Introduction

Steroid hormones for contraception and for hormone replacement therapy (HRT) belong to the most commonly used drugs in the world, especially in the western developed countries. It was estimated that worldwide at least 180 million women had ever taken oral contraceptives (OC) in their life [1] and more than 20 million women were using HRT in the late 1990s [2]; in the USA, more than 10 million young women were current OC users according to a study published in 1998 [3] and 38% postmenopausal women aged 50-74 used HRT in 1995 for the relief of menopausal complaints and for the supposed primary prevention of cardiovascular diseases and osteoporosis [4]. In contrast to drugs used for the treatment of specific diseases, contraceptives and HRT products are taken mainly for the purpose of contraception and supposed disease prevention by healthy fertile women and postmenopausal women (mostly also healthy). For a long time, the health-related outcomes of contraceptives and HRT, either favorable or unfavorable, have been of great concern in the scientific communities as well as in public media due to their extensive use in the general population.

The active agents of contraceptives and products for HRT are solely or jointly used estrogens and progestogens, which are known not only as drugs, but also as important hormones in the female body, or for at least as closely related to these hormones both in chemistry and with aspects to the pharmacological effects. To understand better the effects of estrogen and progestogen, both wanted and unwanted, it is necessary to introduce briefly the physiology of hormones first.

1. What are hormones?

The term 'Hormone' was derived from Greek 'Hormaein', which means 'to urge on', 'to excite'. Hormones, by definition, are signaling molecules produced by endocrine glands or specific cells in the body and transported by the bloodstream to influence distant specific target organ cells expressing specific hormone receptors [5]. Hormones are characterized by high efficacy at low secreted amounts. According to their chemical structures, hormones can be largely divided into three major classes [6], namely:

- Peptide- and proteo-hormones, such as gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), insulin, growth hormone, thyroid-stimulating hormone, adrenocorticotropic hormone etc.. Most of known substances with hormone character in human body belong to this group.

- Amine hormones, which derive from single amino acids, such as thyroxine (T4), triiodothyronine (T3), adrenaline, nor-adrenaline and melatonin.

- Steroid hormones: including corticoids (glucocorticoids and mineralocorticoids) and sex hormones.

2. Steroid hormones

Steroid hormones can be distinguished from the other two hormone groups in many aspects. Steroid hormones are derived from cholesterol after enzymatic cleavage of the side chain (see Fig. 1) [5,7]. Retaining the main chemical structure of cholesterol, steroid hormones are small lipid-soluble molecules with four interconnected rings of carbon atoms, whose basic physico-chemical characteristics enable them to readily cross cellular membranes composed of a lipid bilayer. After entering a cell, steroid hormones cause changes in biologic activity via binding to intracellular (cytoplasmic or nuclear) receptors. The receptor-steroid complex of cytoplasmic receptor then translocates to the nucleus of the cell, where it binds to DNA sequences to activate specific genes, thereby directing the production of specific proteins that will effect the physiological actions in the target tissues [8,9]. However, this is only the 'classic' direct genomic pathway of steroids. There is increasing evidence that the actions of steroids like estrogens are also mediated by indirect genomic pathway as well as nongenomic pathway [10]. A membrane estrogen receptor (ER-α or β) coupling to G proteins may regulate gene expression indirectly via second messenger-regulated DNA binding proteins, which then either converges with the genomic pathway or elicits directly many other intracellular actions on tissues [11,12]. In contrast to the classic pathway of steroid hormones actions, proteohormones are unable to enter the cell directly because of their hydrophilic characteristics, but bind to cell-surface receptors which in turn generate complex intracellular signal transduction inside the target cells without involving gene transcription [8]. Therefore, the classic genomic effects of steroid hormones are delayed in onset and prolonged in duration while the effects of protein hormones are very fast (only seconds to minutes) and last only a short time, namely so-called 'non-genomic effects' [13]. However, steroid hormones

also elicit the rapid non-genomic effects via plasma membrane G-protein-coupled receptors, cell membrane ion channels, or mitogen-activated protein kinase etc.. The non-genomic actions of sex steroid hormones have been found in CNS and cardiovascular system [10].
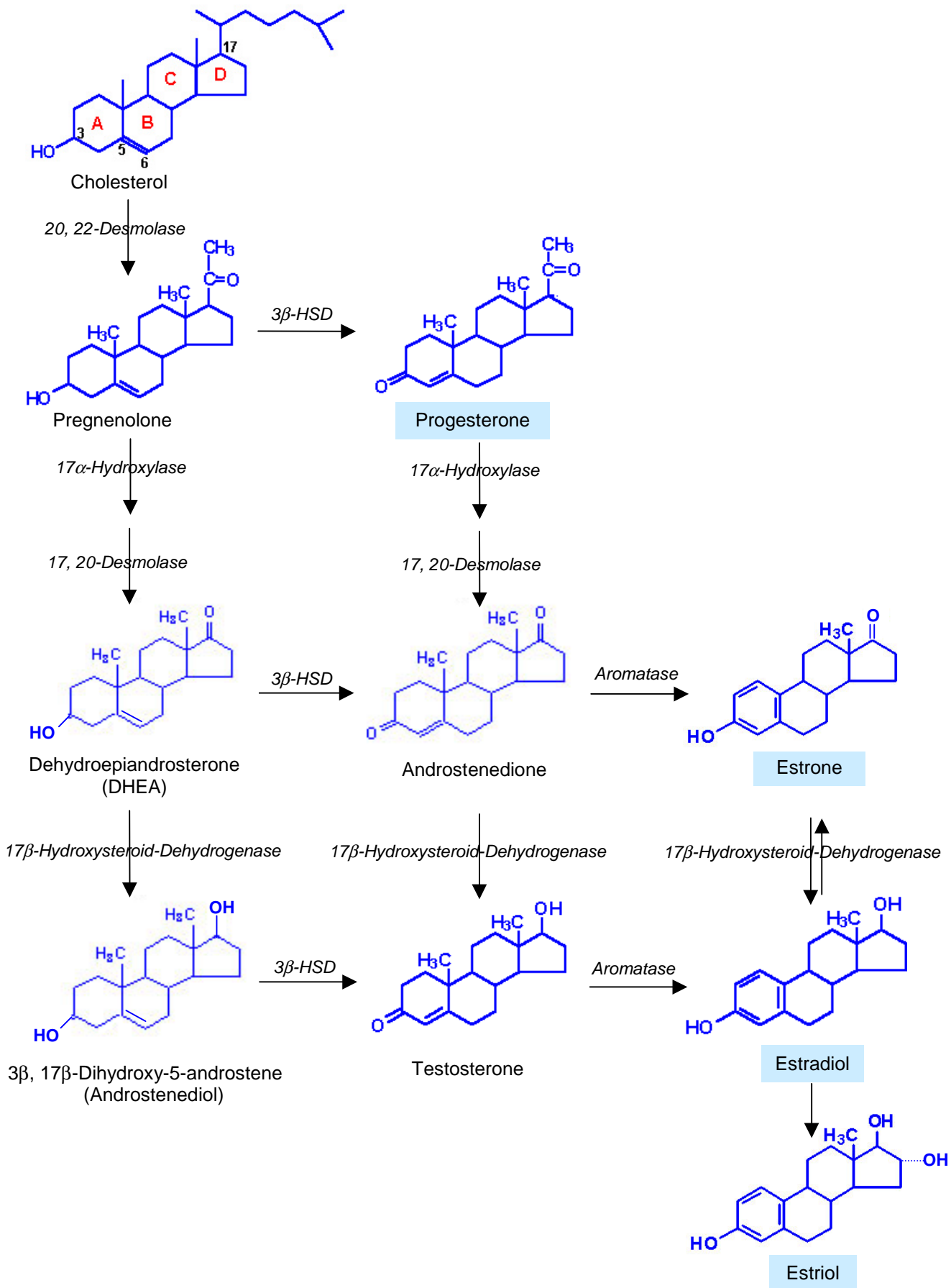
3. Female sex hormones

Sex hormones, by name, act principally on sex organs and play a vital role on sexual development and maturation as well as on reproductive functions, though it was found later that the actions of sex hormones are far beyond the areas related to sex and reproduction.

Sex hormones can be sub-divided into female sex hormones (estrogens and progesterone) and male sex hormones (e.g. testosterone), which are produced mainly in the female ovaries and in the male testes, respectively. However, neither female nor male sex hormones can be found exclusively in the female or in the male body since a minor amount of testosterone can be converted into estradiol (an estrogen) in the testes and the female ovaries can produce testosterone, too [14]. Most of this, however, is converted rapidly into estrogen. In addition, the zona reticularis of adrenal cortex can secrete the androgen dehydroepiandrosterone (DHEA), which may be converted into estrogen in peripheral tissues such as adipose tissues, muscle and skin [15]. This is of particular importance especially for postmenopausal women when the ovaries cease to produce estrogens, in which almost 100% estrogens are synthesized from precursor steroids of adrenal origin [15].

Estrogens and progesterone, the two most important female sex hormones, are functionally antagonistic and synergistic as well on reproductive organs such as the uterus to make a most favorable pregnancy setting and control the menstrual cycle [16]. Though the reproductive system is the classic target of female sex hormones, the physiological effects of female sex hormones, especially of estrogens, are not confined to the area of reproduction only, but extend to the majority of important organs throughout the body [17]. The research related to estrogens has been one of the scientific hot topics since decades due to the widespread biological actions of estrogens found outside and/or inside the reproductive system, which affect the women's health comprehensively [18,19].

Fig. 1: Biosynthesis of sex hormones from cholesterol

3.1 Classification, synthesis and regulation

Formed from androgens, estradiol, estrone and estriol are naturally occurring estrogens in the female body. From Fig. 1, estradiol and estrone can be produced either from androstenedione via a sequence of enzymatic reactions or from testosterone under the action of aromatase that deposits in the ovaries in a large amount but also in the peripheral tissues such as adipose tissues, musculature and skin in a small amount. Aromatization is the final step in estrogen formation from their obligatory precursors androstenedione and testosterone originated from ovaries and adrenal cortex [6]. In this way, estrone and estradiol are formed and can turn into each other under the action of 17β-hydroxysteroid-dehydrogenase. However, the efficacy of these three estrogens differs. 17β-Estradiol is the most potent agent as it has the highest affinity for estrogen receptors. Estrone is less potent with approx. 30% biological activity of estradiol, and estriol owns only ca. 10% biological activity of estradiol, thus it is the least potent [5].

While the primary sources of estradiol in women are the theca and granulosa cells of the ovaries and the luteinized derivatives of these cells, estrone and estriol are primarily formed in the liver from estradiol. In fertile women, 25-100 µg (90-350 nmol) estrogens are produced daily depending on the phase of menstrual cycle, which makes circulating estradiol levels fluctuating from 40 to 200-400 pg/ml. At the end of pregnancy, as much as 30 mg estrogens can be secreted even in one single day [5,7]. After menopause, the secretion of estrogens drops abruptly to approx. 5-10 µg per day and circulating estradiol levels are less than 20 pg/ml, as most of estrogens are synthesized by extragonadal conversion of DHEA and testosterone following the atrophy of ovaries [15,17] and act locally as a paracrine or even intracrine factor at sites such as adipose tissues, osteoblasts and chondrocytes of bone, vascular endothelium and many sites in the brain [20].

Besides estradiol, various other steroids including progesterone, pregnenolone, 17α-hydroxyprogestrone etc. can be produced by human ovaries. Activated by 3β-hydroxy-steroid-dehydrogenase, pregnenolone turns into progesterone, which is the precursor of estrogens and androgens and by far the most important progestogen in female (Fig. 1). Approximately 20 mg progesterone can be produced daily by corpus

luteum at the second half of menstrual cycle in the ovary. During pregnancy, 250 mg progesterone can be synthesized in placenta every single day [5].

Female sex hormones have a short half-life in bloodstream and are not very suitable for oral administration because of the significant first-pass effect (>90% inactivated for orally ingested estradiol). Therefore, different chemical groups have been introduced into 17β-estradiol and progesterone structure to retard the hepatic metabolism. Now a large number of synthetic estrogens and progestogens with an almost identical pharmacological profile to the natural ones are available, yet the half-life is greatly extended to allow the clinical use.

The synthesis and secretion of female sex hormones are regulated by the hypothalamus-pituitary-ovary axis and controlled directly by FSH and LH secreted by anterior pituitary, which is stimulated by GnRH secreted by the hypothalamus. Female sex hormones (endogenous and exogenous) in bloodstream, when reaching certain high levels, inhibit hypothalamus and pituitary by feedback regulation.

3.2 Physiological functions

Like all other steroid hormones, estrogens and progesterone exert their physiological effects mainly through intracellular receptors expressed in varying amount all over the body.

3.2.1 Estrogen

Estrogen receptor (ER)

Currently we are aware of two intracellular estrogen receptors, ER-α [21] and ER-β [22], which are structurally highly homologous. Both subtypes of ERs have several isoforms and splice variants. Stimulated ERs activate the gene transcription (gene expression) [23]. Several studies have suggested that ER-α and ER-β may have quite distinct biological roles in reproductive tissues [24] and ER-β appears to oppose the effects of ER-α in some tissues [25].

Table 1: Distribution of estrogen receptors and physiological functions of estrogens

Target tissues and/or organs	Estrogen receptor	Actions of estrogen	Clinical significance
Urogenital organs (Ovaries, testes, uterus)	ER-α, ER-β	Stimulating the development of female reproductive organs, epithelial lining and the growth of smooth muscle in uterus and vagina	Involved in regulation of menstrual cycle in premenopausal women
Breast (breast cancer cells)	ER-α ER-β	Stimulating the growth of breast epithelial and stromal tissues, the proliferation of glandular and ductal tissues	Developing of breast tissue in puberty; relevance for breast cancer therapy by measuring ER-α and ER-β expression
Bone	ER-α, ER-β	Maintaining the balance of bone metabolism; stimulating the growth of bone-forming osteoblasts; increasing bone mineral density	Increased risk of osteoporosis after menopause; protective effects of HRT against bone loss and osteoporosis
Cardiovascular system (vascular tissues, myocardium)	ER-α, ER-β	Modulating NO, prostaglandin synthase and endothelin gene expression in vascular tissue; suppressing smooth muscle cell proliferation and intima thickening	Maintaining normal cardio-vascular function; putative protective effects against atherosclerosis and/or cardio-vascular diseases
Blood and coagulation	ER-α, ER-β	Promoting blood clotting by enhanced synthesis of factors V, VII and decreasing protein C; increasing platelet adhesion	Risks for thromboembolic events
Liver (lipid synthesis and metabolism)	ER- α	Down-regulating apolipoprotein (a); increasing the expression of angiotensinogen; regulating the level of HMG CoA reductase; increasing synthesis of triglycerides and HDL, decreasing LDL	Improving lipid profile; beneficial effects on cardiovascular diseases
CNS (Pituitary, hypothalamus, neocortex, hippocampus basal forebrain)	ER- α ER- β	Modulating the expression and secretion of LH, FSH as well as the functions of serotonergic, dopaminergic and cholinergic neurons	Regulating overall endocrine homeostasis; various influences on learning, memory, cognitive functions, fine motor skills and mood; potential protective benefits against Parkinson's disease and depression
Skin and muscle	Mainly ER- α	Increasing water and hyaluronic acid concentrations; altering collagen metabolism and de-creasing epithelial proliferation	Estrogen decrease induces wrinkles and vaginal atrophy in postmenopausal women
Gastro-intestinal tract	ER- β	Regulating gastric physiology	Possible therapeutic relevance for stomach adenocarcinoma

Distribution of ERs and physiological functions of estrogens

The physiological functions of estrogens depend on the distribution of two subtypes of estrogen receptors in the body. A high ER-α expression is found in classic target tissues of estrogens such as endometrium, ovarian stroma, pituitary, hypothalamus [26]. In contrast, some non-classic target tissues including the kidney, intestinal mucosa, lung parenchyma, bone, brain, endothelial cells and prostate gland etc. contain mostly ER-β [27]. However, both ER-α and ER-β are present in ovaries, testes and uterus with high levels [26].

Up to now, more than 400 different cellular actions of estrogens have been described [28]. Some important physiological functions and possible clinical significance are summarized in Table 1 [17,23,29,30].

In addition, the important antioxidant effects of estrogens do not require the presence of estrogen receptors and may offer protection from neurodegenerative disorders caused by oxidative stress or atherogenesis due to excess uptake of oxidized low-density lipoprotein cholesterol in vascular walls [31]. Estrogen receptors can localize at the cell membrane levels, eliciting non-transcriptional effects by regulating cell membrane ion channels, G-protein-coupled receptors, activating adenylate cyclase production and triggering phospholipase C activation etc. [10].

3.2.2 Progesterone

Progesterone exerts its effects also by binding to intracellular receptors with subsequent DNA-directing RNA and protein synthesis. There are two subtype progesterone receptors, too: progesterone receptor-α (PR-α) and progesterone receptor-β (PR-β) [8,32]. Both subtypes of PRs have an identical nuclear translocation domain and are expressed in the female reproductive tract, the mammary gland, the CNS and the pituitary etc.. Progesterone acts not only on progesterone receptors, but also on other steroid receptors, thereby mediating other effects such as androgenic/antiandrogenic, estrogenic/antiestrogenic and glucocorticoid-like effects.

Steroid receptors, e.g. ER and PR, share common structure and function domains: 1) an amino-terminal (N-terminal) domain containing ligand-independent transcriptional

activating functions; 2) a centrally located DNA-binding domain with two zinc fingers; 3) a linker region; and 4) a ligand-binding C-terminal region containing a ligand-dependent transcriptional activating function. The ligand-binding domains of ER and PR are capacious and promiscuous so that many other chemical compounds, besides estrogen and progesterone, such as phyto-estrogens, environment chemicals like xeno-estrogens and SERMS (selective estrogen receptor modulators) can also bind to these receptors and act as agonists/antagonists or both, which has been attributed to the unique three-dimentional conformation induced by the binding of the ligand to the ER [33]. Thus, the affinities of different ligands (estrogens and progestogens) to steroid receptors, but also the intrinsic activity to activate the receptor, determine their specific profiles with respect to certain effects.

While estrogens have mainly growth-stimulating effects, the actions of progesterone are directed more towards differentiation. Compared with the widespread effects of estrogens, the physiological actions of progesterone, which are described up to now, are more uniform (Table 2) [32,34-36].

Table 2: Physiological functions and possible clinical significance of progesterone

Target organs	Actions of progesterone	Clinical significance
Endometrium	Inhibition of endometrium proliferation; down-regulating estrogen receptors; stimulating the development of secretory endometrium	Prevent the hyperplasia of endometrium; decreasing the risk of endometrial cancer
Breast Mammary glands	Stimulating the tubuloalveolar growth.	Increasing the risk of breast cancer
Cervix	Increasing the viscosity of cervical mucus	Inhibiting sperm motility, contraception
CNS Hypothalamus, pituitary	Inhibiting secretion of GnRH and LH	Inhibiting ovulation; contraception
Bone	Synergistic actions for estrogen on bone	Preventing bone loss, protective effects against osteoporosis

3.3 Clinical uses

In clinical practice female sex hormones are widely used for the treatment of various gynecological diseases such as primary or secondary amenorrhea, dysmenorrhea

and endometriosis etc.. They are most frequently taken, however, as OCs and as HRT agents by healthy young women and postmenopausal women (mostly also healthy).

3.3.1 Oral contraception

Because of their high effectiveness, safety, reversibility and convenience in use, OCs are the preferred contraceptive method by young women. The most common preparations are combined oral contraceptives, which are composed of an estrogen (ethinyl estradiol or, more rarely, mestranol) and a progestogen. Mestranol will be metabolized into the active estrogen ethinyl estradiol in the liver by approx. 70%. For the progestogen component, there are several groups of progestogens available, namely: 1) 17α-hydroxyprogesterone-derivatives such as cyproterone acetate, megestrol acetate, chlormadinone acetate etc.; 2) 19-nor-testosterone derivatives, for instance norethisterone, levonorgestrel, norgestimate and gestodene etc.; and 3) pro-drugs of 19-nor-testosterone, lynestrenol and desogestrel, for example [7]. These progestogens may show androgen or antiandrogen effects depending on specific tissue sensitivity and concentrations of drugs. While the 19-nor-testosterone deratives such as norethisterone and lynestrenol have an androgenic effect, which may result in side effect like acne, the 17α-hydroxyprogesterone-derivative cyproterone acetate shows a strong antiandrogenic property, which may be used (in the OCs) for the treatment of acne.

Combined oral contraceptives decrease the secretion of GnRH from hypothalamus through negative feedback regulation, which subsequently decreases secretion of FSH and LH from the pituitary. Suppression of FSH by estrogen prevents the selection and emergence of a dominant follicle whereas prevention of LH surge by progestogen inhibits the occurring of ovulation even if a dominant follicle had emerged. Therefore, the action of combined oral contraceptives can be described as creating a cycle that is entirely a luteal phase with high levels of estrogens and progestogens and low levels of GnRH, FSH and LH. However, progestogen alone, namely so-called progestogen-only contraceptives (minipills), can also prevent pregnancy. Progestogens generally don't inhibit ovulation, instead, cause decreased, thick cervical mucus to impede sperm penetration and transportation, by which they

prevent fertilization. Thus, the contraception failure rate of progestogen-only contraceptive is higher than that of combined oral contraceptives.

The side effects of OCs are mild. Common side effects include nausea and vomiting, similar to the early reactions following pregnancy. Serious, but very rare, side events such as myocardial infarction [37-39], ischemic stroke [40,41] and venous thromboembolism [42,43] may occur, which, however, were often associated with the higher estrogen dose contained in the earlier preparations and were predominantly to be found in smokers. Progestogen-only contraceptives have generally a greater safety compared with combined oral contraceptives for adverse conditions known to be associated with estrogen.

According to the development of OCs, by adjusting the dose of estrogens and addition of newly developed progestogens in an effort to lower the adverse reactions, currently three generations of contraceptives can be classified [44,45]:

First generation oral contraceptives: high-dose estrogen preparations (ethinyl estradiol >=50 µg or mestranol >=75 µg) with or without synthetic progestogens. High dose estrogens may cause unacceptable adverse reactions.

Second generation oral contraceptives: low-dose estrogens (ethinyl estradiol <50 µg or mestranol <75 µg) with progestins other than gestodene or desogestrel. They were firstly marketed in the 1970s.

Third generation oral contraceptives: low-dose estrogens (ethinyl estradiol <50 µg or mestranol <75 µg) combined with the new progestogens: gestodene or desogestrel. Such as Marvelon and Minulet, which were firstly marketed in 1982 and 1987, respectively.

According to the effect pattern of combined oral contraceptives on levels of estrogens and progestogens in the body, OCs can also be classified into [5]:

Monophasic contraceptives: namely classical OCs, which are combined fixed doses of estrogens and progestogens and should be taken continuously for 21 days;

Biphasic contraceptives: only estrogens, sometimes with very low-dose progestogens, are given in the first half of menstrual cycle, then followed by combined estrogens and progestins up to day 21. The former formulations (only

estrogens) are also called sequence contraceptives and the latter (estrogens with very low-dose progestogens) two-step contraceptives;

Triphasic contraceptives: by which different doses of estrogens and progestogens are given in three periods similar to the physiological fluctuation in women. In the first 6 days after bleeding, estrogens and progestogens are given with low dose; then in the following 5 days with a higher dose; for the last 10 days estrogens are recalled to the first level while progestogen dose in general increases once more;

Progestogen-only contraceptives: composed of low-dose progestogens only, also called 'minipills'.

3.3.2 Hormone replacement therapy

Unlike men, women will experience markedly a special stage in their late life: the climacteric is a transition period of women from fertility to non-fertility characterised by decreasing estrogen levels because of the atrophy of female ovaries. Decrease of estrogen levels in turn may result in climacteric symptoms such as hot flash, sweating, urogenital symptoms and some affective disorders like irritation, stress and depression, etc. [46]. To compensate for the age-related estrogen decrease and to relieve climacteric symptoms, estrogens, alone or combined with progestogens, are given to replace the decreased estrogens in the female body. Therefore, one regimen of HRT is called:

Unopposed estrogen replacement therapy (estrogen replacement therapy, ERT), in which only estrogens are given. This was especially true in the early stage of hormone replacement therapy in the mid-1970s. Later ERT was found to be associated with increased risks of endometrial cancers. To counteract the stimulatory effects of estrogens on endometrium, progestogens are added to the ERT. So, there's another regimen of hormone replacement therapy called:

Combined estrogen-progestagen replacement therapy, in which estrogens and progestogens are given continuously or sequentially. Postmenopausal women with intact uterus who want to use HRT should be offered this form of HRT. Now it is generally accepted that exogenous administration of estrogen alone is contraindicated in postmenopausal women with an intact uterus.

Estrogens used in ERT or HRT can be either natural ones such as conjugated equine estrogens (CEE) or synthetic ones such as estradiol. Extracted from the urine

of pregnant mares, CEE are composed of more than 10 estrogens and some progestins, androgens and other products in small amounts [47]. The pharmacological effects of CEE are therefore a sum of these single entities, effects may vary depending on the amount of these compounds contained and on women's individual difference. Because of the excellent efficacy for the relief of menopausal symptoms and minor side effects, CEE are the most commonly used estrogens in the HRT/ERT preparations. Another source of natural estrogens are phytoestrogens, which are non-steroidal plant compounds with estrogen-like biological activities [48]. Common sources of phytoestrogens are soybeans, red clover, flaxseeds and yams etc., extracts of these plants are rich in isoflavones, flavones, lignans (enterodiol and enterolactone) and coumestrol, which are the active ingredients of phytoestrogens [48]. Because phytoestrogens have a much weaker pharmacological efficacy than human endogenous estrogens or synthetic estrogens, they can be even used as dietary supplements. Though an increasing number of women is using phytoestrogens as an alternative for management of menopausal symptoms [49], phytoestrogens have not been fully studied yet.

Synthetically derived estrogens such as estradiol valerate are structurally close to the naturally produced estrogens. These drugs exhibit a high affinity for estrogen receptors, and therefore, are much more efficacious than CEE or phytoestrogens.

4. Health-related outcomes of oral contraceptives and hormone replacement therapy

The potential risks of OCs and HRT have been investigated in detail. The results of large epidemiological studies are reported in the following.

4.1 Oral contraceptives

4.1.1 Breast cancer

According to a large population-based case-control study (4575 women with breast cancer and 4682 controls) published in 2002, OC use was not associated with a significantly increased risk of breast cancer among women aged 35 to 64 years [50]. The relative risks amounted to 1.0 (95% CI: 0.8-1.3) and 0.8 (95% CI: 0.8-1.0) for current and former OC users, respectively, and did not increase consistently with longer periods of use or with higher dose of estrogens [50]. Importantly, the use of

OCs by women with a family history of breast cancer was not associated with an increased risk of breast cancer, nor was the initiation of OC use at a young age. This was considered to be good news for OC use [51]. However, a meta-analysis of data from 54 epidemiological studies of OC use and the risk of breast cancer showed that users had a slightly increased risk of breast cancer with RR 1.24 (1.15-1.33) compared with nonusers [52,53]. In a nested case-control study, 309 breast cancer patients and 610 controls were examined from the total population of 12,184 women who were followed up for an average of 7.5 years; the authors concluded that long duration of oral contraceptive use (more than 10 years) increased the risk of breast cancer in women over 55 years of age with OR 2.1 (95% CI: 1.1-4.0) [54].

4.1.2 Ovarian cancer and endometrial cancer

Contrary to the increased risk of breast cancer following use of OCs, a decreased risk of ovarian cancer [55,56] and endometrial cancers [57,58] has consistently been found to be associated with OC use. These associations are increased with the duration of OC use, which was bolstered by repeated findings from non-experimental studies. The overall estimated protection for ovarian cancer from cohort and case-control studies was approximately 40% in ever OC users, and increased with duration of use to more than 50% for users of 5 years or longer [59]. According to CASH study (Cancer and Steroid Hormone Study), a 40% reduction in the risk of ovarian cancer could be observed after as short a period as 3 to 6 months of use [60] and 10 or more years of use was associated with an 80% reduction in the risk (OR 0.2; 95% CI:0.1-0.4). The same was true for endometrial cancer [57,58,61]. The risk of endometrial cancer declined by 40% (OR 0.6, 95% CI 0.3-0.9) after 12 months of OC use [62].

4.1.3 Cardiovascular diseases

Venous thromboembolism

Soon after OCs were introduced to the market, their use was associated with a substantial increase in risk for thromboembolic diseases [63]. Many studies suggested that OC use was associated with increased risks of venous thromboembolism [43,64]. However, the increased risk of thromboembolic diseases appeared to be associated with the higher dose of estrogens used in the early OC formulations [65]. A dose-response relation between estrogens and venous

thromboembolism was observed in a large cohort study. A total of 2,739,400 OC prescriptions received by 234,218 women were analyzed with RR 1.5 and 1.7 for intermediate-dose (estrogens 50 μg) and high-dose formulations (estrogens >50 μg), respectively, compared with the low-dose formulations (estrogens<50 μg) [66]. Yet, for OCs containing ethinyl estradiol < 50 μg, no differences in risk have been found [42].

Myocardial infarction

In a recent meta-analysis concerning OC use and myocardial infarction, odds ratios were calculated for seven studies published between 1996 and 2001 and estimates were synthesized using medians and range as a historical point of reference for 22 studies published between 1965 to 1996 [37]. Results showed that the point estimates for third generation versus second generation OCs ranged from 0.44 (0.24-0.80) to 0.62 (0.38-0.99), and the aggregated ORs were 2.18 (1.62-2.94) and 1.13 (0.66-1.92) for second and third generation OCs, respectively, compared with nonusers [37]. The same results concerning the association of myocardial infarction with use of second or third generation OCs were also observed in a recent population-based case-control study with adjusted OR 2.5 (95% CI:1.5-4.1) and 1.3 (95% CI:0.7-2.5) for second and third generation OCs, respectively [38]. However, the association between OC use and myocardial infarction may be confounded by cigarette smoking. Use of OCs and smoking may have a striking synergistic interaction on myocardial infarction [67]. Generally, OC users who were younger than 40 years of age and didn't smoke had little or no increase in risk for myocardial infarction.

4.2 Hormone replacement therapy

A large body of cross-sectional studies suggested that, besides the relief of climacteric symptoms, HRT may prevent cardiovascular diseases. Results of meta-analysis for more than 40 observational studies in the past three decades showed that current HRT users have a lower risk of coronary heart disease (CHD) than nonusers [68,69]. The large Nurses' Health Study, in which 70533 postmenopausal women were followed up from 1976 to 1996, reported that current use of HRT was associated with a RR of 0.61 (95% CI: 0.52-0.71) for a major coronary event [70] and a RR of 0.65 (95% CI, 0.45 to 0.95) for a recurrent major coronary event in women

with previous myocardial infarction or documented atherosclerosis [71]. Despite the strong biological plausibility of HRT, randomized, placebo-controlled clinical trials, however, did not support the findings from observational studies.

Women's Health Initiative (WHI) was a well-designed, double-blinded, placebo-controlled large clinical trial that enrolled 16608 postmenopausal women in the age range from 50 to 79 years between 1993 and 1998 [72]. Originally, the trial was planned to be followed-up 8.5 years for the two regimes tested, however, for the regime of estrogen plus progestin, it was terminated prematurely on May 31. 2002 after 5.2 years following-up because of overall more risks than benefits [73] and for the regime of estrogen alone in women with hysterectomy, it was also terminated prematurely on February 29, 2004 after almost 7 years follow-up [74]. Following the publication of overall principal results in 2002 for the regime of estrogen plus progestin [73], specific endpoints of WHI results were described in detail recently, including CHD with a harzardous ratio (HR) 1.24 (95% CI: 1.00-1.54) [75], stroke with a HR 1.31 (1.02-1.68) [76], breast cancer with a HR 1.24 (1.02-1.50) [77], colorectal cancer with a HR 0.56 (0.38-0.81) (protective effect) [78], dementia with a HR 2.05 (1.21-3.48) [79], while global cognitive function [80] and health-related quality of life [81] appeared not to be influenced by HRT. In women with hysterectomy, use of estrogen alone increased the risk of stroke with a HR 1.39 (95% CI: 1.10-1.77), decreased the risk of hip fracture with HR 0.61 (95% CI: 0.41-0.91) and did not influence the risks of CHD (HR 0.91, 95% CI 0.75-1.12) and colorectal cancer (HR 1.08, 95% CI 0.75-1.55) whereas the risk of breast cancer might be reduced with HR 0.77 (95% CI: 0.59-1.01) [74]. WHI study is the first large clinical trial to directly examine the overall effects of HRT for primary prevention against cardiovascular diseases in predominatly healthy postmenopausal women, the results of which are of importance and often considered as a clinical reference by gynecologists and postmenoposal women if HRT should be initiated.

In contrast to WHI for primary prevention study of HRT, another double-blinded, placebo-controlled clinical trial (Heart & Estrogen-progestin Replacement Therapy Study, HERS) focused on the secondary prevention for postmenopausal women with established CHD. HERS did not indicate an overall reduction of CHD events but an apparent increased risk of thromboembolic events in the first year of treatment [82].

Prolonged following-up in HERS II study got the same null effects of HRT on CHD events and an increased risk of gallbladder disease [83,84].

While the WHI and HERS exclusively studying the preparation of CEE with or without continuous administration of medroxyprogesterone acetate (MPA) failed to show any benefits on cardiovascular diseases, another small, doubled-blind, placebo-controlled clinical trial recruiting 226 postmenopausal women who had at least one coronary-artery lesion had examined the effects of 17-β estradiol, alone or with sequential administrated MPA, on the progression of atherosclerosis. After 3.3 years following-up, no significant change of percent stenosis could be found [85].

The largest cohort study so far, the Million Women Study, which recruited 1084110 UK women aged 50-64 years between 1996 to 2001, has published its findings that HRT use was associated with an increased morbidity and mortality of breast cancer with the RR 1.66 (95% CI: 1.58-1.75) and RR 1.22 (95% CI: 1.00-1.48), respectively [86].

Soon after publication of the results of WHI, a rapid review focused on 4 large randomized clinical trials including the results of WHI concluded that HRT users had overall a significantly increased incidence of breast cancer, stroke and pulmonary embolism; a significantly reduced incidence of colorectal cancer and fractured neck of femur; but no significant change in endometrial cancer and coronary heart disease [87]. Two meta-analyses, in which only studies with good and fair quality were enrolled, showed that the benefits of HRT included the prevention of osteoporotic fracture and colorectal cancer; harms included CHD, stroke, thromboembolic events, breast cancer (with 5 or more years of use) and cholecystitis [69,88].

5. Issues concerned in this dissertation

Therefore, results from recent studies conflict with previous findings, especially for the supposed preventive effects of HRT against cardiovascular diseases. How could it be explained? On behalf of the European Menopause & Andropause Society (EMAS), European experts, for example, had different explanations and opinions on the results of WHI [89], in which only one regimen of CEE plus MPA was tested after all, as said also by authors of WHI [73].

In contrast to US, where HRT consists predominantly of CEE plus MPA, the European HRT tradition is mainly based on 17-β estradiol and norethisterone acetate or other progestogens [90]. Therefore, whether the results from the US completely reflect the situations in other countries is unsure yet. Currently, it is estimated that ca. 5 million women in Germany take HRT for disease prevention [91]. The health-related outcomes following HRT use in German women, especially after publishing the results of WHI, are of major concern among clinicians and postmenopausal women. And OC use meaning hormone intake most often for years by healthy women should be critically and carefully reflected from time to time. This holds true especially before the background with recent experiences with HRT in the (post) menopause [73,74,82,83], revealed unexpected risks obviously outweighing positive effects of hormone replacement. The real use profiles of steroid hormones for oral contraceptives and for HRT and their overall influence on women's health in Germany, however, so far is insufficiently specified. A large study focussed on the association of breast cancer and HRT, in which 3500 breast cancer patients aged 50-74 years and 7000 controls should be enrolled, is currently ongoing in Germany, but first results will be available only after 2004 [92].

Fortunately, since 1984, five cross-sectional National Health Surveys had been performed in Germany (T0, T1 and T2 in the German Federal Republic, T3 in the previous German Democratic Republic and T4 in the unified Germany). The overall sample population of the five National Health Surveys covered approximately 25,000 German representative residents under ambulant care, which allowed to investigate the use profile of steroid hormones for contraception and for HRT, and to evaluate their influence on women's health in the every day life situations from a relatively long period of time.

In this dissertation, the utilization of steroid hormones for oral contraception and for HRT in the German women population are evaluated. Epidemiological profiles and trends in the use of steroid hormones and the differences from nonusers, the overall possible health-related outcomes and profiles including disease prevalence, utilization of health service, laboratory measurements and quality of life are investigated.