COMBINED ESTROGEN–PROGESTOGEN MENOPAUSAL THERAPY

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These substances were considered by a previous Working Group, in June 1998 (IARC, 1999), under the title 'Post-menopausal hormonal therapy'. Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Introduction

Estrogen–progestogen menopausal therapy involves the co-administration of an estrogen and a progestogen to peri- or menopausal women. While it is indicated most clearly for control of menopausal symptoms, the use of estrogens with or without progestogens has expanded to include the treatment or prevention of a range of chronic conditions that are associated with ageing. Such widespread, long-term use was often perceived as a 'replacement', in that it physiologically reconstituted vital functions that were lost with menopausal ovarian failure. This pattern was propitiated by the 'medicalization' of the menopause, which was perceived as pathological rather than as an expected and natural event in life. Evidence from the Women's Health Initiative, which showed a clearly harmful effect of the use of estrogen–progestogen combinations, has modified this attitude; as a result, use of the term 'replacement' has diminished. Patterns of exposure are also changing rapidly as the use of hormonal therapy declines, the indications are restricted and the duration of the therapy is reduced.

Combined estrogen–progestogen formulations are available for oral and transdermal administration, although separate administration of each component is still frequent. Progestogens are available orally, while estrogen may be administered orally, transdermally or transvaginally. The timing of exposure to these hormones may be continuous (both estrogen and progestogen at set daily doses), sequential (estrogen daily with progestogen for the last 10–14 days of the cycle) or cyclical (as with sequential, but including 7 days without hormonal exposure).

Chemical and physical data and information on the production and use of individual ingredients used in formulations of combined estrogen–progestogen therapy are given in Annex 1. Trade names and composition of combined products used in hormonal menopausal therapy are presented in Annex 4.

1.2 Historical overview

The earliest forms of hormone used for the treatment of the effects of natural ovarian failure or surgical removal of the ovaries were natural extracts of ovarian tissue, placenta and urine from pregnant women. These extracts contained both estrogen and progestogen, as well as other substances. Experiments in the late nineteenth century demonstrated the clinical benefit of injecting these extracts to alleviate menopausal symptoms, particularly in women who had premature natural or surgically induced menopause (IARC, 1999).

The identification and purification of ovarian hormones in the late 1920s and 1930s enabled wider clinical use of hormonal menopausal therapy. Esterone, estriol and progesterone were identified in 1929, followed by estradiol in 1936 (IARC, 1979). Progesterone was isolated in crystalline form in 1934. Although the use of estrogen and progesterone injections was reported in the 1930s (Hirvonen, 1996), for several subsequent decades, menopausal symptoms were treated mainly with estrogen alone rather than with combined estrogen–progestogen therapy. The extraction of conjugated estrogens from the urine of pregnant mares led to the marketing in 1943 of Premarin, the first orally active and readily available estrogen (IARC, 1999).

Further developments followed the production of the orally active progestogens, norethisterone (also known as norethindrone in the USA) in 1950 and norethynodrel in 1952, which were ultimately used in combined oral hormonal contraceptives (see the monograph on ‘Combined estrogen–progestogen contraceptives’). During the 1960s and early 1970s, hormonal menopausal therapy was most common in the USA and usually comprised estrogen therapy without a progestogen (Davis *et al.*, 2005). Estrogen–progestogen therapy was used by some clinicians, particularly in Europe, primarily for better control of uterine bleeding during treatment (IARC, 1999). Doses in hormonal menopausal therapy at that time were relatively high compared with current standards, and 1.25 mg conjugated equine estrogens were reportedly used in the USA (Pasley *et al*., 1984). Use of hormonal menopausal therapy increased through the 1960s until the mid-1970s, particularly in women who experienced natural menopause.

An association between estrogen therapy and endometrial cancer described in 1975 (Smith *et al*., 1975; Ziel & Finkle, 1975) led to a rapid decline in levels of estrogen use, and by 1980 reached those noted in the mid-1960s (Kennedy *et al.*, 1985). Many clinicians and researchers advocated that a progestogen be added to estrogen when treating menopausal women with a uterus to offset the proliferating action of estrogen with the differentiating action of progestogen. Studies that began in 1979 (Thom *et al*., 1979; Whitehead *et al*., 1981) demonstrated that progestogens attenuated the risk for endometrial cancer associated with the use of estrogen alone. In the early 1980s, the use of combined estrogen–

progestogen became more common, while greater attention to endometrial monitoring was recommended for users of estrogen only (American College of Physicians, 1992).

Ultimately, prescriptions for menopausal estrogens began to rise again with a significant increase in the use of combined estrogen–progestogen that continued throughout the 1990s. However, regional differences persisted; for example, combined therapy remained less common in the USA compared with the United Kingdom (Kennedy *et al.*, 1985; Townsend, 1998).

As use expanded in the 1980s and 1990s, the menopause was increasingly defined as a hormone deficiency that could be treated through 'replacement' of the missing hormones. Not only was estrogen established as a preventive therapy for osteoporosis in oophorectomised women (Aitken *et al*., 1973; Lindsay *et al*., 1980), but it was suggested that 'hormone replacement therapy' could reduce the risk for a range of related conditions, including cognitive decline (Campbell & Whitehead, 1977) and cardiovascular disease (Ross *et al*., 1981; Greendale *et al.*, 1999). A variety of social and medical factors stimulated an increase in use, including evidence of supporting benefits, corporate promotion of hormonal therapy (Palmlund, 1997) and increasing interest in women's health issues.

Estrogen–progestogen therapy became increasingly used for longer periods by older women and for indications far removed from menopausal symptoms. Combined therapy also became the norm for women with a uterus whereas estrogen therapy alone was largely limited to women who had surgically induced menopause. Use continued to increase despite reports of a greater risk for breast cancer associated with hormonal therapy (Hoover *et al*., 1976; Colditz *et al*., 1993), perhaps because of uncertainties in the estimation of the magnitude of this risk (Grady *et al*., 1992).

During this time, prevalence of current use remained lower in non-white women and lower socioeconomic groups (Stafford *et al.*, 1998). Increase in the use of hormonal therapy was greater outside of than within the USA (IARC, 1999).

In response to the increase in use of concomitant estrogens and progestogens, a number of combined formulations were developed in the mid-1990s, including both continuous combinations (fixed daily dose of estrogen and progestogen) and cyclical combinations (fixed daily dose of estrogen with a progestogen component on a given number of days per month). Intermittent administration of progestogen, as with the cyclical formulations, generally results in withdrawal uterine bleeding, whereas continuous administration does not. A transdermal patch that contained estrogen and progestogen was marketed in 1998.

There were some indications that the benefit of hormonal therapy was uncertain, and observational studies that suggested this benefit were unable to rule out confounding. The assumptions that were fundamental to the expansion of hormonal therapy came under particular scrutiny following the publication of the Heart and Estrogen/Progestin Replacement Study (HERS) in 1998. HERS showed no protective effect against recurrent events of cardiovascular disease in women with known cardiovascular problems who were randomized to conjugated equine estrogens and medroxyprogesterone (Hulley *et al*., 1998). The initial suggestion of a temporal pattern of early harm and later benefit that emerged in this study was not confirmed on further follow-up (Grady *et al*., 2002a). As a result of dampened

enthusiasm for hormonal therapy, levels of use peaked in 2000 and plateaued in subsequent years (Hersh *et al*., 2004).

A more dramatic change in patterns of practice followed the results of the Women's Health Initiative (WHI) trial in July 2002. Women with no history of known cardiovascular disease were randomized to receive combined hormonal therapy. Contrary to expectations based on observational data, WHI showed that rates of cardiovascular events were higher in women exposed to conjugated equine estrogens and medroxyprogesterone than in those exposed to placebo (Rossouw *et al*., 2002; Manson *et al*., 2003; Majumdar *et al*., 2004). In addition, it was reported that conjugated equine estrogens and medroxyprogesterone increased the risk for other adverse events (Chlebowski *et al.*, 2003; Rapp *et al*., 2003; Shumaker *et al*., 2003; Wassertheil-Smoller *et al.*, 2003) that were not offset by reduced risks for fractures and colorectal cancer. Furthermore, overall quality of life was not improved by treatment compared with placebo (Hays *et al*., 2003a). While the results were not as dramatic, publication of the second WHI trial that involved administration of estrogen alone (Women's Health Initiative Steering Committee, 2004) reinforced a new consensus on the increase in adverse vascular outcomes associated with hormonal menopausal therapy.

Although some doubts were raised regarding the reliability and generalizability of the WHI results (Shapiro, 2003; Strickler, 2003), practice patterns changed tremendously. Prescriptions in the USA fell by 50% during the 18 months that followed the results of the WHI (Hersh *et al*., 2004; Majumdar *et al*., 2004). Internationally, similar reductions occurred in western Europe and most of the Western Pacific (Table 1). The decline in the use of hormonal therapy was particularly marked for combined estrogen–progestogen therapy.

Use has begun to shift towards lower-dose formulations (e.g. 0.30 mg conjugated equine estrogens and 1.5 mg medroxyprogesterone). Simultaneously, there is less use of hormonal menopausal therapy among older women. Patterns of use will probably change further as numerous professional organizations continue to recommend the use of lower doses, shorter durations of use and limiting use to more severe menopausal symptoms (US Preventive Service Task Force, 2002; North American Menopause Society, 2004; Wathen *et al*., 2004).

1.3 Preparations of estrogen–progesterone menopausal therapy

A variety of products are available for use in combined estrogen–progestogen menopausal therapy, either as individual estrogen and progestogen components that can be co-administered or as a combined product. The use of individual components may allow better tailoring compared with combined products. A number of combined formulations are described in Annex 4.

Available estrogen products can be defined by their estrogen form, dose and mode of delivery. The most common estrogens available for hormonal menopausal therapy are conjugated equine estrogen, conjugated plant-based estrogen, estradiol and ethinylestradiol. A range of three to five different doses are often available for each product, varying from

low-dose (0.3–0.5 mg orally) to high-dose (2.5–5 mg). Estrogen products are available in oral form, transdermal patches and intravaginal rings. These products can be used either for estrogen-only therapy (e.g. in women who have had a hysterectomy) or in conjunction with a progestogen to provide combined hormonal therapy.

Table 1. Trends in sales of combined estrogen–progestogen menopausal therapy products for selected years (millions of standard units[a])

Regions[b]	1994	1999	2004
Africa	21.0	29.9	27.7
South Africa	20.0	28.9	26.7
West Africa	1.1	1.0	1.1
Eastern Mediterranean	8.9	21.5	27.7
Europe	1 269.5	1 858.3	1 078.4
Eastern Europe	36.2	184.8	159.8
Western Europe	1 233.3	1 673.5	918.6
North America	39.7	1 089.4	421.8
South America	100.2	284.5	190.4
South East Asia	20.0	36.9	67.5
India	0	0	1.2
Korea	7.8	16.8	43.9
Rest of South East Asia	10.5	17.3	20.5
Western Pacific	100.8	219.7	107.0
Australia/New Zealand	17.5	75.8	34.3
China/Hong Kong	0.8	10.8	4.5
Japan	67.6	54.6	36.9
Taiwan, China	4.6	58.5	24.3
Rest of Western Pacific	10.4	20.0	7.1
Total	1 560.1	3 550.2	1 920.6

From IMS Health (2005)

[a] Standard units are sales in terms of standard dose units; the standard dose unit for oral products is one tablet or capsule

[b] *West Africa* includes: Benin, Burkina, Cameroon, Congo, Gabon, Guinea, Ivory Coast, Mali, Senegal, Togo;

Eastern Mediterranean includes: Egypt, Jordan, Kuwait, Lebanon, Morocco, Saudi Arabia, Tunisia, United Arab Emirates;

Eastern Europe includes: Belarus, Bulgaria, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Russian Federation, Slovakia, Slovenia, Ukraine;

Western Europe includes: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, Turkey, UK;

North America includes: Canada, Central America (Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama), Mexico, Puerto Rico, USA;

Rest of South East Asia includes: Indonesia, Pakistan, Thailand;

South America includes: Argentina, Brazil, Chile, Colombia, Dominican Republic, Ecuador, Peru, Uruguay, Venezuela;

Rest of Western Pacific includes: Malaysia, Philippines, Singapore.

A range of progestogens are available for use in combined hormonal therapy. Those most commonly used are medroxyprogesterone acetate, norethisterone and levonorgestrel. Several doses of each progestogen are usually available. For example, medroxyprogesterone acetate is often available in doses of 1.5, 2.5, 5 and 10 mg. While oral forms predominate, progestogens also are available as a vaginal pessary, a systemically absorbed vaginal gel, a transdermal patch and an intrauterine device. Administration of progestogen may follow one of three types of schedule. In continuous combined therapy, the same dose of both estrogen and progestogen is administered each day. In sequential therapy, 10–14 days of progestogen is provided per cycle in addition to daily estrogen. In cyclical therapy, a cycle consists of estrogen alone, followed by progestogen with estrogen and then 5–7 days with no hormones.

Combined oral products that contain both estrogen and progestogen provide greater convenience to users, as only one rather than two tablets are taken. The various preparations available differ in their estrogen component, their progestogen component, the dose of these components, and the schedule and mode of drug administration. Despite the potential for a plethora of combinations, a relatively small number are manufactured. Continuous dose schedules during which the same doses are taken on a daily basis are most common. Less commonly, progestogens may be delivered for only a portion of a monthly cycle (e.g. Premphase). Combined products are frequently available at two dose levels. Oral forms of combined therapy predominate, but a combined transdermal patch and a vaginal ring are also available.

The selection of a specific regimen for menopausal therapy depends on the preferences and needs of each women. Further, evidence regarding long-term risk may motivate physicians to recommend a specific formulation. A number of the products available for hormonal menopausal therapy have only recently been introduced and their long-term effects have not been evaluated fully.

1.4 Patterns of use

A number of studies have provided information on patterns of use of hormonal menopausal therapy, most of which is related to women in developed countries and does not differentiate between use of estrogen alone or in combination with progestogen. Data from individual studies are summarized in Table 2. Most of the available information reflects use in the late 1990s when hormonal therapy had reached its peak. Another set of studies examined more recent use and provided an indication of the extent to which use has declined since the results of the WHI Study.

1.4.1 *Patterns of use in 1990–2000*

Table 2 summarizes the prevalence of current use of estrogen–progestogen menopausal therapy during the years 1997–2003. The section below details those studies that provide additional information on patterns of use during this period.

Table 2. Selected studies of the prevalence of current use of estrogen–progestogen menopausal therapy, 1997–2003

Reference	Country	Year(s) of study	Age group (years)	Prevalence of current use		Comments
				Combined estrogen–progestogen therapy	Any current hormonal therapy	
Pre-2002						
MacLaren & Woods (2001)	USA	1998	40–65	NR	39%	
Progetto Menopausa Italia Study (2001)	Italy	1997–99	45–75	NR	8.5%	
Banks *et al.* (2002) (EPIC)	Denmark Germany Greece Italy Netherlands Spain United Kingdom	1993–97	50–64	NR	29.0% 38.6–40.7% 2.1% 4.4–11.5% 14.3% 4.5–11.5% 28.1–30.3%	 2 centres 2 centres 2 centres 2 centres
Benet Rodriguez *et al.* (2002)	Spain	1989–99	≥ 40	NR	> 3.19%	Detail by 5-year age group and by year
Merom *et al.* (2002)	Israel	1998	45–74	NR	16.8%	
Million Women Study Collaborators (2002)	United Kingdom	1996–2000	50–64	17%	33%	
Mueller *et al.* (2002)	Germany	1985 1990 1995	45–64	0.1% 4.0% 13.9%	3.0% 8.8% 22.6%	
Bakken *et al.* (2004)	Norway	1996–98	Postmenopausal 45–54	24%	35%	

Table 2 (contd)

Reference	Country	Year(s) of study	Age group (years)	Prevalence of current use		Comments
				Combined estrogen–progestogen therapy	Any current hormonal therapy	
Buist *et al.* (2004)	USA	1999	40–80	14.6%	27.2%	
Heng *et al.* (2004)	Singapore	1994–97	45–69	NR	21%	Ever use
Hersch *et al.* (2004)	USA	1995 2001	50–74 50–74	 16%	33% 42%	
Lundberg *et al.* (2004)	20 countries	1989–97	45–64	NR	0–56%	
Manzoli *et al.* (2004)	Italy	1999–2001	50–70	2.9%	6.9%	
Rachoñ *et al.* (2004)	Poland	April 2002	45–64	[9.3%]	12%	
Carney *et al.* (2006)	USA	1996–99	> 40	13%	43%	
Post-2002						
Strothmann & Schneider (2003)	France Germany Spain United Kingdom	2003	45–75	NR	23% 19% 5% 19%	
Bilgrami *et al.* (2004)	New Zealand	December 2002	45–64	3%	11%	
Buist *et al.* (2004)	USA	December 2002	40–80	8%	17%	
MacLennan *et al.* (2004)	Australia	2003	> 50	7%	19%	

NR, not reported

Based on sales data, Jolleys and Olesen (1996) compared the use of hormonal therapy in the USA and Europe and found three strata of prevalence of use: the USA were in the highest stratum (20% of women); the United Kingdom and Scandinavian countries were in the intermediate group (9–16%); and continental Europe had the lowest prevalence (< 5%). The authors noted that use in France, however, was increasing towards levels found in the intermediate group. A later review on the use of hormonal therapy and risk for cancer by Beral *et al.* (1999) estimated that at least 20 million women in developed countries were currently using hormonal therapy.

Based on sales data, Benet-Rodriguez *et al.* (2002) estimated that prevalence of the use of hormonal therapy among women aged 40 years or over increased from 0.7% in 1989 to 3.4% in 1999. In 1998, prevalence was highest in the age group 50–54 years (10.8%) and was below 1% in women over 65 years of age.

Buist *et al.* (1999) examined patterns of long-term use of hormonal therapy in women aged 50–80 years in Seattle, WA, USA. Long-term users (> 10 years) and short-term users were significantly younger than never users. Compared with never users, long-term users were also more likely to be married, to have had surgically induced menopause, to have experienced menopausal symptoms, to see their family doctor and have mammograms and were less likely to smoke. Estrogen alone was the predominant therapy; combined therapy was more common among short-term (< 10 years) users than among long-term users.

Donker *et al.* (2000) reported on first-time users of hormonal therapy in a survey in the Netherlands. The number of prescriptions for such therapy increased from 2 to 3% between 1995 and 1998. Between 1987–88 and 1995–98, sequential therapy was prescribed more frequently than continuous therapy, but there has been a gradual shift from sequential to continuous therapy in the last few years. There was also a trend in prescriptions from estrogen towards combinations of estrogen and progestogen.

MacLaren and Woods (2001) found that, among peri- or postmenopausal women aged 40–65 years in the USA, use of hormonal therapy was lower among women who experienced natural menopause (31%) than among those who had surgically induced menopause (56%). The median duration of use was 5 years, and 25% reported taking hormones for 10 years or more.

In a study of over 40 000 women aged 45–75 years in Italy (Progetto Menopausa Italia Study Group, 2001), 12% were ever users, among whom 74% were current users. Mean duration of use was approximately 20 months in both current and former users. Ever users were more likely to have a higher education, be nulliparous, have had an early menopause, have ever used oral contraceptives and have a history of osteoporosis, and less likely to have cardiovascular disease or diabetes.

The EPIC [European Prospective Investigation into Cancer and Nutrition] Working Group (Banks *et al.*, 2002) examined the patterns of use of hormonal therapy in women aged 50–64 years in several European countries. Current use varied from 2% in Greece to 41% in Heidelberg, Germany, and ever-use varied from 7 to 55%, respectively. In all centres (except in Germany), the most frequent duration of use among ever users was less than 1 year; long-term use (> 10 years) varied from 26% in Denmark to 2% in southern Italy.

Merom *et al.* (2002) examined Israeli women aged 45–74 years in 1998 who had had a natural menopause, among whom 17% were current users and 13% were past users. The prevalence of current use was higher among post- than among perimenopausal women (15% versus 7%). The rates of current and ever use were highest in the 55–59-year age group and lowest in the 70–74-year age group. Current users were more likely to be more highly educated, to work outside the home and be married (compared with divorcees or widows), to have used contraceptives, to make regular visits to a gynaecologist, to be lean, to have regular physical activity and ever to have smoked.

The Million Women Study Collaborators (2002) examined patterns of use in women in the United Kingdom aged 50–64 years in 1996–2000. Of this cohort, 50% had ever used hormonal therapy, of whom 33% reported current use and 17% reported past use. Average age at initiation of therapy was 49.1 years; 38% started at 45–49 years and 37% started at 50–54 years of age. The most common duration of treatment was 1–4 years (37%) followed by 5–9 years (33%); the mean duration of use was 4.9 years.

Mueller *et al.* (2002) reported trends in use of hormonal therapy in Germany in 1984–95, based on a survey of women aged 45–64 years who were included in the WHO Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study. The highest prevalence of use (29.8%) was among women aged 55–59 years. Use of combined hormonal therapy increased from almost non-existent levels in 1985 to 4.0% in 1990 and 13.9% in 1995.

Ekström *et al.* (2003) examined patterns of use of hormonal therapy in women aged 45, 50, 55 and 60 years in Sweden and found that 50–52% of women aged 55 and 60 years had ever used hormonal therapy; the mean length of treatment was 4.4 years. Current users were more likely to be on antidepressive medication and/or cardiovascular drugs, to report psychological and physical menopausal symptoms and to have visited a psychotherapist.

Bakken *et al.* (2004) reported on over 35 000 postmenopausal women aged 45–64 years from the Norwegian Women and Cancer (NOWAC) cohort study, among whom 80% of ever-users of hormones were current users.

From a sample of women in the USA, Haas *et al.* (2004) found that use of hormones in 1997 was highest among white women (53%) and lowest among African-American, Latina, Chinese and Philipina women (30–34%); it was also much higher among women who had had a hysterectomy (60% versus 36%).

By 2001 in the USA, almost half (42%) of all postmenopausal women under the age of 65 years were being treated with hormonal therapy (Hersh *et al.*, 2004). It was reported that 38% of users were taking combined therapy, either as a single preparation or as separate estrogen and progestogen components.

Based on a sample population for a case–control study, Newcomb *et al.* (2002) reported that 25–28% of all postmenopausal women in the USA had ever used hormonal therapy in 1992–95. Of these users, 30% had used combined therapy.

Bromley *et al.* (2004) reported on the proportion of women who used hormonal therapy from 1992 to 1998. Women who started hormonal therapy during this period were less likely to have a history of a range of diseases but were more likely to have a history

of osteoporosis, hysterectomy, hyperlipidaemia and prior oral contraceptive use than non-users.

Lundberg *et al.* (2004) reported data collected from the MONICA study. Prevalence of current use in women aged 45–64 years varied enormously from 0% in Moscow, Russian Federation, to 42% in Newcastle, Australia, and Canada. Low prevalence of use (< 10%) was noted for central, eastern and southern Europe, the Russian Federation and China, while the highest prevalence of use was reported in populations in western and northern Europe, North America and Australia. Ever use in Perth, Australia, was estimated at 66% of women aged 50–54 years. Regional differences within the same country were generally modest compared with inter-country variations. The highest prevalence was in the age group 45–49 years in 12 populations, in the age group 50–54 years in nine populations and in the age group 55–59 years in four populations.

Rachon *et al.* (2004) examined use of hormonal therapy among Polish women over 45 years of age in April 2002. Overall current use was 12% in women aged 45–64 years and was 16% in the age group 45–54 years; ever use was in the range of 25 and 20% for women aged 45–54 years and 55–64 years, respectively. Women with a medium or higher level of education were more likely to be current users than those who had had a basic education.

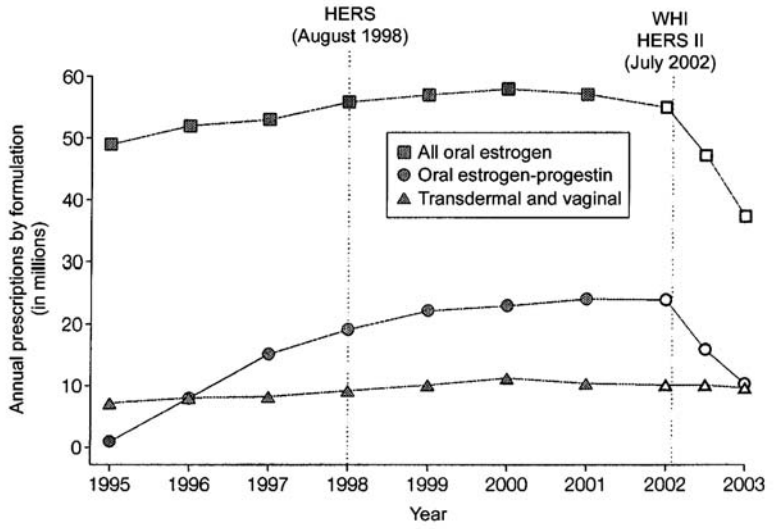
Fournier *et al.* (2005) reported that, among women born between 1925 and 1950 and followed-up between 1990 and 2000, users were more likely than non-users to have had an early menarche, an early menopause, to be parous, to have a personal history of benign breast disease, to have no familial history of breast cancer in first-degree relatives, to be lean, to have a higher level of education, to have used oral contraceptives and to have used oral progestogens before the menopause.

1.4.2 *Recent trends in hormonal menopausal therapy*

Large and rapid changes in the use of combined hormonal menopausal therapy took place in 2002 as a consequence of the publication of the results of the WHI. International data (IMS Health, 2005) suggest that sales of combined hormonal therapy (estrogen and progestogens in a single preparation) declined substantially worldwide (Table 1). Decreases between 1999 and 2004 were noted in Europe (42% decline), North America (61%), South America (33%) and the western Pacific (51%). Increased or stable sales of combined hormonal therapy were noted in Africa, the eastern Mediterranean and South-East Asia during the same period.

In the USA, overall use of hormonal therapy fell by about 38% and that of combined estrogen–progestogen therapy by 58% between 2001 and the first half of 2003 (Hersh *et al.*, 2004) (Figure 1). As use continued to decrease 18 months after the WHI results (Rossouw *et al.*, 2002), sales of Prempro (conjugated equine estrogens plus methoxyprogesterone acetate) had fallen by 80% (Majumdar *et al.*, 2004). Haas *et al.* (2004) found similar time trends from survey data.

Figure 1. Annual number of US prescriptions for hormonal therapy by formulation, 1995–July 2003



Modified from Hersh *et al.* (2004)
HERS, Heart and Estrogen/Progestin Replacement Study; WHI, Women's Health Initiative
Data for January to June 2002, July to December 2002 and January to July 2003 are included (open symbols). Data are from the National Prescription Audit Plus, IMS Health.

Strothmann and Schneider (2003) analysed data from France, Germany, Spain and the United Kingdom in women aged 45–75 years in 2003 and found that in all four countries the number of former users was relatively similar to that of current users.

Bilgrami *et al.* (2004) presented data from New Zealand. Based on survey information, current use of hormone therapy dropped from 15% in June 2002 to 11% in December 2002. The majority of women who had stopped using hormonal therapy specifically identified the results of the WHI trial as their reason. Further data from the New Zealand Pharmacy Management Agency (Metcalfe, 2004) showed a decline of 65% in use of hormonal therapy between 2001 and 2004. Examination of monthly data showed a continued decline through to March 2005.

MacLennan *et al.* (2004a) specifically examined changes in use patterns in Australia and found that prevalence of current use had declined from 22.5% in 2000 to 14.4% in 2003 among women aged > 40 years. Over the same period, duration of use decreased by an average of 10 months among current users. Unlike in studies in the 1990s, the number of past users exceeded the number of current users.

No data were available to the Working Group on changes in use in developing countries.

2. Studies of Cancer in Humans

2.1 Breast cancer

2.1.1 *Background*

In the previous evaluation (IARC, 1999), most of the epidemiological evidence derived from studies that assessed the use of estrogen alone in relation to subsequent risk for breast cancer. The evidence related to combined therapy with estrogen plus a progestogen was considered to be insufficient to reach any firm conclusion. However, in relation to hormonal menopausal therapy with estrogen alone, the evidence was summarized as follows.

A pooled analysis from 51 studies and a review that included 15 cohort studies and 23 case–control studies showed a small increase in risk for ever use, which increased with longer duration of use (5 years or more), and an increased risk in current and recent users. Some information was available on women who used and then stopped using menopausal estrogen therapy; based on this evidence, the increased risk appeared to disappear several years after cessation of use. There was also evidence to suggest that the increase in risk was predominantly for small, localized tumours of the breast. The data were, however, insufficient to determine whether the risk varied with type of compound or the dose of various compounds used.

This evaluation relied heavily on the pooled analysis from the collaborative group in Oxford (Collaborative Group on Hormonal Factors in Breast Cancer, 1997), which had compiled and re-analysed the original data of 51 studies, 22 of which provided information on the hormonal constituents of the preparations. In the re-analysis, data on hormonal constituents were available for 4640 women; 12% (557) of these women had received combined estrogens and progestogens, and 249 women with breast cancer had used combined treatment. The results showed that, among women who were currently using combined therapy, the relative risk was 1.2 (95% confidence interval [CI], 0.8–1.5; based on 136 exposed cases) for less than 5 years of use and 1.5 (95% CI, 0.9–2.2; based on 58 exposed cases) for more than 5 years of use.

These limited data did not provide a firm basis for any conclusion regarding the effects of the use of combined estrogen–progestogen therapy on the risk for breast cancer. Subsequently, many studies, including clinical trials, have assessed the risk for breast cancer in relation to the use of combined hormonal therapy by menopausal women.

2.1.2 *Randomized clinical trials* (Table 3)

The WHI investigators conducted two large, randomized, double-blind, placebo-controlled trials that evaluated the effects of estrogen alone and estrogen plus progestogen on the prevention of chronic disease in 27 347 postmenopausal women aged 50–79 years (Women's Health Initiative Study Group, 1998; Anderson *et al.*, 2003; Stefanick *et al.*, 2003). The incidence of coronary heart disease was the primary outcome and the incidence of invasive breast cancer was the primary safety outcome. Both trials were interrupted prematurely because of adverse effects.

In these two trials, postmenopausal women were recruited between 1993 and 1998 from 40 US clinical centres mainly by mass mailing (Hays *et al.*, 2003b). All women had baseline mammograms and clinical breast examinations. A total of 16 608 eligible women with a uterus at baseline were randomized in equal proportions to treatment with continuous combined conjugated equine estrogens (0.625 mg per day) plus medroxyprogesterone acetate (2.5mg per day) in a single tablet or to a matching placebo. A total of 10 739 women who had had a hysterectomy were randomized with equal probability to conjugated equine estrogens (0.625 mg per day) or placebo. The intervention period was planned to end in 2005, giving a projected mean follow-up of 8.5 years. Participants were followed every 6 months; annual visits to the clinic and mammography were required. Designated outcomes were ascertained by self-reporting at every 6-month contact and documented by medical records that were locally and centrally adjudicated. These outcome procedures were performed by study staff who were blinded to treatment assignment. Vital status was known for 96.7 and 94.7% of the participants in the estrogen plus progestogen (mean follow-up, 5.6 years) and estrogen alone trials (mean follow-up, 6.8 years), respectively (Chlebowski *et al.*, 2003; Anderson *et al.*, 2004).

In May 2002, the Independent Data and Safety Monitoring Board recommended that the estrogen plus progestogen trial be stopped on the basis of an adverse effect on the incidence of breast cancer and an overall assessment that risks exceeded benefits. The protocol-specified weighted log-rank statistic for breast cancer (p-value = 0.001) had crossed the pre-defined monitoring boundary for adverse effects (p-value = 0.02) (Rossouw *et al.*, 2002). The weights in this log-rank statistic were defined by time since randomization, and rose linearly from 0 at time of randomization to 1 at year 10 and thereafter; this effectively down-weighted early differences that were thought to be less probably related to treatment. For simplicity, the initial publication presented unweighted hazard ratios for comparisons of all outcomes, based on the locally adjudicated data available on outcomes at the time that the trial was stopped. These analyses did not acknowledge the anticipated time-dependent effect for breast cancer.

The final unweighted hazard ratio of estrogen plus progestogen for invasive breast cancer was 1.24 (95% CI, 1.04–1.50; weighted $p = 0.003$; 349 cases) (Chlebowski *et al.*, 2003). There was a statistically significant interaction with time since randomization that suggested an effect of duration of exposure. In women who took estrogen plus progestogen, tumours were slightly larger, and more likely to be node-positive and to be at regional

Table 3. Randomized clinical trials of combined hormone therapy and the risk for breast cancer[a]

Reference, name of trial	Country	Age at recruitment	Size of trial	Period of trial	Mean duration of follow-up (years)	No. of exposed women	No. (%) of women using placebo	Total no. of breast cancer cases	Histological type of breast cancer	Cases in exposed women	Cases in placebo women	Hazard ratio (95% CI), treated versus placebo
Hulley *et al.* (2002), HERS	USA	44–79	2 763	1993–2000	4.1	1 380	1 383	88	Invasive	34	25	1.38 (0.82–2.31)
Chlebowski *et al.* (2003), WHI	USA	50–79	16 608	1993–98	5.6	8 506	8 102	822	Invasive + *in situ*	245	185	1.24 (1.02–1.50)
									Invasive	199	150	1.24 (1.01–1.54)
									In situ	46	37	1.18 (0.77–1.82)

CI, confidence interval; HERS, Heart and Estrogen/Progestin Replacement Study; WHI, Women's Health Initiative

[a] In both studies, the treated group received 0.625 mg conjugated equine estrogens and 2.5 mg of medroxyprogesterone acetate.

or advanced stages than those diagnosed in women who took placebo. The incidence of in-situ cancers was not significantly elevated (hazard ratio, 1.18; 95% CI, 0.77–1.82; weighted $p = 0.09$; 84 cases). Mammography rates were high (≥ 88.6% in each year) and did not differ between groups (Chlebowski *et al.*, 2003). Limitations of the study included the proportion of women who discontinued study medications (42% for estrogen plus progestogen and 38% for placebo), the proportion who initiated hormonal therapy outside of the trial (6% versus 11%, respectively) and the proportion of women for whom unblinding of clinical gynaecologists was required (40% versus 7%), primarily to manage vaginal bleeding (Rossouw *et al.*, 2002).

The HERS was a randomized, double-blind, placebo-controlled trial designed to test the effects of continuous combined hormonal therapy (0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate daily) for the prevention of recurrent coronary heart disease among 2763 women aged 44–79 years with a uterus and with documented coronary disease at enrolment. The trial ended after a mean duration of follow-up of 4.1 years and reported no overall effect on coronary heart disease. No significant effect was found on the incidence of breast cancer (relative risk, 1.38; 95% CI, 0.82–2.31; 88 cases) (Hulley *et al.*, 2002).

2.1.3 *Cohort studies* (Table 4)

Persson *et al.* (1999) assessed the use of combined hormonal menopausal therapy and subsequent risk for breast cancer in a prospective study of 10 472 women in Sweden. Information on use of hormonal therapy was obtained at recruitment to the study through prescription records in pharmacies. The cohort was followed for over 6 years by linkage to the Swedish Cancer Registry, and 198 women were registered with incident breast cancer during that time. The relative risk associated with ever use of combined hormonal menopausal therapy was not specified. However, the relative risk for 1–6 years of use at entry to the study was 1.4 (95% CI, 0.9–2.3) compared with never use or use for less than 1 year, and that associated with use for more than 6 years was 1.7 (95% CI, 1.1–2.6). These results were adjusted for age, length of follow-up, age at first full-term pregnancy, body mass index, education and age at menopause. The results also showed that the estimated relative risks were higher for recent or current use than for past use. Recent or current use was associated with a relative risk of 2.8 (95% CI, 0.8–10.0) and use in the past with a relative risk of 1.9 (95% CI, 0.6–6.1).

In a cohort study in the USA, Schairer *et al.* (2000) investigated whether the use of combined hormonal menopausal therapy increased the risk for breast cancer. The cohort of 46 355 postmenopausal women was recruited from a mammography screening programme and followed for 10 years. During follow-up, 2082 women were diagnosed with breast cancer. Ever use of combined hormonal menopausal therapy was associated with a relative risk of 1.3 (95% CI, 1.0–1.6), but the increase in risk was largely restricted to current users or use within the last 4 years (relative risk, 1.4; 95% CI, 1.1–1.8). These results were adjusted for age, mammography screening, age at menopause, body mass index and level of

Table 4. Cohort studies of the use of combined hormone therapy and the risk for breast cancer

Reference	Country	Age at recruitment (years)	Size of cohort	Period of cohort	Average of follow-up (years)	Total no. of cases	Histological diagnosis	Sub-sites	Hormone therapy (type/regimen)	No. of cases	Relative risk (95% CI)	Comments
Person *et al.* (1999)	Sweden	65 (mean)	10 472	1987–93	5.7	198	Invasive		Never	48	1.0	Adjusted for age, follow-up time, age at first full-term pregnancy, body mass index, education, menopausal age/status
									1–6 years	28	1.4 (0.9–2.3)	
									≥ 6 years	44	1.7 (1.1–2.6)	
Schairer *et al.* (2000)	USA	Not specified	46 355	1980–95	10.2	2802	All	All	Never	761	1.0	Adjusted for age, education, body mass index, age at menopause, mammographic screening
									Ever	101	1.3 (1.0–1.6)	
							Invasive	Ductal/lobular	Never	145	1.0	
									Ever	33	[1.73[a]]	
							Invasive	Ductal only	Never	128	1.0	
									Ever	26	[1.55[a]]	
Beral *et al.* (2003)	UK	50–64	1 084 110	1996–2001	2.6	9364	Invasive		Never	2894	1.0	Adjusted for age, region, socio-economic status, body mass index, alcoholic beverage consumption, ever use of oral contraceptives, time since menopause, parity
									Current	1934	2.00 (1.91–2.09)	
									Duration[b]			
									< 1 year	97	1.45 (1.19–1.78)	
									1–4 years	582	1.74 (1.60–1.89)	
									5–9 years	850	2.17 (2.03–2.33)	
									≥ 10 years	362	2.31 (2.08–2.56)	
									All continuous combined			
									< 5 years	243	1.57 (1.37–1.79)	
									≥ 5 years	388	2.40 (2.15–2.67)	
									All sequential combined			
									< 5 years	403	1.77 (1.59–1.97)	
									≥ 5 years	778	2.12 (1.95–2.30)	

Table 4 (contd)

Reference	Country	Age at recruitment (years)	Size of cohort	Period of cohort	Average of follow-up (years)	Total no. of cases	Histological diagnosis	Sub-sites	Hormone therapy (type/ regimen)	No. of cases	Relative risk (95% CI)	Comments
Jernström *et al.* (2003a)	Sweden	50–64	6 586	1995–2000	4.1	101	NR		Never	2422	1.0	Adjusted for age at entry and time of follow-up; continuous combined formula only
									Ever	NR	3.3 (1.9–5.6)	
									Duration			
									≤ 2 years	NR	3.7 (1.8–7.4)	
									3–4 years	NR	2.2 (0.84–5.9)	
									> 4 years	NR	3.7 (1.8–7.7)	
Olsson *et al.* (2003)	Sweden	25–65	29 508	1990–92	Not specified	556	NR		Never		1.0	Adjusted for age, age at menarche, age at first full-term pregnancy, parity, age at menopause, family history of breast cancer
									Ever combined continuous therapy	622	2.45 (1.61–3.71)	
									Never		1.0	
									Ever combined sequential therapy	655	1.22 (0.74–2.00)	
Bakken *et al.* (2004)	Norway	45–64	31 451	1996–98	Not specified	331	NR	All	Never	130	1.0	Adjusted for age, body mass index, age at menarche, ever use of oral contraceptives, time since menopause, family history of breast cancer, mammography, parity, age at first delivery
									Current	116	2.5 (1.9–3.2)	
									Ever use			
									< 5 years	63	2.3 (1.7–3.2)	
									≥ 5 years	51	2.8 (2.0–4.0)	
									Sequential regimen			
									< 5 years	19	1.7 (1.0–2.8)	
									≥ 5 years	14	2.2 (1.3–3.8)	
									Continuous regimen			
									< 5 years	44	2.6 (1.9–3.7)	
									≥ 5 years	37	3.2 (2.2–4.6)	

Table 4 (contd)

Reference	Country	Age at recruitment (years)	Size of cohort	Period of cohort	Average of follow-up (years)	Total no. of cases	Histological diagnosis	Sub-sites	Hormone therapy (type/ regimen)	No. of cases	Relative risk (95% CI)	Comments
Stahlberg *et al.* (2004)	Denmark	≥ 45	10 874	1993–99	Not specified	244	In situ/ invasive		Never	110	1.0	Adjusted for age at menopause, age at menarche, parity, age at first birth, use of oral contraceptives, history of benign breast disease, smoking, night work, body mass index, height, physical activity, alcoholic beverage intake
									Current	75	2.70 (1.96–3.73)	
									Continuous	23	4.16 (2.56–6.75)	
									< 5 years	4	1.96 (0.72–5.36)	
									5–9 years	6	4.96 (2.16–11.39)	
									≥ 10 years	10	6.78 (3.41–13.48)	
									Current cyclical	52	1.94 (1.26–3.00)	
									< 5 years	10	1.58 (0.79–3.17)	
									5–9 years	9	2.47 (1.23–4.95)	
									≥ 10 years	10	2.18 (1.09–4.33)	
Tjønneland *et al.* (2004)	Denmark	50–60	23 618	1993–97	4.8	423	Invasive	Lobular only	Never	15	1.0	Adjusted for parity, age at first birth, history of benign breast tumour surgery, education, alcoholic beverage consumption, body mass index
									Current	41	3.53 (1.94–6.41)	
								Ductal only	Never	109	1.0	
									Current	158	2.10 (1.64–2.7)	
Ewertz *et al.* (2005)	Denmark	50–67	48 812	1989–2002	10	869	NR		Never	561	1.0	Adjusted for age, age at first birth, parity
									Sequential progestogen-derived progestogen	6	0.57 (0.26–1.28)	
									Sequential testosterone-derived progestogen	80	1.52 (1.21–1.93)	
									Continuous testosterone-derived progestogen	13	0.99 (0.57–1.72)	

Table 4 (contd)

Reference	Country	Age at recruit-ment (years)	Size of cohort	Period of cohort	Average of follow-up (years)	Total no. of cases	Histo-logical diagnosis	Sub-sites	Hormone therapy (type/ regimen)	No. of cases	Relative risk (95% CI)	Comments
Fournier *et al.* (2005)	France	52.8 (mean)	54 548	Non-specified	5.8	NR	Invasive		Never Current use	NR 323	1.0 1.3 (1.1–1.5)	Adjusted for time since menopause, body mass index, age at menopause, parity, age at first full-term pregnancy, family history of breast cancer, personal history of benign breast disease, use of oral contraceptives, mammography screening

NR, not reported
[a] No confidence intervals were provided.
[b] Data on duration missing for 43 women

education. When the data were stratified by body mass index, no increased risk related to the use of hormonal therapy was observed in women with an index > 24.4. However, in women with an index of 24.4 or less, the relative risk associated with 5 years of use or more was 2.0 (95% CI, 1.3–3.0). Thus, hormonal therapy that comprised estrogen plus a progestogen exerted its effects primarily, if not solely, among lean women. The investigators also studied the effect of duration of combined therapy and histological subtypes of breast cancer. The results suggested a similar increase in risk with increasing duration of use for ductal and lobular carcinoma, but the number of cases within the subtypes of breast cancer was small and the results should be interpreted with caution.

Risk for breast cancer and the use of hormonal menopausal therapy was also evaluated in the Million Women Study (Beral *et al.*, 2003). More than a million women in the United Kingdom between 50 and 64 years of age were recruited into the study between 1996 and 2001 and provided detailed information on their use of hormonal menopausal therapy. They were followed up for cancer incidence and death. Half of the women had used some type of hormonal menopausal therapy and nearly 150 000 women were current users of combined hormonal therapy. During 2.6 years of follow-up, 9364 women were diagnosed with invasive breast cancer, and current users were more likely than never users to develop the disease. The relative risk for current compared with never use of combined hormonal therapy at the time of recruitment was 2.00 (95% CI, 1.91–2.09), but the association varied according to duration of use. Among current users, use for 1–4 years was associated with a relative risk of 1.74 (95% CI, 1.60–1.89; 582 exposed cases) compared with never users, and use for 10 years or more was associated with a relative risk of 2.31 (95% CI, 2.08–2.56; 362 exposed cases). In relation to past use, the relative risk was 1.04 (95% CI, 0.94–1.16). The relative risks were adjusted for age, region of residence, socioeconomic status, body mass index, alcoholic beverage consumption, ever use of oral contraceptives, time since menopause and parity. Little variation in the associations was observed among women who used different preparations of combined regimens. Thus, among women who had used a treatment containing medroxyprogesterone acetate for less than 5 years, the relative risk was 1.60 (95% CI, 1.33–1.93), and that for women who had taken it for 5 years or more was 2.42 (95% CI, 2.10–2.80). Similarly, treatment for less than 5 years with a therapy containing norethisterone was associated with a relative risk of 1.53 (95% CI, 1.35–1.75); when use of norethisterone lasted for 5 years or more, the relative risk was 2.10 (95% CI, 1.89–2.34). Different modes of administration were also compared and broadly similar relative risks related to daily (relative risk, 1.57; 95% CI, 1.37–1.79) and cyclical (relative risk, 1.77; 95% CI, 1.59–1.97) use of combined hormonal therapy were found. Among women with a body mass index < 25, the relative risk for breast cancer associated with the use of combined hormonal therapy was 2.31 (95% CI, 2.12–2.53) and that in women with a body mass index of ≥ 25 was 1.78 (95% CI, 1.64–1.94). An attempt was made to assess the association between use of hormonal menopausal therapy and mortality from breast cancer, but, at the time of publication, the data did not allow reliable estimates of this. However, in a letter (Banks *et al.*, 2004), it was reported that, for all types com-

bined, current users had a 30% higher risk for mortality from breast cancer than never users (relative risk, 1.30; 95% CI, 1.11–1.53).

The association of the use of combined hormonal menopausal therapy with an increased risk for breast cancer was assessed in a prospective study in southern Sweden (Jernström *et al.*, 2003a) in a cohort of 6586 women aged 50–64 years at baseline. During 4 years of follow-up by linkage to the Swedish Cancer Registry, 101 women were registered with incident breast cancer. Ever use of combined hormonal menopausal therapy was associated with a relative risk of 3.3 (95% CI, 1.9–5.6) compared with never use. In relation to duration of use, the relative risk associated with use for 2 years or less (relative risk, 3.7; 95% CI, 1.8–7.4) was not substantially different from that associated with use for 5 years or more (relative risk, 3.7; 95% CI, 1.8–7.7).

Another prospective study, conducted in the same region in Sweden as the above study, was based on more than 29 000 women (Olsson *et al.*, 2003). The women were followed up by linkage to the Swedish Cancer Registry, and 556 cases of breast cancer were registered. The analyses focused on the duration of use of combined hormonal menopausal therapy and whether the mode of administration had different effects on the risk for breast cancer. The relative risk associated with daily ever use of combined hormonal menopausal therapy was 2.45 (95% CI, 1.61–3.71), and sequential administration was associated with a relative risk of 1.22 (95% CI, 0.74–2.00) compared with never users. The relative risk increased with recency and duration of use. Compared with never users, those who reported daily use of combined preparations for 4 years or more had a relative risk of 4.60 (95% CI, 2.39–8.84) and those who had taken combined sequential therapy for 4 years or more had a relative risk of 2.23 (95% CI, 0.90–5.56). These results were adjusted for age, age at menarche, age at first full-term pregnancy, parity, age at menopause and family history of breast cancer.

In the NOWAC Study, the association between use of combined hormonal menopausal therapy and the risk for breast cancer was assessed in a prospective follow-up of 31 451 postmenopausal women who were aged 45–64 years at entry (Bakken *et al.*, 2004). Information on the use of hormonal menopausal therapy was collected at recruitment by self-administered questionnaires; during follow-up by linkage to the Norwegian Cancer Registry, 331 women were registered with incident breast cancer. The association for ever use versus never use of combined preparations was not reported, but current users of combined hormonal therapy at study entry had a relative risk of 2.5 (95% CI, 1.9–3.2; 116 exposed cases) compared with never users. For current users of combined therapy for less than 5 years, the relative risk was 2.3 (95% CI, 1.7–3.2; 63 exposed cases); for longer duration of use, the relative risk was 2.8 (95% CI, 2.0–4.0; 51 exposed cases). These results were adjusted for age, body mass index, age at menarche, ever use of oral contraceptives, time since menopause, family history of breast cancer, history of mammography screening and age at first birth. The investigators also studied the effect of daily versus sequential use of progestogens in the combined treatment. Daily treatment for less than 5 years was associated with a relative risk of 2.6 (95% CI, 1.9–3.7; 44 exposed cases); for longer duration of daily treatment, the relative risk was 3.2 (95% CI, 2.2–4.6; 37 exposed cases) compared with

the risk of never users. In comparison, the relative risk associated with a cyclical regimen was 1.7 (95% CI, 1.0–2.8; 19 exposed cases) for less than 5 years of use and 2.2 (95% CI, 1.3–3.8; 14 exposed cases) for 5 years or more.

A cohort study from Denmark investigated whether different progestogens in combined hormonal menopausal therapy exert different effects on the risk for breast cancer (Stahlberg *et al.*, 2004). Brands of combined hormonal menopausal therapy were coded as containing either 'progesterone-like' (typically medroxyprogesterone acetate) or 'testosterone-like' (typically norethisterone or levonorgestrel) progestogens. More than 23 000 nurses received a questionnaire in 1993, of whom nearly 20 000 responded and returned information on their use of combined hormonal menopausal therapy. After exclusions, 10 874 women were eligible for breast cancer follow-up through the Danish Cancer Registry and, among these, 244 women were registered with incident breast cancer during 6 years of follow-up. The association with ever use or with past use of combined hormonal therapy was not specified in the report. However, the relative risk associated with current use at entry to the study was 2.70 (95% CI, 1.96–3.73) compared with the risk of never users. Among current users of combined treatment with 'testosterone-like' progestogens, the relative risk was also increased. When these progestogens were administered daily, the relative risk was 4.16 (95% CI, 2.56–6.75) and when they were given less than daily (termed cyclically or sequentially), the relative risk was 1.94 (95% CI, 1.26–3.00) compared with never users. The report did not provide details on the number of days during a cycle that sequential treatment was given.

Another Danish cohort study (The Diet, Cancer and Health Study) assessed type of hormonal menopausal therapy used in relation to the risk for breast cancer, and specified the association according to histological subtypes (Tjønneland *et al.*, 2004). Among 23 618 women with information on hormonal therapy who were assumed to be postmenopausal, 423 incident cases of breast cancer were identified through the Danish Cancer Registry over a median follow-up of 4.8 years. The results for ever use or past use were not reported. However, the effects of daily and cyclical regimens of combined preparations were compared, and whether these modes of administration exterted different effects on the risk for lobular and ductal breast carcinoma was examined. In relation to lobular carcinoma, rates of breast cancer associated with the use of daily and cyclical regimens were essentially identical, whereas the risk for ductal carcinoma was slightly higher when the progestogens were administered daily compared with sequentially.

In a cohort of 48 812 Danish women who were aged 50–67 years at baseline, Ewertz *et al.* (2005) linked information from the Danish Prescription Database to information on incident cases of breast cancer registered by the Danish Cancer Registry during 10 years of follow-up. Altogether, 869 women were registered with breast cancer during the study period. The effects of different progestogens were studied: combined therapy that contained either levonorgestrel, norethisterone, norgestimate, desogestrel or gestodene was classified as combined treatment with 'testosterone-derived' progestogens, and treatment containing medroxyprogesterone [acetate] as combined treatment with 'progesterone-derived' progestogens. Results related to ever use versus never use of combined preparations were not

reported, but the association with current use was specified for various types of combined regimens. Current cyclical use of estrogen plus a progesterone-derived progestogen was associated with a relative risk of 0.57 (95% CI, 0.26–1.28; six exposed cases). Current daily use of estrogen plus a testosterone-derived progestogen was associated with a relative risk of 0.99 (95% CI, 0.57–1.72; 13 exposed cases); among current users of cyclical regimens of estrogen plus a testosterone-derived progestogen, the relative risk was 1.52 (95% CI, 1.21–1.93; 80 exposed cases). These results were adjusted for age, age at first birth and parity.

Fournier *et al.* (2005) assessed the use of different types of hormonal menopausal therapy in relation to risk for breast cancer among 54 548 French women; 948 primary invasive breast cancers were diagnosed during 5.8 years of follow-up. Average use of combined hormonal menopausal therapy was 2.8 years. The association for ever use versus never use with breast cancer was not specified in the report, but women who were current users of combined hormonal therapy had a relative risk of 1.3 (95% CI, 1.1–1.5) compared with never users. The main aim of this study was to examine the effects of different types of progestogens that were used in the combined treatment. Current use of treatment that contained micronized progesterone (only given transdermally) was associated with a relative risk of 0.9 (95% CI, 0.7–1.2; 55 exposed cases). In contrast, current use of synthetic progestogens was associated with a relative risk of 1.4 (95% CI, 1.2–1.7; 268 exposed cases). These results were adjusted for a range of factors, including age, age at menopause, body mass index, parity, age at first birth, family history of breast cancer and previous use of oral contraceptives.

2.1.4 *Case-control studies* (Table 5)

A large population-based case–control study in Sweden (Magnusson *et al.*, 1999) included 3345 women aged 50–74 years who had been diagnosed with invasive breast cancer and 3454 controls of similar age. The main objective was to assess whether the use of combined hormonal therapy is associated with risk for breast cancer, with particular reference to long duration of use. For ever use of combined therapy, the relative risk for breast cancer was 1.63 (95% CI, 1.37–1.94) compared with never use. Risk increased with duration of use: the relative risk for 2–5 years of use was 1.40 (95% CI, 1.01–1.94), that for 5–10 years of use was 2.43 (95% CI, 1.72–3.44) and that for 10 or more years of use was 2.95 (95% CI, 1.84–4.72). These results were adjusted for age, parity, age at first birth, age at menopause, body mass index and height. The results from two sub-analyses were also presented; however, these analyses did not include only women who had exclusively used combined treatment, but also women who had used estrogen-only treatment at some time. The results suggested that combined preparations that contain testosterone-derived progestogens may confer higher risk (relative risk, 1.68; 95% CI, 1.39–2.03; 324 exposed cases) than combined therapy that contains progesterone-derived progestogens (relative risk, 1.14; 95% CI, 0.69–1.88; 32 exposed cases). The results also showed that

Table 5. Case–control studies of the use of combined hormone therapy and the risk for breast cancer

Reference, location	Study period	Age (years)	Histology	Sub-site	Therapy (type/regimen)	Cases	Controls	Odds ratio	95% CI	Duration			Time since last use		
										Years	Odds ratio	95% CI	Years	Odds ratio	95% CI
Magnusson *et al.* (1999), Sweden	1993–95	50–74	Invasive	All	Never	1738	2201	1.0							
					Ever	409	295	1.63	1.37–1.94	> 2–5	1.40	1.01–1.94			
										> 5–10	2.43	1.72–3.44			
										> 10	2.95	1.84–4.72			
					E + T[a]	324	229	1.68	1.39–2.03	≤ 5	1.33	1.05–1.68			
										> 5	2.74	1.99–3.78			
					Cyclic	102	76	1.48	1.08–2.04	> 2–5	1.34	0.71–2.54			
										> 5–10	1.89	0.88–4.09			
					Continuous	139	124	1.41	1.09–1.83	> 2–5	1.26	0.76–2.09			
										> 5–10	2.89	1.66–5.00			
					E + P[b]	32	34	1.14	0.69–1.88	≤ 5	1.41	0.80–2.51			
										> 5	0.79	0.26–2.39			
Li, C.I. *et al.* (2000), USA (King County, WA)	1988–90	50–64	Invasive and *in situ*	All	Never	180	187	1.0							
				Ductal	Ever	35	55	0.70	0.50–1.20	NR			Current	0.70	0.50–1.10
				Lobular	Ever	12	55	2.50	1.10–4.60	NR			Current	2.60	1.10–5.80
			Invasive	All	Never	159	187	1.0							
				Ductal	Ever	30	55	0.70	0.40–1.20	NR			Current	0.70	0.40–1.10
				Lobular	Ever	9	55	2.60	1.00–6.70	NR			Current	2.60	0.80–5.80
Ross *et al.* (2000), USA (Los Angeles, CA)	1987–96	55–72	Invasive and *in situ*	All	Never	873	784			1.0					
					Ever	425	324	NR		NR					
					Cyclical	218	166			1–5	1.19	NR			
						75	48			> 5–10	1.58	NR			
						27	14			> 10	1.79	NR			
					Daily	59	58								
						23	18			1–5	0.88	NR			
						23	20			> 5–10	1.28	NR			
										> 10	1.23	NR			

Table 5 (contd)

Reference, location	Study period	Age (years)	Histology	Sub-site	Therapy (type/regimen)	Cases	Controls	Odds ratio	95% CI	Duration			Time since last use		
										Years	Odds ratio	95% CI	Years	Odds ratio	95% CI
Kirsh & Kreiger (2002), Canada	1995–96	20–74	Invasive	All	Never	272	283	1.0							
					Ever	48	33	1.22	0.72–2.06	< 1	0.86	0.26–2.82			
										1–4	0.96	0.39–2.39			
										5–9	0.84	0.31–2.24			
										≥ 10	3.48	1.00–12.1			
Newcomb *et al*. (2002), USA (New Hampshire, Wisconsin, Massachusetts)	1992–94	50–79	Invasive	All	Never	3827	4132	1.0							
					Ever	315	286	1.43	1.18–1.74	< 5	1.36	1.07–1.73	Current	1.39	1.12–1.71
										≥ 5	1.57	1.15–2.14	< 5	1.71	0.92–3.18
													≥ 5	2.38	0.82–6.87
				Ductal	Ever	208	286	1.43	1.14–1.79						
				Lobular	Ever	32	286	2.01	1.25–3.22						

Table 5 (contd)

Reference, location	Study period	Age (years)	Histology	Sub-site	Therapy (type/regimen)	Cases	Controls	Odds ratio	95% CI	Duration			Time since last use		
										Years	Odds ratio	95% CI	Years	Odds ratio	95% CI
Weiss *et al.* (2002); Daling *et al.* (2002), USA (Atlanta, GA; Detroit, MI; Philadelphia, PA; Los Angeles, CA; Seattle, WA)	1994–98	35–64	Invasive	All	Never	672	655	1.0							
					Ever	689	630	[1.13]		2–< 5	1.3	0.96–1.63	Current	1.22	0.99–1.50
										≥ 5	1.2	0.92–1.48	≥ 0.5	0.76	0.60–0.97
					Sequential	287	267	[1.05]		2–< 5	1.1	0.73–1.58	Current	0.91	0.67–1.24
										≥ 5	1	0.69–1.32	≥ 0.5	1.07	0.80–1.41
					Continuous	419	352	[1.20]		2–< 5	1.20	0.88–1.65	Current	1.29	1.02–1.64
										≥ 5	1.4	0.98–1.85	≥ 0.5	0.78	0.57–1.06
				Ductal	Never	515	655	1.0							
					Ever	448	534	1.00	0.80–1.30	2–< 5	1.00	0.80–1.30	≥ 5	0.70	0.50–1.10
										≥ 5	1.00	0.80–1.30	> 0–0.5	1.20	0.90–1.50
					Sequential	230	284	1.00	0.80–1.30	0.5–< 5	1.00	0.80–1.40	≥ 5	0.90	0.60–1.40
										≥ 5	1.00	0.70–1.30	> 0–0.5	0.90	0.70–1.30
					Continuous	268	280	1.20	0.90–1.50	0.5–< 5	1.20	0.90–1.50	≥ 5	0.70	0.40–1.30
										≥ 5	1.20	0.90–1.50	> 0–0.5	1.30	1.00–1.70
				Lobular	Never	75	655	1.0							
					Ever	112	534	1.80	1.20–2.60	0.5–< 5	1.60	1.00–2.40	≥ 5	0.90	0.40–2.10
										≥ 5	2.00	1.30–3.20	> 0–0.5	2.20	1.40–3.30
					Sequential	53	284	1.40	0.90–2.20	0.5–< 5	1.30	0.80–2.30	≥ 5	1.30	0.60–2.70
										≥ 5	1.50	0.80–2.60	> 0–0.5	1.40	0.80–2.50
					Continuous	75	280	2.20	1.40–3.50	0.5–< 5	2.10	1.30–3.30	≥ 5	1.60	0.60–4.10
										≥ 5	2.50	1.40–4.30	> 0–0.5	2.40	1.50–3.80

Table 5 (contd)

Reference, location	Study period	Age (years)	Histology	Sub-site	Therapy (type/regimen)	Cases	Controls	Odds ratio	95% CI	Duration			Time since last use		
										Years	Odds ratio	95% CI	Years	Odds ratio	95% CI
Li *et al.* (2003), USA (3-county Puget Sound, WA)	1997–99	65–79	Invasive	All	Never	284	339	1.0							
					Ever	136	964	1.80	1.30–2.50	0.5–< 5	1.30	0.80–2.20	Current	1.9	1.6–2.6
					Sequential	80	55	1.80	1.20–2.70	5–< 15	2.00	1.30–3.20			
					Continuous	159	116	1.60	1.20–2.20						
				Ductal	Never	199	339	1.0							
					Ever	89	96	1.60	1.10–2.30	< 5	1.40	0.8–2.5	Former	2.00	1.1–3.7
										5–< 15	1.60	1.0–2.7	Current	1.70	1.2–2.4
										≥ 15	1.90	1.1–3.2	0.5–< 5	1.30	0.8–2.3
													5–< 15	1.70	1.1–2.7
					Sequential	52	55	1.70	1.10–2.60						
					Continous	102	116	1.50	1.10–2.00						
				Lobular	Never	47	339	1.0							
					Ever	29	96	2.50	1.40–4.30	< 5	1.40	0.8–2.5	Former	2.00	0.7–5.7
										5–< 15	3.40	1.7–7.0	Current	3.10	1.9–5.2
										≥ 15	2.40	1.1–5.5	0.5–< 5	1.30	0.5–3.6
													5–< 15	4.60	2.5–8.5
					Sequential	19	55	2.80	1.50–5.40						
					Continous	40	116	2.70	1.60–4.40						

CI, confidence interval; NR, not reported

[a] Estrogen + testosterone-like progestogen

[b] Estrogen + progesterone-like progestogen

the positive association between the use of hormonal menopausal therapy and risk for breast cancer may be confined to women with a body mass index lower than 27 kg/m^2.

Li, C.I. *et al.* (2000) conducted a case–control study in the USA that involved 537 women who had breast cancer and were 50–64 years of age and 492 controls selected at random from the population. The aim of the study was to investigate whether the use of combined hormonal menopausal therapy has different effects on different histological subtypes of breast cancer. For women who had used combined hormonal therapy for at least 6 months, the relative risk for ductal breast carcinoma was 0.7 (95% CI, 0.5–1.2; 35 exposed cases) and that for lobular breast carcinoma was 2.5 (95% CI, 1.1–4.6; 12 exposed cases). Using a likelihood ratio test, the difference between these two estimates of relative risk was statistically significant ($p = 0.007$). The relative risk associated with current use of combined hormonal therapy for at least 6 months was 2.6 (95% CI, 1.1–5.8) for lobular breast carcinoma compared with the risk in never users. These results were adjusted for age and type of menopause (natural or surgical). In comparison, there was no increase in the risk for ductal breast carcinoma (relative risk, 0.7; 95% CI, 0.5–1.1) related to current use of combined hormonal menopausal therapy. A similar comparison between the estimates suggested that the difference was statistically significant ($p < 0.03$).

The specific aim of a case–control study in the USA (Ross *et al.*, 2000) was to investigate whether daily administration of combined hormonal therapy exerts a different effect on risk for breast cancer than sequential administration. The study included 1897 postmenopausal women with breast cancer and 1637 postmenopausal population controls. The age range of the participants was 55–72 years. The relative risk for ever use versus never use of combined preparations was not reported, but the risk for breast cancer increased with duration of use. For every 5 years of use of combined therapy, the relative risk was 1.24 (95% CI, 1.07–1.45). The risk related to combined regimens with cyclical progestogens was slightly higher than that found for regimens in which progestogens were given daily, but the difference was not statistically significant: for 5 years of use, the odds ratio for the cyclical regimen was 1.38 (95% CI, 1.13–1.68; 320 exposed cases) versus 1.09 (95% CI, 0.88–1.35; 105 exposed cases) for the daily regimen. These results were adjusted for age, age at menarche, family history of breast cancer, age at first full-term pregnancy, parity, age at menopause, previous use of oral contraceptives, body weight and consumption of alcoholic beverages.

A population-based case–control study in Canada on data from the Enhanced Cancer Surveillance Project (Kirsh & Kreiger, 2002) included 320 incident cases of breast cancer and 316 controls (with information or hormonal therapy use) who were frequency-matched by age. A self-administered questionnaire was used to collect information on the use of combined hormonal menopausal therapy between 1995 and 1997. Long duration of use (10 years or longer) of combined estrogen–progestogen therapy was associated with an increased risk (odds ratio, 3.48; 95% CI, 1.00–12.11) compared with never use.

Another large case–control study in the USA (Newcomb *et al.*, 2002) investigated the type and duration of use of combined hormonal menopausal therapy in relation to the risk for breast cancer. The study included 5298 postmenopausal cases of breast cancer aged

50–79 years of age and 5571 control women who were randomly selected from population lists. The relative risk for ever use versus never use of combined regimens was 1.43 (95% CI, 1.18–1.74; 315 exposed cases). Women who used regimens with daily progestogens had a relative risk of 1.45 (95% CI, 1.06–1.99; 115 exposed cases), but the association was similar for women who used the different types of sequential therapy. The relative risk for breast cancer increased with duration of use: the increase per year of combined treatment was approximately 4% (relative risk, 1.04; 95% CI, 1.01–1.08) and that for recent use for more than 5 years was 1.57 (95% CI, 1.15–2.14).

The association between the use of combined hormonal menopausal therapy and the risk for breast cancer was also studied in the CARE [Contraceptive and Reproductive Experience] multicentre case–control study in the USA. Weiss *et al.* (2002) included 1870 postmenopausal women with breast cancer aged 35–64 years and 1953 controls identified by random-digit dialling. Current users for 5 or more years of daily combined hormonal menopausal therapy were at increased risk for breast cancer (odds ratio, 1.54; 95% CI, 1.10–2.17) compared with never users. Among current users, increasing duration of use was associated with increasing risk (p for trend = 0.01). Whether different regimens of combined hormonal menopausal therapy may have different effects on different histological subtypes of breast cancer was also studied within the same study (Daling *et al.*, 2002). Cases were 1749 postmenopausal women under 65 years of age with a diagnosis of breast cancer; the 1953 controls were those included in the study of Weiss *et al.* (2002). The aim was to assess whether combined hormonal therapy increases the risk for lobular breast carcinoma. The tumours were grouped into three histological categories: 1386 patients had ductal carcinoma, 148 had lobular carcinoma and 115 women were diagnosed with a mixture of these histological subtypes. Another 100 patients were divided among less prevalent histological types of breast cancer. The association with ever use (≥ 6 months) versus never use of combined menopausal therapy was not reported, but current daily use of combined treatment was associated with an increased risk for invasive lobular disease (odds ratio, 2.2; 95% CI, 1.4–3.5; 75 exposed cases). The relative risks were adjusted for age, race, study site and age at menopause.

A case–control study in the USA (Li *et al.*, 2003) assessed duration and patterns of use of combined hormonal therapy in relation to histological subtypes and hormonal receptor status of breast cancer. The study included 975 women aged 65–79 years who had invasive breast cancer classified according to histology and hormone receptor status and 1007 population controls. For women who had ever used combined hormonal therapy only, the relative risk for breast cancer was 1.8 (95% CI, 1.3–2.5) compared with the risk in never users. When examined by histological subtype, ever users of combined hormonal menopausal therapy had an increased risk for both invasive ductal carcinoma (relative risk, 1.6; 95% CI, 1.1–2.3; 89 exposed cases) and invasive lobular carcinoma (relative risk, 2.5; 95% CI, 1.4–4.3; 29 exposed cases). The increased risk for lobular carcinoma was greater in women who had used combined therapy for a relatively long time. For lobular carcinoma, the relative risk for use for between 5 and 15 years was 3.4 (95% CI, 1.7–7.0) and that for use for longer than 15 years was 2.4 (95% CI, 1.1–5.5). Both current and former

use for at least 6 months were associated with an increased risk for all histological subtypes. With regard to different hormone receptor properties, the results showed that, among ever users, the relative risk for estrogen and progesterone receptor-positive tumours was 2.0 (95% CI, 1.5–2.7). The risk increased with duration of use, but did not differ according to whether progestogens were given sequentially (relative risk, 1.8; 95% CI, 1.2–2.7) or daily (relative risk, 1.6; 95% CI, 1.2–2.2). In relation to estrogen or progesterone receptor-negative breast cancer, no increase in risk was found, but low statistical power related to hormone receptor-negative disease limited the ability of the study to evaluate this subtype of breast cancer reliably.

2.2 Endometrial cancer

Postmenopausal women who use estrogen-only therapy are at an increased risk for endometrial cancer, and the risk increases with increasing duration of use (IARC, 1999). To counteract this risk, many women use combined estrogen–progestogen regimens. At the time when the previous evaluation on this topic was made, only four published studies provided information on the effects of the combined regimens on the risk for endometrial cancer, and the limited available evidence suggested that the addition of progestogens reduced the elevated risk associated with estrogen.

2.2.1 *Descriptive studies*

Using data from the Southern California Kaiser Foundation Health Plan, Ziel *et al.* (1998) reported patterns of prescription of hormonal menopausal therapy among women aged over 45 years in 1971–93 and related them to trends in the incidence of endometrial cancer. Use of combined estrogen–progestogen regimens began to increase during the mid-1980s. A log-linear model fitted to the data indicated that, since about 1984, the prescription of progestogens together with estrogens was negatively associated with the incidence rates of endometrial cancer. The authors concluded that their observation was consistent with the hypothesis that progestogens administered in conjunction with estrogens can protect against much of the increased risk for endometrial cancer associated with the use of estrogens alone.

2.2.2 *Randomized trials*

In a trial in which 168 institutionalized women were randomized to receive estrogen–progestogen menopausal therapy or placebo, no case of endometrial cancer occurred in the treated group and one case occurred in those who received placebo (Nachtigall *et al*., 1979).

The HERS randomized 2763 women with previous coronary heart disease to either placebo or a daily regimen of 0.625 mg conjugated equine estrogen and 2.5 mg medroxyprogesterone acetate. The women were then followed up for 4.1 years on average (Hulley

et al., 1998). During the follow-up period, two endometrial cancers were diagnosed in the treated group and four were diagnosed in the placebo group to give a relative risk of 0.49 (95% CI, 0.09–2.68) for use of continuous combined therapy compared with placebo.

In the WHI Trial, 16 608 women who had not had a hysterectomy were randomized to receive placebo or a daily regimen of 0.625 mg conjugated equine estrogen and 2.5 mg medroxyprogesterone acetate. After an average follow-up of 5.6 years, Anderson *et al.* (2003) reported that 27 incident cases of endometrial cancer had occurred among those randomized to continuous combined hormonal therapy and 31 cases among those randomized to placebo. The relative risk was 0.81 (95% CI, 0.48–1.36) for the use of continuous combined therapy compared with placebo.

2.2.3 *Cohort studies*

Cohort studies that presented relative risk estimates for endometrial cancer associated with the use of estrogen–progestogen menopausal therapy published from 1999 onwards are summarized in Table 6.

Hammond *et al.* (1979) followed up approximately 600 women, approximately half of whom had used either estrogen-only or estrogen–progestogen preparations and half of whom had not used hormones. No cases of endometrial cancer were observed among the 72 women who received estrogen–progestogen therapy, whereas three cases were observed among women who did not. No person–years or age-adjusted relative risks were reported.

Gambrell (1986) reported that the incidence of endometrial cancer among women who had used combined hormonal therapy (eight cases in 16 327 woman–years) was lower than that among women who did not use any hormonal therapy (nine cases in 4480 woman–years). No age-adjusted relative risks were reported.

Persson *et al.* (1999) updated their earlier report on the follow-up of a cohort of Swedish women who had used hormonal menopausal therapy (Persson *et al*., 1989). The cohort had initially been identified through pharmacy records; in 1987–88, the women were mailed a follow-up questionnaire requesting further details on their use of hormonal therapy and other personal characteristics. The 8438 women who replied were linked to the National Swedish Cancer Registry; 66 endometrial cancers were identified in the cohort up to December 1993. In comparison with the population rates in the Uppsala health care region, the relative risk for endometrial cancer associated with use of estrogen–progestogen therapy was 1.4 (95% CI, 0.9–2.3; six exposed cases) for 1–6 years of use and 1.7 (95% CI, 1.1–2.6; 11 exposed cases) for more than 6 years of use. There was no significant difference according to duration of use. Estimates of relative risk were not given according to the number of days per month that progestogens were added to estrogen therapy or by time since last use of the therapy.

Pukkala *et al.* (2001) linked prescription records for hormonal menopausal therapy to cancer registry data in Finland and compared incidence rates of endometrial cancer in users of combined therapy with those in the general population in Finland. Among 78 549 women who were taking progestogens added to estrogen therapy for 10–12 days every month, the

Table 6. Cohort studies of the use of estrogen–progestogen menopausal therapy use and risk for endometrial cancer by number of days that progestogens were added to estrogen therapy per month, duration of use and type of progestogen

Reference, location	Study period	Age range (years)	Source population	Type/measure of combined therapy	No. of cases	Relative risk (95% CI)	Comments
Persson *et al.* (1999), Sweden	1987–93	65 (median)	8438 women	None	12	1.0	Adjusted for age, length of follow-up, age at first full-term pregnancy, body mass index, education, menopausal age/status
				Any progestogen added to estrogen			
				Duration			
				≤ 6 years	6	1.4 (0.9–2.3)	
				> 6 years	11	1.7 (1.1–2.6)	
Pukkala *et al.* (2001), Finland	1994–97	Any age	94 505 women	Progestogens 14 days every 3 months	61	2.0 (1.6–2.6)	Standardized incidence ratios, using the female Finnish population
				Progestogens 10–12 days per month	141	1.3 (1.1–1.6)	
Bakken *et al.* (2004), Norway	1991–NR	45–64	67 336 women	None	45	1.0	Adjusted for age, body mass index, smoking, ever use of oral contraceptives, time since menopause, parity, age at last birth
				Any	11	0.7 (0.4–1.4)	

Table 6 (contd)

Reference, location	Study period	Age range (years)	Source population	Type/measure of combined therapy	No. of cases	Relative risk (95% CI)	Comments
Beral *et al.* (2005), United Kingdom	1996–2002	50–65	716 738 women	None	773	1.0	Adjusted for age, region of residence, socioeconomic status, body mass index, alcoholic beverage consumption, ever use of oral contraceptives, time since menopause, parity
				Progestogens, every day/month			
				Any	73	0.71 (0.59–0.90)	
				Duration			
				< 5 years	28	0.55 (0.37–0.80)	
				≥ 5 years	44	0.90 (0.66–1.22)	
				Type of progestogen			
				Norethisterone	46	0.76 (0.57–1.03)	
				Medroxyprogesterone acetate	27	0.63 (0.43–0.93)	
				Progestogens, 10–14 days/ month			
				Any	242	1.05 (0.90–1.22)	
				Duration			
				< 5 years	95	0.90 (0.72–1.12)	
				≥ 5 years	140	1.17 (0.97–1.41)	
				Type of progestogen			
				Norgestrel	183	1.09 (0.93–1.29)	
				Norethisterone	53	0.93 (0.70–1.23)	

CI, confidence interval; NR, not reported

standardized incidence ratio (SIR) for endometrial cancer was 1.3 (95% CI, 1.1–1.6; 141 cases); among 15 956 women who used progestogens added to estrogen for 14 days every 3 months, the standardized incidence ratio was 2.0 (95% CI, 1.6–2.6; 61 cases).

Bakken *et al*. (2004) followed 67 336 Norwegian women aged 45–64 years who were recruited in 1991–97. Information on use of hormonal therapy was obtained from self-completed questionnaires and incident cancers were determined by linkage to data from the Cancer Registry of Norway. Among 7268 women who were using estrogen–progestogen menopausal therapy at the time of recruitment, 11 incident endometrial cancers were diagnosed. The associated relative risk was 0.7 (95% CI, 0.4–1.4), adjusted for age, body mass index, tobacco smoking, use of oral contraceptives, time since menopause, parity and age at first birth. Estimates of relative risk were not given according to the number of days per month that progestogens were added to estrogen therapy or by time since last use of the therapy.

In 1996–2001, the Million Women Study Collaborators (Beral *et al.*, 2005) recruited over a million women in the United Kingdom aged 50–65 years through the National Health Service Breast Screening Programme. Information was collected on the last formulation of hormonal therapy used and the total duration of use of such therapy or any type of hormonal therapy. This self-reported information showed 97% agreement with prescription records on whether combined or estrogen-only menopausal therapy was currently used (Banks *et al*., 2001). At recruitment, 716 738 members of the cohort were postmenopausal and had not had a hysterectomy or previous diagnosis of cancer. Follow-up of these women via national cancer registries over an average of 3.4 years identified 1320 women with incident endometrial cancer. Compared with never users of hormonal therapy (773 cases), the relative risks for endometrial cancer were 0.71 (95% CI, 0.56–0.90; 73 exposed cases) for any use of continuous estrogen–progestogen therapy and 1.05 (95% CI, 0.91–1.22; 242 exposed cases) for any use of cyclical estrogen–progestogen therapy (usually including progestogens for 10–14 days per month). The relative risks were adjusted for age, region of residence, socioeconomic status, body mass index, alcoholic beverage consumption, ever use of oral contraceptives, time since menopause and parity. The difference between the effects of continuous and cyclical estrogen–progestogen therapy was highly significant ($p = 0.006$). Most women were current or recent users of these therapies at the time of recruitment into the study and, although there was no significant difference in the findings between current and past users, there was limited power to detect any difference, since the average time since last use was only 1–3 years among former users. Among women who had last used a combined therapy (both continuous and cyclical), there were no significant differences according to duration of use or the constituent progestogen. Nine factors that could potentially modify the effects of hormonal therapy on endometrial cancer were examined, and only body mass index consistently showed a significant interaction. Among women with body mass indices of < 25, 25–29 and ≥ 30 kg/m^2, respectively, the relative risks for endometrial cancer were 1.07 (95% CI, 0.73–1.56), 0.88 (95% CI, 0.60–1.30) and 0.28 (95% CI, 0.14–0.55) for use of continuous combined therapy and 1.54 (95% CI, 1.20–1.99), 1.07 (95% CI, 0.82–1.40) and 0.67 (95% CI, 0.49–0.91) for use of cyclical combined therapy.

2.2.4 *Case–control studies*

The case–control studies that presented relative risk estimates for endometrial cancer associated with the use of estrogen–progestogen menopausal therapy are summarized in Table 7.

A multicentre study was conducted with 300 menopausal women who had been diagnosed with endometrial cancer at seven US hospitals located in five different areas of the country and 207 age-, race- and residence-matched control women from the general population (Brinton & Hoover, 1993). Use of any estrogen–progestogen therapy for 3 months or longer was reported by 11 (4%) of the case women and nine (5%) of the control women (odds ratio, 1.8; 95% CI, 0.6–4.9 adjusted for age, parity, weight and years of oral contraceptive use).

Jick *et al.* (1993) studied women who were members of a large health maintenance organization in western Washington State, USA. Women with endometrial cancer were identified from the tumour registry of the organization and control women were other members; both groups included only women who used the pharmacies of the organization and who had previously completed a questionnaire sent to all female members for a study of mammography. Use of hormonal menopausal therapy was ascertained from the pharmacy database. Relative to women who had never or briefly (≤ 6 months) used menopausal hormones, those who had used any estrogen–progestogen therapy within the previous year had a non-significant increased risk (odds ratio, 1.9; 95% CI, 0.9–3.8; 18 cases), after adjustment for age, calendar year, age at menopause, body mass and history of oral contraceptive use. Former users (last use ≥ 1 year earlier) had no significant increase in risk (odds ratio, 0.9; 95% CI, 0.3–3.4; six incident cases), but the statistical power to compare current and past users was limited.

Beresford *et al.* (1997) expanded the study population originally investigated by Voigt *et al.* (1991) and evaluated the risk for endometrial cancer among women who had used estrogen–progestogen therapy exclusively. Women who had been diagnosed with endometrial cancer in 1985–91 were identified from a population-based cancer registry and their characteristics were compared with control women from the general population in western Washington State, USA. The analysis included 394 cases and 788 controls. Relative to women who had never or briefly (≤ 6 months) used menopausal hormones, women who had used only estrogen–progestogen therapy had a borderline increased risk for endometrial cancer (odds ratio, 1.4; 95% CI, 1.0–1.9), after adjustment for age, body mass and county of residence. For women who had used estrogen–progestogen therapy for ≤ 10 days per cycle for at least 5 years, the odds ratio was 3.7 (95% CI, 1.7–8.2; five exposed cases); among women who had used combined therapy with progestogens added cyclically for more than 10 days each month for at least 5 years, the relative risk was 2.5 (95% CI, 1.1–5.5). Statistical power to compare current and past users was limited. Using data from the same study population, McKnight *et al.* (1998) reported that the relative risk associated with the use of cyclical progestogens added for 10–24 days per month was 2.6 (95% CI, 1.3–5.5; 14 exposed cases) among women who had never used estrogen-only previously,

Table 7. Case–control studies of estrogen–progestogen therapy and endometrial cancer risk, by number of days progestogen was added per cycle, duration, and type of progestogen

Reference, location	Study period	Age range (years)	Source of controls	Type/measure of combined therapy	No. of subjects		Adjusted odds ratio (95% CI)	Comments
					Cases	Controls		
Brinton & Hoover (1993), USA (seven hospitals in five areas)	1987–90	20–74	General population	No use	222	176	1.0	Adjusted for age, parity, weight, years of oral contraceptive use
				Any use for ≥ 3 months[a]	11	9	1.8 (0.6–4.9)	
Jick *et al.* (1993), USA (Washington State)	1989–89	50–64	Members of health maintenance organization	No use or use ≤ 6 months	97	606	1.0	Adjusted for age, calendar year, age at menopause, body mass index, oral contraceptive use
				Current/recent	18	83	1.9 (0.9–3.8)	
				Duration (years)				
				< 3	NR	NR	2.2 (0.7–7.3)	
				≥ 3	NR	NR	1.3 (0.5–3.4)	
Beresford *et al*. (1997), USA (Washington State)	1985–91	45–74	General population	No use or use ≤ 6 months	337	685	1.0	Adjusted for age, body mass index, country of residence
				Any use	67	134	1.4 (1.0–1.9)	
				Progestogen ≤ 10 days/month				
				Duration (months)				
				6–35	12	14	2.1 (0.9–4.7)	
				36–59	3	7	1.4 (0.3–5.4)	
				≥ 60	15	12	3.7 (1.7–8.2)	
				Progestogen > 10 days/month				
				Duration (months)				
				6–35	10	31	0.8 (0.4–1.8)	
				36–59	5	23	0.6 (0.2–1.6)	
				≥ 60	12	16	2.5 (1.1–5.5)	
				Progestogen every day/month	9	33	0.6 (0.3–1.3)	

Table 7 (contd)

Reference, location	Study period	Age range (years)	Source of controls	Type/measure of combined therapy	No. of subjects		Adjusted odds ratio (95% CI)	Comments
					Cases	Controls		
Pike *et al.* (1997), USA (California)	1987–93	50–74	General population (neighbours)	*Any use, progestogen < 10 days/month*[b]				Adjusted for age at menarche, time to regular cycle, parity, weight, duration of breast feeding, amenorrhoea, tobacco smoking, oral contraceptive use, age at menopause
				Duration (months)				
				0	759	744	1.0	
				1–24	35	22	1.4 (NR)	
				25–60	12	12	1.5 (NR)	
				≥ 60	27	13	3.5 (NR)	
				Any use, progestogen ≥ 10 days/month				
				Duration (months)				
				0	754	703	1.0	
				1–24	37	30	1.0 (NR)	
				25–60	19	25	0.7 (NR)	
				≥ 60	23	33	1.1 (NR)	
				Any use, progestogen every day/month				
				Duration (months)				
				0	739	710	1.0	
				1–24	45	41	1.1 (NR)	
				25–60	25	15	1.4 (NR)	
				≥ 60	24	25	1.3 (NR)	
Weiderpass *et al.* (1999), Sweden	1994–95	50–74	General population	No use	573	2798	1.0	Adjusted for age, age at menopause, parity, age at last birth, body mass index and duration of previous menopausal hormone use
				Any use[a]	119	477	1.3 (1.0–1.7)	
				Progestogen ~10 days/month, ever	90	300	2.0 (1.4–2.7)	
				Duration (years)				
				< 5	38	191	1.5 (1.0–2.2)	
				≥ 5	40	78	2.9 (1.8–4.6)	
				Progestogen, every day/month, ever	41	237	0.7 (0.4–1.0)	
				Duration (years)				
				< 5	32	162	0.8 (0.5–1.3)	
				≥ 5	2	53	0.2 (0.1–0.8)	

Table 7 (contd)

Reference, location	Study period	Age range (years)	Source of controls	Type/measure of combined therapy	No. of subjects		Adjusted odds ratio (95% CI)	Comments
					Cases	Controls		
Jain *et al*. (2000), Canada (Ontario)	1994–98	> 48	Property assessment list of the Ontario Ministry of Finance	No use	292	316	1.0	Adjusted for age, weight, menarche age, age at menopause, period disorders, education, parity, smoking and physical activity
				Ever use of combined therapy	128	136	1.37 (0.99–1.89)	
				Progestogen for ~10 days/month, ever	65	87	1.05 (0.71–1.56)	
				Duration (years)				
				< 3	18	40	0.57 (0.31–1.06)	
				≥ 3	47	47	1.49 (0.93–2.40)	
				Progestogen every day/month only	15	14	1.51 (0.67–3.42)	
Mizunuma *et al*. (2001), Japan	1995–97	62.0 (mean)	63 hospitals	Never use of therapy	934	1188	1.0	Adjusted for age, parity, body mass index, height
				Ever use of combined therapy				
				Duration (months)				
				< 12	6	6	0.9 (0.3–3.0)	
				≥ 12	2	6	0.6 (0.1–3.1)	
Newcomb & Trentham-Dietz (2003), USA (Wisconsin)	1991–94	40–79	Medicare beneficiaries	No use	402	1667	1.0	Adjusted for age, parity, body mass index, tobacco smoking, oral contraceptive use
				Ever use of any combined therapy	48	166	1.69 (1.15–2.47)	
				Progestogen added for				
				< 10 days/month	8	21	2.43 (1.00–5.92)	
				10–21 days/month	14	71	1.10 (0.59–2.07)	
				> 21 days/month	20	62	2.26 (1.27–4.00)	
				Progestogen for ≤ 21 days/month				
				Medroxyprogesterone acetate				
				< 10 mg	6	24	1.29 (0.49–3.36)	
				> 10 mg	10	54	1.11 (0.53–2.32)	
				Progestogen for > 21 days/month				
				Medroxyprogesterone acetate				
				< 10 mg	12	45	1.68 (0.82–3.43)	
				> 10 mg	2	8	5.75 (1.75–18.9)	

CI, confidence interval; NR, not reported

[a] Women taking estrogen only included

[b] Use of estrogen only and other combined therapy adjusted for in the analysis

but only 0.21 (95% CI, 0.07–0.66; four exposed cases and controls) among women who had used estrogen-only therapy previously. In a study that included the same study subjects, Hills *et al.* (2000) reported that the relative risk associated with the use of progestogens added to estrogen on a daily basis was 0.6 (95% CI, 0.3–1.3; nine exposed cases, 33 exposed controls).

Pike *et al.* (1997) identified 833 women with endometrial cancer from a population-based cancer registry in Los Angeles County, CA, USA, and matched them to control women of similar age and race (white) who lived in the same neighbourhood as the matched case or to 791 women randomly identifed from the US Health Care Financing Administration computer tapes. The risk for endometrial cancer was investigated among women who had used estrogen–progestogen with progestogen added for fewer than 10 days per cycle, for ≥ 10 days per cycle and continuously. The relative risks were [1.9 (95% CI, 1.3–2.6)] for fewer than 10 days per cycle and [0.96 (95% CI, 0.69–1.34)] for ≥ 10 days per month [the referent group for each analysis was women who had never used that type of therapy]. The odds ratios for every additional 5 years of use were 1.9 (95% CI, 1.3–2.7) and 1.1 (95% CI, 0.8–1.4), respectively, after adjustment for age at menarche, time to regular cycles, parity, weight, duration of breast-feeding, amenorrhoea, tobacco smoking, duration of oral contraceptive use and age at menopause. No significant increase in the odds ratio was found for daily use of progestogens together with estrogens (relative risk, 1.23; 95% CI, 0.88–1.71; 94 exposed cases, 81 exposed controls); for every additional 5 years of use, the odds ratio increased by 1.1 (95% CI, 0.8–1.4). No comparisons were made between current and past users of these therapies.

Weiderpass *et al.* (1999) conducted a population-based case–control study in Sweden of 709 women aged 50–74 years who were diagnosed with endometrial cancer in 1994–95 and 3368 matched controls. When users of estrogen–progestogen menopausal therapy were compared with never users of any type of therapy, the overall relative risk for endometrial cancer was 1.3 (95% CI, 1.0–1.7; 119 exposed cases, 477 exposed controls). All analyses were adjusted by age, age at menopause, parity, age at last birth, body mass index and duration of previous use of various types of menopausal hormones. The odds ratio was 2.0 (95% CI, 1.4–2.7) for use of progestogens added cyclically for an average of 10 days each month and 0.7 (95% CI, 0.4–1.0) for use of continuous combined therapy. Among the users of therapy with progestogens added cyclically, the relative risk was significantly higher in women who had used hormonal therapy for more than 5 years (odds ratio, 2.9; 95% CI, 1.8–4.6) than in those who had used them for shorter durations (odds ratio, 1.5; 95% CI, 1.0–2.2). Among users of continuous combined therapy, the risk was lower in women who had used the therapy for more than 5 years (odds ratio, 0.2; 95% CI, 0.1–0.8) than in those who had used them for shorter durations (odds ratio, 0.8; 95% CI, 0.5–1.3). There were no significant differences in risk according to the specific progestogenic constituent of the therapy.

Jain *et al.* (2000) conducted a population-based case–control study in Ontario, Canada, on 512 women with endometrial cancer and 513 controls. Cases identified through the Ontario Cancer Registry were diagnosed between 1994 and 1998. Controls were identified

from property assessment lists maintained by the Ontario Ministry of Finances. Subjects were interviewed at home. For women who reported that they had used estrogen–progestogen menopausal therapy compared with those who had never used any type of therapy, the relative risk for endometrial cancer was 1.37 (95% CI, 0.99–1.89). All analyses were adjusted by age, education, parity, weight, age at menarche, tobacco smoking, past oral contraceptive use, education, and calorie intake and expenditure. Among the users of combined therapy, there were no significant differences according to duration of use, recency of use or the number of days each month that progestogens were added to estrogen therapy but statistical power to compare such patterns of use was limited.

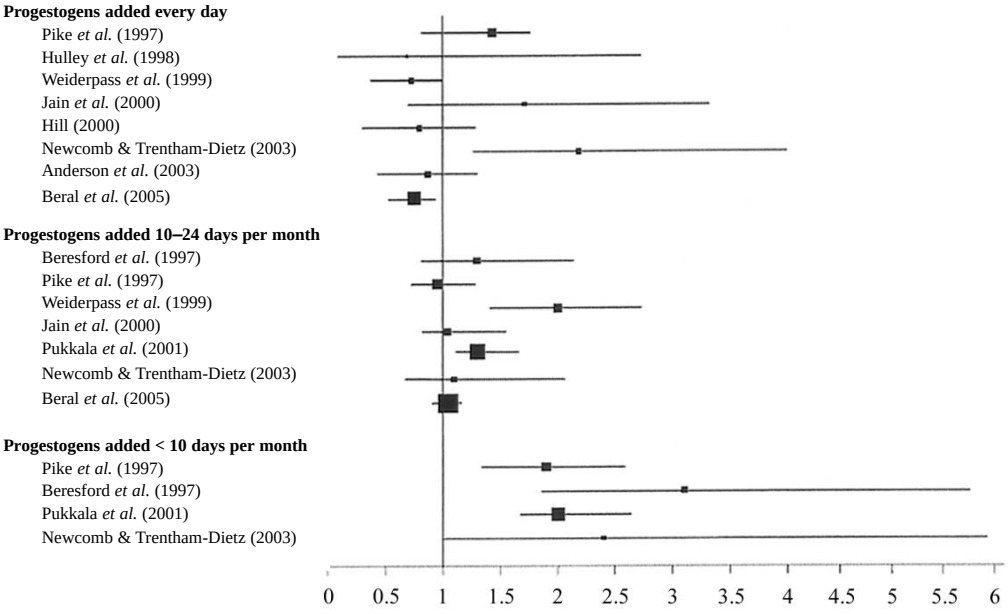
Mizunuma *et al*. (2001) conducted a hospital-based case–control study in Japan of 1025 women who were diagnosed with endometrial cancer in 1995–97 and 1267 matched controls from 63 hospitals. Women who used estrogens with progestin for ≥ 12 months had an odds ratio of 0.6 (95% CI, 0.11–3.11), and those who used estrogens without progestin for ≥ 12 months had an odds ratio of 2.6 (95% CI, 0.23–28.2). Among the users of combined therapy, there were no significant differences according to duration of use; data on risk were not given according to the number of days per month that progestogens were added to estrogen therapy.

Newcomb and Trentham-Dietz (2003) conducted a population-based case–control study in Wisconsin, USA, of 591 women aged 40–79 years who were diagnosed with endometrial cancer in 1991–94 and 2045 matched controls. For ever use of any type of estrogen–progestogen menopausal therapy compared with never use, the odds ratio for endometrial cancer was 1.69 (95% CI, 1.15–2.47). All analyses were adjusted for age, parity, body mass index, tobacco smoking and past oral contraceptive use. For progestogens added cyclically for fewer than 10 days each month, the odds ratio for endometrial cancer was 2.43 (95% CI, 1.00–5.92); for progestogens added cyclically for 10–21 days each month, the relative risk was 1.10 (95% CI, 0.59–2.07); and for daily use of progestogens, the relative risk was 2.26 (95% CI, 1.27–4.00). There were no significant differences in risk according to recency of use, duration of use or the dose of progestogen used, but the power to detect such differences was low.

2.2.5 *Overview*

Two randomized trials, four cohort studies and eight case–control studies have reported relative risks for endometrial cancer associated with the use of combined estrogen–progestogen therapy. Most investigators found that the fewer days each month that progestogens were added to estrogen therapy, the higher was the relative risk for endometrial cancer. Figure 2 summarizes the overall findings. Five of eight studies, including the Million Women Study, reported risks below unity for the addition of progestogen every day. Five of six studies on progestogens added for 10–24 days per month and all four studies on progestogens added for < 10 days per month reported an increased risk for endometrial cancer (Million Women Study Collaborators, 2005).

Figure 2. Summary of published studies on the relation between use of combined estrogen–progestogen hormonal therapy and endometrial cancer, according to the number of days per month that progestogens are added to estrogen therapy



Adapted from Million Women Study Collaborators (2005)

Among the eight studies that reported on the effect of progestogens added to estrogen therapy on a daily basis, only one (Newcomb & Trentham-Dietz, 2003) found that the risk for endometrial cancer was significantly higher in never users of any type of hormonal therapy.

Overall, no consistent trend was found with increasing duration of use of continuous combined therapy (Table 8), and no significant differences were found according to the specific type of progestogen used (Beral *et al.*, 2005) or according to progestogen dose (Newcomb & Trentham-Deitz, 2003).

In the seven studies that reported on the effect of progestogens added to estrogens for 10–21 days per month, all found that the risk for endometrial cancer was similar to or slightly higher than that seen in never users of any type of hormonal therapy (Table 9). Five of the seven studies presented results separately according to duration of use of the therapy and, in every study, the relative risk tended to be higher with longer use. Among users of hormonal therapy with progestogens added for 10–21 days per month, no significant differences were found according to the specific type of progestogen used (Beral *et al.*, 2005).

Table 8. Summary of results on the association of endometrial cancer with the daily addition of progestogens to estrogen therapy

Reference, location	Exposure category	No. of cases	No. of controls/ population at risk	Relative risk/ odds ratio (95% CI)
Observational studies				
Pike *et al.* (1997), USA	No use	739	710	1.0
	Any use	94	81	1.07 (0.80–1.43)
	Duration ≥ 5 years	24	33	1.34 (NR)
Weiderpass *et al.* (1999), Sweden	Never	641	3 014	1.0
	Ever	41	237	0.7 (0.4–1.0)
	Duration ≥ 5 years	2	32	0.2 (0.1–0.8)
Hill *et al.* (2000), USA	No use of any hormonal therapy	392	793	1.0
	Ever continuous hormonal therapy	9	33	0.6 (0.3–1.3)
Jain *et al.* (2000), Canada	No use	292	316	1.0
	Exclusive use of continuous hormonal therapy	15	14	1.51 (0.67–3.42)
Newcomb & Threntham-Dietz (2003), USA	No use	402	1 667	1.0
	Any use	20	62	2.26 (1.27–4.00)
Beral *et al.* (2005), United Kingdom	Never users	763	395 786	1.0
	Any use	73	69 577	0.71 (0.56–0.90)
	Duration ≥ 5 years	44	33 600	0.90 (0.66–1.22)
Randomized trials				
Hulley *et al.* (1998), USA	Placebo[a]	2		1.0
	Estrogen-progestin[a]	4		0.49 (0.09–2.68)
Anderson *et al.* (2003), USA	Placebo[b]	27		1.0
	Estrogen-progestin[b]	31		0.81 (0.48–1.36)

CI, confidence interval; NR, not reported
[a] 2763 women were randomized.
[b] 16 608 women were randomized.

All four studies that reported on the risk for endometrial cancer associated with use of combined hormonal therapy with progestogens added for less than 10 days per month found an increased risk for endometrial cancer associated with such use, although the risk was lower than that associated with the use of estrogen-only therapy (Beresford *et al*., 1997; Pike *et al*., 1997; Pukkala *et al.*, 2001; Newcomb & Trentham-Dietz, 2003). The two studies that reported results according to duration of use found that the risk tended to be higher with longer use (Table 10).

Table 9. Summary of results from studies of endometrial cancer and the addition of progestogens cyclically to estrogen therapy for 10–21 days each month

Reference, location	Exposure category	No. of cases	No. of controls/ population at risk	SIR/odds ratio (95% CI)
Beresford *et al.* (1997), USA	Never	270	593	1.0
	Any use	25	64	1.3 (0.8–2.2)
	Duration ≥ 5 years	12	16	2.5 (1.1–5.5)
Pike *et al.* (1997), USA	No use	754	703	1.0
	Any use	79	88	1.07 (0.82–1.41)
	Duration > 5 years	23	33	1.09 [NR]
Weiderpass *et al.* (1999)[a], Sweden	Never	597	2 963	1.0
	Ever	90	300	2.0 (1.4–2.7)
	Duration ≥ 5 years	40	78	2.9 (1.8–4.6)
Jain *et al.* (2000)[b], Canada	No use	292	316	1.0
	Any use	65	87	1.05 (0.71–1.56)
	Duration ≥ 3 years	47	47	1.49 (0.93–2.40)
Pukkala *et al.* (2001), Finland	Any use	141	105[c]	1.3 (1.1–1.6)
Newcomb & Threntham-Dietz (2003), USA	No use	402	1 667	1.0
	Any use	14	71	1.10 (0.59–2.07)
Beral *et al.* (2005), United Kingdom	No use	763	395 785	1.0
	Any use	242	145 486	1.05 (0.91–1.22)
	Duration ≥ 5 years	140	75 000	1.17 (0.97–1.41)

CI, confidence interval; NR, not reported; SIR, standardized incidence ratio
[a] The average duration of use of progestogens was about 10 days each month.
[b] All but six cases used progestogens for 10 or more days each month.
[c] Expected number of cases, based on incidence rates of endometrial cancer in Finland

Taken together, the results are consistent with the view that the addition of progestogens to estrogen therapy lessens the risk associated with the use of estrogens alone, and that the greater the number of days per month that progestogens are added, the greater is the reduction in risk. The addition of progestogens for less than 10 days per month is associated with a clear increase in the risk for endometrial cancer. To reduce the rates of endometrial cancer in menopausal women to levels that are found in never users of hormonal therapy, progestogens may need to be added to estrogens most of the time and possibly on a daily basis. Since the use of combined estrogen–progestogen therapy began relatively recently, there is as yet little information on the effects of combined estrogen–progestogen therapy on the risk for endometrial cancer many years after cessation of use.

Table 10. Summary of results of studies of endometrial cancer and the addition of progestogens to estrogen therapy cyclically for < 10 days each month

Reference, location	Exposure category	No. of cases	No. of controls/ population at risk	Relative risk/ odds ratio (95% CI)
Beresford *et al*. (1997), USA	Never	270	593	1.0
	Any use	25	26	3.1 (1.7–5.7)
	Duration ≥ 5 years	15	12	3.7 (1.7–8.2)
Pike *et al*. (1997), USA	No use	759	744	1.0
	Any use	74	49	1.9 (1.3–2.6)
	Duration > 5 years	27	13	3.49 (NR)
Pukkala *et al.* (2001), Finland	Any use	61	30	2.0 (1.6–2.6)
Newcomb & Threntham-Dietz (2003), USA	No use	402	1667	1.0
	Any use	8	21	2.4 (1.0–5.9)

CI, confidence interval; NR, not reported

2.3 Cervical cancer

Persistent infection by certain types of human papillomavirus (HPV) is generally considered to be a necessary cause of cervical cancer (IARC, 2007). However, only a small proportion of women who are infected by these viruses develop a cervical neoplasm, which clearly indicates that co-factors probably play an etiological role. Since the uterine cervix is responsive to estrogens and progestogens, these hormones could act to modify the carcinogenic potential of an HPV infection. Combined estrogen–progestogen hormonal therapy at menopause is one exogenous source of these hormones. Their possible role in cervical carcinogenesis has not been studied adequately in humans. Combined estrogen–progestogen hormonal therapy has not been widely used for a sufficiently long period of time for adequate epidemiological study of the risk for cervical cancer in relation to long-term use or to use a long time after initial or most recent exposure.

2.3.1 *HPV infection*

Two randomized trials have provided some initial information of relevance (Smith *et al.*, 1997; Anderson *et al.*, 2003). In a study from Iowa, USA, among women who were enrolled in the Postmenopausal Estrogen/Progestin Intervention trial (Smith *et al*., 1997), 105 women aged 45–64 years were initially tested for nine high-risk types of HPV DNA (16, 18, 31, 33, 35, 39, 45, 51, 52) in cervical scrapings on enrolment and two years later using polymerase chain reaction (PCR)-based technology. Table 11 shows the results at

Table 11. Summary of results from a randomized trial of estrogen and estrogen–progestogen combinations that show percentages of women who were HPV-positive or HPV-negative at baseline and who were HPV-positive after 2 years of treatment

Treatment	HPV-negative at baseline			HPV-positive at baseline		
	Total no. of women	HPV-positive at 2 years		Total no. of women	HPV-positive at 2 years	
		No.	%		No.	%
Placebo	17	3	17.6	5	1	20.0
CEE[a]	12	3	25.0	8	3	37.5
CEE + progestogen (all combinations)	36	7	19.4	27	7	25.9
CEE/2.5 MPA[b]	11	2	18.2	8	3	37.5
CEE/10 MPA[c]	12	2	16.7	10	2	20.0
CEE/200 MP[d]	13	3	23.1	9	2	22.2
Any hormone treatment	48	10	20.8	35	10	28.6

From Smith *et al.* (1997)
CEE, conjugated equine estrogens; HPV, human papillomavirus; MP, micronized progesterone; MPA, medroxyprogesterone acetate
[a] CEE, 0.625 mg daily
[b] 0.625 mg CEE plus 2.5 mg MPA daily
[c] 0.625 mg CEE daily plus 10 mg MPA daily on days 1–12 of cycle
[d] 0.625 mg CEE daily plus 200 mg MP daily on days 1–12 of cycle

2 years in women who initially tested HPV-positive or HPV-negative. Among women who initially tested negative for HPV DNA, the percentage that became positive was not significantly higher in any of the treatment groups than in the placebo group. The treatment groups included one estrogen-only group and three estrogen–progestogen groups. When these three groups were combined, the percentage that were HPV DNA-positive after 2 years of follow-up was also not statistically significantly different in the combined group than in the placebo group. Thus, the incidence of HPV (or recrudescence of existing infection missed on enrolment) was apparently not influenced by estrogen–progestogen treatment. Among women who were initially positive for HPV DNA, the percentage that remained positive at 2 years did not vary significantly by treatment, and the percentage in the three estrogen–progestogen groups combined was not significantly different from that in the placebo group. In any individual woman, the type of HPV at 2 years was not always the same as the type at baseline. The infections at 2 years thus represented a mixture of new and persistent infections. The results did not provide evidence to suggest that estrogen–progestogen therapy alters the risk for either new or persistent infection. Five women were found 2 years after enrolment to have an abnormal Papanicolaou (Pap) smear; four had

atypical cells of undetermined significance and one had atypical squamous cells. No such cells occurred in women with a positive HPV DNA test at baseline or concurrently with a suspicious Pap smear; their relevance to cervical carcinoma and the sensitivity of the HPV DNA assays used were therefore questioned. Abnormal Pap smears were not associated with treatment group. At baseline, the prevalence of HPV DNA was 22.7% in the placebo group and varied from 40.0 to 45.5% in the four treatment groups, suggesting that women in the placebo group may have been at lower risk for HPV infection than those in the treatment groups. If this were the case, it would bias the results towards higher rates of HPV being observed in the treatment groups than in the placebo group at follow-up, and this, in addition to chance, could explain the slightly higher rates of HPV in some of the treatment groups than in the placebo group as shown in Table 11. [However, this study was of low statistical power, so that true differences in rates of HPV infection among study groups could have been missed. Larger studies of longer duration will be needed to determine more definitively whether estrogen–progestogen therapy alters the risk for acquisition or persistence of HPV.]

2.3.2 *Cervical neoplasia*

Table 12 summarizes results relevant to cervical cancer from the WHI (Anderson *et al*., 2003). Between October 1993 and October 1998, women who had not had a hysterectomy aged 50–79 years in 40 participating clinics in the USA were randomized to either treatment

Table 12. Summary of results from a randomized trial of estrogen–progestogen combination showing percentages of women at follow-up with LGSIL, HGSIL and cervical cancer

Treatment	Total no. of women[a]	Results of Pap smears						Reported cervical cancer[b,c]	
		LGSIL		HGSIL		Cancer[b]			
		No.	%	No.	%	No.	%	No.	%[d]
Placebo	7599	420	5.5	29	0.4	3	0.04	8	0.02
CEE/MPA[e]	7950	619	7.8	25	0.3	2	0.03	5	0.01

From Anderson *et al.* (2003)
CEE, conjugated equine estrogen; HGSIL, high-grade squamous intrepithelial lesion; LGSIL, low-grade squamous intraepithelial lesion; MPA, medroxyprogesterone acetate
[a] 503 women in the placebo group and 556 women in the estrogen–progestogen group with no follow-up smears excluded
[b] Whether *in situ* or invasive not stated in published report
[c] Not stated whether reported cervical cancer cases include those detected at Papanicolaou smear screening.
[d] Annualized %
[e] 0.625 mg CEE plus 0.25 mg MPA daily

with 0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate daily (n = 8506) or placebo (n = 8102). Most women had Pap smears every 3 years. After a mean follow-up period of 5.6 years, the incidence of cervical cancer as reported from the 40 participating clinics did not differ significantly between the treatment and placebo groups (hazard ratio, 1.4; 95% CI, 0.5–4.4). It was not indicated whether the cancers were invasive or *in situ*. There were significantly ($p < 0.001$) more low-grade squamous intraepithelial lesions in the treatment group (7.8%) than in the placebo group (5.5%), but the relationship of these lesions to cervical neoplasia is uncertain. Furthermore, this may result from more women in the treatment group having had Pap smears as part of a clinical evaluation for vaginal bleeding than those in the placebo group. There was no significant difference in rates of high-grade squamous intraepithelial lesions (HSIL) or of cervical cancer (presumably carcinoma *in situ*) detected by Pap smears in the two groups of women. Although this study provides little cause for concern that combined continuous estrogen–progestogen therapy for over 5 years alters the risk for cervical cancer, the statistical power to detect an alteration in risk of any type of cervical carcinoma was low, and the duration of follow-up was too short to determine whether risk is increased a long time after initial or last use. The increased risk for HSIL in the treated group warrants further investigation.

2.3.3 *Overview*

There is little evidence from these two randomized trials to suggest that combined estrogen–progestogen therapy alters the risk for persistent HPV infection, HSIL or cervical cancer, but both studies were of limited statistical power to detect true increases in risks in women who are exposed to these treatments.

2.4 Ovarian cancer

2.4.1 *Background*

Major findings of cohort and case–control studies published before the last evaluation (IARC, 1999), including two meta-analyses (Garg *et al*., 1998; Coughlin *et al.*, 2000), and a re-analysis of individual data on hormonal therapy and risk for ovarian cancer indicate that long-term use of hormonal therapy is associated with a moderate, but consistent excess risk for ovarian cancer (IARC, 1999; Negri *et al*., 1999; Bosetti *et al.*, 2001). In a meta-analysis of 10 published studies (nine case–control, one cohort), the overall risk for invasive ovarian cancer for ever users of hormonal therapy was 1.15 (95% CI, 1.05–1.27), with no difference in risk for hospital-based and population-based case–control studies (Garg *et al*., 1998). Another meta-analysis of 15 studies (Coughlin *et al*., 2000), however, found no significant overall association (relative risk, 1.1; 95%, CI, 0.9–1.7). The studies that have been published since the last evaluation (IARC, 1999) are summarized below.

2.4.2 *Controlled clinical trials*

The WHI, a randomized, controlled primary prevention trial, included 8506 women aged 50–79 years who were treated with combined hormonal therapy and 8102 untreated women (Writing Group for the Women's Health Initiative Investigators, 2002). In the group that received combined hormonal therapy, 20 cases of ovarian cancer occurred versus 12 in the placebo group, which corresponded to a multivariate relative risk of 1.58 (95% CI, 0.77–3.24). Nine deaths from ovarian cancer occurred in the combined hormonal therapy group versus three in the placebo group (relative risk, 2.70; 95% CI, 0.73–10.00) (Anderson *et al.*, 2003).

2.4.3 *Cohort studies*

One cohort study (Pukkala *et al*., 2001) provided data on combined hormonal therapy and ovarian cancer. In this Finnish record linkage study, 15 956 women who received long-cycle hormonal therapy (with added progestogen every 2nd or 3rd month) and 78 549 who used monthly cycle therapy were identified from the medical reimbursement register of the national Social Insurance Institution (between 1994 and 1997). Cancer incidence was ascertained through the files of the population-based country-wide Finnish Cancer Registry. By the end of follow-up, 23 cases of ovarian cancer in the long-cycle cohort and 104 in the monthly cycle cohort were observed, to yield SIRs of 1.0 (95% CI, 0.63–1.5) and 1.1 (95% CI, 0.93–1.4), respectively.

A cohort study based on the Breast Cancer Detection Demonstration Project included 329 incident cases of ovarian cancer (Lacey *et al.*, 2002). Compared with never use of any type of hormonal therapy, the relative risk for exclusive use of combined hormonal therapy was 1.1 (95% CI, 0.64–1.7; 18 cases), in the absence of any duration–risk relation (relative risk for ≥ 2 years of use, 0.80; 95% CI, 0.35–1.8). The relative risk for use of combined hormonal therapy after that of estrogen-only therapy was 1.5 (95% CI, 0.91–2.4; based on 21 cases).

2.4.4 *Case–control studies* (Table 13)

In a population-based study of 793 incident cases of epithelial ovarian cancer diagnosed between 1990 and 1999 in Queensland, New South Wales and Victoria, Australia, and 855 controls (Purdie *et al*., 1999), the relative risk adjusted for age, education, area of residence, body mass index, hysterectomy, tubal sterilization, use of talc, tobacco smoking, oral contraceptive use, parity and family history of breast or ovarian cancer was 1.34 (95% CI, 0.83–2.17) for the use of estrogens and progestogens in combination. There was no consistent relation with duration of use, time since last use or any other time factor.

In a case–control study from Sweden of 193 epithelial borderline cases, Riman *et al*. (2001) reported an odds ratio of 0.98 (95% CI, 0.57–1.68) for estrogens with cyclic progestogens and 0.87 (95% CI, 0.46–1.64) for estrogens and continuous progestogens compared with never users. None of the trends in risk with duration of use were significant.

Table 13. Case–control studies of the use of combined hormonal therapy and the risk for ovarian cancer

Reference, location	No. of cases	No. of controls	Odds ratio[a] (95% CI)		
			Ever use	Longest use (duration)	Current/recent use
Purdie *et al.* (1999), Australia	793	855	1.34 (0.83–2.17)	1.33 (0.88–2.00) (> 3 years)	1.24 (0.73–2.09)
Riman *et al.* (2001), Sweden (borderline neoplasms)	193	3899	0.98 (0.57–1.68) sequential	0.91 (0.44–2.03) (≥ 2 years)	–
			0.87 (0.46–1.64) continuous	0.89 (0.35–2.28) (≥ 2 years)	–
Riman *et al.* (2002), Sweden (invasive neoplasms)	655	3899	1.41 (1.15–1.72)	2.03 (1.30–3.17) (≥ 10 years)	–
Sit *et al.* (2002), USA	484	926	1.06 (0.74–1.52) conjugated estrogens 1.08 (0.59–2.00) non-conjugated estrogens	–	–
Glud *et al.* (2004), Denmark	376	1111	1.14 (1.01–1.28)[b] 1.00 (0.95–1.06)[c]	–	–
Pike *et al.* (2004), USA	477	660	–	0.90 (0.55–1.48)[d] (≥ 5 years)	–
			–	1.13 (0.15–8.3)[e]	–

CI, confidence interval

[a] Reference category was never use of combined hormonal therapy.

[b] Per additional gram of estrogen intake

[c] Per additional gram of progestogen intake

[d] Natural menopause

[e] Hysterectomy

In the same study that included 655 cases of ovarian cancer and 3899 controls aged 50–74 years, the odds ratio was 1.41 (95% CI, 1.15–1.72) for ever use of combined hormone therapy (Riman *et al*., 2002). For longest use (≥ 10 years), the odds ratio was 2.03 (95% CI, 1.30–3.17). There was no consistent pattern for time since last use. Adjustment was made for age, parity, body mass index, age at menopause, hysterectomy and duration of oral contraceptive use. The results were similar for serous, mucinous and endometrioid ovarian cancers. No information was presented on sequential or combined hormonal therapy.

A study conducted between 1994 and 1998 in Delaware Valley, USA, included 484 cases of ovarian cancer aged 45 years or over and 926 community controls frequency-matched by age and area of residence (Sit *et al*., 2002). Adjustment was made for age, parity, oral contraceptive use, family history of ovarian cancer and history of tubal ligation. The hormonal therapy formulation was classified as estrogen plus progestogen or estrogen alone. The relative risk was 1.06 (95% CI, 0.74–1.52) for progestogen with conjugated estrogens and 1.08 (95% CI, 0.59–2.00) for progestogen with non-conjugated estrogens.

A nationwide case–control study was conducted in Denmark between 1995 and 1999 and included 376 cases of ovarian cancer and 1111 population controls (Glud *et al*., 2004). The results were presented in terms of groups of estrogen or progestogen intake, with adjustment for parity, use of oral contraceptives, family history of ovarian cancer and infertility. The odds ratio per additional gram of intake was 1.14 (95% CI, 1.01–1.28) for estrogens and 1.00 (95% CI, 0.95–1.06) for progestogens and was similar for estrogen only (odds ratio, 1.05; 95% CI, 0.97–1.14) and combined estrogen–progestogen therapies (odds ratio, 1.08; 95% CI, 1.01–1.16). There was no relationship with duration of use independent from cumulative dose.

A case–control study was conducted between 1992 and 1998 in Los Angeles County, CA, USA, on 477 cases of invasive epithelial ovarian cancer and 660 populations controls aged 18–74 years (Pike *et al*., 2004). Participation rates were approximately 80% of cases and 70% of controls approached. Multivariate relative risks were adjusted for age, ethnicity, socioeconomic status, education, family history of ovarian cancer, tubal ligation, use of talc, nulliparity, age at last birth, menopausal status, age at menopause and use of oral contraceptives. Among women with natural menopause, the odds ratios per 5 years of use were 1.16 (95% CI, 0.92–1.48) for estrogen-only therapy and 0.97 (95% CI, 0.77–1.23) for combined hormonal therapy. Corresponding values for women with surgical menopause were 1.11 (95% CI, 0.92–1.35) and 1.30 (95% CI, 0.63–2.67).

2.5 Liver cancer

Persson *et al.* (1996) studied cancer risks after hormonal menopausal therapy in a population-based cohort of 22 579 women aged 35 years or more who lived in the Uppsala health care region in Sweden. Women who had ever received a prescription for hormonal menopausal therapy between 1977 and 1980 were identified and followed until 1991; information on use of hormones was obtained from pharmacy records. The expected numbers of cases

were calculated from national incidence rates. There was no information on tobacco smoking or alcoholic beverage consumption. There were 43 cancers of the hepatobiliary tract that comprised 14 hepatocellular carcinomas, five cholangiocarcinomas, 23 gallbladder cancers and one unclassified. The expected number was 73.2, to give an SIR of 0.6 (95% CI, 0.4–0.8) for any type of hormonal menopausal therapy. The SIRs for treatment with estradiol combined with levonorgestrel were 0.6 (95% CI, 0.1–2.3) for hepatocellular carcinoma, 0.7 (95% CI, 0.0–3.8) for cholangiocarcinoma and zero (six cases expected) for gallbladder cancer. There was no information on infection with hepatitis viruses.

2.6 Colorectal cancer

2.6.1 *Background*

The previous monograph (IARC, 1999) reported details from three cohort studies and one case–control study on the use of combinations of estrogens and progestogens. Since then, new data have been published on the risks and benefits of estrogen plus progestogen treatment in menopausal women, including two randomized trials (the WHI Trial and the HERS Follow-up Study) (Hulley *et al.*, 2002; Writing Group for the Women's Health Initiative Investigators, 2002), one cohort study (Pukkala *et al.*, 2001) and two case–control studies (Jacobs *et al.*, 1999; Prihartono *et al.*, 2000). Other studies have focused on estrogen only or did not provide separate information for estrogen only and combined hormonal therapy (Paganini-Hill, 1999; Csizmadi *et al.*, 2004; Hannaford & Elliot, 2005; Nichols *et al.*, 2005).

2.6.2 *Randomized trials*

Two large randomized clinical trials have been published that provided information on combined hormonal therapy and colorectal cancer (Table 14).

The HERS was a randomized trial of the use of estrogen plus progestogen in which 2763 menopausal women under 80 years of age at baseline who had coronary artery disease and no prior hysterectomy were recruited at 20 outpatient and community settings between 1993 and 2000 in the USA (Hulley *et al.*, 2002). Of these, 1380 women were allocated to the treatment group (0.625 mg per day conjugated estrogens plus 2.5 mg per day medroxyprogesterone acetate) and 1383 to the placebo group. After a mean of 4.1 years of follow-up, 11 cases of colon cancer were observed in the combined hormonal therapy group versus 16 in the placebo group, which corresponded to a relative risk of 0.69 (95% CI, 0.32–1.49) (Hulley *et al.*, 2002).

The WHI Study was a randomized, controlled, primary prevention trial (that was planned to continue for 8.5 years) in which 16 608 menopausal women aged 50–79 years who had a uterus at baseline were recruited at 40 clinical centres between 1993 and 1998 in the USA. Of these, 8506 women were allocated to the treatment group (0.625 mg per day conjugated estrogens plus 2.5 mg per day medroxyprogesterone acetate) and 8102 to the placebo group (Writing Group of the Women's Health Initiative, 2002). At the end of

Table 14. Randomized clinical trials on the association between the use of combined hormonal therapy and the risk for colorectal cancer

Reference, location	Participants Outcome No. cases/group size	Relative risk (95% CI)	Comments
Chlebowski *et al.* (2004), USA	Healthy postmenopausal women with intact uterus Colorectal cancer Treatment group: 43/8506 Placebo group: 72/8102	0.56 (0.38–0.81)	WHI study; treatment: 0.625 mg/day conjugated estrogens plus 2.5 mg/day medroxy-progesterone acetate; multi-centre study; terminated early
Hulley *et al.* (2002), USA	Postmenopausal women with previous heart disease Colon cancer Treatment group: 11/1380 Placebo group: 16/1383	0.69 (0.32–1.49)	HERS; treatment: 0.625 mg/day conjugated estrogens plus 2.5 mg/day medroxy-progesterone acetate; multi-centre study; terminated early

CI, confidence interval; HERS, Heart and Estrogen/Progestin Replacement Study; WHI, Women's Health Initiative

active intervention (mean follow-up, 5.6 years), 43 cases of invasive colorectal cancer were observed in the combined hormonal therapy group versus 72 in the placebo group (relative risk, 0.56; 95% CI, 0.38–0.81) (Chlebowski *et al.*, 2004). The reduction in the risk for colorectal cancer in the hormonal therapy group was largely confined to local disease (relative risk, 0.26; 95% CI, 0.13–0.53), rather than regional or metastatic disease (relative risk, 0.87; 95% CI, 0.54–1.41). Within the category of regional or metastatic disease, the cancers in the hormonal therapy group were associated with a greater number of positive nodes than the corresponding types of cancer in the placebo group (Chlebowski *et al.*, 2004).

2.6.3 *Cohort studies* (Table 15)

In addition to the three cohort studies reviewed previously (IARC, 1999), one cohort study (Pukkala *et al.*, 2001) provided new data on the potential association between the use of combined hormonal therapy and the risk for colorectal cancer. In this Finnish record linkage study, 15 956 women who took long-cycle hormonal therapy (administered orally on a 3-month basis: 70 days 2 mg estradiol valerate, 14 days 2 mg estradiol valerate plus 20 mg medroxyprogesterone acetate and 7-day tablet-free period) and 78 549 who took monthly or short-cycle (11 days 2 mg estradiol valerate, 10 days 2 mg estradiol valerate and 0.25 mg levonorgestrel and 7-day tablet-free period) hormonal therapy were identified from the medical reimbursement register of the national Social Insurance Institution (between 1994 and 1997); cancer incidence was ascertained through the files of the population-based country-wide Finnish Cancer Registry. SIRs were computed by

Table 15. Cohort studies on the association between the use of combined hormonal therapy and the risk for colorectal cancer

Reference, location	No. cases (or deaths)/cohort size	Follow-up (years)	Relative risk (95% CI) (ever versus never use)	Comments
Risch & Howe (1995), Canada	230/32 973	14	Colon, 1.07 (0.58–1.99) Rectum, 1.16 (0.53–2.52)	Linkage study (cancer registry–drug database); age-adjusted
Persson *et al.* (1996), Sweden	295/22 597	13	Colon, 0.6 (0.4–1.0) Rectum, 0.8 (0.4–1.3)	Relative risk for incident cancer (age-adjusted); no effect among 5573 hormone users (fixed combined brand); relative risk for mortality from colon cancer adjusted for age, 0.6 (95% CI, 0.2–1.1)
Troisi *et al.* (1997), USA	313/33 779	7.7	Colon, 1.4 (0.7–2.5)	Relative risk adjusted for age (unaltered when adjusted for education, body mass index, parity or use of oral contraceptives); no differences right/left colon; no trend with duration of use
Pukkala *et al.* (2001), Finland	11/15 956[a] 50/78 549[b]	5	Colon, 0.67 (0.34–1.20) Colon, 0.85 (0.63–1.10)	Linkage study (Social Insurance Institution drug database and Cancer Registry); relative risk adjusted for age; recency and duration of use not assessed

CI, confidence interval

[a] Long cycle (2 or 3-month) administration of combined hormonal therapy

[b] Short cycle (1-month) administration of combined hormonal therapy

comparing the observed number of cases in the assembled cohort with those expected using national incidence rates. By the end of follow-up, 11 cases of colon cancer were observed in the long-cycle cohort and 50 cases in the monthly cycle cohort, to yield an age-adjusted SIR for colon cancer of 0.67 (95% CI, 0.34–1.20) and 0.85 (95% CI, 0.63–1.10), respectively (Pukkala *et al.*, 2001).

2.6.4 *Case–control studies* (Table 16)

Since the previous evaluation (IARC, 1999), a nested case–control study of more than 1400 women aged 55–79 years who were enrolled from the Group Health Cooperative, a health maintenance organization in Washington State, USA, has been published (Jacobs *et al.*, 1999). Between 1984 and 1993, 341 incident cases of colon cancer and 1679 controls matched by age and length of enrolment in the cooperative were identified. From the records of prescriptions for progestogen tablets, the authors identified 268 cases and 1294 controls who had used combined hormonal therapy during a 5-year period (progestogen-only users and estrogen-only users excluded). The age-adjusted odds ratio for colon

Table 16. Case–control studies on the association between the use of combined hormonal therapy and the risk for colorectal cancer

Reference, location	No. cases/ controls	Odds ratio (95% CI)[a]	Comments
Newcomb & Storer (1995), USA	694/1622	Colon, 0.54 (0.28–1.0)[a] Rectum, 1.1 (0.51–2.5)[a]	Adjustment for age, alcoholic beverage consumption, body mass index, family history of cancer, sigmoidoscopy
Jacobs *et al.* (1999), USA	268/1294	Colon < 180 tablets[b], 0.59 (0.28–1.24) ≥ 180 tablets[b], 1.04 (0.59–1.82)	Nested case–control study in a health maintenance organization; adjustment for age; further adjustment for smoking, height, weight, body mass index, oral contraceptive use, parity, age at first birth, age at menopause and hysterectomy status did not alter the odds ratios.
Prihartono *et al.* (2000), USA	404/404	Colon Last use < 1 year, 0.9 (0.4–2.2) Duration ≥ 5 years, 0.7 (0.2–2.5)	Adjusted for fat, fruit and vegetable intake, physical activity, body mass index, history of screening for colorectal cancer

CI, confidence interval

[a] Ever use versus never use

[b] Progestogen tablet counts: assuming 100% compliance and 10 progestogen tablets per month, consumption of < 180 tablets is equivalent to 1.5 years of use and consumption of ≥ 180 tablets is equivalent to ≥ 1.5 years of consumption.

cancer was 0.59 (95% CI, 0.28–1.24) for those who consumed less than 180 progestogen tablets [assuming 100% compliance and 10 progestogen tablets per month, consumption of 180 tablets is equivalent to 1.5 years of use] and 1.04 (95% CI, 0.59–1.82) for those who consumed > 180 tablets [or used combined hormonal therapy for more than 1.5 years] compared with never users. Adjustment for other covariates did not substantially change these estimates. Duration of use and analysis of colon subsite was not presented for users of combined hormonal therapy.

Prihartono *et al.* (2000) conducted a matched population-based case–control study among women aged 20–69 years in Massachusetts, USA, between 1992 and 1994, and included 515 incident cases of colon cancer (out of 1847 potential eligible cases) and 515 matched controls. The final analysis was restricted to pairs of women with natural menopause or who had had a hysterectomy (404 cases, 404 matched controls). Recent use (interval since last use, < 1 year) of combined hormonal therapy showed an odds ratio of 0.9 (95% CI, 0.4–2.2; 13 exposed cases, 15 exposed controls). Longer duration of use (> 5 years) of combined hormonal therapy showed an odds ratio of 0.7 (95% CI, 0.2–2.5; seven exposed cases, nine exposed controls). The odds ratio was adjusted for fat, fruit and vegetable intake, physical activity, body mass index and history of screening for colorectal cancer.

2.7 Lung cancer

The large population-based mortality study in Sweden (Persson *et al.*, 1996) found no association with lung cancer in users of combined hormonal therapy, and a similar study in Finland found non-significant associations for long (SIR, 1.2; 95% CI, 0.69–1.9) or monthly (SIR, 0.75; 95% CI, 0.53–1.0) cycles of hormonal therapy (Pukkala *et al.*, 2001).

A case–control study in Texas, USA, in which 60 cases of lung cancer and 78 controls reported use of combined hormonal therapy reported a multivariate odds ratio of 0.61 (95% CI, 0.40–0.92) (Schabath *et al.*, 2004).

The HERS (Hulley *et al.*, 2002) and WHI (Writing Group for the Women's Health Initiative Investigators, 2002) trials showed a hazard ratio of 1.39 (95% CI, 0.84–2.28) and 1.04 (95% CI, 0.71–1.53) for lung cancer, respectively.

2.8 Other cancers

Data on other cancers were inadequate for an evaluation as nearly all studies failed to report the type of hormonal therapy used.

3. Studies of Cancer in Experimental Animals

Only one study on the carcinogenicity of conjugated equine estrogens plus progestogens was reviewed in the previous monograph (IARC, 1999; Sakamoto *et al.*, 1997a).

3.1 Oral administration

3.1.1 *Mouse*

In a study to compare estrogen therapy with combined estrogen–progestogen therapy that used conjugated equine estrogens or conjugated equine estrogens plus medroxyprogesterone acetate, three groups of 14 female SHN mice (a strain that has a high spontaneous rate of mammary tumours and uterine adenomyosis), 71 days [about 10 weeks] of age, were fed 0 (controls) or 1.875 mg/kg of diet conjugated equine estrogens (Premarin®) with or without 7.5 mg/kg of diet medroxyprogesterone acetate (Provera®) for 230 days. Based upon a daily dietary intake of 2–3 g per mouse weighing 20–30 g, the daily intakes of conjugated equine estrogens and medroxyprogesterone acetate were calculated to be 0.19 and 0.75 mg/kg bw per day, respectively. Mice were killed 20 days after the appearance of a palpable mammary tumour or at 300 days of age. The significance of differences was evaluated by the χ^2 test. The incidence of mammary tumours [of unspecified histopathology] did not differ in the three groups (control, 4/14; estrogen alone, 6/14; estrogen–progestogen, 5/14). However, treatment with estrogen–progestogen shortened the latent period of mammary tumorigenesis by 44 days ($p < 0.05$ versus controls; not statistically significantly different from estrogen only). Treatment with estrogen–progestogen completely suppressed the development of uterine adenomyosis (0/14 versus 5/14 controls or 6/14 estrogen only-treated mice, $p < 0.01$) (Sakamoto *et al.*, 1997b). [These results are somewhat confounded by the potential influence of endogenous ovarian hormones that are reduced in the postmenopausal state. Endogenous estradiol levels in controls (3.91 ± 1.16 pg/mL) were significantly lower ($p < 0.01$) than those in estrogen only-treated (28.15 ± 2.91 pg/mL) and estrogen–progestogen-treated (20.15 ± 1.37 pg/mL) mice. However, the levels in hormone-treated mice were physiological and did not exceed that observed on day 1 of the estrus cycle (Raafat *et al*. 1999).]

3.1.2 *Monkey*

In one study, ovariectomized cynomolgus monkeys (*Macaca fascicularis*), 5–13 years of age, were treated for 2.5 years with either conjugated equine estrogen alone (Premarin®) (equivalent to 0.625 mg per woman per day; 22 animals) or in combination with medroxyprogesterone acetate (Cycrin®) (equivalent to 2.5 mg per woman per day; 21 animals) in

the diet or were untreated (26 animals). Determination of serum hormone levels of estradiol and medroxyprogesterone acetate confirmed the completeness of ovariectomy. The experiment was terminated at the end of the treatment phase. Mammary gland atrophy was seen in control animals. Eighty-six per cent (18/21) of the estrogen–progestogen-treated animals had mammary hyperplasia, defined as greater mammary gland development than that seen in animals with normal cycles ($p = 0.0065$). Forty-one per cent (9/22) of the animals given estrogen only had mammary hyperplasia. No neoplasms were observed (Cline *et al*., 1996).

In a subsequent, similar study, a progestogen-only group was added and the treatments were administered in the diet for 3 years. Ovariectomies were carried out 3 months before the start of treatments. The treatment groups were controls (no treatment; 27 animals), conjugated equine estrogen-treated (0.625 mg per woman per day equivalent; 27 animals), medroxyprogesterone acetate-treated (2.5 mg per woman per day equivalent; 26 animals) and estrogen–progestogen-treated (0.625 mg per woman per day equivalent conjugated equine estrogen plus 2.5 mg per woman per day equivalent medroxyprogesterone acetate; 26 animals); mean age at the end of the study was 7.5 years. The effective number of control animals was 20. Mammary gland lobuloalveolar hyperplasia was increased with estrogen-only treatment (effective number of animals, 25) and the effect was further increased with estrogen–progestogen treatment (effective number of animals, 26) [incidence not provided]; this development exceeded that usually seen in premenopausal animals with normal cycles. Progestogen-alone treatment (effective number of animals, 19) did not increase hyperplasia. No neoplasms, ductal hyperplasia or atypia were observed (Cline *et al*., 1998).

In a third study (Cline *et al.*, 2002a) designed to assess the effect of tibolone, a similar experimental protocol and the same doses were used as those described by Cline *et al.* (1996). Twenty-eight to 31 ovariectomized monkeys (6–8 years of age) per group were treated for 2 years. Mammary lobuloalveolar hyperplasia was observed in 19/30 (63%) control, 27/28 (96%, $p < 0.001$) estrogen only-treated and 28/29 (97%, $p < 0.001$) estrogen–progestogen-treated animals. No neoplasms were observed.

3.2 Administration with a known carcinogen

Rat

7,12-Dimethylbenz[*a*]anthracene

Female Sprague-Dawley rats, 48 days [about 7 weeks] of age, were divided into four groups of seven rats per group and were administered: 7,12-dimethylbenz[*a*]anthracene (DMBA) alone (as a single intravenous injection of 5 mg); DMBA and were oophorectomized; DMBA plus conjugated estrogens (Premarin®) at a concentration of 1.875 mg/kg of diet and were oophorectomized; or DMBA plus Premarin® plus medroxyprogesterone acetate (Proveza®) at a concentration of 7.5 mg/kg of diet and were oophorectomized. The animals were autopsied at 285 days [about 41 weeks] of age. Mammary tumours were found in 6/7 rats given DMBA, 0/7 given DMBA plus oophorectomy, 5/7 given DMBA plus Premarin® plus oophorectomy and 5/7 given Premarin® plus medroxyprogesterone acetate

plus oophorectomy. Thus, oophorectomy completely inhibited mammary tumour development, but conjugated estrogen with or without medroxyprogesterone acetate markedly stimulated mammary carcinogenesis in the ovariectomized rats (Sakamoto *et al*., 1997a).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The distribution of progestogens is described in the monograph on Combined estrogen–progestogen contraceptives. That of estrogens is described below.

4.1.1 *Humans*

Little more has been discovered about the absorption and distribution of estrone, estradiol and estriol products and conjugated equine estrogens in humans since the previous evaluation (IARC, 1999). Greater progress has been made in the identification and characterization of the enzymes that are involved in estrogen metabolism and excretion. The various metabolites and the responsible enzymes, including genotypic variations, are described below (see Figures 3 and 4). Sulfation and glucuronidation are the main metabolic reactions of estrogens in humans.

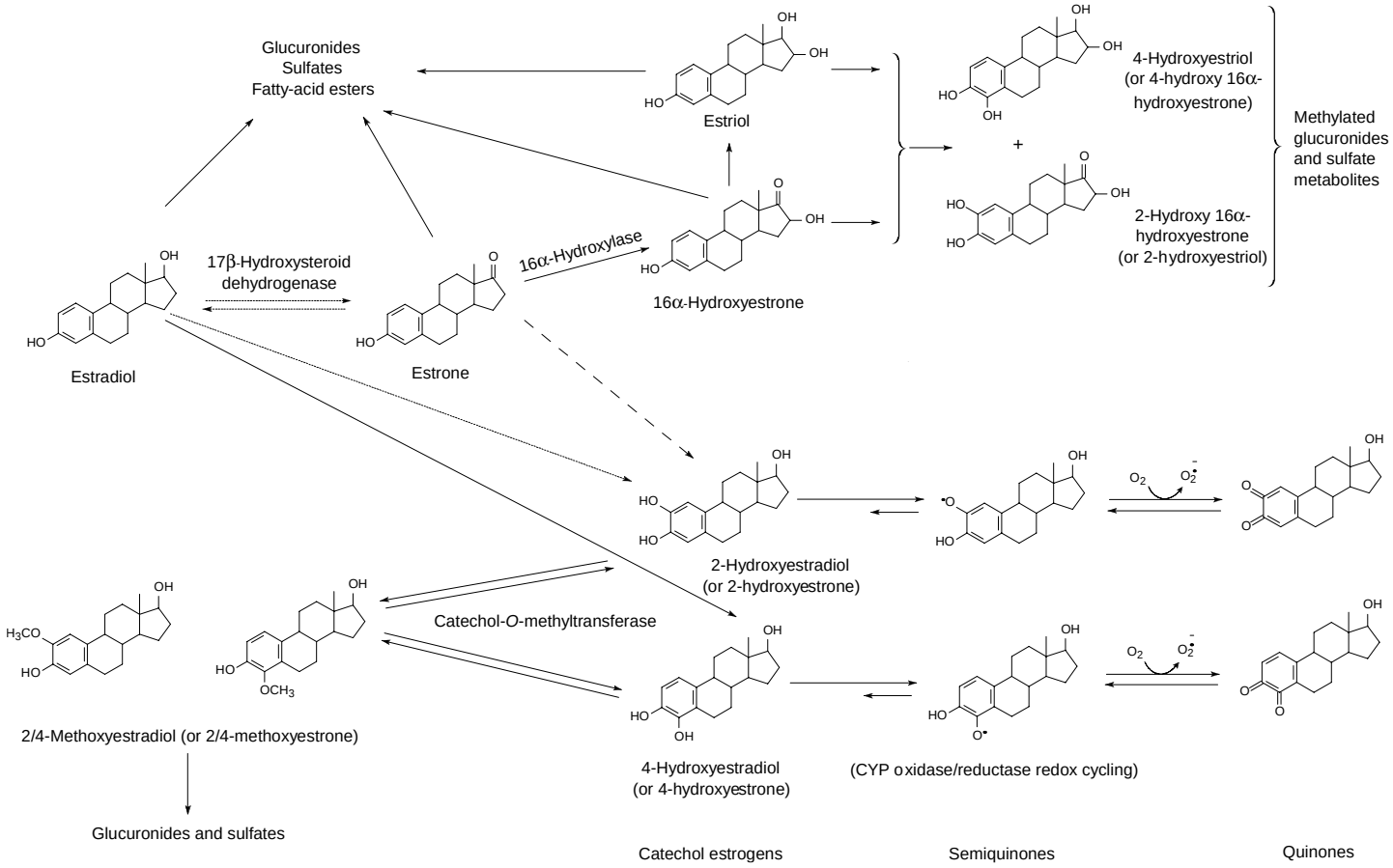
(*a*) *Metabolites*

(i) *Estrogen sulfates*

Several members of the sulfotransferase (SULT) gene family can sulfate hydroxysteroids, including estrogens. The importance of SULTs in estrogen conjugation is demonstrated by the observation that a major component of circulating estrogen is sulfated, i.e. estrone sulfate (reviewed by Pasqualini, 2004). In addition to the parent hormones, estrone and estradiol, SULTs can also conjugate their respective catechols and also methoxyestrogens (Spink *et al*., 2000; Adjei & Weinshilboum, 2002). The resulting sulfated metabolites are more hydrophilic and can be excreted.

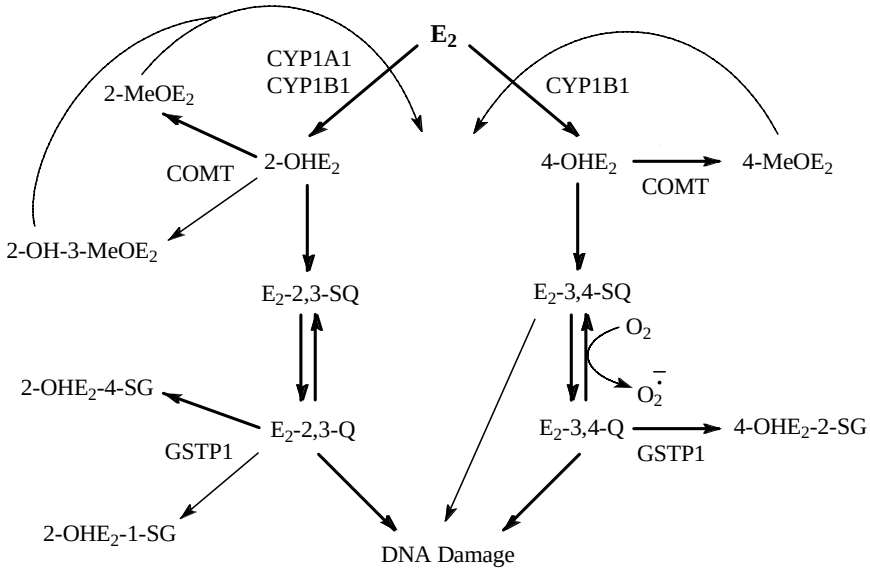
In postmenopausal breast cancers, levels of estrone sulfate can reach 3.3 ± 1.9 pmol/g tissue, which is five to nine times higher than the corresponding plasma concentration (equating gram of tissue with millilitre of plasma) (Pasqualini *et al*., 1996). In contrast, levels of estrone sulfate in premenopausal breast tumours are two to four times lower than those in plasma. Since inactive estrone sulfate can serve as a source for biologically active estradiol, it is of interest that various progestogens caused a significant decrease in the formation of estradiol when physiological concentrations of estrone sulfate were incubated with breast cancer cells MCF-7 and T47D (reviewed by Pasqualini, 2003).

Figure 3. Pathways of the metabolism and redox cycling of estradiol, estriol and estrone



Modified from Yager & Liehr (1996)

Figure 4. The estrogen metabolism pathway is regulated by oxidizing phase I and conjugating phase II enzymes



Adapted from Dawling *et al.* (2003)

COMT, catechol-*O*-methyltransferase; CYP, cytochrome P450; E_2, estradiol; GSH, glutathione; GST, glutathione *S*-transferase; $MeOE_2$, methoxyestradiol; OH, hydroxy; OHE_2, hydroxyestradiol; Q, quinone; SG, *S*-glutathione (oxidized); SQ, semiquinone

CYP1A1 and CYP1B1 catalyse the oxidation of E_2 to the catechol estrogens 2-OHE_2 and 4-OHE_2. The catechol estrogens are either methylated by COMT to methoxyestrogens (2-$MeOE_2$, 2-OH-3-$MeOE_2$, 4-$MeOE_2$) or further oxidized to semiquinones (E_2-2,3-SQ, E_2-3,4-SQ) and quinones (E_2-2,3-Q, E_2-3,4-Q). The methoxyestrogens exert feedback inhibition on CYP1A1 and CYP1B1, as indicated by the curved arrows, and reduce the formation of oxidative E_2 metabolites. The estrogen quinones are either conjugated by GSTP1 to GSH-conjugates (2-OHE_2-1-SG, 2-OHE_2-4-SG, 4-OHE_2-2-SG) or they form quinone–DNA adducts (e.g. 4-OHE_2-N7-guanine) or oxidative DNA adducts via quinone–semiquinone redox cycling (e.g. 8-OH-deoxyguanosine). The same pathway applies to estrone. The thicker arrows indicate preferential reactions.

(ii) *Estrogen glucuronides*

Estradiol and estrone and their respective catechols are recognized as substrates by various isoforms of the uridine-5′ diphosphate (UDP)-glucuronyltransferase (UGT) enzyme family. Several isoforms were more active towards catechol estrogens than towards the parent hormones (Albert *et al.*, 1999; Turgeon *et al.*, 2001). The resulting glucuronidated metabolites are more hydrophilic and can be excreted in bile and urine.

(iii) *Estrogen fatty acid esters*

Several steroids, including estradiol, have been shown to undergo esterification to long-chain fatty acids in a number of mammalian tissues (Hochberg, 1998). The responsible enzyme, fatty acyl-coenzyme A (CoA):estradiol-17β-acyltransferase, has a pH optimum of

5–5.5, which distinguishes it from the related enzyme, acyl-CoA:cholesterol acyltransferase (optimal pH ~7.0) (Xu *et al*., 2001a,b). The fatty acyl-CoA:estradiol-17β-acyltransferase shows specificity for the D-ring, especially the C-17β group of the estrogen molecule. The vicinity of a bulky 16α-hydroxy group appears to hamper the accessibility to the C-17β hydroxyl, which results in a reduced rate (28%) of esterification of estriol compared with estradiol (Pahuja *et al*., 1991). The D-ring esterification of estradiol has two effects: (i) the bulky fatty acid moiety prevents the binding of estradiol fatty acid to the estrogen receptor; and (ii) the fatty acid moiety shields the D-ring from oxidative metabolism to estrone. Thus, estradiol fatty acid may play a role in the action of estrogen by affecting the intracellular equilibrium between estrone and estradiol.

In the circulation, estradiol fatty acids are mainly bound by plasma lipoproteins; the majority (54%) are recovered in the high-density lipoprotein (HDL) and 28% in the low-density lipoprotein (LDL) fractions (Vihma *et al*., 2003a). They are present in very small amounts in the blood of premenopausal women, although their concentration increases 10-fold during pregnancy, from 40 pmol/L in early pregnancy to 400 pmol/L in late pregnancy (Vihma *et al*., 2001). Treatment of postmenopausal women with either oral or transdermal estradiol for 12 weeks resulted in a differential effect on serum estradiol fatty acids and non-esterified estradiol. Both types of application led to similar median concentrations of free (non-protein-bound) estradiol but only the oral therapy caused an increase (27%) in median serum estradiol fatty acid (Vihma *et al*., 2003b). The change during treatment in serum concentrations of estradiol fatty acid, but not those of non-esterified estradiol correlated positively with enhanced forearm blood flow responses *in vivo*. These data suggest that an increase in serum estradiol fatty acid may contribute to the effects of oral treatment with estradiol, compared with those of an equipotent transdermal dose.

(iv) *Oxidative metabolism*

Estradiol and estrone undergo extensive oxidative metabolism via the action of several cytochrome P450 (CYP) monooxygenases. Each CYP favours the hydroxylation of specific carbons, altogether, the CYP enzymes can hydroxylate virtually all carbons in the steroid molecule, with the exception of the inaccessible angular carbons 5, 8, 9, 10 and 13 (Badawi *et al*., 2001; Lee *et al*., 2001, 2002, 2003a,b; Kisselev *et al*., 2005). The generation of hydroxyl and keto functions at specific sites of the steroid nucleus markedly affects the biological properties of the respective estrogen metabolites, i.e. different hydroxylation reactions yield estrogenic, non-estrogenic or carcinogenic metabolites. Quantitatively and functionally, the most important reactions occur at carbons 2, 4 and 16.

Catechol estrogens

2- and 4-Hydroxyestrone, -estradiol and -estriol have been shown to serve a physiological function, to have some hormonal activity and to be substrates in the oxidative estrogen metabolism pathway. In their physiological function, they mediate the activation of dormant blastocysts for implantation into the receptive uterus. Specifically, 4-hydroxyestradiol produced in the uterus from estradiol mediates blastocyst activation for implanta-

tion in a paracrine manner. This effect is not mediated by the estrogen receptor but via prostaglandin synthesis (Paria *et al*., 1998, 2000). The oxidative metabolism of estrogens to catechol estrogens is generally thought to terminate the estrogenic signal, although catechol estrogens retain some binding affinity to the estrogen receptor. Treatment of MCF-7 cells with 2- and 4-hydroxyestradiol increased the rate of cell proliferation and the expression of estrogen-inducible genes such as the progesterone receptor (*PR*) gene and *pS2*. Relative to estradiol, 2- and 4-hydroxyestradiol increased proliferation rate, level of PR protein and *pS2* mRNA expression by 36 and 76%, 10 and 28% and 48 and 79%, respectively (Schütze *et al*., 1993, 1994).

Catechol estrogens occupy a key position in the oxidative pathway of estrogen metabolism (see Figures 3 and 4). They are products as well as substrates of CYP1A1 and CYP1B1 (Hachey *et al*., 2003; Dawling *et al*., 2004). Specifically, CYP1A1 converts estradiol firstly to 2-hydroxyestradiol and then to the estradiol-2,3-semiquinone and estradiol quinone. CYP1B1 converts estradiol firstly to 2- as well as to 4-hydroxyestradiol and then to the corresponding semiquinones and quinones. Estrone is metabolized in a similar manner by CYP1A1 and CYP1B1 (Lee *et al*., 2003a). The catechol estrogens also serve as substrates for catechol-*O*-methyltransferase (COMT), which catalyses *O*-methylation by forming monomethyl ethers at the 2-, 3- and 4-hydroxyl groups. Conjugated equine estrogens are also substrates for COMT (Yao *et al.*, 2003). COMT generated two products from 2-hydroxyestrogens, but only one product from 4-hydroxyestrogens (Dawling *et al*., 2001; Lautala *et al*., 2001; Goodman *et al*., 2002). With 2-hydroxyestradiol and 2-hydroxyestrone, COMT catalysed the methylation of the 2- and 3-hydroxy groups, which resulted in the formation of 2-methoxyestradiol and 2-hydroxy-3-methoxyestradiol and 2-methoxyestrone and 2-hydroxy-3-methoxyestrone, respectively. In contrast, for 4-hydroxyestradiol and 4-hydroxyestrone, methylation occurred only at the 4-hydroxyl group, which resulted in the formation of 4-methoxyestradiol and 4-methoxyestrone, respectively. 3-Methoxy-4-hydroxyestradiol and -estrone were not produced by COMT.

The observation that catechol estrogens are carcinogenic in animal experiments (IARC, 1999) has prompted studies in human tissues. Examination of microsomal estradiol hydroxylation in human breast cancer showed significantly higher 4-hydroxy-:2-hydroxyestradiol ratios in tumour tissue than in adjacent normal breast tissue (Liehr & Ricci, 1996), while the breast cancer tissue samples contained fourfold higher levels of 4-hydroxyestradiol than normal tissue from benign breast biopsies (Rogan *et al*., 2003). Comparison of intra-tissue concentrations of estrogens (estrone, estradiol, estriol), hydroxyestrogens (16α-hydroxyestrone, 2-hydroxyestrone, 2-hydroxyestradiol, 4-hydroxyestrone, 4-hydroxyestradiol) and methoxyestrogens (2-methoxyestrone, 2-methoxyestradiol, 4-methoxyestrone, 4-methoxyestradiol) in normal and malignant breast revealed the highest concentration of 4-hydroxyestradiol in malignant tissue (Castagnetta *et al*., 2002). The concentration (1.6 nmol/g tissue) determined by combined high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) was more than twice as high as that of any other compound. [The Working Group noted that such high levels in neoplastic mammary

tissue suggests a mechanistic role of 4-hydroxyestradiol in tumour development; see also Section 4.4.]

16α-Hydroxyestrogens

An analysis of 15 CYP isozymes showed that CYP1A1, 3A4, 3A5 and 2C8 catalysed the 16α-hydroxylation of both estrone and estradiol (Badawi *et al*., 2001; Lee *et al*., 2003a,b). In contrast, CYP3A7 distinguished the two estrogen substrates with > 100 times higher maximum velocity of the enzyme:Michaelis-Menten constant (V_{max}:K_m) ratio for the 16α-hydroxylation of estrone than that of estradiol. The difference in reaction rates is most probably due to the difference in structure at the C-17 position of estrone and estradiol. The presence of the 17-ketogroup in estrone appears to be essential for recognition of the substrate and 16α-hydroxylation by CYP3A7 (Lee *et al*., 2003b).

Similarly to catechol estrogens, 16α-hydroxylated estrogens are hormonally active, chemically reactive and potentially mutagenic. 16α-Hydroxyestrone possesses the unique property of binding covalently to the estrogen receptor and other nuclear proteins, such as histones. Mechanistically, a Schiff base is formed from 16α-hydroxyestrone by a reaction with amino groups in proteins. The Schiff base, in turn, undergoes Heyns rearrangement to result in the formation of a stable 16-keto-17β-amino estrogen adduct (Miyairi *et al*., 1999).

Bradlow *et al.* (1996) proposed that increased formation of 16α-hydroxyestrone and estriol may be associated with an increased risk for developing breast cancer. They presented the hypothesis that the ratio of the two urinary metabolites 2-hydroxyestrone:16α-hydroxyestrone is inversely correlated with the risk for breast cancer. They chose the numerator 2-hydroxyestrone to reflect the 'good' C-2 hydroxylation and the denominator 16α-hydroxyestrone to reflect the 'bad' C-16α hydroxylation pathways of estrogen metabolism. Enzyme immunoassays for simultaneous quantitation of 2- and 16α-hydroxyestrone levels in urine have been developed and improved to correlate with results obtained by GC–MS (Falk *et al*., 2000). The enzyme immunoassay has been applied to the analysis of blood samples from premenopausal women. Current users of oral contraceptives had a significantly lower plasma 2-hydroxyestrone:16α-hydroxyestrone ratio than non-users ($p = 10^{-21}$) (Jernstrom *et al*., 2003b).

Results from epidemiological studies on the association between 2- and 16α-hydroxylation and breast cancer are inconsistent. Several case–control studies found an increased risk for breast cancer associated with a lower 2-hydroxyestrone:16α-hydroxyestrone ratio (Ho *et al*., 1998; Zheng *et al*., 1998), while other groups did not observe a difference in this ratio between controls and patients (Ursin *et al*., 1999). All of these studies measured metabolites after the diagnosis of breast cancer, which raises the possibility that the results may have been affected by the tumour. Two prospective studies addressed this issue but also yielded inconsistent results. The first was carried out in women on the island of Guernsey, United Kingdom. Urine samples were collected and stored in the 1970s when all women were healthy. Almost 20 years later, Meilahn *et al*. (1998) analysed the samples and

reported a median of 1.6 for the 2-hydroxyestrone:16α-hydroxyestrone ratio in 42 postmenopausal women who had developed breast cancer and 1.7 in 139 matched control subjects. Compared with women in the lowest tertile category of 2:16α-hydroxyestrone ratio, women in the highest tertile had an odds ratio for breast cancer of 0.71, but the 95% CI was wide and was not statistically significant (95% CI, 0.29–1.75). Analysis of premenopausal women in the Guernsey cohort showed no difference between cases and controls. The second prospective study of Italian women had a shorter average follow-up of 5.5 years (Muti *et al*., 2000). The odds ratio in postmenopausal women was 1.31 (95% CI, 0.53–3.18). In the premenopausal group, women in the highest quintile of the 2:16α-hydroxyestrone ratio had an adjusted odds ratio of 0.55 (95% CI, 0.23–1.32). A third type of epidemiological study examined urinary metabolites in women of different ethnic groups that are known to have different rates of breast cancer. One study examined healthy postmenopausal women randomly selected from the Singapore Chinese Health Study (67 subjects) and the Los Angeles Multiethnic Cohort Study (58 subjects). Although the incidence of breast cancer is substantially lower in Singaporean women than among American women, there were no significant differences between the groups in urinary 16α-hydroxyestrone levels or 2:16α-hydroxyestrone ratios (Ursin *et al*., 2001). Finally, no differences were found in premenopausal women with or without a family history of breast cancer (Ursin *et al*., 2002).

(v) *Methoxyestrogens*

Methoxyestrogens are methyl ether metabolites of catechol estrogens produced by COMT. In addition, 2-methoxyestradiol is not just a by-product of estrogen metabolism but is also endowed with antiproliferative activity. It has been shown to inhibit the proliferation of both hormone-dependent and hormone-independent breast cancer cells (LaVallee *et al*., 2003). The antiproliferative effect is not limited to breast cancer cells but extends to leukaemia, and pancreatic and lung cancer cells (Schumacher *et al*., 1999; Huang *et al*., 2000). Human xenograft studies in animal models have demonstrated the oral bioavailability and a high therapeutic index of methoxyestrogens with no sign of systemic toxicity. These features and their broad antitumour activity against a variety of tumour cells have led to the current testing of methoxyestrogens as potential therapeutic agents in clinical trials (Pribluda *et al*., 2000; Schumacher & Neuhaus, 2001). Several synthetic analogues were equally as effective as 2-methoxyestradiol or were even more potent than the endogenous compound (Wang *et al*., 2000; Brueggemeier *et al*., 2001; Tinley *et al*., 2003).

The antiproliferative effect of 2-methoxyestradiol appears to be concentration-dependent and to involve several mechanisms. At nano- and micromolar concentrations, 2-methoxyestradiol disrupted microtubule function, induced apoptosis and inhibited angiogenesis (Klauber *et al*., 1997; Yue *et al*., 1997; Huang *et al*., 2000; LaVallee *et al*., 2003). At concentrations ≥ 1 μM , it caused chromosome breaks and aneuploidy (Tsutsui *et al*., 2000).

Methoxyestrogens are also substrates for CYP1A1 and CYP1B1, which catalyse their *O*-demethylation to catechol estrogens, and thus effectively reverse the COMT reaction by

which they were formed (Dawling *et al*., 2003). Specifically, both CYP1A1 and CYP1B1 demethylated 2-methoxy- and 2-hydroxy-3-methoxyestradiol to 2-hydroxyestradiol, and CYP1B1 additionally demethylated 4-methoxyestradiol to 4-hydroxyestradiol. Thus, CYP1A1 and CYP1B1 recognize as substrates both the parent hormone estradiol and the methoxyestrogens, 2-methoxy-, 2-hydroxy-3-methoxy- and 4-methoxyestradiol. Kinetic analysis showed that estradiol and the methoxyestrogens are alternate substrates, each of which is catalysed by the same enzyme but by a different type of reaction (Dawling *et al*., 2003). Because they are converted to identical catechol estrogen products, each inhibits formation of 2- and 4-hydroxyestradiol from the other substrate in a non-competitive manner. It has been proposed that methoxyestrogens exert feedback inhibition on CYP1A1 and CYP1B1, which affects the entire oxidative metabolic pathway of estrogen in several ways. First, CYP1A1 and CYP1B1 generate catechol estrogen substrates for COMT and, at the same time, compete with COMT by converting the catechol estrogens to estrogen quinones. In turn, the methoxyestrogens generated by COMT are alternate substrates for CYP1A1 and CYP1B1 and inhibit oxidation of the parent hormone estradiol (and most probably also that of the catechol estrogens). Second, the inhibition occurs at a strategic point in the pathway where it branches into 2- and 4-hydroxycatechol estrogens. This may be important in view of the apparent difference in carcinogenicity of these two substances (Liehr & Ricci, 1996; Cavalieri *et al*., 2000). Third, all three products of the COMT-mediated reaction (i.e. 2-methoxy-, 2-hydroxy-3-methoxy- and 4-methoxyestradiol) act as inhibitors, and thereby maximize the feedback regulation (Dawling *et al*., 2003). Fourth, the feedback regulation occurs at the step in the pathway that precedes the conversion to estrogen semiquinones and quinones, and thereby reduces the formation of reactive oxygen species during semiquinone–quinone redox cycling and the potential for estrogen-induced DNA damage (Dawling *et al.*, 2003).

(vi) *Estrogen–glutathione conjugates*

The labile estrogen quinones react with a variety of physiological compounds, including amino acids such as lysine and cysteine and the tripeptide, glutathione (γ-glutamyl-cysteinyl-glycine, GSH) (Cao *et al*., 1998). MS analysis of the GSH–estrogen isomers revealed that the catechol estrogen attachment is at the cysteine moiety of GSH, and the cysteine sulfur binds to an A-ring carbon vicinal to the catechol carbons, i.e. C-1 or C-4 in 2-hydroxyestradiol and C-2 in 4-hydroxyestradiol (Ramanathan *et al*., 1998). Thus, the point of attachment of the -*S*-glutathione (-SG) moiety is always directly adjacent to an oxygen-bearing carbon, in line with all other known quinone–GSH conjugates (Bolton *et al*., 2000).

GSH is the most abundant intracellular non-protein thiol and is found at concentrations that range from 0.1 to 10 mM. In an in-vitro study, Hachey *et al*. (2003) used 0.1 mM GSH and recombinant, purified glutathione *S*-transferase P1 (GSTP1) and estradiol and observed a faster rate of estrogen quinone conjugation in the presence of GSTP1 than in the absence of the enzyme. 2-Hydroxyestradiol and 4-hydroxyestradiol did not form conjugates with GSH alone or in the presence of GSTP1. These data indicate that the enzy-

matic conversion of catechol estrogens to estrogen quinones by CYP1B1 is a necessary step for the subsequent GSH conjugation reaction. The enzymatic reaction with GSTP1 yielded only mono-conjugates, i.e. 2-hydroxyestradiol-1-SG, 2-hydroxyestradiol-4-SG and 4-hydroxyestradiol-2-SG. There was no evidence of bis-conjugates, such as 2-hydroxyestradiol-1,4-bisSG and 4-hydroxyestradiol-1,2-bisSG. GSTP1 is also a target for equine catechol estrogens (Yao *et al.*, 2002). Equine catechol significantly decreased GSH levels and the activity of GSTP1-1 in human breast cancer cells.

All GSH conjugates are catabolized via the mercapturic acid pathway. First, the glutamyl moiety is removed from the GSH conjugate by transpeptidation, which is catalysed by γ-glutamyl transpeptidase. The resulting cysteinylglycine conjugate is then hydrolysed by cysteinyl-glycine dipeptidase to yield the cysteine conjugate. The final step entails acetylation to the *N*-acetylcysteine conjugate, a mercapturic acid compound. Estrogen–GSH conjugates are excreted in the urine mostly as *N*-acetylcysteine conjugates but also as cysteine conjugates (Todorovic *et al.*, 2001). Thus, estrogen quinones are detoxified in tissues by GST-mediated GSH conjugation and the resultant GSH conjugates are catabolized to *N*-acetylcysteine conjugates that are readily excreted.

(*b*) *Enzymes*

(i) *CYP1A1*

Although other CYP enzymes, such as CYP1A2 and CYP3A4, are involved in hepatic and extrahepatic hydroxylation of estrogen, CYP1A1 and CYP1B1 display the highest level of expression in breast tissue (reviewed by Jefcoate *et al*., 2000; Lee *et al*., 2003a). The human gene for CYP1A1 is polymorphic. Apart from the wild-type (*CYP1A1*1*), 10 alleles have been described in different populations. However, several are very rare and of unknown functional significance (Karolinska Institutet, 2007). The most common alleles that result in amino acid substitutions are *CYP1A1*2* (462Ile → Val) and *CYP1A1*4* (461Thr → Asn). Kisselev *et al*. (2005) expressed and purified CYP1A1.1, CYP1A1.2 and CYP1A1.4 proteins and performed enzymatic assays of estrogen hydroxylation in reconstituted CYP1A1 systems. All three CYP1A1 isoforms catalysed the hydroxylation of estradiol and estrone to 2-, 15α-, 6α- and barely detectable 4-hydroxylated estrogen metabolites. The CYP1A1.2 variant had a significantly higher catalytic activity, especially for 2-hydroxylation. The catalytic efficiencies for 2-hydroxyestradiol and 2-hydroxyestrone were 5.7- and 12-fold higher, respectively, compared with the wild-type enzyme. Several studies found no overall association between the risk for breast cancer and the polymorphisms in codons 461 and 462 (Huang *et al*., 1999; reviewed by Mitrunen & Hirvonen, 2003).

In addition to genetic variation, there is a striking interindividual variation in *CYP1A1* expression. For example, Goth-Goldstein *et al*. (2000) measured *CYP1A1* mRNA expression in 58 non-tumour breast specimens from 26 breast cancer patients and 32 cancer-free individuals by reverse transcription-PCR. *CYP1A1* expression varied between specimens by ~400-fold and was independent of *CYP1A1* genotype and age of the patient.

A second study used quantitative immunoblotting of normal and malignant breast tissues and observed ~150-fold differences in CYP1A1 protein expression between individuals (El-Rayes *et al*., 2003). Attempts to explain such a high degree of interindividual variation in CYP1A1 expression have focused primarily on genetic polymorphisms within the *CYP1A1* gene with inconsistent results. Since the expression of CYP1A1 is induced via the aryl hydrocarbon receptor (AhR), Smart and Daly (2000) extended the investigation to the *AhR* gene, and observed that *AhR*-mediated induction of CYP1A1 appears to be influenced by the 1721G → A (554Arg → Lys) polymorphism in exon 10 of the *AhR* gene. The 554Arg residue lies close to the transactivation domain of the AhR protein. Individuals who had at least one copy of the variant 1721A allele showed significantly higher levels of CYP1A1 activity compared with individuals who were negative for the polymorphism ($p = 0.0001$). Levels of 3-methylcholanthrene-induced CYP1A1 activity in lymphocytes also varied by sex: women exhibited significantly lower activity than men (Smart & Daly, 2000). The authors suggested that interindividual variation in levels of CYP1A1 activity appears to be associated more with regulatory factors than with polymorphisms in the *CYP1A1* gene.

(ii) *CYP1B1*

CYP1B1 is the main enzyme that converts estradiol to 4-hydroxyestradiol (Jefcoate *et al*., 2000). Since animal studies have implicated 4-hydroxyestradiol in the development of cancer, the expression of CYP1B1 in hormone-responsive tissues such as the breast has attracted interest. Murray *et al.* (2001) performed several immunohistochemical studies of CYP1B1 expression in the breast. Breast cancer tissue but not normal breast tissue expressed CYP1B1. Forty-six of 60 (77%) invasive breast cancers showed cytoplasmic staining of tumour cells, which ranged from strong in 10 to moderate in 12 and weak in 24 cases. There was no relationship between the presence of CYP1B1 and the histological type or grade of the tumour, the presence of lymph node metastasis or estrogen receptor status (McFadyen *et al*., 1999). Immunohistochemical analysis of CYP1B1 expression also revealed cytoplasmic staining in a wide range of other cancers of different histogenetic types, including cancers of the colon, oesophagus, lung, brain and testis. Similar to the breast, no immunostaining occurred in corresponding normal tissues (Murray *et al.*, 1997). These findings contradict the observation that normal human mammary epithelial cells isolated and cultured from reduction mammoplasty tissue of seven individual donors expressed significant levels of CYP1B1 (< 0.01–1.4 pmol/mg microsomal protein) as determined by immunoblot analysis (Larsen *et al*., 1998). The discrepancy between the studies regarding the presence of CYP1B1 protein in normal mammary epithelium may be due to the use of different antibodies, to the induction of CYP1B1 as a result of the isolation of the mammary epithelial cells from mammoplasty tissue or to their in-vitro culture over 6 days (Murray *et al*., 2001).

Several polymorphisms have been identified in the *CYP1B1* gene, four of which are associated with amino acid substitutions: 48Arg → Gly, 119Ala → Ser, 432Val → Leu and 453Asn → Ser (Stoilov *et al*., 1998; McLellan *et al*., 2000). There is considerable ethnic variation in the frequency of these polymorphisms. For example, the 432Val allele

is present in approximately 70% of African-Americans, 40% of Caucasians and less than 20% of Chinese (Bailey *et al*., 1998; Tang *et al*., 2000).

Several investigators have examined the effect of *CYP1B1* polymorphisms on enzyme function (Shimada *et al*., 1999; Hanna *et al*., 2000; Li, D.N. *et al*., 2000; McLellan *et al*., 2000; Lewis *et al*., 2003). Although all studies analysed the 4- and 2-hydroxylation of estradiol by CYP1B1, a comparison of the results needs to take into account differences in expression systems (bacteria, yeast), assay conditions (microsomal membranes, purified proteins) and the type of analysis of estrogen metabolites (HPLC, GC–MS). Some studies also provided an incomplete definition of constructs, i.e. only two or three of the four amino acids were listed. For these reasons, the results are inconsistent, although it appears that there is at best a two- to threefold difference in catalytic activity between wild-type CYP1B1 and any variant isoform.

Several studies have examined the association of *CYP1B1* polymorphisms with the risk for breast and endometrial cancer. Two case–control studies that involved 1355 Caucasian and African-American women found no association with the risk for breast cancer (Bailey *et al*., 1998; De Vivo *et al*., 2002). Another case–control study of 186 Asian cases of breast cancer and 200 Asian controls found that women with the 432Leu/Leu genotype had a 2.3-fold (95% CI, 1.2–4.3) elevated risk for breast cancer compared with women with the 432Val/Val genotype (Zheng *et al*., 2000). Sasaki *et al*. (2003) examined 113 Japanese patients with endometrial cancer and 202 healthy controls. Women who had the homozygous 119Ser/Ser and 432Val/Val genotypes had relative risks for endometrial cancer of 3.32 (95% CI, 1.38–8.01) and 2.49 (95% CI, 1.10–5.66) compared with those who had wild-type *CYP1B1*. McGrath *et al*. (2004) examined codons 432 and 453 in women who had endometrial cancer within the Nurses' Health Study (222 cases, 666 controls). Carriers of the 453Ser allele had a significantly decreased risk for endometrial cancer (odds ratio, 0.62; 95% CI, 0.42–0.91), and there was no association with the 432Val $\rightarrow$ Leu polymorphism. A case–control study of postmenopausal Swedish women (689 cases, 1549 controls) examined polymorphisms at codons 119, 432 and 453 and found no evidence for an association between *CYP1B1* genotype and risk for endometrial cancer (Rylander-Rudqvist *et al*., 2004). However, two studies observed an association of the 432Val/Val genotype with expression of estrogen receptor in breast cancer patients (Bailey *et al*., 1998; De Vivo *et al*., 2002). Another study noted a significant association between the 119Ser/Ser genotype and expression of estrogen receptors α and β in endometrial cancer patients (Sasaki *et al*., 2003). One study of postmenopausal women found that carriers of the 432Leu and 453Ser alleles had modestly higher plasma levels of estradiol but similar levels of estrone and estrone sulfate (De Vivo *et al*., 2002), while another study found no such association (Tworoger *et al*., 2004). The 432Leu $\rightarrow$ Val polymorphism was also investigated in relation to other cancers and showed no association with lung cancer but increased risks for ovarian cancer associated with the 432Leu allele and for prostate cancer associated with the 432Val allele (Tang *et al*., 2000; Watanabe *et al*., 2000; Goodman, M.T. *et al*., 2001).

(iii) *Catechol*-O-*methyltransferase (COMT)*

The enzymatic activity of recombinant, purified COMT has been determined for methylation of the catechol estrogen substrates 2- and 4-hydroxyestradiol and 2- and 4-hydroxyestrone (Dawling *et al*., 2001; Lautala *et al*., 2001; Goodman *et al*., 2002). COMT catalysed the formation of monomethyl ethers at the 2-, 3- and 4-hydroxyl groups. Dimethyl ethers were not observed. The rates of methylation of 2-hydroxyestradiol and 2-hydroxyestrone yielded typical hyperbolic patterns, whereas those of 4-hydroxyestradiol and 4-hydroxyestrone exhibited a sigmoid curve pattern (Dawling *et al*., 2001). Thus, COMT interacts differently with the 2- and 4-hydroxyestrogen substrates. Methylation of 2-hydroxyestrogen substrates exhibits Michaelis-Menten saturation kinetics and yields two products, i.e. 2- and 3-methoxyestrogens. In contrast, the methylation of 4-hydroxyestrogen substrates displays sigmoid saturation kinetics that indicates cooperative binding and yields only a single product, i.e. 4-methoxyestrogen. The main structural difference between 2- and 4-hydroxy catechol estrogens is the proximity of the 4-hydroxyl group to the B-ring of the steroid. The 2- and 3-hydroxyl groups in 2-hydroxyestrogen appear to be similar in reactivity, whereas the 3- and 4-hydroxyl groups in 4-hydroxyestrogen differ in reactivity to the point that, in the latter, only the 4-hydroxyl group becomes methylated.

Dawling *et al*. (2001) compared the enzymatic activity of wild-type (108Val) COMT with that of the common variant (108Met). The 108Met variant, unlike wild-type COMT, was thermolabile, and led to two- to threefold lower levels of production of methoxyestrogen. These results differ from those of Goodman *et al*. (2002) but are in agreement with two other studies (Lachman *et al*., 1996; Syvänen *et al*., 1997). Dawling *et al*. (2001) developed an enzyme-linked immunosorbent assay to quantify COMT in breast cancer cell lines and determined that ZR-75 and MCF-7 cells contain similar amounts of COMT, but differ in genotype and enzymatic activity. The catalytic activity of variant COMT in MCF-7 cells was two- to threefold lower than that of wild-type COMT in ZR-75 cells. Since COMT is expressed ubiquitously, it appears that the *COMT* genotype significantly affects levels of catechol estrogens throughout the body. However, Goodman *et al*. (2002) found no difference between breast cancer cell lines of different *COMT* genotypes (MCF-10A and ZR-75-1 with high activity allele $COMT^{HH}$, and NCF-7 and T47D with low activity allele $COMT^{LL}$), except for the formation of 2-methoxyestradiol.

Immunohistochemical analysis of benign and malignant breast tissue revealed the presence of COMT in the cytoplasm of all epithelial cells. Immunoreactive COMT was also observed in the nucleus of some benign and malignant epithelial cells. There was no correlation between histopathology and the number of cells with nuclear COMT, size of foci that contained such cells or intensity of nuclear COMT immunostaining. Staining of both intra- and interlobular stromal cells was always of a much lower intensity than that of epithelial cells in the same tissue sections (Weisz *et al.*, 2000).

Several epidemiological studies have examined the association of *COMT* genotype with the risk for breast cancer. A meta-analysis of 13 studies published through to July 2004 did not support the hypothesis that the low-activity variant of COMT, as a single factor, leads to increased risk for breast cancer (Wen *et al*., 2005). However, Goodman, J.E. *et al*.

(2001) observed an association between the risk for breast cancer, *COMT* genotype and micronutrients in the folate metabolic pathway. These micronutrients (i.e. cysteine, homocysteine, folate, vitamin B12, pyridoxal 5′-phosphate) are known to influence levels of the methyl donor *S*-adenosylmethionine, and *S*-adenosylhomocysteine, a COMT inhibitor that is generated by the demethylation of *S*-adenosylmethionine. High-activity homozygous *COMT*1* cases of breast cancer had significantly lower levels of homocysteine ($p = 0.05$) and cysteine ($p = 0.04$) and higher levels of pyridoxal 5′-phosphate ($p = 0.02$) than homozygous *COMT*1* controls. In contrast, low-activity homozygous *COMT*2* cases had higher levels of homocysteine ($p = 0.05$) than low-activity homozygous *COMT*2* controls. An increase in the number of *COMT*2* alleles was significantly associated with an increased risk for breast cancer in women with levels of folate below the median (p for trend = 0.05) or levels of homocysteine above the median (p for trend = 0.02). No association was seen between vitamin B12, *COMT* genotype and risk for breast cancer (Goodman, J.E. *et al*., 2001). These findings are consistent with a role of certain folate pathway micronutrients in the mediation of the association between *COMT* genotype and the risk for breast cancer. At the same time, these results illustrate the complex interaction of genetic and nutritional factors in the development of breast cancer. Equally complex is the interaction of the C*OMT* genotype with other risk factors such as mammographic density (Hong *et al*., 2003).

Lavigne *et al*. (2001) examined the effect of estrogen metabolism on oxidative DNA damage (8-hydroxy-2′-deoxyguanosine [8-OH-dG]) in 2,3,7,8-tetrachlorodibenzo-*para*-dioxin-pretreated MCF-7 cells exposed to estradiol with and without Ro41-0960, a specific inhibitor of COMT. Administration of the COMT inhibitor blocked the formation of 2-methoxyestradiol and, at the same time, increased the levels of 2-hydroxyestradiol and 8-OH-dG. During inhibition of COMT, increased oxidative DNA damage was detected in MCF-7 cells exposed to concentrations of estradiol as low as 0.1 μM, whereas, when COMT was not inhibited, no increase in 8-OH-dG was detected at concentrations of estradiol ≤ 10 μM. These results demonstrate that COMT activity is protective against oxidative DNA damage associated with catechol estrogen metabolites. In the absence of COMT activity and methoxyestrogens, a linear relation was observed between levels of 2- plus 4-hydroxyestradiol and 8-OH-dG. However, this relationship did not remain under experimental conditions that allowed limited formation of methoxyestrogens (when cells were treated with a lower concentration of COMT inhibitor), i.e. 8-OH-dG levels were lower than those expected for a given concentration of 2- plus 4-hydroxyestradiol in the presence of 2-methoxyestradiol. The authors suggested that 2-methoxyestradiol may reduce the formation of 8-OH-dG.

(iv) *Glutathione* S-*transferases*

Hachey *et al*. (2003) determined that GSTP1 and CYP1B1 are coordinated in sequential reactions, i.e. 4- and 2-hydroxyestradiol did not form GSH conjugates in the presence of GSTP1 unless they were first oxidized by CYP1B1 to their corresponding quinones. CYP1B1 metabolized estradiol to two products, 4- and 2-hydroxyestradiol, and further to

estradiol-3,4-quinone and estradiol-2,3-quinone, while GSTP1 formed three products, 4-hydroxyestradiol-2-SG, 2-hydroxyestradiol-4-SG, and 2-hydroxyestradiol-1-SG, the last of which in smaller amounts. The rate of conjugation was in the order 4-hydroxyestradiol-2-SG > 2-hydroxyestradiol-4-SG >> 2-hydroxyestradiol-1-SG, which indicated a difference in the regiospecific reactivity of the two quinones. Estradiol-2,3- and estradiol-3,4-quinones are products of CYP1B1- and substrates of GSTP1-mediated reactions but also react non-enzymatically with other nucleophiles, as indicated by a 10-fold concentration gap between catechol estrogens and GSH–estrogen conjugates. It has been suggested that, although both reactions are coordinated qualitatively in terms of product formation and substrate utilization, the quantitative gap would enable the accumulation of estrogen quinones and their potential for DNA damage.

Based on protein levels, GSTP1 is the most important member of the GST family expressed in breast tissue (Kelley *et al.*, 1994; Alpert *et al*., 1997). However, two other GST isoforms, GSTM1 and GSTA1, are also expressed in mammary epithelium, although at lower levels. About 50% of Caucasian and 30% of African women possess the *GSTM1* null genotype and therefore completely lack GSTM1 expression in all tissues including the breast (Garte *et al*., 2001). GSTs are known to have selective as well as overlapping substrate specificities. It is unknown at present whether GSTM1 and GSTA1 are capable of conjugating estrogen quinones similarly to GSTP1 (Hachey *et al*., 2003).

The *GSTP1* gene also possesses two polymorphisms in codons 104 (Ile → Val) and 113 (Ala → Val) that are associated with altered catalytic activity towards polycyclic aromatic hydrocarbons (Hu *et al*., 1997; Ji *et al*., 1999). It is unknown at present whether the GSTP1-mediated conjugation of estrogen quinones varies between the *GSTP1* wild-type and its variants.

Several epidemiological studies found no overall association between polymorphism in *GSTP1* codon 104 and the risk for breast cancer (reviewed by Mitrunen & Hirvonen, 2003). The polymorphic allele in codon 113 showed a tendency for an increased risk in one study and a protective effect in another (Krajinovic *et al.*, 2001; Maugard *et al.*, 2001). A comprehensive review of 15 studies of *GSTM1* published through to 2002 found no overall evidence for an association of the *GSTM1* null genotype with risk for breast cancer (Mitrunen & Hirvonen, 2003).

(v) *Uridine-5′ diphosphate (UDP)-glucuronosyltransferases*

The UDP-glucuronosyltransferase (UGT) superfamily currently consists of 16 functional genes that are organized into two families of enzymes, UGT1 and UGT2 (King *et al*., 2000; Tukey & Strassburg, 2000). The study of UGTs was initiated by the hypothesis that UGT-mediated estrogen conjugation reduces catechol estrogen levels and thereby decreases the risk for breast cancer (Raftogianis *et al*., 2000). Similarly to the sulfotransferase (SULT) superfamily, several UGT isoforms are capable of estrogen conjugation, i.e. UGT1A1, -1A3, -1A7, -1A8, -1A9, -1A10, -2B4, -2B7, -2B11 and -2B15 (Lévesque *et al*., 1999; King *et al*., 2000; Turgeon *et al*., 2001). Although a comprehensive study of all known UGTs has not yet been performed, individual studies indicate that, of those tested,

UGT1A1, -1A3, -1A8, -1A9 and -2B7 have the highest activity toward estrogens (Albert *et al*., 1999; Tukey & Strassburg, 2000; Turgeon *et al*., 2001; Vallée *et al*., 2001). The parent hormones, estradiol and estrone, and their respective catechols are recognized as substrates, but individual isoforms display distinct differences in substrate specificity and conjugation efficiency. Comparison of UGT1A3 and -2B7 showed regioselective conjugation of estradiol, i.e. UGT1A3 only conjugated the C-3 hydroxyl group of the A-ring, whereas UGT2B7 conjugated the 17β-hydroxyl in the D-ring, to yield estradiol-3 and 17β-glucuronides, respectively (Gall *et al*., 1999). Several isoforms, including UGT1A1, -1A9 and -2B7, were more active towards the catechol estrogens than the parent hormones. In contrast, comparison of catechol estrogen substrates revealed that UGT1A1 and -1A3 were more active toward 2-hydroxyestradiol, while UGT1A9 and -2B7 conjugated 4-hydroxyestradiol more efficiently (Cheng *et al*., 1998; Albert *et al*., 1999). Although the catechols derived from estradiol and estrone are generally metabolized with similar efficiencies, UGT2B7 displayed seven- to 12-fold higher activity (1320 pmol/min/mg microsomal protein) towards 4-hydroxyestrone than 4-hydroxyestradiol, in spite of similar apparent K_m values (Cheng *et al*., 1998; Turgeon *et al*., 2001). The highest activity was recorded for the UGT1A9-mediated conjugation of 4-hydroxyestradiol (2500 pmol/min/mg) (Albert *et al*., 1999).

Few studies have examined UGT expression in breast tissue, and have usually been limited to the detection of the transcript. Of the isoforms with the highest activity toward estrogen conjugation, *UGT1A9* mRNA was detectable in breast tissue whereas *UGT1A1* mRNA was not detected (Albert *et al*., 1999; Vallée *et al*., 2001). UGT2B7 appears to be the only isoform that has been examined for both transcript and protein. *UGT2B7* transcript was present in normal mammary tissue, but not in T47D and ZR-75 breast cancer cells (Turgeon *et al*., 2001). A detailed immunohistochemical study (Gestl *et al*., 2002) demonstrated expression of UGT2B7 protein in normal mammary epithelium obtained from either reduction mammoplasties or tissue distant from invasive cancer in mastectomy specimens. In contrast, expression of UGT2B7 protein was significantly reduced in malignant cells. The observed difference in UGT2B7 expression between benign and malignant cells is consistent with the hypothesis that UGT-mediated conjugation of catechol estrogens prevents the formation of potentially carcinogenic estrogen quinones. Based on the efficiency of estrogen conjugation and expression in breast tissue, UGT1A9 and -2B7 may be considered to be the predominant isoforms in mammary metabolism of estrogen.

To date, polymorphisms have been described in seven of the 16 functional human UGT genes, namely UGT1A1, -1A6, -1A7, -1A8, -2B4, -2B7 and -2B15 (Lévesque *et al*., 1999; Huang *et al*., 2002; Miners *et al*., 2002). Altered catalytic activity has been shown for variants of UGT1A6, -1A7, -1A8 and -2B15, but the biological significance has yet to be proven (Huang *et al*., 2002; Miners *et al*., 2002). A polymorphism in UGT2B7 (268His → Tyr) exhibited similar efficiencies for the glucuronidation of a number of substrates for the wild-type and variant enzymes (Bhasker *et al*., 2000). Functional significance has only been convincingly demonstrated for a polymorphism in a TA repeat

element, $(TA)_{5-8}TAA$, of the UGT1A1 promoter. The length of the TA repeat appears to influence *UGT1A1* transcription, i.e. *UGT1A1* gene expression decreases with increasing number of repeats, and results in impaired glucuronidation of bilirubin in Gilbert syndrome. The *UGT1A1* polymorphism was associated with a marginal effect ($p = 0.06$) on the risk for breast cancer in premenopausal but not in postmenopausal African-American women (Guillemette *et al*., 2000). No association with risk was observed in a larger study of Caucasian women, and levels of circulating estradiol and estrone were not affected by the polymorphism (Guillemette *et al*., 2001).

(vi) *Sulfotransferases*

The SULT superfamily currently consists of 10 distinct enzymes that are classified into three families (SULT1, -2 and -4) based on the identity of amino acid sequence (Falany *et al*., 2000; Glatt *et al*., 2000; Adjei & Weinshilboum, 2002). Growing recognition of the carcinogenic potential of catechol estrogens has led to increased interest in the role of SULTs in the intracellular metabolism of estrogen (Raftogianis *et al*., 2000). These studies were initiated by the hypothesis that SULT-mediated estrogen conjugation reduces catechol estrogen levels and thereby decreases the risk for breast cancer.

The identification of new SULT isoforms during the past few years (Falany *et al*., 2000) has shown that earlier tissue studies frequently encompassed unrecognized isoforms, which obscured the issue of SULT specificity in estrogen conjugation. In a comprehensive study, Adjei and Weinshilboum (2002) prepared the known 10 recombinant SULT isoforms and determined that seven (1A1, 1A2, 1A3, 1E1, 2A1, 2B1a, 2B1b) catalysed the sulfate conjugation of catechol estrogens, whereas three (1B1, 1C1, 4A1) did not.

Although seven SULT isoforms were shown to conjugate estrogens, they differ significantly in their substrate affinity. There is consensus among investigators that only SULT1E1 can conjugate estradiol, and 2- and 4-hydroxyestradiol at nanomolar concentrations, in contrast to the micromolar concentrations observed for SULT1A1, -1A2, -1A3 and -2A1 (Faucher *et al*., 2001; Adjei & Weinshilboum, 2002). However, there is disagreement with respect to the sulfation of methoxyestrogens at nanomolar concentrations (Spink *et al*., 2000; Adjei *et al*., 2003).

Immunocytochemical studies have shown that SULT1E1 is the principal isoform in normal mammary epithelial cells derived from reduction mammoplasties, the non-tumour-derived cell line 184A1 and epithelial cells in normal breast tissues (Spink *et al*., 2000; Suzuki *et al*., 2003). Other isoforms, such as SULT1A1, were not detectable immunohistochemically in normal mammary epithelium, although reverse transcription-PCR revealed SULT1A1 and -1A3 mRNA in 184A1 cells (Spink *et al*., 2000). The expression pattern of SULT was almost converse in breast cancer cell lines and tissues. Virtually every malignant cell line expresses one or more members of the SULT1A subfamily. For example, SULT1A1 protein and mRNA levels were particularly high in BT-20, MCF-7, T47D and ZR-75 cells. In contrast, SULT1E1 was present in trace amounts or undetectable in most malignant cell lines (Spink *et al*., 2000; Falany *et al*., 2002). However, SULT1E1 was detected by immunohistochemistry in 50/113 (44.2%) invasive ductal carcinomas (Suzuki

et al., 2003). A subgroup analysis of 35 cases showed a significant correlation ($p < 0.01$) between the immunohistochemical SULT1E1 score and *SULT1E1* mRNA levels that was semiquantified by reverse transcriptase-PCR or with SULT1E1 enzymatic activity. Women who had SULT1E1-positive tumours had a better prognosis (longer disease-free interval [$p = 0.0044$] and overall survival [$p = 0.0026$]) than their SULT1E1-negative counterparts (Suzuki *et al*., 2003). Both the expression of SULT1E1 in normal mammary epithelium and the poor clinical outcome of SULT1E1-negative breast cancers support the view that SULT1E1-mediated conjugation is important in limiting long-term exposure of the mammary glands to carcinogenic catechol estrogens.

The *SULT1A1* and *-1E1* genes contain polymorphisms that are associated with decreased enzyme activity and thermal stability (Carlini *et al*., 2001; Adjei *et al*., 2003). Two *SULT1A1* polymorphisms have been described in codons 213Arg → His and 223Met → Val, which result in three alleles, *SULT1A1*1* (213Arg, 223Met), *SULT1A1*2* (213His, 223Met) and *SULT1A1*3* (213Arg, 223Val). Allele frequencies for *SULT1A1*1*, *-*2* and *-*3* were 65, 33 and 1% for Caucasians and 48, 29 and 23% for African-Americans, respectively (Carlini *et al*., 2001). A kinetic analysis of 2-methoxyestradiol showed similar K_m values for SULT1A1*1 and SULT1A1*2 (0.90 ± 0.12 and 0.81 ± 0.06 μM, respectively) (Spink *et al*., 2000). Three *SULT1E1* polymorphisms cause amino acid substitutions in codons 22Asp → Tyr, 32Ala → Val and 253Pro → His (Adjei *et al*., 2003). Kinetic studies with estradiol and the recombinant *SULT1E1* variant 22Tyr revealed an increase in apparent K_m, which resulted in a 40-fold lower activity compared with the wild-type enzyme (Adjei *et al*., 2003) and is consistent with the location of residue 22 at the entrance of the substrate-binding pocket (Pedersen *et al*., 2002). The striking decrease in enzyme activity and concentration observed for 32Ala → Val and 22Asp → Tyr are expected to have considerable impact on the mammary metabolism of estrogen. However, the allele frequency of these *SULT1E1* variants is < 1% (Adjei *et al*., 2003), which is much lower than the variant *SULT1A1* allele frequency, and raises the question whether they are indeed polymorphisms or mutations. One epidemiological study found an increased risk for breast cancer associated with the *SULT1A1*2* genotype (213Arg → His) (155 cases, 328 controls; odds ratio, 1.8; 95% CI, 1.0–3.2; $p = 0.04$) (Zheng *et al*., 2001). However, another study reported the lack of an association (444 cases, 227 controls; $p = 0.69$) (Seth *et al*., 2000).

(vii) *Steroid (estrone) sulfatase*

In contrast to the many SULTs, only one steroid sulfatase hydrolyses several sulfated steroids, including estrone sulfate, estradiol sulfate, dehydroepiandrosterone sulfate and cholesterol sulfate (Burns, 1983). Steroid sulfatase is not expressed in normal endometrium but was observed in 65/76 (86%) endometrial carcinomas (Utusunomiya *et al*., 2004). In contrast, the enzyme is expressed in both normal and malignant breast tissues (Chapman *et al*., 1995; Utsumi *et al*., 1999; Miyoshi *et al*., 2001). Utsunomiya *et al*. (2004) found a positive correlation ($p < 0.05$) between the steroid sulfatase:estrogen SULT ratio and shorter survival in patients with endometrial carcinomas, and suggested

that increased steroid sulfatase and decreased estrogen SULT expression may result in increased availability of biologically active estrogens.

Several studies have shown that progestogens can act as 'selective estrogen enzyme modulators' in hormone-responsive breast cancer cells (reviewed by Pasqualini, 2004). Specifically, several progestogens exert an inhibitory effect on estrone sulfatase, which produces estradiol, in conjunction with a stimulatory effect on SULT, which forms the inactive estrogen sulfate. These data help to explain the antiproliferative effect of progestogens in breast tissue. It was also shown in MCF-7 and T47D cells that estradiol inhibited estrone sulfatase in a dose-dependent manner (IC_{50}: concentration of estradiol that inhibits the activity of the enzyme by 50%, 8.8×10^{-10} M and 1.8×10^{-9} M, respectively) and thereby decreased its own formation by blocking the conversion of estrone sulfate to estradiol (Pasqualini & Chetrite, 2001).

(viii) *17β-Hydroxysteroid dehydrogenase*

17β-Hydroxysteroid dehydrogenase (17β-HSD) enzyme is responsible for the interconversion of 17-ketosteroids and their active 17β-hydroxysteroid counterparts, such as estrone, estradiol, androstenedione and testosterone. Six human genes that encode isozymes of 17β-HSD have been cloned (Peltoketo *et al.*, 1999). These isozymes are designated types 1–6 or HSD1–HSD6.

17β-HSD1 is a key enzyme in estrogen metabolism because it catalyses the conversion of estrone into the biologically more active estradiol. It is abundantly expressed in ovarian granulosa cells and placental syncytiotrophoblasts (Peltoketo *et al.*, 1999). 17β-HSD1 is detected in certain peripheral tissues, such as breast and endometrium, in addition to steroidogenic cells in the ovary and placenta. However, the degree of expression reported is quite variable. In breast cancers, for example, the detection of *17β-HSD1* mRNA varies from 16 to 100% (Gunnarsson *et al.*, 2001; Oduwole *et al.*, 2004) and the immunohistochemical staining of 17β-HSD1 ranges from 20 to 61% of cases (Poutanen *et al.*, 1992a,b; Sasano *et al.*, 1996; Suzuki *et al.*, 2000; Oduwole *et al.*, 2004). A positive, inverse or no correlation was observed between 17β-HSD1 expression and estrogen receptor-positive status in breast cancers (Sasano *et al.*, 1996; Suzuki *et al.*, 2000; Oduwole *et al.*, 2004). One study observed significantly higher expression of 17β-HSD1 in postmenopausal cancers while another study found no correlation with menopausal status (Suzuki *et al.*, 2000; Miyoshi *et al.*, 2001). Correlation between 17β-HSD1 expression and prognosis has been inconsistent, and either no association or a shorter overall and disease-free survival have been found in breast cancer patients (Suzuki *et al.*, 2000; Oduwole *et al.*, 2004). One reason for the discrepant data on 17β-HSD1 expression could be the amplification of the *17β-HSD1* gene, which was observed in 14.5% of postmenopausal breast cancers (Gunnarsson *et al.*, 2003). However, 17β-HSD1 is not expressed in normal or malignant endometrium (Utsunomiya *et al.*, 2001, 2003).

The *17β-HSD1* gene contains several polymorphisms, including a common one in exon 6 that results in the amino acid substitution 312Ser → Gly (Normand *et al.*, 1993). Several studies found no association of this polymorphism with either breast or endo-

metrial cancers (Feigelson *et al*., 2001; Wu *et al*., 2003; Setiawan *et al*., 2004). This is consistent with experimental data that show no difference in the catalytic activity of recombinant wild-type and variant 312 alleles (Puranen *et al*., 1994). Nevertheless, one study observed higher plasma levels of estradiol in lean women with the homozygous 312Gly/Gly genotype ($p = 0.01$) (Setiawan *et al*., 2004).

17β-HSD2 catalyses the conversion of estradiol into less potent estrone. In contrast to 17β-HSD1, expression studies of 17β-HSD2 yielded more consistent results. 17β-HSD2 is expressed in normal mammary epithelium but is frequently absent in breast cancer cells (Miettinen *et al*., 1999; Ariga *et al*., 2000; Suzuki *et al*., 2000; Gunnarsson *et al*., 2001; Oduwole *et al*., 2004). *17β-HSD2* mRNA was found in 10–31% of tumours and 17β-HSD2 protein was absent in all breast cancers (Suzuki *et al*., 2000; Gunnarsson *et al*., 2001). In contrast, 17β-HSD2 was regularly expressed in normal endometrium and was detected in 75% of endometrial hyperplasias and 37–50% of endometrial carcinomas (Utsunomiya *et al*., 2001, 2003). Since 17β-HSD2 preferentially catalyses the oxidation of estradiol to less active estrone, it has been suggested that the expression of 17β-HSD2 in proliferative glandular cells of endometrial disorders may represent an in-situ defence mechanism that modulates unopposed estrogenic effects (Utsunomiya *et al*., 2003).

17β-HSD5 (also known as aldo-keto reductase, AKR1C3) is expressed in normal breast and prostate (Penning *et al*., 2000). The level of 17β-HSD5 expression in breast cancer specimens was higher than that in normal breast tissue and 65% of 794 tumours labelled 17β-HSD5-positive (Oduwole *et al*., 2004). Since 17β-HSD5 recognizes a wide range of substrates, including estrogens, androgens, progestogens and prostaglandins, its role in breast tissue is uncertain.

Progestogens have a complex effect on 17β-HSD activity and can direct the interconversion of estrone to estradiol in both directions (reviewed by Pasqualini, 2004). Studies with the hormone-dependent breast cancer cells MCF-7 and T47D have shown that some progestogens stimulate the reductive activity of estrone to estradiol and thereby enhance cell proliferation (Coldham & James, 1990; Poutanen *et al*., 1990, 1992b; Peltoketo *et al*., 1996). Other progestogens favour the oxidation of estradiol to estrone and may thereby inhibit cell growth (Chetrite *et al*., 1999a,b).

(*c*) *Tobacco smoke*

Several compounds in tobacco smoke might affect estrogen metabolism by the induction of CYPs (Zeller & Berger, 1989). An epidemiological study of 27 premenopausal women (14 smokers, 13 nonsmokers) showed a significant increase in urinary excretion of 2-hydroxyestrone that is a result of 2-hydroxylation of reversibly oxidized estradiol (Michnovicz *et al*., 1986). [The concentration of 4-hydroxylated estrogen metabolites was not assessed by this assay.]

Berstein *et al.* (2000) used GC–MS to measure urinary excretion of catechol estrogens in six smoking and 10 nonsmoking postmenopausal women who received 2 mg/day estradiol valerate for 1 month. Before administration of estradiol valerate, smokers had significantly lower excretion of 16-epiestriol and 4-hydroxyestrone than nonsmokers. After

administration of estradiol valerate, much higher excretion of 2-hydroxyestrone and 4-hydroxyestradiol was observed in smokers compared with nonsmokers. These data indicate that only the combination of estradiol valerate and smoking (and not smoking itself) leads to an increase in potentially genotoxic catechol estrogens.

4.1.2 *Experimental systems*

(*a*) *Estrogen fatty acid esters*

Chronic treatment of ovariectomized rats with 0.5 or 5 nmol/day estradiol stearate for 10 or 23 days had a stronger stimulatory effect on mammary gland cell proliferation than treatment with equimolar doses of estradiol (Mills *et al*., 2001). Two commonly prescribed hypolipidaemic drugs, clofibrate and gemfibrozil, increase the size and number of hepatic peroxisomes upon administration to rodents. Treatment of rats with clofibrate caused a multifold increase in the hepatic microsomal formation of estradiol fatty acids (Xu *et al*., 2001a). The stimulatory effect of clofibrate on hepatic fatty acid esterification of estradiol was paralleled by enhanced estradiol-induced increases in the formation of lobules in the mammary gland and by increased incorporation of bromodeoxyuridine, a marker of cell proliferation, into these lobules (Xu *et al*., 2001b).

(*b*) *Catechol estrogens*

Catechol estrogens are carcinogenic in animal experiments. The experimental evidence was reviewed by Cavalieri *et al*. (2000) and showed that 4-catechol estrogens are more carcinogenic than the isomers 2-hydroxyestrogens. In addition to the induction of renal cancer in hamsters (Liehr *et al*., 1986), 4-hydroxyestradiol induces uterine adenocarcinoma, a hormonally related cancer, in mice. Administration of estradiol, 2-hydroxyestradiol and 4-hydroxyestradiol induced endometrial carcinomas in 7, 12 and 66%, respectively, of neonatally treated CD-1 mice (Newbold & Liehr, 2000). However, in adult ACI rats, administration of estradiol but not that of 2- or 4-hydroxyestradiol or 4-hydroxyestrone induced mammary tumours (Turan *et al*., 2004).

4.2 Receptor-mediated effects

As indicated in the monograph on Combined estrogen–progestogen contraceptives, there is evidence that not all of the effects of estrogens and progestogens used in hormonal therapy for the menopause are mediated through nuclear or other receptors. In addition, the effects of these steroids probably involve several molecular pathways and cross-talk between receptor- and/or non-receptor-mediated pathways. During the past decade, extensive growth in research on the mechanisms of action of hormones and on hormones and cancer has taken place, and several steroid hormone receptor subtypes and non-genomic mechanisms of action have been determined.

Hormonal therapy with ‘estrogens only’ is effective in the treatment of many aspects of the menopause, but the increased risk for endometrial cancer renders the prescription

of combined estrogen–progestogen products for women with a uterus essential. In this context, an ideal progestogen would prevent endometrial cancer and maintain the protective benefits of estrogens, which means that it should have no significant anti-estrogenicity, except in the endometrium.

The various components of hormonal therapy for the menopause have received increased attention in recent years. Information has become available on the progestogens used and on their hormonal activities and their binding affinities to various receptors and proteins. This information is summarized in Tables 17 and 18, which were compiled on the basis of information gathered by Sitruk-Ware (2002), Schindler *et al*. (2003), Shields-Botella *et al.* (2003), Sitruk-Ware (2004a,b) and Wiegratz and Kuhl (2004).

There has also been tremendous growth in research on the effects of postmenopausal hormonal therapy on a variety of non-cancer end-points related to endometrial function (vaginal bleeding), postmenopausal vasomotor symptoms, treatment of problems with the menstrual cycle, skin, bone and related calcium metabolism, the cardiovascular system and lipid metabolism. Many of these effects are probably at least in part mediated by mechanisms of steroid receptors. This topic is reviewed in Section 4.3.

4.2.1 *Combined estrogen–progestogen therapy*

(*a*) *Humans*

(i) *Breast*

No data were available on the effects of exposure to combined estrogen–progestogen therapy on the human breast in the previous evaluation (IARC, 1999). During the past 6 years, several reports have been published that are pertinent to this issue.

Hargreaves *et al*. (1998) obtained archival paraffin-embedded breast tissue samples from women who underwent surgery for benign (n = 61) or malignant (n = 124) breast disease and stained sections from these for the proliferation marker Ki-67 and the progesterone receptor. The median percentage of normal epithelial cells that stained for Ki-67 [using an unspecified antibody] was 0.19% (range, 0–3.66%) in breast samples of 111 women who did not receive hormonal therapy. This was not significantly different from the percentages in normal epithelial cells from 35 women who took estrogen only (0.22%; range, 0–1.44%) or 39 women who took combined estrogen plus progesterone therapy (0.25%; range, 0–2.80%). However, the median percentage of normal epithelial cells that stained for the nuclear progesterone receptor significantly increased from 4.8% (range, 0–39%) in 100 untreated women to 10.2% (range, 0.2–40%) in 31 women who took estrogen only and 6.7% (range, 0–44%) in 36 women who took combined estrogen plus progesterone therapy. This increased expression of the progesterone receptor is consistent with an effect of estrogen on breast cells.

Hofseth *et al*. (1999) obtained breast biopsies from women who were taking oral estrogen–progestogen therapy that contained conjugated equine estrogens (0.3–2.5 mg/day) or micronized 17β-estradiol (0.5–1.0 mg/day) plus medroxyprogesterone acetate (2.5–5.0 mg/day), from women who were taking these estrogens only or from women who did

Table 17. Overview of the spectrum of hormonal activities of progestogens used in hormonal menopausal therapy

Progestogen	Progesto-genic	Anti-estrogenic	Estrogenic	Androgenic	Anti-androgenic	Glucocorticoid	Antimineralo-corticoid
Chlormadinone acetate	+	+	–	–	+	+	–
Cyproterone acetate	+	+	–	–	+, +	+	–
Desogestrel	+	+	–	+	–	±, –	–
Dienogest	+	+, ±	–, ±	–	+	–	–
Drospirenone	+, +	+	–	–	+	?, –	+
Dydrogesterone	+	+	–	–	–, ±	?	±
Etonogestrel [3-keto-desogestrel]	+	+	–	+	–	±, –	–
Gestodene	+	+	–	+	–	±, +	+
Levonorgestrel/norgestrel	+	+	–	+	–	–	–
Medroxyprogesterone (acetate)	+	+	–	±	–	+	–
Norethisterone (acetate)	+, +	+	+	+	–	–	–
Progesterone	+, +	+	–	–	±	+	+
Trimegestone	+	+	–	–	±	–	±

Adapted from Wiegratz and Kuhl (2004); second value, for progestogenic activity only from Sitruk-Ware (2002); second value, except for progestogenic activity from Schindler *et al*. (2003)

+, effective; ±, weakly effective; –, ineffective; ?, unknown

Data are based mainly on animal experiments. The clinical effects of the progestogens are dependent on their tissue concentrations.

No comparable data were available for ethynodiol diacetate.

Note: This information should be viewed as only an indication of the hormonal activity and its order of magnitude of the various progestogens.

Table 18. Relative binding affinities of progestogens used in hormonal therapy for the menopause to steroid receptors and serum binding globulins[a]

Progestogen	PR	AR	ER	GR	MR	SHBG	CBG
Chlormadinone acetate	134	5	0	8	0	0	0
Cyproterone acetate	180	6	0	6	8	0	0
Desogestrel (as 3-keto-desogestrel)	300	20	0	14	0	15	0
Dienogest	10	10	0	1	0	0	0
Drospirenone	70, 19	65, 2	0, < 0.5	6, 3	230, 500	0	0
Dydrogesterone	150	0	?	?	?	?	?
Etonogestrel (3-keto-desogestrel)	300	20	0	14	0	15	0
Gestodene	180, 864	85, 71	0, < 0.02	27, 28	290, 97	40	0
Levonorgestrel/norgestrel	300, 323	45, 58	0	1, 7.5	75, 17	50	0
Medroxyprogesterone acetate	130, 298	5, 36	0, < 0.02	29, 58	160, 3.1	0	0
Norethisterone acetate	150, 134	15, 55	0, 0.15	0, 1.4	0, 2.7	16	0
Norgestimate/nomegestrol acetate	30	0	0	1	0	0	0
Progesterone	100	0	0	10	100	0	36
Trimegestone	660, 588	1, 2.4	0, < 0.02	9, 13	120, 42	?	?
Reference compounds (100%)	Progesterone	Metribolone (R1881)	17β-Estradiol	Dexamethasone (or cortisol)	Aldosterone	5α-Dihydro-testosterone	Cortisol

Adapted from Wiegrazt and Kuhl (2004a,b); second value from Sitruk-Ware (2004)
?, unknown; AR, androgen receptor; CBG, corticoid-binding globulin; ER, estrogen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; SHBG, sex hormone-binding globulin
[a] Values were compiled by these authors by cross-comparison of the literature. Because the results of the various in-vitro experiments depend largely on the incubation conditions and biological materials used, the published values are inconsistent. These values do not reflect the biological effectiveness, but should be viewed as only an indication of the order of magnitude of the binding affinities of the various progestogens.
No comparable data were available for ethynodiol diacetate.

not take hormonal treatment. Compared with untreated women (n = 16–19), the percentage of epithelial cells in the inter- and intralobular ducts and the duct-lobular units that stained positive for proliferating cell nuclear antigen were significantly ($p < 0.01$) increased by approximately twofold in women who took estrogen alone (n = 21) and those who took estrogen plus progestogen (n = 15; ducts only); staining was increased by almost threefold in the duct-lobular unit cells of women who took estrogen plus progestogen (n = 19; $p < 0.01$). When another marker of proliferation (Ki-67) was examined, similar differences were found, but only the difference (approximately sixfold) for the duct-lobular unit cells of women who took estrogen plus progestogen was statistically significant ($p < 0.05$). There was a positive correlation between the percentage of epithelial breast cells that stained for markers of proliferation with the duration of both types of hormonal treatment, but this was only statistically significant for the treatment with estrogen plus progestogen when the Ki-67 marker was considered ($p = 0.03$). There was also a significant increase ($p \leq 0.01$) in the percentage of breast tissue occupied by epithelium, i.e. twofold for women who took estrogen only and threefold for women who took estrogen plus progestogen. The percentage of epithelial cells that were positive for the nuclear staining for the progesterone receptor was increased three- to fourfold ($p < 0.01$) in women who took estrogen only and approximately twofold in the lobular units of women who took estrogen plus progestogen ($p < 0.05$); again, the observed increase in the expression of the progesterone receptor is consistent with an effect of estrogen on these cells. No differences were observed in nuclear staining for the estrogen receptor.

Conner *et al*. (2001) studied 12 women who were treated continuously with 17β-estradiol (50 μg per day by skin patch) and either oral (5 mg per day) medroxyprogesterone acetate or vaginal (8 mg every 2 days) progesterone on days 15–26 of each cycle. They obtained fine needle aspiration biopsies during the last 2 days of the estrogen part of the cycle and during day 25 or 26 at the end of the estrogen plus progestogen part of the cycle after 6–8 weeks and after 14–16 weeks of treatment. The percentage of epithelial cells that stained for Ki-67 (using the MIB-1 antibody) was 1.4% at the end of the estrogen phase and 2.1% at the end of the estrogen plus progestogen phase of the cycle, but this was not statistically significant. There was no difference in Ki-67 staining between the two progestogen treatments. In follow-up studies, Conner *et al.* (2003, 2004a) examined women who received continuous oral 17β-estradiol (2 mg per day) plus norethisterone acetate (1 mg per day) or 17β-estradiol valerate (2 mg per day) plus dienogest (2 mg per day) for 6 months. In the first study, two groups of 13–17 women received estrogen plus either norethisterone acetate or estrogen plus dienogest. For both treatments combined, the mean percentage of epithelial cells that stained for Ki-67 in fine needle aspiration was statistically significantly increased ($p < 0.001$) from 2.2% (n = 28; median, 1.4%; range, 0–11.7%) at baseline to 9.1% (n = 30; median, 7.6%; range, 0–27.1%) after 3 months and 8.0% (n = 31; median, 5.7%; range, 0–25.9%) after 6 months of treatment. One woman who had a high baseline proliferation index showed a decrease in proliferation after treatment. The increases in cell proliferation index were similar for both hormonal treatments (Conner *et al.*, 2003). In the second study of 83 women who were treated with 17β-estradiol (2 mg per day) plus

norethisterone acetate (1 mg per day), the mean percentage of epithelial cells that stained for Ki-67 in fine needle aspiration biopsies was significantly ($p < 0.01$) increased from 2.2% (median, 1.9%; range, 0–11.9%) at baseline to 6.4% (median, 5.0%; range, 0–20.4%) after 6 months of treatment. There was a negative correlation between the rate of epithelial proliferation in this study and total and free serum testosterone levels (Conner *et al*., 2004a).

Valdivia *et al*. (2004) obtained breast core biopsies from 19 women at baseline and after treatment for 12 months with continuous conjugated equine estrogen (0.625 mg per day) and medroxyprogesterone acetate (5 mg per day). Of these women, 15 responded with an increase in percentage of epithelial cells that stained for Ki-67, one exhibited a decrease and three women had no change in this parameter; the increase from baseline was probably statistically significant, but this was not clear. Expression of the apoptosis marker Bcl-2 was increased in nine women, decreased in five and unchanged in five.

The results of all but one of these studies indicate that combined estrogen–progestogen menopausal therapy increases the rate of cell proliferation in the breast. The addition of progestogens appears to enhance significantly the modest increase in the rate of breast cell proliferation caused by estrogen-only therapy. This is consistent with the notion of an increase in risk for breast cancer associated with combined estrogen–progestogen menopausal therapy over that associated with estrogen-only menopausal therapy (see also IARC, 1999). Only the study of archival surgical specimens of women with breast disease by Hargreaves *et al*. (1998) did not show an increase in breast cell proliferation associated with combined estrogen–progestogen menopausal therapy. The other studies used either fine needle aspirates or core biopsies from women without breast disease, which may explain the discrepancy.

Mammographic density is a strong identifier of risk for sporadic breast cancer that exceeds the risk associated with elevated circulating levels of 17β-estradiol (Santen, 2003). Risk for breast cancer was increased in a number of case–control studies in which mammographic density was not only subjectively evaluated by radiologists but was also assessed by observer bias-free, automated, computer-assisted techniques. The relative risks were in the order of 4–6 for subjective evaluations and 3–4 for computer-assisted evaluations (Byng *et al*., 1997; Yaffe *et al*., 1998; Boyd *et al*., 1999; Li *et al*., 2005). A number of recent studies have reported on the effects of estrogen–progestogen therapy on mammographic density. Valdivia *et al*. (2004) (see study details above) observed an increase in mammographic density (BI-RADS method) in 11/19 women (58%) who took conjugated equine estrogen plus medroxyprogesterone acetate for 12 months, while density was decreased in only one woman and was unchanged in seven.

Conner *et al*. (2004b) randomized women to continuous oral treatment for 6 months with either 17β-estradiol (2 mg per day) plus norethisterone acetate (1 mg per day) (22 women) or 17β-estradiol valerate (2 mg per day) plus dienogest (2 mg per day) (23 women). Both treatments resulted in an increase in mammographic density (Wolfe method) over baseline values in 50–60% of these women; this change was statistically significant.

Christodoulakos *et al*. (2003) randomized 94 women to continuous oral treatment for 12 months with conjugated equine estrogen alone (0.625 mg per day) (25 women), equine estrogen (0.625 mg per day) plus medroxyprogesterone acetate (5 mg per day) (34 women) or 17β-estradiol (2 mg per day) plus norethisterone acetate (1 mg per day) (35 women); 27 untreated control women were also included. Mammographic density (Wolfe classification method) increased in 12% of women who took equine estrogen plus medroxyprogesterone acetate, 31% of women who took 17β-estradiol plus norethisterone acetate and in 8% of women who took estrogen only, whereas density did not increase in any of the control women. Density decreased in 26% of control women but in none of the women who took hormonal treatment. The difference from controls was statistically significant for all three treatment groups.

Georgiev and Manassiev (2002) found that breast density increased in 16% of 19 women who were treated with continuous oral 17β-estradiol (2 mg per day) plus dienogest (2 mg per day) or 17β-estradiol (2 mg per day) plus norethisterone acetate (1 mg per day) and were followed annually by mammography for 4 years using the Wolfe method.

Sendag *et al*. (2001) compared women who received continuous oral treatment with 17β-estradiol (2 mg per day) plus norethisterone acetate (1 mg per day) (44 women), conjugated equine estrogen (0.625 mg per day) plus medroxyprogesterone acetate (5 mg per day) (17 women), equine estrogen only (0.625 mg per day) (20 women) or transdermal 17β-estradiol (3.9 mg per week) (56 women) and 44 women who received a variety of sequential treatments with estrogen and estrogen plus progestogen. The mean follow-up was 20 months (range, 12–96 months) and the Wolfe method was used to assess breast density. Density was increased in 31% of women who took continuous estrogen plus progestogen and in 4% of women who took estrogen only, but did not change in women who received the sequential treatments with estrogen plus progestogen. More women (34%) who took the continuous treatment with 17β-estradiol plus norethisterone acetate had increased breast density than those who took continuous estrogen plus medroxyprogesterone acetate (24%).

Colacurci *et al*. (2001) randomized women to continuous treatment with transdermal 17β-estradiol (0.05 mg per day) plus nomegestrol acetate at one of two doses (5 mg per day, 26 women; or 2.5 mg per day, 25 women), 17β-estradiol only (23 women) or no treatment (controls; 23 women). Mammographic density (Wolfe method) after 12 months of treatment was increased in 35% and 43% of the women who took estrogen plus the high and low dose of nomegestrol acetate, respectively, in 21% of women who took estrogen only and in none of the control women. The differences from the control group were statistically significant.

Erel *et al*. (2001) assigned women to continuous oral treatment with conjugated equine estrogen (0.625 mg per day) plus medroxyprogesterone acetate (2.5 mg per day) (26 women), continuous treatment with estrogen (0.625 mg per day) plus medroxyprogesterone acetate (10 mg/day) for the last 10 days of the 28-day cycle (21 women) or continuous treatment with estrogen only (0.625 mg per day) (23 women). Women were followed by mammography for 4 years using the Wolfe method to assess breast density. Density was increased in 35% of women who took continuous estrogen plus progestogen,

in 19% of women who took estrogen plus cyclic progestogen and in 22% of women who took estrogen only. Although the differences between these three groups were not statistically significant, the results suggest that treatment with continuous estrogen plus progestogen is more likely to increase breast density than treatment with continuous estrogen only or estrogen plus sequential progestogen.

Lundström *et al.* (2001) studied women who took continuous oral conjugated equine estrogen (0.625 mg per day) plus medroxyprogesterone acetate (5 mg per day) (52 women) or estriol (2 mg per day) (51 women) or used a transdermal patch of 17β-estradiol (0.05 mg per day) (55 women) and were followed every 2 years by mammography using the Wolfe method to assess breast density. Density increased over baseline at the first 2-year visit in 40% of women who took continuous estrogen plus progestogen, in 6% of women who took oral estrogen only and in 2% of women who used a transdermal patch of estrogen only.

Collectively, these studies consistently show that approximately one third of women treated with continuous estrogen (by any route) plus oral progestogen respond with increased mammographic breast density. Treatment with continuous estrogen plus sequential progestogen resulted in fewer women developing increased breast density than treatment with continuous estrogen plus progestogen. Estrogen-only treatment appeared to result in increased breast density in fewer women. These findings correspond to the supposition that continuous estrogen plus progestogen therapy results in an increased risk for breast cancer.

(ii) *Uterus*

In the previous evaluation (IARC, 1999), it was concluded that the addition of progestogens reduces the increased rate of cell proliferation in the endometrium that is seen with estrogen-only therapy. The effects of estrogen only and their reduction by progestogens were dose-related. Two previous studies from the 1980s on cell proliferation concerned combined treatment with conjugated equine estrogens (Premarin®) and norethisterone. At least nine additional studies have been conducted with norethisterone, all but one of which combined the treatment with 17β-estradiol. In addition, studies have been carried out on six other progestogens combined with estrogen therapy. Many of these studies include histopathological analysis of endometrial biopsies. Although many studies have taken care to standardize this analysis, it should be noted that there is considerable potential for significant inter-observer and inter-study variation (Wright *et al.*, 2002)

Norethisterone (acetate) plus estrogen

Cameron *et al.* (1997) followed 14 postmenopausal women for 3 months during which they were treated with a dermal patch that released 0.05 mg per day 17β-estradiol for 7 days alternated with a patch that released 0.05 mg per day 17β-estradiol plus 0.25 mg per day norethisterone acetate for 3 days. End-of-study endometrial biopsies were obtained at the end of an estrogen-only period and at the end of an estrogen plus norethisterone acetate period. Staining for the proliferation marker Ki-67 was reduced at the end

of the estrogen plus norethisterone acetate period compared with the estrogen-only period, but staining for estrogen (α) and progesterone receptors and histological endometrial thickness did not differ. No endometrial hyperplasia was found.

Johannisson *et al*. (1997) randomized postmenopausal women in an open-label dermal patch study to continuous 0.05 mg per day 17β-estradiol plus doses of norethisterone acetate of 0.17 or 0.35 mg per day either continuously or sequentially on days 14–28. A reference group (not randomized) was treated with a continuous 0.05-mg per day 17β-estradiol patch and orally with either 1 mg per day norethisterone acetate or 20 mg per day dydrogesterone during the last 14 days of each cycle. End-of-study endometrial biopsies were obtained from 107–124 women per group after 13 cycles of 28 days. No significant differences were observed in the percentage of women with atrophic or proliferative endometrial histology and no malignancies occurred; only one case of endometrial hyperplasia developed in the group that received 17β-estradiol plus sequential norethisterone acetate at 0.35 mg per day.

Habiba *et al*. (1998) studied 103 postmenopausal women who were treated orally with 2 mg per day 17β-estradiol valerate continuously and 1 mg per day norethisterone on days 16–28 of the cycle. The women received a baseline and end-of-study endometrial biopsy after 6 months of therapy. Most women had inactive or non-secretory endometrial histology at baseline whereas over 90% had secretory morphology after 6 months of treatment. No cases of endometrial hyperplasia or carcinoma occurred.

Dahmoun *et al*. (2004) assigned postmenopausal women to continuous treatment with either 2 mg per day 17β-estradiol plus 1 mg per day norethisterone acetate or 0.625 mg per day conjugated equine estrogens plus 5 mg per day medroxyprogesterone acetate. The two treatment groups were analysed in combination after 1 year of treatment. Staining for the proliferation marker Ki-67 was increased in stromal cells, but was not affected in epithelial cells. Staining for estrogen (α) receptor was reduced in the epithelium but was only slightly reduced in stromal cells. Staining for a marker of apoptosis (TUNEL) and the progesterone receptor in stroma and epithelium were not affected by the treatments, nor was endometrial thickness as assessed by ultrasound.

Kurman *et al*. (2000) conducted a double-blind clinical trial in which postmenopausal women were randomized to continuous oral treatment with 1 mg per day 17β-estradiol only or 1 mg per day 17β-estradiol plus 0.10, 0.25 or 0.50 mg per day norethisterone acetate. End-of-study endometrial biopsies were obtained from 241–251 women per group after 12 months of treatment. In the estrogen-only group, 14.6% of women had endometrial hyperplasia, whereas only 0.8% of women who took estrogen plus 0.10 mg norethisterone acetate and 0.4% of women who took the two higher doses of norethisterone acetate had such lesions. In women over 65 years of age, 4/21 (19%) who took estrogen only had endometrial hyperplasia, while none of the women who received estrogen plus either of the doses of norethisterone acetate had this lesion (0/18, 0/18 and 0/19 women).

Iatrakis *et al*. (2004) conducted an open-label prospective study of continuous oral treatment with 1 mg per day 17β-estradiol and 0.5 mg per day norethisterone acetate of 124 postmenopausal women for up to 3 years. A concurrent control group (not randomized) of

130 untreated women was available. Endometrial thickness, as assessed by ultrasound, was virtually unaffected. In end-of-study endometrial biopsies, no differences in the percentage of women with atrophic, secretory or proliferative histology were observed between the treated and control women. No cases of endometrial hyperplasia or carcinoma occurred.

Wells *et al*. (2002) conducted an open-label prospective study of postmenopausal women who were given continuous oral treatment with 2 mg per day 17β-estradiol plus 1 mg per day norethisterone acetate for up to 5 years; the mean follow-up was 4.4 years. Endometrial biopsies were obtained at baseline, between 24 and 36 months and at the end of the study. Inactive or atrophic endometrium was found in 68/164 (41%) women who had had no hormonal treatment at baseline, in 157/465 (34%) women after 24–36 months of treatment and in 185/398 (46%) women at the end of the study. (The entire cohort consisted of a mixture of women who had had no hormonal treatment and women who had already been taking either sequential or estrogen-only hormonal therapy.) At baseline, 14/164 women had a secretory endometrial histology (9%); this increased to 162/465 (35%) and 102/398 (26%) women after 24–36 months of treatment and at the end of the study, respectively. No cases of endometrial hyperplasia or carcinoma occurred. Sturdee *et al*. (2000) reported on 9 months of follow-up in this study. At baseline the prevalence of complex hyperplasia was 5.3% and that of atypical hyperplasia was 0.7% in the entire group of 1196 women who completed 9 months of treatment, many of whom had previously taken hormonal therapy that may have induced these hyperplastic lesions. None of these women had endometrial hyperplasia after 9 months of treatment and no new cases arose.

Neven *et al*. (2004) reported results of the EURALOX (European double-blind clinical trial on raloxifene) in which postmenopausal women were randomized to continuous oral treatment with 2 mg per day 17β-estradiol plus 1 mg per day norethisterone acetate or 60 mg per day raloxifene alone. End-of-study endometrial biopsies were obtained after 12 months of treatment. The conclusion of a detailed histopathological analysis was that more endometrial pathology occurred in 261 women on estrogen plus norethisterone acetate than in 73 women on raloxifene (polyps, 4.3% versus 2.0%; $p < 0.05$; endometrial proliferation/hyperplasia, 8.8% versus 1.2%; $p < 0.001$; cystic atrophy, 5.5% versus 1.2%; $p < 0.001$). Very few cases of malignant or premalignant endometrial histology occurred in either group.

Portman *et al*. (2003) conducted a double-blind placebo-controlled clinical trial in which postmenopausal women were randomized to continuous oral treatment with placebo, ethinylestradiol at 0.005 mg per day without or with 0.25 or 1.0 mg per day norethisterone acetate, or ethinylestradiol at 0.01 mg per day without or with 0.5 or 1.0 mg per day norethisterone acetate. In addition, an open-label comparison group was given 0.625 mg per day conjugated equine estrogens plus 2.5 mg per day medroxyprogesterone acetate. After 12 months of therapy, 114–121 women in each group received an end-of-study endometrial biopsy. Endometrial hyperplasia was found in 23/118 (19%) of the women who took 0.01 mg per day ethinylestradiol only, but only in a maximum of one woman in each of the other groups. In the estrogen-only groups, 80–90% of women had

proliferative endometrial morphology, including the 19% of women with hyperplasia in the group who took 0.01 mg per day ethinylestradiol only. The occurrence of this morphology was reduced to 30–45% of women in all groups who were given co-treatment with norethisterone acetate, but was found in 70% of women who took estrogens plus medroxyprogesterone acetate. The differences between the co-treatment with norethisterone acetate and that with estrogen only or estrogen plus medroxyprogesterone acetate were statistically significant.

Other progestogens plus estrogens

Ferenczy and Gelfand (1997) conducted an open-label prospective study of postmenopausal women who were given continuous oral treatment with 2 mg per day 17β-estradiol and 10 mg dydrogesterone on days 15–28. Baseline biopsies from 146 women who completed the 12-month course of treatment showed predominantly atrophic endometrium, whereas biopsies taken after 12 months of treatment showed that endometrial histology was predominantly secretory. One endometrial hyperplasia was found, but no endometrial carcinomas.

Hänggi *et al*. (1997) randomized 35 postmenopausal women per group to oral treatment with either placebo, 2 mg per day of continuous 17β-estradiol plus 10 mg dydrogesterone on days 15–28 or 0.05 mg per day continuous 17β-estradiol by dermal patch plus 10 mg oral dydrogesterone on days 15–28. Ultrasound assessment revealed a 2.5- to threefold increase over baseline of endometrial thickness after 12 and 24 months of hormonal treatment. Biopsies taken at the same time-points showed a shift from a predominantly inactive or atrophic endometrial histology to a predominantly secretory morphology.

Ross *et al*. (1997) conducted a double-blind clinical trial in which postmenopausal women were randomized to continuous oral treatment with 2 mg per day 17β-estradiol plus 0.1, 0.25 or 0.5 mg per day trimegestone on days 15–28. In each of the three groups, 10–11 women were available for evaluation. After three cycles, biopsies were taken and 90–100% of the women in all groups had a secretory endometrial morphology; no hyperplasia was found in any of the groups.

Suvanto-Luukkonen *et al*. (1998) conducted an open-label clinical trial in which postmenopausal women were randomized to continuous treatment with a skin gel that released 0.15 mg per day 17β-estradiol into the circulation plus either an intrauterine device that released 0.02 mg per day levonorgestrel for up to 5 years, 100 mg per day oral micronized progesterone on days 1–25 or 100–200 mg per day vaginal progesterone on days 1–25. After 12 months of treatment, endometrial thickness (assessed by ultrasound) was not significantly changed from baseline. No change in endometrial histology was observed in end-of-study biopsies in the group that received 17β-estradiol plus intrauterine levonorgestrel but, in the groups that received 17β-estradiol plus oral or vaginal progesterone, morphology changed from predominantly atrophic at baseline (46/50 cases; 92%) to predominantly proliferative (13/18 cases [72%] in the oral progesterone-treated group and 8/14 cases [57%] in the vaginal progesterone-treated group).

Byrjalsen *et al*. (1999) conducted a double-blind clinical trial in which 55–56 postmenopausal women per group were randomized to placebo or continuous oral treatment with 2 mg per day 17β-estradiol plus 0.025 or 0.05 mg per day gestodene sequentially (days 17–28), 1 mg per day 17β-estradiol plus 0.025 mg per day gestodene sequentially (days 17–28) or 1 mg per day 17β-estradiol plus 0.025 mg per day gestodene continuously. After 2 years of follow-up, end-of-study biopsies were obtained and examined histologically. Treatment with continuous estrogen plus gestodene did not change the high percentage of women with atrophic endometrium (83%) compared with placebo (81%) but, in women who received sequential treatments, the majority (54–79%) had a secretory type of endometrial histology. In the latter groups, endometrial thickness, the histochemical expression of secretory markers and staining for estrogen and progesterone receptors in the endometrium were increased. One endometrial carcinoma developed in the group who took 1 mg 17β-estradiol and 0.025 mg gestodene sequentially. No cases of endometrial hyperplasia occurred.

van de Weijer *et al.* (1999) randomized 151 women to continuous oral treatment with 1 mg 17β-estradiol plus 5 or 10 mg dydrogesterone on days 15–28. Biopsies at baseline and after 13 cycles revealed that 98% of these women had no endometrial lesions; only one woman in each group developed either proliferative changes or hyperplasia.

Wahab *et al*. (1999) conducted a double-blind clinical trial in which postmenopausal women were randomized to continuous oral treatment with 2 mg per day 17β-estradiol plus 0.05, 0.1, 0.25 or 0.5 mg per day trimegestone on days 15–28. After 6 months of follow-up, biopsies were taken and compared with those of untreated control women who were not randomized. Extensive morphometric analysis of the endometrium was carried out. In the endometrium of treated women compared with that of untreated women, there was evidence of somewhat smaller glands and a clearly significantly reduced area occupied by glands, but no change in the number of glands per unit area. Glands with evidence of secretion were less frequent in the high-dose group only. Discriminant analysis revealed a significant relation with dose for the all histomorphometric parameters combined.

Jondet *et al*. (2002) randomized postmenopausal women to treatment with a skin gel that released 1.5 mg per day 17β-estradiol on days 1–24 plus oral administration of either 10 mg per day chlormadinone acetate (42 women) or 200 mg per day progesterone (63 women) on days 10–24. Endometrial biopsies were taken at baseline and at the end of the study (18 months). There was a shift in atrophic morphology from 92% of women who were affected at baseline to 20–27% who were affected after 18 months of treatment; at the same point in time, 63–77% of women had a secretory endometrial morphology versus 3% at baseline.

Drospirenone (1, 2 or 3 mg) in combination with 1 mg 17β-estradiol is a continuous combined product used in hormonal therapy. Phase II/III trials of these combinations have demonstrated that, at all three doses of drospirenone, the combination is associated with a highly favourable safety profile, with excellent endometrial protection after 1 and 2 years (no cases of hyperplasia or cancer) (Rubig, 2003).

The combination of 2 mg estradiol valerate with 2 mg dienogest is the first continuous combined hormonal menopausal therapy preparation to contain a progestogen with substantial anti-androgenic activity. This combination was compared with a continuous combination of 2 mg estradiol plus 1 mg norethisterone acetate. In a large-scale study (1501 women) (Von Schoultz, 2003), biopsy and ultrasound assessment demonstrated that estradiol valerate plus dienogest quickly and effectively achieved endometrial atrophy in the vast majority of subjects, which indicates a protective effect on the endometrium.

Fugère *et al.* (2000) conducted a double-blind clinical trial in which postmenopausal women were randomized to continuous treatment with 0.625 mg per day conjugated equine estrogens plus 2.5 mg per day medroxyprogesterone acetate (69 women) or 150 mg per day raloxifene (67 women). Endometrial thickness, as assessed by ultrasound, increased slightly but significantly after 1 and 2 years of follow-up in the group given estrogen plus progestogen but no change was observed in the raloxifene-treated group. In the former group, more women developed benign proliferative endometrial changes (19–24%) when biopsies were taken 1 and 2 years after the start of treatment, whereas in the raloxifene-treated group only 6% of women developed such changes, which did not differ from baseline (4–6%).

Chang *et al*. (2003) conducted a double-blind clinical trial in which postmenopausal women were randomized to 0.625 mg per day conjugated equine estrogens on days 1–25 plus 5 (102 women) or 10 (66 women) mg per day medroxyprogesterone acetate or 20 mg per day dydrogesterone (73 women) sequentially on days 12–25. After 10–12 cycles, no statistically significant changes in endometrial thickness were observed by ultrasound assessment. End-of-study biopsies were taken and flow cytometric analysis was performed on endometrial tissue. No differences in cell-cycle distribution were observed among the three treatment groups, in all of which 61–81% of women had secretory or proliferative endometrial morphology. Endometrial hyperplasia was found in two cases in the group that took 0.625 mg equine estrogens plus 5 mg medroxyprogesterone acetate. No cases of endometrial carcinoma occurred.

Overall, these studies confirm that addition of progestogens to estrogen therapy for the menopause prevents the development of endometrial hyperplasia and reduces the increased rate of endometrial cell proliferation caused by estrogen only. This beneficial effect was found for all progestogens studied, regardless of the route of administration and dose. Norethisterone was the most frequently studied progestogen and, even at the lowest dose examined in randomized studies (in the range of 0.1 mg per day), there was a maximal protective effect for both estrogen-induced hyperplasia and cell proliferation. Treatment with some, but not all, progestogens given sequentially in combination with continuous estrogen treatment resulted in increased endometrial thickness, but this has not been studied in a sufficiently rigorous fashion to draw any conclusion. Most women treated with estrogen only and, to a lesser extent, women who took the combined therapy had a proliferative or secretory type endometrial histology, whereas most untreated postmenopausal women have atrophic or inactive endometrial morphology.

(iii) *Other effects of hormonal therapy*

Conner *et al*. (2004b) (see above for study details) found statistically significant reductions in free and total serum testosterone and increases in sex hormone-binding globulin (SHBG) caused by the combination of 17β-estradiol and norethisterone, but no change in insulin-like growth factor (IGF)-I levels. However, the combination of 17β-estradiol and norethisterone (acetate) or dienogest did not alter total serum testosterone levels in another study (Conner *et al*., 2003; see above for details), although it also increased SHBG but did not affect IGF-I. Dören *et al*. (2001) and Hofling *et al*. (2005) reported essentially the same findings in postmenopausal women treated with 17β-estradiol (2 mg per day) and norethisterone acetate (1 mg per day) for 6 or 12 months but found a decrease in the circulating levels of IGF-binding protein-1 and -3, no effects on dehydroepiandrosterone or its sulfate and only minor effects on androstenedione. However, Chatterton *et al.* (2005) found a reduction in the level of dehydroepiandrosterone sulfate in women who took Prempro® (conjugated estrogens plus medroxyprogesterone acetate).

Other studies have investigated the effects of treatment with progestogen plus estrogen on the IGF axis in more detail and have found that a variety of progestogens in combination with 17β-estradiol result in decreases in total and free IGF-I and IGF binding protein-3, increases in IGF-binding protein-1 and no effect on IGF-II (Heald *et al*., 2000; Campagnoli *et al*., 2002). The magnitude of these effects appears to depend on the type of progestogens used (Biglia *et al*., 2003; Campagnoli *et al*., 2003), but apparently not on the route of administration of the estrogen and progestogens (Raudaskoski *et al*., 1998). However, in another study, significant differences were found between the effects of transdermal and oral treatment on IGF-I, SHBG and growth hormone-binding protein (Nugent *et al.*, 2003).

Another issue is the possibility that the various regimens used in hormonal therapy for the menopause may affect the metabolism of the hormonal agents used, as suggested by studies of estrogen metabolism (Seeger *et al*., 2000; Mueck *et al*., 2001, 2002) (see also Section 4.1).

These studies may suggest reduced androgenic stimulation, e.g. of the breast, and changes in the IGF axis. However, several of the progestogens used, such as norethisterone, have androgenic activity themselves and antigonadotropic effects reported for progestogens such as norethisterone may not be mediated by androgen receptor mechanisms (Couzinet *et al*., 1996). Furthermore, there is a lack of consistency in many observations, such as the inconsistent effects reported on the IGF axis. Nevertheless, these studies raise the possibility of complex interactions of the agents used in hormonal therapy for the menopause with various hormonal systems.

(*b*) *Experimental systems*

(i) *Animal studies*

Three studies of the effects of hormonal therapy regimens in cynomolgus monkeys that had been surgically rendered postmenopausal are summarized in Section 3.1.2 (Cline *et al*.,

1996, 1998; 2002a,b). In these studies, continuous treatment with conjugated equine estrogens slightly increased the rate of cell proliferation in the mammary gland after 2–3 years of exposure, but this increase was not statistically significant. Addition of medroxyprogesterone acetate to the continuous treatment with estrogen increased the rate of cell proliferation in the lobuloalveolar mammary tissue by 50–100% over control values. Staining of mammary tissue for progesterone receptor, an indicator of estrogenic activity, was markedly increased by treatment with estrogen only and this effect was reduced by the addition of progestogen to the treatment. A preliminary study (Isaksson *et al*., 2003) explored the effect of the same regimens on the immunohistochemical mammary expression of progesterone receptor-A and -B subtypes in cynomolgus monkeys. Treatment with progestogen alone did not significantly affect cell proliferation or expression of the progesterone receptor-A and -B. However, when ethinylestradiol plus norethisterone acetate was used as a regimen for only 1 year, cell proliferation was not increased (Suparto *et al*., 2003). One study of short duration in mice injected with 17β-estradiol and progesterone also found increased mammary cell proliferation in the combined estrogen plus progestogen group compared with the control groups (Raafat *et al*., 2001).

[These results are consistent with the observations in breast tissue of women who took conjugated equine estrogen plus medroxyprogesterone acetate as hormonal menopausal therapy but not those who took ethinylestradiol plus norethisterone.]

(ii) *Cell culture and other studies*

Studies of the effects of estrogen–progestogen combinations on breast cell proliferation *in vitro* were carried out with 17β-estradiol and a variety of progestogens, doses and treatment regimens. Lippert *et al*. (2000, 2001, 2002), Mueck *et al*. (2003) and Seeger *et al*. (2003a,b) determined the in-vitro effects of a range of progestogens on the proliferation of MCF-7 breast cancer cells induced by 10 nM 17β-estradiol, either combined for 5–7 days or sequentially using estrogen only for 4–5 days followed by combined exposure for 3–5 days. Although the results of these studies are not completely identical, they generally showed that norethisterone, medroxyprogesterone acetate, progesterone, chlormadinone acetate, dienogest, 3-keto-desogestrel, gestodene and levonorgestrel counteracted the cell proliferation induced by 17β-estradiol in these estrogen receptor-positive cells. The effects were stronger when exposure to the estrogen and progestogens occurred simultaneously for 5–10 days than when the progestogens were added 4–5 days after the start of estrogen treatment for 3–5 days in some, but not in all studies (Lippert *et al*., 2000, 2001). Although the consistency across these studies was not perfect, the continuous regimen with progesterone generally produced the strongest counteraction to the 17β-estradiol-induced stimulation of cell proliferation; medroxyprogesterone acetate gave an intermediate and norethisterone gave the weakest counteraction (Seeger *et al*., 2003a). The inhibitory effect required concentrations of progestogens greater than 1 nM. The equine estrogens, equilin and 17α-dihydroequilin, induced cell proliferation to a lesser extent than 17β-estradiol and progestogens inhibited their activity to a lesser extent than that of 17β-estradiol (Mueck *et al*.,

2003). [These findings suggest a protective effect on breast cancer of combined exposure to estrogen–progestogen but this does not correlate with the epidemiological data.]

Franke and Vermes (2002, 2003) and Franke *et al*. (2003) observed similar effects, but the potencies of progestogens to inhibit 17β-estradiol-induced cell proliferation differed from those found previously (Lippert *et al.*, 2001; Mueck *et al.*, 2003; Seeger *et al.*, 2003a,b), which may be related to the fact that they measured an indicator of cell proliferation, cyclin D, and not proliferation *per se*. They also found that apoptosis (measured by flow cytometry) was induced by 17β-estradiol and enhanced by progestogens, but the actual data were not presented in their reports. However, they used single high doses of both hormones (1 μM) and one time-point — 6 days of continuous treatment. Treatment of T47D human breast cancer cells with 17β-estradiol and medroxyprogesterone acetate resulted in a variety of changes in gene expression patterns (Mrusek *et al*., 2005). [Although the biological significance of these findings is not clear at present, the changes in gene expression patterns differed between treatments with estrogen only and those with estrogen plus progestogen.]

The endometrial effects of 17β-estradiol (10 nM) with or without medroxyprogesterone acetate (100 nM) were studied by Bläuer *et al*. (2005) in an organotypic culture system of primary human endometrial cells. 17β-Estradiol significantly doubled the percentage of cells that stained for Ki-67 over control values, whereas addition of the progestogen significantly reduced the percentage of Ki-67-positive cells to 50–70% of control values. The apparent effect of 17β-estradiol on cell proliferation required the presence of stromal cells and raised the possibility that the effect is indirect and stroma-mediated. These findings correlate with the supposition that the addition of progestogens to estrogen therapy confers a protective effect for the endometrium. Treatment of primary human endometrial cells with 17β-estradiol in the presence or absence of norethisterone acetate resulted in a variety of changes in gene expression patterns that differed depending on the presence of progestogen (Oehler *et al*., 2002). [Although the biological significance of these findings is not clear at present, the differences in gene expression patterns between treatment with estrogen only and estrogen plus progestogen may, once confirmed and extended, provide a mechanistic basis for differences in the known biological effects of the two treatments.]

4.2.2 *Individual estrogens and progestogens*

(*a*) *Humans*

No new data were available to the Working Group.

(*b*) *Experimental systems*

(i) *Estrogens*

Only one new study of estrogenic compounds that are used in hormonal therapy for the menopause (conjugated equine estrogens, ethinylestradiol or mestranol) that is rele-

vant to the evaluation of the carcinogenic risk of such therapy via the oral or other routes has been carried out since the previous evaluation (IARC, 1999).

17β-Estradiol has been shown to increase the generation of reactive oxygen species through anchorage- and integrin-dependent signalling to mitochondria. The 17β-estradiol-induced reactive oxygen species increased the phosphorylation of c-Jun and cyclic adenosine monophosphate-response element-binding protein and increased the transcriptional activity of redox-sensitive transcription factors, activator protein 1 and the phosphorylated element-binding protein; these are involved in growth of estrogen-dependent cancer cells (Felty *et al.*, 2005a). Inhibitors of protein synthesis, transcription and replication and function of mitochondria, as well as antioxidants, effectively reduced the estrogen-induced growth of breast cancer cells by blocking the estrogen-induced G_1/S transition of G_0-arrested MCF-7 cells (Felty *et al*., 2005b). These authors suggested that, in addition to the receptor activity of estrogens, other factors such as reactive oxygen species may be involved in the early growth of cancer cells (Felty *et al*., 2005b).

(ii) *Progestogens*

New studies of progestogens, including those most recently introduced, have been conducted that may be relevant to an evaluation of the carcinogenic risk of combined hormonal therapy via the oral or other routes, but no new studies were available on chlormadinone acetate, ethynodiol diacetate or norethynodrel.

Many studies described the influence of substituting active groups on the basic molecule of several progestogens — desogestrel, (3-keto-)desogestrel (etonogestrel), gestodene, levonorgestrel, norethisterone and drospirenone — on receptor binding, receptor transactivation and in-vivo hormonal activities (Deckers *et al*., 2000; Schoonen *et al*., 2000a; Garciá-Becerra *et al*., 2004); these are summarized in Section 4.2.3(*b*) of the monograph on Combined estrogen–progestrogen contraceptives in this volume.

A few studies have examined the role of estrogen and progesterone receptor subtypes on the activities of progestogens and their divergent tissue-specific effects. Estrogen receptor α but not estrogen receptor β appears to be activated by the A-ring 5α-reduced metabolites of both norethisterone and gestodene which have weak estrogenic activity (Larrea *et al*., 2001). However, Pasapera *et al.* (2002) found that the same metabolites of norethisterone activated both estrogen receptors α and β, and Rabe *et al.* (2000) obtained similar results for norethisterone but not for gestodene. These divergent findings may be related to the fact that the former study used HeLa and Chinese hamster ovary cells, whereas the latter studies used CV-1 monkey kidney cells and T-47D breast cancer cells or COS7 cells. Progesterone, norethisterone, levonorgestrel, desogestrol and gestodene are progestogens that are used in hormonal therapy and contraception. They bind with approximately equal affinity to the progesterone receptor subtypes A and B in MCF-7 cells, in Chinese hamster ovary cells stably transfected with these receptor subtypes and in in-vivo assays (Schoonen *et al*., 1998). However, after supertransfection of these receptor subtypes in different Chinese hamster ovary cell subclones, differences among the progestogens tested were found in the stimulation of reporter genes for the two receptor subtypes in diffe-

rent clones (Dijkema *et al*., 1998). These studies illustrate the critical roles of both metabolism and receptor-subtype specificity in the various hormonal effects of progestogens, while tissue or cell specificity appears to be another critical determinant of the activities of progestogens on receptor subtypes.

Progestogens may potentially affect not only factors that are related to tumour development and tumour cell growth. Some new evidence suggests that they may also affect factors that are related to tumour progression, such as angiogenesis. However, this is an emerging field of research that does not allow any conclusions to be drawn at present. For example, medroxyprogesterone acetate, progesterone, norethisterone, norgestrel and norethynodrel are mediated by the progesterone receptor B and have been shown to induce vascular endothelial growth factor in human breast cancer cells (Wu *et al*., 2004). Dienogest, on the contrary, was shown to inhibit tumour cell-induced angiogenesis (Nakamura *et al*., 1999) (See also Section 4.2.3(*b*) of the monograph on Combined estrogen–progestogen contraceptives).

Dydrogesterone inhibits the activity of estrogen sulfatase and 17β-HSD in the human breast cancer cell lines MCF-7 and T-47D and inhibits the conversion of estrone to 17β-estradiol in these cells (Chetrite *et al*., 2004).

Medroxyprogesterone acetate stimulated proliferation of the progesterone receptor-positive breast cancer cell line T-47D in a time-dependent manner with a biphasic dose–response (Thuneke *et al*., 2000). Induction of cyclin D1 was found to parallel the stimulation of cell proliferation at the same (fairly high) dose of 250 nM. In addition, medroxyprogesterone acetate appears to inhibit the induction of apoptosis by serum depletion of several human breast cancer cell lines at a non-cytotoxic dose of 10 nM. However, this effect was only found in progesterone receptor-positive cell lines and not in the progesterone receptor-negative cell line MDA-MB-231, which suggests that this is a progesterone receptor-mediated effect (Ory *et al*., 2001).

A study of norethisterone by Schoonen *et al*. (2000b) indicates that some of its various 3β- and 5α-reduced metabolites are much stronger estrogens or androgens *in vivo* than the parent compound. Their respective receptor-binding affinities and receptor-transactivation activities correlate with this observation. Rabe *et al.* (2000) found that norethisterone moderately transactivated estrogen receptor α in COS7 cells in a manner that was inversely related to dose. It transactivated estrogen receptor β somewhat more strongly; a concentration of 0.1 nM was strongly estrogenic (85% that of ethinylestradiol, which is 100% estrogenic) but higher (1 nM) and lower (0.01 nM) concentrations were far less estrogenic.

Nomegestrol acetate is a strong progestogen that is relatively devoid of other hormonal activities. Its properties have been reviewed by Shields-Botella *et al*. (2003).

Nestorone and trimegestone are newly synthesised progestogens that are less progestogenic than nomegestrol acetate but have activities that are in the same range as those of progesterone itself and are also relatively devoid of other hormonal activities. The properties and activities of nestorone have been reviewed and described by Kumar *et al*. (2000), Tuba *et al*. (2000) and Sitruk-Ware *et al*. (2003), and those of trimegestone by Zhang *et al*. (2000), Lundeen *et al*. (2001) and Winneker *et al*. (2003).

Progesterone is a natural progestogen that is used in a highly bioavailable, micronized form in hormonal therapy in combination with estrogens (de Lignières, 1999). Differential metabolism occurs in normal and malignant human breast tissue and results in 4-pregnene and 5α-pregnane metabolites that have opposite effects on MCF-7 breast cancer cell proliferation *in vitro* (Wiebe *et al*., 2000). The 5α-pregnane metabolites stimulate, whereas the 4-pregnene metabolites inhibit cell proliferation. Progesterone and 17β-estradiol affect IGF-I and IGF-binding protein -2 and -3 in a complex but non-synergistic manner, and not all of these effects can be blocked by the anti-estrogen tamoxifen or the anti-progestogen RU486 (mifepristone) (Milewicz *et al*., 2005a). Similarly, progesterone has been shown to stimulate local production of growth hormone in human breast cancer explants, which cannot be counteracted by RU486 (Milewicz *et al.*, 2005b). Progesterone inhibits the 17β-estradiol-stimulated proliferation of MCF-7 cells more strongly than either medroxyprogesterone acetate or norethisterone (Seeger *et al*., 2003a); this may have implications for the risk for breast cancer in women who are treated with either progesterone or a synthetic progestogen in combination with estrogen (Fournier *et al.*, 2005).

Medroxyprogesterone acetate or synthetic progestogen R5020 (as surrogate of progesterone) induced distinctly different changes in gene expression in progesterone receptor-negative Ishikawa endometrial cells that had been stably transfected with either progesterone receptor A or B (Smid-Koopman *et al*., 2005). In both cases, however, the cells responded to medroxyprogesterone acetate or synthetic progestogen R5020 by growth inhibition and induction of apoptosis, which suggests that there is no difference between the two subtypes in the molecular pathways involved in these responses. In a rat endometrial cell line that expresses progesterone receptor, however, progestogen R5020 prevented apoptosis; this was counteracted by RU486 (Pecci *et al*., 1997). [These results indicate that the progesterone receptor is required for these types of response of endometrial cells to progestogens, but suggest that the responses are highly cell type-specific.]

4.3 Side-effects other than genetic or cancer-related effects

Estrogen–progestogen therapy was designed to provide estrogen to women in order to relieve the vasomotor effects of the menopause and progestogen to modulate the adverse effects of estrogen on the uterus. The actual effects of the combination of these two types of hormone may differ from those of estrogen alone, depending on the target tissue considered. The addition of progestogen may ameliorate the adverse effects of estrogen at some sites, but counteract its possible beneficial effects at other sites. The complexity and interactions of estrogen–progestogen combinations should be borne in mind when considering these therapies.

4.3.1 *Cardiovascular effects*

It is generally believed that women are relatively protected from the development of coronary artery disease until the menopause: the incidence of cardiovascular disease in

women lags behinds that in men by approximately 20 years (Colditz *et al.*, 1987; Grundy *et al*., 1999). Ovarian hormones appear to be involved in the maintenance of these lower rates, since ovariectomized women who do not take hormonal therapy have an incidence of cardiovascular disease similar to that of men of the same age and, at any given age, postmenopausal women have a higher incidence of cardiovascular disease than women who menstruate normally (Wuest *et al.*, 1953; Gordon *et al.*, 1978).

A large body of evidence from observational studies has suggested that postmenopausal hormonal supplementation is associated with a 35–50% reduction in cardiovascular mortality and morbidity (Stampfer & Colditz, 1991). However, the majority of case–control and cohort studies on this subject have been conducted on women who used estrogen-only therapy. These hypothetical benefits have not been confirmed by several recent randomized trials of generally asymptomatic postmenopausal women (Hulley *et al.*, 1998; Herrington *et al.*, 2000; Manson *et al.*, 2003; Anderson *et al.*, 2004). Progestogens that are added to estrogens in combined hormonal therapy to reduce the risk for uterine malignancy have a number of potential adverse effects on the cardiovascular system, which may alter their efficacy in postmenopausal women. [Because combined hormonal therapy is so widely used, it is of pivotal importance to know whether or not it has an effect on cardiovascular disease.] Progestogens can have various effects on the vasomotor system, which are dependent on the agent and the dose regimen, and may also induce vasoconstriction of estrogenized vessels (Horwitz & Horwitz, 1982; Lin *et al.*, 1982).

It should be noted that hormonal therapy is designed to address symptoms that are related to reduced production of female hormones in the peri- and postmenopausal intervals. The menopause may involve changes other than cessation of estrogen and progestogen production, and the use of exogenous hormones may not mimic premenopausal physiology.

(*a*) *Biological effects of estrogens on the cardiovascular system*

The putative protective effect of estrogens on the cardiovascular system has for a long time been associated with their beneficial effect on the metabolism and deposition of cholesterol, which contributes to the inhibition of the formation of atherosclerotic plaque in the arterial walls (Bush & Barrett-Connor, 1985; Bush *et al.*, 1987). Although early reports suggested that up to 50% of the protective effect of estrogens on coronary artery disease was attributable to favourable changes in plasma lipids, it is now believed that the changes in lipids induced by estrogens are probably not relevant (Rossouw, 2000; Rossouw *et al.*, 2002).

Estrogen deprivation has been associated with an increased risk for coronary artery disease and poorer vascular functions in women (Wuest *et al.*, 1953; Gordon *et al.*, 1978; Colditz *et al.*, 1987). Acute and chronic administration of estrogens to estrogen-deficient individuals restores the endothelium-dependent vasodilatation of coronary arteries that is lost after the menopause (Herrington *et al.*, 1994; Collins *et al.*, 1995; Volterrani *et al.*, 1995).

In the past decade, it has become clear that ovarian hormones have significant effects on arterial blood flow (Gilligan *et al.*, 1994a; Reis *et al.*, 1994; Collins *et al.*, 1995). The vascular effects of ovarian hormones may differ according to their chemical structure, and it is important not only to differentiate the effects of estrogens from those of progestogens but also to distinguish between the effects of different estrogens. Estrogens induce vasodilation while estrogen depletion leads to vasomotor instability, diminished vasodilatory activity and enhanced sensitivity to vasoconstrictor stimuli (Kronenberg *et al.*, 1984; Penotti *et al.*, 1993; Gilligan *et al.*, 1994a; Herrington *et al.*, 1994; Reis *et al.*, 1994; Collins *et al.*, 1995). Ovarian hormones act at all levels of the arterial structure — the endothelium, the vascular smooth muscle and the nerve endings in the adventitia in almost all the arterial systems; they act very rapidly and at both non-genomic and genomic levels (Kronenberg *et al.*, 1984; Jiang *et al.*, 1991, 1992a; Penotti *et al.*, 1993; Gilligan *et al.*, 1994a; Herrington *et al.*, 1994; Reis *et al.*, 1994; Collins *et al.*, 1995).

(i) *Calcium-antagonistic action of estrogens*

Early in-vitro studies showed that 17β-estradiol has a relaxing effect on isolated rabbit and human coronary artery rings and cardiac myocytes contracted both by activation of receptor-operated and potential-operated calcium channels, due to a calcium-antagonistic effect (Jiang *et al.*, 1991, 1992a,b; Chester *et al.*, 1995). Subsequent in-vitro studies in animals and humans produced further evidence that estrogens have calcium-antagonistic properties, which account for a new non-endothelium-dependent mechanism of relaxation of coronary and peripheral arteries. The calcium-antagonistic property of estrogen was confirmed in coronary vascular myocytes by measuring cytosolic concentration, contraction and calcium current. Sudhir *et al.* (1995) demonstrated that estrogens cause dilation of coronary conductance and resistance arteries in dogs when administered acutely into the coronary circulation. This in-vivo effect was shown to be endothelium-independent and partially mediated by effects on calcium channels. Calcium-antagonistic properties of estrogen have also been demonstrated in uterine arteries, cardiac myocytes and vascular smooth muscle cells (Stice *et al.*, 1987; Jiang *et al.*, 1991, 1992a; Sudhir *et al.*, 1995). Since it has been proposed that calcium channel blockers may reduce the progression of atherosclerosis in animals, it has been suggested that estrogens may reduce the progression of coronary artery disease by a similar mechanism in humans (Collins *et al.*, 1993).

(ii) *Endothelial action of estrogens*

Another important component of the effect of estrogens on the vascular system is mediated through the endothelium. In-vivo studies have demonstrated that estrogens potentiate the endothelium-dependent vasodilator response to acetylcholine in the coronary arteries of animals and humans (Gilligan *et al.*, 1994a; Reis *et al.*, 1994; Collins *et al.*, 1995). The effect of estrogens on the restoration of altered endothelial function was demonstrated *in vitro*, and in animals and humans *in vivo* in different vascular beds. Williams *et al.* (1990) reported that a reversal of acetylcholine-induced vasoconstriction was produced by subcutaneous implants of 17β-estradiol in ovariectomized monkeys fed

an atherogenic diet for 30 months. Volterrani *et al.* (1995) showed that 17β-estradiol reduces peripheral vascular resistance and increases peripheral blood flow in menopausal women. Collins *et al.* (1995) showed that acute administration of 17β-estradiol reverses the coronary constrictor effect of acetylcholine in postmenopausal women with coronary artery disease and that the effect is gender-dependent. Reis *et al.* (1994) demonstrated an increase in coronary blood flow and epicardial cross-sectional area and decreased resistance in postmenopausal women 15 min after an intravenous infusion of ethinylestradiol. Abnormal coronary vasomotor responses to acetylcholine were also attenuated. Similar results were obtained by Gilligan *et al.* (1994a,b) in women who received continuous infusions of estrogens to achieve physiological concentrations of intracoronary 17β-estradiol. These studies indicated that estrogens influence vascular tone by enhancing the production of an endothelium-derived relaxant factor (nitric oxide). Estrogen receptors have been identified in endothelial cells from human aorta, and coronary and umbilical arteries (Kim-Schulze *et al*., 1996; Venkov *et al*., 1996). Caulin-Glaser *et al.* (1997) demonstrated that estradiol induced a rapid (within 30 min) increase in the production of nitric oxide in human umbilical vein endothelial cells grown in cell culture and that this action was inhibited when the estradiol receptor was blocked. Estrogen can induce calcium-dependent nitric oxide synthase activity, which results in an increased release of nitric oxide. Using inhibitors of nitric oxide synthase, Tagawa *et al.* (1997) demonstrated that estrogens considerably improve both nitric oxide-mediated and non nitric oxide-mediated vasodilation in the peripheral vasculature of the forearm in humans.

Since coronary artery tone plays a significant role in the pathogenesis of ischaemic cardiac syndromes, the effect of estrogens on the reactivity of coronary arteries may be important to the cardiovascular effects of these hormones. Rosano *et al.* (2006) showed that intracoronary administration of 17β-estradiol attenuates the vasoconstrictor effect of methylergometrine in women with coronary artery disease, which suggests that 17β-estradiol has a direct effect on the smooth muscle of coronary vessels in humans.

(iii) *Inflammatory effects of estrogens*

C-Reactive protein is a marker of inflammation that has been associated with the risk for coronary heart disease in menopausal women (Ridker *et al.*, 2000; Pradhan *et al.*, 2002). Estrogen administered with or without progestogen rapidly and substantially increases plasma concentrations of C-reactive protein in menopausal women (van Baal *et al*., 1999; Cushman *et al.*, 1999; Ridker *et al.*, 1999). The effects of hormonal therapy on other markers of inflammation are not consistent with those on C-reactive protein, which suggests that they may be related to metabolic hepatic activation (Silvestri *et al.*, 2003). [The extent to which hormonal therapy affects cardiovascular risk through inflammation is unknown.]

(iv) *Route of administration*

To establish differences in alteration of some risk factors for cardiovascular disease (such as lipoproteins, fibrinogen) according to the route of administration of hormonal

menopausal therapy, a study was carried out among women aged 50–65 years who received hormones orally, transdermally or by implantation. Initial therapy consisted of oral or transdermal estrogen alone for 3 months, followed by concomitant cyclical, continuous oral or transdermal administration of norethisterone, as appropriate, for a further 3 months. A separate group of women received an implant of estrogen only followed by an implant of estrogen and testosterone combined. All regimes lowered LDL cholesterol; the oral route was more potent than the parenteral route. Risk factors for cardiovascular disease were significantly reduced in women who used both oral and transdermal estrogen–progestogen therapy compared with untreated menopausal women (controls), although some of the benefit of estrogen alone on fibrinogen and HDL were attenuated (Seed *et al.*, 2002).

(*b*) *Biological effects of progestogens on the cardiovascular system*

Progesterone receptors are present in the arterial wall, and the effects of progestogens on arteries are therefore probably mediated by these receptors as well as by down-regulation of the estradiol receptor. Progestogen therapy has various effects on arterial function; it can stabilize arteries that are in a state of vasomotor instability, but may also induce vasoconstriction of estrogenized vessels (Mercuro *et al.*, 1999). Because progestogens have estrogenic effects in some systems and also have progestational mineralo-corticoid and androgenic effects, there has been some concern that combined estrogen–progestogen therapy may modify some of the effects of estrogens on the cardiovascular system (Williams *et al.*, 1994; Adams *et al.*, 1997).

(i) *Progestogens and lipid profile*

Estrogen-only therapy lowers total serum cholesterol and LDL cholesterol, increases HDL cholesterol and produces an effect upon plasma triglycerides, which is dependent upon the route of administration of the estrogens and the baseline plasma levels of lipids (Walsh *et al.*, 1991). It also stimulates the removal of cholesterol from the systemic circulation, which results in an increase in reverse cholesterol transport (Tikkanen *et al.*, 1982).

In contrast to estrogens, progestogens induce hepatic lipase activity, which increases the degradation of HDL-cholesterol. Accordingly, the addition of a progestogen to estrogens tends to attenuate the increase in serum HDL-cholesterol and the decrease in LDL-cholesterol that are obtained with estrogen only, an effect that may be related to the biochemical structure, dose, androgenic potency and regimen of the progestogen. Progestogens that have pure progestogenic activity do not alter lipid metabolism; 19-nortestosterone derivatives reduce HDL cholesterol, while 17α-hydroxyprogesterone derivatives and non-androgenic progestogens (19-norpregnane derivatives) seem to have little effect and progesterone has no detrimental effect on plasma lipids (Tikkanen *et al.*, 1986; Rijpkema *et al.*, 1990; Walsh *et al.*, 1991; The Writing Group for the PEPI Trial, 1995).

(ii) *Progestogens and coronary atherosclerosis*

The vasodilatory and anti-atherogenic effects of estrogens on normal and diseased arteries are well known. Estrogens may influence the progression of coronary atherosclerosis and, when administered either acutely or chronically, may reverse acetylcholine-induced vasoconstriction in both animals and humans. When administered in combination with estrogens, progestogens may interfere with these effects.

Adams *et al.* (1997) evaluated the effect of estrogen-only and estrogen–progestogen therapy in ovariectomized monkeys fed an atherogenic diet and found that estrogens alone or in association with progesterone or medroxyprogesterone acetate significantly reduced (by 50–70%) the degree of coronary atherosclerosis when therapy began soon after oophorectomy, but not when it began later (Williams *et al.*, 1994, 1995; Adams *et al.*, 1997; Clarkson *et al.*, 1998, 2001). Nevertheless, it should be noted that the effect of progestogens on the arteries of non-human primates may be mediated by different metabolic pathways than those present in human arteries. Therefore, the effects of progestogens on atherosclerosis and vascular function cannot be extrapolated fully from animals to humans.

In a randomized, placebo-controlled trial, Herrington *et al.* (2000) compared the effect of hormonal supplementation with conjugated equine estrogens alone or in combination with medroxyprogesterone acetate on the progression of coronary atherosclerosis in normocholesterolaemic postmenopausal women (aged ≥ 55 years) with proven coronary artery disease. After a mean follow-up of 3.2 years, no significant difference in mean coronary artery stenosis was found between women allocated to estrogen alone, estrogen plus progestogen or placebo.

The evaluation of intima-media thickening of arteries may help to identify initial stages of atherosclerosis. Several studies have shown that long-term estrogen therapy or combined hormonal therapy are effective in delaying the progression of early stages of atherosclerosis by reducing the intimal thickening in users of hormone compared with non-users (Espeland *et al.*, 1995a; Liang *et al.*, 1997; Hodis *et al.*, 2001).

At present, no role has been established for combined hormonal therapy in the prevention of the progression of atherosclerosis in postmenopausal women. From a preventive aspect, the inhibition of plaque formation and progression of small plaques is more important than a reduction in the size of pre-existing atherosclerotic plaques.

(iii) *Progestogens and vascular reactivity*

Progestogens have vasoactive properties that are partly mediated by non-nuclear receptors. Since the expression of these receptors on the cell surface is influenced by levels of circulating estrogen, exposure to estrogens may affect the response of the vascular tree to progestogens.

Several studies have evaluated the effect of progesterone and other progestogens on coronary arteries *in vitro* and have demonstrated an endothelium-independent mechanism of relaxation that differs minimally between a variety of substances (Jiang *et al.*, 1992c). Miller and Vanhoutte (1991) assessed relaxation in coronary artery strips from ovariecto-

mized dogs treated with estrogen, progesterone or estrogen plus progesterone. The relaxation response was similar in the coronary arteries of animals that received estrogen and in those that received progesterone, while it was minimally reduced in the group treated with the combined therapy. Therefore, it seems that there is little or no detrimental effect of progesterone on vasomotility, at least *in vitro* (Rosano *et al*., 2003). However, pure progesterone is not commonly used in combined hormonal therapy.

Studies carried out *in vivo* suggested that synthetic progestogens may antagonize the dilator effect of estrogens in experimental animals. Two studies evaluated the separate and combined effects of conjugated equine estrogens and medroxyprogesterone acetate on the coronary reactivity of atherosclerotic monkeys. Exposure to estrogen increased coronary dilator responses and blood flow reserve, while co-administration of the progestogen resulted in a 50% reduction in dilation (Williams *et al.*, 1994; Adams *et al*., 1997). Again, the effect of synthetic progestogens cannot be fully translated to women, due to the different metabolic pathways that operate in animals and humans.

Androgenic progestogens have been reported to reduce the beneficial effect of estrogens on vascular reactivity to a greater extent than progesterone and less androgenic progestogens; similar results were found in studies of carotid artery stiffness (Vitale *et al.*, 2001; Rosano et *al*., 2001; Gambacciani *et al*., 2002; Rosano *et al*., 2003).

(*c*) *Biological effects of hormones on risk for thrombosis*

Estrogens have many different effects on the coagulation system. These include increases in the levels of prothrombin fragments 1 + 2 and reductions in those of the anticoagulant factors, protein S and antithrombin, and may also occur after transdermal administration (Teede, *et al*., 2000; Post *et al.*, 2003). These modifications predict a change towards a more pro-coagulant state (which was confirmed in studies that examined activated protein C resistance or thrombin generation) that is not counterbalanced by an increase in fibrinolytic activity (Teede *et al*., 2000). It is currently unclear how these effects are induced at the molecular level of the estrogen receptor. At the cellular level, these effects are probably under genetic control, because the haemostatic system of some women appears to be more sensitive to the effect of estrogens than that of others. How estrogens and progestogens interact in their effect on thrombosis is also unclear. It appears that estrogens are pro-thrombotic rather than pro-atherogenic, which explains an increase in risk for thrombosis in former users (Bloemenkamp et *al*., 1998; Koh *et al.*, 1999); again genetic factors may play an important role (Bloemenkamp *et al.*, 2002).

Selective modulators of the estrogen receptor, such as tamoxifen and raloxifene, have anti-estrogenic effects on breast and endometrial tissue and are used in the treatment and prevention of breast cancer. However, these drugs have estrogenic effects on blood clotting (Peverill, 2003).

(*d*) *Effects of the use of estrogen and estrogen plus progestogen on the risk for cardiovascular disease*

(i) *Coronary heart disease*

The effects of hormonal therapy on coronary heart disease have been evaluated in two randomized trials. The HERS and the WHI have provided critical data on the effect of hormonal therapy on primary and secondary prevention in postmenopausal women with or without coronary artery disease (Hulley *et al.*, 1998; Rossouw *et al.*, 2002). Neither study found a protective effect of fixed-dose hormonal therapy. For continuous combined hormones, the WHI reported a hazard ratio for coronary heart disease of 1.24 (95% CI, 1.00–1.54) over an average of 5.2 years of follow-up; most of the apparent risk occurred during the first year (hazard ratio, 1.81; 95% CI, 1.09–3.01). These effects did not differ by age (Manson *et al.*, 2003). In the HERS secondary prevention trial, the hazard ratio for coronary heart disease for the same combined hormonal therapy over an average of 4.1 years of follow-up was 0.99 (95% CI, 0.80–1.22) with some evidence of an increased risk in the first year (Hulley *et al.*, 1998). The WHI found no significant effects on the risk for coronary heart disease of estrogen alone over an average of 6.8 years of follow-up (hazard ratio, 0.91; 95% CI, 0.75–1.12). The data suggested the possibility that younger women experience a reduction in risk (*p* value for interaction, 0.14; hazard ratio, 0.56; 95% CI, 0.30–1.03) (Anderson *et al.*, 2004).

Few epidemiological studies have investigated the effect of combined hormones, but all have suggested that estrogen plus progestogen therapy may be more effective than estrogen therapy alone in the reduction of cardiovascular events. The majority of the observational studies suggested that the risk for coronary artery disease was reduced in women who received both estrogen only and estrogen–progestogen therapy (Stampfer & Colditz, 1991).

All of the observational studies were conducted in healthy women who used hormonal therapy for reasons other than the menopause and who were generally at low risk for coronary heart disease. The effect of the adjunct of a progestogen to estrogens in women who are at increased risk for cardiovascular disease may differ. The discrepancies with the randomized trials require that the observational studies be viewed cautiously. Biases inherent in cohort and case–control studies and variability of dosing, duration and other time-dependent effects of hormonal therapy must be taken into account in order to present a balanced view of the results. Careful control for confounding and an allowance for an adverse effect during the first 2 years of exposure, with attenuation of this effect in subsequent years, has been shown to align the results from observational studies with those of randomized trials (Prentice *et al.*, 2005). Petitti (1994) argued that compliance bias could account for some of the observed benefits. It has been suggested that the women who were included in the hormonal therapy groups were of higher socioeconomic class, had healthier habits and exercised regularly. Barrett-Connor (1991) demonstrated that a healthy women bias exists at least for the women who were included in the Rancho Bernardo (CA, USA) study population (Barrett-Connor *et al.*, 1989). Not all study populations were restricted to upper class

retirement areas, however, and no socioeconomic differences were noted in the Nurses' Health Study (Grodstein *et al.*, 2000) or other studies. Selection bias probably exists in nearly all studies, since women who take hormonal therapy tend to exercise more and have healthier habits. Therefore, estimates of risk reduction in women who take ovarian hormones may be biased towards finding a protective effect that may be due in part to a healthier lifestyle (Nelson *et al.*, 2002a).

(ii) *Stroke*

The WHI trial reported an increase in risk for stroke from estrogen plus progestogen therapy (hazard ratio, 1.31; 95% CI, 1.02–1.68) that was restricted to ischaemic stroke (hazard ratio, 1.44; 95% CI, 1.09–1.90) (Wassertheil-Smoller *et al.*, 2003). The WHI trial of estrogen alone was interrupted because of the observed increase in risk for stroke (hazard ratio, 1.39; 95% CI, 1.10–1.77) (Anderson *et al.*, 2004). The HERS trial reported a hazard ratio for estrogen plus progestogen of 1.18 (95% CI, 0.83–1.66) for non-fatal stroke and 1.61 (95% CI, 0.73–3.55) for fatal stroke during an average follow-up of 4.1 years (Simon *et al.*, 2001a). The Women's Estrogen for Stroke Trial conducted on 664 postmenopausal women who had had a recent ischaemic stroke or a transient ischaemic attack found no beneficial effect of 17β-estradiol (1 mg per day) for stroke (hazard ratio, 1.1; 95% CI, 0.8–1.6) or mortality (hazard ratio, 1.2; 95% CI, 0.8–1.8) after a mean follow-up of 2.8 years (Viscoli *et al.*, 2001). A meta-analysis of randomized trials found a significant increase in risk for stroke (relative risk, 1.44; 95% CI, 1.10–1.89) with no substantial variation between studies (Gabriel-Sanchéz *et al.*, 2005).

A meta-analysis of nine observational studies suggested that hormonal therapy is associated with a small increase in risk for stroke (relative risk, 1.12; 95% CI, 1.01–1.23) that is primarily confined to thromboembolic stroke (relative risk, 1.20; 95% CI, 1.01–1.40) (Nelson *et al.*, 2002b).

(iii) *Thrombosis*

The risk for venous thrombotic disease was increased twofold (hazard ratio, 2.06; 95% CI, 1.57–2.70) for estrogen plus progestogen in the WHI trial (Cushman *et al.*, 2004) and almost threefold in the HERS trial (hazard ratio, 2.89; 95% CI, 1.50–5.58) (Hulley *et al.*, 1998). Pulmonary embolism and deep vein thrombosis were similarly affected. The WHI trial reported a smaller increase with estrogen alone (hazard ratio, 1.33; 95% CI, 0.99–1.79) (Anderson *et al.*, 2004).

(*e*) *Estrogen–progestogen therapy in postmenopausal women*

The divergent results of observational and randomized studies on cardiovascular endpoints have led many authors to stress the superiority of randomized clinical trials over observational studies, but have not solved the dilemma of the effect of hormonal therapy on the cardiovascular system. The discrepancies in the results of observational and randomized studies are related to methodological issues and to differences between study populations, hormonal regimens and time- and age-dependent biological effects of hormones

during different periods of the lives of women. Observational studies typically did not distinguish between the hormonal regimens used, whereas the randomized studies examined a fixed dose of continuous combined conjugated equine estrogens plus medroxyprogesterone acetate. Although the treatment regimens in the two types of study differed, the dose of estrogen and progestogen used probably played an important role. More recent progestogens that have fewer androgenic and mineralo-corticoid effects may influence the risks for cardiovascular disease differently. Several varied opinions on the potential cardiovascular effects — beneficial and adverse — of hormonal therapy for postmenopausal women are still emerging, and the selection of patients, dose regimen and timing of treatment are probably critical (Rosano *et al.*, 2004).

It is possible that factors other than methodological differences may explain the divergent effects of hormonal therapy noted in observational and randomized studies. The randomized clinical studies were conducted exceptionally well and methodological design is not an issue. A key difference between the observational and randomized studies is the women under study: in the observational studies, the exposed women chose to take hormonal therapy for menopausal symptoms and represented long-term compliers while, in the randomized studies, the absence of severe menopausal symptoms was a prerequisite for inclusion in the study. This seemingly small difference may have important implications, since symptomatic women are younger and have clinical symptoms that suggest an effect of a lack of estrogen on several organs or systems. The low prevalence of symptoms may indicate a physiological adaptation to lower levels of ovarian hormone in these women, due to a slow decline in estrogen levels or the long time lapse since menopause, and therefore a new homeostasis. These and other biological explanations for the divergent results of observational and randomized studies should be considered in detail (Rosano *et al*., 2004).

(i) *Ageing and cardiovascular response to estrogen–progestogen therapy*

Several studies that evaluated the effect of hormonal therapy on intermediate markers of coronary heart disease in women and in non-human primates indicated substantial benefits, although they also suggested some adverse effects (Scarabin *et al.*, 1999; Walsh *et al.*, 2000; Silvestri *et al.*, 2003). Clinical and experimental evidence suggests that the putative cardio-protective and anti-atherogenic effects of ovarian hormones are receptor-mediated and endothelium-dependent (Caulin-Glaser *et al.*, 1997; Mikkola *et al.*, 1998). Both estrogen receptors and endothelial function are markedly influenced by the time at which estrogen deprivation and progression of the atherosclerotic injury occur. Evidence indicates that expression of the estrogen receptor in the arterial wall is sharply diminished with increasing age, which might be related to an age-related increase in methylation of the promoter region of the estrogen receptor gene in vascular areas with atherosclerosis (Post *et al.*, 1999).

Time since menopause and the presence of atherosclerosis are associated with a reduced cardio-protective effect of estrogens, while the unfavourable effects of hormones

on coagulation remain unaltered. Therefore, in early postmenopausal women such as those included in observational studies, hormonal therapy may be cardio-protective because of the responsiveness of the endothelium to estrogens while, in late postmenopausal women, hormonal therapy has either no effect or even a detrimental effect because of the predominance of the pro-coagulant or plaque-destabilizing effects over the vasculo-protective effects. It is possible that hormonal therapy inhibits atherosclerosis in younger women but may not be able to inhibit progression of atherosclerosis and complicated plaques that lead to coronary events in older women. This hypothesis has also been suggested by randomized studies of postmenopausal cynomolgus monkeys in which estrogens had no effect on the extent of coronary artery plaque in those assigned to estrogen alone or to estrogen combined with medroxyprogesterone acetate beginning 2 years (approximately 6 human years) after oophorectomy (Williams *et al*., 1995). When given to younger monkeys soon after oophorectomy, hormonal treatment resulted in a 50% reduction in the extent of plaques (Clarkson *et al*., 2001; Mikkola & Clarkson, 2002).

The effect of atherosclerosis and ageing on the vascular responsiveness to hormonal menopausal therapy has been also analysed in several clinical studies (Herrington *et al.*, 2001). In the Cardiovascular Health Study, women (over 65 years of age) who had established cardiovascular disease had a flow-mediated vasodilator response that was equal among women who used hormones (estrogen alone or estrogen combined with progestogen) and those who did not; among women (over 65 years of age) who had no cardiovascular disease, users of hormones had a 40% better response than non-users (Herrington *et al.*, 2001). In the Estrogen Replacement and Atherosclerosis Trial, a randomized trial that involved women (aged ≥ 55 years) who had documented coronary disease, no effect of estrogen alone or of estrogen combined with progestogen was found on the diameter of coronary arteries (Herrington *et al*., 2000). In contrast, in the Estrogen in the Prevention of Atherosclerosis Trial, in which younger women (aged ≥ 45 years) who had no cardiovascular disease were randomly assigned to 17β-estradiol or placebo, the average rate of progression of carotid atherosclerosis was lower in women assigned to estrogen (Hodis *et al.*, 2001).

(ii) *Characteristics of the study populations*

Women recruited in the randomized studies comprised a broader age range and were representative of individuals for whom prevention interventions would be contemplated. Observational studies recruited women who were exposed to hormonal therapy primarily for short-term relief of menopausal symptoms and longer-term use for the prevention or treatment of osteoporosis. Recently, a new class of progestational agents with anti-aldosteronic properties has been developed: the prototype of this class is drospirenone, a progestational agent that can reduce water retention, body weight and blood pressure (Keam & Wagstaff, 2003). The addition of this newer progestogen to estrogens in hormonal therapy schemes may help to minimize the side-effects of estrogen therapy that are related to water and salt retention and may represent a new strategy for the treatment of postmenopausal women (Pollow *et al.*, 1992; Krattenmacher, 2000).

In a randomized trial of 230 hypertensive postmenopausal women, treatment with drospirenone plus 17β-estradiol was not associated with a higher incidence of hyperkalaemia than treatment with placebo in patients with and without type-2 diabetes mellitus and concomitant use of angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists or ibuprofen. Drospirenone plus 17β-estradiol was found to reduce both systolic and diastolic blood pressure compared with the placebo group (Preston *et al.*, 2005).

Body mass index is an important marker of endogenous estrogen in postmenopausal women and has been associated with risk for cardiovascular disease especially when it is ≥ 25 kg/m^2 (Wilson *et al.*, 2002). In a very large cohort of 290 827 postmenopausal women, the coronary benefits of hormonal therapy were found exclusively in women with a lower body mass index (< 22 kg/m^2) (Rodriguez *et al.*, 2001). In the WHI, there was no significant interaction with body mass index, which mean was 28.5 (standard deviation [SD], 5.9) (Manson *et al.*, 2003).

Randomized studies of hormones in younger women who seek treatment for menopausal symptoms are not informative with regard to hormonal effects on rates of cardiovascular disease, because of the very low incidence rates in this population.

4.3.2 *Other effects*

(*a*) *Established benefits*

(i) *Control of vasomotor symptoms*

The primary indication for use of hormonal therapy during the menopause is vasomotor symptoms. Numerous studies have documented the beneficial effects of estrogen, either alone or combined with progestogen, for the relief of hot flushes/flashes and night sweats. MacLennan *et al.* (2004b) recently reviewed the randomized, double-blind placebo controlled trials of hormonal therapy and reported a summary measure of the reduction in the frequency of hot flushes of 75% (95% CI, 64.3–82.3%) relative to placebo, accompanied by similarly important reductions in the severity of symptoms.

(ii) *Prevention of osteoporosis and fractures*

Hormonal therapy has also been shown to be effective for the prevention or treatment of osteoporosis and bone fractures. In the WHI trial, the risk for hip fractures was reduced by both estrogen alone (hazard ratio, 0.61; 95% CI, 0.41–0.91) and by estrogen plus progestogen (hazard ratio, 0.67; 95% CI, 0.47–0.96) (Cauley *et al.*, 2003; Anderson *et al.*, 2004). Beneficial effects on bone mineral density have been shown for other hormonal preparations (Recker *et al.*, 1999; Lees & Stevenson, 2001; Arrenbrecht & Boermans, 2002; Civitelli *et al.*, 2002).

(*b*) *Overview of evidence for other effects*

The efficacy of hormonal therapy for the above indications has typically been established in randomized trials of up to a few hundred women and a follow-up of a few months for vasomotor symptoms and 3 years for osteoporosis. These trials lack sufficient

power to establish rates of disease or rarer side-effects with adequate precision. Further, because of the strong evidence for beneficial effects on vasomotor symptoms, such trials often lack a placebo group, which obscures any inference of hormonal effects on other outcomes. Two large randomized trials, the WHI (Rossouw *et al*., 2002) and the HERS (Grady *et al.*, 1998) are the primary exceptions. Both of these were randomized, double-blinded, placebo-controlled trials of hormonal therapies that tested chronic disease prevention strategies in the USA. The WHI trial involved separate placebo-controlled comparisons of estrogen plus progestogen (conjugated equine estrogens plus medroxyprogesterone acetate) in 16 608 postmenopausal women aged 50–79 years with an uterus and estrogen alone in 10 739 postmenopausal women in the same age range with prior hysterectomy (Stefanick *et al*., 2003). The HERS trial tested the same combined hormonal regimen in 2764 postmenopausal women under 80 years of age who had a uterus and documented coronary artery disease. Additional details for both trials are provided in Section 2. Because of the strength of the evidence derived from these two studies, most of the information summarized here on other effects of hormonal therapy relies on data from these trials. For many outcomes, important additional information derives from the Postmenopausal Estrogen/Progestin Interventions (PEPI) study, another randomized, double-blinded, placebo-controlled trial conducted in the USA to evaluate the effects of three combined hormonal regimens and one estrogen-alone therapy on biomarkers of cardiovascular disease in 840 postmenopausal women aged 45–65 years (Espeland *et al.*, 1995b). Data from other randomized trials are described only when they contradict these findings or introduce different inferences. There is also an immense body of data from observational studies that include many features of health that cannot be summarized adequately here. Mention is made of these data only when significant controversy exists between the findings from trials and observational studies.

(i) *Quality of life and symptoms associated with menopause or ageing*

Quality of life

Improvement in other symptoms that are commonly associated with ageing or menopause have been reported, occasionally in conjunction with overall quality of life. The reported benefits vary across studies; the differences are probably attributable to the populations studied and the dimensions of symptoms and quality of life used. No clinically significant effects on measures of general quality of life were observed with estrogen plus progestogen therapy in the WHI trial (Hays *et al.*, 2003a) despite significant improvements in vasomotor symptoms and vaginal or genital dryness (Barnabei *et al.*, 2005). In the HERS trial, estrogen plus progestogen decreased hot flushes, vaginal dryness and sleep troubles (Barnebei *et al.*, 2002) and improved depression scores, but was associated with worsening of some health measures (i.e. more rapid decline in physical function scores and in energy/fatigue) (Hlatky *et al.*, 2002). Trials designed specifically to test the effect of hormonal therapy on vasomotor symptoms as their primary objective generally reported an

improvement in quality of life, derived primarily from the relief of hot flushes, night sweats and related conditions (e.g. Wiklund *et al.*, 1993).

Vaginal bleeding

Common side-effects of hormonal therapy are vaginal bleeding, breast tenderness, urinary incontinence and headaches. Among women with a uterus, bleeding rates vary by type of hormonal therapy, schedule of progestogen, age and time since initiation of therapy. The prevalence of bleeding with both sequential and continuous formulations has led to numerous attempts to identify hormonal therapy regimens that provide good relief of vasomotor symptoms while minimizing bleeding (e.g. Saure *et al.*, 1996; Al-Azzawi *et al.*, 1999; Cano *et al.*, 1999; Saure *et al.*, 2000; Mendoza *et al.*, 2002). Intermittent and continuous treatment with progestogen has been used to reduce bleeding, especially in older women. In the WHI estrogen plus progestogen trial, 51% of women randomized to continuous combined therapy but less than 5% of women who took placebo reported vaginal bleeding within the first 6 months. The proportion who reported bleeding in the active hormonal therapy group declined thereafter but remained above 11% throughout the study (Barnabei *et al.*, 2005). These estimates do not take into account the non-compliance to the therapy (approximately 42% by year 5) (Rossouw *et al.*, 2002), some of which was a result of bleeding. Most of the bleeding in the active hormonal therapy group was classified as spotting (Barnabei *et al.*, 2005). Bleeding, especially after the first few months of use, causes concern and may lead to significantly increased rates of endometrial biopsy (Anderson *et al.*, 2003). Intermittent treatment provides only a marginal improvement in bleeding rates (Cano *et al.*, 1999), but some studies have found reduced bleeding with higher doses of progestogen (Al-Azzawi *et al.*, 1999). Many smaller randomized trials lacked either a placebo control or a common active treatment control group against which to judge the relative effects. Nevertheless, few significant differences in symptom control or vaginal bleeding were reported.

Breast symptoms

The frequency of breast symptoms, variously reported as breast tenderness, discomfort, pain or mastalgia, is also significantly increased by hormonal therapy. In the WHI trial, 9.3% of asymptomatic women randomized to combined hormones reported breast tenderness at year 1 compared with 2.4% who took placebo, and this proportion remained elevated at year 3 (odds ratio, 2.55; 95% CI, 0.98–6.64) (Barnabei *et al.*, 2005). In the PEPI trial, the risk for greater breast discomfort was doubled by all three combined hormonal regimens relative to placebo (range of odds ratios, 1.92–2.33). Corresponding risks for estrogen alone were not increased over those for placebo (odds ratio, 1.16; 95% CI, 0.70–1.93) (Greendale *et al.*, 1998), which suggests that these breast symptoms may be an effect of protestogen.

Mammographic screening

Use of exogenous hormones interferes with mammographic screening. In the WHI trial, Chlebowski *et al.* (2003) reported that 9.4% of women assigned to combined hormones had an abnormal mammogram during the first year of use compared with 5.4% of placebo-treated women ($p < 0.001$), a period during which there was no excess incidence of breast cancer. This pattern of increased incidence of mammographic abnormalities persisted throughout the follow-up. In a study ancillary to the PEPI trial, Greendale *et al.* (2003) reported increased breast density with all three combined hormonal regimens (change from baseline in adjusted mean mammographic per cent density ranged from 3.1 to 4.8%), which was significantly different from the rate of change in the placebo group (–0.07%; 95% CI, –1.50–1.38%). Use of estrogen alone resulted in a non-significant 1.17% (95% CI, –0.28–2.62%) adjusted mean change, which again suggests that progestogen is the active agent in these breast changes. However, in a randomized, double-blinded, placebo-controlled multi-arm trial of raloxifene and estrogen, the mean breast density in women who took estrogen was significantly greater than that in the other arms (Freedman *et al.*, 2001).

Urinary incontinence

Urinary incontinence is adversely affected by hormonal therapy. Asymptomatic women randomized to estrogen alone in the WHI Trial experienced an increased risk for self-reported urinary incontinence at 1 year, including all subtypes: stress (hazard ratio, 2.15; 95% CI, 1.77–2.62), urge (hazard ratio, 1.32; 95% CI, 1.10–1.58) and mixed urinary incontinence (hazard ratio, 1.79; 95% CI, 1.26–2.53). The addition of medroxyprogesterone acetate did not alter these effects substantially. Among women who reported urinary incontinence at baseline, the risk for worsening the self-reported frequency of incontinence, amount of leakage, limitation in activities and bother associated with the symptoms was significantly elevated by both hormonal regimens at 1 year (Hendrix *et al.*, 2005). In a 3-year randomized, double-blind, placebo-controlled osteoporosis prevention trial (Goldstein *et al.*, 2005), 7% of 158 women randomized to conjugated equine estrogens reported new or worsening urinary incontinence compared with 1.3% of the 152 women randomized to placebo ($p \leq 0.02$). In the HERS trial, Grady *et al.* (2001) found that 39% of women who took estrogen plus progestogen reported worsening symptoms compared with 27% of women who took placebo ($p = 0.001$). Several smaller, short-term, randomized, double-blind, placebo-controlled trials of hormonal therapy have been carried out in incontinent women (Wilson *et al.*, 1987; Fantl *et al.*, 1996; Jackson *et al.*, 1999); some of these used objective measurements of response, but none identified any significant therapeutic response.

Headaches

Frequency and duration of headaches may be affected by hormonal therapy. In the WHI trial, the incidence of headaches or migraines in the placebo-treated group was 4.7% and was modestly increased by estrogen plus progestogen at 1 year to 5.8% (odds ratio,

1.26; 95% CI, 1.08–1.46) (Barnabei *et al.*, 2005). Vestergaard *et al.* (2003) did not find an effect on the occurrence of headache after 5 years in the Danish Osteoporosis Prevention Study. In women who are known to suffer from migraine headaches, however, there is evidence of an increase in the frequency of attacks, the number of days with headache and analgesic consumption over 6 months of observation during continuous combined, continuous sequential or cyclical sequential hormonal therapy (Facchinetti *et al.*, 2002).

(ii) *Incidence of disease*

In addition to the more common symptoms associated with hormonal therapy, there is increasing evidence of hormonal effects on other clinical outcomes.

Gallbladder disease

Randomized trials have shown a significant increase in the rates of gallbladder disease and biliary tract surgical procedures following hormonal therapy. In the WHI trial, Cirillo *et al.* (2005) reported a hazard ratio for estrogen alone of 1.67 (95% CI, 1.35–2.06) for the incidence of hospitalized gallbladder disease or related surgical procedures over an average of 7.1 years of follow-up and a hazard ratio for estrogen plus progestogen of 1.59 (95% CI, 1.28–1.97) over a mean 5.6 years of follow-up (attributable risk of 31 and 20 cases per 10 000 person–years, respectively). The HERS results on estrogen plus progestogen for the same combined outcome over the initial mean 4.1 years of follow-up were similar (hazard ratio, 1.38; 95% CI, 1.00–1.92) (Simon *et al.*, 2001b) and became significant (hazard ratio, 1.48; 95% CI, 1.12–1.95) during the 6.8-year average open-label extended follow-up period (Hulley *et al.*, 2002). [The Working Group noted that these results suggest that the effect is primarily a function of estrogen alone and may be dependent on duration.] Several observational studies have also found evidence of an adverse effect of hormonal therapy on gallbladder disease (e.g. Mamdani *et al.*, 2000; Boland *et al.*, 2002).

Dementia and cognitive function

Despite preliminary evidence of improved cognitive function following hormonal therapy, randomized trials have not provided evidence of a benefit. On the contrary, data from these trials suggest an increased risk for dementia and a negative impact on cognitive function in women randomized to hormones. The strongest evidence derives from the WHI Memory Study, which is an ancillary study of women over 65 years of age at randomization into one of the WHI hormone trials. In this subset of older participants, the combined hormonal therapy group experienced a significantly increased risk for probable dementia relative to the placebo-treated group (hazard ratio, 2.05; 95% CI, 1.21–3.48) over an average of 4 years of follow-up (Shumaker *et al.*, 2003). A somewhat smaller increase was observed with estrogen alone relative to placebo (hazard ratio, 1.49; 95% CI, 0.83–2.66) over an average of 5 years (Shumaker *et al.*, 2004). The increases in the incidence of probable dementia were not explained by those observed in the incidence of stroke. Rapp *et al.* (2003) and Espeland *et al.* (2004) found an adverse effect of hormonal therapy on global cognitive function for both combined hormones and estrogen alone.

Grady *et al.* (2002b) reported no significant differences between the effects of estrogen plus progestogen and placebo among women at 10 HERS centres from six end-of-study measurements of cognitive function (mean age at time of testing, 71 ± 6 years). One measurement, verbal fluency, was significantly worse ($p = 0.02$) in women who were randomized to estrogen plus progestogen. In the PEPI trial, in which the average age of participants was 56 years (SD, 4.3 years; somewhat younger than those in the WHI or HERS), no significant differences were found between the placebo-treated group and the four hormonal therapy groups at 12 or 36 months for self-reported forgetfulness, concentration or distraction (Reboussin *et al.*, 1998). No randomized trial has been conducted to examine the risk for dementia in younger women. Several very small, short-term randomized trials of hormonal therapy in women with dementia have been conducted (Henderson *et al.*, 2000; Mulnard *et al.*, 2000; Asthana *et al.*, 2001), each of which reported some modest improvements in a subset of the cognitive measurements examined but no consistent pattern of effects.

In contrast, observational studies have mostly reported substantially lower rates of dementia associated with hormonal therapy but with variable relationships between duration and recency of use. In a Cache County Study report, women who had ever used hormonal therapy had a reduced risk for Alzheimer disease compared with non-users (odds ratio, 0.59; 95% CI, 0.35–0.96); the reduction was concentrated in former users or those who had more than 10 years of exposure. The risk for Alzheimer disease in current users of hormonal therapy was not affected (odds ratio, 1.08; 95% CI, 0.59–1.91). According to the authors, this pattern of effects suggests a limited window of time in which a beneficial effect of hormonal therapy on the risk for Alzheimer disease exists (Zandi *et al.*, 2002). Baldereschi *et al.* (1998) reported a reduced risk for Alzheimer disease with ever use of estrogen (odds ratio, 0.28; 95% CI, 0.08–0.98) in the Italian Longitudinal Study on Aging, which was an analysis of data from 1582 postmenopausal women in eight Italian cities. A similar reduction in risk for Alzheimer disease was reported in the Baltimore Longitudinal Study of Aging cohort of 472 peri- or postmenopausal women (mean age at enrollment, 61.5 years). After 16 years of follow-up, the hazard ratio for Alzheimer disease for ever use of oral or transdermal estrogen was 0.46 (95% CI, 0.21–1.00), but no effect of duration was observed (Kawas *et al.*, 1997). Paganini-Hill and Henderson (1994) reported a reduced risk for dementia in users of estrogen (odds ratio, 0.69; 95% CI, 0.46–1.03) and evidence of a stronger effect with higher dose and longer duration of use in a nested case–control study within the Leisure World cohort of southern California.

[Whether hormonal therapy used early in the menopausal period has a beneficial effect on dementia rates or whether these observed reductions arise from subtle patterns of prescription, adherence, survival or other biases remains unanswered.]

Diabetes

The risk for non-insulin-dependent diabetes has been found to be reduced by use of estrogen plus progestogen. In the WHI trial, rates of self-reported diabetes were reduced by 21% (95% CI, 7–33%) (Margolis *et al.*, 2004), and the observed reduction in the HERS

trial was 35% (95% CI, 11–52%) (Kanaya *et al.*, 2003), each of which was accompanied by corresponding changes in fasting glucose and insulin levels.

Other diseases or conditions

The potential impact of hormonal therapy on other age-related health conditions (e.g. osteoarthritis, rheumatoid arthritis, lupus, macular degeneration, cataract, Parkinson disease) has been examined in observational studies and in some randomized trials. The evidence for substantial effects on these disease processes is limited at this time, but additional studies would provide much needed clarification (Cooper *et al.*, 2002; d'Elia *et al.*, 2003; Abramov *et al.*, 2004; Currie *et al.*, 2004; Freeman *et al.*, 2004).

Mortality

Randomized trials have not shown a statistically significant effect on mortality during average intervention periods that ranged from 5.2 to 6.8 years (Hulley *et al.*, 2002; Roussouw *et al.*, 2002; Anderson *et al.*, 2004). Additional follow-up of the WHI cohorts will be of particular interest. A recent meta-analysis of 30 trials also found no effect of hormonal therapy on mortality (odds ratio, 0.98; 95% CI, 0.87–1.18). Further analyses suggested a possible reduction in mortality due to factors other than cancer or cardiovascular disease (Salpeter *et al.*, 2004).

4.4 Genetic and related effects

Estrogen may contribute to the promotion of uterine, breast, ovarian and cervix tumours. It is thought to sustain the growth of preneoplastic and malignant cells by acting through estrogen receptor-mediated signalling pathways that regulate the production of growth factors that maintain clonal growth of both cell types. Epidemiological observations and mathematical models derived therefrom have suggested that most malignant cells accumulate several genetic changes in the genes or chromosomes as they evolve into cancer. Since the previous evaluation (IARC, 1999), many studies in experimental systems on the possible direct genetic and genotoxic effects of steroid sex hormones as a factor in carcinogenesis have been published, and these are summarized in Tables 19 and 20. The data that are now available indicate more strongly that some of these hormones and their metabolites can cause DNA damage, which can potentially induce genetic alterations in cells.

As described in detail in Section 4.1 of this monograph, estrogens, i.e. estrone, 17β-estradiol, estriol and 17α-ethinylestradiol, are activated by aromatic hydroxylation at the C-2 and C-4 positions that is catalysed by CYPs. Subsequently, peroxidase enzymes convert them into catechol estrogens, i.e. 2-hydroxyestrogens and 4-hydroxyestrogens (Roy & Liehr, 1999; Cavalieri *et al.*, 2000; Roy & Singh, 2004). Catalytic oxidation of these two catechol estrogens gives rise to the corresponding estrogen-2,3-quinone and estrogen-3,4-quinone, which react with DNA to form adducts (Roy & Liehr, 1999;

Table 19. Genetic and related effects of estrogens in experimental systems

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Estradiol				
Gene mutation, Chinese hamster V79 cells, *Hprt* locus, *in vitro*	+	NT	0.01 nM [0.0027 ng/mL]	Kong *et al.* (2000)
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	1 µg/mL	Tsutsui *et al.* (2000a)
DNA single-strand breaks, comet assay, human peripheral blood lymphocytes *in vitro*	+	NT	50 µM [13.6 µg/mL]	Anderson *et al.* (1997)
DNA single-strand breaks, comet assay, human sperm *in vitro*	+	NT	10 µM [2.7 µg/mL]	Anderson *et al.* (1997)
DNA single-strand breaks, human MCF-7 cells *in vitro*	+	NT	1 nM [0.27 ng/mL]	Yared *et al.* (2002)
DNA single-strand breaks, human MCF-7 cells *in vitro*	+	NT	10 nM [2.7 ng/mL]	Rajapakse *et al.* (2005)
Sister chromosome exchange, human peripheral blood lymphocytes *in vitro*	+	+	25 µg	Ahmad *et al.* (2000)
Micronucleus formation, human MCF-7 cells *in vitro*	+	NT	1 nM [0.27 ng/mL]	Yared *et al.* (2002)
Chromosomal aberrations, human peripheral blood lymphocytes *in vitro*	+	+	25 µg	Ahmad *et al.* (2000)
Chromosomal aberrations, human peripheral blood lymphocytes *in vitro*	–	+	10 µg	Ahmad *et al.* (2000)
Aneuploidy, human MCF-7 cells *in vitro*	–	NT	3.6 µM [1 µg/mL]	Fernandez *et al.* (2005)
Micronucleus formation, mouse bone-marrow cells *in vivo*	–		150 mg/kg bw ip	Ashby *et al.* (1997)
Micronucleus formation, rat and mouse bone-marrow cells *in vivo*	–		1250 mg/kg bw ip	Shelby *et al.* (1997)
2-Hydroxyestradiol				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	–	NT	3 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	3 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	1 µg/mL	Tsutsui *et al.* (2000a)

Table 19 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks, human MCF-7 cells *in vitro*	+	NT	100 nM [30 ng/mL]	Rajapakse *et al.* (2005)
Aneuploidy, human MCF-7 cells *in vitro*	–	NT	3.6 µM [1 µg/mL]	Fernandez *et al.* (2005)
4-Hydroxyestradiol				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	+	NT	1 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	3 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	1 µg/mL	Tsutsui *et al.* (2000a)
DNA single-strand breaks, human MCF-7 cells *in vitro*	+	NT	100 nM [30 ng/mL]	Rajapakse *et al.* (2005)
Aneuploidy, human MCF-7 cells *in vitro*	–	NT	3.6 µM [1 µg/mL]	Fernandez *et al.* (2005)
2-Methoxyestradiol				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *in vitro*	+	NT	0.1 µg/mL	Tsutsui *et al.* (2000b)
Gene mutation, Syrian hamster embryo cells, *Hprt* locus, *in vitro*	+	NT	0.3 µg/mL	Tsutsui *et al.* (2000b)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	0.3 µg/mL	Tsutsui *et al.* (2000b)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	0.3 µg/mL	Tsutsui *et al.* (2000b)
Estrone				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	30 µg/mL	Tsutsui *et al.* (2000a)
DNA single-strand breaks, comet assay human MCF-7 cells *in vitro*	+	NT	0.1 nM [0.03 ng/mL]	Yared *et al.* (2002)

Table 19 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, human MCF-7 cells *in vitro*	+	NT	0.1 nM [0.03 ng/mL]	Yared *et al.* (2002)
2-Hydroxyestrone				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	3 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	0.3 µg/mL	Tsutsui *et al.* (2000a)
4-Hydroxyestrone				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *in vitro*	+	NT	3 µg/mL	Tsutsui *et al.* (2000a)
Gene mutation, Syrian hamster embryo cells, *Hprt* locus, *in vitro*	+	NT	1 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	3 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	3 µg/mL	Tsutsui *et al.* (2000a)
2-Methoxyestrone				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	10 µg/mL	Tsutsui *et al.* (2000a)
16α-Hydroxyestrone				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	10 µg/mL	Tsutsui *et al.* (2000a)

Table 19 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Estriol				
Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase, *Hprt* locus, *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
Aneuploidy, Syrian hamster embryo cells *in vitro*	–	NT	10 µg/mL	Tsutsui *et al.* (2000a)
DNA single-strand breaks, human MCF-7 cells *in vitro*	+	NT	10 nM [3 ng/mL]	Yared *et al.* (2002)
Micronucleus formation, human MCF-7 cells *in vitro*	–	NT	0.1 mM [28.9 µg/mL]	Yared *et al.* (2002)
Ethinylestradiol				
Salmonella typhimurium TA100, TA1535, TA98, TA97a, reverse mutation	–	–	10 mg/plate	Hundel *et al.* (1997)
Unscheduled DNA synthesis, rat hepatocytes *in vitro*	+	NT	1 µM [0.3 µg/mL]	Martelli *et al.* (2003)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	50 µM [15 µg/mL]	Martelli *et al.* (2003)
Sister chromosome exchange, human peripheral blood lymphocytes *in vitro*	+	+	1 µg/mL	Hundal *et al.* (1997)
Chromosomal aberrations, human peripheral blood lymphocytes *in vitro*	+	NT	1 µg/mL[c] 48 h	Hundal *et al.* (1997)
Chromosomal aberrations, human peripheral blood lymphocytes *in vitro*	–	+	10 µg/mL[d] 6 h	Hundal *et al.* (1997)
Sister chromosome exchange, mouse bone-marrow cells *in vivo*	+		1 mg/kg bw ip	Hundal *et al.* (1997)
Micronucleus formation, mouse bone-marrow cells *in vivo*	+		1 mg/kg bw ip	Hundal *et al.* (1997)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; ip, intraperitoneal
[c] Tested for 48 h without exogenous metabolic system only
[d] Tested with exogenous metabolic system only for 6 h. The result was negative without exogenous metabolic system after an exposure of 6 h.

Table 20. Genetic and related effects of progestogens in experimental animals

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Progesterone				
Unscheduled DNA synthesis, rat hepatocytes *in vitro*	–	NT	50 µM [15.7 µg/mL]	Martelli *et al.* (2003)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	50 µM [15.7 µg/mL]	Martelli *et al.* (2003)
Micronucleus formation, rat liver *in vivo*	+		100 mg/kg bw po	Martelli *et al.* (1998)
Medroxyprogesterone				
Unscheduled DNA synthesis, rat hepatocytes *in vitro*	–	NT	50 µM [17.2 µg/mL]	Martelli *et al.* (2003)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	50 µM [17.2 µg/mL]	Martelli *et al.* (2003)
Norethisterone				
Unscheduled DNA synthesis, rat hepatocytes *in vitro*	±	NT	10 µM [3 µg/mL]	Martelli *et al.* (2003)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	50 µM [15 µg/mL]	Martelli *et al.* (2003)
Sister chromosome exchange, human peripheral blood lymphocytes *in vitro*	–	–	75 µg/mL	Ahmad *et al.* (2001)
Chromosomal aberrations, human peripheral blood lymphocytes *in vitro*	–	–	75 µg /mL	Ahmad *et al.* (2001)
Micronucleus formation, rat liver *in vivo*	–		100 mg/kg bw po	Martelli *et al.* (1998)
Norgestrel				
Sister chromosome exchange, human peripheral blood lymphocytes *in vitro*	+	+	25 µg/mL	Ahmad *et al.* (2001)
Chromosomal aberrations, human peripheral blood lymphocytes *in vitro*	+	+	25 µg/mL	Ahmad *et al.* (2001)
Chromosomal aberrations, human peripheral blood lymphocytes *in vitro*	–	+	10 µg/mL	Ahmad *et al.* (2001)

Table 20 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cyproterone acetate				
DNA single-strand breaks, comet assay, rat hepatocytes *in vitro*	+	NT	10 µM [4 µg/mL]	Mattioli *et al.* (2004)
Unscheduled DNA synthesis, male rat hepatocytes *in vitro*	–	NT	10 µM [4 µg/mL]	Mattioli *et al.* (2004)
Unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	10 µM [4 µg/mL]	Mattioli *et al.* (2004)
Gene mutation, Big Blue™ transgenic Fischer 344 rats, *LacI* gene, *in vivo*	+		75 mg/kg bw × 1 po	Krebs *et al.* (1998)
Levonorgestrel				
Gene mutation, mouse lymphoma L5178Y cells *in vitro*	–	NT	NG	Jordan (2002)
Chromosomal aberrations, Chinese hamster ovary fibroblasts *in vitro*	–	NT	NG	Jordan (2002)
Desogestrel				
Salmonella typhimurium [strains NG], reverse mutation	–	–	NG	Jordan (2002)
Micronucleus formation, female rat liver *in vivo*	–		NG	Jordan (2002)
Potassium canrenoate				
DNA single-strand breaks, rat hepatocytes *in vitro*	+	NT	10 µM [3.7 µg/mL]	Martelli *et al.* (1999)
Unscheduled DNA synthesis, rat liver *in vitro*	+	NT	30 µM [11 µg/mL]	Martelli *et al.* (1999)
Micronucleus formation, rat hepatocytes *in vitro*	+	NT	30 µM [11 µg/mL]	Martelli *et al.* (1999)
DNA single-strand breaks, human hepatocytes *in vitro*	+	NT	30 µM [11 µg/mL]	Martelli *et al.* (1999)
DNA single-strand breaks, human peripheral blood lymphocytes *in vitro*	–	NT	90 µM [33 µg/mL]	Martelli *et al.* (1999)
Unscheduled DNA synthesis, human liver *in vitro*	–	NT	90 µM [33 µg/mL]	Martelli *et al.* (1999)
Micronucleus formation, human hepatocytes *in vitro*	–	NT	90 µM [33 µg/mL]	Martelli *et al.* (1999)
Micronucleus formation, human peripheral blood lymphocytes *in vitro*	–	NT	90 µM [33 µg/mL]	Martelli *et al.* (1999)

Table 20 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks, male rat liver *in vivo*	+		325 mg/kg bw po	Martelli *et al.* (2002)
DNA single-strand breaks, female rat thyroid and bone marrow *in vivo*	+		325 mg/kg bw po	Martelli *et al.* (2002)
DNA single-strand breaks, rat testes and ovary *in vivo*	+		81 mg/kg bw po	Martelli *et al.* (2002)
Micronucleus formation, rat liver *in vivo*	–		325 mg/kg bw po	Martelli *et al.* (2002)
Micronucleus formation, rat bone marrow polychromatic erythtocytes *in vivo*	–		325 mg/kg bw po	Martelli *et al.* (2002)
Drospirenone				
Unscheduled DNA synthesis, rat hepatocytes *in vitro*	+	NT	1 µM [0.36 µg/mL]	Martelli *et al.* (2003)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	50 µM [18.3 µg/mL]	Martelli *et al.* (2003)

[a] +, positive; –, negative; ±, equivocal; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; NG, not given; po, oral

Cavalieri *et al.*, 2000). These adducts can form stable modifications that remain in the DNA unless they are removed by repair. Alternatively, the modified bases can be released from DNA by destabilization of the glycosydic bond and result in the formation of depurinated or depyrimidinated sites (Cavalieri *et al.*, 2000).

4.4.1 *Humans*

At present, only two studies have reported the presence of catechol estrogen adducts in human breast tissue. Embrechts *et al.* (2003) analysed estrogen adducts in the DNA from 18 human samples: five malignant breast tumour samples, five samples of tissues adjacent to the tumour and eight alcohol-fixed and paraffin-embedded malignant breast tumour samples. Almost every DNA sample showed the presence of deoxyguanosine adducts of 4-hydroxyestradiol and 4-hydroxyestrone. In four patients who had used conjugated equine estrogens, 4-hydroxyequilenin–DNA adducts, derived from conjugated equine estrogen metabolites, were detected. In seven patients, deoxyadenosine adducts of 4-hydroxy-17α-ethinylestradiol were observed. The formation of catechol estrogen quinone-derived DNA adducts has also been reported in two breast samples that were collected from one woman with and one woman without breast cancer (Markushin *et al.*, 2003). The catechol quinone-derived adducts identified were 4-hydroxyestradiol-1-N3-adenosine, 4-hydroxyestrone-1-N3-adenosine and 4-hydroxyestradiol-1-N7-guanine.

4.4.2 *Experimental systems* (Tables 19 and 20)

Liquid chromatographic–tandem MS analysis of mammary fat pads of rats injected with 4-hydroxyequilenin showed a dose-dependent increase in DNA single-strand breaks and formation of alkylated guanine adducts that are prone to depurination; stable cyclic deoxyguanosine and deoxyadenosine adducts and other oxidized bases were also observed (Zhang *et al.*, 2001). Injection of 4-hydroxyestradiol or estradiol-3,4-quinone into the mammary glands of female ACI rats resulted in formation of the depurinating adducts 4-hydroxyestradiol-1-N3-adenosine and 4-hydroxyestradiol-1-N7-guanosine (Li *et al.*, 2004). 4-Hydroxyestradiol-GSH conjugates were also detected. Recently, 4-hydroxy catechol estrogen conjugates with GSH or its hydrolytic products (cysteine and *N*-acetylcysteine) were detected in picomole amounts both in tumours and hyperplastic mammary tissues from ERKO/Wnt-1 mice and demonstrated the formation of estrogen-3,4-quinones (Devanesan *et al.*, 2001). DNA adducts derived from 2-hydroxyestrogen-quinone have been shown to be mutagenic, and primarily produced G→T and A→T mutations in simian kidney (COS-7) cells (Terashima *et al.*, 2001). Estradiol-3,4-quinone reacted rapidly to form 4-hydroxyestradiol-1-N3-adenosine adducts that are depurinating adducts. Numerous A→G mutations in H-*ras* DNA were observed in SENCAR mouse skin treated with estradiol-3,4-quinone (Chakravarti *et al.*, 2001). [These studies indicate that certain estrogen metabolites can react with DNA to form adducts. Such adducts or the apurinic sites they generate in DNA can give rise to mutations and these, in turn, could contribute to the development of tumours.]

Recently, it was reported that estrogen-induced mammary gland tumours in female ACI rats show losses and gains in chromosomes. Cells with an increased copy number of the *c-myc* gene (7q33), one on each of the three homologues of a trisomy of chromosome 7, were observed. A frequency of aneuploidy of 61% in sporadic invasive human ductal breast cancers and 71% in ductal carcinoma *in situ* was also observed. The authors asserted that the estrogen-induced mammary tumours in female ACI rats resembled human ductal carcinoma *in situ* and invasive ductal breast cancer because they are aneuploid and exhibit a high frequency of *c-myc* amplification (Li *et al.*, 2002).

Mitochondria are significant targets of estrogen (reviewed by Roy *et al.*, 2004; Felty & Roy, 2005a,b). Recently Felty *et al.* (2005a) reported that physiological concentrations of 17β-estradiol stimulate a rapid production of intracellular reactive oxygen species which, in epithelial cells, depends on cell adhesion, the cytoskeleton and integrins. Induction of the production of reactive oxygen species by estradiol occurs much more rapidly than the estrogen receptor-mediated interaction with the genome. Furthermore, estradiol-stimulated production of reactive oxygen species does not depend on the presence of the estrogen receptor in breast cancer cells because it was equal in both estrogen receptor-positive cell lines MCF7, T47D and ZR75.1 and the estrogen receptor-negative cell line MDA-MB 468. Exposure of human mammary epithelial cells to 2- or 4-hydroxyestradiol has been shown to produce reactive oxygen species and a subsequent increase in the formation of 8-OHdG (Hurh *et al.*, 2004; Chen *et al.*, 2005). This finding shows that formation of reactive oxygen species following exposure to estradiol could explain oxidative damage in hormone-dependent tumours and subsequent genetic alterations reported earlier (Malins & Haimanot, 1991; Musarrat *et al.*, 1996; Yamamoto *et al.*, 1996; Malins *et al.*, 2001). Mutations have recently been reported to occur following exposures to physiological and pharmacological concentrations of estrogens (Kong *et al.*, 2000; Singh *et al.*, 2005).

It has been shown that catechol estrogens can induce aldehydic DNA lesions in calf thymus DNA (Lin *et al.*, 2003). Equilin and equilenin are major constituents of Premarin®, a widely prescribed drug used in estrogen therapy for the menopause. These equine estrogens are metabolized, respectively, to 4-hydroxyequilin and 4-hydroxyequilenin, which, in turn, are oxidized to products that react with DNA. Mutations induced by 4-hydroxyequilin have been identified in supF plasmid shuttle vectors that were transfected in human fibroblast cells (Yasui *et al.*, 2003).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Combined estrogen–progestogen menopausal therapy involves the co-administration of an estrogen and a progestogen to peri- or postmenopausal women. These hormones may be given as individual compounds administered simultaneously or as combination preparations. Early treatment regimens included estrogen only. After a substantial increase

in the 1960s and early 1970s, the use of these regimens declined after 1975 when a strong association with endometrial cancer was found. When the addition of a progestogen was introduced as a strategy to reduce this risk, the use of hormonal menopausal therapy again increased steadily in the 1980s, particularly in developed countries. Combined estrogen–progestogen menopausal therapy is now administered to women who have not undergone a hysterectomy, whereas estrogen-only menopausal treatment tends to be prescribed to hysterectomized women. Although combined hormonal therapy was initially indicated for the control of menopausal symptoms, its application was expanded in the 1990s to include the treatment or prevention of a range of conditions related to ageing. However, since 2002, dramatic declines in use followed the report of a broad range of adverse effects in the Women's Health Initiative Estrogen Plus Progestin Trial in the USA. Reflecting this new evidence, practices are returning to a narrower set of indications directed at the short-term treatment of menopausal symptoms.

Combined estrogen–progestogen formulations are frequently used in hormonal menopausal therapy, although separate administration of each hormonal component is still prevalent. Commercial preparations are available for oral, vaginal and transdermal administration. Currently, continuous exposure to both hormones (both estrogen and progestogen at fixed daily doses) is common, particularly in the USA, whereas cyclical dosing, in which progestogen is added periodically to daily estrogen, is prevalent in other countries. Other scheduling strategies are also used occasionally. Some formulations and doses that are currently available for combined hormonal therapy are new and their possible long-term adverse effects have not been evaluated.

Combined hormonal therapy is much more commonly used in developed than in developing countries. At the peak of use in 1999, approximately 20 million women in developed countries used combined hormonal therapy, including 50% of women aged 50–65 years in the USA. Use has fallen by more than 50% since 2002, particularly for continuous combined hormonal therapy. Use in some developing countries also has declined modestly, although the data are more limited. Among peri- and postmenopausal women in developed countries, current users of combined hormonal therapy tend to be younger and more highly educated, to have a lower body mass and to use health care more regularly than non-users. The characteristics of users are known to vary between countries and to change over time.

5.2 Human carcinogenicity data

Breast cancer

Two large randomized trials, 10 cohort studies and seven case–control studies reported on the relationship between the use of combined estrogen–progestogen menopausal therapy and breast cancer in postmenopausal women. The studies consistently reported an increased risk for breast cancer in users of combined estrogen–progestogen therapy compared with non-users. The increased risk was greater than that in users of estrogen alone. The available evidence was inadequate to evaluate whether or not the risk for breast cancer

varies according to the progestogenic content of the therapy or its dose, or according to the number of days each month that the progestogens are added to the estrogen therapy. Observational studies showed that the relative risk was greater for lobular than for ductal cancers. The increase in the risk for breast cancer was largely confined to current or recent users, and the risk increased with increasing duration of use of the combined hormonal therapy.

Endometrial cancer

One randomized trial, four cohort studies and eight case–control studies reported on the relationship between use of combined estrogen–progestogen menopausal therapy and the risk for endometrial cancer in postmenopausal women. The risk for endometrial cancer was inversely associated with the number of days per month that progestogens were added to the regimen. The addition of progestogens to estrogen therapy for less than 10 days per month was associated with a significantly higher risk for endometrial cancer than never use of hormonal therapy, and the risk increased with increasing duration of use of that regimen. Estrogen therapy with daily progestogens was associated with a risk for endometrial cancer similar to, and possibly lower than, that found in women who had never used hormonal therapy. In contrast, the use of estrogens alone was associated with a considerably higher risk than that of any combined estrogen–progestogen regimen. Use of combined estrogen–progestogen menopausal therapy began relatively recently and, as yet, there is little information on its effects on the risk for endometrial cancer many years after cessation of use. The available evidence was inadequate to evaluate whether or not the risk for endometrial cancer varies according to the type or daily dose of progestogen.

Cervical cancer

The data from two randomized trials were inadequate to suggest that combined estrogen–progestogen hormonal therapy alters the risk for human papillomavirus infection or cervical cancer, and are of limited statistical power.

Ovarian cancer

Data from one randomized trial and two cohort and four case–control studies were inadequate to evaluate an association between ovarian cancer and combined estrogen–progestogen hormonal therapy.

Colorectal cancer

Two randomized trials and four cohort and three case–control studies provided information on the use of combined estrogen–progestogen hormonal therapy and the risk for colorectal cancer. None showed significantly elevated risks in women who had used these preparations for any length of time. Seven studies showed relative risks below 1.0 and the risk was significantly reduced in two, which suggests a potential protective effect. The

reduced risk tended to be observed among recent users and did not appear to be related to duration of use.

Other cancers

Large randomized trials provided the only substantial data on risk for lung cancer, which was slightly but not significantly elevated in users of combined estrogen–progestogen hormonal therapy. Observational data on lung cancer include both slightly increased and slightly reduced rates in users of such combined hormonal therapy. Data on cancer at other sites, including the liver, were too limited for evaluation.

5.3 Animal carcinogenicity data

Relatively few studies have been carried out to examine the tumorigenic effects of combined hormonal therapy in animals.

Oral administration of combined hormonal therapy in mice that are prone to develop mammary tumours resulted in similar incidences of mammary tumours in controls and in animals treated with conjugated equine estrogens alone and with conjugated equine estrogens plus medroxyprogesterone acetate. However, tumour latency was reduced in animals treated with conjugated equine estrogens plus medroxyprogesterone acetate. Conjugated equine estrogens plus medroxyprogesterone acetate suppressed the development of uterine adenomyosis.

Oral administration of conjugated equine estrogens alone or with medroxyprogesterone acetate to ovariectomized rats pretreated with the carcinogen 7,12-dimethylbenz[*a*]-anthracene increased the incidence of mammary tumours with equal frequency and to a level equal to that in non-ovariectomized controls.

5.4 Other relevant data

Absorption, distribution, metabolism and excretion

Various combinations of estrogens and progestogens are used for hormonal menopausal therapy. Since steroids penetrate normal skin easily, a variety of systems have been developed that deliver estrogens and progestogens parenterally (e.g. transdermal patches), thus by-passing the liver.

While the mechanisms of absorption and distribution of estrogens and progestogens have been known for a number of years, only recently has an understanding of the genes that encode the enzymes which control the enzymatic steps involved in steroid metabolism been acquired. This applies especially to the oxidative metabolism of estrogen. The phase I enzymes cytochrome P450 1A1 and 1B1 catalyse the production of catechol estrogen and metabolites of estrogen quinone that can induce the formation of DNA adducts. This is counteracted by the phase II enzymes, catechol-*O*-methyltransferase and glutathione

S-transferase P1, which reduce the levels of catechol and quinones by forming methoxyestrogens and glutathione conjugates. Polymorphic variants of these and other enzymes occur frequently in the population and several are associated with altered enzyme function. A large body of epidemiological data has failed to identify a consistent association between exposure to hormones and risk for cancer with any single enzyme variant. However, possible interactions between these genes need to be examined.

Progestogens are discussed in the monograph on Combined estrogen–progestogen contraceptives.

Receptor-mediated effects

The use of combined estrogen–progestogen menopausal therapy increases the rate of cell proliferation in the postmenopausal human breast, and appears to enhance significantly the modest increase in breast-cell proliferation induced by estrogen alone. Oral administration of conjugated equine estrogens alone or in combination with medroxyprogesterone acetate to ovariectomized monkeys resulted in an increase in epithelial cell proliferation and epithelial density in the mammary gland, as determined histologically, whereas the combination of conjugated equine estrogens and norethisterone acetate did not. The effects were greater with conjugated equine estrogens plus medroxyprogesterone acetate than with conjugated equine estrogens or medroxyprogesterone alone. Subcutaneous implantation of 17β-estradiol alone or in combination with progesterone for 3 days into ovariectomized monkeys resulted in a slight increase in epithelial cell proliferation in the mammary gland as did intraperitoneal administration of 17β-estradiol alone or in combination with progesterone to ovariectomized mice; the effect in mice was greater with 17β-estradiol plus progesterone than with 17β-estradiol alone. Approximately one-third of women treated with daily estrogen (by any route) plus daily oral progestogen develop increased mammographic breast density. In contrast, following treatments with estrogen daily plus progestogen less frequently than daily, a smaller proportion of women develop increased breast density. The addition of progestogens to estrogen therapy for the menopause prevents the development of endometrial hyperplasia and reduces the increased rate of endometrial cell proliferation induced by treatment with estrogen only. This effect has been found for all progestogens studied, regardless of the route of administration or dose. Inadequate data were available to the Working Group on duration of treatment or time since cessation of treatment.

Cardiovascular effects of estrogen and progestogen

Randomized trials that studied combined hormonal menopausal therapy did not show a protective effect of a fixed single dose of conjugated equine estrogens with or without medroxyprogesterone acetate on the incidence of coronary heart disease, although a large body of literature from observational studies suggests that such treatment confers benefits for this disease. These discrepancies have not been fully resolved but may arise from methodological limitations in some observational studies. Randomized trials have consis-

tently reported a small adverse effect of combined hormonal therapy on the incidence of stroke, which is generally supported by observational studies. Evidence of an increase in the incidence of venous thromboembolism from hormonal therapy, particularly with estrogen plus progestogen, has been found in both randomized trials and observational studies, and is supported by mechanistic studies. The overall evidence relies heavily on studies of conjugated equine estrogens and medroxyprogesterone acetate, the data from which suggest a small increase in risk for broadly defined cardiovascular disease as a whole. The extent to which these results apply to other estrogens and progestogens, doses or routes of administration is not known.

Other effects

The beneficial effects of combined hormonal menopausal therapy have been established unambiguously for vasomotor symptoms, osteoporosis and fractures, with moderate evidence for a reduced risk for non-insulin-dependent diabetes. The evidence for an increase in breast density and an increase in the prevalence of breast tenderness and vaginal bleeding is also unambiguous. There is strongly suggestive evidence of interference in mammographic screening associated with breast density and an increase in problems of urinary incontinence. There is consistent evidence for an increase in the risk for gallbladder disease. Results for cognitive function and dementia are less clear. In women who initiate therapy later in life (≥ 65 years of age), randomized trials have provided evidence of a small deleterious effect on cognitive function and an increased risk for dementia. The cognitive effects in women who initiate therapy at younger ages are still uncertain. Randomized trials did not show substantial effects on mortality or on the quality of life, other than the relief of symptoms related to the menopause.

Genetic and related effects

Data on the genetic effects of estrogens and their derivatives indicate that these compounds give rise to reactive metabolites and reactive oxygen species that can induce DNA damage. The evidence reported since the previous evaluation further substantiates the premise that these mechanisms could contribute to the induction of cancer by estrogens. New evidence demonstrates that DNA adducts that are expected to result from the metabolites of catechol estrogen are found in humans, experimental animals and in-vitro systems and that exposure to estrogens generates reactive oxygen species. While these new findings increase the plausibility of these pathways as mechanisms of estrogen-related carcinogenesis, they do not prove that these are the major pathways to estrogen-related cancers. The way in which progestogens might influence the genotoxicity of estrogens is not known.

Receptor-mediated responses to hormones are a plausible and probably necessary mechanism for hormonal carcinogenesis. The results of research over the past few years add considerable support for a direct genotoxic effect of hormones or their associated by-

products such as reactive oxygen species. Current knowledge does not allow a conclusion as to whether either of these mechanisms is the major determinant of hormonally induced cancer. It is entirely possible that both mechanisms contribute to and are necessary for carcinogenesis. Cessation of hormonal treatment may reduce the receptor-mediated effects while gene damage may be more persistent.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of combined estrogen–progestogen menopausal therapy in the breast.

There is *evidence suggesting lack of carcinogenicity* in humans for combined estrogen–progestogen menopausal therapy in the colorectum.

There is *sufficient evidence* in humans for the carcinogenicity of combined estrogen–progestogen menopausal therapy in the endometrium when progestogens are taken for fewer than 10 days per month, and there is *evidence suggesting lack of carcinogenicity* in the endometrium when progestogens are taken daily. The risk for endometrial cancer is inversely associated with the number of days per month that progestogens are added to the regimen.

There is *limited evidence* in experimental animals for the carcinogenicity of conjugated equine estrogens plus medroxyprogesterone acetate.

Overall evaluation

Combined estrogen–progestogen menopausal therapy is *carcinogenic to humans (Group 1).*

6. References

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