antibody to vascular endothelial growth factor and has been approved for non-small cell lung cancer treatment showing improvement in overall survival when added to chemotherapy (Sandler et al. 2006a, b). It is known to have a 17–26% lower clearance in women (Brahmer et al. 2011). Interestingly, while women overall showed a higher overall survival compared to men, the addition of bevacizumab to chemotherapy added no improvement and additionally showed increased toxicity (higher rate of hypertension, constipation, and abdominal pain in females) (Sandler et al. 2006b; Brahmer et al. 2011). However the hematological toxicity was not biased to sex while the duration of response was increased in women after addition of bevacizumab (Brahmer et al. 2011). After adaption to other risk factors which were slightly different in males and females in a multivariable analysis, the survival after treatment was no longer biased to gender (Brahmer et al. 2011). The reason for the initially described lack of overall survival benefit is therefore likely due to different biological behavior of the tumor with tiny differences in tumor stage (e.g., more liver involvement in women) cumulating to simulate a sex-associated lack of response in women. Also no gender-related differences on outcome have been reported with bevacizumab in the treatment of colon cancer (Saltz et al. 2008). This demonstrates that careful randomization, subgroup analyses, and adaptation for cumulative minor differences in the groups have to be performed in gender-adapted studies.

6 Gender Differences in Supportive Care

No gender-related differences in the response to NSAIDs, benzodiazepines, or proton pump inhibitors have been established (Marosi 2006).

6.1 Pain Management

Women tend to have an increased reactivity of opioid receptors (42 nM/l in females vs. 71 nM/l in males) and therefore need less morphine to relieve pain while they have an increased risk of respiratory depression (Dahlstrom et al. 1982; Burns et al. 1989; Gear et al. 1996; Dahan et al. 1998; Chia et al. 2002; Pleym et al. 2003). Both, the endogenous analgesic system and the opioid receptors have been shown to be differently active. In addition, gender-related variables like psycho-social and cultural experiences have been shown to result in a different degree of pain perception (Wiesenfeld-Hallin 2005).

6.2 *Anti-Emesis*

Nausea and vomiting during cancer therapy pose a significant challenge—more in women than in men (Gralla et al. 1999; Liaw et al. 2001). Increased incidences of nausea and vomiting can also be found in women in the post-operative period and after non-operative pain treatment with opioids (Gralla et al. 1999; Cepeda et al. 2003). Additionally, the rate of chemotherapy-induced nausea is higher in women, while standard anti-emetics like metoclopramide, serotonin-receptor-modulators, or neurokinin-1-antagonists are less effective than in men (Osoba et al. 1997; Jann et al. 1998; Tsavaris et al. 1998; Friedman et al. 2000; Liaw et al. 2003; Hesketh et al. 2006). Also new generation 5-HT3-antagonists like palonosetron are less effective in women (Petru et al. 2008). Only NK-1 inhibitors seem to significantly improve response rates in women during the first cycle of cisplatin therapy (Hesketh et al. 2006). NK-1 inhibitor addition prevented emesis in about 20% of the patients independent of gender.

One study demonstrated that in-family conflicts increase the incidence of nausea in women, but not in men (Kim and Morrow 2003). Other studies also showed that women rather benefit from social and psychological support compared to men (Reynolds and Kaplan 1990; Ell et al. 1992).

However, cisplatin induces more therapy-resistant hiccups in men which can persist for several days (Liaw et al. 2001; Marosi 2006).

6.3 *Antihistamines*

Antihistamines are often used as premedication for antibodies or other biologicals, taxanes, blood products, and as anti-emetic drugs. Women have a higher plasma level, especially the elderly, which can lead to severe cardiac arrhythmias (Ebert et al. 1998; Timmer et al. 2000).

7 Conclusions and Clinical Implications

Complex sex-related differences in pharmacokinetics exist in many chemotherapeutic drugs. These often affect the probability of side effects and survival. Reasons can be different expression/activity of enzymes and transporters in various tissues/tumors or can be based on differing body composition like muscle/fat ratios. Even if detailed data are available, only rare gender-adjusted dosing of cytostatic drugs has been described. In contrast, clinical studies are now performed to see whether outcome can be influenced by increasing the dose of monoclonal antibodies, e.g., rituximab.

Take Home Messages

- Women often have increased side effects during cancer therapy
- Men show reduced response rates during treatment
- Gender differences are variable for each cytostatic drug
- Side effects and response rates often correlate with pharmacokinetics
- Vomiting and oral mucositis are increased in women
- Women are less responsive to medical treatment
- Men have higher incidence of gut-associated side effects
- Men are less likely to benefit from psychological care

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Sex Differences in Effects and Use of Anti-inflammatory Drugs

Svitlana Demyanets and Johann Wojta

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Abstract Gender accounts for important differences in the incidence, prevalence, and course of many immunoinflammatory diseases. However, similar treatment strategies, such as the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and tumor necrosis factor-α (TNF-α) inhibitors, have been advocated for both genders. Experimental studies found that molecular mechanisms of inflammation differ in males and females. In our chapter we summarize the data concerning gender-specific aspects about prevalence of use, drug survival, responsiveness, and adverse drug effects of NSAIDs and TNF-α inhibitors. Gender-related differences in the prevalence and course of many autoimmune diseases as well as differences in effects of anti-inflammatory drugs should be considered for the tailored treatment options for these patients.

Keywords Inflammation • Gender-specific • Sex • Nonsteroidal anti-inflammatory drugs • Aspirin • COX-2 inhibitors • TNF-α inhibitors

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Abbreviations

COX	Cyclooxygenase
ERK	Extracellular signal-regulated kinase
FDA	Food and Drug Administration
IFN	Interferon
IL	Interleukin
IL-1Ra	IL-1 receptor antagonist
NF-κB	Nuclear factor-κB
NSAIDs	Nonsteroidal anti-inflammatory drugs
RA	Rheumatoid arthritis
TNF-α	Tumor necrosis factor-α
UGIB	Upper gastrointestinal bleeding

1 Introduction

Inflammation is the immunologic response against damage and pathogens to protect the body and to start the healing process at the compromised site. Thus, the inflammatory process is a key to survival but at the same time can lead to the development of degenerative diseases (Medzhitov 2008). Anti-inflammatory refers to the property of a medication that reduces inflammation. At present, anti-inflammatory medications are prescribed irrespective of patients' gender. The area of gender differences in inflammation appears to offer potential indications for the future development of better strategies of intervention.

Generally, females have more potent inflammatory and immune reactions than males and lymphocytes/monocytes from female subjects show higher immune-inflammatory reactivity (Chrousos 2010; Cutolo et al. 2009). Males and females show differences in the prevalence of many major diseases that have important inflammatory components to their etiology (Table 1).

Rheumatoid arthritis (RA) affects women about three times more often than men (Joseph et al. 2010). The prevalence of RA in women has been associated with sex hormone levels, major histocompatibility complex genetic background, and cytokine production (Bouman et al. 2005; Whitacre 2001). Estrogen has a dichotomous impact on the immune system by downregulating inflammatory immune responses and upregulating immunoglobulin production (Carlsten 2005). Sex hormones—in particular estrogens—may also regulate the immune response by favoring the survival of forbidden autoreactive clones and the prevalence of autoimmunity in women. Accordingly, estrogens have been suggested to be associated with the development of RA (Gerosa et al. 2008). Most pregnant RA patients experience a remission. This has been closely related to a switch from T-lymphocytes helper1 to T-lymphocytes helper2 immune responses and to a decreased production of proinflammatory cytokines, at least in part supported by the changes of the hormonal profile in pregnancy (Gerosa et al. 2008). Women are not only more prone than men

Table 1 Gender prevalence in immunoinflammatory diseases

Women	Men
Rheumatoid arthritis (RA)	Reactive arthritis (Reiter's syndrome)
Systemic lupus erythematosus	Inclusion body myositis
Antiphospholipid syndrome	Hepatocellular carcinoma
Polymyositis and dermatomyositis	
Giant cell arteritis (temporal arteritis)	
Takayasu's arteritis	
Primary biliary cirrhosis	
Autoimmune hepatitis	

to develop RA, but recent data suggest that they also suffer greater disability than men with this disease (Kovacs and Olsen 2011). Female sex is among the most common variables associated with future disability in RA that greatly affects patients' quality of life (Toussirot 2010). Females with RA below the age of 45 years had a fourfold increased risk of work disability compared to men in a Scandinavian study (Wallenius et al. 2009). Ahlmén et al. found that despite a similar degree of radiographic joint destruction in RA women had, compared with men, worse scores in the 28-joint Disease Activity Score and the Health Assessment Questionnaire (Ahlmen et al. 2010). Male sex has been shown to be a major predictor of remission in patients with early RA (Forslind et al. 2007). In the large multinational cross-sectional cohort including 6,004 patients, RA disease activity measures appear to be worse in women than in men. Women had poorer scores than men in all American College of Rheumatology Core Data Set measures (Sokka et al. 2009). Pregnancy planning is required in RA in order to avoid unwanted complications. In particular, the need to control the disease requires safe use of antirheumatic drugs during pregnancy and in the lactation period. Another point which has to be considered by clinicians and RA female patients is that hormonal treatment for contraception is contraindicated in the case of positivity for antiphospholipid antibodies owing to the increased thrombophilic risk (Gerosa et al. 2008).

Other autoimmune diseases that affect women more that men include systemic lupus erythematosus, antiphospholipid syndrome, polymyositis and dermatomyositis, giant cell arteritis (temporal arteritis), and Takayasu's arteritis (Table 1) (Joseph et al. 2010; Richards et al. 2010). Autoimmune hepatitis and primary biliary cirrhosis are autoimmune inflammatory diseases of the liver with a female preponderance (Bhandari et al. 2011). In a study of Al-Chalabi et al., women with autoimmune hepatitis were significantly more likely to die or require liver transplantation. Men with autoimmune hepatitis have significantly better long-term survival and outcomes than women (Al-Chalabi et al. 2008).

However, female sex can also protect against inflammatory disease. Hepatocellular carcinoma, the most common liver cancer, which is linked to chronic liver inflammation, is less common among females because of estrogen inhibiting secretion of interleukin (IL)-6 from Kupffer cells (Naugler et al. 2007). As opposed to polymyositis and dermatomyositis, inclusion body myositis affects men twice as often as women. Spondyloarthropathies (ankylosing spondylitis (previously known

as Bekhterev's disease) and reactive arthritis (Reiter's syndrome)) are also more common in men than in women (Joseph et al. 2010). However, sex differences in Bekhterev's disease are now supposed to be much lower than earlier believed with a male-to-female ratio of approximately 2–3:1 (Gran and Husby 1993; Will et al. 1990).

2 Gender-Specific Aspects of Inflammation

With respect to gender dichotomies in inflammatory diseases, relatively little is known about the molecular mechanisms involved. Gender-specific diseases (Table 1) are largely considered to reflect the actions of sex hormones on the susceptibility to inflammatory stimuli (Duma et al. 2010). Sex steroids—estrogens, progesterone, and testosterone—differ between gender and within different reproductive stages (Bouman et al. 2005). Evidence suggests that estrogens affect the immune system and the processes associated with inflammation (Demyanets et al. 2006; Nilsson 2007). However, there is still an unresolved paradox with respect to the immunomodulating role of estrogens. Estradiol-17β at periovulatory to pregnancy levels most often has anti-inflammatory effects by inhibiting many proinflammatory pathways of innate and adaptive immunity (Straub 2007). In chronic inflammatory diseases, where monocytes, macrophages, dendritic cells, T-cells, and neutrophils play a dominant role, estrogens demonstrate a dual role: at low concentrations estrogens stimulate, and, at high levels, estrogens inhibit the disease process (Straub 2007). Thus, a uniform concept for the action of estrogens cannot be found for all known chronic inflammatory diseases. However, inflammation reflects a balance between pro- and anti-inflammatory signals, and investigation of gender-specific responses to these signals is an important aspect of gender-related medicine.

Leukotrienes are a class of eicosanoids formed from arachidonic acid and play a role in both innate and adaptive immune response. By interacting with their receptors, leukotrienes promote the accumulation and function of virtually all subgroups of leukocytes at sites of inflammation. Leukotrienes mediate a variety of inflammatory and allergic conditions, including RA, atherosclerosis, inflammatory bowel disease, psoriasis, allergic rhinitis, and bronchial asthma (Peters-Golden and Henderson 2007). Leukotriene formation in stimulated whole blood or neutrophils from males was shown to be substantially lower as compared with females (Pergola et al. 2008). The differences are directly related to variant male/female testosterone plus 5α-dihydrotestosterone levels, and addition of 5α-dihydrotestosterone to female blood or neutrophils reduced the high female leukotriene biosynthesis capacity to low male levels. Male neutrophils were found to have higher levels of extracellular signal-regulated kinase (ERK) activity than female cells. 5α-dihydrotestosterone caused phosphorylation of ERK in female neutrophils. Incubation of female neutrophils with male, but not female, plasma caused activation of ERKs. Because ERKs are central signaling kinases, these androgen

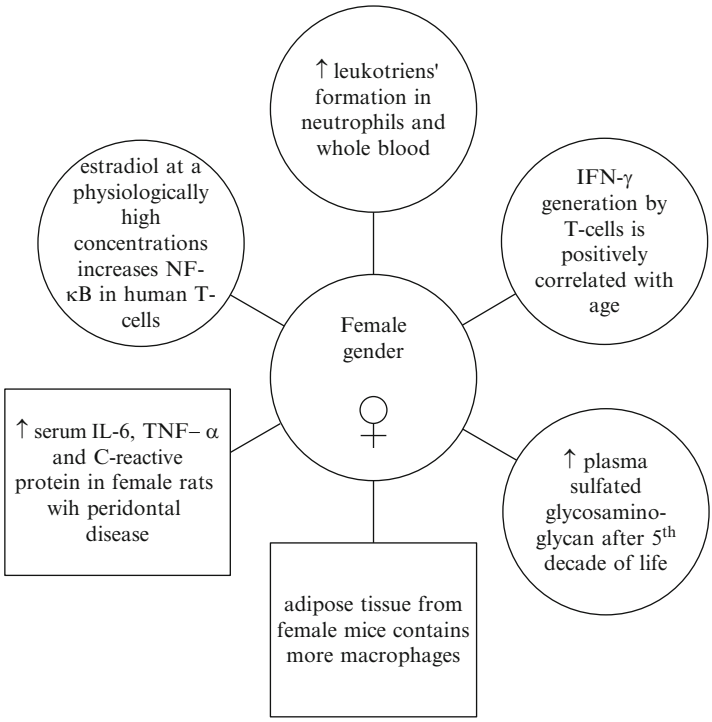

Fig. 1 *Possible targets for anti-inflammatory drugs in women*. The figure shows possible molecular and cellular targets for anti-inflammatory drugs in female gender. Respective references are cited in text (see chapter "Gender-specific aspects of inflammation"). *Circles* indicate studies in humans; *squares* show data from animal studies

actions may have profound effects on neutrophil and other inflammatory cells biology (Pergola et al. 2008). Pergola et al. conclude that regulation of ERKs and leukotriene formation by androgens constitutes a molecular basis for gender differences in the inflammatory response. Therefore, inflammatory responses in general, for which leukotrienes are of relevance, may be more vigorous in females (Fig. 1) (Pergola et al. 2008).

Gender also accounts for important differences in the incidence and prevalence of a variety of age-related diseases (Candore et al. 2006). Considering people of far-advanced age, demographic data document a clear prevalence of females compared to males. Gender differences in the health status of centenarians are also reported, because male centenarians are healthier than female centenarians (Franceschi et al. 2000). Goetzl et al. examined cytokine generation by T-cells and macrophages in old subjects ($\geq$65 years) as compared with matched young controls (Goetzl et al. 2010). Interferon (IFN)-γ and IL-17 generation by stimulated T-cells was significantly lower in healthy old as compared with young men. Generation of IFN-γ and IL-17 by stimulated T-cells was higher or not different for healthy old women as compared with young woman, respectively (Goetzl et al. 2010). $CD8^+$ T-cells were

the major source of increased IFN-γ generation by T-cells in old women and the predominant site of decreased IFN-γ generation by T-cells in old men (Goetzl et al. 2010). These investigators suggest that estrogens may be the determinant of gender differences in the altered cytokines' generation by T-cells in aging (Goetzl et al. 2010). In support of this hypothesis, another study found that low concentration of estradiol, but not progesterone or high levels of estradiol, augmented generation of IFN-γ by mitogen-activated human mononuclear leukocytes in whole blood (Matalka 2003). Therefore, a low concentration of estrogen in old women is the possible explanation for higher levels of IFN-γ production by their T-cells (Goetzl et al. 2010). The analysis of the cytokine generation by T-cells in patients with inflammatory or immune diseases revealed that for old men mean old-to-young ratios of T-cell-generated IFN-γ and IL-17 increased with disease severity; however, they did not change for old women with similar diseases (Goetzl et al. 2010). The relevance of these findings to anti-inflammatory therapy is that gender determines the array of T-cells cytokines that are potential targets for such a therapy (Fig. 1) (Goetzl et al. 2010).

Immune modulators such as cytokines and growth factors exert their biological activity through high-affinity interactions with cell surface receptors, thereby activating specific signaling pathways. However, many of these molecules also participate in low-affinity interactions with another class of molecules, referred to as proteoglycans. Proteoglycans consist of a protein core to which glycosaminoglycan chains are attached (Handel et al. 2005). A linear age-related decline in plasma sulfated glycosaminoglycan was found during the first 5 decades of life, followed by an increase occurring only in females (Fig. 1). Circulating TNF-α concentrations were inversely correlated with age over the lifetime, and the observed changes were gender-specific (Komosinska-Vassev et al. 2011). Female rats with experimental periodontal disease had higher serum concentrations of IL-6, TNF-α and C-reactive protein, and liver C-reactive protein as compared with male rats (Fig. 1). This study suggests that females with periodontal disease have a greater risk for inflammatory-based systemic diseases than males (Bain et al. 2009). Diversity in T-cell recognition of antigens is determined by diverse usage of T-cell receptor repertoire. The T-cell receptor repertoire varies according to sex in normal BALB/c mice as the percentage of T-cell receptor BV15-1-bearing cells was significantly higher in males than in females (Kitaura et al. 2009).

IL-1β and its endogenous antagonist IL-1 receptor antagonist (IL-1Ra) play an important role in various inflammatory responses. The production of IL-1 and IL-1Ra is different between men and women (Bessler et al. 2007; You et al. 2007). Bessler et al. found gender difference in IL-1Ra gene polymorphism (e.g., variable number of tandem repeats) that may affect IL-1β and IL-1Ra levels. This diversity might be one of the causes for the sex differences in immune response observed in autoimmune diseases (Bessler et al. 2007). A polymorphism in the second intron of the IL-1Ra gene and two single nucleotide polymorphisms at positions −511 and +3,954 of the IL-1β gene may be associated with an increased risk of RA. The frequencies of the IL-1β + 3954 allele and genotype in female patients were significantly different compared with the controls; but in males, only

the frequency of the IL-1β + 3,954 allele was different. The association of these three polymorphisms in the IL-1Ra or IL-1β genes with the susceptibility to RA appears to be significantly affected by gender (You et al. 2007).

The known association of pregnancy with remission of some autoimmune diseases and exacerbation of others suggests that physiological fluctuation in estrogen levels could affect the immune responses in humans. The transcription factor nuclear factor-κB (NF-κB) plays a role in the orchestration, amplification, and perpetuation of the inflammatory response (Lawrence 2009). Estrogen and progesterone have been shown to modulate NF-κB activity (Dale et al. 2006). Physiologically high concentrations of estradiol enhance NF-κB activity in human T-cells stimulated by TNF-α or phorbol 12-myristate 13-acetate/ionomycin (Fig. 1). Overexpression and ribonucleic acid interference experiments suggested that the effects were mediated through estrogen receptor β. Estradiol at a physiologically high concentration also affected T-cell survival. The results of Hirano et al. suggest that these actions of estradiol may underlie the gender differences in autoimmune diseases (Hirano et al. 2007).

Numbers and functions of inflammatory cells seem to be also different in males and females. Adipose tissue from female mice has been shown to contain significantly more macrophages than tissue from male mice (Fig. 1). These leukocyte populations within adipose tissue may be involved in the development of heightened inflammation that is characteristic of obesity (Robker et al. 2004). In women, but not in men, a significant association between white blood cell count and the risk for chronic kidney disease was found in the 2007 Korean National Health and Nutrition Examination Survey (Na et al. 2011).

Mucin-2 is expressed in certain states of mucosal damage in gastrointestinal tract, and cyclooxygenase (COX)-2 is an enzyme known to contribute to mucosal repair (Allen et al. 1998; Peskar 2005). Healing of acetic acid-induced gastric ulcers was significantly impaired in male mucin-2-deficient mice (Wallace et al. 2011). Induction of COX-2 in the stomach, in response to indomethacin- or acetic acid-induced ulceration, was significantly reduced in male mucin-2-deficient mice. These results demonstrate an impairment of gastric mucosal repair in male mucin-2-deficient mice that may be related to an insufficient induction of COX-2 (Wallace et al. 2011).

In conclusion, experimental and clinical studies revealed sex-specific differences in molecular mechanisms of the inflammatory reaction. These differences are considered to reflect the actions of sex hormones, at least in part.

3 Nonsteroidal Anti-inflammatory Drugs

NSAIDs are commonly used for the treatment of inflammation, with millions of people using NSAIDs daily. The principal target of NSAIDs is COX, an enzyme for synthesis of lipid mediators called prostanoids (Vane and Botting 1987). COX

exists in two isoforms: COX-1 appears to be constitutively expressed in many tissues and is responsible for homeostatic production of prostanoids. In contrast, COX-2 is often considered an inducible isoform and a principal contributor to peripheral inflammation via production of prostaglandin E2 (Demyanets et al. 2012; Smith et al. 2000).

Chillingworth et al. used knockout mice lacking COX-1 or COX-2 to investigate the contribution of these enzymes to the development of arthritis and inflammatory nociception (Chillingworth et al. 2006). To do this, they delivered Freund's complete adjuvant around the tibiotarsal joint. Although COX-2 disruption eliminated the development of adjuvant-induced thermal hyperalgesia and mechanical allodynia in both males and females, it reduced edema and joint destruction only in females. Moreover, although COX-1 disruption had little influence on ipsilateral thermal hyperalgesia and mechanical hyperalgesia in either sex, it reduced contralateral allodynia, as well as edema and joint destruction only in females. These results suggest that the mechanisms by which COX inhibitors influence inflammation differ in males and females in ways that need further exploration (Chillingworth et al. 2006).

3.1 Nonselective NSAIDs

Gender differences in patterns of NSAID use were shown in some studies in patients with rheumatic diseases as well as in general population (Dominick et al. 2003; Fosbol et al. 2008; Ngo et al. 2010; Stuck et al. 1995). Women were significantly more likely to be prescribed an NSAID than men (37% vs. 30%) and had a greater total days' supply of NSAIDs among older ($>$ or $=$ 65 years) adults with osteoarthritis (Dominick et al. 2003). In a nationwide study on 4.6 million people in Denmark, female gender was associated with increased use of NSAID, and ibuprofen and diclofenac were the most frequently used nonselective NSAIDs (Fosbol et al. 2008). In community-dwelling elderly subjects, the average number of different medications in women was higher than in men (4.0 vs. 3.5). A major gender difference was revealed in the pattern of NSADs used as women had a higher use of NSADs compared to men. Twenty-four percent of all women and 15% of all men reported use of NSADs for which safer medication and nonmedication alternatives would be available in many cases. These gender differences in medication use can be explained by the fact that compared to men, women have a higher prevalence of nonlethal chronic conditions. However, additional factors such as gender-specific differences in patient or physician behavior are likely to contribute to the observed differences in medication use as well (Stuck et al. 1995).

Many NSADs are available over the counter and may be used by patients without medical advice. High level of use of nonprescription anti-inflammatory drugs among the general public has been reported in many European countries and in the USA. Many patients are unaware that nonprescription analgesics can cause potentially serious adverse effects when used in combination with other common

medications. In a study population in Australia, 60% of patients, predominantly females, were currently on other medications, and 65% of patients did not seek medical advice before using nonprescription ibuprofen 200 mg (Ngo et al. 2010). The majority of patients reported rarely or never reading manufacturer's printed warning instructions on the potential drug interactions or adverse effects associated with the use of the product (Ngo et al. 2010). Patient–pharmacy staff communication about treatment is important for the choice of medication. Among 687 patients participating in the Alabama NSAID Patient Safety Study 72.8% were women and only 31.2% discussed use of medications with pharmacy staff. Discussing use of prescription pain/arthritis medications with pharmacy staff differed by race and gender (white men: 40.3%, white women: 34.6%, black men: 30.2%, black women: 19.8%). Thus, black women had the lowest odds of discussing their medications with pharmacy staff compared with white men. Given the complex risks and benefits of chronic NSAID use, pharmacists, pharmacy staff, and patients are missing an important opportunity to avoid unsafe prescribing (LaCivita et al. 2009). NSAIDs were the drugs most often involved in adverse drug events in regional surveillance of emergency department visits for outpatient adverse drug events in Italy. Adverse drug events were significantly more frequent in women (Capuano et al. 2009).

Most often side effects associated with the use of NSAIDs are gastrointestinal symptoms, and gender was shown to be the factor influencing susceptibility and prevalence of these complications. The gastroduodenal adverse effects include dyspepsia without endoscopically proven damage, asymptomatic endoscopic lesions of submucosal hemorrhage, erosions and ulcers, and ulcer complications. Earlier studies established female gender as a risk factor for NSAID-associated upper gastrointestinal symptoms and ulcer complications (Aalykke and Lauritsen 2001; Pilotto et al. 2006). During evaluation of 5,515 elderly outpatients in Italy a significantly higher prevalence of upper gastrointestinal symptoms was observed in females. The use of NSAIDs and steroids was significantly associated with upper gastrointestinal symptoms (Pilotto et al. 2006). However, when data from eight databases in four countries (Denmark, Italy, Netherlands, the UK) were pooled, there were consistently higher incidence rates of upper gastrointestinal bleeding (UGIB) in males overall compared to females, and the rates of UGIB were significantly increased during use of NSAIDs (Coloma et al. 2011) (Table 2). This increase in risk of UGIB in men compared to women is consistent with the literature (van Leerdam 2008). Male gender, history of complicated peptide ulcer disease, and current use of steroids were risk factors for UGIB in a study of Gutthann et al. Simultaneous use of multiple NSAIDs as well as use of a single individual NSAID at high doses greatly increases the risk of complicated peptic ulcer disease (Gutthann et al. 1997). Male gender, drinking alcohol, aspirin/antiplatelet use, and history of peptic ulcer disease were independent risk factors for bleeding in other studies (Kang et al. 2011; van Oijen et al. 2006) (Table 2).

Diverticular bleeding is a common cause of lower gastrointestinal hemorrhage. Several factors, including use of NSAIDs, could be risk factors. In a study of Tsuruoka et al. (2011), more men than women suffered from diverticular

Table 2 Gender differences in adverse effects of anti-inflammatory drugs

Adverse drug effect	Association with male gender	Association with female gender	No gender association
Nonselective NSAIDs			
Upper gastrointestinal symptoms	Aalykke and Lauritsen (2001), Pilotto et al. (2006)	Coloma et al. (2011), Gutthann et al. (1997), Kang et al. (2011), van Leerdam (2008)	
Lower gastrointestinal symptoms	Tsuruoka et al. (2011), Yamada et al. (2008)	Stollman and Raskin (2004)	Jansen et al. (2009)
Gastroesophageal reflux disease		Ruszniewski et al. (2008)	
Renal impairment	Murray et al. (1990), Perez Gutthann et al. (1996)		
Adverse skin reactions (Stevens–Johnson syndrome and toxic epidermal necrolysis)		Ward et al. (2010)	
Aspirin			
Upper gastrointestinal symptoms	Ahsberg et al. (2010), Sostres and Lanas (2011)		
Lower gastrointestinal symptoms			Ahsberg et al. (2010), Okada et al. (2010)
Selective COX-2 inhibitors			
Gastroduodenal ulcers			Goldstein et al. (2001)
Nimesulide			
Liver damage		(2011)	
TNF-α antagonist therapy			
Infections	Burmester et al. (2007), Takeuchi et al. (2008)		
Hematological cancer	Pallavicini et al. (2010)		
Nonmelanoma skin cancer	Amari et al. (2011)		
Infliximab-related hepatitis		Mancini et al. (2010)	
Lupus-like syndrome		Williams and Cohen (2011)	

hemorrhage. The sex difference was similar to that in another Japanese study (Yamada et al. 2008). However, many Western studies of diverticular hemorrhage have indicated a female predominance or no sex difference at all (Jansen et al. 2009; Stollman and Raskin 2004). Although the associations between NSAIDs and peptic ulcer disease or dyspepsia are well established, fewer data exist concerning the relationship between NSAIDs and gastroesophageal reflux disease. NSAID use, female gender, and age were independent predictors of gastroesophageal reflux disease symptoms in a study of 10,000 French adults (Ruszniewski et al. 2008) (Table 2).

Murray et al. determined the incidence of ibuprofen-associated renal impairment and risk factors for its development in 1,908 patients treated with ibuprofen. Renal impairment occurred in 18% of patients. Elderly male patients were at risk for ibuprofen-associated renal impairment and therefore should have their renal function monitored when ibuprofen and possibly other NSAIDs are prescribed (Murray et al. 1990). Current exposure to NSAIDs and male gender were found to be independent risk factors for acute renal failure (Perez Gutthann et al. 1996). Provocation tests with the suspected compounds are considered the "gold standard" for establishing or excluding a diagnosis of hypersensitivity to NSAIDs. Male gender was a significant risk factor for a positive provocation tests (Viola et al. 2011). The risk of severe adverse skin reactions such as Stevens–Johnson syndrome and toxic epidermal necrolysis in patients receiving NSAIDs is extremely low. However, women, older patients, and patients within the first month of treatment initiation appear to have the greatest risk (Ward et al. 2010) (Table 2).

The findings suggest that NSAIDs may confer a protective effect against cancer development or progression. Total NSAID use was associated with a moderately reduced risk of lung cancer, which was strongest for men, adenocarcinoma, and long-term former smokers (Slatore et al. 2009). Evidence from a pooled analysis in the International Lung Cancer Consortium suggests that NSAID use in men confers a modest protection for lung cancer, especially amongst ever-smokers (McCormack et al. 2011). The reduction in risk for lung carcinoma was greater for men than for women taking NSAIDs regularly (Muscat et al. 2003). After stratification by smoking status, regular use of NSAIDs was not found to be associated with lung carcinoma risk in never-smokers. An inverse association was observed in ever-smokers. The odds ratio for regular use of NSAIDs was 0.37 in male current smokers and 1.77 in female current smokers. In former smokers, a protective effect was found for both men and women (Muscat et al. 2003). Small aggressive non-small cell lung cancers, which metastasize while relatively small, were inversely associated with ibuprofen use and were significantly associated with female gender, younger age, and family history of lung cancer. The inverse association between small aggressive non-small cell lung cancers and ibuprofen did not differ by gender and was similar in current and former smokers (Tammemagi et al. 2007). In an epidemiologic case control study, the inverse trend of lung cancer risk with increasing NSAIDs use was highly significant. Results were similar for men and women, and for aspirin and ibuprofen (Harris et al. 2002). A dramatic increase in the incidence of esophageal adenocarcinoma has occurred among men in the USA. Most adenocarcinomas arise in a metaplastic epithelium termed Barrett's esophagus.

An abdominal distribution of body fat, which is more common in men and is termed male-pattern obesity, may be a strong predictor of risk of neoplastic progression among persons with Barrett's esophagus. Vaughan et al. found that NSAID use may reduce the risk of progression to cancer in this population (Vaughan et al. 2002). Some (Garcia Rodriguez and Gonzalez-Perez 2004; Harris et al. 2003; Terry et al. 2004) but not other (Cook et al. 2005; Jacobs et al. 2005; Marshall et al. 2005) studies have found an association between use of NSAIDs and reduced risk for breast cancer. In order to reveal possible explanatory mechanisms, associations among medication use and levels of estrogens, androgens, and prolactin, which have been reported to relate to risk of breast cancer, were evaluated in a cross-sectional analysis from a random sample of 274 postmenopausal women selected from the Women's Health Initiative Dietary Modification Trial (McTiernan et al. 2008). Women who reported regular use of NSAIDs had higher dehydroepiandrosterone concentrations and lower prolactin concentrations compared with nonusers (McTiernan et al. 2008). The association of NSAID use with some hormones suggests one possible mechanism for the association between use of NSAIDs and reduced risk for breast cancer, as elevated prolactin concentrations have been associated with increased risk for breast cancer (Hankinson et al. 1999; Tworoger et al. 2004). One possible mechanism for the negative association of regular NSAID use with prolactin concentrations is through blunting of pituitary response to stress since prostaglandins modulate the hypothalamus–pituitary axis (Di Luigi et al. 2001).

A positive influence of NSAIDs on cognitive function in the elderly was proposed. In population-based sample of 7,671 subjects, cognitive function at the end of the observation period was significantly higher in chronic NSAID users than in controls. Female gender was independent predictor of a lower Short Portable Mental Status Questionnaire score (Rozzini et al. 1996). Female gender may confer an additional risk factor for Alzheimer's disease. Protective factors include a history of use of NSAIDs (Cummings et al. 1998). When data from 11 studies were included in the meta-analysis, NSAIDs as a class did not seem to modify the risk of Parkinson disease. However, ibuprofen may have a slight protective effect in lowering the risk of Parkinson disease. Although the risk ratios of Parkinson disease in male and female NSAID users were similar, the 95% confidence interval for men was suggestive of a slight risk reduction (Samii et al. 2009).

3.1.1 Aspirin

Aspirin is being used as an effective anti-inflammatory agent and analgesic at doses >325 mg daily. At low doses, aspirin is the key antiplatelet drug in the pharmacological prevention of cardiovascular diseases. Aspirin is one of the most prescribed drugs worldwide and its clinical impact is huge. Gender-specific differences in the pharmacokinetics of acetylsalicylic acid have been known for many years. The bioavailability of acetylsalicylic acid is greater in women than in men, owing to slower clearance and prolongation of half-life (Ho et al. 1985). There were

statistically significant differences in metabolites excreted between sexes in healthy men and women following oral treatment with 650 mg aspirin. Men excreted more salicyluric acid; women more aspirin, salicylic acid, salicylic acid acyl glucuronide, and salicylic acid phenolic glucuronide (Navarro et al. 2011). Low-dose aspirin has a gender-dependent impact on anti-inflammatory 15-epi-lipoxin A4 formation as was shown in a randomized human trial (Chiang et al. 2006). In female subjects, a positive correlation between age and aspirin-triggered 15-epi-lipoxin A4 was found, and in men a negative correlation was observed. Formation of aspirin-triggered lipoxin A4 may provide a molecular rationale for low-dose aspirin therapies in elderly women to reduce inflammation-related disorders and may shed light on gender-dependent therapeutics of aspirin (Chiang et al. 2006).

Aspirin use is often associated with mucosal damage in the upper and lower gastrointestinal tract. The risk of upper gastrointestinal bleeding with aspirin was found to be increased with male sex, old age, ulcer history, and concomitant medication with NSAIDs, COX-2-selective inhibitors, or corticosteroids (Sostres and Lanas 2011) (Table 2). In a retrospective Swedish review of 731 patients, there was a male preponderance for upper gastrointestinal bleeds and an equal gender distribution among lower gastrointestinal bleeds patients. Aspirin use and male gender were associated with peptic ulcer bleeds. There were no gender differences in fatal outcome during hospitalization for upper gastrointestinal bleeds and lower gastrointestinal bleeds (Ahsberg et al. 2010). In Japanese patients a history of peptic ulcer was found to be the risk factor for low-dose (81–100 mg daily) aspirin-associated peptic ulcer common to both sexes (Okada et al. 2010) (Table 2). In female patients, age greater than 70 years was found to be another significant risk factor, and the time to diagnosis as having low-dose aspirin-associated peptic ulcer by endoscopy was significantly shorter than that in the male patients. The authors concluded that special attention should be paid to aged female patients taking low-dose aspirin (Okada et al. 2010).

Although only women face the hemostatic challenges of menstruation, pregnancy, and childbirth, there is only limited information on whether other bleeding symptoms differ between men and women (Mauer et al. 2011). Mauer et al. investigated bleeding symptoms in healthy adults. When all symptoms were considered, women reported more bleeding symptoms than men. However, after removal of sex-specific bleeding symptoms, men and women both reported a median of one symptom without difference. When individual symptoms were analyzed together by logistic regression, however, differences by sex were noted. Women reported easier bruising and venipuncture-related bruising. Paradoxically, infrequent aspirin users (those who used aspirin less than once a week) reported more bruising and heavy menses than frequent users (those who used aspirin once a week or more often). Thus, bleeding scores that are based on the number of bleeding symptoms need to be adjusted for sex (Mauer et al. 2011).

Animal experiments found that the antineoplastic action of aspirin is gender dependent. Using a murine model of T-cell lymphoma, Kumar et al. indicate that aspirin administration to male and female tumor-bearing hosts resulted in gender-dependent differential tumor growth retardation, which was associated with a

differential impact on cell cycle progression, expression of cell survival regulatory molecules, contents of glucose, lactate, and cell growth regulatory cytokines. Aspirin was found to reverse estrogen-dependent augmentation of tumor cell survival in vitro (Kumar et al. 2011). A large Dutch population-based case–control study investigated the potential prophylactic properties of NSAIDs on the incidence of cutaneous melanoma (Joosse et al. 2009). Cutaneous melanoma incidence was not significantly associated with ever acetylsalicylic acid use or ever non-acetylsalicylic acid NSAID use. However, continuous use of low-dose acetylsalicylic acid was associated with a significant reduction of cutaneous melanoma risk in women but not in men. A significant trend from no use and noncontinuous use to continuous use was observed in women. Thus, continuous use of low-dose acetylsalicylic acid may be associated with a reduced incidence of cutaneous melanoma in women, but not in men (Joosse et al. 2009). Regular use of aspirin ($\geq$4 times/month) in the past year was inversely associated with colorectal polyps in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (Johnson et al. 2010). Decreased risk was more evident in males and persons over 70 years of age. The protective effects of NSAIDs for females were apparent only among those with body mass index $<$25 (Johnson et al. 2010). A report by Chan et al. (2004) on women in the Nurse's Health Study revealed that regular, short-term use of aspirin is inversely associated with risk for colorectal adenoma. Adenomas were decreased among male regular ($\geq$2 times/week) aspirin users in the Health Professionals Follow-up Study to the same degree (Giovannucci et al. 1994). In a clinical trial of aspirin with a follow-up at 3 years, Baron et al. (2003) showed that protection for advanced adenomas was more evident in females. Tangrea et al. (2003), in their analyses of data from the Polyp Prevention Trial, found that NSAIDs protected against the recurrence of polyps in both men and women. Studies investigating the association between aspirin use and gastric cancer risk have reported conflicting results. In meta-analyses of the data for males and females, summary odds ratios were 0.71 and 0.67, respectively, and the test for heterogeneity was not statistically significant (Yang et al. 2010). Rothwell et al., in their meta-analysis of randomized trials, show that low-dose long-term aspirin use reduces risk of death due to several cancers (Rothwell et al. 2011). The benefit was unrelated to aspirin dose (75 mg upwards) or sex (Rothwell et al. 2011). Further to COX inhibition, aspirin has several additional mechanisms of action that may contribute to its anticancer effect. It also influences cellular processes such as apoptosis and angiogenesis that are crucial for the development and growth of malignancies (Langley et al. 2011). By analyzing the data from the first and second National Health and Nutrition Examination Study, Ratnasinghe et al. found that aspirin use offered protection from lung cancer among men. In women aspirin use was associated with increased risk of bladder and brain cancer. However, the number of female bladder and brain cancer cases was small in this cohort (Ratnasinghe et al. 2004). Aspirin as a preventive therapy against lung carcinoma was found to be effective only in men and not in women (Muscat et al. 2003). In former smokers, a protective effect was found for both men and women (Muscat et al. 2003). In a California retirement community cohort, the incidence of lung carcinoma in daily aspirin users was reduced among

women but not among men (Paganini-Hill et al. 1989). The Iowa Women's Health Study did not suggest that aspirin or other NSAIDs reduce risk of lung cancer in the cohort of postmenopausal women (Hayes et al. 2006). However, the New York University Women's Health Study suggests that regular aspirin use might be inversely associated with risk of lung cancer in women, particularly the non-small cell subtype (Akhmedkhanov et al. 2002). Among 635,031 adults who were followed for 6 years, there was no reduction in lung carcinoma mortality rates among aspirin users, except for a subgroup of women who took 1–15 tablets a month (Thun et al. 1993).

The data of Wahner et al. suggested a decreased risk of Parkinson disease among regular ($\geq$2 pills/week for at least 1 month) aspirin users. The aspirin effect estimates differed by gender, showing a protective effect only in women, especially among long-term ($\geq$24 months) regular users (Wahner et al. 2007). In Alzheimer's disease female gender appears to be an important risk factor associated with the aberrant production of beta amyloid peptides. Although decreased levels in plasma docosahexaenoic acid concentration are associated with cognitive decline in healthy elderly and Alzheimer's patients, pretreatment with docosahexaenoic acid significantly reduced the survival of cortical neurons incubated with beta amyloid. Hence, in the presence of an increasing amount of beta amyloid, paradoxically women who have higher plasma levels of docosahexaenoic acid are more likely to develop Alzheimer's disease. Aspirin converts COX-2 into a form that generates new neuroprotective docosanoids from docosahexaenoic acid; therefore, aspirin might positively resolve the paradoxical effect of the concomitant presence of docosahexaenoic acid and beta amyloid (Pomponi et al. 2011).

3.2 Selective COX-2 Inhibitors

Therapy with COX inhibitors is associated with a number of side effects including gastrointestinal damage and renal and hepatic insufficiency. These adverse reactions are thought to be mostly dependent on COX-1 inhibition. In order to reduce adverse effects of nonselective NSAIDs, selective COX-2 inhibitors, such as celecoxib, have been developed. COX-2 inhibition became a mainstay therapy in treatment for RA, osteoarthritis, and other chronic inflammatory conditions (Croom and Siddiqui 2009; Suleyman et al. 2007).

However, selective COX-2 inhibitors have been shown to increase the risk for heart attack, thrombosis, and stroke by a relative increase in thromboxane, which contributes to platelet aggregation and vasoconstriction. Rofecoxib was taken off the market in 2004 because of an increase in the risk for cardiovascular events (Solomon et al. 2004). Also lumiracoxib and valdecoxib were recently withdrawn from the market. Although etoricoxib available in many countries in Europe, Latin America, the Asia-Pacific region, the Middle East, and North Africa, this medication was not permitted in USA, as was parecoxib. As of December 2011, only 1 COX-2 specific inhibitor namely Celebrex BRAND (celecoxib is the respective

genericum) is commercially available in the US market. Setakis et al. investigated the changes in the characteristics of patients prescribed selective COX-2 inhibitors after the 2004 withdrawal of rofecoxib. Patients receiving selective COX-2-inhibitors after September 2004 were younger and included more men compared with those receiving therapy before September 2004 (Setakis et al. 2008).

Although COX-2 inhibitors were developed to cause less gastrointestinal damage than nonselective NSAIDs, contradictory data in this field still exist. Use of celecoxib as compared to naproxen was associated with lower rates of gastric, duodenal, and gastroduodenal ulcers but had comparable efficacy in patients with arthritis (Goldstein et al. 2001). In the celecoxib group, gastroduodenal ulcers were not associated with gender in this double-blind, parallel-group, multicenter study of 537 patients with arthritis (Goldstein et al. 2001) (Table 2). In patients with arthritis, the most important risk factors for abdominal pain, dyspepsia, and/or nausea were nonspecific NSAID (naproxen, ibuprofen, or diclofenac sodium) use, female gender, and age <65 years. The treatment with valdecoxib, a COX-2-specific inhibitor, was associated with a decrease in dyspepsia and an improvement in upper gastrointestinal tolerability compared with those taking nonspecific NSAIDs over 12 weeks (Eisen et al. 2005). However, in the evaluation of 35,007 pairs of COX-2 inhibitor and nonselective NSAID users the risk of gastrointestinal bleed was not significantly different for COX-2 users compared with nonselective NSAID users (Stockl et al. 2005). In a cohort study of 2,061 patients with the first hospitalization for peptic ulcer perforation, the 30-day mortality was 25% overall, and 35% among current NSAID users. The mortality increase associated with the use of COX-2 inhibitors was similar to that of traditional NSAIDs (Thomsen et al. 2006).

Women are the greatest consumers of COX-2 inhibitors (over 85%) as stated by postmarketing studies (Dominick et al. 2003; Solomon et al. 2006, 2008). However, the design of clinical trials on efficacy seems to underrepresent them according to their prevalence of consumption (Cascales Perez et al. 2003; Chilet-Rosell et al. 2009). The Food and Drug Administration (FDA) Guideline for the Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs and the Sex, Gender and Pain Special Interest Group Consensus Working Group Report was used to analyze the data from the clinical trials on rofecoxib (Cascales Perez et al. 2003) and etoricoxib (Chilet-Rosell et al. 2009). A review of 28 rofecoxib clinical trails, which were published between 1999 and 2001, revealed that 80% of the trials did not describe efficacy results by sex, and only one reported side effects by sex. Only 8% of the clinical trials considered the influence of hormonal variation in the results. Pregnancy as exclusion criteria was only considered in 50% of the trials (Cascales Perez et al. 2003). A review of 58 etoricoxib trials determined that women formed 70% of a total of 49,835 subjects included in the etoricoxib trials, but only 31% of the subjects were in Phase I. About 85.7% of trials did not show sex-stratified data. About 90.6 and 93.3% did not provide efficacy and adverse effects data by sex, respectively (Chilet-Rosell et al. 2009). With regard to the safety of etoricoxib by sex, the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme detected greater risks of thrombosis in women, although differences were not statistically significant (Cannon et al. 2006).

The summary of product characteristics for etoricoxib approved by the European Medicines Agency describes the interaction of this drug with contraceptives and hormone replacement therapy. The clinical consequences for women remain unknown (Chilet-Rosell et al. 2009). The potential risk of etoricoxib in pregnant human subjects is also unknown, although a certain level of toxicity has been reported in pregnant animals (Chan 2004; Chilet-Rosell et al. 2009). Furthermore, the underrepresentation of women in clinical trials and the lack of sex stratification reflect the methodological problems of clinical research from a gender perspective.

Selective COX-2 inhibitors represented the class of NSAIDs less frequently reported as responsible of adverse reaction. There is a higher risk to find a positive challenge test to a nonselective COX-2 inhibitor than to a selective one in patients with previous adverse reactions to a nonselective COX-2 inhibitor. Females could have a higher risk compared to males to develop an adverse reaction to selective COX-2 inhibitors (Trombetta et al. 2009).

Cherney et al. examined the renal hemodynamic role of prostaglandins by assessing the response to COX-2 inhibition in young men and women with type 1 diabetes mellitus. COX-2 inhibition (200 mg celecoxib daily for 14 days) was associated with an increase in filtration fraction and renal vascular resistance and a decline in renal blood flow in women compared with men. Before COX-2 inhibition, women exhibited a decline in glomerular filtration rate in response to angiotensin II. COX-2 inhibition abolished this effect, whereas the response was not altered in men. Hemodynamic effects associated with COX-2 inhibition differed based on gender, and the results of Cherney et al. are consistent with experimental data suggesting augmented female prostanoid dependence (Cherney et al. 2008).

Nimesulide is a relatively COX-2-selective NSAID. The European pharmacovigilance database shows that nimesulide is associated with more cases of severe liver damage than other NSAIDs. Young women are particularly at risk. In early 2008, 17 cases of nimesulide-induced liver damage requiring transplantation had been reported in Ireland, Italy, Spain, Finland, and France. In the vast majority of cases of liver damage, the dose of nimesulide used was that recommended in the summary of product characteristics (2011).

Melnikova et al. investigated whether increased activity of COX-2 potentiates disease progression in a transgenic mouse model of Alzheimer's disease. Overexpression of COX-2 in trigenic COX-2/APPswe-PS1dE9 mice resulted in specific deficits in spatial working memory in female but not male mice. These sex-specific deficits were abolished by pharmacological inhibition of COX-2 activity. These findings suggest that pathological activation of COX-2 may potentiate the toxicity of amyloid beta peptides, particularly in females (Melnikova et al. 2006).

In conclusion, gender differences in patterns of NSAID use were shown in patients with immune-inflammatory diseases and in the general population. Women had a higher risk of inappropriate NSAID use than men. Further research is needed to examine reasons for these gender variations, as well as their impact on the quality of symptom management. Gender appears to predispose to some adverse drug reactions associated with the use of NSAIDs. Animal experiments suggest that the antineoplastic action of aspirin is gender dependent and should be considered in

designing gender-specific therapeutic applications of aspirin. Additional research is needed regarding the possible effects of duration, dose, and type of NSAIDs on cancer and whether effect modification by smoking status or sex exists. In experimental studies, COX-2-derived vasodilatory prostaglandins play a more prominent role in arterial vasoregulation in females. Important recommendations on the specific situation related to gender, outlined by FDA Guideline for the Study and Evaluation of Gender Differences in the Clinical Evaluation Drugs, have not been followed in clinical trials with selective COX-2 inhibitors until now. Sex-stratified data on efficacy and adverse effects are scarce in trials with selective COX-2 inhibitors.

4 TNF-α Antagonist Therapy

Ongoing progress in understanding the pathogenic mechanisms regulating different immuno-inflammatory diseases has led to the development of new drugs. Among these, TNF-α inhibitors, such as infliximab, adalimumab, golimumab, certolizumab pegol, and etanercept are now available for clinical use. Three of these antagonists are anti-TNF-α monoclonal antibodies, which are either chimeric (infliximab) or human (adalimumab, golimumab). The other two are certolizumab, a single, humanized anti-TNF-α Fab' conjugated with polyethylene glycol and without the Fc domain, and etanercept, a soluble TNF receptor, composed of a protein fusion between the extracellular domain of human TNF receptor2 and the Fc domain of a human immunoglobulin G1 (Demyanets et al. 2011; Desroches et al. 2010; Murdaca et al. 2011). In the last years, the number of patients with chronic inflammatory diseases being treated with TNF-α antagonist has increased dramatically (Poddubnyy and Rudwaleit 2011).

There is concern that women might be less likely to be treated aggressively for RA compared with men. A report from the Netherlands indicates a longer delay of referral of females to an early arthritis clinic compared with men (Lard et al. 2001). However, a recent study by Sokka et al. did not find significant differences in the proportion of females and males who were taking biologic agents in the QUEST-RA Study. Furthermore, the delay to initiation of therapies was similar for females and males within countries (Sokka et al. 2009). In a large US observational cohort including 1,545 patients there were no significant differences with respect to sex concerning initiation of biologic agents for the treatment of RA (DeWitt et al. 2009). The cohort enrolled in a British Society for Rheumatology Biologics Register includes 82% males (Lord et al. 2010). Mahic et al. retrieved data from the nationwide Norwegian Prescription Database for all individuals who were dispensed etanercept or adalimumab from pharmacies during 2005–2009. The 1-year prevalence for etanercept and adalimumab was 1.59‰ ($n = 3{,}840$) for men and 1.85‰ ($n = 4{,}483$) for women in 2009, an increase from 0.76‰ ($n = 1{,}752$) for men and 1.21‰ ($n = 2{,}830$) for women from 2005 (Mahic et al. 2011). Among 262 patients in the Arthritis and Biologicals in Children Register, which includes all Dutch juvenile idiopathic arthritis patients who used biologic agents, 185 (71%) were female (Otten et al. 2011).

Drug survival is considered a surrogate marker for effect. Ankylosing spondylitis patients in a study of Kristensen et al. have an excellent 2-year drug survival rate of 74%. Male sex and the presence of peripheral arthritis were found to be significant predictors of better anti-TNF-α therapy survival in ankylosing spondylitis patient (Kristensen et al. 2010). The finding that male sex entails a lower risk of premature TNF inhibitor withdrawal in ankylosing spondylitis is supported by the results from a Czech database (Pavelka et al. 2009), as well as from a large observational study of patients with various arthritic rheumatic diseases (Carmona and Gomez-Reino 2006). Survival of anti-TNF treatment was shown to be superior in ankylosing spondylitis and psoriatic arthritis patients compared with RA patients. Female sex and higher baseline disease activity were associated with a higher risk of treatment termination (Heiberg et al. 2008; Saad et al. 2009). The significant positive predictors of drug survival in patients with psoriasis vulgaris were male sex, the anti-TNF-α agent, and the previous response to the anti-TNF-α agent. The major predictors of poor drug survival were female sex, the type of anti-TNF-α agent, and a previous experience of inefficacy with one or more anti-TNF-α agent (s) (Gniadecki et al. 2011). The reasons for why women exhibit shorter times of treatment retention than men can only be a subject of speculation.

Quality of life and the physical consequences associated with ankylosing spondylitis have a direct relationship with a patient's ability to work. Adalimumab sustains improvements in work outcomes in patients with ankylosing spondylitis. Male sex was significantly and independently associated with working patients with ankylosing spondylitis (Maksymowych et al. 2010). In RA, several publications have indicated male sex as a predictor of better responsiveness, mainly a greater chance to achieve remission, to treatment with biologics (Atzeni et al. 2009; Forslind et al. 2007; Hyrich et al. 2006; Kvien et al. 2006; Yamanaka et al. 2007). It has been speculated that male patients could have greater benefit of anti-TNF blockers due to influence of these medications on the neuroendocrine axis including increased levels of anti-inflammatory androgens in the synovial tissue in males as compared with females (Cutolo et al. 2006). This, however, remains inconclusive and may partly be explained by how remission is defined in combination with the generally lower baseline disease activity seen in male RA patients (Kristensen et al. 2008). Thus, Kristensen et al. (2008) did not find any association between gender and response to anti-TNF treatment in the South Swedish Arthritis Treatment Group Register. The differences found might reflect genetic or environmental variations between RA patients in Sweden and other countries. The Arthritis and Biologicals in Children Register, which includes all Dutch juvenile idiopathic arthritis patients who used biologic agents, found that the achievement of a poor treatment response (less than 50% improvement from baseline or discontinuation earlier due to ineffectiveness or intolerance) 15 months after initiation of etanercept was associated with female sex and systemic juvenile idiopathic arthritis (Otten et al. 2011). Improvement in the Bath Ankylosing Spondylitis Disease Activity Index and the Bath Ankylosing Spondylitis Functional Index after 6 months of therapy in 261 patients enrolled in a British Society for Rheumatology Biologics Register was described. A strong association between gender and improvement in

function was found, with a significantly greater improvement in women (Lord et al. 2010). Treatment with TNF inhibitors was found to be associated with a reduced mortality in women but not men with RA from a regional register in Sweden (Jacobsson et al. 2007). This coincides well with the results that only female patients showed increases in plasma adiponectin levels after treatment with anti-TNF agents (Nagashima et al. 2008) as adiponectin exhibits antiatherogenic, antidiabetic, and anti-inflammatory effects (Mangge et al. 2010).

TNF-α antagonist therapy is not successful in all cases. Some patients suffer from either adverse events or intolerance (Desroches et al. 2010). Gender influences the half-life of TNF-α antagonists with shorter half-lives in women. It is suggested that the recommended doses of TNF-α antagonists, derived from rates of response in clinical studies, may not be optimal for every patient. An insufficient dose and serum concentration may expose patients to failure, whereas excessive concentrations may favor adverse events and infections (Desroches et al. 2010). Men have been shown to experience a greater number of adverse effects, particularly serious infections during biologic treatments (Burmester et al. 2007; Takeuchi et al. 2008) (Table 2). As was already mentioned, TNF inhibitors alter sex hormone metabolism in the synovial tissue (Cutolo et al. 2007). The beneficial effects include restored levels of synovial androgens although restored androgenic (immunosuppressive) activity may explain, in part, the higher likelihood of men to develop serious infections during biologic treatments (Burmester et al. 2007; Takeuchi et al. 2008).

The overall cancer risk in RA patients treated with anti-TNF-α seemed to be similar to that in the general population in a study in Italy, but the risk of hematological cancer was significantly greater. The demographic and clinical factors associated with a higher risk of cancer in this cohort were male gender and an age of >65 years (Pallavicini et al. 2010). TNF-α antagonist therapy in veterans with RA, derived from the Department of Veterans' Affairs national administrative database, may be associated with an increased risk of nonmelanoma skin cancer, compared with therapy with nonbiologic disease-modifying antirheumatic drugs. Risk factors for nonmelanoma skin cancer included male gender, older age, NSAID and glucocorticoid use, and a history of prior malignancies (Amari et al. 2011) (Table 2).

However, female gender was also shown to predispose to some adverse reactions associated with the treatment with TNF inhibitors (Mancini et al. 2010; Williams and Cohen 2011). Despite its rarity, infliximab-related hepatitis constitutes a challenging problem. In December 2004, a drug warning was issued by the FDA to alert healthcare professionals to the risk of hepatotoxicity in course of infliximab therapy. Infliximab may provoke both immunomediated and direct liver injury. Infliximab immunomediated liver dysfunction resembles that of autoimmune hepatitis type I with elevation of antinuclear, antismooth muscle, anti-double-strand deoxyribonucleic acid antibodies, and a clear preference for female sex (Mancini et al. 2010) (Table 2). Drug-induced lupus-like syndrome has been reported with the use of adalimumab, certolizumab pegol, etanercept, and infliximab. It occurs most often in women in the fifth decade of life. Onset of symptoms ranges from less than 1 month to more than 4 years (Williams and Cohen 2011) (Table 2).

Due to limited human pregnancy experience safety issues in regard to children exposed antenatally to biological drugs are still under debate. Case reports confirm that monoclonal antibodies expose the child to the full adult dose when administered in late pregnancy with a risk for adverse effects in the newborn. Differences in molecular structure of TNF-α inhibitors may turn out to favor the use of agents that are not complete monoclonal antibodies in women who consider pregnancy. At present use of biological agents throughout pregnancy cannot be recommended (Ostensen and Forger 2011).

5 Conclusions and Clinical Implications

The immune-inflammatory response is generally more pronounced in females than in males although the molecular mechanisms are still not completely understood. Unraveling these mechanisms may open up new avenues for gender-tailored therapies of inflammatory diseases. Different gender patterns of NSAID and TNF-α inhibitor use were reported in different countries and for different nosologies. Targets for the therapy such as severity of the symptoms, work disability rates, and probability to achieve the remission seem to be gender dependent. Similar treatment strategies, however, have been advocated for both genders. When initiating and evaluating treatment with anti-inflammatory agents such as NSAIDs and TNF-α blocking agents, the underlying dissimilarities between men and women should be kept in mind.

Take Home Messages

- Immune-inflammatory reactions are different between males and females.
- A prevalence of female patients among populations taking NSAIDs is reported, and NSAIDs are often involved in adverse drug reactions in the general populations. However, NSAID use is shown to be effective as a preventive therapy in cancer and is associated with positive effects on cognitive functions. A reduction in the incidence of lung cancer during NSAID use is strongest for men. The data about the use of NSAIDs as a preventive therapy for breast cancer are still controversial.
- Selective COX-2 inhibitors should be prescribed under the evaluation of patients' individual cardiovascular risk profile. Women are the greatest consumers of COX-2 inhibitors; however, they are underrepresented in clinical trials on COX inhibitors.
- Male gender is a predictor of better anti-TNF-α therapy survival in patients with ankylosing spondylitis, RA, and psoriasis vulgaris. However, men are more likely to develop serious infections during biologic treatments. Women are more predisposed to develop infliximab-mediated liver dysfunction and TNF-inhibitor-induced lupus-like syndrome.

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Treatment of Irritable Bowel Syndrome: Sex and Gender Specific Aspects

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Abstract Patients with functional gastrointestinal disorders constitute the majority of patients seeking healthcare for gastrointestinal symptoms in primary and secondary care. Of these disorders irritable bowel syndrome (IBS) is one of the most common and affects 10–20% in the Western world. IBS is a functional bowel disorder characterized by chronic abdominal pain, discomfort, bloating, and alteration of bowel habits in the absence of any detectable organic cause. Sex and gender aspects

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are important in understanding differences between men and women in their risk and experience of IBS. Relative to men, women are diagnosed more frequently with IBS. Female patients are more likely to be constipated, complain of abdominal distension and of certain extracolonic symptoms. Given the variability of IBS, the most successful treatment will be comprehensive, involving multiple strategies. Efficacy, safety and tolerability are important in the evaluation of IBS therapies, as patients are likely to require long-term treatment. Laxatives, antidiarrheals or antispasmodics are common in the treatment of IBS but the majority of patients receive antispasmodics followed by prokinetic agents. In treatment of IBS there appears to be a grater clinical response to serotonergic agents developed for IBS in women compared to men. There is an absence of drugs licensed specifically for the treatment of IBS. Further studies with novel agents are needed, to evaluate new approaches to IBS management including gender specific behavioral therapies and better characterization of patient subgroups with regard to drug therapy so that personalized therapy can be tested.

Keywords Antidepressants • Gender • Hormones • Irritabel bowel syndrom • Laxatives • Prostaglandins • Probiotics • Serotonin

Abbreviations

5-HT	Serotonin
$5\text{-HT}_{1/3/4}$	Serotonin receptor subtypes
IBS	Irritable bowel syndrome
IBS-A/M	-C, -D, mixed/alternating, constipation, diarrhea predominant IBS
$NK_{1/2/3}$	Neurokinin receptor subtypes
SERT	Serotonin transporter
SSRI	Serotonin reuptake inhibitor
TCA	Tricyclic antidepressants

1 Introduction

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by chronic abdominal pain, discomfort, bloating, and alteration of bowel habits in the absence of any detectable organic cause. In some cases, the symptoms are relieved by bowel movements. Diarrhea or constipation may predominate, or they may alternate. IBS may begin after an infection, a stressful life event, or onsets of maturity without any other medical indicators. Up to 60% of persons with IBS also have a psychological disorder, typically anxiety or depression (Talley 2006; Whitehead et al. 2002).

The clinical importance of IBS is increasing, and many gastroenterologists and basic scientists have begun to take an interest in this disorder, including sex and gender differences. To separate chronic conditions from transient gut symptoms, they must have started at least more than 6 months ago and have occurred on more than 3 days

a month during the last 3 months. Previous diagnostic criteria presumed the absence of a structural or biochemical disorder. However, research will likely confirm that functional gut disorders exhibit such findings. Moreover, IBS, functional bloating, functional constipation, and functional diarrhea may have multiple etiologies (Longstreth et al. 2006), and it is recognized that psychological, social, and biological factors can all play a part, although the impact of each of these factors is likely to be different in patients and may differ over time within the same patient (Payne 2004; Koloski et al. 2001).

Studies including patients with IBS which have explored gender differences have focused their investigations on prevalence and health seeking behavior, physical and psychological symptomatology, and abuse history (Toner and Akman 2000). Women reported functional gastrointestinal complaints two to three times more often than men, and increasingly with higher age (Hochstrasser and Angst 1996). With increased awareness of gender differences in perception and treatment of visceral pain, there has been new interest in research on gender differences with functional gastrointestinal disorders. Attention has mainly focused on IBS and dyspepsia, whereas gender differences in other gastrointestinal disorders are less well described (Ahlawat et al. 2006). However, because IBS does affect women and men, it is important to examine and to understand gender similarities and differences in the expression and treatment of this complex disorder.

2 Sex Differences in the Pathophysiology of IBS

IBS is a common functional bowel disorder characterized by severe pain, discomfort, changes in bowel consistency and movements, and bloating in the absence of any detectable organic cause. IBS is diagnosed by the presence of symptoms outlined the Rome III criteria and by exclusion of organic diseases. (Chang 2006; Drossman 2006; Layer et al. 2011). Throughout the world, about 10–20% of adults and adolescents have symptoms consistent with IBS, and most studies find a female predominance (Saito et al. 2002). In addition, IBS is most commonly diagnosed between the ages of 15–44 years. The incidence depends on used diagnostic criteria and varies between 5 and 22% in Western countries and 2–17% in Eastern countries (Kang 2005; Layer et al. 2011). More women than men have IBS in the United States, the United Kingdom, and Europe: Women outnumber men by $\geq$2:1 (Borum 1998). The extent of female overrepresentation in IBS, however, varies, which suggests that part of the differences between men and women is related to factors, such as perception of symptoms, interfering with daily life, differential access to healthcare or medical beliefs (Payne 2004). An additional explanation is the overrepresentation of women with IBS in clinical and epidemiological studies (Heitkemper and Jarrett 2008a).

According to the symptoms IBS can be classified into different subgroups: constipation predominated IBS (IBS-C), diarrhea predominated IBS (IBS-D), and IBS with mixed or alternating constipation and diarrhea (IBS-A/M) (Chang 2006; Drossman 2006).Women predominantly suffer from IBS-C while men develop IBS-D or IBS-A/M more often (Herman et al. 2010). Bloating, chronic functional

abdominal pain, and pelvic floor dysfunction can be seen more often in women than men (Adeyemo et al. 2010; Cain et al. 2009; Lee et al. 2001).

Within the past decade there has been increasing evidence supporting the concept that IBS is a multi-symptom disorder of brain-gut function in which emotional and cognitive areas of the brain modulate the symptoms (Toner 2005). A bi-directional interaction along the brain-gut axis could explain that social and psychological stressors and associated alterations in mood alter the function of the gut and IBS symptoms (Levy et al. 2006). The sympathetic and parasympathetic pathways seem to be altered in IBS. Several studies revealed an over-activation of the sympathetic nervous system possibly due to different causes. Patients with IBS-D showed adrenergic abnormalities, while IBS-C seems to be linked with parasympathetic dysfunctions. Furthermore, there are some gender differences. An increased sympathetic activation and a decreased parasympathetic activation could be shown in men compared to women (Heitkemper et al. 2001; Layer et al. 2011; Tillisch et al. 2005). The altered autonomic nervous system may be reasonable for the reported influence of stress on symptom severity (Heitkemper and Jarrett 2008a; Chang 2011).

Another reason for the hypersensitivity may be an immune balance disturbance and inflammation in the mucosa. Several studies revealed an increased number of mast cells, often located near nerve fibers and enhanced release of mast cell mediators, especially histamine and proteases, in patients with IBS. These conditions are proposed to correlate with visceral pain intensity and frequency. Although, an increased pro-inflammatory cytokine profile could be revealed, cytokine profile as well as visceral hypersensitivity seems to be linked to bacterial infections (Spiller et al. 2007; Barbara et al. 2004; Cremon et al. 2009; Park et al. 2006). IBS often develops after a gastrointestinal infection (post-infective IBS). Especially women have a higher risk, but also a genetic predisposition is discussed as a cause as shown in twin and family studies (Spiller et al. 2007; Heitkemper and Jarret 2008a; Clark and DeLegge 2008).

Additionally, compared with men, women suffering from IBS show a higher comorbidity with other diseases like somatization disorders and other functional syndromes like functional dyspepsia, fibromyalgia, and chronic-fatigue-syndrome (Ouyang and Wrzos 2006; Sainsbury and Ford 2011). There is also a high prevalence of psychological diseases like depression, anxiety, and panic attacks in women with IBS (Garakani et al. 2003; Lydiard et al. 1993; Mayer et al. 2004) (Table 1).

In general, the impact of both, sex or biological factors and gender, or socially structured factors, on development and maintenance of IBS is well documented and now well accepted. Sex differences in visceral perception, cardio-autonomic responses, gastrointestinal motility, and brain activation patterns to visceral stimuli may exist in IBS patients (Chang et al. 2006). Gender differences in social factors, psychological symptoms, and response to psychological treatments have been also studied (Chang et al. 2006). However, it is known that most differences between males and females are a function of the interaction between biology and environment.

Table 1 Comorbid extraintestinal pain conditions associated with IBS (adapted from Heitkemper and Jarrett 2008a, b)

Affect women more than men
Interstitial cystitis
Dyspareunia
Chronic pelvic pain
Migraine headache with aura
Temporomandibular joint disorder
Fibromyalgia
Depression or anxiety
Affect women with IBS more than women without IBS
Premenstrual syndrome or premenstrual dysphoric disorder
Dysmenorrhea

Table 2 The effect of sex and age on the prevalence of functional gastrointestinal disorders (adapted from Chang et al. 2006)

Functional gastrointestinal disorders	Effect of sex	Change with age
Esophageal		
Globus	F > M	↓
Rumination	F = M	↓
Functional chest pain	F = M	↓
Functional heartburn	F = M	=
Dysphagia	F > M	↑
Gastroduodenal		
Dyspepsia	F = M	↓
Aerophagia	M > F	↓
Functional vomiting	F = M	↓
Biliary tract	F	↑
Lower gastrointestinal tract		
IBS	F	↓
Functional constipation	F	↑
Functional diarrhea	M > F	↓
Functional bloating	Discordant	Discordant
Chronic functional abdominal pain	F > M	↓
Fecal incontinence	F > M (at home) M > F (nursing homes)	↑
Functional anorectal pain	F > M	↓
Outlet delay	F	

F female, *M* male

3 IBS and Sex Hormones

Meta-analysis of Adeyemo et al. (2010) reveals that women more frequently report individual IBS symptoms than men (Table 2).

These findings are consistent with studies which showed an enhanced perception of pain or discomfort to distension in the colon and rectum in women vs. men. Women in

generally have a slower colonic transit than men. There are two sex-linked factors related to understanding why IBS differs in symptoms between women and men. The first is that women and men have physiologic differences for example in gastrointestinal motility, pain perception or central nervous system responses. The second is the reproductive system, particularly hormonal factors. Variation in intestinal motility is gender specific and seems to be regulated by hormones.

A systematic review found that IBS symptoms are heightened at time of menses. In female patients with IBS gastrointestinal symptoms are elevated across all menstrual phases compared to healthy women (Heitkemper and Jarrett 2008b; Heitkemper et al. 2003; Houghton et al. 2002). Enhanced visceral perception at menses is supported by the finding of decreased sensory thresholds to rectal distension compared with other phases of the menstrual cycle (Houghton et al. 2002). At late luteal phase or early state of menses often a shift from increased constipation to increased diarrhea was reported marked by an increased stool frequency, looser stools, and bloating. One plausible mechanism that is supported by some animal and human studies is that declining or low ovarian hormone levels at time of menses may underlie the increased gastrointestinal symptoms and discomfort across the menstrual cycle. Laessle et al. (1990) showed that progesterone levels negatively correlated with pain-related symptoms. This supports the above-mentioned clinical observations that lower levels of female sex hormones are associated with greater somatic pain. It is conceivable that women have more prevalent and severe IBS symptoms at time of menses when the hormones decline from high to low levels. There are other possible mechanisms involved in gastrointestinal function to explain these findings. Patients with IBS are more sensitive to a rectally placed bloated balloon (Clark and DeLegge 2008; Gunnarsson and Simrén 2009; Mayer et al. 2004). This enhance in visceral sensibility is possibly caused by alterations in central nervous system progressing of visceral input or descending inhibitory effects (Clark and DeLegge 2008; Heitkemper and Jarret 2008a; Tillisch et al. 2005). Interestingly, several studies revealed an activation of different brain areas after rectal distension in patients with IBS compared to controls. The brain activation differs between genders. Women with IBS showed activation predominantly of limbic and paralimbic regions including the amygdala, the anterior cingulate cortex, and the cingulate cortex after rectal distension. In contrary, in men the lateral prefrontal cortex, the dorsal anterior cingulate cortex, the dorsal pons, and the periaqueductal gray are activated (Labus et al. 2008; Mayer et al. 2006; Naliboff et al. 2003).

Female sex hormones seem to influence visceral sensibility. Rectal perception in female patients with IBS is enhanced compared to men and the colonic visceral threshold in women is significantly lower at menses. The reason could be that the rectal sensitivity is affected by ovarian steroids in women with IBS. This effect could not be detected in healthy female persons (Chang et al. 2006; Labus et al. 2008). The influence of female sex hormones on visceral sensitivity and pain processing was also demonstrated in animal studies. In ovariectomized rats which show a lower visceral sensitivity, decreased neuronal responses, and a reduced

primary afferent excitability during colorectal distension, an increase of symptoms was elicited by systematic administration of estradiol. One region in the central nervous system involved in these processes seems to be the amygdala. Female sex hormones placed to the amygdala nuclei in ovariectomized female or male rats increased visceral pain behavior (Ji et al. 2003; Myers et al. 2011).

4 IBS and Gender

As mentioned, IBS has a substantial impact on patient's quality of life. Psychological factors are significantly associated with health-related quality of life in patients with IBS in primary care. Important factors in gendered explanations involved stress and poor metal health (Payne 2004). A number of researchers have attempted to explain how gender might influence IBS, and the nature of these explanations illustrates the difficulty in disentangling gender as discrete factor in the etiology of IBS. Chang and co-workers published in 2006 a systematic review about gender and psychological factors (Chang et al. 2006) indicating that relatively few studies have examined gender differences in psychological symptoms (Table 3).

Two studies including a large number of patients did not find differences between men and women in patients seeking psychological treatment for IBS using a psychological self-report measure or a diagnostic interview (Blewett et al. 1996; Lee et al. 2001). Two other studies with few patients have reported differences between men and women with IBS (Corney and Stanton 1990; Fock et al. 2001) with women reporting higher rates of psychological distress than men. Additionally, a Scandinavian group evaluated the quality of life of male and female IBS patients. The results of this study indicated that quality of life was reduced in hospital outpatients compared to primary care patients, but only in females. Moreover the authors reported that women had more fatigue and depression and less positive well-being and self-control compared with men with IBS (Simrén et al. 2001). In general, there are relatively few studies examining gender aspects in psychological symptoms. It could not be excluded that the differences between men and women represent differences in the general population in relation to psychological symptoms, rather than to specific IBS phenomenon (Chang et al. 2006).

Another important factor that impacts on IBS is stress. Stress has been described as an important trigger in the onset or worsening of IBS symptoms as well as in the decision to seek consultants (Blanchard et al. 1990; Koloski et al. 2001). Interestingly, apart from a study created in 1982 in which prolonged exposure to chronic or threatening life stress was described as highly predictive of symptom intensity and clinical outcome in IBS (Drossman et al. 1982) there are no systematical studies to date that have assessed gender differences in stress related to IBS.

Table 3 Studies in functional gastrointestinal disorder reporting gender differences on psychological measures (adapted from Chang 2006)

Study	Number (% women)	Criteria	Findings
Corney and Stanton (1990)	42 (74%)	IBS—Abdominal pain and alteration in bowel habit for 6 month	Women reported more psychological distress and were more likely to have psychiatric diagnosis than men
Blewett et al. (1996)	76 (66%)	Manning criteria	No difference in prevalence of psychiatric disorder between men and women
Simrén et al. (2001)	343 (73%)	IBS—Rome I Women	Women > men for fatigue, depression, and anxiety, differences more marked for outpatients than primary care
Fock et al. (2001)	43 (63%)	Manning criteria	62% of women had psychiatric diagnoses compared to 17% of women with organic gastrointestinal illness, but no difference between men with IBS or organic disease
Lee et al. (2001)	714 (67%)	IBS—Rome I	No difference in psychological symptoms
Blanchard et al. (2001)	341 (70%)	IBS—Rome I (retrospective)	Women were more depressed and showed greater trait anxiety than men. No difference on state anxiety or percent of patients with an Axis I psychiatric disorder
Westbrook et al. (2002)	748 (54%)	Dyspepsia—Rome I	Women had poorer physical and mental well-being than men

5 Treatment of IBS

A good patient–physician relationship is essential for the treatment of IBS. Effective patient–physician relationships have been associated with improved health status and increased efficiency of care. In order to establish an effective and interactive relationship with patients, healthcare providers should obtain a medical history by a patient-centered interview, conduct a cost-efficient investigation, including a physical examination, provide a clear explanation of IBS and the patient's symptoms that takes into consideration the patient's beliefs, address the patient's expectations, involve the patient in the treatment, and establish a long-term relationship with the patient (Drossman 2006; Adeyemo and Chang 2008).

Management of IBS is complex and depends on a confident diagnosis, explanation of why symptoms occur, and suggestions for coping with them. Education about healthy lifestyle behavior, reassurance that the symptoms are not due to

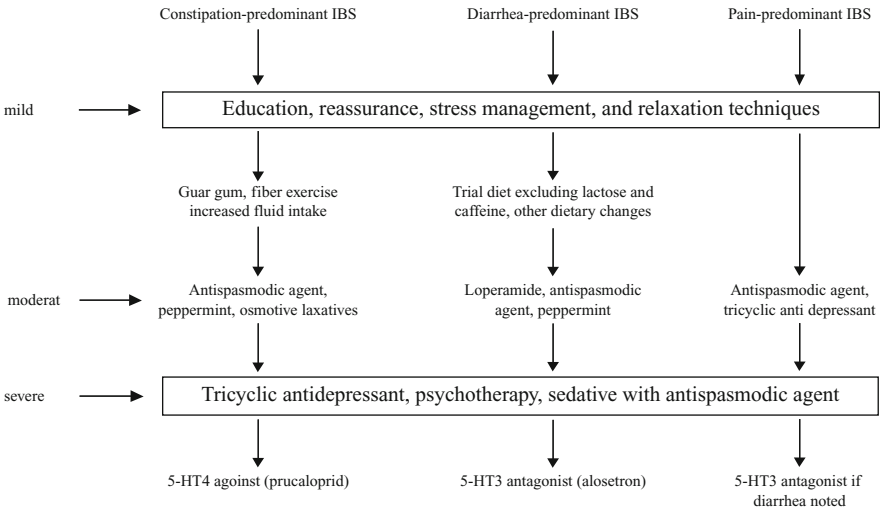

Fig. 1 Algorithm for the management of patients with IBS. (5-HT3 = serotonin receptor subtype 5-hydroxytryptamine-3; 5-HT4 = serotonin receptor subtype 5-hydroxytryptamine-4 (Adapted from Mertz 2003)

cancer and establishment of a therapeutic relationship are essential. Combined pharmacological and non-pharmacological strategies relief discomfort and enhance quality of life.

Because of the manifold causes and occurrences of IBS, traditional treatment is carried out according to symptomatology, focusing on relaxing the smooth muscles. Nevertheless, there has recently been a shift in the treatment of patients evolving into attempts to alter gut transit and to modulate the perception of visceral afferent information in the central nervous system. Various pharmacological agents have been tried in the management of IBS, but these have proved of limited efficacy for the cardinal symptoms of abdominal pain and bloating. Additionally, the placebo response of up to 40–50% in IBS trials confounds interpretation of many drug studies (Spiller 1999; Enck and Klosterhalfen 2005; Pitz et al. 2005). Another important fact for evaluation of the therapies in IBS is a significant lack of data on safety and tolerability of the therapies currently used routinely to treat IBS. In 2002, the American College of Gastroenterology published a systematic review on the management of IBS in North America, which includes efficacy and safety data from randomized controlled trials (American college of Gastroenterology Functional Gastrointestinal Disorders Task Force 2002; Brandt et al. 2002). Two comparable systematic reviews with European perspectives in safety and tolerability (Heading et al. 2006) as well as efficacy of treatments (Tack et al. 2006) were published in 2006.

According to the "Guidelines on the irritable bowel syndrome: mechanisms and practical management" (Spiller et al. 2007) and the "German consensus Guidelines on Definition, Pathophysiology and Management of IBS" (Layer et al. 2011) mainly five drug categories were proposed for the treatment of IBS (Fig. 1).

Additionally, some novel drugs of different chemical structures were integrated in the therapy of IBS during the last years. The guidelines did not differentiate between therapy strategies for men and women. Drug therapy is directed toward the dominant symptoms. Their changeable nature and the complex interaction between central and enteric nervous system influence the effectiveness of the therapy (Longstreth et al. 2006). It is notable to emphasize that drugs relieve only some symptoms in selected patients.

5.1 Laxatives

Laxatives are widely used to relieve constipation in patients with IBS-C or IBS-A/M. Three classes of laxatives are available, bulking agents, osmotic laxatives, and stimulant laxatives. Fiber supplementation with naturally derived concentrated non-starch polysaccharides such as bran, ispaghula husk, methylcellulose, and sterculia increases fecal mass and may accelerate transit. Especially, soluble fibers like psyllium or ispaghula husks are more effective and tolerated than treatment with insoluble fibers (e.g., wheat bran) which is often accompanied by bloating and additional abdominal pain. Fibers may also be effective in the treatment of diarrhea or pain associated with IBS (Bijkerk et al. 2009; Shen and Nahas 2009). Polyethylene glycol is a good alternative in case of bloating during fiber treatment. Daily therapy with polyethylene glycol laxative for 14 days resulted in a significant improvement in bowel movement frequency in constipated patients relative to placebo by the second week of treatment (Cleveland et al. 2001). Fibers as well as polyethylene glycol can be used in long-term treatment. Other osmotic or stimulant laxatives could be effective in the treatment of constipation but should be avoided because of gastrointestinal side effects like bloating and cramping. Although widely used in clinical practice, there are no clinical trials in which laxatives or bulking agents are beneficial used in the treatment of IBS (Quartero et al. 2005; Adeyemo and Chang 2008; Layer et al. 2011). In general, they do not help to relieve other IBS symptoms than constipation. In fact, they may actually worsen other symptoms. Side effects include diarrhea, dehydration, and bloating. They may offer limited relief and can be harmful if taken regularly for weeks.

5.2 Antispasmodic Agents

Beside fiber intake to regulate defecation another traditional strategy includes the use of antispasmodic agents. Agents like mebeverine, hyoscine, otilonium bromide or peppermint oil inhibit intestinal smooth muscle contractions and relieve pain via antimuscarinic or direct effects (Adeyemo and Chang 2008). One meta-analysis of 23 trials found an advantage over placebo in global improvement, pain, and abdominal distension, but no difference regarding constipation. However, the

studies involved in meta-analysis were generally of poor quality (Poynard et al. 2001). A recently published large randomized controlled clinical trail including 71.4% females reported the efficacy of antispasmodics alverine citrate and simeticone combination which has been used for almost 20 years in IBS. The combination was significantly more effective than placebo in relieving abdominal pain/discomfort in patients with IBS. The duration of follow-up was only 4 weeks (Wittmann et al. 2010). Constipation and the anticholinergic effects of antispasmodics limit their use, especially in IBS-C and IBS-A/M patients during long-term treatment. These results emphasize the need for more effective therapies for the long-term treatment of IBS.

5.3 *Prostaglandin Derivatives*

Lubiprostone is approved for the treatment of chronic constipation and for IBS-C in the USA and Switzerland (Layer et al. 2010; Lunsford and Harris 2010). It is a bicyclic fatty acid compound obtained from a prostaglandin E1 metabolite that activates chloride channels type-2. Chloride channels type-2 expressed throughout the gastrointestinal tract are involved in fluid transport and secretion. Activation of chloride channels type-2 in epithelial cells stimulates intestinal fluid secretion into the intestinal lumen, which loosens stool consistency and increases motility and frequency of defecation. Furthermore, chloride channels type-2 activation seems to play a role in repairing tight junctions that are often disturbed in patients with IBS (Lacy and Chey 2009; Lunsford and Harris 2010; Shekhar and Whorwell 2009). Two Phase III studies revealed a significant improvement of IBS symptoms in women treated with lubiprostone compared to placebo. No conclusion could be made about the efficacy of lubiprostone in men because there were only a small number of men involved in the two studies. Lubiprostone in general seems to be well tolerated with lower prevalence of side effects like nausea and diarrhea in patients with IBS-C (Drossman et al. 2008).

5.4 *Antidiarrheal Agents*

Loperamide, a synthetic piperidine derivative, is an opioid drug and acts on μ-opioid receptors in the myenteric plexus of the large intestine. It is used for the treatment of diarrhea resulting from gastroenteritis or IBS-A/M. It slows intestinal transit, increases intestinal water absorption, and increases resting sphincter tone. Constipation is the most common side effect (Adeyemo and Chang 2008). Loperamide was shown in clinical trials to be effective in decreasing stool frequency and improving stool consistency, although it provided no significant improvement in other IBS symptoms compared with placebo (Efskind et al. 1996). Furthermore, there is no evidence that loperamide improves abdominal pain or discomfort. Because loperamide does not cross the blood–brain barrier,

side effects are less than of other opioids. Nevertheless, loperamide has been shown to cause a mild physical dependence during preclinical studies, specifically in mice, rats, and rhesus monkeys. Symptoms of mild opiate withdrawal have been observed following abrupt discontinuation of long-term therapy with loperamide (Yanagita et al. 1979). Therefore, it should be used with caution. The treatment with loperamide is not recommended in patients with IBS-C since the drug could exacerbate the symptoms of constipation. As well, the enkephaliase inhibitor racecadotril (acetorphan) which is an efffective and safe treatment for acute diarrhoea in adults and children is not recommendet for IBS patientw (Layer et al. 2011)

5.5 Serotonergic Agents

Serotonin (5-HT) is a critical signaling molecule in the gut. 5-HT released from enterochromaffin cells initiates peristaltic, secretory, vasodilatory, vagal, and nociceptive reflexes via activation of seven 5-HT receptors with 5-HT_1, 5-HT_3, and 5-HT_4 subtypes as the most important (Spiller 2011; Shekhar and Whorwell 2009). Mucosal 5-HT, tryptophan hydroxylase 1 messenger RNA, serotonin transporter (SERT) messenger RNA, and SERT immunoreactivity were all significantly reduced in IBS-C and IBS-D (Coates et al. 2004). Several specific ligands were developed for the treatment of IBS. Especially, 5-HT_4 agonists like cisapride and tegaserod were effective in the treatment of motility disorders, but only tegaserod showed significant efficacy in the treatment of chronic constipation or IBS-C. At the doses used, tegaserod was proved less effective in men (Müller-Lissner et al. 2001). Both drugs were withdrawn from the market because of cardiovascular side effects mediated by interaction with 5-$HT_{1B/D}$ and the hERG potassium channel receptors (Camilleri 2010; Shekhar and Whorwell 2009). Tegaserod still can be received from the FDA in case of emergency.

Prucalopride, a first in class dihydrobenzofurancarboxamide, is a selective, high affinity serotonin (5-HT_4) receptor agonist with enterokinetic activities. It improves gastric emptying, bowel frequency, stool consistency, and overall symptoms of constipation. Prucalopride differs from other 5-HT_4 agonists such as tegaserod and cisapride. It does not interact with 5-$HT_{1B/D}$ and hERG potassium channel receptors (De Maeyer et al. 2008). Clinical trials evaluating the effect of prucalopride on QT interval and related adverse events have not demonstrated significant differences compared with placebo. Headache, abdominal pain, and diarrhea were the common side effects. It is approved for the treatment of chronic constipation and opioid-induced constipation in females. Because of the insufficient number of men in clinical trials there is no clear evidence for the treatment of male patients. Prucalopride has not been tested in IBS so far, but because of its benefits in the treatment of constipation prucalopride seems to be applicable to patients with IBS-C (Adeyemo and Chang 2008; Sainsbury and Ford 2011; Shekhar and Whorwell 2009).

Since stimulation of 5-HT_3 receptors is positively correlated with gastrointestinal motility, 5-HT_3 antagonists slow the movement of fecal matter through the large intestine, increase the extent to which water is absorbed, and decrease the moisture and volume of the remaining waste products. Several studies revealed an improvement in abdominal pain, abnormal bowel habits, and overall IBS symptoms for 5-HT_3 antagonists (Spiller 2011). As a result, 5-HT_3 receptor antagonists such as alosetron have been tested in IBS-D patients (Delvaux et al. 1998; Houghton et al. 2000). Alosetron acts as an antagonist on 5-HT_3 receptors in the enteric nervous system and was effective in IBS (Ford et al. 2009). While being a 5-HT_3 antagonist like ondansetron, it was not classified or approved as an antiemetic. In 2000 alosetron was withdrawn from the market because of the risk of severe gastrointestinal side effects like ischemic colitis. Due to inconsistent data for male and the side effects alosetron was reintroduced 2002 under a risk management plan for the treatment of severe IBS-D in women who are less than 55 years old (Adeyemo and Chang 2008; Shekhar and Whorwell 2009). Another promising 5-HT_3 antagonist is ramosetron. It is approved for the treatment of nausea and vomiting in Japan and South-East Asia. Ramosetron significantly suppressed restraint stress-induced decrease in colonic pain threshold, an effect not observed with loperamide. Therefore it seems that ramosetron is also indicated for a treatment of patients with IBS-D symptoms (Hirata et al. 2007).

The question arises why women are more responsive than men to serotonergic agents developed for IBS. Two possible mechanisms are discussed in the literature. First, the enterochromaffin cells in which approximately 90% of the 5-HT is found in the gastrointestinal tract are modulated by estrogen, progesterone or testosterone. This hypothesis is speculative and needs additional research (Heitkemper et al. 2003) but is supported by findings that fluctuations in estrogen levels over the lifespan and during ovarian cycles cause predictable changes in 5-HT systems in female mammals (Rybaczyk et al. 2005). Secondly, enterocytes terminate the effects of 5-HT by removing it from the interstitial space by activation of the re-uptake SERT. Thus, changes in 5-HT content and release, expression of 5-HT receptors or changes in SERT expression/activity may contribute to motor function in IBS (Coates et al. 2004). Data suggest that there may be a genetic component in IBS including polymorphisms of genes controlling down-regulation of SERT. It is hypothesized that the genetic polymorphisms at the SERT promoter influence response to a 5-HT_3 antagonist in IBS-D and may influence benefit–risk ratio with this class of compounds (Camilleri et al. 2002).

5.6 Antidepressants

Antidepressants are purported to be beneficial and are often used in patients with chronic refractory symptoms. Indeed, these drugs are particularly helpful for patients with comorbid depressive and anxiety disorders. Especially in female patients, depression, anxiety, and pain are very common. These people have what is called visceral hypersensitivity resulting in symptoms with even the slightest

disturbance. The two main groups of antidepressants used for IBS are tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). Studies have shown that TCAs are much better than placebo at relieving abdominal discomfort. Interestingly, the most benefit was observed in patients with IBS-D without severe symptoms and no psychiatric disorder. The medication is started at low doses and then slowly increased if there has been no response (Layer et al. 2011). Amitriptyline reduced pain-related cerebral activation in females with IBS and was found to be helpful in people who had disturbed sleep patterns (Adeyemo and Chang 2008; Quartero et al. 2005). Desipramine is sometimes used for IBS-C. A randomized, controlled study has shown that this drug improves pain in women with IBS who can tolerate the drug (Drossman et al. 2003). SSRIs (e.g., paroxetin) enhance extra-cellular serotonin centrally and peripherally and affects pain and gut motility. It may be useful in the treatment of IBS-C (Adeyemo and Chang 2008; Sainsbury and Ford 2011). Nevertheless, antidepressants should only be prescribed in the treatment of IBS, if there is any evidence of an associated psychological disorder or severe pain. Common side effects are dizziness and drowsiness. Additionally, TCAs may cause constipation and therefore, they should be used with caution in patients with IBS-C (Heading et al. 2006).

5.7 Probiotics and Other Pharmacological Therapies

There are evidences that changes within the intestinal flora are associated with IBS. Patients with IBS show a great homogeneity in the fecal flora with a decrease in coliforms, lactobacilli, and bifidobacteria compared to healthy individuals (Balsari et al. 1982). Up to now it is unclear which role, if any, antibiotics containing single or mixed cultures of living microbes might play in treating IBS. In some clinical trials probiotics reduced pain and bloating by altering gut flora, enhanced gut barrier function, and reduced mucosal permeability. Additionally, a reduction of visceral hypersensitivity and immunomodulatory effects could be shown. Most potent effects were seen for bifidobacteria (e.g. B. infantis, B. animalis) or lactobacilla (Camilleri 2006; McFarland and Dublin 2008; Moayyedi et al. 2010; Spiller 2008). Other studies did not demonstrate a significant difference in IBS symptoms (Bausserman and Michail 2005; Drouault-Holowacz et al. 2008).

There is a variety of other agents with reported advantages in treating IBS symptoms. Different plant extracts can ameliorate bloating, pain as well as motility disturbances. There are several studies suggesting the benefit of traditional Chinese medicine as well as the combinations STW 5 (Iberogast®) and Padma Lax in the treatment of IBS. Although, studies are very heterogeneous, herbal medicine seems to be a good alternative in IBS-D (Wu 2010; Shen and Nahas 2009; Liu et al. 2006). There is growing evidence that STW 5, a fixed combination of hydroethanolic herbal extracts, besides being effective in functional dyspepsia, also improves symptoms in IBS. Studies in experimental models demonstrated a broad action profile of STW 5 including modulation of muscle activity (Michael et al. 2009),

radical scavenging (Schempp et al. 2006), anti-inflammatory (Michael et al. 2009), and antioxidative properties (Germann et al. 2006). As for other multi-herbal drugs, the concept of multi-targeting may improve treatment options for IBS. This is supported by clinical studies, which demonstrated that STW 5 improved the overall symptoms score in IBS patients (Madisch et al. 2004; Liu et al. 2006).

The non-absorbable broad-spectrum antibiotic rifaximin can be helpful in the treatment of bloating and meteorism in IBS. In a study of more than 600 patients who received medication or a placebo, 40.7% taking rifaximin reported relief of IBS symptoms that endured for weeks after the medication was stopped (Pimentel et al. 2011). Rifaximin is well tolerated in several doses but should only be applied in case of refractory IBS symptomatology (Adeyemo and Chang 2008; Layer et al. 2011).

In general, most of these studies were not evidence-based and did not use the Rome criteria for selection of patients.

5.8 New Drugs for IBS

The treatment of IBS is an area of ongoing research. As the pathophysiology of IBS is better understood, compounds with novel mechanisms of action are increasingly seen in clinical development. To investigate the efficacy of new therapeutic approaches for the treatment of IBS studies were performed mainly with women, because of the high number of female patients seeking for healthcare. However, several novel drugs aimed at normalizing bowel movements have produced clinical effects, not only on the primary target, but also on pain and discomfort.

Different receptor systems influencing intestinal motility, secretion, and sensitivity represent possible targets for further therapy opportunities in IBS. Several novel substances were developed, whose efficacies are examined in different clinical studies. For example, similar to prucalopride two highly selective 5-HT_4 receptor agonists, naronapride and velusetrag, showed significant improvement in bowel movements in humans with low risk of cardiovascular side effects in clinical trials phase II. Both seem to be promising substances for the treatment of IBS-C, although there are only data for chronic constipation (Camilleri 2010; Shekhar and Whorwell 2009). Ramosetron, a 5-HT_3 receptor antagonist, is currently in trials for IBS-D in Europe. The partial 5-HT_3 receptor agonist pumosetrag offers prokinetic properties. It enhances intestinal motility and delays gastric emptying. Therefore, pumosetrag is examined in clinical trials phase II for patients with IBS-C and gastroesophageal reflux disease (Spiller 2011; Shekhar and Whorwell 2009). Because of enhanced serotonin levels in patients with IBS-D and the risk of ischemic colitis during 5-HT_3 treatment, an orally available tryptophan hydroxylase inhibitor, LX1031, was developed. LX1031 was well tolerated in phase II studies and improved intestinal transit and stool consistency in non-constipated patients with IBS (Camilleri 2010; Brown et al. 2011).

Renzapride and E-3620, compounds with combined 5-HT_4 agonist and 5-HT_3 antagonist activity, are in late-stage of clinical trials for IBS-C treatment and

DDP225, a single small molecule with combined noradrenaline reuptake inhibition and 5-HT_3 antagonist properties, is currently in Phase IIa trials for IBS-C and IBS-D, respectively (Ashburn and Gupta 2006).

Crofelemer is a proanthocyanidin from the bark latex of Croton lecheri undergoing investigations in clinical studies phase III. It acts as an antagonist at the cystic fibrosis trans-membrane regulator chloride channel in the apical intestinal membrane. Thereby, crofelemer reduces fluid secretion and should improve secretory diarrhea. There are conflicting results pointing out that crofelemer does not influence stool frequency and consistency, but increases pain- and discomfort-free days in female patients with IBS-D (Mangel and Chaturvedi 2008, http://www.napopharma.com).

Other potential treatment options in clinical development currently include opioid modulators, chloride channel modulators, and neurokinin antagonists.

Asimadoline is a potent high selective k-opioid receptor agonist which is tested in phase III trials for relief of pain in IBS. It is mainly acting peripherally with a low permeability across the blood brain barrier. In several animal studies asimadoline activated ion channels and influenced secretional processes. In humans asimadoline was well tolerated and effective in reducing moderate pain in patients with IBS-D or IBS-A/M in low doses of 0.5 or 1 mg per day. Higher doses showed no significant benefit on pain relief but more side effects like nausea and vomiting (Sainsbury and Ford 2011; Shekhar and Whorwell 2009; Mangel et al. 2008).

Guanylate cyclase type-C receptors are enriched in intestinal epithelium. Activation of the guanylate cyclase type-C receptors leads to an increase in cyclic guanosine monophosphate that induces a stimulation of chloride and bicarbonate secretion via different signaling pathways stimulating cystic fibrosis trans-membrane regulator channel-dependent mechanisms. Linaclotide, a 14 amino acid peptide, acts as an agonist on guanylate cyclase type-C receptors and improves bowel transit time, stool consistency, and frequency. In clinical trials phase II and III most common side effect was diarrhea. Linaclotide is a promising compound for the treatment of chronic constipation and IBS-C in both genders (Camilleri 2011; Bharucha and Linden 2010; Shekhar and Whorwell 2009).

Sodium-hydrogen exchanger is important for intracellular pH regulation and Na^+ homeostasis. The isoform 3 is expressed at the apical intestinal membrane and is necessary for sodium chloride reabsorption followed by water influx into the gut (D'Souza et al. 1998). RDX5791 is a minimally systemic sodium-hydrogen exchanger 3 antagonist which increases intestinal sodium that leads to an increase of intestinal fluid and transit. In animal studies RDX5791 was effective to influence stool consistency and bowel transit. A clinical study phase I is performed to examine safety and efficacy of RDX5791 in humans for the treatment of IBS-C (http://www.clincicaltrials.gov, neurourogastro.typepad.com).

There are three types of neurokinin receptors, NK_1, NK_2 and NK_3. Depending on the receptor type, distribution is on sensory neurons in the central or enteric nervous system. For all three receptor subtypes antagonist drugs are under investigation. The drug which has undergone the most study is the NK_3 antagonist talnetant. Talnetant is a selective, orally active NK_3 antagonist that is chemically derived from 4-quinolinecarboxamide. It is currently studied for the treatment of various conditions, including IBS. Although NK_3 receptors are known to exist in the spinal

cord, these receptors also exist peripherally in the intestinal tract and may mediate intestinal motility and inhibit intestinal nociception. Because of these actions, talnetant and similar agents are currently investigated as therapeutic agents for IBS (Houghton et al. 2007).

Nepadutant, a glycosylated bicyclic peptide, is a NK_2 receptor antagonist (Catalioto et al. 1998). In healthy volunteers nepadutant reduced contraction frequency and amplitude in the small intestine (Camilleri 2011). In first clinical trials phase I nepadutant seems to be safe and well tolerated in the treatment of children with colic or functional gastrointestinal disorders. Phase II studies for the efficacy of nepadutant in the treatment of infantile colic are performed at the moment (http://www.clinicaltrials.gov).

Ibodutant, a small, non-peptide molecule, is a selective NK_2 receptor antagonist. Ibodutant applied orally or intravenously prevented alterations in intestinal motility mediated by NK_2 receptors. In phase I studies ibodutant was well tolerated and safe. The efficacy of ibodutant in patients with IBS-D is examined currently in phase II studies (Guliani et al. 2008, http://www.clinicaltrials.gov).

DNK 333 is a dual tachykinin NK_1/NK_2 receptor antagonist and was tested in two randomized clinical phase II studies in women with IBS-D. The small peptide DNK 333 was safe and well tolerated with headache as most common side effect. DNK 333 reduced pain and abdominal or overall symptoms (Zakko et al. 2011, http://www.clinicaltrials.gov).

ROSE-010 is a dipeptidyl peptidase-IV resistant peptide mimicking the gut hormone glucagon-like peptide-1. It is applied intravenously or subcutaneously to reduce abdominal pain. One clinical phase II trial was performed showing a relief of pain but some side effects like nausea were measured. Despite the subcutaneous application ROSE-010 could be a potential therapeutic for patients with IBS that suffer from episodes of acute abdominal pain resistant to other treatments (Hellström 2011; Suares and Ford 2011; Hellström et al. 2008).

6 Clinical Implications

The heterogeneity in the pathogenesis of IBS creates unique challenges when designing and assessing the efficacy of novel therapies. For the foreseeable future, it is unlikely that there will be a "magic bullet" for IBS patients. Given our current level of understanding, the practice of subgrouping IBS patients on the basis of predominant bowel pattern is a good first step, but clearly it is not the final answer to define which drugs will be most effective for which patients. As the process of new drug discovery proceeds, emphasis should be placed not only on how often or how much a drug improves global and individual symptoms in IBS sufferers, but also on ways in which the clinician can identify the subset of patients most likely to benefit from a specific drug. This strategy will not only allow the clinician to more effectively treat his/her patients, but should allow scientists to add further pieces to the ever evolving puzzle of IBS.

Most published clinical trials tend to include small number of patients and do not systematically differentiate between males and females. Therefore, it is difficult to

Table 4 Sex/gender differences in existing drugs

Drug/substance	Class	Sex specific features	Comments	References
Psyllium, Ispaghula, polyethylene glycol	Laxatives	None	For IBS-C and IBS-A/M, long-term treatment is possible	Layer et al. (2010)
Hyoscine, mebeverine, peppermint oil	Antispasmodic agents	None	Constipation and anticholinergic side effects limit long-term treatment in IBS-C and IBS-A/M	Adeyemo and Chang (2008)
Lubiprostone	Prostaglandines	More effective in women	For IBS-C, insufficient number of men included in clinical trials	Drossman et al. (2008)
Loperamide	Antidiarrheal agents	None	For IBS-D and IBS-A/M, constipation as side effect	Adeyemo and Chang (2008)
Tegaserod/Prucalopride	Serotonergic agents/ 5-HT_4 agonists	More effective in women	For IBS-D, insufficient number of men included in clinical trials, tegaserod only in case of emergency, prucalopride admitted for chronic constipation	Müller-Lissner et al. (2001), Shekhar and Whorwell (2009)
Alosetron	Serotonergic agents/ 5-HT_3 antagonists	Only for women <55 years under risk management	For IBS-D, severe GI side effects, inconsistent data for men	Adeyemo and Chang (2008)
Amitriptyline/paroxetin	Antidepressant (TCA, SSRI)	Effective for treatment of pain in females	Only for treatment of psychological comorbidities, second line therapy, TCA's may cause constipation	Sainsbury and Ford (2011)

draw conclusions regarding effectiveness of these agents in the treatment of IBS (Table 4).

7 Conclusion

Effects of gender and sex on gastrointestinal diseases are well known and have hardly been investigated during the last decades. Differences are clearly an important factor in the epidemiology, clinical presentation, management, and outcome of IBS. Female patients report higher levels of a variety of intestinal and non-intestinal sensory symptoms. Because IBS is a multi-symptom disorder, an effective treatment must impact on the global symptoms of IBS. Various pharmacological agents have been tried in the management of IBS, but these have proved of limited efficacy for the cardinal symptoms of abdominal pain and bloating. The guidelines for management of IBS favor mainly five drug categories. Although many data demonstrate evidence for sex- or gender-related differences in IBS the guidelines do not consider this aspect. Beside a number of sex-related factors that may impact the clinical symptoms and response to treatment of IBS, both men and women have psychological issues that may need to be addressed. Further studies are needed, to evaluate new approaches to IBS management including gender specific behavioral management therapies and better characterization of male and female subgroups with regard to drug therapy, so that personalized therapy can be implemented.

Take Home Messages

- IBS is one facet of a more general condition of illness behavior which includes an unallertiveness in expressing personal feelings.
- IBS patient status is associated with depressive and anxiety symptoms; in addition IBS patients have more severe depressive symptoms and depressive dysfunctional attitudes.
- Sex and gender aspects are important in understanding differences between men and women in their risk and experience of IBS. Relative to men, women are diagnosed more frequently with IBS.
- Laxatives, antidiarrheals or antispasmodics are common in the treatment of IBS but the majority of patients receive antispasmodics followed by prokinetic agents. Additionally, different plant extracts e.g. STW 5 (Iberogast) can ameliorate bloating, pain as well as motility disturbances.
- In treatment of IBS there appears to be a grater clinical response to serotonergic agents developed for IBS in women compare to men.

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Sex Differences in Prophylaxis and Therapeutic Treatments for Viral Diseases

Sabra L. Klein

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Abstract The intensity and prevalence of viral infections are typically higher in males than in females. In contrast, disease outcome can be worse for females. Males and females also differ in their responses to prophylaxis and therapeutic treatments for viral diseases. In response to vaccines against herpes viruses, hepatitis viruses, influenza viruses, and others, females consistently mount higher humoral immune responses and experience more frequent and severe adverse reactions than males. Males and females also differ in the absorption, metabolism, and clearance of antiviral drugs. The pharmacological effects, including toxicity and adverse reactions, of antiviral drugs are typically greater in females than males. The efficacy of antiviral drugs at reducing viral load also differs between the sexes, with antiviral treatments being better at clearing HIV and hepatitis C virus in females, but

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showing greater reduction of herpes simplex virus and influenza A virus loads in males. Biological variables, including hormone and genes, as well as gender-specific factors related to access and compliance to drug regimens must be considered when evaluating male–female differences in responses to treatments for viral diseases. Clinicians, epidemiologists, and basic biomedical scientists should design experiments that include both males and females, develop a priori hypotheses that the sexes will differ in their responses to and the outcome of vaccines and antiviral treatments, and statistically analyze outcome data by sex. Knowledge that the sexes differ in response to prophylaxis and therapeutic treatments for viral diseases should influence the recommended course of treatment differently for males and females.

Keywords Acyclovir • Antiretroviral therapy • HIV • Hepatitis • Herpes • Influenza • Oseltamivir • Ribavirin

Abbreviations

E2	Estradiol
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
HBV/HCV	Hepatitis B or C virus
P4	Progesterone
T	Testosterone

1 Introduction

Males and females differ in the intensity, prevalence, and pathogenesis of viral infections (Table 1). Although behavioral factors can influence exposure to viruses, several studies illustrate that physiological differences between males and females cause differential responses to infection. Females display reduced susceptibility to viral infections because they typically mount stronger immune responses than males. The innate recognition and response to viruses as well as downstream adaptive immune responses differ between males and females during viral infections. This results in sex differences in cytokine responses to infection that play a critical role in determining susceptibility to viruses. Immune responses to viruses can vary with changes in hormone concentrations naturally observed over the menstrual or estrous cycle, following contraception use, and during pregnancy. As a result of heightened immunity to viruses, both the intensity (i.e., viral load within an individual) and prevalence (i.e., number of infected individuals within a population) of viral infections are often lower for females than males (Table 1). There is growing awareness, however, that much of the disease attributed to viral infection results from aberrant host inflammatory responses (Meier et al. 2009;

Table 1 Sex differences in the intensity (I), prevalence (P), severity of disease (D), or mortality following viral infections in humans

Virus	Dependent measure	Sex difference	References
Cytomegalovirus	P	M < F	Villacres et al. (2004)
Dengue virus	P	M > F	Guha-Sapir and Schimmer (2005)
Epstein–Barr virus	D	M > F	Murphy et al. (2009)
Hantaviruses (multiple species)	P M	M > F M < F	Klein et al. (2011b), Armien et al. (2004), White et al. (1996), Williams et al. (1997), Ferrer et al. (1998), Hjertqvist et al. (2010)
Hepatitis B virus	I, P, D	M > F	Tsay et al. (2009), Wang et al. (2009), Yu et al. (2000), Yu et al. (2001), Farza et al. (1987), DeLoia et al. (1989)
Hepatitis C virus	P, I	M > F	Balogun et al. (2009), Burguete-Garcia et al. (2011)
Herpes simplex virus type 2	I, P	M < F	Langenberg et al. (1999), Fleming et al. (1997), Obasi et al. (1999), Mertz et al. (1992), Gillgrass et al. (2003)
Human immunodeficiency virus	I D	M > F M < F	Sterling et al. (2001), Napravnik et al. (2002), Farzadegan et al. (1998), Katagiri et al. (2006), Gandhi et al. (2002), Meditz et al. (2011)
Human T-cell leukemia virus type 1	P	M < F	Eshima et al. (2009)
Influenza A viruses	D, M	M < F	Zarychanski et al. (2010), Chen et al. (2009), Sedyaningsih et al. (2007), Kandun et al. (2008), Fasina et al. (2010), Fielding et al. (2009), Kumar et al. (2009), Oliveira et al. (2009), Randolph et al. (2011)
Measles	M	M < F	Garenne (1994)
West Nile virus	I	M > F	Jean et al. (2007), O'Leary et al. (2004)

Theiler et al. 2008; Robinson et al. 2011). Consequently, heightened antiviral, inflammatory, and cellular immune responses in females, though essential for virus clearance, might underlie increased development of symptoms of disease among females as compared with males following infection (Table 1) (Klein and Huber 2009; Anker 2007; Klein et al. 2011a).

Although sex and gender differences have been well documented for viral infections, considerably less attention has been paid to the differences between males and females in prophylaxis and therapeutic treatments for viral diseases. The goals of this chapter will be to (1) document the immunological differences that exist between males and females and the factors that mediate these differences; (2) examine how immunological differences between males and females contribute to dimorphic disease pathogenesis and responses to vaccines; and (3) evaluate how the efficacy of antiviral drug treatments differ between the sexes. The contribution of gender-related differences in access, compliance, and acceptance of treatments for viral diseases will be addressed where data exist; the primary focus throughout this chapter, however, will be on the biological differences between males and females

and how these sex differences contribute to differential responses to treatments for disease. All too often sex differences are either ignored or understudied in clinical and basic biomedical research. As a result, significant gaps exist in our understanding of the profound biological differences between males and females in the efficacy of treatments for viral infections.

2 Sex Differences in Immune Function

Males and females differ in their innate immune responses, suggesting that some sex differences may be germ-line encoded. For example, innate detection of viral and bacterial nucleic acids by pattern recognition receptors (PRRs) differs between the sexes (Berghofer et al. 2006; Marriott and Huet-Hudson 2006). There are differences between the sexes in the induction of genes associated with toll-like receptor (TLR) pathways and antiviral type I interferon (IFN) responses (Hannah et al. 2008; Klein et al. 2010), with cells from females showing a tenfold greater level of expression than cells from males (Klein et al. 2010). Studies of both humans and rodents illustrate that the number and activity of innate immune cells, including monocytes, macrophages, and dendritic cells (DCs) as well as inflammatory immune responses in general are higher in females than in males (Boissier et al. 2003; Xia et al. 2009; Melgert et al. 2010).

In rodents and humans, females exhibit greater humoral and cell-mediated immune responses to antigenic stimulation, vaccination, and infection than do males (Klein et al. 2010; Klein and Roberts 2010). Both basal levels of immunoglobulin (Butterworth et al. 1967) as well as antibody responses to viruses and vaccines are consistently higher in females than in males (Klein et al. 2010; Klein and Roberts 2010; Cook 2008). Clinical studies reveal that males have lower $CD3^+$ and $CD4^+$ cell counts, $CD4^+$:$CD8^+$ cell ratios, and inflammatory helper T-cell type 1 (Th1) responses than females (Wikby et al. 2008; Villacres et al. 2004; Amadori et al. 1995; Das et al. 2008). Studies in mice further reveal that cytokine responses of $CD4^+$ T cells often differ between males and females with females reportedly exhibiting higher inflammatory Th1 (i.e., IFN-γ), anti-inflammatory Th2 (i.e., IL-4), and regulatory T-cell (i.e., IL-10) responses than males depending on stage of infection or type of antigen encountered (Roberts et al. 2001; Araneo et al. 1991; Barrat et al. 1997; Pinzan et al. 2010). Female mice also have higher proportions of regulatory T cells than males, at least in response to certain viruses (Frisancho-Kiss et al. 2007). Females exhibit higher cytotoxic T-cell activity along with upregulated expression of antiviral and proinflammatory genes, many of which have estrogen response elements in their promoters (Hewagama et al. 2009).

The prevailing hypothesis for immunological differences between the sexes is that sex steroids, particularly testosterone (T), estradiol (E2), and progesterone (P4), influence the functioning of immune cells. Sex steroids alter the functioning of immune cells by binding to specific receptors, which are expressed in various lymphoid tissue cells as well as in circulating lymphocytes, macrophages, and

dendritic cells (Kovats et al. 2010). The binding of sex steroids to their respective steroid receptors directly influences cell signaling pathways, including NF-κB, cJun, and interferon regulatory factor (IRF) 1, resulting in differential production of cytokines and chemokines (Kovats et al. 2010). Although direct effects of gonadal steroids cause many sex differences in immune function, some sex differences might be caused by the inherent imbalance in the expression of genes encoded on the X and Y chromosomes (Arnold and Chen 2009). Polymorphisms or variability in sex chromosomal genes as well as in autosomal genes that encode for immunological proteins can contribute to sex differences in immune responses (Poland et al. 2008). For example, sex-based differences in human leukocyte antigen alleles contribute to the higher antibody responses of females than males to vaccination (Gordeeva et al. 2006). Gene polymorphisms are associated with sex differences in susceptibility to infection with viruses, including human immunodeficiency virus (HIV) (Siddiqui et al. 2009). These examples illustrate some of the diverse responses that differ between males and females and the role that hormones and genes play in modulating these differential immune responses. The impact that dimorphic immune responses can have on susceptibility to viral infection, the outcome of viral infection, and the efficacy of treatments for viral infection requires greater consideration.

3 Sex Differences in Response to Viral Infection, Antiviral Treatment, and Vaccination

3.1 Human Immunodeficiency Virus

3.1.1 Disease Pathogenesis

Human immunodeficiency virus replication exhibits a sexually dimorphic pattern. The amount of circulating HIV RNA in plasma is one marker of progression to acquired immunodeficiency syndrome (AIDS). HIV RNA levels are consistently lower in women than in men (Sterling et al. 2001; Napravnik et al. 2002; Farzadegan et al. 1998). A meta-analysis of published studies revealed that women have approximately 41 % less HIV RNA in circulation than do men (Napravnik et al. 2002). HIV loads in women often are below the cutoff value for initiation of antiretroviral therapy (Sterling et al. 2001). In addition to having lower HIV loads, women have higher antiviral responses to HIV than men. Plasmacytoid DCs (pDCs), which are significant initiators of antiviral immunity, from women produce more IFN-α in response to HIV-1 encoded TLR7 ligands than pDCs derived from males, resulting in higher levels of $CD8^+$ T-cell activation in women (Meier et al. 2009). HIV-infected women also have higher baseline $CD4^+$ T-cell counts than males (Meditz et al. 2011). Progesterone can modulate the function of pDCs and women with higher plasma P4 concentrations have greater

numbers of IFN-α-producing pDCs in response to the HIV TLR7 ligand than women with lower P4 concentrations (Meier et al. 2009). Use of P4-based hormone contraceptives also is associated with increased acquisition of HIV-1, loss of $CD4^+$ T cells, disease progression, and death rates among women (Hel et al. 2010).

In men, HIV infection causes hypogonadism (i.e., reduced androgen concentrations), which is associated with wasting syndrome, loss of bone mass, and depression (Grinspoon 2005). Treatment of patients with anabolic steroids improves muscle mass, bone density, and quality of life in both men and women (Grinspoon 2005); the immunological consequence of androgen treatment, however, has not been reported. In parallel with reduced androgen concentrations, estrone and E2 concentrations increase with the progression of HIV (Christeff et al. 1996; Teichmann et al. 2003). Consequently, E2 augments transcription of HIV in vitro and this effect can be reversed by exposure to the estrogen receptor (ER) antagonist ICI 182,780 (Katagiri et al. 2006).

3.1.2 Antivirals

Sex-specific differences in HIV load and progression to AIDS raise the question as to whether responses to antiretroviral therapies (ART) differ between the sexes as well. Women are less likely to receive ART and there is some indication that initiation of ART is lower in women than men, but may involve racial and geographic variables as well (Meditz et al. 2011; Gebo et al. 2005). There is growing knowledge that women experience more adverse reactions to antiretroviral drugs, including nonnucleoside reverse transcriptase inhibitors and protease inhibitors, than men (Ofotokun 2005) (Table 2). In particular, treatment with antiretroviral drugs results in more frequent rashes, lactic acidosis, gastrointestinal intolerance, metabolic disorders, and abnormal fat distribution in women (Ofotokun 2005; Ofotokun and Pomeroy 2003). As a result of more frequent adverse reactions to antiretroviral drugs, women are more likely than men to reduce drug dosage or discontinue use (Currier et al. 2000). To illustrate the magnitude of this problem, in one trial of nevirapine, a nonnucleoside reverse transcriptase inhibitor, women were seven times more likely to develop a rash and three to five times more likely to discontinue drug therapy than men (Bersoff-Matcha et al. 2001). With antiretroviral therapies containing protease inhibitors, gastrointestinal intolerance is two to three times higher among women than men (Ofotokun and Pomeroy 2003). The mechanisms mediating how adverse reactions to antiretroviral drugs are greater in women than in men are not known but might include body mass, fat distribution, drug metabolism, drug bioavailability, and gastric motility (Ofotokun 2005). Pharmacokinetic studies suggest that antiretroviral drugs, including zidovudine and lamivudine triphosphate, reach higher intracellular concentrations in women than in men, which might explain the higher rate of antiretroviral drug toxicity in women (Anderson et al. 2003). Disease progression, however, is reportedly four times

Table 2 Sex differences in adverse reactions, immune responses, and efficacy of vaccines and antiviral drugs in humans

Virus	Antiviral drug/ vaccine	Sex-specific features	Comments	References
HIV	HAART	M < F	CD4+ T-cell count; adverse reactions; fat accumulation; drug concentration; virus clearance; hepatitis	Hawkins et al. (2011), Emery et al. (2010), Lucas et al. (1999), Ofotokun and Pomeroy (2003), Anderson et al. (2003), Ofotokun et al. (2007), Bersoff-Matcha et al. (2001)
	HAART	M > F	Fat loss; survival	Andany et al. (2011), Emery et al. (2010)
HSV-2	HSV-2 gD vaccine	M < F	Humoral immune responses; cell-mediated immune responses; vaccine efficacy	Stanberry et al. (2002), Straus et al. (1994), Zhang et al. (2008)
	Acyclovir	M < F	Frequency of prescription; adverse reaction	Theng and Chan (2004), Reichman et al. (1983)
	Acyclovir	M > F	Reduction of virus shedding	Reichman et al. (1983)
HBV	HBV vaccine	M < F	Humoral immune responses	Fang et al. (1994), Hess et al. (1992), Morris et al. (1989), Zeeshan et al. (2007)
HCV	Pegylated interferon alpha/ribavirin	M < F	Adverse reaction; sustained virological response[a]	Narciso-Schiavon et al. (2010), Villa et al. (2011), Yu et al. (2011)
Seasonal influenza viruses	TIV vaccine	M < F	Humoral immune responses; adverse reactions	Engler et al. (2008), Cook et al. (2006), Beyer et al. (1996), Nichol et al. (1996)
	Oseltamivir	M < F	Drug clearance and metabolism[b]	Maltezou et al. (2011)
	Oseltamivir	M > F	Alleviation of symptoms; reduction of viral load	Blanchon et al. (2011)
	Zanamivir	M = F	Alleviation of symptoms; reduction of viral load	Blanchon et al. (2011)

HAART highly active antiretroviral therapy, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *HIV* human immunodeficiency virus, *HSV* herpes simplex virus, *TIV* trivalent inactivated influenza virus

[a]Premenopausal females only

[b]Tested in neonates only

lower for women than men on antiretroviral therapy (Currier et al. 2000). Virological suppression also is more rapid in women than in men on antiretroviral drug therapy (Moore et al. 2001). Future studies must determine how to reduce the adverse reactions and still maintain low disease progression and viral loads in HIV-infected women on ART. There is also interest, but no available data, in the role that sex hormones play in modulating the pharmacokinetics of antiretroviral drugs in women and men (Ofotokun 2005). As the incidence of HIV infection continues to grow more rapidly in women than in men (Quinn and Overbaugh 2005), enrollment of women in clinical trials evaluating the safety and efficacy of antiretroviral drugs or future HIV vaccines must continue, with data from these trials being statistically analyzed to evaluate whether the responses of males and females differ.

3.2 Herpes Simplex Viruses

3.2.1 Disease Pathogenesis

Herpes simplex virus-type 2 (HSV-2), the causative agent of genital herpes infections and infection of the female reproductive tract, is influenced by ovarian sex hormones, including E2 and P4. The prevalence of HSV-2 typically is higher in women than in men (Wald 2004). In HSV-2 seropositive women, oral contraceptive use is associated with increased genital tract shedding of HSV-2 (Cherpes et al. 2005). Ex vivo E2 treatment of primary genital epithelial cells co-cultured with stromal cells increases HSV-2 shedding, whereas pretreatment of cells with P4 decreases HSV-2 shedding (MacDonald et al. 2007). In female mice, susceptibility to HSV-2 varies with stage of the estrous cycle (Gallichan and Rosenthal 1996). High concentrations of P4 are associated with reduced survival, increased viral titers in the vagina, vaginal pathology, inflammation, infiltration of leukocytes (e.g., neutrophils), and the expression of chemokines and chemokine receptors in vaginal tissue (Gillgrass et al. 2005a). Conversely, administration of E2 increases survival and reduces signs of inflammation and vaginal pathology during primary HSV-2 infection, at least in mice (Gillgrass et al. 2005a).

Mortality rates following exposure to HSV-type 1 (HSV-1) also differ between the sexes, in which male mice exhibit more severe pathology following corneal infection and are more likely to die from infection than are females; treatment of female mice with dihydrotestosterone prior to infection significantly increases morbidity and mortality (Han et al. 2001). HSV-1 can infect the central nervous system (CNS), and in mice inoculated intraperitoneally with HSV-1 and examined during the first week post-inoculation, HSV-1 loads are higher in the midbrain, ventricles, cortex, and cerebellum of female than male mice and are associated with worse outcome in females during the acute phase of infection (Burgos et al. 2005). In addition to having more virus in the CNS, females show greater dissemination of virus to peripheral tissues, including the gonads, spinal cord, and trigeminal ganglia than males (Burgos et al. 2005). Following ocular infection with HSV-1, treatment

of latently infected ovariectomized female mice with E2 induces viral reactivation in the trigeminal ganglia, which is mediated by signaling through estrogen receptor alpha (ERα) (Vicetti Miguel et al. 2010).

3.2.2 Vaccines and Antivirals

Responses to vaccines against HSV-2 differ between the sexes, in which the vaccine provides protection against the development of symptoms associated with genital herpes in women, but not in men (Stephenson 2000). For example, in phase 1 and 2 studies of a recombinant gD-based HSV-2 vaccine, there was no significant protection from acquisition of HSV-2 infection in HSV-1 and HSV-2 seronegative subjects when data were combined for males and females (overall efficacy 38 %). When data were partitioned by sex, a significant sex bias in protection was observed, in which the efficacy was 73 % in females and only 11 % in males (Stanberry et al. 2002).

In ovariectomized female mice, immunization with an attenuated strain of HSV-2 only protects against challenge with wild-type HSV-2 when females are treated with P4, but not E2. Progesterone, administered either alone or in combination with E2, reduces HSV-2 replication in the reproductive tract by increasing the number of DCs and T cells in the vaginal lamina propria and increasing titers of gB-specific vaginal IgA (Gillgrass et al. 2005b). Cessation of E2 treatment for 5 days, but not 1–3 days, prior to challenge with HSV-2 can increase protection in ovariectomized mice suggesting that the effects of E2 can be reversed and are dependent on E2 clearance (Gillgrass et al. 2010). Immunization of female mice with regular estrous cycles with a recombinant adenovirus vector expressing HSV gB results in higher titers of gB-specific vaginal IgA during estrus than during either diestrus or proestrus and exogenous administration of P4 to female mice at the time of immunization protects females from lethal intravaginal HSV-2 challenge (Gallichan and Rosenthal 1996), indicating that sex steroids affect induction of protective immunity following vaccination against HSVs.

Despite significant differences between males and females in the prevalence, shedding, and healing of lesions caused by HSV (Reichman et al. 1983; Wald 2004), there is a paucity of data pertaining to differences between males and females in the efficacy of antiviral drugs for reducing symptoms of disease (Table 2). Acyclovir (Zovirax) is a synthetic nucleoside analogue administered orally or topically to reduce pain and increase healing of herpes sores and blisters. The available data suggest that topical treatment of HSV-2 sores with acyclovir reduces virus shedding in male, but not female, patients (Reichman et al. 1983). In some regions of the world, oral acyclovir is prescribed more frequently for female than male HSV patients (Theng and Chan 2004). Considerably more research is required to determine whether the efficacy of acyclovir treatment consistently differs between the sexes. From the available data, vaccines may be more efficacious in females, whereas antiviral drugs may be more effective in males at reducing symptoms of HSV disease.

3.3 Hepatitis Viruses

3.3.1 Disease Pathogenesis

Hepatitis B and C viruses (HBV and HCV) cause chronic infections and are a major risk factor for the development of liver cancer and hepatocellular carcinoma. The prevalence of serum HBV surface antigen (HBsAg) is consistently higher in men than in women (Koulentaki et al. 1999; Robinson et al. 2005; Tsay et al. 2009). Male sex also is an independent factor associated with elevated HBV DNA titers (Chen et al. 2006). Development of hepatocellular carcinoma occurs at a 2:1 to 4:1 ratio of males to females (El-Serag and Rudolph 2007). Increased rates of exposure to HBV in males do not completely explain why men are more likely to develop liver cancer than women. Studies show that among HBsAg-positive individuals, males are more than twice as likely to experience mortality from liver cancer as are females, suggesting that men may be more sensitive to the effect of HBV infection on the development of liver cancer (Wang et al. 2009).

Hormones mediate sex differences in susceptibility to liver cancer following infection with HBV. Among HBsAg-positive men, elevated concentrations of T and expression of certain androgen receptor (AR) gene alleles (*SRD5A2* and *V89L*) correlate with increased risk of hepatocellular carcinoma (Yu et al. 2000, 2001). The development of chemically induced hepatocellular carcinoma is delayed in androgen receptor male knockout mice as compared with wild-type male mice (Ma et al. 2008). In HBV transgenic mice, castration of males reduces, whereas replacement of T or treatment with a T agonist (R1881) in castrated males increases, serum HBsAg concentrations (DeLoia et al. 1989; Farza et al. 1987; Tian et al. 2012). Male HBV transgenic mice have higher concentrations of HBsAg than females after, but not before, puberty (Tian et al. 2012). The effect of androgens on HBV is mediated by the AR because male *Tfm* mice (i.e., mice with a mutation in the AR) do not show elevated concentrations of HBsAg as do wild-type males following HBV infection (Breidbart et al. 1993). One mechanism by which androgens affect HBV replication is through direct binding to androgen response elements that have been identified in the enhancer I of HBV (Wang et al. 2009). In addition to direct modulation of virus transcription, hormones can alter host immune responses to infection. For example, chemically induced hepatocellular carcinoma is more severe in male than in female mice, which is mediated by increased IL-6 production by Kupffer cells in the livers of male mice (Naugler et al. 2007). These studies further reveal that E2 reduces the synthesis of IL-6 by Kupffer cells through inhibition of Myd88-dependent induction of NF-κB (Naugler et al. 2007). Thus, sex steroids modulate sex differences in the prevalence of HBV and development of liver cancer through effects on the transcription of virus and host immune responses to HBV.

For HCV, injection drug use is the single most important risk factor for acquiring HCV. Male sex is an independent risk factor for HCV prevalence as measured by antibody or detection of HCV RNA (Burguete-Garcia et al. 2011). The odds ratio of

being HCV-positive is 3.47 (95 % confidence interval 2.48–4.87) for males compared with females (Balogun et al. 2009). Females are more likely to spontaneously clear HCV than males (Grebely et al. 2007). When risk factors, like injection drug use, are considered, then females are at an increased risk of exposure to HCV because they appear to be more like to share needles and other drug equipment than males (Iversen et al. 2010). In people chronically infected with HCV, the risk of developing cirrhosis and the time to onset of cirrhosis is shorter for males than females by 8–11 years (Rodriguez-Torres et al. 2006). Sex differences in chronic HCV disease appear to be mitigated after menopause, with postmenopausal women showing accelerated rates of cirrhosis and fibrotic progression, which can be reversed by hormone replacement therapy (Di Martino et al. 2004). Genetic variation can impact the outcome of HCV infection. Male HCV patients are more likely to carry detrimental IL-6 promoter polymorphisms associated with development of chronic HCV infection (Cussigh et al. 2011). CTLA4 is an inhibitory T-cell receptor. Certain polymorphisms in *Ctla4* are associated with resolution of HCV infection and are more common in women than men (Schott et al. 2007).

3.3.2 Vaccines and Antivirals

Following vaccination against HBV, among both children and adults, anti-HBV antibody titers are higher in females than males (Fang et al. 1994; Hess et al. 1992; Jilg et al. 1984; Morris et al. 1989; Bock et al. 1996). In multivariate analyses, being male is a significant predictor of being "nonresponsive" to the HBV vaccine; thus, adult females show higher rates of seroconversion following exposure to the HBV vaccine than do males (Zeeshan et al. 2007). Greater efficacy of the HBV vaccine in females also may contribute to reduced prevalence of HBV and development of liver cancer among females compared with males.

HCV infections are treated with antiviral drugs. Sex differences in the efficacy and responses to antiviral treatment, including the standard combined treatment with pegylated interferon alpha and ribavirin, are only consistently observed between men and premenopausal women (Table 2). Among patients of reproductive ages, females experience more adverse reactions (e.g., anemia, weight loss, nausea, vision impairment, reduced thyroid function, and infections) to the combined antiviral drug treatment, are more likely to modify the doses of the antiviral drugs, and are more likely to interrupt or suspend treatment than males (Bhattacharya et al. 2010; Narciso-Schiavon et al. 2010). In HCV patients of reproductive ages who maintain at least 80 % of the planned antiviral drug doses, the sustained virological response is greater for females than males (Yu et al. 2011). Sex differences in the sustained virological response to antiviral therapy are not apparent in analyses that include women who have entered menopause (Villa et al. 2011). Among HCV-infected women who have entered menopause, baseline liver inflammation, fibrosis, and proinflammatory cytokine concentrations are significantly elevated as compared with premenopausal women and might explain why antiviral therapy is less effective following menopause (Villa et al. 2011).

3.4 Influenza A Viruses

3.4.1 Disease Pathogenesis

Sex differences in the incidence influenza A viruses have been documented in humans (Wingard 1984; Noymer and Garenne 2000; Chen et al. 2007). Although exposure rates are often higher in men (Noymer and Garenne 2000), fatality following exposure to pathogenic influenza A viruses is reportedly higher in women (Sedyaningsih et al. 2007; Kandun et al. 2008; Fasina et al. 2010; Zarychanski et al. 2010). Hospitalization with severe disease from 2009 H1N1 was higher in young women (Chen et al. 2009; Kumar et al. 2009; Oliveira et al. 2009; Fielding et al. 2009; Zarychanski et al. 2010), with data from Canada and the United States, indicating that females had an over twofold higher risk of death than males (Randolph et al. 2011; Zarychanski et al. 2010). In addition to pregnancy, which is a critical factor associated with increased severity of disease (Jamieson et al. 2009), many cases involve comorbid conditions, including chronic lung disease (e.g., asthma), which is typically more severe in females than males (Singh et al. 1999; Schatz and Camargo 2003; Schatz et al. 2006; Moorman et al. 2007).

Although sex differences in the incidence of influenza virus infection may reflect differences in exposure to these viruses, differential disease severity between the sexes may involve biological differences in response to infection. The extent to which immune responses differ between males and females during influenza virus infection requires assessment as this might contribute to differential severity of disease between the sexes. Disease associated with highly pathogenic influenza viruses and the clinical manifestations that ensue in humans is hypothesized to be mediated by the profound proinflammatory cytokine and chemokine response (referred to as the "cytokine storm") initiated by the host in response to infection (Guan et al. 2004; de Jong et al. 2006). Humans, macaques, and mice infected with highly pathogenic strains of influenza virus, including the 1918 H1N1 or avian H5N1, produce excessively high concentrations of proinflammatory cytokines and chemokines, which correlate with elevated mortality (Guan et al. 2004; Cook et al. 1995; Szretter et al. 2007; Kobasa et al. 2007; Kash et al. 2006; de Jong et al. 2006). Studies in mice reveal that adult females experience greater morbidity and mortality in response to H1N1 infection than males and this appears to be mediated, not by altered levels of virus replication, but by greater pulmonary proinflammatory immune responses (Robinson et al. 2011). Administration of E2 or an ERα agonist to females reduces virus-induced lung proinflammatory immune responses, morbidity, and mortality (Robinson et al. 2011). Elevated immunity in females against influenza A viruses might represent a delicate balance between immune responses conferring protection through clearance of virus or causing pathology through increased production of proteins and an influx of immune cells into the lungs.

3.4.2 Vaccines and Antivirals

In addition to influenza virus pathogenesis, males and females differ in response to influenza virus vaccines (Table 2). Rates of immunization against influenza are reportedly either similar between the sexes or lower in women (Merrill and Beard 2009; Qureshi et al. 2004; Opstelten et al. 2008; Jimenez-Garcia et al. 2008; Endrich et al. 2009) and may be influenced by greater negative beliefs about the risks of vaccination (Santibanez et al. 2010) and lower acceptance of vaccines (Schwarzinger et al. 2010; Chor et al. 2009) among women. Antibody responses to the seasonal trivalent inactivated vaccines (TIV) are higher in women than in men (Edwards et al. 2007; Cook 2008; Engler et al. 2008; Cook et al. 2006). Whether antibody responses to the live attenuated influenza vaccine differ between the sexes has not been reported, to date. Women also report more frequent and severe local and systemic reactions to the seasonal TIV than men (Engler et al. 2008; Beyer et al. 1996; Nichol et al. 1996).

Like women (Edwards et al. 2007; Cook 2008; Engler et al. 2008; Cook et al. 2006), female mice mount higher neutralizing and total antibody responses against a sublethal primary infection/vaccination with influenza viruses than males (Lorenzo et al. 2011). Following vaccination, female mice are better protected against lethal challenge with heterosubtypic (i.e., novel) strains of influenza viruses than males (Lorenzo et al. 2011). Although elevated immunity afforded females greater protection than males against lethal challenge with heterosubtypic viruses, both sexes are equally protected against lethal challenge with homologous virus (i.e., the strain of virus in vaccine) (Lorenzo et al. 2011). Estradiol, at physiological concentrations, can stimulate antibody production by B cells (Lu et al. 2002; Franklin and Kutteh 1999; Pauklin et al. 2009), including antibody responses to an inactivated influenza vaccine administered in mice (Nguyen et al. 2011).

Following infection, neuraminidase inhibitors can be administered to alleviate symptoms of disease and virus shedding (Burch et al. 2009). Oseltamivir (Tamiflu) is administered orally, absorbed in the gastrointestinal tract, and converted to the active metabolite, oseltamivir carboxylate, by an esterase in the liver (De Clercq 2006). Zanamivir (Relenza) is an inhaled powder delivered as the active compound directly into the respiratory tract (De Clercq 2006). In patients with confirmed influenza A virus infection and treated with oseltamivir, alleviation of symptoms of disease is faster (Fig. 1) and the reduction of nasal virus load is greater among males than females (Blanchon et al. 2011). In contrast, in influenza A virus-infected patients treated with zanamivir, no sex differences in either alleviation of symptoms or virus load are observed, suggesting that male–female differences in drug absorption or metabolism may contribute to the dimorphic outcome of treatment with oseltamivir, but not zanamivir (Blanchon et al. 2011). Data also suggest that females are better able to clear oseltamivir more rapidly than males, at least in newborns (Maltezou et al. 2011). Male–female differences in the outcome of oseltamivir treatment do not appear to be due to gender differences in treatment compliance (Fig. 1) (Blanchon et al. 2011). Future clinical studies must continue to

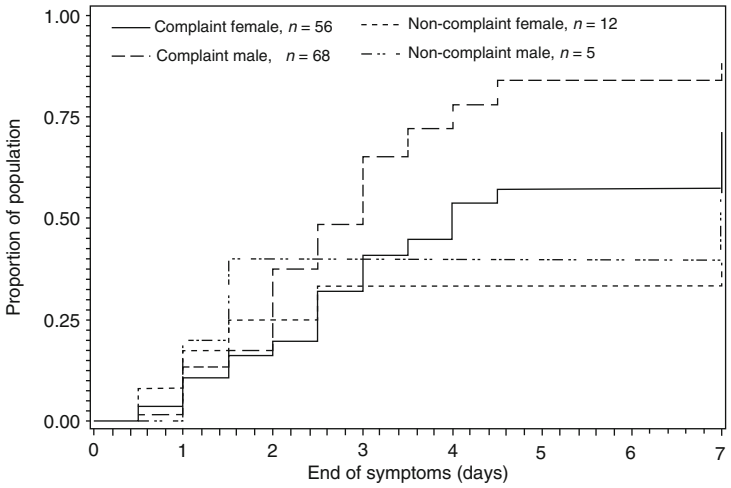

Fig. 1 Proportion of male and female influenza A virus-infected patients ($n = 141$) with alleviation of symptoms following treatment with oseltamivir. Patients were further subdivided into those who were compliant or noncompliant with the two days of treatment. Alleviation of symptoms was defined as reduced nasal stuffiness, sore throat, cough, muscle aches, fatigue, feverishness, and headache. Data adapted with permission from Blanchon et al. 2011

partition and analyze antiviral outcome data by sex and establish the biological mechanisms mediating how oseltamivir is more effective in males than females.

4 Conclusions and Clinical Implications

The pathogenesis of viral diseases and treatments for these diseases differ between males and females. Adverse reactions to vaccines and antiviral drug treatments are consistently greater for females than males (Table 2). Male–female differences in the pharmacological effects of antiviral drugs do not solely involve differences in body mass or fat distribution, but also involve differences in the pharmacokinetics and pharmacodynamics of these drugs. There is growing evidence that the absorption, metabolism, and clearance of antiviral drugs differ between the sexes (Ofotokun et al. 2007). The importance of sex differences in pharmacokinetics is highlighted by the observation that the efficacy of anti-influenza drugs that are processed and metabolized in the liver results in sex differences in clinical and virological responses, whereas the efficacy of anti-influenza drugs delivered directly to the lungs does not cause significant differences between the sexes (Blanchon et al. 2011). Sex differences in the efficacy of antiviral drug treatments are not as consistent as are the differences in adverse reactions. Antiviral drug treatments appear to resolve infection better for females infected with HIV or hepatitis C virus, whereas antiviral treatments for infection with herpes simplex

viruses or influenza A viruses result in better clinical and virological outcomes in males (Table 2). These observations are based on a limited number of studies; thus, restraint must be shown before drawing strong conclusions. There is a significant need for additional basic biomedical research in this area.

Sex steroid hormones influence the outcome of infection and the efficacy of antiviral drug treatments. Future studies should continue to consider the age and reproductive status of females as well as whether females are using exogenous hormones (either through contraceptives or replacement therapy) at the time of infection, drug treatment, or both. The observation that sex differences in the outcome of infection and in responses to antiviral treatments for hepatitis depend on the hormonal status, and not merely the age, of women (Villa et al. 2011) is an important observation that should be pursued for other diseases (Ofotokun 2005). Additionally, whether the hormonal milieu at the time of vaccination influences immune responses and long-term protection against disease should be examined.

As interest in personalized medicine continues to grow, it will likely become increasingly apparent that one of the most fundamental ways that individuals differ in their responses to prophylaxis and therapeutic treatments for disease is based on their sex. How the hormonal milieu, genes, and genetic polymorphisms affect responses to vaccines and antiviral drug treatments differently between the sexes must be considered. The end goal should be that clinicians and researchers alike consider the sex of their patients when designing and administering treatments for diseases.

Take Home Messages

- Although the intensity and prevalence of viral infections are often higher in males than in females, disease outcome can be worse for females.
- Females are biologically different from males, mounting higher immune responses to vaccines and absorbing, metabolizing, and clearing drugs faster than males.
- Humoral immune responses and adverse reactions following vaccination are higher in females than males.
- The pharmacological effects, including toxicity and adverse effects, of antiviral drugs are greater in females than males; the efficacy of these antiviral drugs at reducing viral load differs between the sexes, with the direction of the dimorphism being drug-specific.
- Clinicians, epidemiologists, and basic biomedical scientists should design studies that include both males and females, develop a priori hypotheses that the sexes will differ in their responses to and the outcome of vaccine and antiviral treatments, and statistically analyze outcome data by sex.
- Knowledge that the sexes differ in response to prophylaxis and therapeutic treatments for viral diseases should influence the recommended course of treatment differently for males and females.

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Gender Differences in Anticoagulation and Antithrombotic Therapy

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Abstract Cardiovascular disease is the main cause of mortality and morbidity worldwide. The rate of thromboembolic events has increased in women but not in men. Large clinical studies support the use of a variety of antithrombotic drugs for the treatment of patients with different cardiovascular diseases. The heterogeneous patient population included in these trials affects the attempt to generalize the study results to subgroups, which are not sufficiently represented in the study population, such as women and other minorities. Gender-related differences in the clinical presentation and outcome seem to relate to differences in platelet biology and coagulation reactions, resulting in different rates of thromboembolic and bleeding events. The effectiveness of antithrombotic therapies and the occurrence of adverse events define the clinical benefit of the treatment for each patient. This chapter gives an overview of the currently available data on gender-differences in anticoagulation and antithrombotic therapy.

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Abbreviations

ACS Acute coronary syndrome
AF Atrial fibrillation
INR International normalized ratio
MI Myocardial infarction

1 Introduction

Thromboembolic events are important contributors to mortality and morbidity in clinical settings. A large variety of anticoagulant and antithrombotic drugs are currently available for the treatment of patients with different cardiovascular diseases. Profound differences have been delineated in the clinical outcomes among different patient groups. Especially women have been characterized by significantly higher mortality and morbidity rates (Lawesson et al. 2012; Kim and Menon 2009). Gender-related disparities in clinical symptom presentation, diagnostic tools, clinical management and clinical outcome seem to relate to biological differences in platelet function and blood coagulation. The effectiveness of antithrombotic therapy and the rate of adverse complications determine the clinical benefit of each patient. Understanding gender-related differences with regard to antithrombotic therapy and anticoagulation should result in an individualized therapeutic approach for prevention and treatment of different cardiovascular diseases.

2 Thrombosis and Coagulation: Gender-Related Considerations of Pathophysiology

Gender-related differences in platelet function and coagulation are a possible reason for the worse clinical outcome in female patients after acute coronary syndromes or atrial fibrillation. In the literature, it is well described that the thrombosis rate varies between women and men (Capodanno and Angiolillo 2010). Several studies in humans have documented gender-related differences with regard to the number of circulating platelets, the degree of platelet adherence to injured vasculature and agonist-induced platelet activation and aggregation (Schwertz and Penckofer 2001; Eshel-Green, et al. 2009; Johnson, et al. 1975). A higher baseline platelet activation and adenosine diphosphate- or collagen-induced platelet reactivity were observed in female than in male patients (Haque, et al. 2001). Several agonists have been used to induce platelet aggregation in

in vitro studies. Gender differences were identified dependent on the agonist used for platelet stimulation (Bailey et al. 2009; Blais et al. 2009). The increased baseline platelet aggregability present in women was found to be independent of platelet morphology and surface adhesion molecule expression (Stevens and Alexander 1977; Leng et al. 2004). Moreover, several platelet agonists were documented to activate the glycoprotein IIb/IIIa receptor to a larger extent in females than in male individuals (Faraday et al. 1997).

Sex hormones have been suggested to cause the gender difference in platelet reactivity. The risk for cardiovascular events in female patients is well known to increase after menopause (Gordon et al. 1978). Estrogens have been reported to induce the synthesis of prostacyclins, thereby increasing nitric oxide synthesis and inhibiting platelet aggregation (Caulin-Glaser et al. 1997; Herman et al. 1997). Thus, platelets obtained from premenopausal women are less reactive than platelets from men of the same age. However, it has to be stressed that postmenopausal hormone replacement therapy has not been proved to improve the clinical outcome (Rossouw et al. 2002; Grady et al. 2000). Vice versa, the use of contraceptives or postmenopausal hormone replacement has been associated with an increased rate of thromboembolic events in female patients (Manson et al.; 2003; Langer et al. 2005).

Coagulation factors and parameters of fibrinolysis are affected by sex hormones. Estrogens have been shown to reduce levels of fibrinogen, antithrombin III, protein S and plasminogen activator inhibitor 1 (PAI-1) (Mendelsohn and Karas 1999). In contrast, testosterone was found to correlate to plasminogen levels, implicating that testosterone may exhibit fibrinolytic properties (Bonithon-Kopp et al. 1988; Bain and Forester 1980). Moreover, a negative correlation was observed between testosterone and fibrinogen as well as PAI-1 levels. Several studies have looked at the haemostatic balance in women compared to men. These studies do not support the hypothesis that a reduced coagulation reaction and/or better fibrinolysis exists in men compared to women (O'Brien 1951; Bain and Forester 1980). In contrast, women have been shown to exhibit a longer in vivo bleeding time than men (O'Brien 1951; Bain and Forester 1980). Nevertheless, the unopposed administration of estrogens in postmenopausal women has been shown to modulate the tissue factor and factor VII expression (Vlotides et al. 2007; De Valk-de Roo et al. 2000). In addition, the tissue factor pathway inhibitor was significantly reduced under these conditions, pointing to an elevated risk of thromboembolic complications under external administration of anticoagulation therapy (Peverill et al. 2001). These data explain the increased thromboembolic risk of postmenopausal women when receiving hormone replacement therapy.

3 Gender Differences in Acute Coronary Syndromes and Antithrombotic Therapy

Cardiovascular disease harms approximately 50% of men and women and constitutes the leading cause of death (Rauch et al. 2001; Chesebro et al. 1997). Acute coronary syndromes are important contributors to mortality and morbidity. Probably due to the later onset of the disease in females, fewer women than men

have been included into the early studies on ACS (Alfredsson and Swahn 2010; Meinert 1995; Lee et al. 2001). Therefore, less evidence is currently available for the treatment strategies in women than in men. In the year 1993, the National Institutes of Health (NIH) Revitalization Act established guidelines to reinforce the existing policies for inclusion of women in clinical research (U.S. Congress Public Law No 103–43 1993). Although more females have since then been included into NIH-sponsored phase trial 3, the general trend towards a substantial under-representation of women in clinical trials sponsored by pharmaceutical companies has not changed (Capodanno and Angiolillo 2010). Nevertheless, evidence exists that women have less often received reperfusion therapies, such as fibrinolysis or percutaneous coronary interventions as well as antithrombotic therapy. In contrast, males have been referred for coronary angiography (Chandra et al. 1998; Gan et al. 2000; Mahon, et al. 2000). However, the differences disappeared after adjustment for comorbidity, such as age and severity of the disease (Alfredsson et al. 2007). Therefore, more attention has lately been given to gender differences in the treatment of ACS. Major differences between the women and men have been identified with regard to the patient characteristics. The profoundly different patient profiles may explain the difference in treatment strategies. The INTERHEART study identified six risk factors that were positively associated with an increased risk for a first MI: hyperlipidemia, smoking, hypertension, diabetes, abdominal obesity and psychosocial stress (Anand et al. 2008; Yusuf et al. 2004). The risks factors identified in this large study were identically present in women and men. Nevertheless, the degree of hypertension, diabetes and physical inactivity as well as the lack of alcohol intake were more strongly associated with an acute coronary syndrome in women than in men. Smoking turned out to be a more important risk factor in women than in men (Jonsdottir et al. 2002).

3.1 Antiplatelet Treatment

A large number of trials showed that aspirin compared to placebo offered a large clinical benefit for patients with an acute coronary syndrome. The risk for death or non-fatal myocardial infarction was reduced by aspirin by approximately 50 %. The mortality after 35 days of treatment was significantly lower with aspirin. No differences were seen when comparing females with males (Woodfield et al. 1997). A meta-analysis of studies examining the effect of aspirin on the primary and secondary prevention of vascular disease also revealed a comparable treatment effect in men and women (Baigent et al. 2009).

The ADP receptor antagonist clopidogrel has been demonstrated to effectively reduce the cardiovascular risk and was more effective in the treatment of ACS (CAPRIE Steering Committee 1996; Yusuf et al. 2001). The CURE study revealed a 20 % risk reduction for the composite endpoint of cardiovascular death, myocardial infarction or stroke (Yusuf et al. 2001). In a recent meta-analysis of large clinical trials comparing clopidogrel and placebo showed no gender

differences in treatment effect. Regarding major bleeding, there were small but real excess risks with clopidogrel therapy in both men and women with no differences between the both groups. (Berger et al. 2009).

In the TRITON TIMI 38 study, prasugrel was compared to clopidogrel in patients with ACS. The primary endpoint defined as a composite endpoint of death from cardiovascular causes, non-fatal myocardial infarction or non-fatal stroke was reduced with prasugrel [9.9 % vs. 12.1 %, HR = 0.81, 95 % CI (0.73–0.90)]. This was accompanied by an increased rate of major bleedings and was significantly higher in patients on treatment with prasugrel (2.4 % vs. 1.8 %, $P = 0.03$) (Wiviott et al. 2007). Gender differences were not seen in the TRITON TIMI 38 study.

The reversible ADP receptor antagonist ticagrelor was studied in the PLATO trial. Ticagrelor was also found to be superior to clopidogrel in patients with ACS (Wallentin et al. 2009a, b). A significant clinical benefit was observed in men and women without difference in treatment effect between the two groups. A detailed description of the existing sex-related features that have been analysed in the literature is given in Table 1.

3.2 Anticoagulation

Anticoagulation with unfractionated heparin is associated with a higher activated partial thromboplastin time in women than in men (Granger et al. 1996). Low-molecular-weight heparin has been documented to be of value in the acute phase of an ACS. The FRICS study (Fragmin and Fast Revascularization during InStability in Coronary artery disease Study) reported a larger absolute and relative risk reduction in the composite endpoint of death and MI in women than in men (FRICS study group 1999). In addition, more minor bleedings were observed in women than in men (Toss et al. 1999). In line, the anti-Xa activity during the acute phase treatment was higher in women than in men. Moreover, it is well known that a prolonged therapy with heparin increases the bleeding risk without adding a convincing advantage. Further gender differences were not observable when planned fibrinolysis was given as add-on to a regimen of enoxaparin or unfractionated heparin (Mega et al. 2007).

The direct thrombin inhibitor and hirudin analogue bivalirudin was recently compared to unfractionated heparin or enoxaparin in ACS patients. Non-inferiority analyses showed that bivalirudin was not inferior to unfractionated heparin or low-molecular-weight heparin with or without GPIIb/IIIa inhibitor regarding the composite ischaemic endpoint death, myocardial infarction or unplanned revascularization at 30 days. Treatment with bivalirudin was associated with reduced bleeding events (Stone et al. 2006; 2007a, b). No gender-related differences were observed with regard to ischaemic outcomes (Manoukian et al. 2007). However, female gender was significantly associated with an increase in the major bleeding (Chacko et al. 2006). In a subgroup analysis on patients with PCI the presence of a gender

Table 1 Gender-related differences of antithrombotic therapies

Drug/substances	Class	Variable analysed for sex-specific features	Gender-related differences	References
Antiplatelets				
Aspirin	COX-1 inhibitor	Platelet reactivity in women treated with aspirin	Higher agonist-induced platelet reactivity in women	Becker et al. (2006) Buchanan et al. (1983)
		Pharmacokinetic properties Drug bioavailability	Quicker absorption and larger distribution volume in women	Ho et al. (1985) Woodfield et al. (1997)
		Clinical outcome for primary and secondary prevention in females versus male patients	Slower drug clearance in females No difference observed in meta-analysis of clinical trials	Baigent et al. (2009)
Clopidogrel	$P2Y_{12}$-inhibitor, thienopyridine	Platelet function inhibition by clopidogrel	No gender-related differences	Ferreiro et al. (2010)
		Reduction of the combined risk of death, MI or stroke at one year	Greater risk reduction among women	Steinhubl et al. (2002)
		Meta-analysis of five large phase III clinical trials focused on sex-related differences in clinical outcome	No statistical significant heterogenicity with regard to gender	Berger et al. (2009)
Prasugrel	$P2Y_{12}$-inhibitor, thienopyridine	Reduction in ischaemic events comparing prasugrel with clopidogrel	No interactions between treatment effect, bleeding and gender	Wiviott et al. (2007)
Ticagrelor	$P2Y_{12}$-inhibitor, cyclo-pentyl-triazolo-pyrimidine	Composite ischaemic event rate comparing ticagrelor with clopidogrel	No interactions between treatment effect, bleeding and gender	Wallentin et al. (2009a, b)
Abciximab	GPIIb/IIIa antagonist	In vitro platelet response to abciximab	No gender-related differences	Coller et al. (1983)
		Meta-analysis of large clinical trials with regard to major adverse events	No differences with regard to adverse outcome, higher major and minor bleeding rates in women	Cho et al. (2000)

Eptifibatide	GPIIb/IIIa antagonist	Composite adverse clinical events and bleeding rate	No differences in death, MI or target vessel revascularization, higher major and minor bleeding rates in women	Fernandes et al. (2002)
Tirofiban	GPIIb/IIIa antagonist	Biomarker in ACS	C-reactive protein and brain natriuretic peptide more likely elevated in women than men	Wiviott et al. (2004)
Anticoagulants				
Unfractionated heparin	Indirect factor Xa and thrombin	aPTT values	Higher in women	Granger et al. (1996)
Low-molecular-weight heparin	Indirect factor Xa and thrombin	Reduction in the composite of death and MI	Larger reduction of the primary endpoint and more minor bleedings in female patients	FRICS study group 1999 Toss et al. (1999)
		Meta-analysis of phase III clinical trials focused on sex-related differences in clinical outcome	No gender-related interactions with regard to triple endpoint, death or MI significantly reduced in women but not men	Cohen et al. (2001)
		Clinical adverse outcome in fibrinolysis plus heparins	No gender-related interactions	Mega et al. (2007)
Warfarin	Vitamin K antagonist	Risk of thromboembolism under treatment with a vitamin K antagonist	Higher benefit in women	Fang et al. (2005)
		Major bleeding risk	No gender-related interactions	Hughes et al. (2007)
Bivalirudin	Direct factor IIa inhibitor	Ischaemic outcomes and major bleeding rates under treatment with bivalirudin plus provisional use of GPIIb/IIIa inhibitor	female gender significantly associated with major bleeding risk, No gender-related differences with regard to ischaemic outcomes	Chacko et al. (2006) Manoukian et al. (2007)
		Ischaemic outcomes and bleeding rates under monotherapy with bivalirudin	No gender-related differences	Mehran et al. (2009)

(continued)

Table 1 (continued)

Drug/substances	Class	Variable analysed for sex-specific features	Gender-related differences	References
Dabigatran	Oral direct factor IIa inhibitor	Thromboembolic events and bleeding risk in patients with atrial fibrillation	No gender-related interactions, safe and effective in women and men	Connolly et al. (2009)
Fondaparinux	Indirect factor Xa inhibitor	Reduction in the composite endpoint of death, MI, refractory ischaemia or major bleeding	Higher absolute reduction of major bleeding in women with a trend for statistical interaction	Yusuf et al. (2006a, b)
		Reduction in the composite of death or re-infarction	No gender-related interactions	Oldgren et al. (2008)
Rivaroxaban	Oral direct factor Xa inhibitor	Thromboembolic events and bleeding risk in patients with atrial fibrillation	No gender-related interactions, safe and effective in women and men	Patel et al. (2011)
Apixaban	Oral direct factor Xa inhibitor	Thromboembolic events and bleeding risk in patients with atrial fibrillation	No gender-related interactions, safe and effective in women and men	Granger et al. (2011)

differences was not confirmed (Stone et al. 2007a, b). Moreover, the HORIZONS-AMI study compared the treatment with bivalirudin to heparin therapy with GPIIb/IIIa. Patients receiving bivalirudin had reduced adverse event rates and a diminished rate of major bleeding. No interactions were present with regard to gender (Mehran et al. 2009).

In the OASIS 5 and OASIS 6 trials, fondaparinux, a indirect factor Xa inhibitor, was compared to unfractionated heparin or low-molecular-weight heparin. Therapy with fondaparinux was non-inferior standard treatment in patients with an ACS. The rates of major bleeding were significantly lower with fondaparinux. Thus, the composite efficacy and safety endpoint was in favour of fondaparinux (Yusuf et al. 2006a, b). Subgroup analyses of the primary endpoint revealed no gender difference in both clinical studies (Yusuf et al. 2006a, b). A detailed description of the existing sex-related features from the literature is given in Table 1.

3.3 Fibrinolysis

Several studies showed a similar benefit with fibrinolytic therapy in men and women who suffered from an acute coronary syndrome (Alfredsson and Swahn 2010; Klein 1996). The GISSI trial comparing streptokinase with placebo reported a sustained benefit over 10 years with fibrinolysis in patients with STEMI and no significant interaction with regard to gender (Franzosi et al. 1998). There is much evidence that women with STEMI are admitted to the hospital after a significantly longer delay since onset of symptoms compared to males. Thus, it is not surprising that women with STEMI have been treated with fibrinolytic treatment more seldom than men. The time from symptom to treatment is crucial for the effectiveness of fibrinolytic therapy and determines its application. Importantly, fibrinolysis for patients with STEMI has been associated with a higher risk of bleeding in women than in men (Alfredsson and Swahn 2010).

3.4 Bleeding Complications

Bleeding complications are frequent complications in patients receiving antithrombotic therapy. The GRACE register demonstrated the occurrence of major bleeding to be between 2.7 and 4.7 % in patients with an acute coronary syndrome (Moscucci et al. 2003). Independent predictors of major bleeding included female sex, age, renal dysfunction and history of bleeding. Multivariate analysis showed that the adjusted OR for bleeding was 1.71 (95 % CI, 1.35–2.17) in women compared to men (Moscucci et al. 2003). Several recent studies pointed to the importance of bleeding complications for the prognosis of patients with ACS (Manoukian et al. 2007; Budaj et al. 2009). An increase in bleeding was shown to be associated with an increase in mortality and ischaemic events (Eikelboom et al.

2006). Female gender was observed to be an independent predictor of bleeding in several trials with different anticoagulation strategies (Manoukian et al. 2007; Eikelboom et al. 2006; Alexander et al. 2005).

The CRUSADE Bleeding Score was developed to help clinicians estimate a patient's baseline risk of in-hospital major bleeding during non-ST-segment elevation myocardial infarction. This score system considers baseline patient characteristic, admission clinical variables and admission laboratory values to estimate the patient's likelihood of having an in-hospital major bleeding event. Female sex is here considered to be risk factor for the occurrence of major bleedings during hospitalization.

Several reasons exist to possibly explain the adverse outcomes associated with severe bleeding. Parameters such as older age, comorbidity and renal failure as well as decreased haemodynamic instability may account for this observation (Alfredsson and Swahn 2010). Moreover, the discontinuation of antithrombotic drugs as a consequence of bleeding led to an increased risk of ischaemic events. Women have well been documented to be at risk for increased doses of antithrombotic medication and, thus, elevated bleeding rate (Alexander et al. 2005; Xang et al. 2005). In addition, an impaired renal function is more frequent among female patients, and this is also associated with bleeding (Xang et al. 2005).

4 Gender Differences in Atrial Fibrillation and Anticoagulation

Atrial fibrillation is the most common clinically relevant arrhythmia and constitutes a major risk for stroke and other thromboembolic events. Women with atrial fibrillation are known to be at higher propensity for stroke independently of other known risk factors. The Stroke Prevention in Atrial Fibrillation and Framingham risk scores as well as CHADS2-VACS scoring system included the feature "female gender" as clinically important parameter for stroke risk assessment (Hart et al. 1999; Wang et al. 2003; Dickstein et al. 2010). The Anticoagulation and risk factors in atrial fibrillation (ATRIA) study confirmed that women are at higher risk than men for atrial fibrillation-related thromboembolic events when not treated with anticoagulation therapies (Fang et al. 2005). Moreover, the risk of death in patients with nonvalvular atrial fibrillation was associated with an odds ratio of 1.5 (95 % CI, 1.2–1.8) in men and 1.9 (95 % CI, 1.5–2.2) in women in up to 40 years of follow-up in the Framingham study (Benjamin et al. 1998). In addition, the Copenhagen City Heart Study found AF to be independently associated with a 2.5-fold higher mortality due to cardiovascular diseases in female compared to male patients (Dagres et al. 2007). Thus, the mortality is greater for women than in men with AF (Michelena et al. 2010). Whether AF is the reason for death or only an indirect marker of increased comorbidities leading to death has not been elucidated yet.

The mechanism behind the gender-related differences in thromboembolic risk associated with atrial fibrillation has not been investigated yet. Higher levels of

prothrombotic factors and increased platelet activation have been observed in patients with atrial fibrillation. Female patients with atrial fibrillation have been documented to exhibit increased concentrations of prothrombin fragment F1.2, von Willebrand factor and tissue plasminogen activator antigen within the circulating blood (Conway et al. 2003; Feinberg et al. 1999; Wang et al. 2001). Thus far, these prothrombogenic factors have not prospectively been linked to an increased risk of stroke in atrial fibrillation. With respect to the increasing incidence of atrial fibrillation, it would be very helpful to understand the underlying mechanisms that contribute to the increased AF-related thromboembolic risk-associated with female sex.

4.1 Oral Anticoagulation

The ATRIA (Anticoagulation and Risk Factors in Atrial Fibrillation) study found rates of thromboembolism in patients not taking warfarin of 3.5 % per year for women compared to 1.8 % per year for men (adjusted rate ratio [RR] = 1.6; 95 % CI, 1.3–1.9) (Fang et al. 2005). Oral anticoagulation led to a reduction in thromboembolic events, which was significantly larger in female than in male patients (adjusted RR = 0.4 versus 0.6, respectively; $p = 0.01$). Another prospective study of 780 patients with AF who received anticoagulation therapy also reported that the stroke risk was significantly more reduced in women than in men (Poli et al. 2009).

For several decades vitamin K antagonists have frequently been prescribed for the primary and secondary prevention of thromboembolic events in patients with atrial fibrillation. The major contributors which determine the bleeding risks in patients on oral anticoagulation have intensively been studies (Dickstein et al. 2010). These are specific patient characteristics, such as age and comorbidity, the intensity of the anticoagulant effects, the length of anticoagulant therapy and the presence of drugs interactions, especially those which interfere with blood haemostasis (Schulman et al. 2008; Ferreiro et al. 2010). Female gender has not been described to be associated with major bleeding. It should be stressed that gender differences do not affect the incidence of life-threatening bleeding events, such as intracranial haemorrhage. No relation between gender and bleeding was found in a systematic review of several studies on patients with atrial fibrillation treated with anticoagulant therapy (Hughes et al. 2007). A retrospective analysis of 18,867 patients with AF found that anticoagulant therapy with warfarin was associated with a 0.47 % annual risk for intracranial haemorrhage compared with 0.15 % without warfarin use. No differences were seen with regard to gender (Shen et al. 2007). However, female sex has been reported to be an independent risk factor for the occurrence of minor bleeding in patients on therapy with vitamin K antagonists (Pengo et al. 2001; van der Meer et al. 1993).

Dabigatran, a new oral direct thrombin inhibitor which is administered twice daily, is characterized by a pharmacologic profile, which is better than that of warfarin. This eliminates the need for regular monitoring of the anticoagulant

drug efficiency, such as measuring the INR level under treatment with vitamin K antagonists. Two fixed doses of dabigatran have been randomly tested against warfarin in 18,113 patients with nonvalvular AF in the RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) study (Connolly et al. 2009). Both dabigatran doses (110 mg twice daily and 150 mg twice daily) were not inferior to warfarin with regard to the primary endpoint, defined as occurrence of stroke or systemic embolism. The low-dose dabigatran was associated with comparable rates of stroke and less bleeding than warfarin. The high-dose dabigatran compared to warfarin was associated with a reduced stroke risk and a comparable bleeding risk. No interaction was observed between women and men (Connolly et al. 2009).

Rivaroxaban, an oral factor Xa inhibitor, has also been documented to provide more consistent and predictable anticoagulation than vitamin K antagonists. In a double-blind trial, we randomly assigned 14,264 patients with nonvalvular atrial fibrillation who were at increased risk for stroke to receive either rivaroxaban (at a daily dose of 20 mg) or dose-adjusted warfarin. In patients with atrial fibrillation, rivaroxaban was noninferior to warfarin for the prevention of stroke or systemic embolism (event rates 1.7 % per year for rivaroxaban versus 2.2 % per year for warfarin, hazard ratio in the rivaroxaban group, 0.79; 95 % confidence interval, 0.66–0.96; $p < 0.001$ for noninferiority). There was no significant between-group difference in the risk of major bleeding. Importantly, intracranial and fatal bleeding occurred less frequently in the rivaroxaban than warfarin group. No differences were found with regard to the gender (Patel et al. 2011).

The efficiency of apixaban, another novel oral direct factor Xa inhibitor with better pharmacokinetic and -dynamic properties than warfarin, for preventing thromboembolic events has also been studied in patients with atrial fibrillation. In patients with atrial fibrillation for whom vitamin K antagonist therapy was unsuitable, apixaban reduced the risk of stroke or systemic embolism without significantly increasing the risk of major bleeding or intracranial haemorrhage (Connolly et al. 2011). The AVERROES study was prematurely terminated due to the superiority of apixaban compared to warfarin. No significant interaction was reported without regard to sex (Connolly et al. 2011). In the ARISTOLE trial, apixaban at a dose of 5 mg twice daily was compared with warfarin at an INR of 2.0 to 3.0 in 18,201 patients with atrial fibrillation and at least one additional risk factor for stroke. The primary outcome was defined as a composite of ischaemic or haemorrhagic stroke or systemic embolism. Apixaban was found to be superior to warfarin in the prevention of the primary endpoint. The annual rate of the primary outcome was 1.27 % in the apixaban group, as compared with 1.60 % in the warfarin group (hazard ratio with apixaban, 0.79; 95 % confidence interval, 0.66 to 0.95; $p = 0.01$ for superiority). Moreover, treatment with apixaban compared to warfarin caused less bleeding and resulted in lower mortality in patients suffering from nonvalvular AF (Granger et al. 2011). No differences were detectable with regard to the gender aspect. A detailed description of the existing sex-related features is given in Table 1.

The new oral anticoagulants are expected to largely replace the vitamin K antagonists for primary and secondary prevention of thromboembolic events. With regard to the safety and efficiency of these new drugs, gender-related differences have not been described to occur in the large phase 3 trials. The superiority of these new oral anticoagulants over the traditional vitamin K antagonists presents a major step forward, enabling efficient and safe anticoagulation for both women and men.

5 Clinical Implications and Conclusions

The presence of gender differences in coagulation and platelet biology translates into sex-related differences in treatment options in a variety of common cardiovascular diseases. For the future, it is of major importance to perform further studies to gain more insight into these differences of cardiovascular diseases and its management. The study design of large randomized studies should take gender-related differences into account to be able to draw proper conclusions from clinical trials to daily clinical practice. Until now, most clinical results regarding gender differences have been based on subgroup analyses without sufficient statistical power. Women have an increased thromboembolic risk and worse clinical outcome without the proper anticoagulation or antithrombotic therapies. On the other hand, bleeding risks on antithrombotic treatment seem to be increased in female patients with coronary artery disease. Whether this is a matter of the drug doses or of differences in metabolism is not clear. With regard to the increased efficiency of the newer antithrombotic drugs, it appears to be a challenge for the future to delineate the underlying mechanisms of gender-related differences and their clinical consequences.

Take Home Messages

- The feature “female gender” has been included into scoring systems for the risk assessment of thromboembolic events in patients with nonvalvular atrial fibrillation.
- Female patients with nonvalvular atrial fibrillation have been documented to exhibit greater benefits from anticoagulation and, therefore, should be treated with anticoagulants rather than aspirin.
- Fewer women than men have been included in the large clinical cardiovascular studies of the last decades. This results in limited knowledge concerning gender-specific difference with regard to antithrombotic treatment options.
- Women had more bleeding complications with thrombolytic therapy than men. This may be due to inadaequate dosing or a true pharmacodynamics effect.
- Several phase III clinical trials have demonstrated the superiority of the new oral factor Xa and IIa antagonists over vitamin K antagonists for primary and secondary prevention of thromboembolic events in atrial fibrillation. No evidence of gender-related differences has thus far been described in these trials with regard to the efficiency and safety of the new oral drugs.

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Pharmacology and Clinical Use of Sex Steroid Hormone Receptor Modulators

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Abstract Sex steroid receptors are ligand-triggered transcription factors. Oestrogen, progesterone and androgen receptors form, together with the glucocorticoid and mineralocorticoid receptors, a subgroup of the superfamily of nuclear receptors. They share a common mode of action, namely translating a hormone—i.e. a small-molecule signal—from outside to changes in gene expression and cell fate, and thereby represent "natural" pharmacological targets.

For pharmacological therapy, these receptors have originally been addressed by hormones and synthetic hormone analogues in order to overcome pathologies related to deficiencies in the natural ligands. Another major use for female sex hormone receptor modulators is oral contraception, i.e. birth control.

On the other side, blocking the activity of sex steroid receptors has become an established way to treat hormone-dependent malignancies, such as breast and prostate cancer.

In this review, we will discuss how the experience gained from the classical pharmacology of these receptors and their molecular similarities led to new options for the treatment of gender-specific diseases and highlight recent progress in medicinal chemistry of sex hormone-modulating drugs.

Keywords Oestrogen receptors • Androgen receptors • Progesterone receptors • Receptor biology • Clinical indications

1 Introduction

Sex steroid hormones have been the subject of intensive studies for the major part of the last century. Haberlandt in the early twentieth century showed the importance of endocrine glands—the organs that synthesise hormones. It was in 1929 that the first sex steroid hormone, oestrone, was isolated and found to be a prototype "bioactive small molecule", i.e. a non-peptidergic structure amenable to synthesis and chemical modifications. These early studies soon allowed the clinical use of hormone preparations which originally, however, relied on natural sources. The discovery of ethynyloestradiol by Inhoffen and Hohlweg in 1934 at the Schering Research laboratories in Berlin started the field of synthetic hormones (Hohlweg and Inhoffen 1947).

A major scientific progress in the post-war era was the isolation of the sex steroid receptors (Jensen et al. 2010; Smith et al. 1975; Tindall et al. 1975) and the clarification of their molecular mechanism of action. This allowed, in turn, the design of systematic assays and, ultimately, of drugs with defined mechanisms of action.

The purpose of this review is to give an overview on sex steroid receptor biology and on the subsequent clinical uses of modulators of these receptors. Recent advances in the development of synthetic sex steroid receptor modulators will be discussed.

1.1 Basics of Steroid Hormone Receptor Agonism and Antagonism

Both male (androgens) and female (oestrogens, progestins) sex hormones are steroid hormones. Starting from cholesterol, they share a common biosynthetic pathway with mineralocorticoids, glucocorticoids and vitamin D (Miller and Auchus 2011). Thus, these compounds have several properties in common: they are small, very lipophilic molecules with the potential to freely diffuse through cell membranes. Their receptors also share important features: in all animals, the receptors for steroid hormones are part of the nuclear receptor superfamily of ligand-triggered transcription factors (Mangelsdorf et al. 1995). Unlike membrane receptors that trigger intracellular signalling pathways, these receptors work by influencing gene expression in the cell. The unique gene expression patterns ultimately bring about changes in cell function as a result of hormone action. A closer look at the molecular structures of these receptors and at their biology reveals that they are exciting targets for drug discovery.

1.2 Structure and Function: Steroid Hormone Receptors Are Ligand-Bound Transcription Factors

Steroid receptors are ligand-triggered transcription factors that, upon ligand binding, control the activities of target genes in the cell nucleus. Four basic functions are needed for their activity (Fig. 1a):

- A sequence-specific DNA-binding function to specifically recognise target gene promoters
- A ligand-binding function for activation by the hormone
- A molecular "switch" that precludes the receptor from being active in the absence of ligand (or when treated with an antagonist) and that converts it into an active transcriptional factor in the presence of cognate ligands
- A domain responsible for transcriptional activation, i.e. which has the capability to recruit the factors needed for stimulation of gene expression

In the canonical structure of nuclear receptors the first function, namely the specific recognition of DNA, is contained in a highly conserved region named the DNA-binding domain (DBD). The ligand-binding domain (LBD) hosts several functions (Fig. 1b). It is located C-terminal to the DBD and has an α-helical structure. The lipophilic core of the LBD is capable of accommodating the ligand (the second function) (Fig. 1c). Also, its helix 12—which makes direct ligand contacts—can adopt different conformations in the presence of hormone (on) or in the absence of hormone as well as in the presence of an antagonistic ligand (off), which is the third function (molecular switch). In the "on" conformation, helix 12 and neighbouring areas of the LBD create a protein interface capable of recruiting

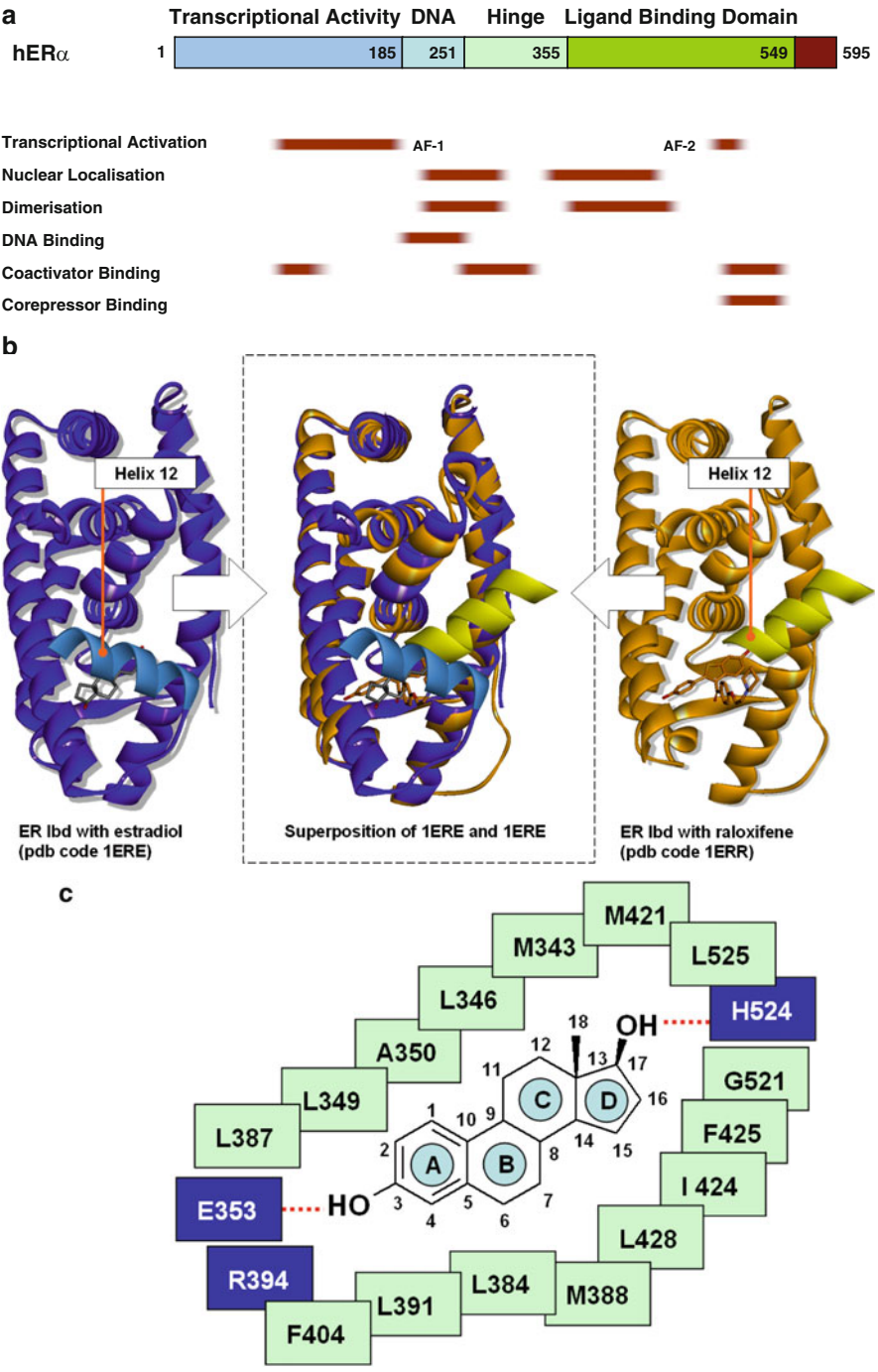


Fig. 1 *Structure and function of nuclear receptors*. (**a**) The modular structure of nuclear receptors. (**b**) A molecular switch upon agonist or antagonist binding. (**c**) The binding of the steroid, oestrogen, to a nuclear receptor, ERα

the transcriptional machinery to the receptor and thus to the target promoter. So, the LBD also contains the fourth function, namely transcriptional activation.

In addition, some nuclear receptors (including all sex steroid receptors) contain another transcriptional activation function at the N-terminus of the molecule, called activation function 1 (AF-1).

1.3 Biology of Steroid Receptors

According to the classical model of steroid receptor action, the unliganded receptor resides in the cell cytoplasm in an inactive complex with heat-shock proteins (hsp) (Pratt et al. 2004). Upon binding of the natural hormone or of another agonistic ligand, the receptor undergoes a conformational change (the helix 12 "switch" mentioned above), leading to dissociation of the hsp, transport into the nucleus and binding to enhancers and promoters of target genes, recruitment of the transcriptional machinery and activation of target genes. In addition, a number of post-translational modifications have been described for all steroid receptors, and in several cases their impact on the fine-tuning of receptor action has been elucidated (Faus and Haendler 2006; Stanisic et al. 2010).

There are, however, considerable variations in this model, several of which can have an impact on pharmacology:

(a) There is variability as to the mechanisms of receptor *location* and *stability*: The receptor may reside in the cytoplasm or in the nucleus (and even, in an inactive complex, already on the DNA), and the transport and/or degradation mechanisms involved may vary (Kumar et al. 2006; Stanisic et al. 2010). A recent modelling study (Kolodkin et al. 2010) suggests that these mechanisms have the potential to influence both receptor sensitivity and the kinetics of the response. The pharmacological consequences of regulating receptor stability will be discussed below.

(b) There is a variability as to the *mechanisms of target gene regulation* by the activated receptor once located on the chromosome. Classical "positive" target genes harbour a binding site (hormone-responsive element, HRE) in their promoters and are activated by receptor binding (Beato et al. 1996). Non-classical target genes lack HREs, but can be activated by a "crosstalk" of the receptor with other transcription factors (Kaarbo et al. 2007; Lange et al. 2007; Osborne and Schiff 2011). In addition, repression of target genes by the activated hormone receptor has been described (Perissi et al. 2010). These mechanisms can be addressed pharmacologically (see below). Very recently, the essential role of pioneering factors in controlling steroid receptor action on target genes has been evidenced (Augello et al. 2011).

(c) There is a variability in *downstream pathways*: several observations describe the triggering of cytoplasmic signal transduction pathways by nuclear receptors located in the cytoplasm or in the cell membrane (Hammes and Levin 2007;

Losel et al. 2003). Many of these studies have however been controversially discussed, and so far no pharmacological route to exploit these pathways has been described.

It is important to consider the cellular and tissue context in this respect. Receptor location and stability mechanisms depend on the localisation and degradation mechanisms in a certain cell type; target gene regulation depends on the transcriptional cofactors present in the cell type and on the promoters being accessible to transcription at all. So, depending on the cell type and tissue considered, hormone receptors can have very different effects. For example, oestrogens trigger cell proliferation in uterine tissue via oestrogen receptor (ER) α, while the very same hormone triggers neuropeptide synthesis (and no cell proliferation) in the hypothalamus via the very same receptor (Wintermantel et al. 2006). Androgens have proliferative effects in the prostate gland; however, their effects on hair follicles are stimulatory during puberty but inhibitory in adults.

1.4 Pharmacological Consequences

By evolution, steroid hormone receptors are the prototypic small-molecule targets and change the fate of the cell after stimulation by their cognate ligand. Thus, they are easily accessible to pharmacological manipulation. Their natural small-molecule ligands are derived from the cholesterol-like steroid structure. Depending on the structural variations, ligands (natural or synthetic) can have different effects on the receptor:

- Full agonists can trigger all activation steps, just as the endogenous hormone does
- Partial agonists can elicit a weaker response, for example, by recruiting the receptor to target promoters but not triggering full activation of HRE-containing target genes. This can be brought about, e.g. by dissociating the hsp and translocation of the receptor to the target gene promoters, but not activating the LBD "switch", leaving transcriptional activation via the N-terminal AF-1 intact. Also, ligands can be agonistic on non-classical target genes but antagonistic on the classical promoters. As these different mechanisms can be relevant in different tissues (see above), partial agonists can elicit tissue-specific effects, i.e. be strongly agonistic in some tissues but weakly agonistic in others. If administered in the presence of a full agonist, they can have an antagonistic dose–response curve, at least up to the level of their partial agonism
- Full antagonists can completely block the response in any cell type and tissue
- Ligands can interfere with the transport and/or degradation pathways and reduce the amount of receptor protein ("destabilisers")
- As the binding sites of all steroid receptors accommodate the same "lead structure" (i.e. pregnenolone derivatives), ligands can modify the activity of

several receptors, e.g. cyproterone acetate, which is a ligand for both AR and PR (see below)

The focus of drug development in the field of steroid hormone receptors is therefore to achieve the desired receptor specificity, to calibrate the agonist/partial agonist/antagonist/destabiliser activity and—finally—to achieve pharmacokinetic properties compatible with a clinical use. It should be remembered that most of the endogenous steroid hormones are rather unstable and not effective when administered orally.

1.5 Classical Indications

1.5.1 Hormone Agonists

An important area where pharmacological activation of hormone receptors is therapeutically used is the compensation of absent or insufficient hormone production by endocrine glands. In females, ovaries physiologically cease to produce steroid hormones during menopause. In males, gonads do not physiologically cease to produce testosterone; however, in cases of male hypogonadism, hormone replacement is indicated.

Female Sex Hormones: Oestrogen and Progesterone

The first sex steroid used as pharmacological agent was Progynon®, first sold by Schering AG in 1928. As the name already disclosed, Progynon was literally a drug "by women for women": It was a mixture of female sex steroids that alleviated the symptoms of menopause, when the endocrine glands had ceased to produce the natural oestrogens and progestins. The lack of oestrogen leads to several impairments in quality of life, such as hot flushes, sleep disturbances and mood changes.

The replacement of female sex hormones after menopause which today is called "Menopause Management" remains an area of active pharmacological research (Shifren and Schiff 2010). It has been shown that oestrogen agonists are able to alleviate most of the symptoms of menopause, such as hot flushes. Furthermore, they have been shown to protect from postmenopausal osteoporosis. When given alone, however, their proliferative action on the uterus is a matter of concern. Therefore, progestins need to be administered that convert the uterus into a non-proliferative, differentiated state. Oestrogen-only hormone replacement therapy is indicated only in patients after hysterectomy (i.e. surgical removal of the uterus).

While the combination of oestrogenic and progestogenic hormones is effective in Menopause Management, large clinical studies have spurred a controversial debate on potential untoward cardiovascular side effects (Samsioe 2003).

Developments in recent years have therefore focussed on different sets of hormone receptor modulators that maintain the beneficial effects of oestrogens while precluding hormone actions that may potentially cause unwanted effects. This can be achieved by using either special receptor modulators with unique profiles or a combination of an agonist with a tissue-selective antagonist (E2 + SERM concept, see below).

Probably the most important pharmacological application of sex steroid hormone agonists is their use in combined hormonal contraception (Bitzer and Simon 2011). Contraception is defined as methods or behaviours for the intentional prevention of pregnancy. Among the available contraceptive methods the "pill" containing an oestrogen and a progestin for oral contraception is most broadly used and characterised by high contraceptive efficacy. As an alternative route of administration, oestrogen/progestin combinations are also applied transdermally by contraceptive patches or intravaginally by contraceptive rings. The contraceptive effect of combined products relies primarily on inhibition of ovulation through interference with the secretion of the pituitary-derived gonadotropins. In addition, effects on the cervical canal and viscosity of the cervical mucus contribute to the contraceptive effects. The progestogenic component of combined contraceptives is in the first line responsible for the contraceptive effect. Synthetic progestins that mimic the activity of the natural hormone progesterone at the progesterone receptor (PR) are in clinical use since the early 1960s and still an area of active research (Schmees and Weinmann 2009). They inhibit the secretion of the gonadotropins LH and FSH and thereby block the menstrual cycle. They exhibit direct effects on the endometrium and keep the cervical canal tight and the mucus viscous to form a natural barrier to sperm. The exogenous oestrogenic component guarantees expression of the PR in the target organs of progestin action. Furthermore, the exogenous oestrogen maintains oestrogen-dependent functions in different organ systems including reproductive organs such as the endometrium, cervix and vagina, and in non-reproductive organs such as the central nervous system, cardiovascular system and bone.

Male Sex Hormones: Androgens

Primary and secondary male hypogonadism are the main indications for androgen replacement therapies. A number of androgen derivatives and application routes have been developed and details are given below.

1.5.2 Hormone Antagonists

A variety of cancers, especially those originating from hormone-responsive tissues, retain responsiveness to hormones. In both male-specific (prostate) and female-specific (breast) glands, sex hormones stimulate cell proliferation. Thus, several cancers of these tissues grow in response to hormones. Antagonism of these

receptors is a proven therapeutic option to treat hormone-responsive cancers: The partial ER antagonist tamoxifen (see below) is widely used in breast cancer therapy, and the AR antagonist bicalutamide is used to treat prostate cancer.

Frequently, however, breast and prostate tumours escape anti-hormonal treatment and grow in spite of the presence of the antagonist. To identify receptor modulators that circumvent this resistance but retain the ability to control tumour growth by the hormone receptors is an ongoing task of drug discovery. Indeed very recent clinical data with novel anti-hormonal compounds used to treat prostate cancer patients indicate that resistant tumours are often still dependent on the AR for their proliferation (see below).

Antagonistic compounds that inhibit the activity of the PR have first been described in the 1980s (see below). However, although such compounds displayed very promising data in diverse clinical settings, their use in patients is still very limited up to now.

2 Examples and Recent Developments

2.1 Progesterone Receptor

2.1.1 Receptor Biology and Physiology

Progesterone is one of the key modulators of normal female reproductive functions. It regulates central processes such as ovulation, uterine and mammary gland development and differentiation, decidualisation, implantation and maintenance of pregnancy. The diverse effects of progesterone on the female reproductive target tissues are mediated via the PR.

The PR exists as two different receptor isoforms, termed PR-A and PR-B (Horwitz and Alexander 1983). These two isoforms originate from the same gene and differ in the presence of an N-terminal region of 165 amino acids in PR-B that is absent in PR-A (Bain et al. 2001; Sartorius et al. 1994; Takimoto et al. 2003; Tung et al. 2001). Both receptor isoforms are expressed in every progesterone target organ. As PR isoform-specific agonists or antagonists are not available, the understanding of their specific physiological function has largely been gained in preclinical model systems. PR-A and PR-B have for instance been analysed by the utilisation of genetically modified mice (Conneely et al. 2002; Fernandez-Valdivia et al. 2005). A null mutation of both receptor isoforms, PR-A and PR-B, leads to pleiotropic reproductive abnormalities (Chappell et al. 1997; Lydon et al. 1995). These alterations include the inability to ovulate, uterine hyperplasia and inflammation, severely limited mammary gland development, and the inability to exhibit sexual behaviour. The PR-A-specific knock-out mouse which only lacks this PR isoform exhibits an infertility phenotype with severely affected ovulation. In addition, the endometrium is unable to mount a decidual response. Furthermore,

the isoform PR-A plays only a minor role in breast tissue functions as females lacking this PR isoform show normal mammary gland development in response to progesterone. The PR-B-specific knock-out mice lacking only the PR-B isoform are fertile but display a markedly reduced pregnancy-associated mammary gland morphogenesis (Conneely et al. 2003; Mulac-Jericevic et al. 2003, 2000). In summary, the PR isoforms mediate different physiological effects in vivo in response to progesterone in a cell- and tissue-specific manner.

2.1.2 Clinical Indications

Due to the diverse physiological functions of the PR, the application of PR antagonists and selective PR modulators (SPRMs), in both gynaecological and non-gynaecological conditions, is an attractive strategy. In the following sections, we will focus on breast cancer, uterine leiomyoma and endometriosis for which clinical efficacy of PR antagonists and SPRMs has been demonstrated and for which ongoing clinical programmes have been reported.

Breast Cancer

In breast cancer cells that express functional PR, progesterone is essential for cell proliferation. Hence, PR antagonists could inhibit the growth of breast tumours and might be promising new tools for breast cancer therapy. To date, the results of five phase II clinical trials with PR antagonists in patients with metastatic breast cancer have been reported (Klijn et al. 2000, 1994, Robertson et al. 1999). Combination of PR antagonists with anti-oestrogens or an aromatase inhibitor showed promising effects.

Uterine Leiomyoma

Uterine fibroids (myoma, leiomyoma) are sex steroid-dependent benign tumours of the uterine muscle (myometrium). They constitute the most common pelvic tumours in women. About 25 % of women of reproductive age are thought to be affected by symptoms evoked through fibroids (Laughlin et al. 2010; Okolo 2008). The exact prevalence of fibroids as observed in pathological examinations of hysterectomised uteri was reported to be even higher (Cramer and Patel 1990). Typical symptoms are abnormal uterine bleeding, dysmenorrhoea, pelvic pain and reproductive dysfunction (Wilson 2011).

Uterine fibroids are the single most prevalent indication for hysterectomy, which is the traditional treatment of these tumours along with myomectomy (Wilcox et al. 1994). Approximately 600,000 hysterectomies are performed annually in the United States, at least one-third of these being due to uterine fibroids.

Currently, there is no effective long-term medication for the treatment of uterine fibroids. The application of gonadotropin-releasing hormone (GnRH) agonists,

which results in an inhibition of ovarian hormone production, leads to a reduction of fibroid size (for an overview, see Olive et al. (2004)). However, because of the induced side effects accompanying the resulting hypooestrogenic status (e.g. loss of bone mass and hot flushes), this regimen is only used as a preparatory treatment prior to surgical excision. In contrast to GnRH agonists, PR antagonists do not suppress oestrogen production, thus avoiding side effects due to hypooestrogenicity.

Evidence that application of PR antagonists and SPRMs has a beneficial effect on benign tumour size and symptoms comes from clinical observations (Steinauer et al. 2004). Treatment with the PR antagonist mifepristone (RU486 from Roussel-UCLAF) led to a shrinkage of fibroids, dependent on dose and duration of treatment, and to a relief of symptoms (Fiscella et al. 2006).

Endometriosis

Endometriosis is characterised by the presence of endometrial tissue outside the uterus in the peritoneal cavity (Seli et al. 2003; Vigano et al. 2004). The most widely accepted cause for the pathogenesis of endometriosis is retrograde menstruation. Endometrial tissue fragments are able to adhere in the peritoneal cavity, attract new blood vessels and proliferate. They can also induce inflammation and at the same time evade clearance by the immune system. Endometriosis is an oestrogen-dependent disease (Garai et al. 2006). It is believed that 10–17 % of women develop symptomatic endometriosis during their reproductive age. The most frequent symptoms are dysmenorrhoea, dyspareunia, chronic pelvic pain and infertility (Jones et al. 2001). Besides laparoscopic ablation of endometriotic lesions and management of the endometriosis-associated pelvic pain with non-steroidal anti-inflammatory drugs, the major goal of current medical treatment is either to block ovarian oestrogen secretion (with the use of GnRH superagonists or antagonists, or danazol, a steroidal compound with weak androgenic activity) or to locally inhibit oestrogenic stimulation of the ectopic endometrium with the help of progestins (Brown et al. 2010; Kappou et al. 2010; Nothnick 2010). However, due to menopausal side effects like bone mass loss and hot flushes caused by the induced hypooestrogenicity under GnRH analogues or breakthrough bleeding under progestin-only therapy, new treatment options with similar efficacy but reduced side effects are needed.

Clinical data demonstrating the efficacy of PR antagonists and SPRMs in the treatment of endometriosis are available. The use of mifepristone in endometriotic patients has been reported in three small clinical trials and an improvement of symptoms was observed (Kettel et al. 1996, 1998, 1991).

2.1.3 Progesterone Receptor Ligands

The isolation and structural elucidation of progesterone, the naturally occurring ligand of the PR in the early 1930s, served as a basis for the identification of

synthetic progesterone analogues (PR agonists) (Fig. 2a). Due to a very low oral bioavailability caused by a rapid metabolic degradation in the liver, the natural PR agonist progesterone was found to be only of limited use as pharmaceutical agent. The extensive search for synthetic analogues led to several compounds with significantly improved oral bioavailability. It was found that modification of the natural ligand in particular by introduction of substituents at C-6 and C-17 improved metabolic stability. Several of these 6,17-modified progesterone analogues (pregnane series) entered the market. As one representative of this class of PR agonists, medroxyprogesterone acetate is shown in Fig. 2a. Furthermore, it was discovered that the insertion of a 17α-ethynyl group into the natural ligand of the androgen receptor (AR), testosterone, surprisingly changed the profile to a potent PR agonist with only weak partial androgenic activity. This compound, named ethisterone, was found to be orally active and entered the market about 70 years ago (Onken and Heublein 1970). Later it was shown that removal of the 19-methyl group as well as introduction of an ethyl group at position C-13 can further increase the potency and selectivity towards the AR. Levonorgestrel is a prominent example of several 19-norandrostane derivatives which are on the market (see Fig. 2) (Benagiano et al. 2008a, b). Due to an increased potency, the daily dosage of levonorgestrel in oral contraceptives is significantly lower compared to the first synthetic PR agonists (150–250 μg compared to daily doses of up to 5 mg in oral contraceptives of the first generation). For several years the major goal of synthetic modifications was a further increase of potency and selectivity, mainly towards the AR. However, the latest invention in the field of PR agonists is a compound called drospirenone which exhibits, in addition to its strong PR agonistic activity, partial antagonistic effects towards the AR and the mineralocorticoid receptor, and therefore has a receptor selectivity profile which is very similar to that of the natural hormone progesterone (Machado et al. 2011; Rapkin and Winer 2007).

Synthetic progesterone analogues were a crucial prerequisite for the discovery of PR antagonists and selective PR ligands, as described in the following section.

Classification of Progesterone Receptor-Modulating Ligands

The activity profile of PR ligands ranges from full agonists, which provide an agonistic activity at the receptor in all target tissues of progesterone to full antagonists, which demonstrate antagonistic activities under all conditions. Compared to these extremes, the SPRMs display an intermediate profile while sharing many PR antagonistic properties. Based on characteristic experimental behaviours, the PR antagonists and SPRMs were classified into three categories: type I antagonists (e.g. onapristone) prevent PR binding to DNA, type II antagonists (e.g. mifepristone and SPRMs) promote DNA binding and act as PR antagonists under most circumstances but can display agonistic behaviour under distinct experimental settings and type III antagonists (e.g. lonaprisan) also promote a strong binding to DNA, but do not display any PR agonistic activity at all. In more recent studies using global gene expression profiling techniques, this classification system

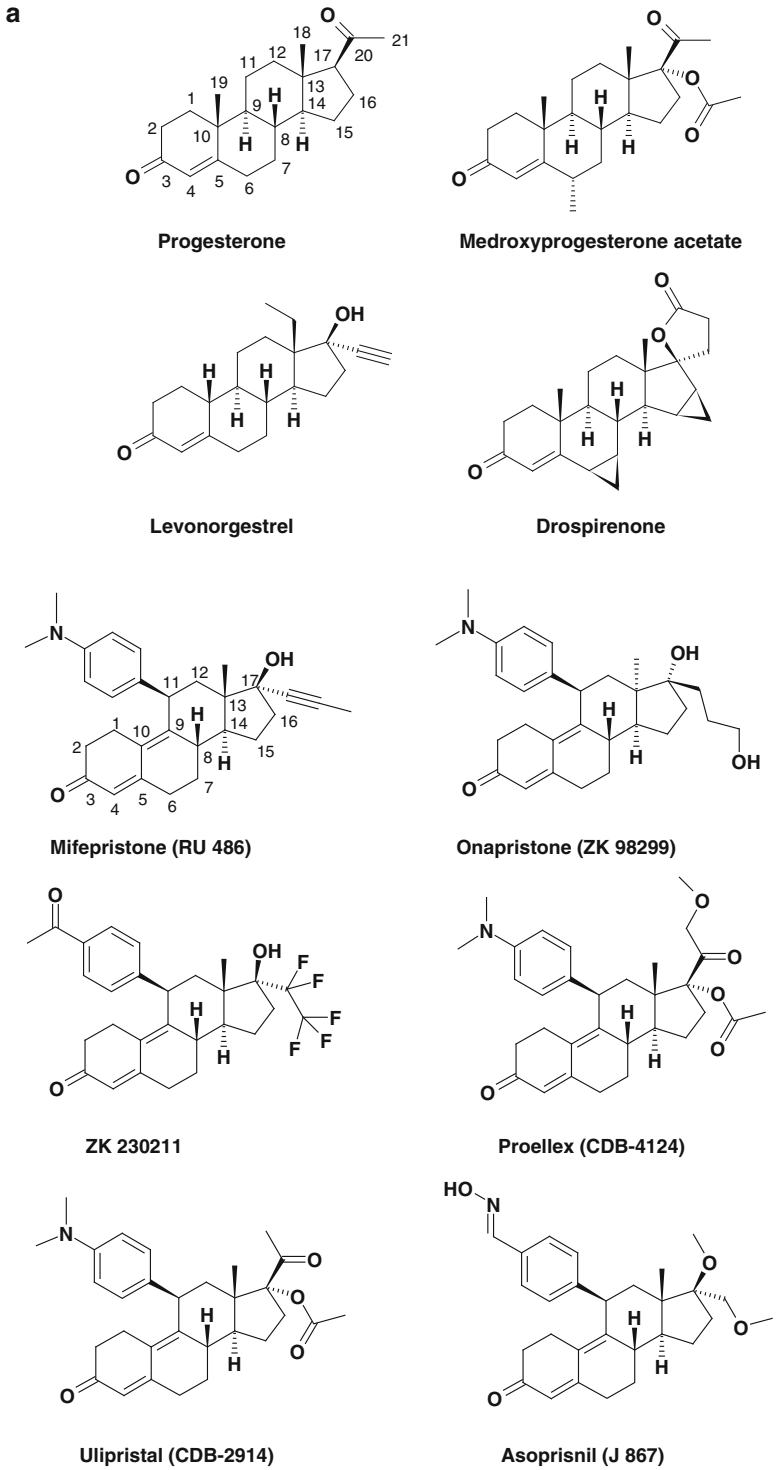


Fig. 2 (continued)

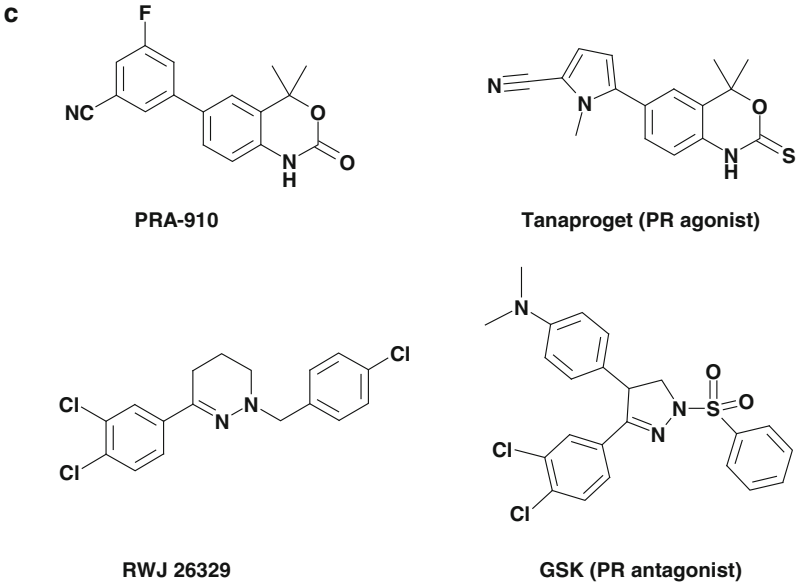


Fig. 2 *Progesterone receptor ligands*. (**a**) Natural PR agonist progesterone and three synthetic analogues. (**b**) Mifepristone and five optimised steroidal PR antagonists/SPRMs which have entered clinical trials. (**c**) Four representative non-steroidal PR ligands

has been refined (Afhuppe et al. 2009). Most interestingly, type III antagonists like lonaprisan demonstrate an enhanced antiproliferative effect in breast cancer cells, underlying their potential utility for the treatment of PR-positive breast cancer (Afhuppe et al. 2010; Busia et al. 2011; Fuhrmann et al. 2000). The clinical relevance of these classifications and characterisations in terms of divergent pharmacological effects has, however, yet to be demonstrated.

Development of Antagonistic Ligands and SPRMs

The first PR antagonist, mifepristone, was discovered in the early 1980s (Baulieu et al. 1987). It is a 19-nor-testosterone derivative that has a 4-(dimethylamino) phenyl group at the 11β-position, a Δ^9-double bond, and a 1-propynyl chain at the 17α-position (Fig. 2b). The 11β-phenyl group was found to be a crucial structural element of all potent steroidal PR antagonists. In contrast to the PR agonists, the pharmaceutical use of PR antagonists is rather limited until now. For many years, mifepristone was the only PR antagonist on the market in several countries and used for induction of abortion (short-term use) (Avrech et al. 1991).

Several clinical trials studying the effect of mifepristone on uterine fibroids have been reported (for a recent overview, please see Spitz (2009)). Overall, mifepristone leads to a shrinkage of fibroids, dependent on dose and duration of treatment, and to a relief of symptoms. Women receiving mifepristone reported an

improvement in leiomyoma-specific quality of life, their rates of anaemia improved and the adjusted uterine size was reduced by 47 % compared to the placebo group.

Mifepristone has also been tested in endometriotic patients in small clinical trials and an improvement of symptoms was reported (Bouchard et al. 2011; Koide 1998; Spitz 2009, 2010).

In postmenopausal women, two studies with mifepristone as second- or third-line treatment for metastatic breast cancer showed an objective response rate (complete response (CR), partial response (PR)) of 10 and 13 %, and stable disease in 54 and 40 % of patients, respectively. A third study was conducted using mifepristone as a first-line treatment. An objective response rate (CR + PR) of 11 % and a stable disease rate of 39 % were reported. However, the strong anti-glucocorticoid side effect of mifepristone requires corticoid substitution, which limits the clinical use of this compound for breast cancer (Klijn et al. 2000; Koide 1998).

Mifepristone also shows relatively strong antagonistic effects towards the glucocorticoid receptor. For long-term application of a PR antagonist to treat chronic conditions, an improvement of selectivity was considered essential. Therefore, considerable effort has been devoted towards optimising the receptor selectivity of steroidal PR antagonists. Since the *para* substituent of the 11β-phenyl group and the 17α-side chain were both found to play a key role for potency and selectivity, these two positions have been the focal points for optimisation.

In addition to mifepristone, five other steroidal PR antagonists have entered clinical trials (Fig. 2b). Recently, one of these compounds, ulipristal (CBD-2914, HRA/Preglem), was launched for emergency contraception (McKeage and Croxtall 2011; Spitz 2009). This compound is currently under clinical evaluation for contraception and for treatment of progesterone-dependent diseases. Ulipristal demonstrated reduction of uterine fibroid size and improvement in quality of life assessments (Bouchard et al. 2011; Orihuela 2007).

Another compound that has entered clinical trials for the indications uterine fibroids and endometriosis is Proellex® (CDB-4124, Repros Therapeutics) (Spitz 2009). This compound is structurally very similar to ulipristal as shown in Fig. 2b. Repros is currently conducting a double-blind phase II study of 12.5, 25 and 50 mg Proellex® in endometriosis and myoma patients. Interim reports related a statistically significant pain reduction with the highest dose tested for up to 6 months (Ioffe et al. 2009).

Onapristone (ZK 98299, Schering AG) is characterised by inversion of stereochemistry at C-13 and C-17 relative to mifepristone as well as by replacement of the propynyl side chain at C-17 by a 3-hydroxypropyl group (Wiechert and Neef 1987). This compound was investigated in the clinic for the treatment of advanced breast cancer and efficacy could be shown in phase II clinical trials where onapristone was investigated as first- and second-line endocrine therapy in patients with breast cancer (Klijn et al. 2000; Robertson et al. 1999). In an explorative phase II clinical trial, 19 patients with primary breast cancer received onapristone at a dose of 100 mg/day. Seventeen of the 19 tumours expressed the ER while 12 of the 18 tumours tested expressed the PR. Of the 18 patients that could be evaluated,

10 (56 %) showed a partial response and 2 (11 %) showed durable static disease (≥6 months), giving an overall tumour remission rate of 67 %. This confirmed that PR antagonists can induce tumour responses in human breast cancer. The median duration of remission was 70 weeks. In a phase II study, onapristone was given at a dose of 100 mg/day to 118 patients with metastatic breast cancer resistant to tamoxifen. The objective response rate was 10 % and in 39 % of the patients there was stable disease for at least 3 months. The overall time to progression was 4 months. After some patients developed abnormalities in liver function tests, development was terminated.

As the 17α-side chain was found to be a key factor for selectivity, this position was chosen for a detailed structure–activity relationship analysis in order to find highly potent PR antagonists with reduced endocrine side effects. As a result of an intensive optimisation programme at Bayer HealthCare, ZK 230211 (lonaprisan) was discovered, with a pentafluoroethyl group as new side chain (Fuhrmann et al. 2000). The compound combines high PR antagonism with low undesirable hormonal activities. Phase I clinical trials have been completed successfully.

The last compound depicted in Fig. 2 is asoprisnil. This compound was originally discovered by the German company Jenapharm (DeManno et al. 2003). In contrast to the PR antagonists described before, asoprisnil exhibits a partial agonistic/antagonistic profile in some in vivo models and is therefore referred to the substance class of SPRMs. The term SPRM refers to compounds with mixed agonist/antagonistic properties. Structurally, asoprisnil is characterised by a modification of the *para* phenyl substituent (the dimethylamino group was replaced by an oxime) and of the C-17 function. Asoprisnil was tested in clinical trials for the treatment of uterine fibroids and endometriosis by Takeda Abbott Pharmaceuticals (Bouchard et al. 2011; Spitz 2009; Wilkens et al. 2008). Recently, results from a randomised, double-blind, placebo-controlled phase II study in fibroid patients treated with asoprisnil were published. Asoprisnil displayed beneficial effects on uterine bleeding, fibroid volume, bloating and pelvic pressure. Furthermore, asoprisnil was associated with follicular-phase oestrogen concentrations and minimal hypooestrogenic symptoms. Two randomised, placebo-controlled phase II studies have been conducted for asoprisnil in subjects with pain derived from endometriosis, and one has been reported in abstract form. Doses tested in these studies ranged from 0.5 to 25 mg for 3 months. Doses of 5, 10 and 25 mg significantly reduced the average daily non-menstrual pelvic/dysmenorrhoea pain scores compared to placebo.

The mechanisms that account for the beneficial effects of PR antagonists and SPRMs in endometriosis are not clearly understood. One probably has to distinguish between direct effects of the PR antagonists and SPRMs on the endometrium and endometriotic lesions, and overall systemic effects. Regarding the latter, it is likely that suppression of oestradiol to proliferative phase levels has a positive effect on overall pathology. The direct endometrial effects of the PR antagonists and SPRMs are unique and do not match traditional classifications. Overall, PR antagonists have been shown to exert an antiproliferative effect on the endometrium, referred to as non-competitive anti-oestrogenic action.

Non-Steroidal Progesterone Receptor Ligands

Following the identification and development of potent steroidal PR agonists and antagonists, a search for non-steroidal PR ligands was begun. Four representative structures are shown in Fig. 2c. Several of these compounds approach the potency and in vivo activity of mifepristone with better selectivity towards other nuclear receptors. However, no non-steroidal PR antagonist or SPRM has significantly advanced towards market approval up to now.

The most advanced non-steroidal PR agonist is tanaproget which originates from a collaboration between Ligand and Wyeth (Fensome et al. 2005; Zhang et al. 2005). First clinical trials have been performed with this compound to determine the human pharmacokinetic and safety profiles (Bapst et al. 2006). Recently, the compound was sublicensed to Pfizer.

Displayed in Fig. 2c are tanaproget and representative structures of further non-steroidal PR ligands. The most extensively reported class of compounds is exemplified by the Wyeth compound PRA-910 which is structurally related to the agonist tanaproget (Zhang et al. 2007). This compound is 50-fold less potent than mifepristone at PR in vitro, but displays comparable potency in vivo. WAY-255348 is a pyrrole oxindole PR antagonist with a novel mechanism of action: it prevents nuclear accumulation and promoter binding (Yudt et al. 2011).

A series of tetrahydropyridazine PR ligands has been originally described by Ortho Pharmaceutical (Combs et al. 1995; Palmer et al. 2000). This class contains agonists, partial agonists and antagonists, but no clear SAR was elucidated that predicts which profile a compound will possess.

GlaxoSmithKline reported on 4-substituted pyrazoline structures with full PR antagonistic activity and containing the dimethylaminoaryl group also responsible for the antagonistic profile of mifepristone (Jones et al. 2005). Pyrrolidinone compounds with partial PR agonistic activity were furthermore described (Washburn et al. 2009). More recently, non-basic pyrrolidine derivatives with an improved safety profile were characterised (Kallander et al. 2010).

2.2 Oestrogen Receptor

2.2.1 Receptor Biology and Physiology

Oestrone and 17β-oestradiol, the natural "oestrogens", i.e. the physiological ligands of the ER, are formed from androstenedione and testosterone by the action of the aromatase enzyme (Boon et al. 2010; Dahlman-Wright et al. 2006; Findlay et al. 2010; Morani et al. 2008). Physiological production of oestrogens mainly takes place in the granulosa cells of the ovarian follicle. Oestrogens have a variety of actions on the organism, primarily by regulating cell proliferation and growth in the accessory sex organs, such as the uterus and the mammary gland. They regulate their own production by several feedback loops: they inhibit the production of the

gonadotropic hormones FSH and LH in the pituitary, and they inhibit hypothalamic GnRH release in the brain. However, during the oestrous cycle, oestrogens can exert a positive feedback that ultimately leads to a peak in gonadotropin hormones and ovulation (Wintermantel et al. 2006).

Apart from these essential functions in regulating mammalian reproduction, oestrogens act on bone by inhibiting resorbtion (Frenkel et al. 2010; Imai et al. 2010), on blood vessels by protecting endothelial cells and inhibiting vascular smooth muscle cell proliferation and on the brain by protecting neurons from apoptosis (Turgeon et al. 2006).

The main physiological receptor, ERα, is a 55 kD protein expressed at high levels in the uterus, liver, bone, anterior pituitary and mammary gland, as well as in ovarian theca cells and in the hypothalamus in the brain (Ascenzi et al. 2006; Pike 2006; Thomas and Gustafsson 2011).

A second isoform, ERβ, has a slightly lower affinity for oestrogen and is mainly expressed in ovarian granulosa cells, colon, prostate (epithelium) as well as in the hypothalamus (Ascenzi et al. 2006; Couse et al. 2005; Deroo and Buensuceso 2010; Harris 2007; Morani et al. 2008). Experiments using genetically modified mice devoid of ERα, ERβ or both reveal that ERα mediates most of the actions of oestrogens in reproductive tissues and endocrine feedback (Emmen and Korach 2003). ERβ is described to have a role in follicle granulosa cell function (Couse et al. 2005; Hegele-Hartung et al. 2004). The contribution of ERβ to other aspects of oestrogen physiology and pathophysiology is still under investigation, and clinical application of therapies addressing ERβ as the target has yet to be demonstrated (Mohler et al. 2010). A role of ERβ in prostate cancer has furthermore been discussed (McPherson et al. 2006, 2010).

Oestrogen-receptor related receptors (ERRs) are proteins highly homologous to ERα and ERβ, yet they are not recognised by endogenous oestrogens (Tremblay and Giguere 2007). Whether or not other non-nuclear receptors mediate physiological responses to oestrogen, such as, e.g. the recently described G-protein-coupled receptor Gpr30, remains a matter of controversy (Langer et al. 2010).

2.2.2 Clinical Indications

The use of oestrogens in oral contraception has already been described above. Further indications for ER ligands can broadly be classified in three clusters:

- Indications where the receptor should be activated to substitute for endogenous hormone deficiency, such as treatment of postmenopausal hot flushes, vaginal dryness, postmenopausal osteoporosis or sleeping disorders. The central task of drug development in these areas has focused on finding compounds and modes of administration that harness oestrogen's positive effects on the desired target tissue, while not activating oestrogen-dependent cell proliferation in breast and uterine tissue.
- Indications where the receptor should be selectively antagonised to prevent oestrogen-mediated cell growth, in particular for the treatment of gynaecological

diseases such as endometriosis and leiomyomata, as described above. Here, drug development focuses on achieving receptor antagonism in uterine tissue, while maintaining residual agonism in tissues where oestrogen has a positive effect, such as in brain or bone.
– Indications where the receptor should be fully antagonised, namely treatment of hormone-dependent breast cancer.

While in the case of breast cancer, the task is to find a full receptor antagonist, in all other indications drug development has focused on achieving tissue selectivity with compounds called "Selective Oestrogen Receptor Modulators" or SERMs.

2.2.3 Oestrogen Receptor Ligands

Depending on their positioning within the ligand-binding domain and the induced conformational changes, ER ligands favour the recruitment of selective sets of cofactors which will ultimately lead to cell- and tissue-specific effects. The elucidation of the crystal structures of ER LBDs complexed with different ligands has greatly improved our understanding of the interactions involved and helped to tailor the pharmacological profile of novel ER ligands (Ascenzi et al. 2006; Egner et al. 2001; Lonard and Smith 2002; Shiau et al. 1998; Wurtz et al. 1996).

Oestrogen Receptor Agonists

The physiological ligand 17β-oestradiol is pharmacologically used to activate the ER. Oral availability and metabolic stability are however low.

A major breakthrough in hormone research was the discovery in the 1930s by Inhoffen and Hohlweg that 17-α-substituted steroids exerted oral activity (Hohlweg and Inhoffen 1947). This paved the way for the development of ethynyloestradiol, which until today is the most widely used oestrogenic component in combined hormonal contraceptives. Marketed oestrogens are shown in Fig. 3a.

The ethynyloestradiol content usually found in combined oral contraceptives (COC) ranges from 15 to 35 μg (Gallo et al. 2011; Maguire and Westhoff 2011). In some countries, COCs containing mestranol, a prodrug of ethynyloestradiol and the first oestrogenic component ever used, are available (McLaurin et al. 1991). Recent focus was on the introduction of the natural hormone oestradiol as the oestrogenic component of COC. In 2009, a COC containing the oestradiol prodrug oestradiol-17-valerate and the progestogen dienogest was introduced by Bayer Healthcare Pharmaceuticals as Qlaira® in Europe and as Natazia® in the United States (Whalen and Rose 2011).

In the 1950s, several approaches have been made to develop non-steroidal ER agonists. Diethylstilbestrol (DES) was used to treat pregnancy complications but was however later shown to be associated with tumours of the reproductive tract in daughters of women who had taken this compound (Baird and Newbold 2005; Stillman 1982).

Following the discovery of the second physiological oestrogen receptor, ERβ, several projects set out to dissect the respective roles of the two receptors with

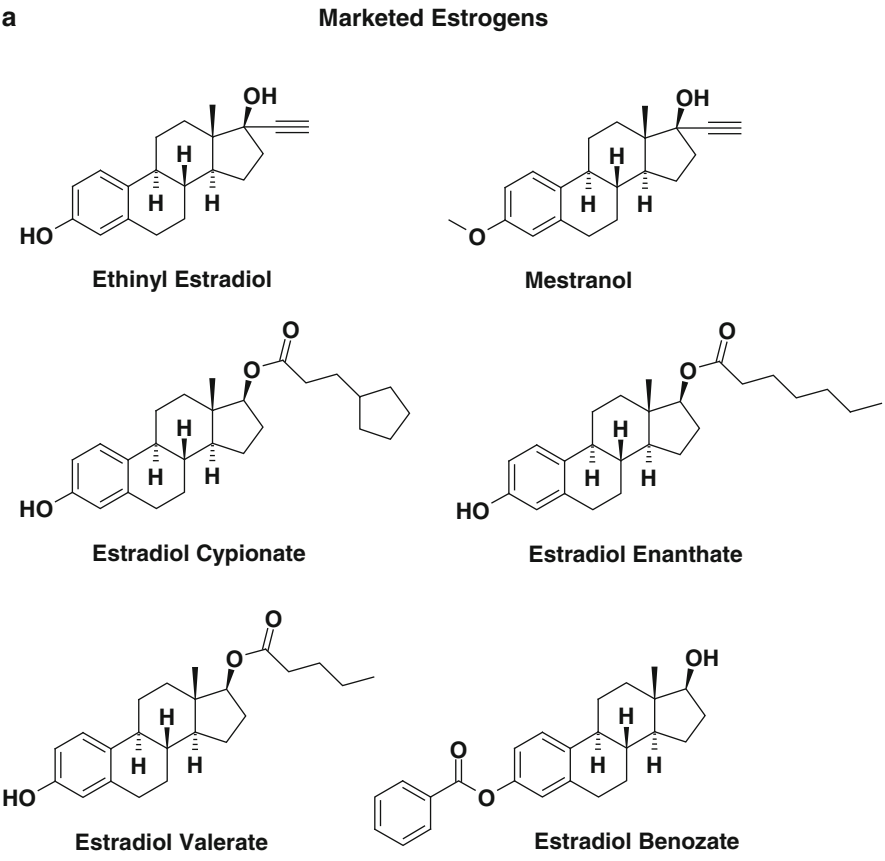
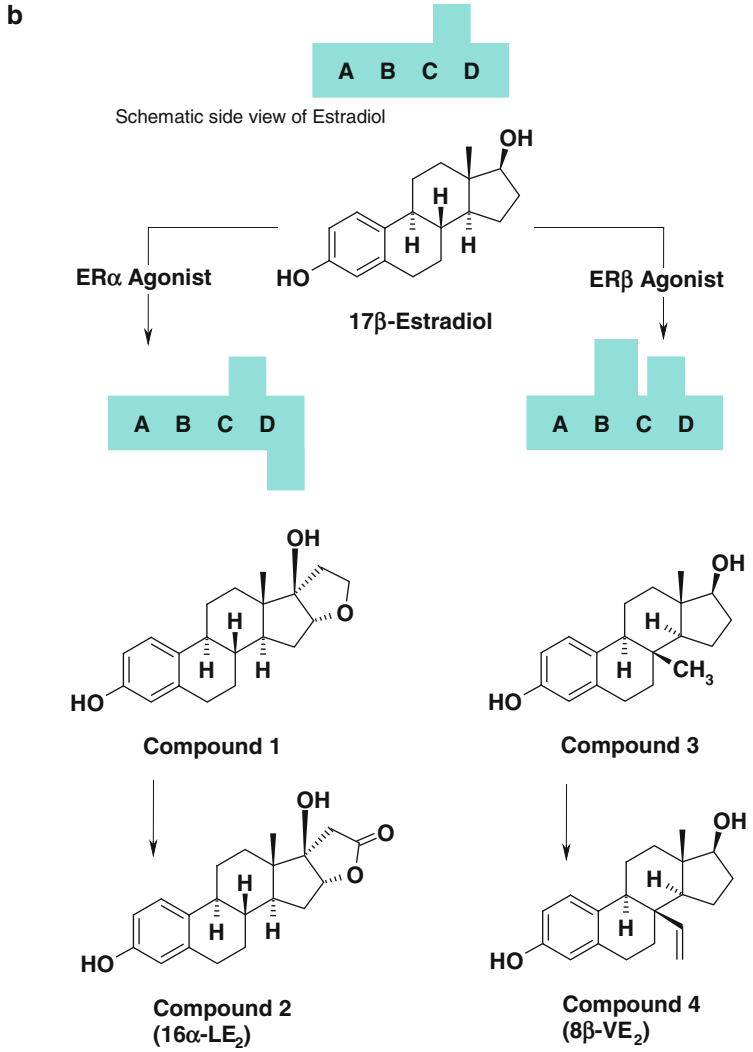


Fig. 3 (continued)

pharmacological means. The LBDs of the two receptors are however highly homologous with only three amino acid differences (Pike 2006). A structure-guided approach revealed that two of these amino acid differences were oriented towards the ligand-binding pocket (Hillisch et al. 2004). This allowed the rational design of two oestradiol derivatives, namely 16α-LE_2 and 8β-VE_2, that probe the different cavities (Fig. 3b). Natural steroidal compounds with preference for ERβ comprise the steroidal metabolites 5-androstenediol, 3β-androstandiol and the metabolite of oestradiol, oestriol (Minutolo et al. 2011; Mishra et al. 2006).

Besides the synthetic steroidal subtype-selective agonists,16α-LE_2 and 8β-VE_2, a number of non-steroidal core structures have been identified as a basis for the synthesis of selective ER agonists (Fritzemeier et al. 2004; Harris 2006; Hillisch et al. 2004; Minutolo et al. 2011). A diphenolic element is common to most compounds with an inter-phenolic distance and geometry imitating the natural 17β-oestradiol. Additional pharmacophore elements were identified throughout the various chemical classes influencing the preference for either ERα or ERβ.



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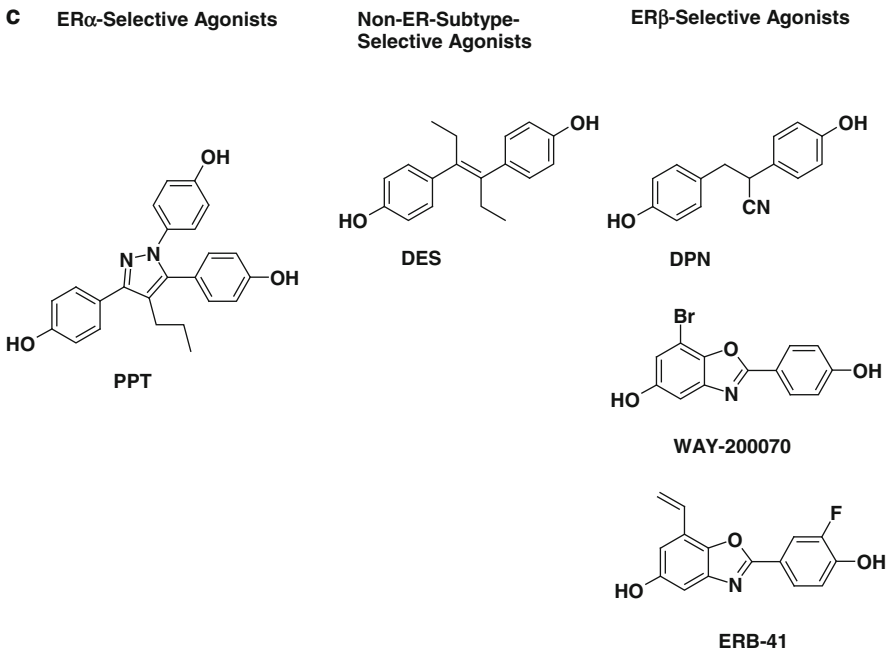
Recent advances in the design of ERβ agonists and their potential applications have been reviewed in comprehensive articles (Minutolo et al. 2011; Mohler et al. 2010; Nilsson and Gustafsson 2011).

Prototypes of non-steroidal ERβ agonists are diarylpropionitril (DPN) identified by J. Katzenellenbogen (Katzenellenbogen et al. 2000) and the benzoxazol derivatives ERB-41 (WAY-202041) and WAY 200070 (Harris 2007) whereas the prototypic non-steroidal ERα agonist is 4,4′,4″-(4-propyl-1*H*-pyrazole-1,3,5-triyl)trisphenol (PPT) again identified in the laboratory of J. Katzenellenbogen (2000). The structures are shown in Fig. 3c.



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Some natural non-steroidal compounds such as coumestrol and genistein (which are referred to as phytooestrogens) exhibit some ERβ preference (Mueller et al. 2004), whereas others such as 8-prenylnaringenin show selectivity for ERα (Schaefer et al. 2005).

Subtype-selective agonists have been applied in a variety of animal models as tools to dissect biological functions of ERα and ERβ (Fritzemeier et al. 2004; Hegele-Hartung et al. 2004) and in animal models of human disease to test their therapeutic potential (Harris 2006, 2007; Katzenellenbogen et al. 2000; McPherson et al. 2006, 2007, 2010; Mohler et al. 2010; Nilsson and Gustafsson 2011; Schleipen et al. 2011)

First clinical studies with the ERβ agonist ERB-041 were so far not successful and the anticipated therapeutic benefit could not be achieved (Roman-Blas et al. 2010).

Partial Agonism: SERMS

Non-steroidal structures were also investigated in the early search for ER antagonists (Fig. 3d). The phenyl-substituted ethylene scaffold, which is similar to DES mentioned above, led to the development of tamoxifen, the first synthetic antagonistic ER ligand (Jordan 1997). It was soon discovered however that tamoxifen is a partial ER antagonist, effectively inhibiting oestrogen signalling in breast cancer

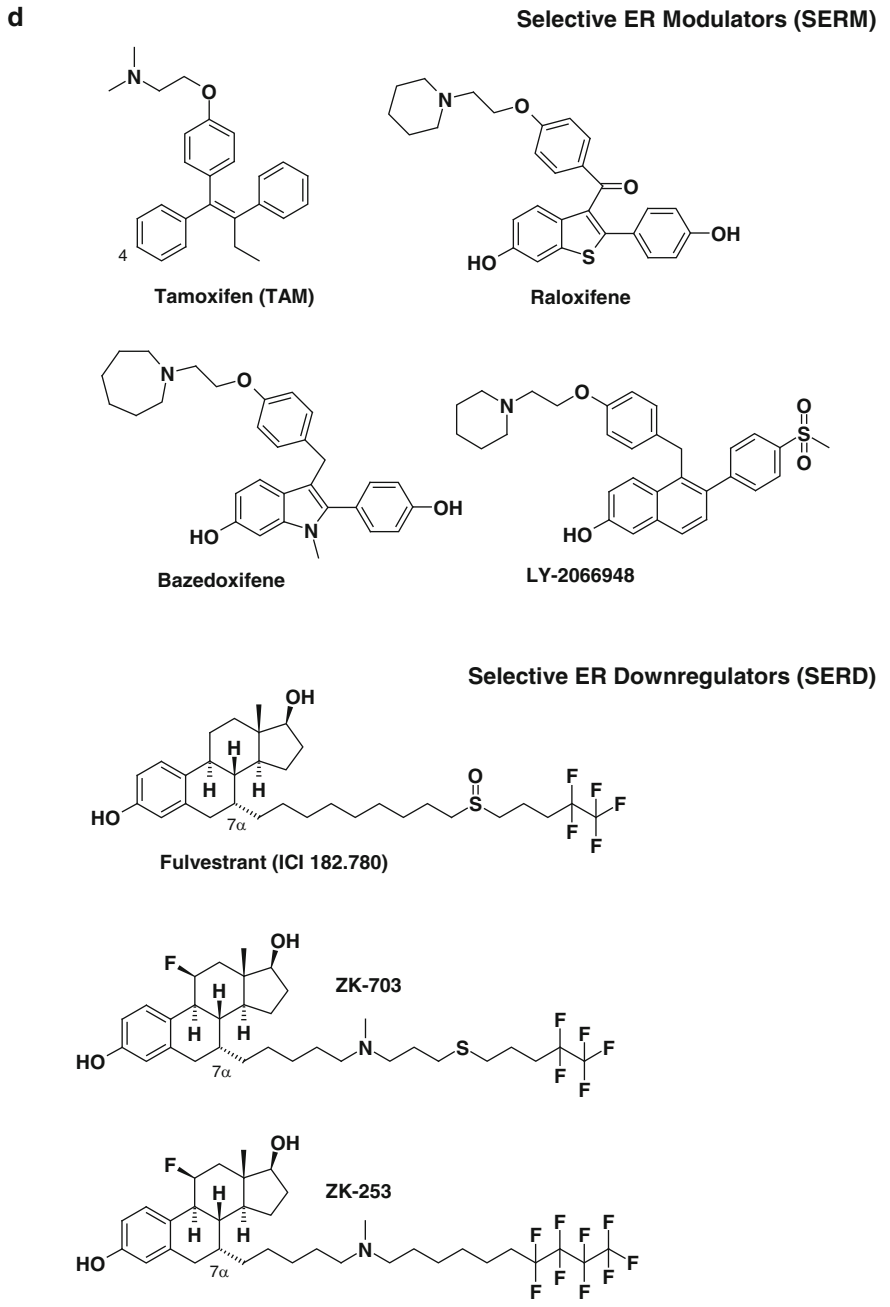

Fig. 3 *Oestrogen receptor modulators*. (**a**) Steroidal oestrogens in clinical use. (**b**) Principle of subtype-selective oestrogen receptor ligands. (**c**) Non-steroidal ER agonists and their subtype selectivity. (**d**) Partial and full oestrogen receptor antagonists: SERMs and SERDs.

cells, but evoking agonist-like actions in uterus and bone (Katzenellenbogen et al. 2000). This discovery has established the concept of selective ER modulation by non-steroidal drugs that displace oestrogen binding to the receptor and show tissue-selective agonism. Actually, first studies on SERM effects on bone were fuelled by the concern that blocking the ER might have negative effects on bone. The discovery that SERMs can actually be agonistic on bone allowed the development of another SERM, raloxifene, specifically for the treatment of postmenopausal osteoporosis (Recker et al. 2011). Selective effects of SERMs on the cardiovascular system have furthermore been described, suggesting that they could be beneficial for the treatment of coronary heart disease (Mishra et al. 2006; Regitz-Zagrosek et al. 2007). In line with the manifold effects of oestrogens on different target tissues, a number of SERMs have been synthesised and analysed for a variety of indications.

Chemically, most of the non-steroidal SERMs follow a modular concept with a lipophilic, planar core structure containing at least one phenylic element mimicking the steroid A ring, thereby providing receptor binding and selectivity. The actual antagonism is brought about by the side chain of varying length and structure. The side chain in experimentally verified 3D structures of the antagonist-bound receptor has been shown to displace helix 12 (see above) and thus switches the receptor to the "off" state, precluding coactivator recruitment (Musa et al. 2007).

However, in most cases, DNA binding of the antagonist-bound receptor is still possible, and therefore residual target gene activation via the AF-1 can lead to partial agonism. The challenge in drug discovery has been to achieve the "right" degree of partial agonism at the molecular level in order to achieve the tissue selectivity in signalling required for the desired clinical indication.

Tamoxifen

The triphenylethylene compound tamoxifen is the first selective ER modulator described (Jordan 1997). Tamoxifen is a prodrug converted into 4-hydroxytamoxifen, which has a 300-fold higher affinity for the receptor than the non-hydroxylated form. X-ray structures show that the hydroxyl group of 4-hydroxytamoxifen, when bound to the receptor, interacts with the same residues (D253, R394 and a conserved water) as does the A-ring hydroxyl group of 17β-oestradiol, while the side chain precludes helix 12 from adopting an agonist conformation (Shiau et al. 1998).

Discovered in 1966 and tested in clinical trials for breast cancer in 1971, tamoxifen is probably the most used and best-researched SERM. Clinical trials have evaluated the use of tamoxifen in osteoporosis (see above), to trigger ovulation in women with fertility problems, to prevent gynecomastia, as well as in neurological indications. Some effects observed when studying non-classical indications have however been speculated to be due to off-target effects, e.g. the inhibition of protein kinase C in the treatment of mania. A transdermal gel formulation of the active metabolite 4-hydroxytamoxifen is currently in clinical studies for the indication mastalgia (Mansel et al. 2007). However, adjuvant breast cancer

treatment remains the most significant use of tamoxifen. Here, tamoxifen therapy for 5 years resulted in a 34 % reduction in death rate and an absolute reduction in mortality of 9.2 % at 15 years (Mouridsen and Palshof 1983). Prior to patent protection expiry in 2001, sales of tamoxifen have been above $1bn.

Raloxifene

The finding that tamoxifen prevented bone loss in postmenopausal women led to the development of specific SERMs for this indication. Ely Lilly developed the benzothiophene raloxifene for the prevention of bone loss (Clemett and Spencer 2000). Raloxifene is a benzothiophene compound with a lipophilic core, a phenylic OH group and a side chain structurally similar to tamoxifen, but with a piperidine nitrogen instead of a dimethylamine.

In addition to its use in osteoporosis prevention, raloxifene was found in clinical studies to be effective in reducing breast cancer risk. Since 2007, raloxifene is approved for prevention of invasive breast cancer in osteoporotic postmenopausal women and in women at high risk (Ko and Jordan 2011).

Bazedoxifene

Wyeth/Pfizer have developed an indole SERM, bazedoxifene, for the prevention of postmenopausal osteoporosis (Duggan and McKeage 2011; Stovall et al. 2011). Bazedoxifene has also been developed as a combination with conjugated oestrogens to treat postmenopausal hot flushes (Duggan and McKeage 2011; Stovall et al. 2011). The seemingly contradictory combination of an ER agonist together with an ER antagonist actually follows a concept proposed by Labrie et al. (2003): As the desired tissue selectivity for the treatment of postmenopausal ailments (significant oestrogen agonism in brain and bone, and antagonism in peripheral tissues such as uterus and breast) is hard to achieve in a single compound, a combination may be beneficial. An agonist is effective in substituting oestrogen in the brain, and a tissue-selective antagonist that blocks untoward agonist effects in all peripheral tissues, with the exception of bone, where oestrogen action is wanted (and the SERM is agonistic) should be effective. Clinical studies have shown that the combination of bazedoxifene with conjugated equine oestrogens reduced hot flushes and increased bone mineral density. The combination product, Aprela®, awaits approval by regulatory agencies (Stovall 2010).

Newer SERMs

While the main indications for tamoxifen and raloxifene—breast cancer and osteoporosis—are mainly or only relevant after menopause, other hormone-dependent diseases such as endometriosis or leiomyoma require a therapeutic principle that is suitable for premenopausal women as well. Most SERMs however lead to ovarian

stimulation, most probably due to their interference with hormonal feedback regulation. Newer developments have focused on SERMs that lack ovarian stimulation such as LY-2066948 (Geiser et al. 2005). The clinical profile of these compounds however needs to be analysed.

Antagonists: SERDs

Pure antagonists of the ER have been found not only to block oestrogen binding, but also to lead to degradation of the ER protein. Therefore, these compounds are also called "Selective oestrogen receptor downregulators" or SERDs (Fig. 3d). In vivo, they exert complete oestrogen antagonism in all tissues (Baumann and Castiglione-Gertsch 2009).

The most prominent example of SERDs is the steroidal compound, fulvestrant (ICI 182,780) first described by Wakeling and colleagues, and today marketed for the therapy of ER-positive metastatic breast cancer under the name Faslodex® (Morris and Wakeling 2002; Osborne et al. 2004). The structure of this compound is directly derived from 17β-oestradiol (E2). The steroid moiety confers receptor specificity and high binding affinity, leading to a higher antioestrogenic potency of fulvestrant compared with other non-steroidal SERMs. The long aliphatic side chain in the 7α-position of the steroid scaffold confers antagonism (as observed in SERMS, see above), and is likely to also be responsible for ligand-triggered receptor degradation. The exact biochemical mechanism of SERD-triggered receptor degradation is unknown, but is likely to be different from the increased receptor turnover described for the oestradiol-activated receptor (Reid et al. 2003).

In the treatment of breast cancer, fulvestrant is administered as a once-monthly injection due to its low oral bioavailability. The size and lipophilicity of this molecular class make it challenging to develop oral steroidal SERDs. In recent times, ZK-703 and ZK-253, both derived from the fulvestrant structure, have been published as potent oral antioestrogens (Hoffmann et al. 2004). Ongoing developments show that modulation of the steroid scaffold can contribute to increased oral bioavailability (NH, TW, unpublished).

2.3 Androgen Receptor

2.3.1 Receptor Biology and Physiology

Testosterone and dihydrotestosterone (DHT) are the main androgenic steroid hormones (Fig. 4) and are involved in a broad range of biological processes (Li and Al-Azzawi 2009; Matsumoto et al. 2008; Wierman 2007; Wilson 1983). Their main function is the development and maintenance of male reproductive organs, and of secondary sexual characteristics. They are also important for regulation of body fat and muscle mass, and for bone turnover. In view of the gender differences

observed in heart failure and atherosclerosis, an implication of sex steroid hormones in the cardiovascular system has furthermore been discussed (Perez-Lopez et al. 2010). The analysis of AR-deficient mice suggests that androgens also play a role in ovarian function and in female reproduction (Matsumoto et al. 2008).

The extensive knowledge gained on the mode of action of the AR makes this receptor a very attractive target for pharmacological intervention (Brinkmann 2011; Ryan and Tindall 2011). AR agonists have been used for many years to replace the natural androgens in early- and late-onset hypogonadism (Bassil 2011; Corona et al. 2011; Dandona and Rosenberg 2010; Ho and Beckett 2011; Nieschlag 2006; Shelton and Rajfer 2012; Traish et al. 2011), selective AR modulators (SARMs) are being clinically tested in patients suffering from frailty and cachexia (Bhasin and Jasuja 2009; Kilbourne et al. 2007; Narayanan et al. 2008b), and antiandrogens have been successfully applied for androgen blockade in prostate cancer patients (Akaza 2011; Barmoshe and Zlotta 2006; Chen et al. 2008; Denmeade and Isaacs 2002).

2.3.2 Clinical Indications

Hypogonadism

Male hypogonadism is due to the failure of testes to produce sufficient testosterone (Bassil 2011; Dandona and Rosenberg 2010; Ho and Beckett 2011; Lenzi et al. 2009). Depending on the timing of the onset, the resulting phenotype will differ. Primary hypogonadism is primarily caused by testicular trauma or genetic disorders such as Klinefelter's syndrome. Secondary hypogonadism may result from dysfunction of the hypothalamus or pituitary gland. Testosterone replacement is used as therapy and the modality is chosen so that it fits the needs of the patient and provides him with physiological testosterone levels for a prolonged time while being safe and easy to use (Coplan et al. 2011; Corona et al. 2011; Nieschlag 2006).

Late-onset hypogonadism is an age-related condition characterised by many symptoms including diminished libido, reduction in lean body mass, increase in visceral fat and decrease in bone mineral density (Bassil and Morley 2010). It is also called andropause, in reference to the gradual decrease in circulating testosterone levels observed in the aging male population. It is estimated that the hormone reduction is about 1 % per year and co-morbidities such as diabetes or metabolic syndrome increase the prevalence of late-onset hypogonadism. Following a definitive diagnosis of hypogonadism, testosterone replacement therapy can be envisaged. Risk factors such as a family history of prostate cancer, high haematocrit or poorly controlled heart failure need to be considered.

Frailty Syndrome and Cachexia

The frailty syndrome is associated with two important pathologies, osteoporosis and loss of skeletal muscle (Clegg and Young 2011; Xue 2011). This is often

observed in elderly people and may greatly affect the normal lifestyle. Since the decrease in steroid hormone levels observed in the aging population may play a role in these ailments, replacement therapies have been evaluated (Travison et al. 2011). Testosterone supplementation has been used to alleviate the symptoms linked to these conditions and to restore skeletal muscle mass and strength (Bhasin and Jasuja 2009; Kilbourne et al. 2007; Mohler et al. 2009; Narayanan et al. 2008b). This may however lead to side effects such as prostate events, leg oedema and erythrocytosis. The identification of SARMs with activity on muscle and bone but with no androgenic effects on reproductive organs could therefore offer new treatment options for these patients.

Cachexia is characterised by body weight loss and is frequently observed in patients suffering from late-stage tumours or AIDS (Evans et al. 2008). The underlying reasons are still not clear, but cytokines are likely to be involved so that anti-inflammatory drugs have been used for treatment (Penna et al. 2010). A link between cachexia and androgen deficiency has been proposed, but is still under debate (Vigano et al. 2010).

Prostate Cancer

Prostate cancer is a major health issue with 382,000 estimated cases in Europe in 2008 and its incidence is rising, partly due to the overall aging of the population (Denmeade and Isaacs 2002). Approximately 90,000 men died of the disease in Europe in 2008, making prostate cancer the third most frequent cause of cancer death. Confined prostate tumour can usually be removed by surgical intervention or treated with local radiation. Advanced, metastatic disease is treated by chemical castration which is achieved by blockade of the hypothalamus/pituitary/testis axis with GnRH ligands (Engel and Schally 2007; Schally 2007). This reduces circulating FSH and LH levels, and consequently serum testosterone levels. Since androgen secretion by the adrenal glands is not affected, AR antagonists are often administered additionally in order to achieve a complete blockade of androgen action (Akaza 2005).

Androgen ablation works in most patients and is associated with only a few side effects (Barmoshe and Zlotta 2006; Bianchini and de Bono 2011; Labrie 2011). Treatment resistance however often occurs after 18–24 months and this disease stage is named castration-resistant prostate cancer (CRPC). It can be treated with cytotoxic chemotherapeutics (e.g., taxanes), but the benefit is limited (Beardsley and Chi 2008; Tannock et al. 2004) and a number of clinical studies are ongoing to identify new compounds addressing the AR axis or other targets.

Numerous recent studies indeed show that the AR pathway is still the major player in CRPC (Chen et al. 2008; Feldman and Feldman 2001; Knudsen and Penning 2010). Analysis of tumour samples reveals that AR gene amplification often leading to overexpression occurs in roughly one-third of CRPC patients (Visakorpi et al. 1995). Then, AR mutations have been identified in tumour biopsies from many patients developing resistance to antiandrogen treatment (Bergerat and Ceraline 2009; Steinkamp et al. 2009). Recent studies with circulating tumour cells

(CTCs) suggest the frequency of such mutations to be fairly high (Jiang et al. 2010). Quite often these mutations make the AR promiscuous with regard to ligand activation so that weak androgens or non-androgenic steroids become relevant AR agonists (Brooke and Bevan 2009; Feldman and Feldman 2001). In addition, mutations that convert antagonists into agonists have been identified, explaining why temporary improvement is sometimes observed when interrupting antiandrogen treatment (Miyamoto et al. 2004). Another mechanism leading to resistance is the increase of intra-tumoural androgen levels due to de novo androgen synthesis or enhanced conversion of adrenal androgens (Montgomery et al. 2008; Waltering et al. 2009). The positive results of clinical studies performed with a compound targeting the steroidal biosynthesis path led to the recent approval of abiraterone acetate (Pal and Sartor 2011; Pezaro et al. 2011). Finally, stimulation by growth factor signalling pathways may lead to ligand-independent AR activation and play a role in therapy resistance (Knudsen and Penning 2010).

Polycystic Ovary Syndrome

Approximately 5–10 % of the female population suffer from polycystic ovary syndrome, which is due to overproduction of androgens by the ovaries (Artini et al. 2010; Saha et al. 2012). Besides clomiphene citrate as treatment for anovulation and infertility, antiandrogens are being used to alleviate the symptoms of hyperandrogenicity such as hirsutism and acne.

2.3.3 Androgen Receptor Ligands

Androgen Receptor Agonists

The treatment of male hypogonadism with testosterone has been in use for about 70 years (Edelstein and Basaria 2010; Nieschlag 2006; Nieschlag et al. 2004). The marketed compounds are derived from the testosterone core (Fig. 4) and can be classified by their route of administration (Bhasin et al. 2010; Edelstein and Basaria 2010). Intramuscular injection of testosterone esters was introduced first. An application is needed every 2 months to achieve the desired hormone levels. A strong, supraphysiological peak in serum concentration is observed after the injection, which may cause mood changes. More recently, the long-acting testosterone undecanoate (Fig. 4) form has been introduced (Edelstein and Basaria 2010). It is usually given every 3 months by slow intramuscular injection. Transdermal gels containing testosterone are available since 2000 (Gooren 2009). They allow consistent maintenance of physiological testosterone levels and have become very popular due to simplicity of use, absence of injections and negligible dermal reactions. Transdermal patches are as effective as gels but less well accepted, due to skin irritation. Buccal tablets containing testosterone or testosterone undecanoate are also available, but their bioavailability is limited due to metabolisation in the liver (Edelstein and Basaria 2010).

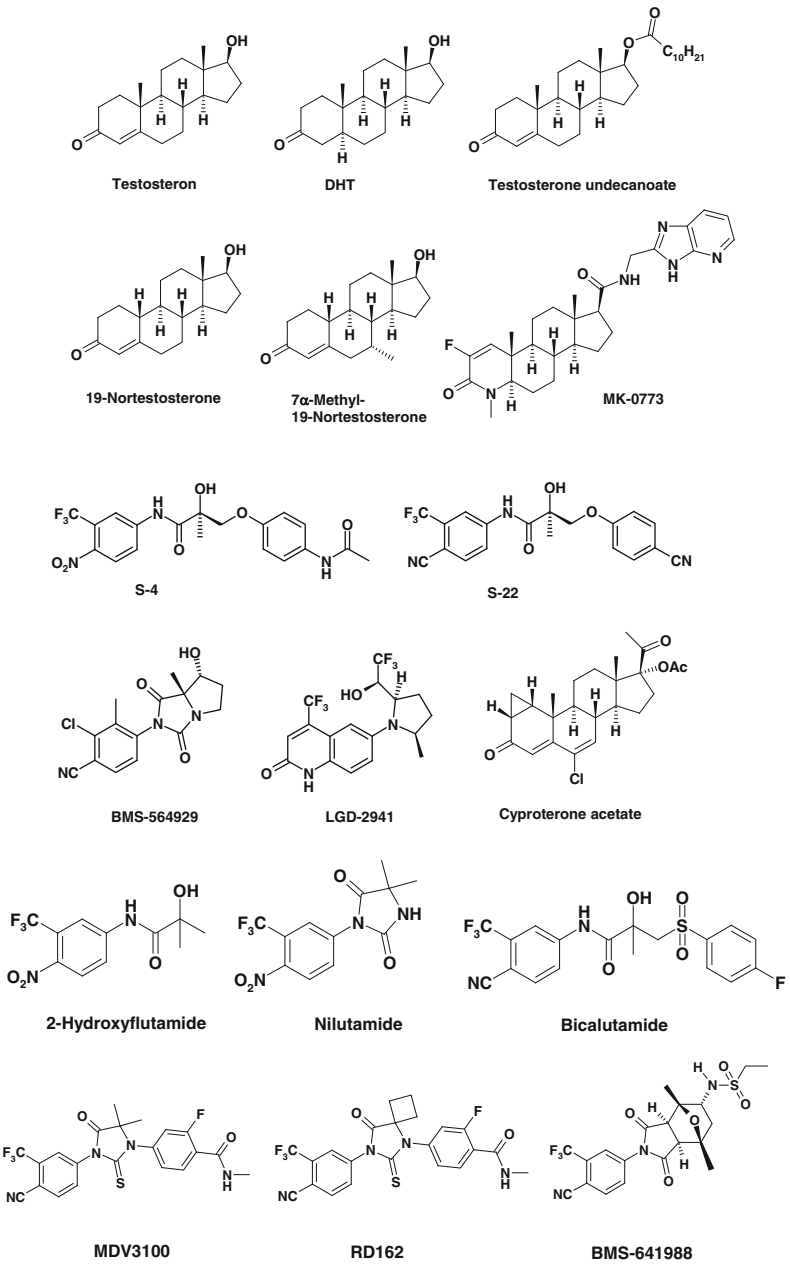

Fig. 4 Androgen receptor ligands

Selective Androgen Receptor Modulators

Frailty syndrome and cachexia can be treated with SARMs. These compounds should have selective androgenic activity on muscle and bone but not on the prostate (Bhasin and Jasuja 2009; Kilbourne et al. 2007; Mohler et al. 2009; Narayanan et al. 2008b). An essential factor for selectivity is the lack of 5α-reduction or aromatisation (Gao et al. 2005). Another possible mechanism may be the differential recruitment of cofactors. Indeed, the crystal structure of the AR LBD complexed to SARMs suggests that a specific AR conformation is brought about by ligand binding (Bohl et al. 2005; Sun et al. 2006).

SARMs were first derived from testosterone, but this has limitations with regard to the application routes and potential side effects. Efforts have therefore been undertaken to identify dissociated, non-steroidal androgens with an improved profile and the most advanced compounds are now in clinical trials (Mohler et al. 2009; Narayanan et al. 2008b; Thevis and Schanzer 2010). It should also be noted that regulatory issues linked to the potential abuse of anabolic drugs exist. SARMs are on the list of substances forbidden by the International Olympic Committee in 2008 and tests to detect SARM metabolites in urine are being developed (Gerace et al. 2010; Mohler et al. 2009; Thevis et al. 2010).

Steroidal SARMs

Steroidal SARMs are derived from testosterone and formulated for dermal patches or injections (Edelstein and Basaria 2010; Nieschlag 2006; Nieschlag et al. 2004). Removal of the methyl group at position 19 gives 19-nortestosterone (Fig. 4), an androgen which is less susceptible to aromatisation but still a substrate for the 5α-reductase. Substitution at the position 7α by alkyl groups, as exemplified by 7α-methyl-19-nortestosterone (Fig. 4), reduces the susceptibility to 5α-reductase activity, thus enhancing anabolic properties and reducing the effects on the prostate. Esterification of the testosterone 17β group increases hydrophobicity and leads to a prolonged duration of action, as exemplified by testosterone undecanoate (Fig. 4).

The 4-aza-steroid MK-0773 has recently been described (Fig. 4). It stimulates lean body mass and bone formation in an ovariectomised female rat model, whereas limited activity is seen on the prostate and seminal vesicles of orchiectomised male rats (Schmidt et al. 2010). The compound was tested in clinical trials and initial phase I results show anabolic effects after a 12-week treatment.

Non-Steroidal SARMs

Aryl propionamides were the first non-steroidal SARMs showing tissue-selective activity, followed soon after by pyrrolidinoquinoline derivatives (Mohler et al. 2009; Narayanan et al. 2008a; Zilbermint and Dobs 2009). Later, several structurally different SARMs were identified, but only those that have advanced to clinical studies are described below (Fig. 4).

S-4 (Andarine) and S-22 (GTx-024, MK-2866) are two related aryl propionamides. Rat studies with S-4 show full agonistic activity for the *levator ani* muscle and partial agonism for the prostate (Gao et al. 2005; Kearbey et al. 2007). Improvement in lean body mass and of skeletal muscle strength and prevention of bone loss were observed. S-4 was tested in clinical phase I studies but side effects were observed, leading to discontinuation. The related S-22 SARM shows preferential anabolic effects on the rat *levator ani* muscle (Narayanan et al. 2008a). Several clinical phase II studies have been performed with S-22 (Zilbermint and Dobs 2009). In one study, 120 elderly men and women were treated and those receiving a 3 mg/day dose had improvement in lean body mass and in muscle function. Another trial recruited patients with cancer cachexia. Here also, improvement in lean body mass and in muscle function was reported following treatment with 1 or 3 mg (Mohler et al. 2009; Narayanan et al. 2008b).

The hydantoin-derived substance BMS-564929 is a subnanomolar AR agonist with high receptor selectivity (Ostrowski et al. 2007). It stimulates muscle but not prostate tissue. It also strongly reduces endogenous LH levels in animal models, which may be an obstacle for further development, if this is also seen in humans. Clinical studies have been started but no results are currently available.

LGD-2941 is a pyrrolidinotrifluoromethylquinolinone with potency and efficacy comparable to DHT on the rat levator ani and with much reduced activity on the prostate (Martinborough et al. 2007). Positive effects on muscle and bone were observed in rats and cynomolgus monkeys. LGD-2941 is currently in a phase I clinical trial for the indications osteoporosis and frailty.

2.3.4 Androgen Receptor Antagonists

Marketed Antiandrogens

Competitive AR antagonists were the first antiandrogens described (Akaza 2011; Haendler and Cleve 2011; Labrie 2011; Sharifi 2009) (Fig. 4). They occupy the same binding niche as the natural ligands and lead to conformational changes that do not favour coactivator recruitment and receptor activity. They generally have only weak affinity for the AR in comparison to DHT and promote nuclear translocation and binding to regulatory regions of androgen target genes. More recently, compounds with a different mode of action and addressing some of these aspects have been described.

Cyproterone acetate (Fig. 4) was the first antiandrogen tested clinically and was originally found in a programme aiming at the identification of hydroxyprogesterone derivatives without androgenic activity (Barradell and Faulds 1994; Habenicht et al. 1988; Neumann 1994). The first clinical use was reported in 1966 and several studies with prostate cancer patients were performed. Cyproterone acetate acts as a competitive antiandrogen which additionally inhibits gonadotropin secretion. Clinical studies showed efficacy and fewer side effects compared to oestradiol undecyclate, which was the standard oestrogen treatment for prostate

cancer at that time. Cyproterone acetate is also one of the active ingredients of a contraceptive pill with positive effects on some acne types.

Flutamide is a non-steroidal, acetanilide-derived compound with pure antiandrogenic properties (Brogden and Chrisp 1991). It is converted in vivo to the active form 2-hydroxyflutamide (Fig. 4). It was approved by the FDA in 1989 for locally confined metastatic prostate cancer in combination with a GnRH agonist.

Nilutamide (Fig. 4) belongs to the nitrotrifluorotoluene group and is related to flutamide (Dole and Holdsworth 1997). It is approved since 1996. However, side effects including pneumonitis and delayed darkness adaptation limit its use.

Bicalutamide is an oral, non-steroidal, fluorophenyl-derived substance (Fig. 4) approved since 1995 for the treatment of metastatic prostate cancer in combination with a GnRH ligand (Cockshott 2004). It is currently used in its racaemic form but post-approval investigation revealed that the *R*-enantiomer is the active principle. The *R*-enantiomer has a better affinity for the AR, a longer half-life and accumulates in the plasma whereas the *S*-enantiomer is inactive. Bicalutamide has a side-effect profile superior to that of other antiandrogens and is currently the standard-of-care antiandrogen for prostate cancer treatment (Akaza 2011). The results of a large-scale study with over 8,000 patients show that a dose of 150 mg/day of bicalutamide may be beneficial in patients with locally advanced prostate cancer, irrespective of the initial therapy (Wirth et al. 2008).

Despite initially good responses, most prostate cancer patients will develop resistance to antiandrogen treatment (Attar et al. 2009b; Bianchini and de Bono 2011; Chen et al. 2008; Feldman and Feldman 2001). There is therefore a high medical need for the identification of novel antiandrogens which delay the occurrence of resistance and show activity in CRPC patients.

Antiandrogens in clinical development

MDV3100 and RD162 (Fig. 4) are diarylthiohydantoins structurally derived from nilutamide and displaying high binding affinity for the AR (Clegg et al. 2012; Tran et al. 2009). In contrast to other antiandrogens, they do not lead to nuclear import of the AR. RD162 is more potent than bicalutamide in reducing expression of several androgen target genes and in preventing the binding to gene regulatory regions. Antitumour effects were observed for RD162 and MDV3100 in different prostate cancer xenografts, including models that express high AR levels and are resistant to treatment with other antiandrogens. MDV3100 was tested in 140 CRPC patients with progressive, metastatic disease at doses varying between 30 and 600 mg daily (Mukherji et al. 2012; Scher et al. 2010). PSA decrease and conversion from unfavourable to favourable CTC count were observed. The maximal tolerated dose was 240 mg and the most common adverse event reported was fatigue. For the randomised phase III study, 160 mg is applied. Two studies were initiated in 2010, one with CRPC patients already treated by chemotherapy and one with chemonaive CRPC patients. Interim analysis of the first study showed median survival to be increased by 4.8 months and death risk to be reduced by 37 %. Filing for approval is planned for 2012 (Mukherji et al. 2012).

Very recently, a related antiandrogen named ARN-509 was described (Fig. 4). It shows in vivo antitumour effects at lower doses than MDV3100 (Clegg et al. 2012). A clinical phase I/II study was initiated with this compound in patients suffering from progressive CRPC.

BMS-641988 is an oxabicyclo-imide-based antiandrogen (Fig. 4) with much higher AR-binding properties and more potent antiandrogen activity in transactivation, compared to bicalutamide (Attar et al. 2009a). It shows efficacy in several prostate tumour xenograft models and delays the time until relapse takes place. A phase I dose-escalation clinical study was initiated in CRPC patients who received between 5 and 100 mg per day (Rathkopf et al. 2010). A partial response was observed in one patient and PSA decline of more than 30 % in 16 % of patients. These modest antitumour effects and the occurrence of an epileptic seizure led to the termination of the trial.

Take Home Messages

- Sex steroid receptors are important molecular targets, not only for hormone replacement but also for a variety of gender-specific diseases and symptoms
- By physiological function, they coordinate specific changes in gene expression and cellular responses in response to a small-molecule signal from outside—thus, they are ideal targets for drug discovery, allowing for the development of potent and highly selective (towards this class of receptor) ligands
- Due to the homology in primary structure of steroid receptors, synthetic steroidal ligands can be designed that target more than one receptor from this family
- Both agonism, selective agonism, and antagonism can be evoked by ligands addressing the same binding site, e.g. steroid derivatives
- The biggest challenge—and opportunity—of current drug discovery approaches is to achieve a desired profile of tissue-specific activities

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