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Pharmacology of estrogens and progestogens: influence of different routes of administration

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ABSTRACT

This review comprises the pharmacokinetics and pharmacodynamics of natural and synthetic estrogens and progestogens used in contraception and therapy, with special consideration of hormone replacement therapy. The paper describes the mechanisms of action, the relation between structure and hormonal activity, differences in hormonal pattern and potency, peculiarities in the properties of certain steroids, tissue-specific effects, and the metabolism of the available estrogens and progestogens. The influence of the route of administration on pharmacokinetics, hormonal activity and metabolism is presented, and the effects of oral and transdermal treatment with estrogens on tissues, clinical and serum parameters are compared. The effects of oral, transdermal (patch and gel), intranasal, sublingual, buccal, vaginal, subcutaneous and intramuscular administration of estrogens, as well as of oral, vaginal, transdermal, intranasal, buccal, intramuscular and intrauterine application of progestogens are discussed. The various types of progestogens, their receptor interaction, hormonal pattern and the hormonal activity of certain metabolites are described in detail. The structural formulae, serum concentrations, binding affinities to steroid receptors and serum binding globulins, and the relative potencies of the available estrogens and progestins are presented. Differences in the tissue-specific effects of the various compounds and regimens and their potential implications with the risks and benefits of hormone replacement therapy are discussed.

INTRODUCTION

The aim of any hormonal treatment of postmenopausal women is not to restore the physiological serum levels occurring in ovulatory cycles of fertile women, but to prevent or improve complaints and symptoms caused by an estrogen deficiency. Nevertheless, the close association between the pharmacokinetics, i.e. the time course of the serum concentrations of sex steroids after administration, and the pharmacodynamics, i.e. the metabolic and biological changes caused by the treatment, indicate the fundamental importance of pharmacological knowledge for an optimal use of hormone therapy.

The history of hormone replacement therapy (HRT) is, however, accompanied by misconceptions and errors concerning the implications of pharmacokinetics for the individual woman. Even though there is a significant correlation between the serum concentrations of estradiol and their clinical effects, e.g. on hot flushes or bone mass, the serum level of an individual woman does not predict the therapeutic effect. As shown in Figure

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REVIEW

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1, the number of hot flushes differs largely in patients who showed identical estradiol levels during transdermal hormone therapy[1]. This casts considerable doubts on the usefulness of regular measurements of hormone levels for the prediction or control of a therapeutic success. Another claim that turned out to be wrong was the story of the advantage of constant hormone levels observed during transdermal estrogen therapy as compared to the rapid rise and fall after oral administration. The good effectiveness and tolerability of intranasal estradiol therapy, which is associated with extremely high peak levels occurring within a few minutes after administration and a rapid fall thereafter, refuted this general opinion.

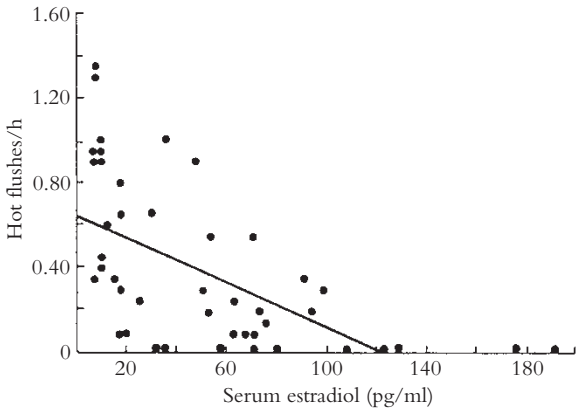

Figure 1 Correlation between the number of hot flushes per hour and the serum levels of estradiol during transdermal treatment of postmenopausal women with various doses of estradiol (after Steingold *et al*., 1985[1])

ESTROGENS

Mechanism of action

The biological effects of estrogens are based on genomic mechanisms mediated by interactions with the nuclear estrogen receptors, ERα and ERβ, but also by rapid non-genomic mechanisms involving cell membrane receptors which are coupled with G-proteins and can activate intracellular signal cascades. Nuclear and membrane ER are biochemically identical and may work in concert[2]. The ERβ is 96% homologous to the ERα in the deoxyribonucleinic acid (DNA)-binding domain and 55% in the C-terminal ligand-binding domain. In many tissues, the ERβ can antagonize

Table 1 Distribution of estrogen receptors ERα and ERβ in various tissues[4–8]

Target tissue	*ERα*	*ERβ*
Ovary	+ (theca cells)	+ (granulosa cells)
Uterus	+	+ (stromal cells)
Vagina	+ (pre- and postmenopause)	+ (premenopause)
Urinary tract	(−)	+
Mammary gland	+	+
Placenta	+	(−)
Ventral prostate	(−)	+
Central nervous system	+	+
Cardiovascular system	+	+
Bone	+	+
Muscle	(−)	+
Adipose tissue	+	+
Intestinal tract	(−)	+
Immune system	(−)	+
Hematopoiesis	+	+
Liver	+	(−)
Lung	(−)	+
Adrenals	(−)	
Pineal gland	(−)	
Thyroid gland	(−)	
Parathyroids	(−)	
Pancreas	(−)	
Gallbladder	(−)	
Skin	+ (sebaceous glands)	+

+, high expression and important function; (−) no or low expression

the genomic actions of ERα, but there are also genes which are regulated by ERα or ERβ, the latter being existent in six isoforms[3,4]. The variability in the expression and distribution of ERα and ERβ in the various tissues and organs may explain the large differences in the response to different estrogens (Table 1)[4–8]. ERα and ERβ are regulated in a tissue- or cell-specific manner, and their expression can be increased or decreased by estradiol[4]. The various estrogens differ in their binding affinity to ERα and ERβ (Table 2)[9]. The binding of estrogens to ERα or ERβ leads to phosphorylation and conformational change of the protein, which results in homodimerization (ERα–ERα or ERβ–ERβ) or heterodimerization (ERα–ERβ)[2]. In the cell nucleus the dimeric ER–steroid complex binds to the specific estrogen responsive elements (ERE) located in the promoter region of estrogen-activated genes, and recruits a coactivator complex to the promoter. There are many proteins (coactivators, corepressors) which may interact with these complexes and regulate the transcription. Moreover, the ERs may also directly interact with various transcription factors, and may bind to other nuclear receptors (e.g. thyroid hormone receptor, retinoic acid receptor)[10]. Therefore, beyond the binding affinity to the ERα or ERβ and the intracellular concentration of the estrogen, many other factors are involved in the control of the estrogen-induced biological response.

Table 2 Relative binding affinity (RBA) of various estrogens for *in vitro* synthesized human estrogen receptors ERα and rat ERβ as determined by competition experiments (ratio of concentration at 50% inhibition (IC_{50}) values of estradiol : competitor)[9]

Steroid	*RBA for ERα*	*RBA for ERβ*
Estradiol-17β	100	100
Estrone	60	37
Estrone-3-sulfate	<1	<1
Estriol	14	21
Estradiol-17α	58	11
4-Hydroxy-estradiol	13	7
2-Hydroxy-estradiol	7	11
Diethylstilbestrol	468	295
Tamoxifen	7	6
4-Hydroxy-tamoxifen	178	339
Clomiphene	25	12
Nafoxidine	44	16
5-androstenediol	6	17
3β-androstanediol	3	7
Coumestrol	94	185
Genistein	5	36

The comparable clinical efficacy of intranasal, transdermal and oral administration of estradiol indicates that the total exposure to the intracellular estradiol (area under the concentration–time curve, AUC) is an important determinant for the biological response. The short-term presence of high concentrations and the long-term presence of low concentrations of estradiol may, therefore, cause a similar expression of estrogen-dependent products during a time interval of 12–48 h. It has been shown that, in normal human epithelial breast cells as well as in ER-positive breast cancer cells, the proliferation rate did not differ between incubation with 1 nmol/l estradiol for 24 h and with 24 nmol/l for 1 h[11].

Structure, activity and metabolism

Structure of estrogens

The classical estrogenic hormones are C18 steroids with a phenolic structure in ring A. The most potent human estrogen is estradiol-17β (estradiol) which has a hydroxy group at position 17β, i.e. above the stiff plane of the steroid skeleton, whereas the hydroxy group of estradiol-17α is below the steroid plane. Estradiol-17α is not produced in the human and cannot be metabolized to estrone. The equine estrogens are characterized by one or two additional double bonds in ring B (equilin and equilenin) which increase considerably the estrogenic activity. Similar to estradiol, the equine estrogens with a 17β-hydroxy group are more potent than their 17α-isomers (Figure 2).

There are also non-steroidal estrogens like diethylstilbestrol and its derivatives, tamoxifen, clomiphene, raloxifene, etc., and the various phytoestrogens. Their structure allows binding to the ERs, but – as they do not fit exactly in the ligand-binding domain of the receptor protein – prevents the correct interaction necessary for exerting the same biological effects as estradiol. These structure-dependent peculiarities and the existence of two different ERs are the basis of the development of selective estrogen receptor modulators (SERMs). The prerequisite of steroid-like actions of non-steroidal compounds is a stiff, planar structure which is brought about by so-called conjugate double bonds (single bond alternating with double bond) which couple the aromatic systems of two rings. Diethylstilbestrol is a potent estrogen, because it is a stiff plane with two hydroxy groups in a distance similar to that of the estradiol molecule. Compounds with no

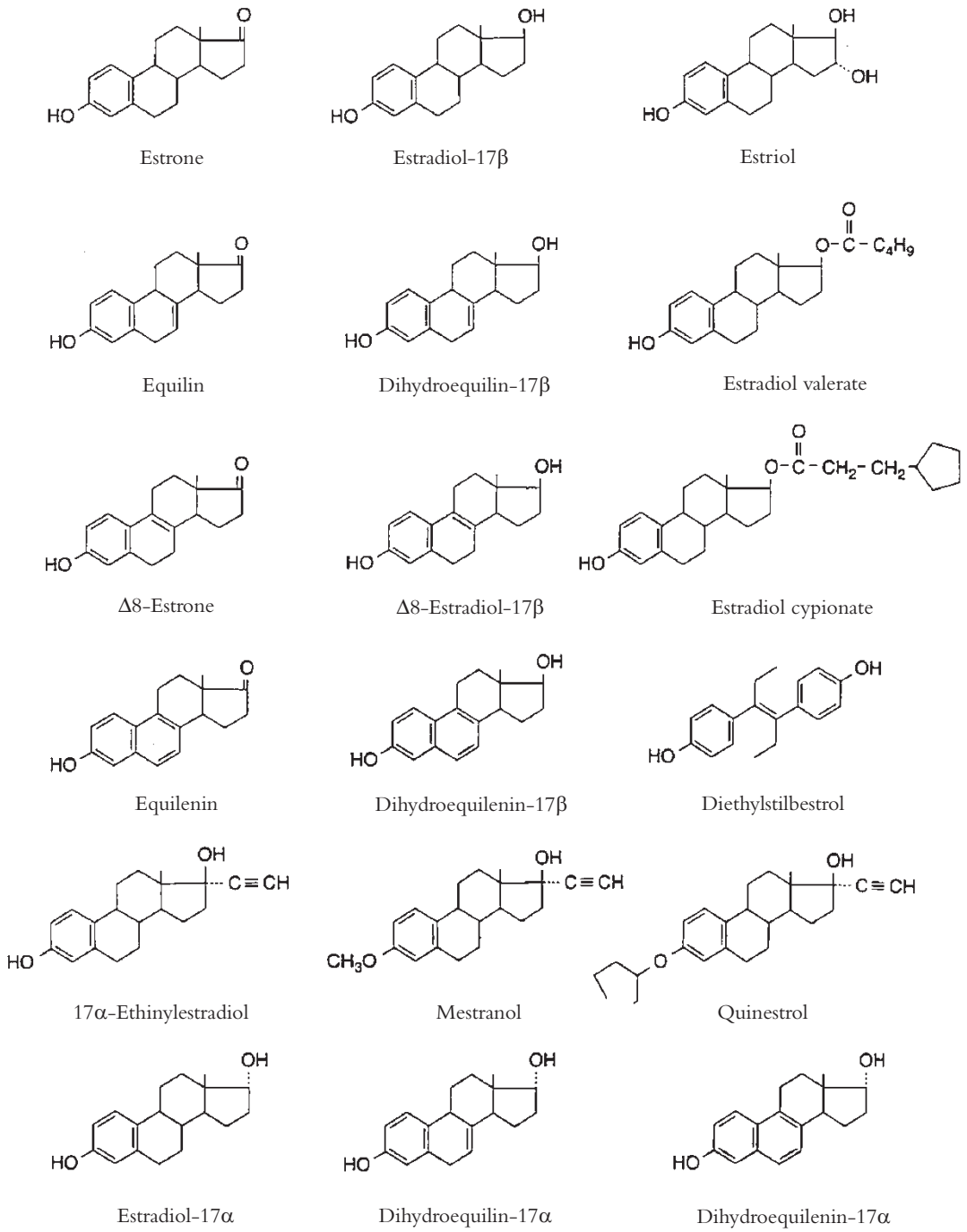

Figure 2 Structural formulae of estrogens

phenolic hydroxy group (e.g. tamoxifen) are prodrugs that are converted to the active estrogen agonist or antagonist (e.g. 4-hydroxy-tamoxifen) in the intestinal tract and liver.

The phytoestrogens differ largely in their estrogenic activity. The less the similarity to the structure of estradiol is, the less is the binding affinity to the ERα or ER*β*, and the weaker is their biological effect.

Esters of estrogens, e.g. estradiol valerate, estriol succinate, or ethinylestradiol sulfonate, and ethers like mestranol or quinestrol are readily hydrolyzed to the active hormones. In contrast to esters and ethers, the introduction of substituents like an ethinyl group at C17α into the estradiol molecule is largely irreversible, and the resulting ethinylestradiol (EE) is a potent orally active estrogen which is neither split to

estradiol, nor inactivated by oxidation of the hydroxy group at C17β. Therefore, it is active in the endometrium despite the stimulation of the 17β-hydroxysteroid-dehydrogenase (HSD) type 2 by progestogens.

Hormonal potency of estrogens

Many attempts have been made to compare the potency of the various estrogens by means of a simple bioassay. As every action of every estrogen is unique for every tissue, generalizing comparisons on the basis of a single parameter is highly questionable. The determination of a so-called 'estrogenicity' which reflects the effect of various estrogens on the hepatic production of, for example, sex hormone-binding globulin (SHBG), cannot be extrapolated to the effect on, for example, hot flushes.

The hormonal potency of the various estrogens is not only determined by their interactions with ERs and EREs on the DNA, but also by the intracellular concentration of the estrogen. The latter is dependent on the bioavailable ('free') fraction of the circulating steroid, the intracellular production and inactivation rate. Estriol is a relatively weak estrogen because it is bound to the ER for a relatively short period of time. EE is much more active than the natural estrogens, because the 17α-ethinyl group prevents the oxidation of the 17β-hydroxy group and is able – after the oxidative formation of a very reactive intermediate – to inhibit irreversibly cytochrome P450 enzymes, which are involved in the metabolism of steroids. The potency of conjugated equine estrogens (CEE) is considerably higher than that of estradiol, particularly concerning the effect on the hepatic production of certain serum parameters, e.g. SHBG, corticosteroid-binding globulin (CBG), thyroxine-binding globulin (TBG) and angiotensinogen (Table 3)[12–14]. In this regard, the route of administration plays an important role, and oral treatment has a considerably stronger impact on hepatic protein synthesis than parenteral therapies.

Interconversion between estradiol-17β and its metabolites

The natural estrogens produced by the human are estradiol, estrone, estriol, and their conjugates, i.e. the sulfuric acid esters (sulfates) and glucuronic acid esters (glucuronides). There is a dynamic mutual conversion system between the hormonally active estradiol-17β, the weak estrogen estrone and the inactive conjugates estrone sulfate and estradiol sulfate which, therefore, can be regarded as both metabolites and precursors (Figure 3).

The same holds true for the equine estrogens equilin, equilenin, and Δ8-estrone which correspond structurally to estrone. Their conversion to dihydroequilin-17β, dihydroequilenin-17β, and Δ8-estradiol-17β is comparable with that of estrone to estradiol and causes an increase in the estrogenic activity. Whereas the balance of the mutual interconversion between estradiol and its metabolites is largely shifted to estrone and estrone sulfate (Figure 3), that between dihydroequilin-17β or dihydroequilenin-17β and the respective metabolites is nearly equal, resulting in a higher hormonal activity of the equine preparations[15–17].

The mutual conversion of estradiol to estrone and vice versa is catalyzed by different 17β-HSDs which occur in various organs and tissues. The

Table 3 Relative potency of various estrogens concerning several clinical (relief of hot flushes) and metabolic parameters (suppression of follicle stimulating hormone (FSH) levels; increase in the serum levels of high density lipoprotein (HDL) cholesterol, sex hormone-binding globulin (SHBG), corticosteroid-binding globulin (CBG) and angiotensinogen). The values are estimated on a weight basis[12–14]

Estrogen	*Hot flushes*	*FSH*	*HDL cholesterol*	*SHBG*	*CBG*	*Angiotensinogen*
Estradiol-17β	100	100	100	100	100	100
Estriol	30	30	20			
Estrone sulfate		90	50	90	70	150
CEE	120	110	150	300	150	500
Equilin sulfate			600	750	600	750
Diethylstilbestrol		340		2 560	2 450	1 950
Ethinylestradiol	12 000	12 000	40 000	50 000	60 000	35 000

CEE, conjugated equine estrogens

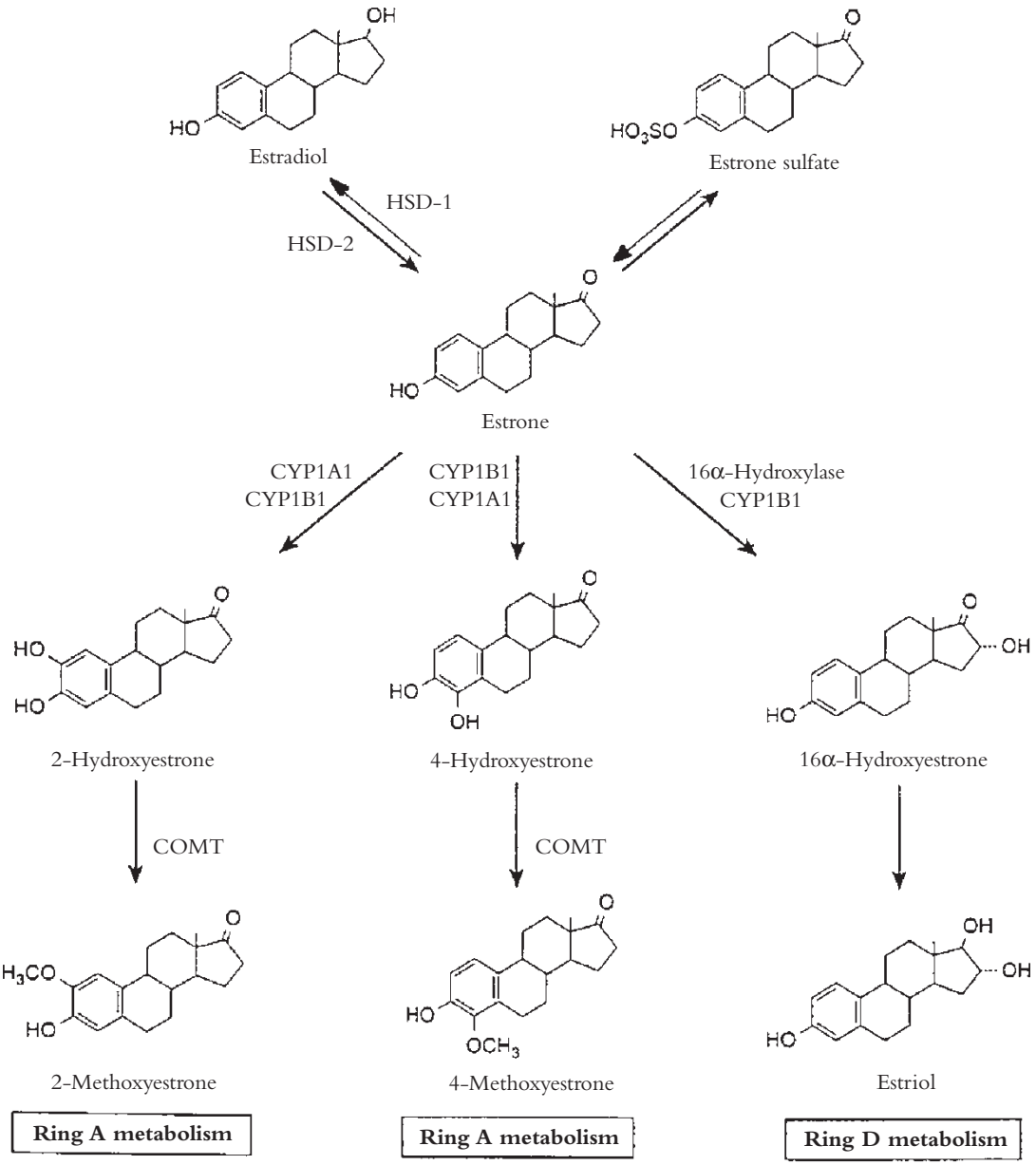

Figure 3 Metabolism of estradiol-17β and estrone

17β-HSD type 1 catalyzes the reduction of the 17-oxo-group of estrone to estradiol, while the 17β-HSD type 2 is responsible for the oxidation of the 17β-hydroxy group of estradiol to estrone. In the endometrium, the latter is stimulated by progesterone and synthetic progestins, resulting in a strong increase in the local inactivation rate of estradiol[18].

Similar oxidative and reductive interconversions occur between the equine estrogens and their metabolites. Moreover, equilin and dihydroequilin-17β which contain one double bond in ring B, can be transformed into equilenin and dihydroequilenin-17β with two double bonds in ring B[15,16].

Metabolism of estrogens

Beyond these reversible conversion reactions at C17 of estradiol, further phase 1 metabolic reactions occur which are irreversible. There are two main pathways of oxidative metabolism; the hydroxylation of ring A at C2 or C4 results in the formation of the so-called catecholestrogens, which can be further converted to the methoxy-metabolites by the catecholestrogen-orthomethyl

transferase (COMT), while the introduction of a hydroxy group at C16α of ring D leads to 16α-hydroxy-estrone and estriol (Figure 3).

In healthy subjects, ring A metabolism outweighs ring D metabolism, and the concentration of 2-hydroxy metabolites is higher than that of 4-hydroxy metabolites. Hyperthyroidism and thyroxine therapy reduce 16α-hydroxylation and enhance 2-hydroxylation, whereas hypothyroidism has the opposite effect. Obesity stimulates ring D metabolism and underweight causes a shift to the ring A metabolism.

Estriol is a weak, short-acting estrogen which cannot be re-transformed to estradiol, but undergoes extensive conjugation. Moreover, estradiol, estrone and estriol are also oxidized and hydroxylyzed in ring B at C6α, C6β, C7α, and C7β, in ring C at C11β and C14α, in ring D at C15α, and at the angular C18 methyl group[18,19].

Both the formation and hydrolysis of conjugates which are catalyzed by intestinal and hepatic enzymes are important factors in the regulation of the pharmacokinetics and pharmacodynamics of estradiol. The conjugation of lipophilic steroids increases largely their water solubility. Therefore, during oral treatment with estradiol or estrone sulfate, high amounts of estrogen sulfates are circulating, while the glucuronides are involved in the excretion of the steroids with the bile and urine. The conjugates dissolved in the bile are extensively hydrolyzed in the colon by bacterial enzymes and re-absorbed, and this enterohepatic circulation is of considerable importance for the serum concentrations of estradiol and estrone.

Besides the liver, hydroxylation reactions catalyzed by cytochrome P450 (CYP) enzymes may occur in many other tissues, e.g. in the uterus, breast, kidney, brain, and pituitary[19]. Some of the metabolites may exert important local functions in target cells, either mediated by ERα or ERβ, or by other mechanisms. This concerns particularly the 2-hydroxy and 4-hydroxy metabolites of estradiol and estrone. Owing to the structural similarity of the catecholestrogens with the catecholamines, they can modulate the activity of, for example, dopamine and noradrenaline, by interacting with their metabolism. As part of a metabolic redox system, the 2- and 4-hydroxy-estrogens can be converted to reactive semiquinones and quinones, which have been suggested to act as carcinogens[19]. The 2- and 4-hydroxy-estrogens are further metabolized to 2- and 4-methoxy-estrogens, which cannot be transferred to quinones. Both the catecholestrogens and their methoxy-derivatives may exert considerable estrogenic effects (Table 4)[19–21]. 16α-Hydroxyestrone has also been suggested to exert carcinogenic effects by covalent binding to the ER, but there is no evidence for a clinical role in cancer development[19].

Pharmacokinetic properties

The natural estrogens estradiol, estrone, estrone sulfate (sodium salt) and estriol are available in different galenic preparations. Estradiol and estrone are lipophilic, but show a sufficient water solubility, which is increased by binding to albumin and SHBG, whereas the sulfates and glucuronides of estrogens are highly water-soluble. For oral treatment, estradiol is used in a microcrystalline form to increase compound surface, which enables an accelerated resorption and increased bioavailability. The smaller the crystals, the more rapid is the absorption and the higher is the bioavailability. The crystals contain some water (hemihydrate, two molecules estradiol are associated with one molecule water), which plays no role after resorption/dissolution. Esterification of the C17-hydroxy group of estradiol with valerianic acid results in estradiol valerate, which prevents metabolism to estrone as long as hydrolysis has not taken place. After hydrolysis in the intestinal tract, the resulting estradiol is rapidly absorbed. Therefore, after oral administration of estradiol valerate or micronized estradiol, the pharmacokinetics of estradiol is similar and the bioavailability is about 5%[22].

Table 4 Relative binding affinity (RBA) for the estrogen receptor (ER) from rat uterine cytosol and estrogenic potency (wet weight of rat uterus) of estradiol metabolites[20,21]

Compound	*RBA for ER*	*Uterine wet weight*
Control		100
Estradiol-17β	100	506
2-Hydroxy-estradiol-17β	24	285
2-Methoxy-estradiol-17β	0.05	101
4-Hydroxy-estradiol-17β	45	
4-Methoxy-estradiol-17β	13	260
16α-Hydroxy-estradiol (estriol)	10	468
2-Hydroxy-estrone	2	130
2-Methoxy-estrone	0.01	
4-Hydroxy-estrone	11	351
4-Methoxy-estrone	0.13	338

The majority of the circulating estrogens is bound to serum proteins. In women, about 37% of estradiol is bound with high affinity (55% of that of testosterone) to SHBG and 61% with low affinity to albumin, while only 2% are free. Both the free and the albumin-bound fractions are regarded as biologically active, but are also subject to metabolism. As during oral treatment with 1 mg estradiol or 0.625 mg CEE, the serum levels of SHBG are significantly increased by 50% and 100%, the proportion of free estradiol decreases from 1.3% to 1.2% and 1.0%, respectively[23]. Estrone is a weak estrogen which has only 4% of the estrogenic activity of estradiol; the proportion bound to SHBG is 16% and to albumin 80%. As much as 99% of the estrogen sulfates is bound with a relatively high affinity to albumin, which causes a relatively long half-life of the conjugates. While the half-life of estradiol and estrone is 20–30 min, that of estrone sulfate is 10–12 h. Similarly, the clearance of equilin and dihydroequilin-17β is much faster than that of the respective sulfates (Table 5).

Owing to the large individual differences in the resorption and metabolism, there are considerable interindividual variations in the course and height of the serum concentrations of sex steroids in women treated with the same preparation. The coefficients of variation are usually in the range between 30 and 60%. The interindividual variations are mainly due to genetic or acquired differences in the intestinal and hepatic metabolism, while the intraindividual variations from day to day may be caused by external factors like diet, alcohol or drug consumption, smoking, physical activity, stress, etc., which may cause rapid and transitory changes in peripheral or splanchnic blood flow, absorption or metabolism. Certain diseases, e.g. disorders of the thyroid gland, may also affect estrogen metabolism[18].

The role of the route of administration

The oral route of administration of estrogens has both advantages and disadvantages. Its use is easy and convenient, non-invasive and rapidly reversible. On the other hand, because of the high rate of metabolism in the gut and liver, which leads to the high estrone/estradiol ratio (Table 6), higher doses are needed than with the parenteral route. Despite this, the use of oral and transdermal or intranasal doses of estradiol which leads to similar systemic estradiol levels over time (AUC), results in comparable clinical effects, e.g. on hot flushes. This confirms that estrone and estrone sulfate, which are circulating in high concentrations during oral therapy, but not after parenteral application, have weak or no estrogenic activity. Estrone has only 4% of the activity of estradiol, and its estrogenic potency measured in animals is mainly due to its conversion to estradiol. Moreover, in contrast to estradiol and estriol, estrone is not accumulated in target tissues[26]. Therefore, the suggestion, that the high serum levels of estrone and estrone sulfate after oral administration may be unfavorable, is not justified.

On the contrary, the pronounced reconversion of these estradiol metabolites to estradiol contributes to the maintenance of elevated estradiol levels for up to 12 h after intake. During oral treatment, the first liver passage of estrogens after the rapid absorption in the gut is characterized by high local hepatic steroid levels, which are about 4-fold higher than the peripheral serum concentrations[27]. Moreover, owing to the high permeability of the hepatic microvasculature and the higher availability of protein-bound estrogen

Table 5 Binding affinity to serum binding proteins and metabolic clearance rate of estrogens[15,16,24,25]

Steroid	*RBA to SHBG*	*% bound to SHBG*	*% bound to albumin*	*MCR* (l/day/m^2)
Estradiol-17β	50	37	61	580
Estrone	12	16	80	1050
Estriol	0.3	1	91	1110
Estrone sulfate	0	0	99	80
17β-Dihydroequilin	30			1250
Equilin	8	26	13	2640
17β-Dihydroequilin sulfate	0			375
Equilin sulfate	0			175
Δ8-estrone				1710

RBA, relative binding affinity (testosterone, 100%); SHBG, sex hormone-binding globulin; MCR, metabolic clearance rate

Table 6 Ratio between the serum concentrations of estrone and estradiol in premenopausal and postmenopausal women as well as in postmenopausal patients treated with estradiol, depending on the route of administration. The values do not reflect the efficacy or tolerability of the therapies

	Estrone : estradiol ratio
Premenopause	1 : 2
Postmenopause	2 : 1
Oral estrone sulfate	5 : 1
Oral estradiol	5 : 1
Transdermal estradiol (patch)	1 : 1
Transdermal estradiol (gel)	1 : 1
Intranasal estradiol	2 : 1
Sublingual estradiol	1 : 3
Vaginal estradiol	1 : 5
Subcutaneous estradiol (implant)	1 : 1.5
Intramuscular estradiol	1 : 2

for influx into the liver as compared to other organs[28,29], the impact on hepatic metabolism of orally applied estrogens is much higher than that of other routes. This may cause desired or undesired effects.

Estradiol-17β (estradiol)

Estradiol is the most important estrogen produced in the human. Beyond its essential role in reproduction, it affects the whole organism and is involved in many metabolic processes. Estradiol is synthesized in the growing ovarian follicles and the corpus luteum, the placenta, adrenals and Leydig cells, but also in the liver, endometrium, brain, muscle and fat tissue. During an ovulatory cycle, the serum concentrations of estradiol vary from 30 pg/ml in the early follicular phase, to 150–350 pg/ml in the preovulatory phase and 100–210 pg/ml in the luteal phase. During pregnancy, the estradiol levels rise 100-fold, reaching concentrations of 20 ng/ml at the end of the third trimester. In postmenopausal women, the estradiol levels are usually below 20 pg/ml.

Oral administration of estradiol or estradiol valerate

After oral administration, the pharmacokinetics of estradiol differs from that of most other sex steroids. While the serum concentrations of EE, estriol and the progestogens are characterized by a rapid rise, up to a maximum after 1–3 h and followed by a rapid decline, the levels of estradiol remain elevated for up to 12 h and decrease slowly during the following time (Figure 4)[30]. This peculiarity is due to the special metabolism of estradiol, which is rapidly and extensively transformed in the intestinal tract and liver to estrone and estrone sulfate. Both metabolites are circulating in high concentrations and serve as a hormonally inert reservoir from which estradiol is continuously delivered after reconversion (Figure 5). This phenomenon is confirmed by the time course of estradiol, estrone and estrone sulfate after oral administration of estrone sulfate, which is similar to that after oral treatment with estradiol valerate[30]. Moreover, pharmacokinetic investigations with frequent blood sampling during the first hours after a single administration of 1 mg estradiol and 0.5 mg norethisterone acetate (NETA) revealed a short-term peak of estradiol – but not estrone – after 15–30 min, which was followed by a transitory fall to a nadir at 1 h. Thereafter, a gradual increase occurred, reaching a second maximum some hours later (Figure 4)[31]. This demonstrates that the decline in the estradiol levels after the first peak is stopped by an increasing supply with estradiol from the circulating estrone reservoir.

Twenty-four hours after a single application of estradiol or estradiol valerate, the serum concentrations of estradiol are still considerably higher than the pretreatment levels. Consequently, daily treatment results in an increase up to a steady state within several days. During oral treatment with 2 mg micronized estradiol or estradiol valerate, the average peak levels of estradiol were 40 pg/ml on day 1 and 80 pg/ml on day 21. The levels of estrone were 4–6-fold and those of estrone sulfate 200-fold higher than those of estradiol (Figure 5)[22,30,31]. The single administration of a combination of 1 mg estradiol with either 0.5 mg or 1 mg NETA caused a peak level of estradiol of approximately 25 pg/ml after 6 h[32]. The addition of a progestin did not influence the pharmacokinetics of estradiol[32,33].

In older postmenopausal women treated with estradiol and medroxyprogesterone acetate (MPA), the estradiol levels were observed to be slightly lower and those of MPA higher than in patients < 60 years[33].

Transdermal/percutaneous administration of estradiol

The efficacy of transdermal hormone therapy is based on a sufficient permeability of the steroid through the skin. The permeability is dependent

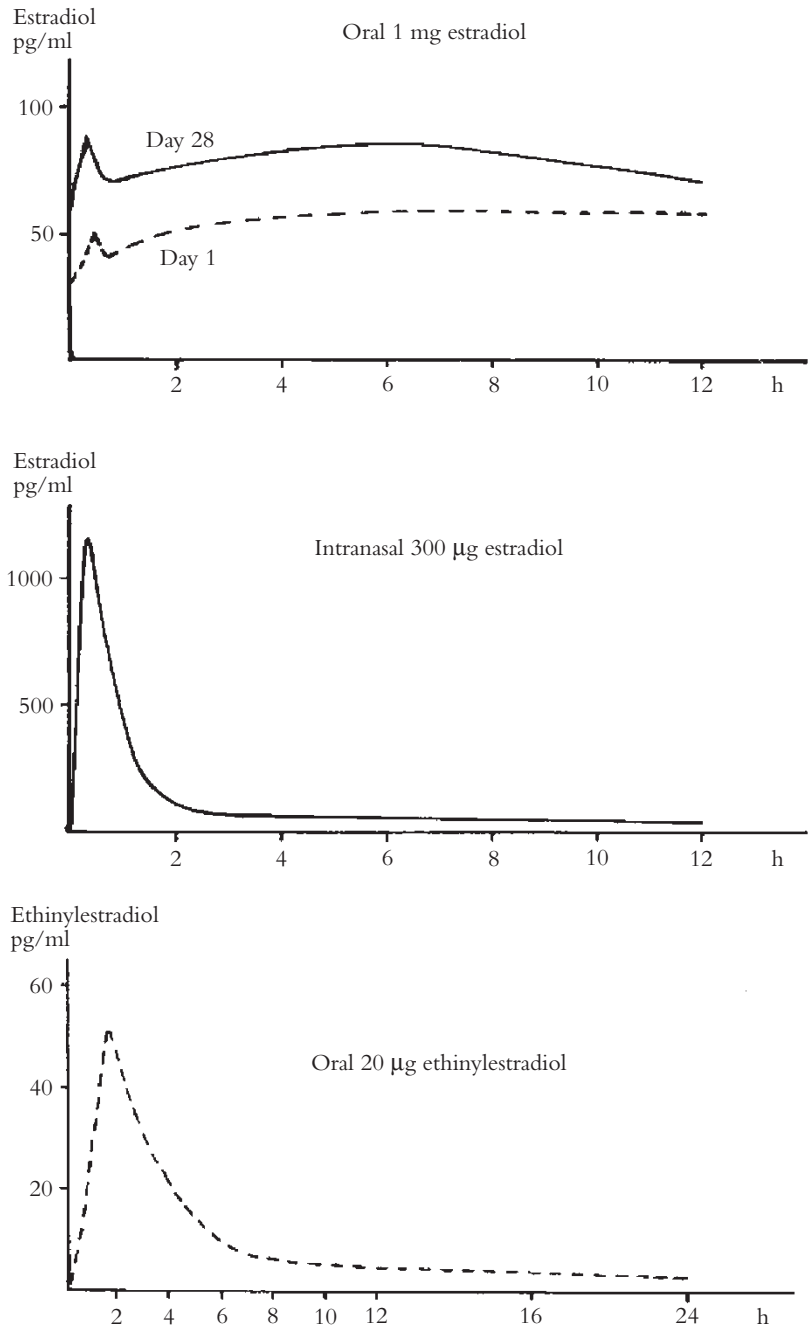

Figure 4 Serum concentrations of estradiol after oral treatment with 1 mg estradiol (after Stadberg *et al.*, 1999[31]) or intranasal treatment with 300 μg estradiol (after Devissaguet *et al.*, 1999[116]) and serum concentrations of ethinylestradiol after oral administration of 20 μg ethinylestradiol and 1 mg norethisterone (after Boyd *et al.*, 2003[190]). Conversion factor for estradiol: 1 pg/ml = 3.676 pmol/l

on lipophilic and hydrophilic properties of the compounds, and is high for progesterone and estrone, moderate for estradiol and low for estriol and cortisol, i.e. the more hydroxy groups are present, the less is the permeability.

Whether administered by means of a patch or gel, estradiol diffuses through the stratum corneum, epidermis and dermis, where it penetrates into the blood capillary system. The continuous estrogen flow is caused by a concentration gradient between the application site and the capillary vessels. The metabolism of estradiol in the skin is low and the serum levels of estrone are similar to those of estradiol. In contrast to the oral

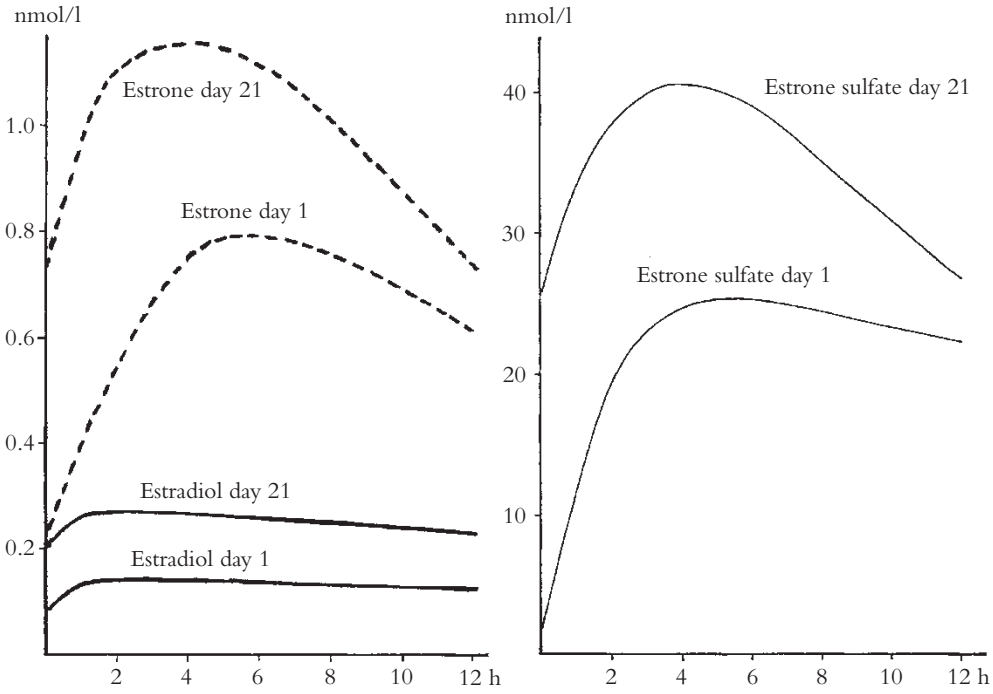

Figure 5 Serum concentrations of estradiol-17β, estrone and estrone sulfate on days 1 and 21 of treatment with 2 mg estradiol valerate (after Aedo *et al*., 1990[30])

treatment with estradiol, there is no accumulation of estrogens or estrogen conjugates in blood.

Patches are available, which are labelled to deliver a daily dose between 25 μg and 100 μg estradiol, even though the actual delivery rate may be different. In contrast to the general belief that is based on the graphic presentation of mean values, a wide range of estrogen serum levels are measured in women treated with the same patch[1,34]. Using patch or gel, there are large interindividual variations in the estradiol levels, which may differ by up to a factor of 10, and, in as much as 30% of the patients treated with a 50 μg patch, the estradiol concentrations are low. There are also considerable short-term intraindividual changes in the estradiol levels (Figure 6)[34,35].

Within 24 h after removal of the patch, the estradiol levels decrease to baseline. Using a matrix patch, it was observed that the serum concentrations of estradiol are higher in the evening and lower in the morning (Figure 6)[36]. This might be due to circadian variations in the dermal blood flow, which is highest in the evening and may enhance absorption.

There are three systems for transdermal treatment with estradiol: the reservoir patch, the matrix patch and estradiol gel. Treatment with patches may cause skin reactions. Dependent on the duration of time of affixation on the same site, mild to moderate skin erythema was recorded in 50–60% of the patients, and, after removal of the patch, some visible adhesive residues can be found[37].

It is not known to what extent the alcohol or other penetration enhancers contained in the patches may cause allergic reactions due to cutaneous sensitization.

Reservoir patch The reservoir patch contains an alcoholic gel with 2, 4 or 8 mg estradiol. Accordingly, between 25 and 100 μg estradiol are diffusing through a rate-limiting membrane per day. After application of a 50 μg patch, a rapid rise in serum estradiol occurred; this reached a maximum of 40–60 pg/ml within 30 h. Thereafter, the estradiol levels decreased continuously to 30 pg/ml after 48 h and to basal levels after 72 h (Figure 6)[38]. The use of patches which deliver daily 25 μg leads to estradiol levels of 30–40 pg/ml, and, with 100 μg daily, to values of 60–110 pg/ml. On the third day after application, the estradiol concentrations decline markedly because of the disappearance of alcohol in the reservoir. Therefore, the patches must be changed every 3.5 days. A steady state in the peak levels of estradiol is reached with the second patch.

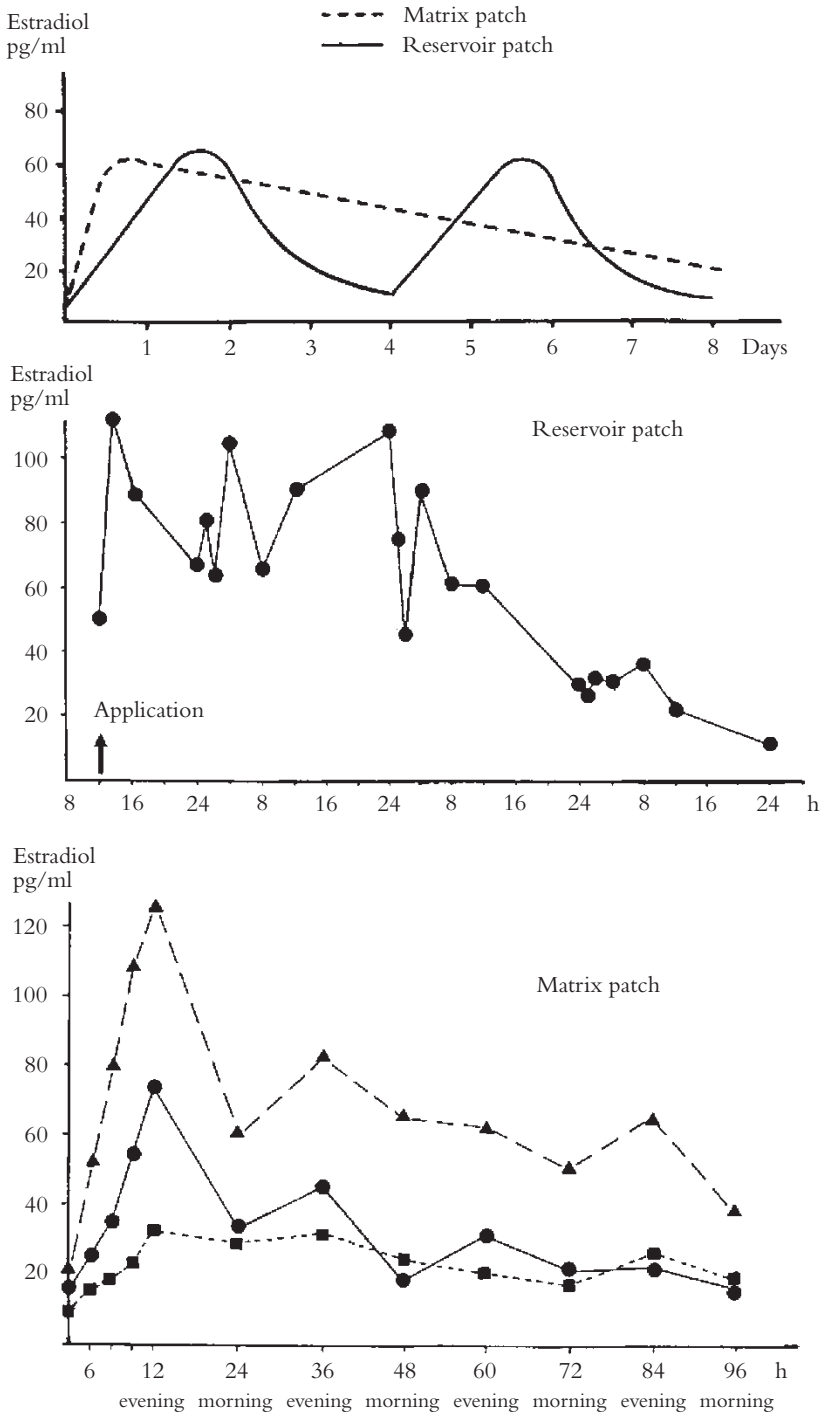

Figure 6 Serum concentrations of estradiol during transdermal treatment with daily 50 μg estradiol using a matrix patch or two reservoir patches (upper graph; after Baracat *et al.*, 1996[38]). Short-term variations in the estradiol level in a postmenopausal woman treated transdermally with estradiol (middle graph). Individual estradiol levels in three postmenopausal women treated with a matrix patch releasing daily 50 μg estradiol (after Rohr *et al.*, 1997[36])

Matrix patch The adhesive layer of the matrix patch consists of polymeric acrylate or vinylacetate in which the estradiol molecules are distributed. Therefore, as the dose delivered daily depends on the size of the patch, it can be individually adjusted by cutting the patch. The diffusion of the estrogen from the patch matrix into the skin is facilitated by the so-called

penetration enhancers (fatty acid esters, oleic acid, lecithin, ethanol). In contrast to the reservoir patch, the diffusion rate remains relatively constant over 7 days, with an only gradual decline[38]. Therefore, the patch is effective for 7 days, even though some producers recommend a change twice a week. After application of the matrix patch, the estradiol levels rise to a maximum within 12 h (Figure 6). During treatment with a 25 μg patch, the peak levels were 30–45 pg/ml, with a 50 μg patch 40–80 pg/ml, and with a 100 μg patch 90–140 pg/ml[39]. After a single application of matrix patches releasing either 25 μg, 37.5 μg, 50 μg or 100 μg estradiol, the serum estradiol concentrations showed a good dose proportionality (24 pg/ml, 35 pg/ml, 50 pg/ml and 96 pg/ml)[40].

Estradiol gel The application of a hydro-alcoholic gel containing estradiol results in a rapid penetration of the estrogens into the stratum corneum; this stops after drying of the gel on the skin. As the absorption is proportional to the surface of application, deviations from the instructions may cause variations in the estrogen level and clinical efficacy. About 10% of the dose is absorbed by the skin during the 2 min until drying. The estradiol is stored in the stratum corneum and permeates through the epidermis into the dermal capillaries according to the concentration gradient between the stratum corneum and blood. This diffusion lasts for 2–14 h. The two available gel preparations differ in their concentration and mode of application.

The one preparation contains 0.06% estradiol, and the generally used dosage is 1.5 mg estradiol in 2.5 g gel. It is applied daily on a distinct area of skin on the upper arm, thigh or abdomen. As this preparation is applied daily to the same skin area, the stratum corneum and adjacent tissues are saturated with estradiol, and the serum concentrations of estradiol correlate with the treated skin area. Daily treatment with 1.5 mg or 3 mg estradiol leads to estradiol levels of 60–90 pg/ml or 100–120 pg/ml, respectively, which are reached within 3–5 days[41]. The estrone levels are 10–30% higher than those of estradiol. After discontinuation of treatment, the estradiol levels decrease slowly and the pretreatment levels are reached after 6 days.

The other preparation contains 0.1% estradiol and is applied daily on thigh or lower abdomen to changing sites of the skin. With a dose of 1.5 mg estradiol contained in 1.5 g gel, a maximal estradiol serum level of 30 pg/ml is reached within 6 h, which thereafter declines slowly. Daily use results in a steady state, with a peak level of 80 pg/ml within 4 h after application (Figure 7)[42]. As there is no saturation with estradiol of the skin at the site of application because of the daily change of the treated area, there is no correlation between estradiol levels and gel-treated skin area. At steady state, the estrone levels are double those after a single dose, and the estradiol/estrone ratio is about 1. The estradiol levels achieved with 1.5 mg estradiol are similar to those with a 50 μg estradiol patch[42].

Pharmacodynamics of transdermal versus oral route of administration

Climacteric symptoms Both oral treatment with 0.625 mg or 1.25 mg CEE and transdermal therapy with 100 μg estradiol per day improved hot flushes and other climacteric symptoms in a similar manner[43]. The beneficial effect is observed at relative low estradiol concentrations, and no relation between the estradiol levels and the Kupperman index was observed[44]. Treatment of postmenopausal women with 1.25 g/day estradiol gel (containing 0.75 mg estradiol) resulted in a rapid improvement of moderate to severe hot flushes by 67% after 5 weeks and 76% after 12 weeks, and with 2.5 g/day (containing 1.5 mg estradiol) by 69% after 5 weeks and by 81% after 12 weeks. The incidence of estrogen-related adverse effects, particularly nausea and bleeding, was higher using the higher dose[45].

Bone Both oral therapy with estradiol or CEE and transdermal estradiol inhibit bone resorption and increase bone mass in postmenopausal women. The effect was demonstrated to correlate with the serum level of estradiol[44]. The estrogenic action on the bone is associated with a significant reduction in the fracture rate[46–48].

Cardiovascular disease Recent randomized trials on the secondary prevention of atherosclerosis or coronary heart disease by oral or transdermal HRT revealed no significant overall effect on the risk[49–53]. There was, however, a transitory increase in the rate of coronary heart disease in the first year of treatment. A similar increase in the first year of HRT was also observed in the Women's Health Initiative (WHI) study for primary prevention of coronary heart disease, which was, in fact, a secondary prevention trial for many participants[47,54]. As there was neither an early nor an overall increase in the rate of

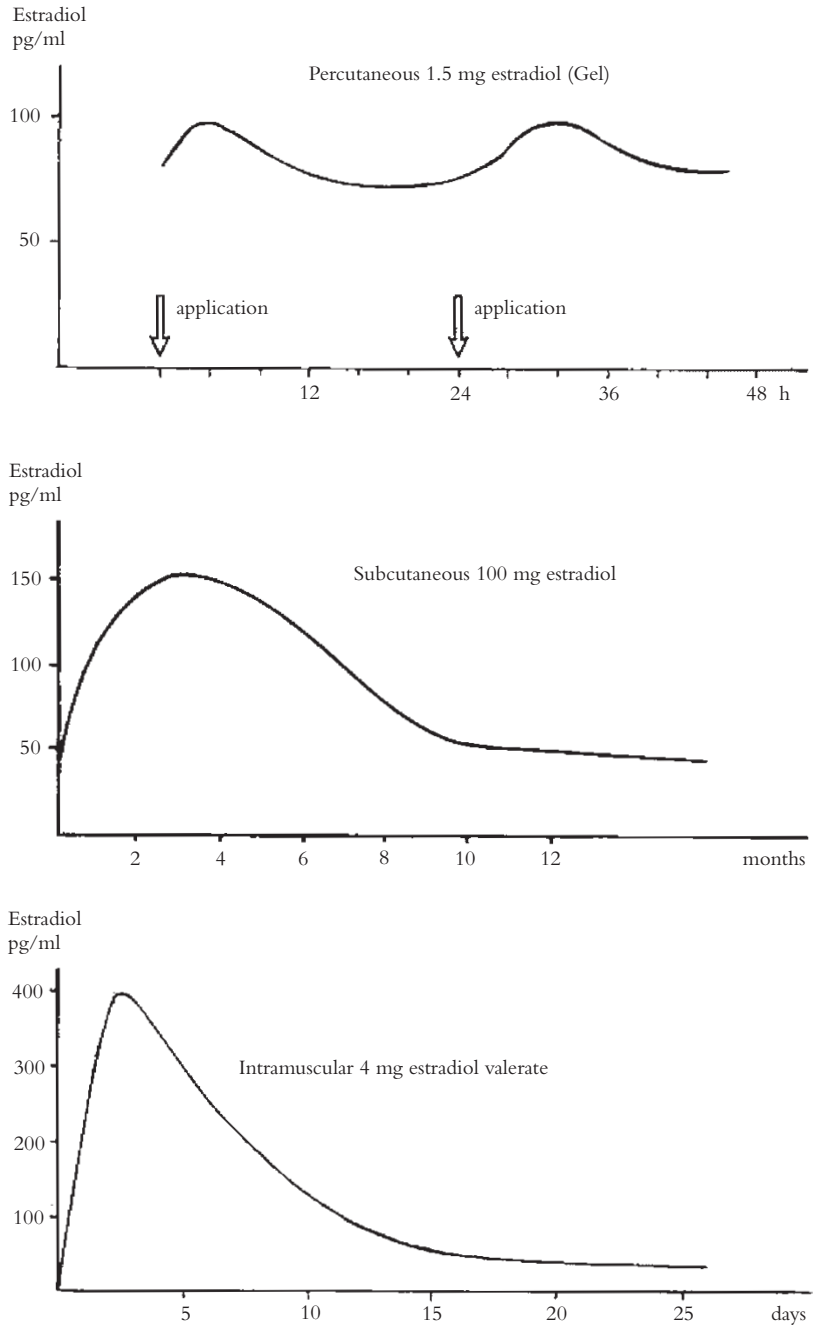

Figure 7 Serum concentrations of estradiol after percutaneous treatment with a gel containing 1.5 mg estradiol, after subcutaneous implantation of a pellet containing 100 mg estradiol or after intramuscular injection of 4 mg estradiol valerate (after Düsterberg and Nishino, 1982[151])

coronary heart disease using estrogens alone[48], the additional progestin can be regarded as a trigger of coronary events. Moreover, in the age group 50–59 years, the relative risk of coronary heart disease was decreased by 44%, narrowly missing statistical significance[48].

Primary prevention of coronary heart disease with estrogens is only possible when no severe lesions of the arterial endothelium exist. The number of ERs in the arterial wall and, hence, the protective effects of estrogens decrease with increasing age and the progression of atherosclerosis[55,56].

If treatment is started in time, estrogens inhibit the development of atherosclerosis caused by estrogen deficiency and protect against coronary

heart disease[57–63]. The available data indicate that both transdermal and oral HRT may prevent the development of atherosclerosis in postmenopausal women, provided that the endothelium has remained functionally intact[64,65].

Oral HRT increases the risk of venous thrombosis which mainly affects predisposed women, particularly in the first year[66]. It may, therefore, facilitate also the development of arterial thromboses. If there are severe lesions of the arterial endothelium, unstable plaques may exist which can rupture and cause thrombosis under the influence of HRT. This might explain the transitorily elevated coronary heart disease risk during the first year of treatment.

The weak effects on hemostasis of transdermal estradiol may contribute to the relative low risk of venous thromboembolic disease observed in a recent case–control study with or without additional progestogen[67]. In another case–control study, oral but not transdermal HRT increased significantly the risk of venous thromboembolic disease, but the number of cases in the transdermal group was low[68]. On the other hand, a third study revealed no difference between oral and transdermal therapy[69].

Hepatic serum parameters The transdermal route of application of estradiol avoids the first passage through the liver, which is characterized by local hepatic steroid levels about 4-fold higher than the peripheral serum concentrations[27]. Consequently, the effect on estrogen-dependent hepatic serum parameters is more pronounced during oral treatment with estradiol than with transdermal therapy. This includes an increase in the levels of SHBG, CBG, TBG, transferrin, ceruloplasmin, angiotensinogen and apolipoprotein A1, and a change in various coagulation and fibrinolysis factors (Table 7)[70]. The effect can be modified by additional progestins, particularly those with androgenic properties. Transdermal estradiol was demonstrated not to change binding globulins and angiotensinogen[70].

Biliary markers of gallstone formation Estrogen is believed to promote the formation of gallstones by increasing cholesterol saturation of bile, changing the composition of bile acids and decreasing bile flow. As oral treatment with estrogens has a more pronounced impact on hepatic metabolism, the transdermal route of administration of estrogens was suggested to be associated with a lower

Table 7 Effects of oral and transdermal estrogen replacement therapy on the cardiovascular system and various surrogate parameters. The effects may vary according to the type and dose of the estrogens, and may be modulated by the addition of progestogens

Parameter	*Oral estrogens*	*Transdermal estrogens*
Risk of thrombosis	increase	possibly smaller increase
Hemostasis	procoagulatory effect	minor effect
APC resistance	increase	minor increase
Atherosclerosis	prevention	prevention
Triglycerides	increase	minor decrease
HDL cholesterol, triglycerides, Apo A	increase	minor increase
LDL cholesterol, remnants, Apo B	reduction	minor reduction
Size of LDL particles	decrease	increase
Activity of metalloproteinases	increase	no effect
Vasodilation	increase	increase
Release of NO, prostacyclin	increase	increase
Release of endothelin-1	reduction	reduction
Angiotensinogen	increase	no effect
C-reactive protein	increase	no effect
Adhesion molecules	decrease	decrease
Cytokines (IL-1, IL-6, TNF-α)	no effect	no effect
PAI-1	decrease	no effect
IGF-1, IGFBP-3	decrease	no effect
IGFBP-1, GH, GHBP	increase	no effect

APC, activated protein C; HDL, high density lipoprotein; LDL, low density lipoprotein; Apo, apolipoprotein; NO, nitric oxide; IL, interleukin; TNF, tumor necrosis factor; PAI-1, plasminogen activator inhibitor-1; IGF, insulin-like growth factor; IGFBP; insulin-like growth factor-binding protein; GH, growth hormone; GHBP, growth hormone-binding protein

risk of developing gallstones. However, treatment of postmenopausal women with either transdermal estradiol 100 μg daily or oral CEE 1.25 mg daily increased the biliary cholesterol saturation index and decreased the nucleation time to the same degree, and the number of patients with cholesterol crystals was similar[71].

Hemostasis Transdermal treatment with estradiol has no effect on hemostatic parameters except a reduction in the plasminogen activator inhibitor-1 (PAI-1) and total and free protein S. In contrast, the oral therapy is associated with a certain change in various hemostatic parameters, e.g. an increase in factor IX, von Willebrand factor, thrombin–antithrombin complex (TAT), fragment 1 + 2 and D-dimer or a decrease in fibrinogen, factor VII, antithrombin, proteins S and C, tissue-plasminogen activator (t-PA) and PAI-1[72–78]. Moreover, the induction of a reversible resistance to activated protein C, possibly associated with the decrease in protein S, was more pronounced during oral estrogen therapy[73–76,79].

Lipid metabolism Oral treatment with CEE or estradiol of postmenopausal women results in a dose-dependent stimulation of the hepatic production of triglycerides, very low density lipoprotein (VLDL) cholesterol, high density lipoprotein (HDL) cholesterol, and apolipoproteins A-1 and A-2. The effects have been shown to correlate with the serum concentrations of estradiol[44]. Moreover, the activity of hepatic lipoprotein lipase (triglyceride hydrolase) which degrades HDL, is inhibited. The levels of low density lipoprotein (LDL) cholesterol and VLDL remnants are reduced due to an estrogen-induced increase in B : E- and E-receptor-mediated clearance in the liver. During oral therapy with estradiol, an increase in total triglycerides, HDL cholesterol, apolipoprotein A1 and A2, and a reduction in total cholesterol, LDL cholesterol, apolipoprotein B, and lipoprotein(a) can be observed. The effects of transdermal estradiol, i.e. a rise in HDL cholesterol and a reduction in LDL cholesterol and lipoprotein(a), are less pronounced, but, in contrast to oral treatment, the total triglyceride levels are reduced[70,72,80–84]. The estrogen-induced increase in HDL cholesterol and triglycerides can be opposed by progestins according to their type and dose, whereas the decrease in LDL cholesterol and total cholesterol is only slightly affected, if at all[82,85].

It is known that elevated triglyceride levels caused by an impaired lipolysis and remnant clearance are an independent risk factor for coronary heart disease, as remnants are highly atherogenic. Contrary to this, an estrogen-induced rise in triglycerides is associated with an elevated hepatic production of VLDLs which are not atherogenic. Moreover, the hepatic clearance of LDL and remnants is enhanced and their residence time in the circulation is shortened and oxidation is reduced. Therefore, the estrogen-induced rise in triglycerides is probably not associated with an elevated risk of atherosclerosis. Rather, the available data indicate that both transdermal and oral HRT may prevent the development of atherosclerosis in postmenopausal women, probably through direct effects on the arterial wall of the circulating estrogens[64,65].

The renin–angiotensin–aldosterone (RAA) system Oral treatment with estradiol or CEE causes a pronounced increase in serum angiotensinogen and plasma renin activity, whereas even high-dosed transdermal estradiol has no effect[70,72,84]. In healthy women, the increased level of renin substrate is without clinical relevance, because an enhanced formation of angiotensin II is counter-regulated by the negative feedback effect of aldosterone on the renin concentration, and a decrease in the activity of serum angiotensin-converting enzyme[70]. Despite a rise in the renin substrate, HRT generally does not increase blood pressure, even in hypertensive women. In fact, oral estradiol may reduce blood pressure in patients with treated hypertension[86]. It must be kept in mind that the additional progestin may exert vasconstrictory effects and contribute to a rise in blood pressure in predisposed patients with disturbed regulation of the RAA system. For safety reasons, the transdermal route of administration is recommended for HRT in symptomatic hypertensive patients who receive antihypertensive therapy[87].

Carbohydrate metabolism In contrast to high-dose CEE, which adversely affected carbohydrate metabolism, oral treatment with 0.625 mg CEE or estradiol has a favorable rather than unfavorable effect. Both oral and transdermal estradiol can reduce fasting glucose and insulin levels, and increase insulin secretion, elimination, sensitivity and glucose tolerance. Orally administered progestins may antagonize the estrogen-induced effects to a certain degree[70,88].

Other serum factors Transdermal/percutaneous treatment with estradiol exerts no or only minor

effect on the hepatic protein synthesis[84]. The effect on the serum levels of SHBG is negligible, whereas the oral therapy with estradiol or CEE causes a dose-dependent, pronounced increase. The effect may be attenuated by the addition of progestins with androgenic activity, e.g. norethisterone (NET)[34,89,90]. The rise of CBG caused by CEE is much less than that of SHBG, whereas standard doses of oral or transdermal estradiol do not influence the CBG levels. Progestins have no or only weak effects on the estrogen-induced changes in CBG. The increase in the serum concentrations of TBG observed during treatment with CEE is between that in the SHBG and CBG levels[91]. The antagonistic effect of nortestosterone derivatives on estrogen-induced change in TBG is relatively weak.

Oral treatment with estrogens caused a reduction in the serum concentrations of insulin-like growth factor (IGF)-1 and IGF-binding protein-1 (IGFBP-1) and an increase in those of IGFBP-3, growth hormone (GH) and GH-binding protein. Contrary to this, transdermal estradiol had no significant effect (Table 7)[90,92–94]. The addition of progestins with androgenic activity may counteract the estrogen-induced effects[90]. The reduction in the levels of the anabolic hormone IGF-1 during oral estrogen therapy was associated with a significant loss of lean body mass, whereas transdermal treatment with estradiol had no unfavorable effect[95].

Arterial wall Estrogens exert a vasodilatory effect which is mostly endothelium-dependent, although an estrogen-induced inhibition of calcium influx into vascular smooth muscle cells may contribute to the dilatory effect of estrogens on arteries. As the effect is caused by a direct interaction of the circulating estrogen with the arterial wall, no difference between oral and transdermal treatment must be expected, provided that the serum levels of estradiol are similar. This phenomenon is brought about by an enhanced release of vasodilatory factors (e.g. nitric oxide, prostacyclin) and a reduction of vasoconstrictors (e.g. endothelin-1) (Table 7)[96–101]. Similarly, the antioxidative effect of estradiol in the arterial wall must be independent from the route of administration. On the other hand, transdermal estradiol but not oral CEE was shown to increase the diameter of LDL particles and, hence, the susceptibility to oxidation[102].

Matrix metalloproteinases (MMP) play a role in the degradation of collagen that is associated with the formation of instable atherosclerotic plaques. In contrast to oral treatment of postmenopausal women with CEE, which caused an increase in MMP and a decrease in the tissue inhibitor of MMP (TIMP-1), transdermal estradiol did not affect the level of MMP but increased TIMP-1[103,104]. Therefore, oral but not transdermal estrogen may contribute to destabilization of vulnerable plaques.

As the development of atherosclerosis is an inflammatory process, the effect of estrogens on C-reactive protein and other inflammation markers may be important. Although oral estrogens, but not transdermal estradiol, caused a pronounced rise in the hepatic production of C-reactive protein, the lack of an increase in other inflammation markers (interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α)), or the reduction in the levels of adhesion molecules (intercellular adhesion molecule-1 (ICAM-1) and E-selectin) suggest that the estrogen-induced rise in C-reactive protein is not associated with an elevated risk of coronary heart disease (Table 7)[72,79,105–113].

Intranasal administration of estradiol

The nasal cavity comprises approximately 160 cm^2 of mucosa that are available for absorption. The tissue is highly vascularized and any absorbed compound is rapidly transported to the systemic circulation. There is also a direct transport pathway of the drugs from the nasal cavity to the cerebrospinal fluid, and the elevated drug concentrations in the cerebrospinal fluid correlate with the lipophilic nature of the compound[114,115].

The problem of intranasal administration of steroids was the dissolution of sufficient amounts of the lipophilic hormones in a very small volume of water. This was achieved by the use of methylated cyclodextrin, which is highly hydrophilic but can bind steroids by forming inclusion complexes. In this way, the solubility of steroids in water is increased 1000-fold.

It was a general belief that a bolus-like exposure to estrogens may be unfavorable with respect to the efficacy and side-effects, and a steady exposure with no large fluctuations of the serum levels should be maintained. The experience with the intranasal route of administration showed that it is, on the contrary, as effective as the transdermal route and may, for example, cause a lower rate of mastalgia.

Concerning the efficacy, the importance of the total dose of estradiol exposure over time on

target cells was demonstrated by experiments with ER-positive breast cancer cell lines[11].

The lower rate of breast tension, which is caused by edema and elevated water storage, might be associated with rapid, non-genomic actions of estrogens, e.g. on the synthesis and polymerization of proteoglycans (hyaluronic acid).

Pharmacokinetics An aqueous spray formulation containing 150 μg estradiol in 70 μl is available for HRT. After application of the spray, estradiol is rapidly absorbed by the highly vascularized nasal mucosa. This leads to a short-term rise in the estradiol levels, up to very high serum levels of estradiol within 10–30 min, followed by a rapid fall. The absorption and appearance in the circulation of the methylated cyclodextrin are negligible.

After intranasal application of 300 μg estradiol in two sprays, a peak serum level of 1150 pg/ml estradiol was reached within 20 min, which subsequently dropped rapidly to 150 pg/ml after 2 h (Figure 4). The maximal serum concentration of estrone was only 350 pg/ml[116]. The systemic exposure to estradiol during a 24-h period, as expressed by the AUC, did not differ between the intranasal therapy with 300 μg and the oral therapy with 2 mg micronized estradiol or the transdermal therapy with 50 μg estradiol[116]. Therefore, the short-term bolus of high estradiol concentrations is as effective as the relatively sustained estradiol levels observed during transdermal or oral HRT. This is based on a rapid accumulation of estradiol in the target cells and a high rate of binding to nuclear ERs. The ligand–receptor complex remains at the ERE of the DNA for more than 24 h and induces all estrogen-specific effects.

Pharmacodynamics After 4 weeks of intranasal treatment of postmenopausal women, a significant reduction of the Kupperman index was observed with 300 and 400 μg estradiol, and of hot flushes with 200–400 μg estradiol. The clinical effects of 200–300 μg intranasal estradiol corresponded to those of 1–2 mg oral estradiol valerate and 50 μg transdermal estradiol[117,118]. Dose-ranging studies with intranasal estradiol revealed an insufficient efficacy with 100 μg, and an excessive estrogen effect with 900 μg estradiol, while daily doses between 200 μg and 600 μg were well accepted by more than 80% of the patients[119].

The intranasal therapy with 300 μg estradiol daily for 56 weeks resulted in a significant increase in bone mineral density at the spine and hip and a normalization of bone turnover, as reflected by various serum bone markers. The effect was similar to that of transdermal treatment with 50 μg estradiol[120]. Intranasal treatment of postmenopausal women with 300 μg estradiol for 12 weeks increased the karypyknotic index and improved all urogenital symptoms in 90% of the patients[114]. In contrast to the 40% reduction in the serum levels of follicle stimulating hormone (FSH), no significant change in lipids and lipoproteins was observed, except a decrease in lipoprotein(a). The intranasal therapy caused a significant rise in the fibrinogen levels and a slight increase in SHBG[114].

Endometrial hyperplasia was reliably prevented by the sequential or continuous addition of progestogens at the usual doses. The frequency of breakthrough bleeding during sequential or continuous addition of progestogens was lower with the intranasal than with the transdermal or oral therapy[114,121]. An interesting finding is the significantly less occurrence of moderate to severe mastalgia during intranasal therapy (7% of the patients) than under treatment with transdermal estradiol 50 μg (15%)[121,122]. The most frequent adverse effects caused by the intranasal therapy were rhinorrhea and excessive sneezing, while prickling and nose bleeds were reported by 4–5% of the women[117].

Oral mucosal administration of estradiol

Sublingual and buccal treatment is a non-invasive and simple route of administration. Owing to the high vascularization of the oral mucosa, estradiol is rapidly absorbed and enters directly the circulation. Although the oral mucosa contains degrading enzymes, the inactivation is much less than after enteral administration, and the avoidance of the gastrointestinal metabolism and the first-pass effect in the liver results in serum levels of estradiol 10-fold higher than after oral ingestion, and the bioavailability is about five times higher[123,124].

Pharmacokinetics The sublingual administration of a tablet with 0.25 mg micronized estradiol caused a rise in the level of estradiol up to a maximum of about 300 pg/ml and of estrone of 60 pg/ml within 1 h. The application of 1 mg estradiol led to a serum maximum of 450 pg/ml estradiol and 165 pg/ml estrone. Thereafter, the estradiol level decreased rapidly to 85 pg/ml within 3 h, while that of estrone declined much slower, reaching a value of 80 pg/ml after 18 h[123].

After the buccal administration of 0.25 mg estradiol in postmenopausal women, a steep rise in the serum concentration of estradiol occurred, reaching a maximum of 500 pg/ml within 1 h. Thereafter, the estradiol level declined rapidly to 70 pg/ml after 4 h. Treatment for 2 weeks with 0.25 mg estradiol each twice a day resulted in peak levels at steady state of 620 pg/ml[125].

Pharmacodynamics The method was well tolerated, and no taste or other sensation was reported. In postmenopausal women with coronary artery disease, a single sublingual administration of 1 or 2 mg estradiol caused vasodilation, improved ischemia and augmented blood flow. The clinical effect occurred some minutes after dosing and suggested a rapid non-genomic effect of estradiol on the arterial wall[126]. In patients with severe postnatal depression, daily sublingual treatment with 1 mg estradiol 3–8 times daily resulted in a rapid improvement of depression symptoms within 1 week[127].

After 4 weeks of buccal treatment of postmenopausal women with daily 400 μg estradiol, hot flushes had significantly decreased by 80% (from 0.8 to 0.15 hot flushes per hour) and the vaginal epithelium (maturation index) was normalized. There were no unusual or severe adverse effects, and mastodynia was reported by only one out of 18 women[128].

Vaginal adminstration of estradiol (systemic treatment)

Estrogens are rapidly absorbed by the vaginal mucosa. As the local metabolism is low, the serum concentrations of estradiol after vaginal administration are 10–20-fold higher than those after oral intake of the same dose.

Pharmacokinetics The administration of a vaginal cream containing 2 mg estradiol resulted in a rapid rise in the serum levels of estradiol up to 530 pg/ml and of estrone to about 100 pg/ml, while the dose of 0.2 mg estradiol led to levels of 80 pg/ml estradiol and 45 pg/ml estrone[129].

After the insertion of intravaginal rings containing estradiol distributed in a silicone elastomer matrix, a sustained release of the hormone occurred. Using vaginal rings releasing 60 and 150 μg estradiol per day, levels of 35 and 85 pg/ml estradiol and 90 and 145 pg/ml estrone were measured. The serum concentrations of estrone sulfate were 8-fold those of estrone[130]. The daily release of 100–200 μg estradiol resulted in estradiol levels between 60 and 150 pg/ml, but a transitory high peak level could be observed on the first day after insertion[131].

The consecutive use of intravaginal rings containing 17β-estradiol-3-acetate, which is rapidly hydrolyzed to estradiol, caused a dose-dependent increase in the serum levels of estradiol. Treatment with 50 μg estrogen daily led to a mean estradiol level of 40 pg/ml, with 75 μg of 45 pg/ml, with 100 μg of 60 pg/ml, with 150 μg of 95 pg/ml, and with 200 μg of 125 pg/ml[132].

Pharmacodynamics Vaginal rings releasing 60 or 140 μg daily caused an 80% reduction in the incidence of hot flushes. They also caused an improvement of mood and vaginal symptoms. Ring expulsions occurred in 14% of the women[130].

Consecutive treatment of ovarectomized/hysterectomized patients for 4 weeks with intravaginal ring devices containing different amounts of 17β-estradiol-3-acetate, which daily released between 50 μg and 200 μg estradiol, resulted in a dose-dependent improvement of vasomotor, psychological and somatic symptoms[132]. The lowest effective dose was 75 μg estradiol/day. The change in bone markers suggested a reduction in bone resorption, whereas no significant change in the lipid metabolism was observed[132].

Vaginal administration of low-dose estradiol (local treatment)

Pharmacokinetics Using very low estrogen doses, a local effect on vaginal tissue is achieved and systemic effects are avoided. After the vaginal application of tablets containing 10 μg or 25 μg estradiol, there is a transitory small rise in the serum concentration of estradiol up to 25 pg/ml or 40 pg/ml which, after 24 h, had returned to baseline. No change in the estrone levels occurred. During the subsequent daily therapy, no increase in the serum estradiol levels was observed after the application of the tablets[133].

After the first insertion of a vaginal Silastic ring releasing 7.5 μg estradiol, there was a transitory initial burst, with relatively high serum levels of estradiol up to 55 pg/ml after 0.5 h, which was lower after the second insertion of the ring. After 24 h, the estradiol levels were very low and remained during the following weeks at the postmenopausal range of 5–10 pg/ml[134,135]. The ring is removed after 12 weeks and replaced with another ring. The daily insertion of three vaginal rings, each releasing 7.5 μg per day, resulted in a

peak level of estradiol of about 250 pg/ml after 1 h[136].

Pharmacodynamics The daily vaginal application of tablets containing 10 μg or 25 μg estradiol improved vaginal atrophy effectively due to the local action of estradiol on the mucosa. With both doses, the vaginal cytology revealed a significant reduction in parabasal cells and an increase in the superficial cells. A significant effect on urethral cytology was observed only with the dose of 25 μg estradiol[133]. Long-term treatment with 25 μg estradiol daily for the first 2 weeks and then twice a week for the remaining 12 months resulted in a significant improvement of all symptoms of vaginal atrophy (vaginal dryness, itching/burning, vaginitis, dyspareunia) and in symptoms of urinary atrophy (dysuria, frequency/nocturia, urinary tract infection). The most common side-effect was increased discharge[137,138]. In contrast to vaginal treatment with daily 50 μg estradiol, the dose of 25 μg for 3 weeks did not cause endometrial proliferation[139]. The daily insertion of three vaginal rings, each releasing 7.5 μg per day, did not affect various hemostatic factors and thrombin formation[136].

A therapy with the low-dose vaginal ring releasing 7.5 μg daily improved the maturation index of the vaginal epithelium, normalized the vaginal pH, and reduced significantly all urogenital symptoms (vaginal dryness, pruritus vulvae, dyspareunia, dysuria and urinary urgency)[135,140]. As, during long-term therapy, the marginal increase in the serum levels of estradiol is far smaller than the variability seen for the serum concentrations in untreated postmenopausal women, a systemic effect is improbable[134]. The effect of this intravaginal ring on the endometrium was negligible – only two patients had a proliferative endometrium and mild bleeding episodes were rare[141].

Subcutaneous implantation of estradiol

Pharmacokinetics The subcutaneous implantation by means of a trocar of crystalline estradiol pellets into the fat of the lower abdomen, lower backs or buttocks represents an estrogen-depot which releases estradiol at an even, slow rate. In contrast to the transdermal treatment with estradiol which showed large intra- and interindividual variations, the administration of estradiol pellets was associated with relatively small fluctuations during the 6 months after implantation[142]. The implantation of 25-mg estradiol pellets caused an estradiol level of 90 pg/ml on average for 6 months[143,144]. Using two 25-mg estradiol pellets, a maximal estradiol level of 180 pg/ml was reached after 24 h, which remained during the following 24 weeks between 100 and 120 pg/ml[142]. The implantation of a single pellet with 50 mg estradiol caused an estradiol level of about 100 pg/ml, with 75 mg of 140 pg/ml, and with 100 mg of 150 pg/ml (Figure 7)[144,145]. The individual estradiol concentrations showed, however, no relation to the dose and varied throughout a wide range[144,145]. Since, after 6 months, the levels are still elevated, reimplantation of new pellets may lead to an accumulation and to higher estradiol levels. The serum concentrations of estradiol were 50% higher than those of estrone[145].

Pharmacodynamics Within 2 months after insertion of a 50-mg estradiol implant into postmenopausal women, severe hot flushes had nearly totally disappeared. Since, after 6 months, an increase in the symptoms occurred, reimplantation was performed every 6 months[145]. Treatment with 25-mg, 50-mg or 75-mg estradiol implants increased bone mineral density in a dose-dependent manner, but did not affect hemostatic parameters[144,146,147]. Implants of 50 mg estradiol showed only minor effects on lipid metabolism, with a slight decrease in LDL cholesterol and a slight increase in HDL cholesterol[148].

The use of two 25-mg estradiol pellets caused a suppression of FSH levels by 60–70% and an increase in HDL cholesterol by about 15%, whereas LDL cholesterol, total cholesterol and triglycerides did not change[142].

There are reports on recurrence of hot flushes within 3–16 weeks after implantation of 50–100 mg estradiol, although (or because?) the estradiol levels were measured in these women to be extremely high (between 400 and 1000 pg/ml)[149]. The underlying mechanism is unknown; perhaps the symptoms are due to a desensitization phenomenon by extremely high estrogen levels causing the recurrence of estrogen deficiency symptoms. In women with supraphysiological estradiol levels during treatment with implants, no adverse effects on lipid metabolism, but a reduction in LDL cholesterol and fasting insulin were observed[150].

Intramuscular administration of estradiol esters

After intramuscular injection of an oily solution of fatty acid esters of estradiol, the solvent is

absorbed, leading to a primary microcrystalline depot at the injection site. Moreover, a secondary depot in fat tissue may also be formed. The ester is released at a slow rate and hydrolyzed in the liver and other organs. The longer the fatty acid chain, the more lipophilic is the ester, the more prolonged is the time course of the serum concentration curve, the more protracted is the duration of action and the lower are the serum concentrations of estradiol. The estrone levels are only half those of estradiol. After the injection of 4 mg estradiol valerate, a maximal serum level of 400 pg/ml is reached after 2 days, which thereafter decreased to 150 pg/ml within 10 days (Figure 7)[151]. The injection of 5 mg of the more lipophilic estradiol cyclopentylpropionate (cypionate) caused a peak level of 340 pg/ml estradiol after 4 days, which was followed by a slow decline to a value of 50 pg/ml not before 2 weeks. The serum maximum of estrone was only 145 pg/ml[152].

Conjugated equine estrogens

The term 'conjugated equine estrogen' (CEE) refers to a natural mixture of water-soluble sodium salts of estrogen sulfates, which are extracted from the urine of horses and blended to represent an average standardized estrogenic activity. Therefore, the composition of the mixture may vary within a certain range. In Table 8, the most important compounds with estrogenic activity are listed, but, beyond this, the urinary extract contains many other substances, e.g. phytoestrogens and non-estrogenic steroids[16].

Composition of conjugated estrogens

CEE contains the sulfates of at least ten estrogens. Besides the compounds also produced in the human (estradiol-17β, estrone, and estradiol-17α), there are the ring B unsaturated equine estrogens equilin, dihydroequilin-17β, dihydroequilin-17α), equilenin, dihydroequilenin-17β, dihydroequilenin-17α, and Δ8-estrone (Figure 2). The human estrogens cannot be converted to the ring B unsaturated estrogens, which are produced in the horse by an alternative pathway[16].

The most potent estrogen is equilin sulfate: at a dose of 0.25 mg it is as effective as 0.625 mg CEE in the improvement of hot flushes, and, at a dose of 0.625 mg, the increase in the levels of SHBG, CBG, and angiotensinogen was 1.5–8 times that observed with estrone sulfate[16]. Due to the different bioavailabilities and pharmacokinetics of the components contained in a tablet of CEE, the ratios between the serum levels of the various compounds may differ from those between the doses. While the proportion of estrone in the total dose of CEE corresponds to the proportion in the circulation (about 50%), the percentage of circulating equilin is higher than that in the tablet, and that of the serum level of Δ8-estrone is 5-fold that in the tablet.

Esterified estrogens

There are other preparations containing artificial mixtures of estrogen conjugates which differ in their composition from that of the CEE. These formulations are named 'esterified estrogens' and

Table 8 Composition of conjugated equine estrogens (CEE) and the relative estrogenic potencies on the vaginal epithelium of ovariectomized rats and the rat uterus, and the relative binding affinity (RBA) for recombinant human estrogen receptors ERα and ERβ[17,153–155]

Compound	*Proportion* (%)	*Relative potency in the vagina* (%)	*Relative potency in the uterus* (%)	*RBA for ERα*	*RBA for ERβ*
CEE	100	38	100		
Estradiol-17β	0.56	100		100	100
Estrone	49.1	30	32	26	52
Equilin	22.8	42	80	13	49
17α-dihydroequilin	13.5	0.06	2.6	42	32
17β-dihydroequilin	1.5	83	200	113	108
Δ8-estrone	3.9			19	32
Δ8-estradiol-17β				68	72
Estradiol-17α	3.7	0.11	3.5	19	42
Equilenin	2.8	1.3	11.4	15	29
17α-dihydroequilenin	1.6	0.018	1.3	20	49
17β-dihydroequilenin	0.7	0.21	9.4	68	90

consist mostly of estrone sulfate (75–85%) and equilin sulfate (6–15%)[16]. As the major part of the dose is estrone sulfate, the activity of the preparation is mainly dependent on its conversion to estradiol.

Mechanism of action of conjugated equine estrogens

As there are large differences in the tissue distribution of the ER and the binding affinity for ERα and ERβ of the various compounds (Table 8), the importance of a single component for the biologic response to the CEE mixture may vary. Moreover, the interindividual variations in the pharmacokinetics of the various components may be associated with large variations in the biologic effects. It is, however, not possible to specifiy the effects of the various components. According to the high amount contained in the CEE mixture and the high potency of their active metabolites (estradiol-17β and dihydroequilin-17β), estrone sulfate and equilin sulfate are the most important compounds in the CEE preparation. In general, the clinical effects correspond to those of estradiol. As prolonged treatment with unopposed CEE is associated with an increased risk of endometrial hyperplasia, it must be combined sequentially or continuously with a progestin[156].

Oral administration of conjugated estrogens

Pharmacokinetics After oral administration, a large proportion of the conjugates is hydrolyzed in the mucosa of the stomach and the small bowel. After resorption, the free steroids are, to a large degree, conjugated once more, particularly during the first liver passage. A certain amount of estrone, equilin and equilenin is converted to the active estrogens estradiol-17β, dihydroequilin-17β and dihydroequilenin-17β (Figure 2). The conversion occurs not only in the liver, but also in the other organs and tissues[14]. During daily treatment, the serum concentrations of the hormones and conjugates increase up to a steady state. Equilin and dihydroequilin-17β can also be converted to equilenin and dihydroequilenin-17β. Similar to estradiol and estrone, the ring B unsaturated equine estrogens can be hydroxylated at C2 and C4 into catechol estrogens, and also at C16α. Although the main proportion of CEE is circulating as sulfate, excretion occurs mainly after transformation to glucuronides.

Owing to the relatively high binding affinity of the steroid sulfates to albumin, the half-life of equilin sulfate in the circulation is considerably longer than that of equilin. After a single oral dose of 1.25 mg CEE, the serum level of estrone reached a maximum of 135 pg/ml after 8.6 h and decreased thereafter slowly, while the peak level of equilin of 70 pg/ml occurred after 7.3 h on average. The peak level of total estrone (including the conjugates) was 5.7 ng/ml after 7.7 h and that of total equilin 4.1 ng/ml after 5.8 h[157]. Using a preparation of esterified estrogens consisting of 75–85% estrone and 6–15% equilin, the levels of estrone were higher and those of equilin lower than after administration of the CEE preparation[157].

Pharmacodynamics Regarding the estrogenic efficacy, the most important compounds are estradiol and dihydroequilin-17β (Table 8). Even though estradiol-17α, dihydroequilin-17α, and dihydroequilenin-17α have only weak effect on the endometrium and vaginal epithelium, it cannot be excluded that the 17α-isomers may act in a non-genomic way, e.g. as vasodilators or antioxidants[153]. HRT with CEE effectively improves climacteric symptoms and atrophic changes in the urogenital tract, prevents osteoporosis and atherosclerosis. Concerning the clinical effects, treatment with 0.3–0.45 mg CEE is comparable with 0.5–1 mg oral estradiol or 25–37.5 μg transdermal estradiol, while 0.625 mg CEE corresponds to 1–2 mg oral estradiol. With respect to the effect on the production of hepatic proteins, CEE has a more pronounced effect than estradiol, as estimated on weight basis, and the ratio between hepatic and clinical effects of CEE is higher than that of oral estradiol (Table 3)[12].

Vaginal administration of conjugated estrogens

Pharmacokinetics Topical applied CEE is rapidly absorbed by an atrophic vaginal mucosa. Vaginal treatment of postmenopausal women with 0.3 mg, 0.625 mg, 1.25 mg or 2.5 mg CEE daily for 4 weeks resulted in a dose-dependent increase in the proportion of superficial cells of the vaginal epithelium up to 37%. Owing to the poor metabolism in the vaginal mucosa, the serum concentrations of unconjugated estrogens and, hence, the systemic effects are relatively low. The mean serum levels of estradiol measured after 4 weeks were 13, 23, 38, and 63 pg/ml, while those of estrone were three times higher[158].

Pharmacodynamcis Vaginal treatment with 0.625 mg CEE, contained in 1 g of cream, did not cause endometrial proliferation. In 8% of the patients, mild intercurrent vaginal bleeding was recorded, but only one woman had a proliferative endometrium[141].

There was a dose-dependent reduction in the levels of FSH and luteinizing hormone (LH). In contrast, the effect on urinary excretion of calcium and hydroxyprolin, and on the serum levels of hepatic proteins, was relatively weak. A significant rise in angiotensinogen by 43% was observed only with a dose of 2.5 mg CEE. A small increase in the levels of SHBG and TBG occurred only with 1.25 mg and 2.5 mg CEE, whereas CBG did not change at all. There was also no significant change in the levels of lipids and lipoproteins[158]. As compared with the oral route of administration, the effectiveness of vaginal CEE was about 4-fold with regard to the vaginal epithelium, but only 15–50% concerning the effect on gonadotropins, calcium excretion, angiotensinogen and TBG, and only 6% concerning SHBG[91,158].

Estradiol-17α

Estradiol-17α is an isomer of estradiol-17β which has a lower binding affinity to the ERα and ERβ than estradiol-17β and only weak estrogenic activity (Figure 2, Table 2). Similar to estriol, it is a short-acting estrogen which, after oral administration of usual doses, has no proliferative effect on the endometrium, breast epithelium and vaginal epithelium, and does not influence the hepatic protein synthesis (e.g. SHBG) and lipid metabolism. Estradiol-17α may, however, improve vasomotor symptoms and inhibit gonadotropin secretion. In the arterial wall, it does not affect proliferation and migration of vascular smooth muscle cells or endothelial production of adhesion molecules, but exerts vasodilatory effects by inhibition of calcium influx into the smooth muscle cells. Moreover, it has an antioxidative activity and shows a favorable local effect on androgenetic alopecia.

In contrast to estradiol-17β, it cannot be metabolized to estrone, but is conjugated much faster. The major conjugate is the 17α-sulfate, whereas estradiol-17β is mainly conjugated to the 3-sulfate. Like estradiol-17β, it is metabolized to 2- and 4-hydroxy-estradiol-17α which affect the central nervous system and are inactivated by the COMT similar to the catecholestrogens derived from estradiol-17β. It is also converted to the 16,17-dihydroxy-metabolite. As it cannot be converted to estrone and estrone cannot be transformed to estradiol-17α, the pharmacokinetics differs from that of estradiol-17β and shows a rapid fall after reaching the peak level.

Estriol

Estriol is an end-product of the estradiol–estrone metabolism. It is mainly produced by the reduction of 16α-hydroxyestrone and can be reconverted to this compound, but not to estrone or estradiol. In non-pregnant women, the serum concentrations of estriol are very low (about 10 pg/ml), but increase during pregnancy up to 12 ng/ml of unconjugated estriol and 210 ng/ml of total estriol[159]. In most tissues, e.g. endometrium or liver, estriol acts as a weak estrogen, but produces full estrogenic responses in the vaginal epithelium[160].

Mechanism of action of estriol

Estriol is a so-called short-acting estrogen. While the association rate constant is similar to that of estradiol, its dissociation from the activated receptor occurs much faster[161]. Moreover, the interaction of the ER with the ERE is impaired with estriol as a ligand[160].

Consequently, the dissociation from the ER is rapid and it is bound in the cell nucleus for a much shorter period of time (6 h) than estradiol (24 h)[161,162]. Therefore, estriol can induce only those biological effects which are based on a short-term interaction with the ERE. Since, for the induction of endometrial mitoses, binding to the nuclear binding sites for at least 9–12 h is necessary, treatment with estriol at the recommended doses does not cause endometrial proliferation. Although it is accumulated in endometrial, myometrial and vaginal tissue to a similar degree as estradiol[26], it exerts pronounced estrogenic effects only in the vaginal epithelium.

On the other hand, if estriol is administered at high doses or if the dose is divided and taken twice or three times a day, estriol will be present within the endometrial cell for a prolonged time interval and, hence, may cause proliferation of the endometrium. The same holds true for the effect of a meal on the pharmacokinetics of orally administered estriol. The estriol conjugates excreted in high amounts in the bile can be hydrolyzed in the colon and re-absorbed. Therefore, stimulation of the enterohepatic circulation of estriol by a meal may cause a second peak of

the serum concentration of estriol, which leads to a prolonged presence of estriol in the target cells and, for example, to endometrial proliferation[163,164]. This might explain the increased risk of endometrial cancer in postmenopausal women treated orally with the recommended dose of 1–2 mg[165]. Therefore, estriol should be taken as a single dose, as low as possible, in the evening.

Oral administration of estriol

Pharmacokinetics The standard dose of estriol is 1–2 mg, preferably taken as a single dose in the evening. After oral administration, estriol is extensively conjugated and then rapidly excreted. Only 10–20% of the dose remains in the circulation and only 2% of the total dose appears as unconjugated estriol in the blood, with a maximum after 1–3 h[163,166,167]. While it has nearly no affinity to SHBG, 91% of the circulating estriol is bound to albumin and 8% is free (Table 5). Within 1 h after a single oral administration of 8 mg estriol to postmenopausal women, maximal serum concentrations of 65 pg/ml estriol and 60 ng/ml estriol conjugates were reached. During daily treatment with 8 mg estriol, the peak levels of estriol increased up to 130 pg/ml (Figure 8), whereas those of estriol conjugates remained at a level of 60 ng/ml[166]. After reaching the serum maximum, the estriol levels decreased rapidly to low levels. The predominant metabolites in the circulation are glucuronides, which are mainly formed in the intestinal mucosa, and the sulfates, which are formed in the liver. Due to the three hydroxy groups, estriol can be conjugated to various glucuronides and sulfates or mixed glucuronides/sulfates. These conjugates can be hydrolyzed to estriol, particularly in the colon by bacterial enzymes, and undergo enterohepatic circulation. Due to the complete hydrolysis of estriol conjugates and reabsorption of estriol in the colon, more than 95% of estriol is excreted by the kidney. The importance of the entero-hepatic circulation was demonstrated in postmenopausal women who took a meal 4 h after oral administration of 8 mg estriol. Two hours later, a second estriol peak of 120 pg/ml occurred, and thereafter the estriol concentration declined slowly to about 25 pg/ml after 24 h (Figure 8)[163].

In estriol succinate (estriol-16α,17β-dihemisuccinate), the hydroxy groups at C16α and 17β are esterified with succinic acid, and a dose of 2 mg contains 1.18 mg estriol. Estriol succinate is nearly not hydrolyzed in the intestinal mucosa and, therefore, absorbed at a slower rate than estriol. The circulating estriol succinate is then rapidly hydrolyzed in the liver, and, within 12 h after a single oral dose of 8 mg, a maximum of serum estriol of 40 pg/ml is reached, which increases during daily administration up to 80 pg/ml[168].

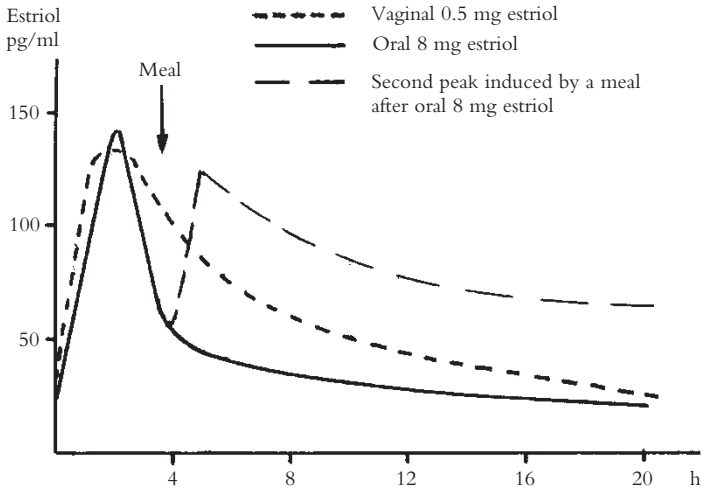

Figure 8 Serum concentrations of estriol after oral administration of 8 mg estriol with or without taking a meal 4 h later, and after vaginal application of 0.5 mg estriol (after Englund *et al*., 1984[163]; Heimer, 1987[164]; Kuhl, 1990[24])

Pharmacodynamics Oral treatment with estriol may improve climacteric symptoms dose- and time-dependently. In a randomized, placebo-controlled trial, treatment of postmenopausal women with daily 2–4 mg estriol resulted in a significant improvement of hot flushes, but it was found to be less effective than 0.625 mg CEE or 2 mg estradiol valerate[169]. Already after 4 weeks of therapy with 2 mg estriol, the Kupperman index was reduced by 39%, and after 12 months by 60%[170]. Estriol also improved effectively atrophic symptoms in the urogenital tract, but had no significant effect on urinary incontinence[171,172]. Oral treatment with 2 mg estriol reversed the postmenopausal decline in skin thickness and collagen content, but had no significant effect on bone resorption and fracture risk[173–175]. Estriol has a proliferative effect on breast epithelium, but does not increase mammographic density. Oral estriol at daily doses of 2–4 mg did not affect hepatic serum proteins, lipid metabolism and hemostatic parameters.

Vaginal administration of estriol

Pharmacokinetics Owing to the rapid absorption and low metabolism in an atrophic vaginal mucosa, the topical administration of estriol causes serum concentrations of estriol which are 10–20 times higher than after the oral application of the same dose. As about 20% of the vaginally administered estriol reaches the circulation as an unchanged steroid, the peak level after topical application of 0.5 mg estriol is 130 pg/ml, which is in the range of that found after oral administration of 8 mg estriol (Figure 8)[24]. Therefore, the daily vaginal treatment with 0.5 mg estriol not only causes high local concentrations in the urogenital tissue and a conspicuous improvement of atrophic disorders, but acts systemically, too. The lacking difference in the local effects of 0.5 mg and 1 mg estriol suggests a saturation effect[173]. With regard to the improvement of atrophic symptoms, the vaginal application of only 30 μg estriol was as effective as 0.5 mg estriol, but was not associated with systemic effects[138]. If a vaginal dose of 0.5 mg estriol is used, the replacement of daily administration by a maintenance dose twice every week largely attenuates the systemic effects of vaginal estriol.

Two hours after a single vaginal application of a pessary containing 1 mg estriol to postmenopausal women, a mean peak serum concentration of estriol of about 480 pg/ml was measured, which was followed by a rapid fall to very low levels after 12 h. Owing to the relatively low metabolism in the vaginal mucosa, the serum concentrations of estriol conjugates measured after vaginal application are similar to those of unconjugated estriol[176].

Pharmacodynamics Vaginal treatment with 0.5 mg estriol resulted in a marked proliferation and maturation of an atrophic epithelium and a relief of vaginal symptoms like itching, burning, dryness and dyspareunia. The treatment also caused proliferation of the urethral epithelium and a reduction in urinary tract infections and alleviated subjective urinary tract sym-ptoms[140,177]. The increase in the vaginal estriol dose to 1 mg did not enhance the local effects, whereas the systemic effects, for example, the improvement of climacteric symptoms and the reduction in the gonadotropin levels, were found to be more pronounced with the higher dose[177]. When administered in the recommended dose and regimen (0.5 mg daily for the first 2–3 weeks, followed by 0.5 mg twice a week), the vaginal estriol therapy did not cause endometrial proliferation[178]. Similar to the oral treatment with estriol, vaginal estriol did not change the serum concentrations of hepatic proteins[179].

Ethinylestradiol

Ethinylestradiol (EE) is much more active than the natural estrogens, because the 17α-ethinyl group prevents the oxidation of the 17β-hydroxy group, as known from the conversion of estradiol-17β to estrone (Figure 2). Moreover, the 17α-ethinyl group can be oxidized, resulting in a very reactive intermediate which may irreversibly inhibit CYP enzymes involved in the metabolism of steroids. It has been demonstrated that EE may inhibit its own hydroxylation at C2 by means of this mechanism-based inactivation of CYP enzymes[180–182]. Therefore, the dose of EE necessary both for relief of climacteric complaints and the prevention of bone resorption is much lower than that of estradiol. On the other hand, the reduced inactivation rate results in a more pronounced hepatic effect of EE as compared to estradiol (Table 3)[183]. This is reflected by, for example, a dose-dependent increase in the serum levels of hormone-binding globulins, hemostatic parameters or lipids and lipoproteins[184–186]. Consequently, the ratio between clinical and hepatic effects is lower during treatment of postmenopausal women with EE than with CEE or estradiol (Table 3).

On the other hand, the increased incidence of bleeding and/or spotting during continuous treatment with combinations of estradiol and progestins is associated with the progestin-

induced stimulation of the endometrial 17β-HSD. The activated enzyme causes an enhanced inactivation of estradiol within the endometrial cells and, hence, an insufficient local estrogen effect. This is not the case when using EE, because the 17α-ethinyl group blocks the action of this enzyme[187]. This explains the lower rate of bleeding and spotting during continuous treatment of postmenopausal women with combinations of EE and progestins, as compared to combinations of natural estrogens and progestins. In the absence of a progestin, the proliferative effect in the endometrium of estradiol is higher than that of EE[188]. It is not known whether this also holds true for the proliferative action of estrogens on the breast epithelium. In ovarectomized cynomolgus monkeys, treatment with an EE/NETA combination did not induce proliferation, whereas a combination of CEE and MPA caused a pronounced proliferation of the mammary epithelium[189].

Oral administration of ethinylestradiol

Pharmacokinetics After a single administration of a combination of 20 μg EE and 1 mg NET to postmenopausal women, a rapid rise in the EE serum levels occurred, reaching a maximum of 50 pg/ml, on average within 1.5 h (Figure 4)[190]. It is known from investigations with oral contraceptives that, during daily treatment, the EE levels increase further by about 50% until reaching a steady state[191]. In contrast to the pharmacokinetics of estradiol, which is characterized by a rapid rise of the estradiol level which remains elevated for many hours, a rapid fall of the serum EE occurs after reaching the maximum (Figure 4). There were large interindividual variations in the EE levels[191].

Owing to the high first-pass metabolism in the intestinal mucosa and the liver, the bioavailability of oral EE is 38–48%. Therefore, most of the circulating compounds are conjugates of EE and EE metabolites. Only 1% of the dose appears in the circulation as EE, of which 98.5% are bound to albumin, because EE has no binding affinity to SHBG. The ratio between the serum levels of EE sulfate and EE varies between 6:1 and 22:1. Consequently, the enterohepatic circulation plays a role in the pharmacokinetics of EE. The metabolism is focused on hydroxylation at C2 and C4, resulting in the formation of catecholestrogens which can be transformed to 2- and 4-methoxy-EE. Further metabolites are 6α- and 16β-hydroxy-EE, whereas the formation of 16α-hydroxy-EE (according to estriol) is sterically hindered by the 17α-ethinyl group.

Pharmacodynamics Treatment of postmenopausal women with EE results in a dose-dependent improvement of hot flushes. The maximal effect was seen with a daily dose of 15 μg EE, which caused a relief in 77% of the patients[192]. A dose-related response was also observed during continuous treatment with combinations of EE and NETA. Using both 5 μg EE and 10 μg EE each combined with 1 mg NETA, a 80–90% reduction in the frequency and severity of hot flushes was recorded[193]. After 4 months of treatment with EE/NETA combinations at doses between 5 μg EE/0.5 mg NETA and 20 μg EE/1 mg NETA, hot flushes had disappeared in nearly all women[194]. At month 3 of treatment with 5 μg EE/1 mg NETA, the incidence of bleeding and spotting was half that observed with CEE/MPA[195].

The effect of EE on postmenopausal bone resorption was also dose-dependent. A net loss of bone mass was observed using doses below 15 μg EE daily, whereas no loss occurred using doses between 15 and 25 μg and a net gain occurred with doses of 25 μg and more EE[196]. Treatment of postmenopausal women with combinations of EE (5–20 μg) with NETA (0.5–1 mg) increased vertebral bone mineral density in a dose-dependent manner[194].

Despite an increase in the angiotensinogen level by 150%, treatment of postmenopausal women with 20 μg EE did not affect day-time blood pressure and even reduced night-time blood pressure[197].

Vaginal administration of ethinylestradiol

Despite bypassing the first liver passage, vaginal treatment with EE caused significant hepatic effects. After a single vaginal application to young women of a tablet containing 50 μg EE and 250 μg levonorgestrel (LNG), the EE level rose to a maximum of 190 pg/ml within 3.5 h, i.e. the peak level was lower and occurred later than after oral administration[198]. Treatment of fertile women with a vaginal ring releasing daily 15 μg EE resulted in serum EE levels of about 20 pg/ml on average. The bioavailability was 55%[199].

Vaginal treatment of postmenopausal women with daily 20 μg EE in suppositories caused an increase in SHBG by 100%, which was similar to that observed under oral treatment with 5 μg EE. Regarding the effects on gonadotropins, SHBG, CBG, and HDL cholesterol, the oral route was 4–5 times more potent than the vaginal route of administration of EE. As this holds true both for hepatic and central parameters, it was concluded

that the vaginal administration did not reduce the hepatic impact of EE as compared to non-hepatic actions[200].

PROGESTOGENS

The only indication for the use of progestogens in HRT is the prevention of estrogen-induced endometrial hyperplasia, because a long-term unopposed estrogen action on the endometrium increases the risk of hyperplasia and cancer of the endometrium. The natural progestogen is progesterone, which plays an important role in the regulation of the function of the cervix, uterus, endometrium, tubes, the central nervous sytem, pituitary and the breast. As progesterone is rapidly metabolized in the intestinal tract, liver and many other tissues, its effectiveness is dependent on the galenic preparation, and – if administered orally or vaginally – on a high dosage. Therefore, most HRT preparations contain a synthetic progestogen (progestin) which can be used at relatively low doses because its inactivation is slowed down owing to structural peculiarities.

Mechanism of action

Originally, progestogens were defined as compounds which maintain pregnancy, but, in the human, only progesterone can maintain pregnancy. The activity and potency of synthetic progestins are mostly evaluated by means of parameters associated with endometrial effects.

The various actions of progesterone and synthetic progestins are brought about by genomic interactions with the progesterone receptors (PRs), which exist in the two isoforms, PRA and PRB, and by rapid non-genomic interactions with membrane binding sites. Moreover, according to their chemical structure, progestogens may bind to other members of the nuclear receptor superfamily. Binding of a progestogen to the receptor results in dimerization and interaction with a hormone responsive element within hormone-regulated target genes. In general, PRA may act as transcriptional repressor and PRB as activator. PRA may repress not only the transcriptional activity of the PRB, but also that of the ER, androgen receptor, and the glucocorticoid and mineralocorticoid receptors[201].

In most tissues, the biological action of progestogens is dependent on the presence of estrogens, as estrogens play a key role in the induction of PR, while progestogens down-regulate the expression of the ER. Progestogens may also reduce the expression of PR in the endometrial epithelium, but not the stromal and myometrial PR. Both PRA and PRB are expressed in the endometrial glands, whereas PRA predominates in the endometrial stroma[202]. In the breast of primates, progestogens may reduce the expression of the ERα and PR, but the estrogen-induced proliferation of the mammary epithelium is not inhibited, but enhanced by progestogens[203].

The primary role of progestogens in HRT is the inhibition of estrogen-induced proliferation of the endometrium. Moreover, they induce secretory changes in a proliferated endometrium. The antiestrogenic effect of progestogens in the endometrium is associated with a suppression of ER and the activation of the 17β-HSD type 2 which converts estradiol to estrone, and the estrone-sulfotransferase which causes conjugation of estrone.

Structure, activity and metabolism

Besides the natural progesterone, four types of orally active, synthetic progestins are available: the progesterone derivatives, 19-norprogesterone derivatives (Figure 9), 19-nortestosterone derivatives and the spirolactone derivative drospirenone (Figure 10). They all exert progestogenic and – in some tissues - antiestrogenic activities, but differ largely in their hormonal pattern. According to their chemical structure, they may act as weak androgens or antiandrogens, glucocorticoids or antimineralocorticoids. This is based on the structural similarity of the respective receptors which belong to the nuclear receptor superfamily. The various progestogens may bind to one or more of these receptors with low or high binding affinity, but there is not necessarily a corresponding biologic response (Tables 9 and 10). Binding to a receptor may be associated with an agonistic, antagonistic or no clinical effect.

The prerequisite of the progestogenic activity of a steroid is the existence of a 3-keto group and a double bond between C4 and C5 in ring A (Δ4-3-keto group). There are some nortestosterone derivatives which lack this characteristic, e.g. desogestrel, norgestimate or tibolone. They are prodrugs which, after oral administration, are rapidly converted to an active progestin with a Δ4-3-keto group.

Besides their effect on the endometrium, synthetic progestins may act on the vaginal epithelium as antiestrogens and reduce the maturation index. In the cervix, they reduce the amount and spinnbarkeit of the mucus, in the

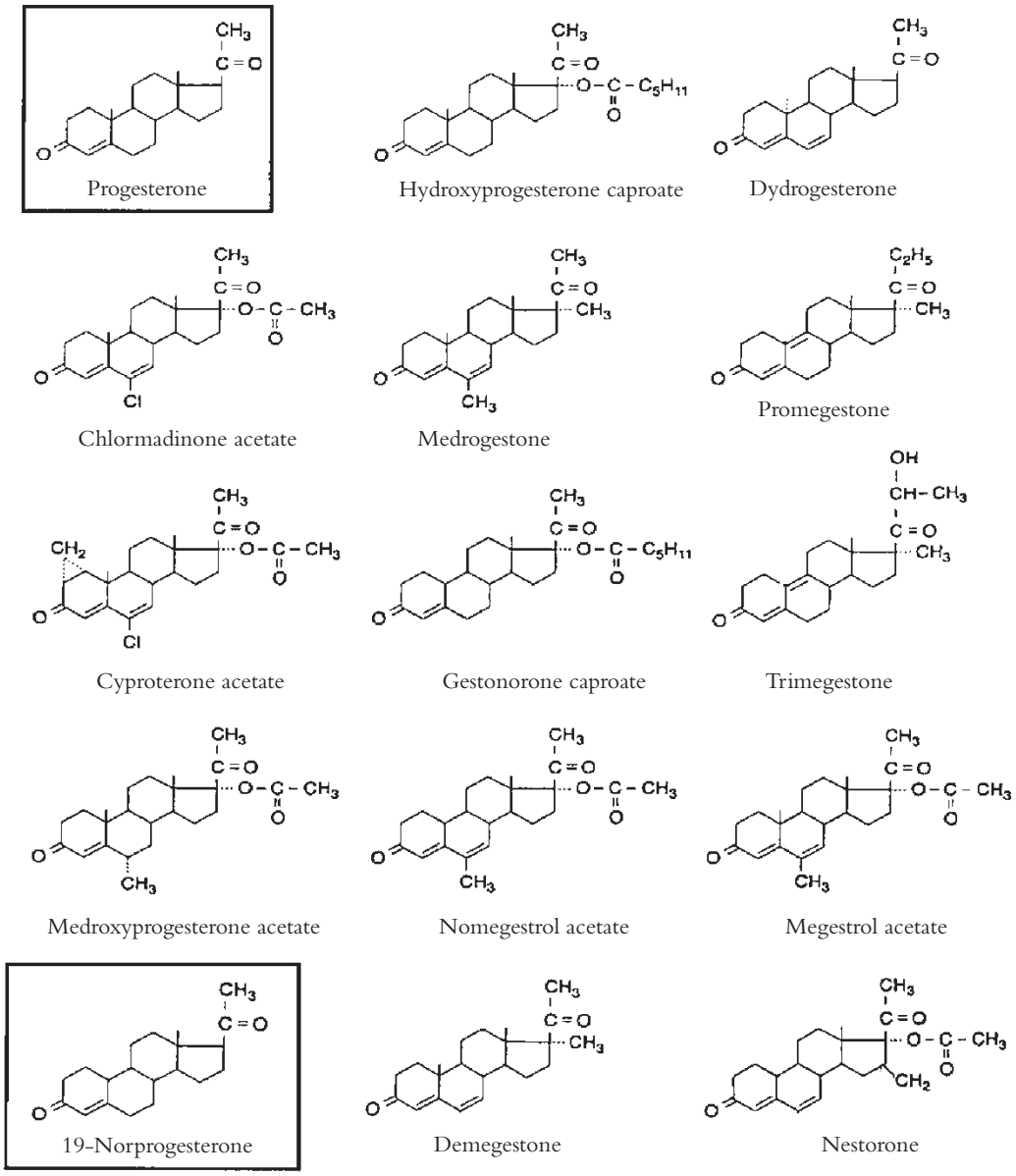

Figure 9 Structural formulae of progesterone derivatives and 19-norpregnane derivatives

tubes they control motility and composition of fluid, and in the breast they enhance estrogen-induced proliferation of mammary epithelium. Except dydrogesterone, the progestogens may influence central nervous system function and psyche, inhibit gonadotropin release, increase body temperature, and antagonize various central effects of estrogens. Progestins with antiandrogenic activity, e.g. cyproterone acetate (CPA), dienogest, chlormadinone acetate (CMA) or drospirenone, may reduce the effects of endogenous androgens, whereas those with androgenic properties, e.g. LNG, NET or tibolone, may cause androgenic effects on the skin and hair, and may antagonize certain estrogen-dependent alterations in lipid metabolism, hemostasis and the synthesis of certain hepatic proteins (e.g. SHBG, TBG, angiotensinogen). Progestogens with glucocorticoid activity may reduce ACTH secretion at higher concentrations or exert glucocorticoid effects on the vessel wall or immune system at the usual concentrations. Some progestogens, e.g. progesterone and drospirenone, may act as an aldosterone antagonist which is accompanied by a compensatory rise in the aldosterone levels. Progestogens may also impair glucose tolerance and cause a slight hyperinsulinemia.

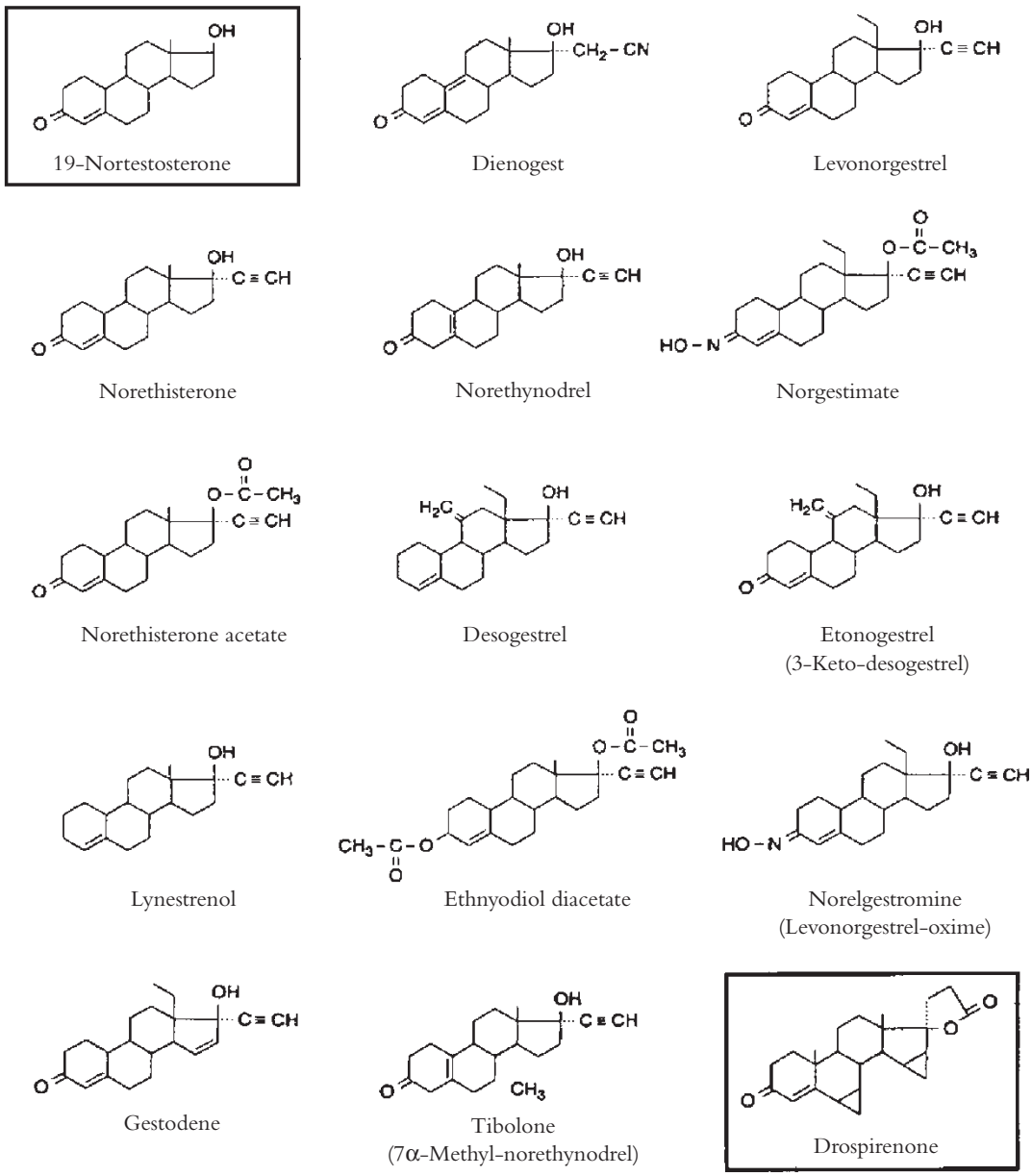

Figure 10 Structural formulae of 19-nortestosterone derivatives and of the spirolactone derivative drospirenone

Due to their antiestrogenic effect, progestogens, including progesterone, may counteract the stimulatory and excitatory effects of estrogens on the brain. Beyond this, progesterone exerts a pronounced sedative effect after conversion to 5α- and 5β-pregnanolone, which bind to the $GABA_A$-receptor. The receptor binding affinity and hormonal activity of metabolites of some synthetic progestins have been investigated (Table 11). It is known that 3α-hydroxy-CMA and 15β-hydroxy-CPA exert a pronounced antiandrogenic effect. Some reduced metabolites of the nortestosterone derivatives show some antiandrogenic or androgenic effects, or even a slight estrogenic activity[202].

Potency of progestogens

Similar to the estrogens, the data on the potency of the various progestogens are tissue-specific and cannot be generalized (Table 12). Moreover, it must be emphasized that the results of various clinical trials differ largely. The transformation dose reflects the typical PR-mediated progestogenic effect in the endometrium. The transformation dose was evaluated in ovarecto-

Table 9 Spectrum of hormonal activities of progestogens. The data are mainly based on animal experiments and are compiled from the literature[202,204,236–244]. The clinical effects of the progestogens are dependent on their tissue concentrations

Progestogen	*A-E*	*EST*	*AND*	*A-A*	*GLU*	*A-M*
Progesterone	+	–	–	(+)	+	+
Chlormadinone acetate	+	–	–	+	+	–
Cyproterone acetate	+	–	–	+	+	–
Medroxyprogesterone acetate	+	–	(+)	–	+	–
Medrogestone	+	–	–	–	?	–
Dydrogesterone	+	–	–	–	?	(+)
Norethisterone	+	+	+	–	–	–
Levonorgestrel	+	–	+	–	–	–
Gestodene	+	–	+	–	(+)	+
Etonogestrel (3-keto-desogestrel)	+	–	+	–	(+)	–
Norgestimate	+	–	+	–	?	?
Dienogest	+	–	–	+	–	–
Tibolone metabolites	+	+	++	–	–	–
Drospirenone	+	–	–	+	–	+
Trimegestone	+	–	–	(+)	–	(+)
Promegestone	+	–	–	–	+	–
Nomegestrol acetate	+	–	–	+	–	–
Nestorone	+	–	–	–	–	

A-E, antiestrogenic; EST, estrogenic; AND, androgenic; A-A, antiandrogenic; GLU, glucocorticoid; A-M, antimineralocorticoid activity
++, strongly effective; +, effective; (+) weakly effective; -, not effective; ?, unknown

Table 10 Relative binding affinities to steroid receptors and serum binding globulins of progestogens. The values were compiled from the literature by cross-comparisons[243,244,252–255]. As the results of the various *in vitro* experiments are largely dependent on the incubation conditions and biological materials used, the values are inconsistent. They do not necessarily reflect the biological effectiveness

Progestogen	*PR*	*AR*	*ER*	*GR*	*MR*	*SHBG*	*CBG*
Progesterone	50	0	0	10	100	0	36
Chlormadinone acetate	67	5	0	8	0	0	0
Cyproterone acetate	90	6	0	6	8	0	0
Medroxyprogesterone acetate	115	5	0	29	160	0	0
Medrogestone	?	?	?	?	?	?	?
Dydrogesterone	75	?	?	?	?	?	?
Norethisterone	75	15	0	0	0	16	0
Levonorgestrel	150	45	0	1	75	50	0
Gestodene	90	85	0	27	290	40	0
Etonogestrel (3-keto-desogestrel)	150	20	0	14	0	15	0
Norgestimate	15	0	0	1	0	0	0
Dienogest	5	10	0	1	0	0	0
Δ4-Tibolone (7α-methyl-norethisterone)	90	35	1	0	2	1	0
Drospirenone	35	65	0	6	230	0	0
Trimegestone	330	1	0	9	120	?	?
Promegestone	100	0	0	5	53	0	0
Nomegestrol acetate	125	42	0	6	0	0	0
Nestorone	136	0	0	38	?	0	?

PR, progesterone receptor (promegestone, 100%); AR, androgen receptor (metribolone R1881, 100%); ER, estrogen receptor (estradiol-17β, 100%); GR, glucocorticoid receptor (dexamethasone, 100%); MR, mineralocorticoid receptor (aldosterone, 100%); SHBG, sex hormone-binding globulin (dihydrotestosterone, 100%); CBG, corticosteroid-binding globulin (cortisol, 100%)

Table 11 Relative binding affinity to steroid receptors of progestin metabolites. The values were compiled from the literature by cross-comparisons[202]. As the results of the various *in vitro* experiments are largely dependent on the incubation conditions and biological materials used, the values are inconsistent. They do not necessarily reflect the biological effectiveness

Progestin	*PR*	*AR*	*ER*
Norethisterone	75	15	0
5α-dihydro-norethisterone	25	27	0
Ethynodiol (3β-hydroxy-norethisterone)	1		18
Levonorgestrel	150	45	0
5α-dihydro-levonorgestrel	50		
Norgestimate	15	0	0
Levonorgestrel-17β-acetate	135		0
Levonorgestrel-3-oxime (norelgestromin)	10	0	
3-keto-desogestrel (etonogestrel)	150	20	0
3β-hydroxy-desogestrel	13	3	2
3-keto-5α-dihydro-desogestrel	9	17	0
Dienogest	5	10	0
9α,10β-dihydro-dienogest	26	13	
3,5α-tetrahydro-dienogest	19	16	
Norethynodrel	6	0	2
Tibolone (7α-methyl-norethynodrel)	6	6	1
Δ4-tibolone (7α-methyl-norethisterone)	90	35	1
3α-hydroxy-tibolone	0	3	4
3β-hydroxy-tibolone	0	4	3

PR, progesterone receptor (promegestone, 100%); AR, androgen receptor (metribolone R1881, 100%); ER, estrogen receptor (estradiol-17β, 100%)

Table 12 Hormonal potency of progestogens and inactivation of hepatic microsomal cytochrome P450-dependent enzymes *in vitro*[180,204,206,208,209,211,212]

Progestin	*TFD* (mg/cycle)	*OID* (mg/day)	*AA activity* (%)	*% Inhibition 5α-R 0.1 μmol/l*	*% Inhibition 5α-R 1 μmol/l*	*IC_{50} CYP* (μmol/l)
Progesterone	4200	300				
Medroxyprogesterone acetate	50					
Megestrol acetate	50					
Chlormadinone acetate	25	1.7	30	0.0	0.0	
Cyproterone acetate	20	1.0	100			
Dienogest	6	1.0	40	0.0	5.0	NE
Tibolone		2.5				
Norethisterone	120	0.4		4.4	20.1	51
Norethisterone acetate	50	0.5				
Norgestimate	7	0.2		3.0	10.3	
Levonorgestrel	5	0.06		2.8	18.5	32
Desogestrel/3-keto-desogestrel	2	0.06		5.7	34.9	24
Gestodene	3	0.04		14.5	45.9	5
Drospirenone	50	2.0	30			
Nomegestrol acetate	100	5.0	90			
Promegestone	10	0.5				

TFD, transformation dose in women; OID, ovulation-inhibiting dose in women (without additional estrogen); AA activity, relative antiandrogenic activity in castrated, androgen-treated rats; 5α-R, hepatic 5α-reductase: % inhibition after 30 min incubation with 0.1 or 1 μmol/l progestin; IC_{50}, progestin concentration at 50% inhibition of CYP (cytochrome P-450 IIIA4 monooxygenase); NE, not effective

mized women who were treated orally with 50 μg EE per day for 14 days and thereafter with EE and a certain dose of a progestin for 10 days. The transformation dose of a progestogen was that daily dose which causes full secretory transformation of the proliferated endometrium.

The high transformation dose of progesterone reflects the low oral bioavailability owing to a rapid inactivation. The relatively high transformation dose of NET and NETA can be explained by the aromatization of a small proportion of NET to EE, which antagonizes the progestogenic effect of NET in the endometrium[204,205].

The ovulation-inhibiting dose was evaluated in ovulatory women who were treated daily with a certain dose of a progestogen between cycle days 5 and 25. The lowest dose which inhibits ovulation in all women is the ovulation-inhibiting dose. It must be kept in mind that the data of most progestogens are evaluated in relatively few subjects. The ovulation inhibition is brought about by a complex mechanism including not only the disturbance of FSH and LH secretion at the hypothalamic and pituitary level and the inhibition of the preovulatory LH peak, but also by direct interactions of the progestogens with ovarian functions. Synthetic progestins may cause a direct inhibition of the ovarian steroid biosynthesis which is more pronounced using compounds with an ethinyl group. After oxidative activation of the 17α-ethinyl group, nortestosterone derivatives may not only inhibit irreversibly cytochrome P450-dependent oxygenases which are involved in the hepatic inactivation of steroid hormones, but may also inhibit ovarian CYP enzymes which play a role in the biosynthesis of endogenous steroids[180,181,206–210]. This may explain the discrepancy between dienogest and LNG or gestodene regarding their potency. Similar to LNG and gestodene, dienogest showed a high endometrial efficacy, as reflected by a low transformation dose, but has a relatively weak ovulation-inhibiting potency due to the lack of a 17α-ethinyl group (Table 12).

Progesterone

Progesterone is an important intermediate in the ovarian and adrenal steroid synthesis, but larger amounts are produced only in the corpus luteum and the placenta. During the luteal phase, serum concentrations of 25 ng/ml are reached, which may increase during pregnancy up to 200 ng/ml. In the human, progesterone is the only progestogen which is able to maintain pregnancy. In the endometrium and cervix, it exerts strong progestogenic and antiestrogenic activities it has a pronounced antimineralocorticoid effect, which causes a compensatory rise in the aldosterone levels, and exerts an 'antiandrogenic' effect which is not associated with binding to the androgen receptor, but a competitive inhibition of the 5α-reductase activity in the skin.

About 17% of the circulating progesterone is bound with high affinity to CBG and 80% with low affinity to albumin. Despite this, the half-lives are only 6 min ($t_{1/2\alpha}$) and 42 min ($t_{1/2\beta}$). Progesterone is rapidly metabolized, predominantly by reduction of the keto groups and the Δ4-double bond, and the pattern of metabolites depends largely on the route of administration.

The oral application of progesterone is associated with an extensive metabolism in the gastrointestinal tract and the liver, which results in high, but individually variable, concentrations of circulating metabolites. Consequently, the investigation of the pharmacokinetics of progesterone by means of radioimmunoassay (RIA) may be hampered by falsely high progesterone levels due to a relative pronounced cross-reactivity of progesterone metabolites. Therefore, either the gas chromatography/mass spectrometry (GC/MS) method or RIA after chromatographic separation are suitable for the measurement of progesterone. This problem is less pronounced after vaginal administration of progesterone owing to the relative low degree of metabolism[213].

Oral administration

After oral administration, progesterone can be metabolized to more than 30 metabolites, among which some exert specific physiological activities. The most important pathway is the formation of 5α- and 5β-pregnanolone which exert considerable sedative effects after binding to the $GABA_A$ receptor. Further metabolites were 20-dihydroprogesterone, which has 25–50% of the progestogenic potency of progesterone, 11-deoxycorticosterone, which is a potent mineralocorticoid, 17α-hydroxyprogesterone, and the inactive end-product pregnanediol (Figure 11).

There are large interindividual differences in the pattern of metabolites circulating after oral administration[214]. The low oral bioavailability could be increased by the use of micronized progesterone suspended in oil and packaged in a gelatine capsule.

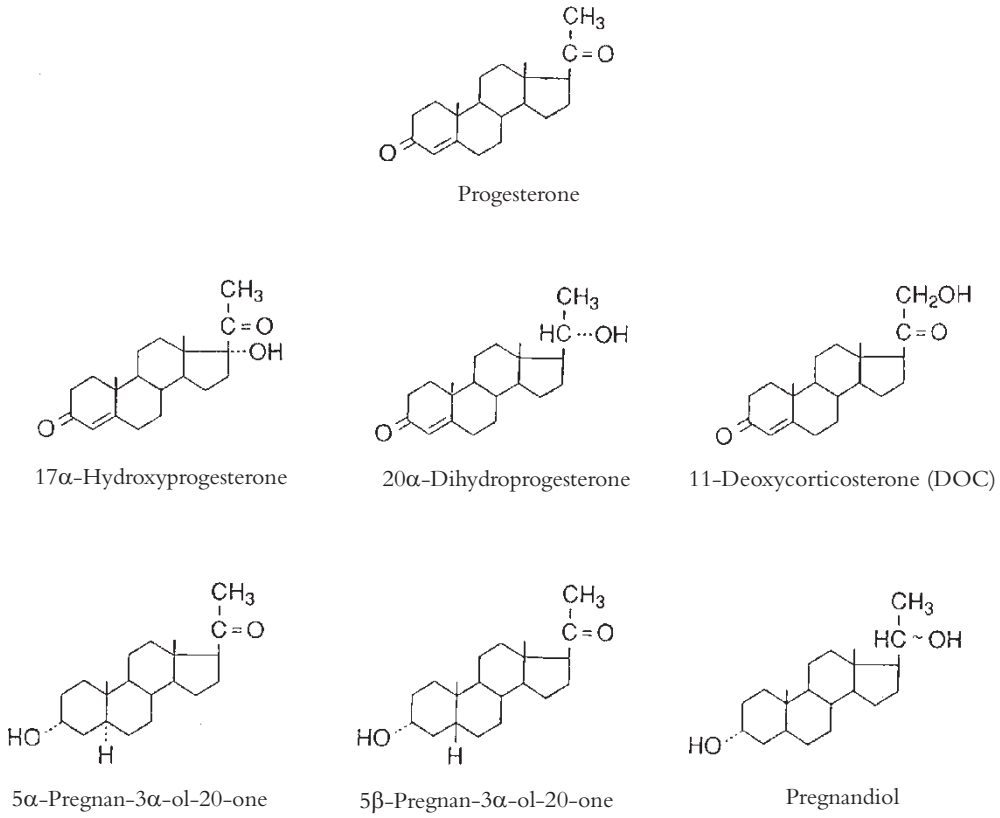

Figure 11 Structural formulae of progesterone and various progesterone metabolites

Pharmacokinetics A single oral dose of 100 mg progesterone contained in a gelatine capsule led to a rapid rise in serum progesterone to a peak level of 1.5–2.2 ng/ml after 1–2 h. Thereafter, the levels decreased rapidly to baseline levels within 4–6 h[213,215]. There was a pronounced rise in the serum levels of 5α- and 5β-pregnanolone up to a maximum of 14 ng/ml and 3.6 ng/ml after 2 h. The 11-deoxycorticosterone levels rose from 120 pg/ml to 680 pg/ml after 2 h and decreased rapidly thereafter[215].

After oral intake of 200 mg progesterone, the peak levels of progesterone, as measured by RIA after 4 h, were 12 ng/ml, while 5α- and 5β-pregnanolone reached serum concentrations of 30 ng/ml and 60 ng/ml[214]. Further metabolites were 20-dihydroprogesterone, 11-deoxycorticosterone, 17α-hydroxyprogesterone and pregnanediol (Figure 11).

Pharmacodynamics Oral treatment with progesterone caused a secretory change in estrogenized postmenopausal endometria in a dose-dependent manner[216]. Generally, the addition of 200 mg progesterone for 12–14 days per cycle to oral or percutaneous estrogens was effective in preventing endometrial hyperplasia, while regular withdrawal bleeding occurred in about 60% and amenorrhea in most of the remaining women[217,218]. As compared with 10 mg CMA, the use of 200 mg progesterone resulted in a similar endometrial protection, but was associated with a worse cycle control and a higher incidence of drowsiness and dizziness, although the drugs were taken at bedtime[217].

The dose of progesterone can be reduced if it is added daily to the estrogen. During treatment of postmenopausal women with a combination of 1.5 mg estradiol percutaneously and 100 mg progesterone orally for 21 or 25 days per cycle, no endometrial hyperplasia occurred, and amenorrhea was recorded in most of the patients[219].

The PEPI trial revealed that the favorable effects of estrogens on lipid metabolism are preserved when progesterone is added orally[220]. Oral treatment with 100–300 mg led to a dose-dependent decrease in blood pressure[221]. Using single oral doses between 300 and 1200 mg, a significant increase in fatigue and a decrease in vigor were recorded. With the highest dose, some women showed a reduced information processing and verbal memory function[222]. In a patient who

ingested 400 mg micronized progesterone, a hypnotic state was induced that lasted for 2 h. In this woman, very high levels of 5α- and 5β-pregnanolone could be measured[214].

Vaginal administration

In contrast to the oral route of administration, the rate of metabolism and the formation of pregnanolones is much lower during vaginal treatment with progesterone. Therefore, the risk of a sedative effect of progesterone is lower than that observed during oral therapy.

Pharmacokinetics Compared with the oral route, the vaginal route of administration of progesterone results in higher serum levels of progesterone, which are sustained for a longer time than after oral treatment. The slow elimination of progesterone might be associated with a direct vagina-to-uterus transport by diffusion (uterine first pass) resulting in a high storage of progesterone in the uterus and a subsequent delayed release of the progestogen[223,224]. Using an *ex vivo* uterine perfusion model, concentrations of 185 ± 155 ng/100 mg endometrial tissue and 254 ± 305 ng/100 mg myometrial tissue were measured[224].

A single vaginal application of a gelatine capsule with 100 mg progesterone led to a rapid rise in serum progesterone, up to a maximum of about 5 ng/ml after 6 h. Thereafter, the concentrations remained at this level for 24 h and were still above baseline levels after 48 h. Among the metabolites, 5α-pregnanolone reached a peak level of 3.5 ng/ml after 2 h, whereas 5β-pregnanolone did not change. The 11-deoxycorticosterone levels differed individually, and a rise from 30 to 100 pg/ml was observed after 4 h only in some of the women[215].

Twelve hours after a single intravaginal administration of a gelatine capsule containing 200 mg progesterone, serum progesterone reached an average peak level of 5.2 ng/ml. Thereafter, the levels declined very slowly to 4.8 ng/ml after 24 h and to 2.5 ng/ml after 72 h[225].

A single vaginal application of a suppository containing 400 mg progesterone resulted in mean peak levels of 16 ng/ml progesterone after 5 h. The maximal level of 5α-pregnanolone was 1.2 ng/ml, of 5β-pregnanolone 0.3 ng/ml, of 5α-dihydroprogesterone 0.8 ng/ml, and of 11-deoxycorticosterone 0.3 ng/ml[226].

A vaginal gel consisting of a water-in-oil emulsion with polycarbophil, which contains either 45 mg (4%) or 90 mg (8%) micronized progesterone, has bioadhesive properties and releases progesterone in a sustained manner[227]. A single dose of 90 mg progesterone resulted in a rise of the progesterone level to a maximum of 10 ng/ml after about 8 h. Thereafter, the levels declined to 3 ng/ml after 24 h[213].

In estrogen-treated postmenopausal women, the progesterone levels may be lower due to an enhanced metabolism in the estrogen-induced proliferated vaginal epithelium. In patients treated with 100 μg transdermal estradiol, the sequential vaginal treatment with a gel containing 45 mg, 90 mg or 180 mg progesterone every other day resulted in peak serum concentrations of progesterone of approximately 4 ng/ml, 6 ng/ml and 7.5 ng/ml after 7 h[227].

Pharmacodynamics In postmenopausal women treated with 100 μg transdermal estradiol, the sequential vaginal treatment with a gel containing 45 mg, 90 mg or 180 mg progesterone every other day from day 15 to day 27 induced in all patients a full secretory transformation of the endometrium[227]. Similarly, in postmenopausal women treated continuously with 0.625 mg CEE for three cycles, cyclic treatment with a vaginal gel containing 45 mg or 90 mg progesterone between days 17 and 27 every other day caused a secretory or atrophic endometrium and prevented endometrial hyperplasia in all women[228]. The high efficacy supports the thesis of a direct transport to the uterus of vaginally applied progesterone. The most frequent side-effects were fatigue and weakness[225].

Intranasal administration

As progesterone is lipophilic, sufficient doses can be applied using a suspension of progesterone in almond oil with a bioavailability of 18%[113]. After intranasal spraying of 11.2 mg progesterone contained in 0.55 ml almond oil, peak levels of serum progesterone of 3.75 ng/ml were reached within 1 h; after a transitory decline, a second peak of 2.7 ng/ml occurred after 4 h[229]. Intranasal treatment with 11.2 mg progesterone three times daily resulted in a progressive increase in the progesterone serum levels up to 6 ng/ml. The endometrial histology revealed a suppressed or late secretory pattern[229].

Similar to estradiol, a sufficient increase in the solubility of progesterone was achieved using methylated cyclodextrin, which is highly hydrophilic but can bind steroids. In this way, the bioavailability of intranasally administered

progesterone was increased to 58%. The intranasal co-administration of 5 mg progesterone and 2 mg estradiol, solubilized by complexing with methylated cyclodextrin, caused maximal serum concentrations of progesterone between 3.9 and 6.7 ng/ml within 15–40 min[229].

Intramuscular administration

A single intramuscular injection of 100 mg progesterone in an oily solution resulted in a rapid increase in the serum levels of progesterone up to a maximum between 40 and 80 ng/ml after 8 h. Thereafter, the levels declined continuously to about 6 ng/ml after 48 h. The maximal serum concentrations of 20-dihydroprogesterone were between 4 and 16 ng/ml, and of 17α-hydroxyprogesterone between 0.8 and 2.7 ng/ml[230].

Buccal administration

After the buccal administration of 100 mg progesterone in postmenopausal women, a steep rise in the serum concentration of progesterone occurred, reaching a maximum of about 8 ng/ml within 1.3 h. Thereafter, the progesterone concentration declined to 1.5 ng/ml after 8 h. Treatment for 2 weeks with 100 mg progesterone each twice daily resulted in peak levels at steady state of 9 ng/ml on average[124].

Transdermal administration

There are several studies on the transdermal use of progesterone. As the serum levels of progesterone achieved by this route of administration are much lower than those measured in the luteal phase, a protective effect on the endometrium must be called in question[231].

The daily administration of a cream containing 40 mg progesterone on an area of 100 cm^2 of the forearm of postmenopausal women resulted in a small rise in the serum progesterone levels. On the first day, the application of either 40 mg once daily or 20 mg twice daily caused only a negligible increase, but, on day 42, the progesterone concentrations reached a mean value of about 1 ng/ml[232].

The efficacy of transdermally administered progesterone is contested. In a study with postmenopausal women, the daily application of a cream containing 32 mg progesterone on the skin increased the serum levels of progesterone only slightly to 0.1–0.3 ng/ml. After 12 weeks of treatment, no effect on climacteric or psychic symptoms, lipids or bone markers was recorded[233]. Contrary to this, daily treatment for 1 year with a cream containing 20 mg progesterone significantly reduced vasomotor symptoms in 83% of the women, whereas no effect was observed concerning bone mineral density or lipids[234]. Moreover, transdermal treatment of postmenopausal women who received daily 0.625 mg CEE, with 15 mg or 40 mg progesterone twice a day, resulted in a significant suppression of endometrial proliferation[235].

It can be assumed that the high 5α-reductase activity in the skin may rapidly inactivate a large proportion of the absorbed progesterone. Therefore, it remains to be clarified to what extent 20-dihydroprogesterone contributes to the antiproliferative effect on the endometrium, and whether 5α- and 5β-pregnanolone, which exert sedative effects, play a role in the relief of vasomotor symptoms.

Progesterone derivatives

The introduction of substituents into the steroid skeleton that sterically hinder the action of metabolizing enzymes resulted in a considerable slowing down of the inactivation rate and an increase in the hormonal potency (Figure 9). A methyl group or chloro atom at C6 reduces or blocks the reduction of the Δ4-3-keto group, and influences the interaction with the androgen receptor. Whereas a chloro atom at C6β causes antiandrogenic properties of the progestin, a methyl group at C6 leads to a weak androgenic activity. An acetyl group or a methyl group at C17α inhibits the reduction of the 20-keto group of progesterone. In contrast to the 17α-esters, 17α-hydroxyprogesterone has no hormonal activity.

If no chromatographic separation is carried out, the measurement of the serum levels of progesterone derivatives by means of RIA may lead to falsely high serum concentrations, owing to the presence of metabolites which interact with the antibody. Therefore, the use of the GC/MS method revealed much lower levels than previously published.

Medroxyprogesterone acetate (MPA)

MPA does not undergo a first-pass inactivation after oral administration and the bioavailability is 100%. Treatment of postmenopausal women for 2 weeks with 1 mg or 2 mg estradiol valerate and 2.5 mg or 5 mg MPA per day resulted in a rapid increase in the serum levels of MPA up to maximum serum concentrations within 1.5 and

2 h. Using 2.5 mg MPA, the mean peak levels were 0.3 ng/ml in the age group < 60 years and 0.45 ng/ml in women > 65 years, and, using 5 mg MPA, 0.60 ng/ml and 0.9 ng/ml, respectively[33]. During daily intake, a steady state is reached after 3 days of treatment.

In the circulation, 88% of MPA is bound to albumin, but not to SHBG or CBG. MPA is, to a certain extent, stored in fat tissue. The half-lives are 2.2 h ($t_{1/2\alpha}$) and 33 h ($t_{1/2\beta}$). The main metabolic steps are hydroxylation reactions, e.g. at C6β and C21, with the preservation of the Δ4-3-keto group, but there are also dihydro- and tetrahydro-derivatives of MPA[202].

MPA antagonizes the estrogen-induced endometrial proliferation. In general, daily doses of 5–10 mg are sufficient for the prevention of endometrial hyperplasia in postmenopausal women during sequential or cyclic HRT, while 2.5 mg MPA have been shown to be protective during continuous combined HRT. Despite a binding affinity to the aldosterone receptor, MPA has no mineralocorticoid or antimineralocorticoid activity. It was, however, demonstrated that MPA exerts considerable glucocorticoid effects mediated by binding to the glucocorticoid receptor, which causes an upregulation of the thrombin receptor and stimulates the procoagulatory activity[236]. MPA has no antiandrogenic effect, but weak androgenic properties. Although MPA does not antagonize the estrogen-induced rise in triglycerides and HDL cholesterol, treatment with depot-MPA every second week may reduce HDL[202]. At doses of 10 mg daily, MPA causes an impairment of glucose tolerance without affecting lipid metabolism[245]. In women with contraindication for estrogens who suffer from vasomotor symptoms, daily treatment with 20–40 mg MPA may improve the complaints.

Megestrol acetate

According to structural similarities, the hormonal pattern of megestrol acetate is similar to that of MPA (Figure 9, Table 9). Three hours after oral administration of 4 mg megestrol acetate, a maximal serum concentration of megestrol acetate of about 7 ng/ml was measured. The bioavailability is 100% and the majority of megestrol acetate in the circulation is bound to albumin, because it has no binding affinity to SHBG or CBG. The main metabolic pathways are hydroxylation reactions at C21, C2α and C6.

Similar to MPA, megestrol acetate has been shown to improve vasomotoric symptoms at doses of 20–40 mg[246]. Continuous combined therapy of postmenopausal women with 2 mg estradiol and 2.5 mg megestrol acetate caused an increase in triglycerides and HDL cholesterol, but did not change LDL cholesterol, whereas the use of 5 mg megestrol acetate resulted in a reduction of HDL cholesterol and LDL cholesterol and no effect on triglycerides[247]. This indicates a moderate androgenic activity of megestrol acetate.

Chlormadinone acetate (CMA)

In contrast to MPA and megestrol acetate, the progesterone derivative CMA has some antiandrogenic activity which corresponds to 20–30% of that of CPA. Owing to the low first-pass metabolism, the bioavailability after oral administration is about 100%. Similar to other progesterone derivatives, CMA accumulates in fat tissue and is stored in the endometrium, myometrium, cervix and tubes. Therefore, the clearance is relatively low, and, 7 days after application, 74% of the dose is excreted[248]. Within 1–2 h after a single oral administration of a combination of 2 mg CMA and 30 μg EE, the serum concentration of CMA reached a maximum of 1.6 ng/ml. During daily intake, the CMA levels increased to a steady state of 2 ng/ml within 2 weeks[249]. CMA has no binding affinity to SHBG and CBG, and 97–99% of the circulating CMA is bound to albumin. The half-lives are 2.4 h ($t_{1/2\alpha}$) and 38 h ($t_{1/2\beta}$)[249,250]. The main metabolic steps are the reduction of the 3-keto group with preservation of the Δ4-double bond, hydroxylation, and deacetylation. Hydroxylation reactions occur at C2α, C3α, C3β, and C15β and the resulting metabolites are conjugated to sulfates and glucuronides. The latter are excreted in the kidney. The conjugates excreted in the bile are hydrolyzed in the colon and reabsorbed. As 3α-hydroxy-CMA has 70% of the antiandrogenic activity, the enterohepatic circulation may be of clinical relevance. At doses of 2–4 mg, CMA has been observed to increase body temperature by 0.2–0.5°C. Using doses of 15–20 mg, CMA can improve hot flushes[250].

Cyproterone acetate (CPA)

CPA is the progestin with the highest antiandrogen activity, as shown in animal experiments. This effect is brought about by competitive inhibition of the binding of endogenous androgens to the androgen receptor, and is, therefore, dose-dependent. CPA has some glucocorticoid properties, the

clinical importance of which is not clarified (e.g. vessel wall, immune system). After oral administration, the bioavailability of CPA is nearly 100%. A single oral dose of 2 mg CPA led to peak serum levels of CPA of about 11 ng/ml. As it has no binding affinity to SHBG and CBG, 93% of the circulating CPA is bound to albumin. CPA accumulates in fat tissue, and the half-lives are 2–8 h ($t_{1/2\alpha}$) and 60 h ($t_{1/2\beta}$)[202]. The accumulation of CPA in fat tissue during daily administration of higher doses of CPA results in a depot effect and may prevent withdrawal bleeding after cessation of intake. The major metabolic steps are hydroxylation and deacetylation, while the Δ4-double bond is preserved. The antiandrogenic activity of 15β-hydroxy-CPA is similar to that of CPA, but the progestogenic efficacy is only 10% of that of CPA[202].

Oral treatment of postmenopausal women with 5 mg CPA daily has no effect on the lipid metabolism[245]. The sequential addition of 1 mg CPA to treatment with 2 mg estradiol valerate resulted in a rise in triglycerides and HDL cholesterol, and a reduction in LDL cholesterol[251].

Medrogestone

In contrast to MPA, CMA and CPA, medrogestone is not an esterified derivative of 17α-hydroxyprogesterone, but has a methyl group at C17α (Figure 9). The bioavailability of medrogestone is 100%, and, after oral administration of a dose of 10 mg, maximal serum concentrations of 10–15 ng/ml are reached. Similar to other progesterone derivatives, the circulating medrogestone is largely bound to albumin (90%), but only to a small degree to SHBG (2%) and CBG (3%).

The half-lives of medrogestone are 4 h ($t_{1/2\alpha}$) and 36 h ($t_{1/2\beta}$). The most important metabolic steps are hydroxylation reactions. As there is no information on the binding affinities of medrogestone to the various steroid receptors, the hormonal pattern of the compound can hardly be estimated. The lack of effect of a sequential addition of 10 mg medrogestone on the estrogen-induced rise in triglycerides and HDL cholesterol suggests that medrogestone has no androgenic properties[202].

Retroprogesterones

The common structure of steroid hormones is the arrangement of the four rings in a plane, which is achieved by the attachment of the rings in the trans-orientation. The hormonal activities are largely determined by substituents that lie either above (β-position) or below the plane (α-position, indicated by dotted lines). Retroprogesterones are characterized by a conspicuous change in the configuration of the steroid molecule. Owing to the attachment of ring B to ring C in the cis-conformation, the plane of rings A/B is orientated in a 60° angle below the rings C/D, and the angular C19 methyl group is in the α-position (Figure 12).

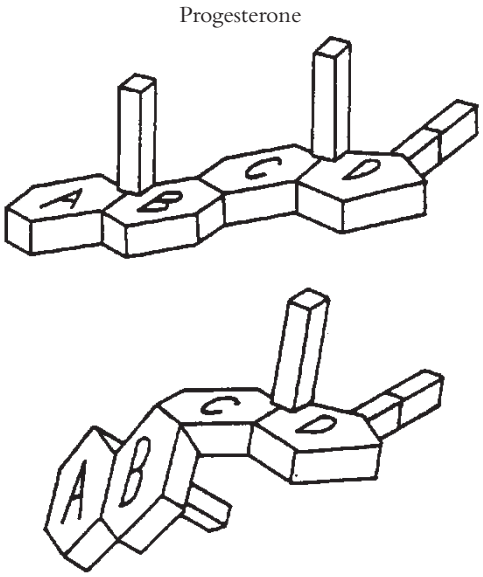

Figure 12 Schematic graph of the configurations of the progesterone and retroprogesterone molecules

Dydrogesterone

Dydrogesterone is a stereoisomer of progesterone with an additional double bond between C6 and C7 (Figures 9 and 12), and its hormonal pattern and metabolism differ largely from those of the natural progestogen. It is an orally active progestin that is non-thermogenetic, non-sedative and does not inhibit gonadotropin release and ovulation. It has weak antimineralocorticoid effects, negligible androgenic and glucocorticoid activities, and no antiandrogenic properties[256]. Oral treatment with 10–20 mg dydrogesterone daily caused a sufficient secretory transformation of a proliferated endometrium. The half-life ($t_{1/2\beta}$) is 5–7 h and 24 h after oral administration, and within 24 h 85% of the dose is excreted. Due to the 9β,10α-retro structure of the molecule, both double bonds cannot be enzymatically reduced. The most important metabolic steps are the

reduction of the 20-keto group, and hydroxylation at C16α and C21. The main metabolite is 20α-dihydrodydrogesterone.

The sequential therapy of postmenopausal women with either 1 mg estradiol and 5 or 10 mg dydrogesterone or 2 mg estradiol and 10 or 20 mg dydrogesterone caused an atrophic or secretory endometrium in most patients and prevented the development of endometrial hyperplasia[257].

The continuous combined oral treatment of postmenopausal women with 1 mg estradiol and 2.5 mg, 5 mg, 10 mg, or 20 mg dydrogesterone daily caused an increase in HDL cholesterol and a decrease in LDL cholesterol. The results suggest that dydrogesterone enhanced the estrogen-induced decrease in LDL cholesterol and attenuated that on HDL cholesterol[258].

Norpregnane derivatives

The 19-norpregnane derivatives are progesterone derivatives that have no angular 19-methyl group (Figure 9). The hormonal pattern of this group of progestins is similar to that of progesterone derivatives.

Promegestone

Promegestone is a potent progestin and antiestrogen and is used in HRT at a daily dose of 0.5 mg. It has weak glucocorticoid, but no antimineralocortcoid effects. It does not bind to the androgen receptor and has no androgenic or antiandrogenic activity. Promegestone is mainly bound to albumin, but not to SHBG and only weakly to CBG. After oral administration, the serum maximum is reached after 1–2 h. The main steps of metabolism are hydroxylation at C21 and other positions of the steroid[237]. The daily administration of 0.5 mg promegestone to postmenopausal women did not change the serum levels of SHBG, angiotensinogen, antithrombin or lipids and lipoproteins. The effect on lipid metabolism of a cyclic therapy with 1.5 mg estradiol percutaneously and 0.5 mg promegestone orally during the last 12 days did not differ from that of estradiol and progesterone[259].

Trimegestone

Trimegestone is the most potent norpregnane derivative which causes a secretory transformation of an estrogen-treated endometrium at a daily dose of 0.1 mg. In cyclic HRT, trimegestone is used at doses of 0.25–0.5 mg daily. After a single oral administration of 1 mg, a maximal serum concentration of trimegestone of 25 ng/ml is reached within 0.5 h. The half-life was measured as 13.8 h[259].

Trimegestone has no glucocorticoid, androgenic or antiandrogenic effect and a weak antimineralocortcoid activity[242,244]. The main metabolic steps are hydroxylation reactions. The 1β- and 6β-hydroxy-trimegestone metabolites showed a considerable progestogenic potency with no binding affinity to the other steroid receptors.

Treatment with daily 2 mg estradiol continuously and 0.5 mg trimegestone on days 15–28 for 13 cycles caused an inactive or secretory endometrium in 85% of the women. The rate of endometrial hyperplasia (without atypia) was 1.9%. The pattern of adverse effects and the cycle control were similar to those of 2 mg estradiol and 0.5 mg norgestrel or 1 mg NETA, except a shorter duration of withdrawal bleeding with trimegestone[260,261]. Trimegestone did not counteract the estrogen-induced changes in the lipid metabolism.

Nomegestrol acetate

Nomegestrol acetate differs from megestrol acetate only by the lack of the angular C19-methyl group. It shows a pronounced antiandrogenic activity, but, for the full transformation of a proliferated endometrium, a daily dose of 5 mg is necessary. The majority of the circulating nomegestrol acetate is bound to albumin. After oral administration of 5 mg nomegestrol acetate, a peak serum level of nomegestrol acetate of 8 ng/ml is reached within 4 h. The half-life ($t_{1/2\beta}$) is 35–50 h[206,259].

Nomegestrol acetate has no glucocorticoid, antimineralocorticoid or androgenic activity, but a pronounced antiandrogenic effectiveness that is between that of CMA and CPA. Treatment of premenopausal women with 5 mg nomegestrol acetate daily did not affect the serum levels of SHBG, CBG, angiotensinogen, HDL cholesterol, LDL cholesterol, fibrinogen or plasminogen, but increased antithrombin and reduced triglycerides[262,263]. The addition of nomegestrol acetate to estrogen therapy did not counteract the estrogen-induced changes in the lipid metabolism[259,263].

Nestorone®

Nestorone is a potent progestin when administered parenterally by means of sustained-release

formulations. After subcutaneous application, it was over 100-fold more potent in rats than by the oral route. Nestorone does not bind to the androgen receptor and has, therefore, no androgenic or antiandrogenic activity. Despite its binding affinity to the glucocorticoid receptor, Nestorone exerts no glucocorticoid effects. In the circulation, Nestorone is not bound to SHBG, but to albumin[243].

After a single oral administration of a solution with 100 μg Nestorone, the serum level of Nestorone increased rapidly to a maximum of 160 pg/ml within 10 min. Thereafter, it decreased, reaching a value of 80 pg/ml after 1 h. Using the oral route, the bioavailability of Nestorone is only 10%, and, due to the rapid metabolism, the half-life is 1–2 h. After 2 years of use of a subdermal implant releasing 100 μg Nestorone daily, the mean Nestorone serum level was 20 pg/ml[264]. After a single transdermal application of a gel containing 2.3 mg Nestorone to fertile women, a continuous rise of the Nestorone levels occurred, reaching a value of 85 pg/ml after 24 h. During daily application of the gel, the levels increased up to 300 pg/ml on the fifth day of treatment. The results suggest a sustained release of Nestorone from the skin[265].

Nortestosterone derivatives

The 19-nortestosterone derivatives are derived from the anabolic nandrolone (19-nortestosterone) which has some affinity to the PR (22% of that of progesterone). The introduction of an ethinyl group at C17α caused a shift from the androgenic to the progestogenic activity, and the resulting NET is an orally potent progestin with weak androgenic properties (Figure 10). Further modifications of the steroid skeleton led to various progestins which differ in their potency and pattern of hormonal activities (Table 9). The substitution of the angular methyl group at C13 by an ethyl group led to an increase in the progestogenic potency, as exemplified by the higher potency of LNG as compared to NET (Figure 12).

The older progestins norethynodrel, lynestrenol, and ethynodiol diacetate are prodrugs and are rapidly transformed after oral administration to the active progestin NET.

Norethisterone and norethisterone acetate

Oral administration NETA is rapidly hydrolyzed to NET in the intestinal tract and liver. Therefore, the pharmacokinetics and pharmacodynamics of NET during treatment with both compounds are similar. The bioavailability of orally administered NET or NETA is 40–80%. The particle size of the administered dose influences the pharmacokinetics of NET, and smaller particles cause higher serum levels because of faster absorption and lower intestinal metabolism[266]. The concomitant intake of the tablets with a high-fat meal caused lower peak levels but higher AUC of NET, as compared with those after administration during fasting[190].

After a single oral administration of 0.5 mg NETA, a maximal serum concentration of NET of about 5 ng/ml on average was reached within 1 h. After intake of 1 mg, maximal serum levels of 5–10 ng/ml were measured. When combined with 1 mg estradiol, the pharmacokinetics of NET were found to be similar, with a maximum of 5–7 ng/ml[31,32]. Using a dose of 2 mg NET, a peak NET level of 12 ng/ml was reached[190]. Since, after 24 h, the NET levels had not yet returned to baseline, multiple administration of the estradiol/NET combination resulted in NET levels which were significantly higher by 38% (AUC) with a mean peak level of 7.4 ng/ml after 30 min[31]. A single oral administration of a combination of 1 mg NETA and 2 mg estradiol resulted in a maximal NET level of 8.5 ng/ml within 1 h[32].

In blood, 36% of NET is bound to SHBG and 61% to albumin. The half-lives are 1.5 h ($t_{1/2\alpha}$) and 9.5 h ($t_{1/2\beta}$)[31]. The main metabolic steps are the reduction of the Δ4-double bond to 5α- or 5β-dihydro-NET and subsequently the reduction of the 3-keto group to the four isomers of 3,5-tetrahydro-NET. The 5α-dihydro-NET has a relatively high binding affinity to the androgen receptor and may play a role in the androgenic activity of NET. The ethinyl group is preserved in 90% of all metabolites[267]. Despite the steric hindrance by the 17α-ethinyl group, conjugation of the 17β-hydroxy group takes place to a certain extent, and may undergo enterohepatic circulation. A small proportion of the NET dose (0.35%) is aromatized to EE, and the concentration–time curve of EE suggest that is formed in the liver[205,268]. Using a dose of 1 mg, the levels of EE are low and, in the presence of a natural estrogen, probably without clinical relevance[268]. Using doses of 5 mg or 10 mg, the EE peak levels are similar to those after ingestion of 30 or 60 μg EE (Figure 14)[205].

NET has no glucocorticoid or antimineralocorticoid activity, but a weak androgenic effect.

Transdermal administration Treatment with a patch releasing daily 0.25 mg NETA leads to serum concentrations of 0.5–1 ng/ml which are reached on the second day after application[269]. This is followed by a continuous decrease to a value of 0.25–0.5 ng/ml until the application of a new patch after 3.5 days.

During transdermal treatment with daily 100 μg estradiol and 0.34 mg NETA (two patches with 50 μg estradiol and 0.17 mg NETA), NET serum levels of 0.65 ng/ml were measured.

With continuous transdermal treatment of postmenopausal women for 12 months with 50 μg estradiol combined with 0.14 mg, 0.25 mg or 0.4 mg NETA, endometrial hyperplasia was prevented. The incidence of uterine bleeding (no bleeding in 50% of the cycles) was lowest in the group using estradiol and 0.14 mg NETA. The improvement of hot flushes was similar in all groups. Application-site reactions, mostly erythema, were reported by 25% of the women[270].

Continuous therapy for 1 year with a patch releasing daily 25 μg estradiol and 0.125 mg NETA prevented endometrial hyperplasia and caused a higher rate of amenorrhea (90%) than 50 μg estradiol and 0.25 mg NETA (65%) or an oral therapy with 2 mg estradiol and 1 mg NETA (79%)[271,272]. Continuous treatment with 25 μg estradiol and 0.125 mg NETA increased significantly bone mineral density in postmenopausal women[273].

The sequential addition of transdermal 0.14 mg, 0.25 mg or 0.40 mg NETA on days 15–28 to the continuous therapy with 50 μg estradiol daily reduced vasomotor symptoms significantly in all three groups[274]. The sequential therapy with patches releasing 50 μg estradiol alone and those combined with NETA 0.25 mg daily resulted in a similar symptom improvement, although a slight reduction in efficacy was noted during the combined phase for some symptoms[269].

Transdermal treatment with 50 μg estradiol continuously and in addition 0.17 mg NETA or 0.35 mg NETA either continuously or sequentially (days 15–28) reduced vasomotor symptoms to a similar degree (by $> 90\%$)[275]. All regimens caused an effective endometrial protection, and no significant difference in the rate of bleeding was observed between the lower and the higher dose of NETA[275].

The sequential transdermal treatment with 50 μg estradiol and 0.25 mg NETA caused regular bleeding in 80%, irregular bleeding in 11% and no bleeding in 9% of the cycles. The rate of endometrial hyperplasia was 2%[276]. The sequential therapy with patches releasing 50 μg estradiol without and with 0.25 mg NETA caused a slight decrease in total cholesterol, LDL cholesterol, HDL cholesterol and apolipoproteins B and A1, and a pronounced reduction in total triglycerides[269]. In contrast to the oral treatment with NETA, transdermal estradiol/NETA does not adversely affect carbohydrate metabolism[269].

Levonorgestrel and norgestrel

Oral administration The racemate D,L-norgestrel consists in equal shares of the potent progestin LNG and the hormonally inactive dextronorgestrel. Therefore, the hormonal activity of 0.5 mg norgestrel is identical to that of 0.25 mg LNG. LNG is a potent progestin exerting some androgenic activity, but no glucocorticoid or antimineralocorticoid properties (Table 9).

After oral administration, the two stereoisomers are metabolized in different ways. The bioavailability of LNG is about 95%. Within 1–2 h after a single oral administration of 150 μg LNG to young women, a maximal serum level of 4.3 ng/ml was measured[277]. Within 1 h after a single ingestion of 50 μg LNG and 30 μg EE, the maximal serum level of LNG was 2.0 ng/ml; with 100 μg LNG and 20 μg EE the maximum serum level was 2.4 ng/ml, and with 125 μg LNG and 30 μg EE, a peak level of LNG of 4.3 ng/ml was measured[278,279]. The administration of 2 mg estradiol and 0.3 mg LNG to postmenopausal women resulted in a peak serum level of LNG of 6.2 ng/ml after 1 h, which declined thereafter with a terminal half-life of 32 h.

In the blood, 48% of LNG is bound to SHBG and 50% to albumin. The half-lives are 1 h ($t_{1/2\alpha}$) and 24 h ($t_{1/2\beta}$). Owing to its androgenic activity, oral treatment with LNG alone may reduce the SHBG levels, whereas a combination with potent estrogens may cause an increase in SHBG. This may influence the pharmacokinetics of LNG. The main metabolic pathways of LNG are the reduction of the Δ4-3-keto group and hydroxylation reactions[206].

Intrauterine administration The T-shaped LNG-releasing intrauterine device (LNG-IUD) is approved for contraception, but offers some advantages if used for endometrial protection in peri- and postmenopausal women. The vertical Silastic arm contains 52 mg LNG, which is released after insertion at a low rate for 5 years. During the first year, it releases 20 μg LNG per

day and in the fifth year 15 μg daily. A small proportion of the daily dose appears in the circulation, and, during the use of the IUD releasing 20 μg daily, mean serum LNG levels of about 0.5 ng/ml were measured after 6 and 12 months[280]. A smaller LNG-IUD releasing only 10 μg daily was developed for postmenopausal women and caused LNG levels of 0.2 ng/ml after 6 and 12 months, respectively[280].

The frameless FibroPlant LNG-IUD is a completely flexible device releasing 14 μg LNG daily. It caused a profound endometrial suppression and amenorrhea in 64% of perimenopausal women and 100% of postmenopausal patients. It is suitable for the reduction of menstrual bleeding in women with menorrhagia[281].

After insertion of the LNG-IUDs, the progestin accumulates in the endometrium and myometrium and causes a profound suppression of the endometrium. Therefore, after transitory spotting and breakthrough bleeding, which occur during the first year after insertion in some women, the endometrium becomes atrophic[280]. In postmenopausal women, the insertion of a LNG-IUD was found to cause pain in approximately 50% and to be difficult in one-third of the patients and may, therefore, need cervical dilatation and/or paracervical blockade[282]. Treatment with the LNG-IUD combined with either 50 μg estradiol transdermally or 2 mg estradiol valerate orally caused a profound suppression of the endometrium for 5 years in all patients, and 64% of the patients were totally amenorrheic[282].

The results of various studies with the 20 μg LNG-IUD demonstrate that the endometrial effects and the safety profile in postmenopausal women using estrogens for HRT are similar to those observed in fertile women. Moreover, the morphological changes in the endometrium are similar to those occurring after oral use of progestins in HRT[283]. In the presence of potent estrogens, the systemic effects, e.g. on metabolic parameters, of the low serum levels of LNG are negligible. There are, however, no data on the effect on breast tissue and breast cancer risk.

In postmenopausal women, the use of a smaller LNG-IUD releasing 10 μg LNG daily was demonstrated to be easier and to cause less pain. During continuous oral treatment with 2 mg estradiol valerate, the use of this LNG-IUD caused a strong endometrial suppression and prevented endometrial hyperplasia. The bleeding pattern was similar to that using the LNG-IUD releasing 20 μg LNG per day[280]. When combined with 2 mg estradiol valerate orally, a significant increase in HDL cholesterol and decreases in total cholesterol, LDL cholesterol and lipoprotein(a) were measured 6 months after insertion of the 20 μg LNG-IUD and the 10 μg LNG-IUD. The favorable effect on HDL cholesterol was maintained after 12 months with the lower-dosed IUD, but was reversed with the 20 μg LNG-IUD[280]. The most frequent adverse effects during use of the LNG-IUDs were bleeding, headache, abdominal pain, mastalgia and vaginal discharge.

Transdermal administration Treatment of postmenopausal women with a 7-day sequential matrix patch realeasing 50 μg estradiol and 10 μg LNG per day resulted in estradiol levels of 30 pg/ml and LNG levels of 120 pg/ml on average. This therapy improved climacteric symptoms significantly, but did not change the serum levels of lipids and lipoproteins. Moderate to severe application-site reactions were observed in less than 10% of the women[284].

After 1 year of sequential treatment of postmenopausal women with 7-day matrix patches releasing daily 50 μg estradiol and 10 μg LNG, 75 μg estradiol and 15 μg LNG or 100 μg estradiol and 20 μg LNG, the rate of endometrial hyperplasia was below 1%[285]. The frequency of cyclic bleeding and of intermittent bleeding was lowest with the 50 μg estradiol/10 μg LNG patch and increased with the hormone dose[286]. Application-site reactions were observed in 5% of the women[285].

Sequential transdermal treatment with daily 80 μg estradiol in the first 2 weeks and 50 μg estradiol plus 20 μg LNG in the following 2 weeks did not alter the SHBG levels, but changed bone markers indicating a reduction of bone resorption and reduced LDL cholesterol[287].

Continuous combined HRT with 7-day patches releasing daily 45 μg estradiol and 15 μg, 30 μg or 40 μg LNG improved climacteric symptoms significantly and prevented endometrial hyperplasia. After 9 months, amenorrhea was achieved in one-third of the patients. Application-site reactions occurred in 30–44%, vaginal hemorrhage in 29–37% and mastalgia in 16–23% of the women[288].

Norgestimate

Norgestimate is a prodrug which is rapidly metabolized after oral administration. Therefore, using an oral dose of 250 μg norgestimate, only low serum levels of norgestimate (70 pg/ml) can be measured. It is rapidly transformed by a two-step metabolism through LNG-3-oxime and

LNG-17β-acetate into LNG. The deacetylation of norgestimate to LNG-3-oxime occurs in the intestinal mucosa and the liver, and the transformation of the LNG-3-oxime to LNG mainly in the liver[206]. As only small amounts of LNG-17β-acetate appear in the circulation, it plays nearly no role in the mechanism of action, despite a high binding affinity to the PR. Consequently, the hormonally active metabolites are LNG and LNG-3-oxime (norelgestromine, deacetylated norgestimate; Figure 10), which differ in their binding affinities to the PR (Table 11). In contrast to LNG, norgestimate and its metabolites LNG-3-oxime and LNG-17β-acetate are not bound to SHBG and CBG. Therefore, the amount of free and albumin-bound LNG-3-oxime was 0.19 nmol/l and 6.5 nmol/l, whereas that of free and albumin-bound LNG was only 0.05 nmol/l and 0.58 nmol/l[289]. The inactivation takes place through reduction and hydroxylation reactions, resulting in the formation of LNG metabolites.

After a single oral administration of 35 μg EE and 250 μg norgestimate, the level of LNG-3-oxime rose to 2.5 ng/ml after 1.5 h and decreased thereafter rapidly, whereas the LNG maximum of 0.5 ng/ml appeared later and was followed by a slow decline[290]. During daily intake, the level of LNG-3-oxime increased up to 3 ng/ml and the half-life ($t_{1/2\beta}$) was 17 h[206]. After multiple oral administration of 1 mg estradiol continuously and 180 μg norgestimate intermittently, a peak level of LNG-3-oxime of only 0.64 ng/ml was measured[291].

The regimen used for HRT is 1 mg estradiol continuously and 90 μg norgestimate intermittently (a 6-day repeating sequence with norgestimate for 3 days, followed by 3 days without norgestimate)[291]. It caused a significant improvement in climacteric symptoms and increased bone mineral density. The rate of adverse effects was similar to those of other continuous combined therapies with 1 mg estradiol and a progestin. The bleeding pattern was not better than that in women treated continuously with a combination of 2 mg estradiol and 1 mg NETA. The data on the risk of endometrial hyperplasia during treatment with the intermittent estradiol/norgestimate regimen are inconsistent[291].

Dienogest

The structure and hormonal pattern of dienogest differ from those of other nortestosterone derivatives in so far as it contains at C17α no ethinyl group but a cyanomethyl group (Figure 10). The lack of an ethinyl group is associated with a lack of an irreversible inhibition of CYP enzymes which is caused by ethinylated steroids through the oxidatively activated ethinyl group[206]. As CYP enzymes are involved both in the ovarian steroid synthesis and the inactivation of steroid hormones, ethinylated progestins – as well as EE – may directly impair follicular activity and inhibit their own degradation. This may explain the relatively low dose of the other nortestosterone derivatives as compared to dienogest.

Dienogest is the only nortestosterone derivative which exerts no androgenic, but an antiandrogenic activity, which is about 30% of that of CPA (Table 12). Despite the relatively low binding affinity to the PR, dienogest shows a strong progestogenic effect on the endometrium. The transformation dose of 6.3 mg per cycle is similar to that of LNG. This is probably due to the high serum levels of dienogest after administration and, hence, high intracellular concentrations, because the proportion of non-protein-bound dienogest in the circulation is 10% due to the lack of binding affinity to SHBG or CBG. Dienogest has also no estrogenic, glucocorticoid or antimineralocorticoid activity, and does not antagonize the estrogen-induced alterations of certain hepatic serum proteins[206].

Orally administered dienogest is rapidly absorbed and the bioavailability is about 95%, but the elimination half-life is relatively short ($t_{1/2\beta}$, 9.1 h). After a single oral administration of 2 mg dienogest and 30 μg EE, a peak level of dienogest of 53 ng/ml is reached within 2 h. This is followed by a rapid decline to 7 ng/ml after 24 h[206]. The main metabolic steps are the reduction of the Δ4-3-keto group, hydroxylation reactions and the elimination of the cyano group.

Tibolone

Pharmacokinetics Tibolone is the 7α-methyl-derivative of norethynodrel, which was used as a progestin component in the first oral contraceptives. Similar to norethynodrel, tibolone is a prodrug and rapidly converted after oral administration in the intestinal tract and the liver to the progestin 7α-methyl-NET (Δ4-tibolone) and some other metabolites (Figure 13). Following a single administration of 2.5 mg tibolone into late postmenopausal women, maximal serum levels of 1.6 ng/ml tibolone, 0.8 ng/ml Δ4-tibolone, 16.7 ng/ml 3α-hydroxy-tibolone, and 3.7 ng/ml 3β-hydroxy-tibolone were found after 1–2 h[292].

Tibolone has only a weak binding affinity to the steroid receptors, while Δ4-tibolone is bound to

the PR and the androgen receptor with high affinity (Table 11). Animal experiments revealed that Δ4-tibolone (7α-methyl-NET) is a relatively weak progestin, but exerts a strong androgenic effectiveness which is comparable to that of testosterone[241,293].

Pharmacodynamics Treatment with tibolone led to a pronounced suppression of the endometrium which is probably caused by Δ4-tibolone originating from the circulation and a local conversion of tibolone[294]. In a minority of the women, endometrial proliferation may occur under treatment with tibolone[295]. In one-third of the patients treated for 3 years with tibolone, endometrial polyps have been found[296]. During the first months of treatment with tibolone, the frequency of irregular bleeding was considerably less than with a combination of 2 mg estradiol and 1 mg NETA, but, after 6 months of treatment, there was no difference between the preparations[297].

The strong androgenic activity of tibolone may account for the reduced proliferation of the breast epithelium, the increase in some parameters of sexuality, for the less unfavorable changes in hemostatic parameters, as compared to estrogen/progestogen combinations, and for the reduction of HDL cholesterol levels by 30%, triglycerides by 20%, and SHBG by 50%[298–302].

Tibolone has been demonstrated to relieve hot flushes and atrophic urogenital complaints, and to inhibit bone resorption[302]. This reflects a strong estrogenic activity of a metabolite of tibolone.

The estrogenic effects have been claimed to be caused by the two metabolites 3α- and 3β-hydroxy tibolone which show only a weak binding affinity to the ER, but are circulating at high concentrations.

Aromatization of tibolone and norethisterone After oral treatment of ovarectomized rats, tibolone was found to be 50 times more estrogenic than NET, and to be more estrogenic than 3α- and 3β-hydroxy-tibolone which were claimed to be responsible for the pronounced estrogenic activity of tibolone (Figure 13). Moreover, the NET prodrug norethynodrel had previously been shown in the Allen-Doisy test to display an estrogenic efficacy 100 times that of NET[204]. Since, in postmenopausal women, NET was demonstrated to be rapidly aromatized to EE after oral administration (Figure 14)[205,268], it was probable that the high estrogenic potency of norethynodrel after oral administration is caused by a pronounced conversion to EE. Consequently, it was assumed that tibolone is also aromatized after oral administration. This was investigated in a pharmacokinetic trial with young women who were treated during

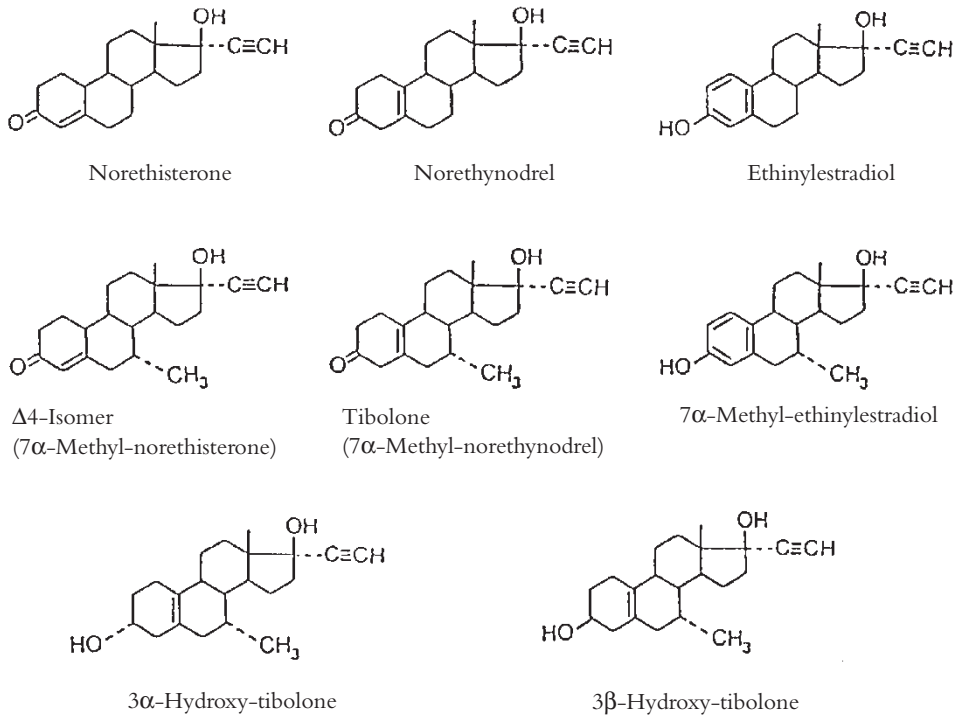

Figure 13 Structural formulae of tibolone, tibolone metabolites and related steroids

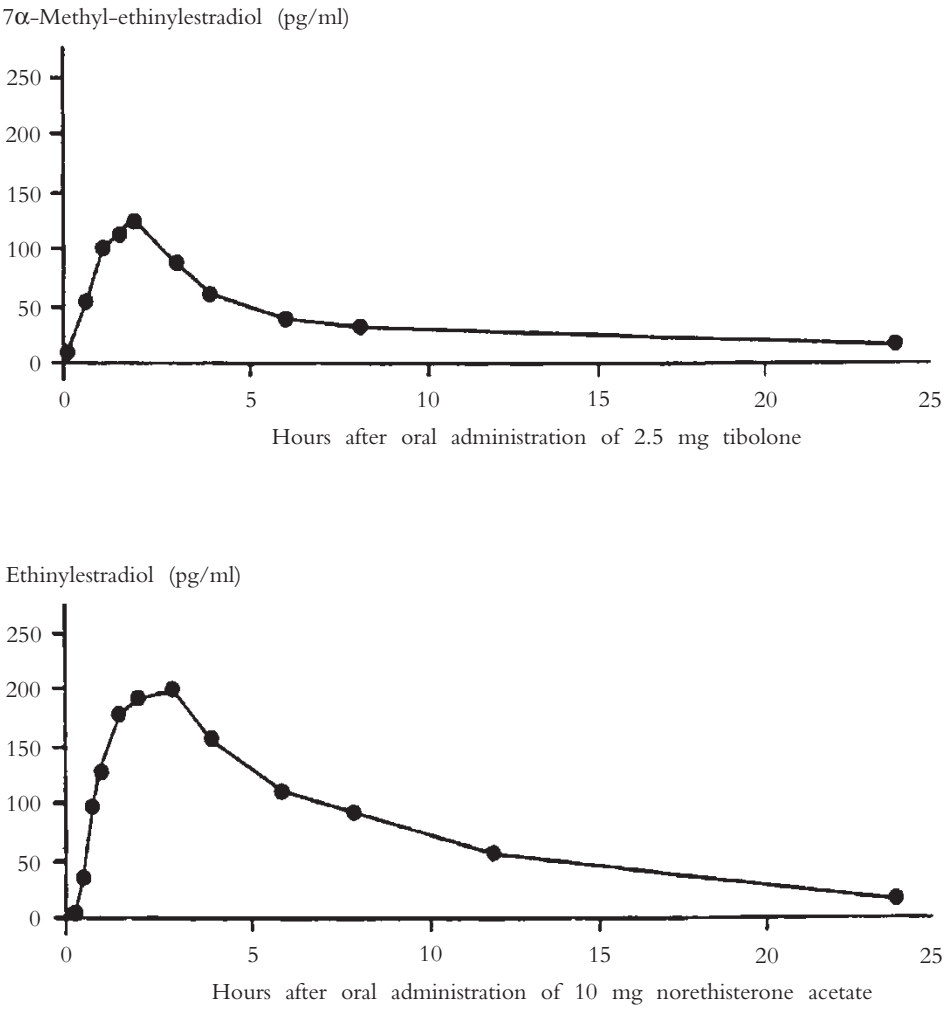

Figure 14 Time course of the serum concentration of 7α-methyl-ethinylestradiol after oral treatment of fertile women with 2.5 mg tibolone (after Wiegratz *et al.*, 2002[303]); time course of the serum concentration of ethinylestradiol after oral treatment of postmenopausal women with 10 mg norethisterone acetate (after Kuhnz *et al.*, 1997[205])

the luteal phase with 2.5 mg tibolone. The analysis of the serum samples by means of the gas chromatography/mass spectrometry method revealed that daily treatment with 2.5 mg tibolone leads to a mean peak serum concentration of 7α-methyl-ethinylestradiol (MEE) of 125 pg/ml after 2 h (Figure 14)[303]. This suggests that the formation of the highly active estrogen MEE occurs during the first liver passage[304].

It has been claimed that the hepatic aromatization of tibolone is not possible because the CYP aromatase encoded by the CYP19 gene is not expressed in the adult human liver. Moreover, using human recombinant CYP aromatase, neither tibolone nor NET could be aromatized *in vitro*. Accordingly, the authors concluded that the formation of EE from NET and of MEE from tibolone must be artifacts caused by heating during gas chromatography[305]. It is known that norethynodrel can be chemically aromatized during the derivatization procedure (trimethylsilylation) before gas chromatography, but this was not the case for NET[306]. Consequently, the results from a trial with postmenopausal women treated orally with NET were correct: after oral administration of 10 mg NET, the mean serum level of EE rose rapidly up to a maximal serum concentration of 200 pg/ml (Figure 14)[205]. Moreover, *in vitro* investigations have clearly demonstrated that healthy human liver is able to aromatize NET to EE (Table 14)[307,308].

Concerning a heat-induced aromatization of tibolone, it was shown that the low rate of conversion of tibolone during the GC/MS proce-

Table 13 Aromatization of norethisterone (NET) to ethinylestradiol (EE) by adult human liver tissue, according to Yamamoto *et al.* (1986)[307]

Liver tissue	*Formation of EE* (fmol/100 mg protein)
Male, 48 years, cirrhosis	157
Male, 54 years, cancer	24
Male, 54 years, healthy	169
Female, 48 years, cancer	54
Female, 40 years, cirrhosis	1121
Female, 69 years, cirrhosis	699
Female, 48 years, cancer	414
Male, 38 years, healthy	302
Male, 51 years, healthy	604

Tritium-labeled NET was incubated for 2 h at 37°C with homogenates of healthy liver, cirrhotic liver, and liver cancer. After extraction and isolation by means of column chromatography and thin layer chromatography, the tritium-labeled EE originating from norethisterone was co-crystallized with EE to constant radioactivity which was measured (EE: 100 fmol = 30 pg)

dure cannot explain the high levels of MEE measured in the serum of women treated with tibolone, and only 3% of the measured MEE might be an artifact (personal communication from the laboratory which performed the GC/MS analysis)[303]. Moreover, the method used for the determination of EE and MEE by GC/MS did not include derivatization by trimethylsilylation (personal communication from the laboratory which performed the GC/MS analysis). Another pharmacokinetic investigation on the aromatization of tibolone was carried out in postmenopausal women, but the manufacturer of tibolone has not published the results to date.

The findings of the formation of EE after administration of NET, as well as that of MEE after intake of tibolone, suggest that CYP enzymes (monooxygenases, hydroxylases) other than the classical aromatase system must be involved in the aromatization during the first passage through the human liver.

It has been argued that the 30% decrease in the serum levels of HDL cholesterol during treatment with tibolone is incompatible with the presence of a potent estrogen like MEE. It must be kept in mind that the progestogenic metabolite Δ4-isomer has a very strong androgenic activity comparable to that of testosterone, which easily counteracts the estrogenic effect on the liver. It is well known that oral contraceptives containing 30 μg EE and 0.25 mg LNG may reduce HDL cholesterol by 20% owing to the androgenic activity of LNG, which is much lower than that of testosterone[309].

Spirolactone derivatives

Drospirenone

The chemical structure of drospirenone, which is a derivative of 17α-spirolactone, is similar to that of the aldosterone antagonist spironolactone (Figure 10). It has a moderate binding affinity to the PR and a high binding affinity to the androgen receptor and mineralocorticoid receptor (Table 10). The progestogenic activity of drospirenone in the endometrium corresponds to 10% of that of LNG. Therefore, daily doses of 3 mg drospirenone are used in HRT preparations. Owing to the strong antimineralocorticoid effect of drospirenone, treatment of fertile women with 2 mg alone during the follicular phase caused an increase in sodium excretion, but this was compensated for by a rise in the plasma renin activity by 100% and the aldosterone serum levels by 65%[310]. The antiandrogenic activity of drospirenone is about 30% of that of CPA. It has no estrogenic and no appreciable glucocorticoid activity[311].

Drospirenone has an oral bioavailability between 76% and 85%. It has no binding affinity to SHBG and CBG and the majority of the circulating compounds are bound to albumin; in the blood about 3–5% are non-protein-bound, free drospirenone.

After a single administration of 3 mg drospirenone, a peak serum level of 35 ng/ml is reached within 1–2 h. Thereafter, the levels decline, but, after 24 h, values of 20–25 ng/ml can be measured. Consequently, drospirenone accumulates in blood during multiple dosing, and treatment with drospirenone in combination with a potent estrogen leads to a peak serum concentration of 60 ng/ml after 7–10 days. The half-lives are 1.6 h ($t_{1/2\alpha}$) and 27 h ($t_{1/2\beta}$). The main metabolic pathways are the opening of the lactone ring leading to an acid group, and the reduction of the Δ4-double bond[311].

Continuous combined treatment of postmenopausal women with 1 mg estradiol and 1, 2 or 3 mg drospirenone was shown to protect efficiently the endometrium, to improve climacteric complaints and to increase bone mineral density. The use of these formulations caused amenorrhea in 80% of the patients within 1 year[312]. Owing to the lack of androgenic activity, the estrogen-induced changes in lipid metabolism

were not counteracted. The slight blood pressure-lowering effect of estradiol/drospirenone combinations is similar to that of other HRT preparations containing estradiol and progestins.

Pharmacodynamics of progestogens

Progestogens may modulate the estrogen-induced effects in many tissues. They can enhance the beneficial effects of estrogens on hot flushes, but may exert unfavorable effects on the risk of cardiovascular disease, breast cancer and mood. As progestogens differ in their hormonal pattern, e.g. glucocorticoid, androgenic or antiandrogenic, or antimineralocorticoid activity, there may be differences between the various progestogens regarding the clinical response to HRT.

Hot flushes

The addition of progestogens to estradiol may enhance the beneficial effect on hot flushes. In postmenopausal women, continuous combined therapy with 1 mg estradiol and 0.5 mg NETA improved hot flushes significantly better than with 1 mg estradiol alone[313]. High-dose progesterone derivatives like MPA or megestrol acetate can effectively improve hot flushes[314]. The effect of 2.5 mg tibolone, which is probably caused by metabolites with estrogenic activity, is comparable with that of estrogens.

Bone

The only two progestins which have been demonstrated to increase bone mineral density and to prevent osteoporosis are NETA and tibolone[315,316]. It remains to be clarified, in as much as the conversion of NETA to EE or of tibolone to 7α-methyl-EE or 3-hydroxy-tibolone is involved in the preservation of bone. The enhancement of the favorable effect of 1 mg estradiol by the addition of 0.25 mg or 0.5 mg NETA was clearly dose-dependent[313]. The results of trials investigating the effect of MPA or other progestins on bone resorption are contradictory, but, if there is a beneficial action, it is small[317].

Skin and hair

Estrogen deficiency and aging may play a role in the development of acne and seborrhea, hirsutism, and alopecia. Low estradiol levels may cause a diffuse loss of scalp hair and a predominance of the action of endogenous androgens on the hair follicles. Estrogen replacement may improve the symptoms. The addition of progestins with androgenic activity, particularly LNG or NET, may counteract the estrogenic effect, whereas progestins with antiandrogenic activity, e.g. CPA, nomegestrol acetate, dienogest, drospirenone and CMA, may bind to the androgen receptor and reduce the interaction of the receptor with endogenous androgens. This may improve the signs of relative hyperandrogenism. As the effect depends both on the binding affinity to the androgen receptor and the intracellular concentration of the antiandrogenic progestin, severe disturbances may need higher doses. Moreover, progesterone and nortestosterone derivatives (e.g. NET), but not progesterone derivatives (e.g. CPA, CMA), may competitively inhibit the activity of 5α-reductase, resulting in a reduced conversion of testosterone to the more active dihydrotestosterone. This may explain the favorable effect of NET-containing ointments on acne. Topically applied CPA has been reported to improve acne, similar to oral treatment with a combination of EE and CPA, whereas topical treatment with a cream containing Δ1-CMA was ineffective[318,319].

Central nervous system and mood

Owing to their antiestrogenic properties, progestogens may antagonize the stimulatory effects on the central nervous system of estrogens. They may reduce the number of ERs and of synaptic connections, attenuate the effect of excitatory amino acids, and increase the inactivation of neurotransmitters. This may explain the unfavorable effect of progestogens on mood of predisposed women, e.g. in women with a history of premenstrual syndrome[320]. It has been shown that MPA may impair the beneficial effects of CEE on depressive mood and other psychological symptoms in postmenopausal women[321]. The mutual interactions between estrogens and progestogens are still controversially discussed[321,322].

In women with sexual dysfunctions, treatment with estrogen/androgen combinations may improve libido, at least in patients with low testosterone levels. Treatment with 2.5 mg tibolone, which has strong androgenic activity, was shown to improve physiological aspects of sexual functions and sexual desire, but not frequency of sexual function and orgasm[323].

The natural progesterone has a special position concerning the central effects of progestogens, as,

in addition to its estrogen-antagonistic actions, it exerts sedative effects. These are mainly brought about by the progesterone metabolites 3α,5α- and 3α,5β-pregnanolone which are formed after oral administration and may interact with the $GABA_A$ receptors.

Endometrium

In the endometrium, progestogens reduce the number of ERs, enhance the inactivation of estrogens and, hence, inhibit estrogen-induced proliferation. According to their structure, progestogens differ largely in their antiestrogenic and progestogenic effects on the endometrium. It is well known that, even using very high doses, the addition of progestogens cannot cause a full secretory transformation in all estrogen-treated women[324]. The indication for the addition of progestogens to estrogen replacement therapy is, however, not the secretory transformation, but the prevention of endometrial hyperplasia. The formulations approved for oral or parenteral HRT fulfil the requirement that the incidence of endometrial hyperplasia is less than 2% during 1–2 years of treatment. Therefore, the composition of the preparations used in HRT varies according to the route of application and type and dose of both the estrogen and progestogen. Using sequential regimens, the number of days with additional progestogen is more important than the dose of the progestogen, and the dose of a progestogen necessary for endometrial protection is generally lower in continuous combined therapies than in sequential/cyclic treatment.

Breast

In contrast to the endometrium, progesterone and most synthetic progestins enhance the proliferative effect of estrogens on breast epithelium. This is believed to be the physiological basis for the increase in breast cancer risk during long-term HRT. Although the mitosis rate in breast cancers was observed to be higher in the luteal phase than in the follicular phase[325], the role of progestogens and estrogens in the development of breast cancer remains obscure. Recent epidemiological studies found an increase in the relative risk by 20–30% during treatment with CEE/MPA, which was in the range of that of other risk factors, but may be due to a selection bias[47,326]. The nearly significant reduction in breast cancer incidence in women treated with CEE alone contradicts the present opinion on the mechanism of action of estrogens[48]. There are no data on the breast cancer risk concerning possible differences between oral and transdermal HRT.

Cardiovascular disease

Epidemiological studies have shown that the development of atherosclerosis is accelerated by a long-term estrogen deficiency and may be prevented if HRT is started early. Recently, a nearly significant protective effect of CEE on the development of coronary heart disease was reported in women with high risk factors[48]. The results with CEE/MPA suggest that MPA counteracts the favorable effect of estrogens[47]. A similar effect was observed in ovariectomized cynomolgus monkeys, in which the addition of MPA (monkey equivalent of 2.5 mg/day) antagonized the protective effect of CEE (monkey equivalent of 0.625 mg/day) against atherosclerosis and vasospasms[327,328]. In a subsequent experiment with the monkey model, the same dose of MPA did not counteract the beneficial effect of CEE on coronary atherosclerosis, but this was possibly due to a change in the dosing regimen: in this trial CEE and MPA were given in divided doses, half in the morning and half in the afternoon[329]. Obviously, it is the serum peak level of MPA rather than the total exposure to MPA which determines the direct effects on the vessel wall, whereas the effect of the estrogen is dependent on the daily exposure. Treatment with CEE/MPA as a single dose was also more effective in inhibiting endometrial proliferation than giving CEE/MPA in two divided doses[329]. In the monkey model, LNG and a NET prodrug did not antagonize the protective effect of EE against atherosclerosis, and, in postmenopausal women, estradiol was shown to prevent atherosclerosis even in combination with 0.15 mg LNG[330,331]. This suggests that it is not the androgenic activity of a progestin which may counteract the direct effects of estrogens on the arterial wall. It is probably the pronounced glucocorticoid activity of MPA that may contribute to the unfavorable cardiovascular effects of CEE/MPA. *In vitro* experiments revealed that MPA at low concentrations (3 ng/ml) may upregulate the thrombin receptor on vascular smooth muscle cells[236]. Consequently, the activation of the thrombin receptor by thrombin may stimulate the extrinsic coagulation and facilitate the development of atherosclerosis. Treatment with progestogens with glucocorticoid effects may, therefore, stimulate thrombin-induced

Table 14 Relative binding affinity (RBA) to the glucocorticoid receptor of various steroid hormones and their *in vitro* effect on the expression of the thrombin receptor (TR) in vascular smooth muscle cells[24,236]

Steroid hormone	*Upregulation of the TR*	*RBA to glucocorticoid receptor* (%)
Dexamethasone	++	100
Medroxyprogesterone acetate	+	29
Gestodene	+	27
3-Keto-desogestrel	+	14
Progesterone	+	10
Levonorgestrel	–	1
Norgestimate	–	1
Norethisterone	–	0
Ethinylestradiol	–	0

–, no effect; +, pronounced effect; ++, strong effect

expression of the tissue factor and upregulate the procoagulatory and vasoconstrictory activity of lesioned arterial walls (Table 14).

This mechanism may also be involved in the increased risk of venous thromboembolic disease during HRT. The increased formation of thrombin induced by progestogens with glucocorticoid activity may enhance the production of the thrombin-activatable fibrinolysis inhibitor and, hence, downregulate fibrinolytic activity. Progestins may also modify the estrogen-induced increase in the intrinsic coagulation via their influence on the hepatic production of hemostatic factors. In this regard, progestins with androgenic activity, e.g. LNG or NET, are more favorable[332]. They may also antagonize the reversible estrogen-induced resistance to activated protein C[333].

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