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Consensus paper

New evidence regarding hormone replacement therapies is urgently required
Transdermal postmenopausal hormone therapy differs from oral hormone therapy in risks and benefits

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Abstract

Controversies about the safety of different postmenopausal hormone therapies (HTs) started 30 years ago and reached a peak in 2003 after the publication of the results from the Women Health Initiative (WHI) trial and the Million Women Study (MWS) [Writing group for the women's health initiative investigations. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. JAMA 2002;288:321–33; Million women study collaborators. Breast cancer and hormone-replacement therapy in the million women study. Lancet 2003;362:419–27]. The single HT formulation used in the WHI trial for non hysterectomized women—an association of oral conjugated equine estrogens (CEE–0.625 mg/day) and a synthetic progestin, medroxyprogesterone acetate (MPA–2.5 mg/day)—increases the risks of venous thromboembolism, cardiovascular disease, stroke and breast cancer. The MWS, an observational study, showed an increased breast cancer risk in users of estrogens combined with either medroxyprogesterone acetate (MPA), norethisterone, or norgestrel. It is unclear and questionable to what extent these results might be extrapolated to other HRT regimens, that differ in their doses, compositions and administration routes, and that were not assessed in the WHI trial and the MWS. Significant results were achieved with the publication of the WHI estrogen-only arm study [Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 2004;291:1701–1712] in which hormone therapy was reserved to women who had carried out hysterectomy. What emerged from this study will allow us to have some important argument to develop.

Keywords: Menopause; Hormone replacement therapy; Transdermal; Lipids; Glucose

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1. Low dose, short duration?

The EMEA has recommended to use HRT in the "lowest effective dose during the shortest period of time whatever the formulation" [4]. This strategy, the simplest recommendation to lower the risks potentially extended to the use of any HTs, seems to be endowed with common sense, but is unlikely to significantly improve the safety of already tested HTs within the frame of doses and durations of HT prescribed in clinical practice [5]. Although there is not a universal accepted definition, we refer to "low-dose estrogen" speaking about the administration of 1 mg/day of estradiol, 0.312 mg/day of equine conjugated estrogen per os, a dose of 0.025 mg/day of estradiol patch or a dose of 0.75 mg/day of estradiol gel transdermally administered.

The combined analysis of the results of four available randomized trials [HERS [6–8], EVTET [9], WEST [10] and WHI [1]] concludes that the tested HTs increased the risk of cardiovascular event as early as the first months of use, regardless of parameters such as age (although the data available on young, healthy, postmenopausal women starting HT are missing), personal medical history, and ethnic origin, [11]. This analysis concluded, moreover, that there was a similar risk for all the estrogens administered orally, CEE or 17β-estradiol, including the use of estradiol alone, without associated progestin, at the lower dose of 1 mg/day [10,11]. Hence, it would seem that use of these HT formulations even at a low dose and during a short duration is not proven to be safe. Similarly, available studies show no dose/effect relationship between different HTs [2,12] and the risk of breast cancer. The risk of breast cancer may start to rise as soon as the first months of use, but the higher incidence may be attribuable to the fact that women under HT undergo more scrupulous screening for breast cancer, thus increasing the probability of detecting malignancies [13].

2. Age dependence of estrogen-mediated cardiovascular risk

Recent WHI estrogen-only arm study [3] has put clearly in evidence that the reduction of the risk mediated from estrogens depends essentially from the age in which women begin to be treated. In this study, in fact, for the age group <60 years, the risk of acute myocardial infarction was significantly reduced from 44%, and the risk for stroke was not increased. This suggests that a "window of opportunity" exists whereby an early initiation with estrogen-only therapy may cause lower cardiovascular risk. For women >59 years, no benefit was observed in terms of the risk for coronary heart disease, and the risk of stroke was increased (as for the combined HRT). This data emphasizes the great importance of the receptor-mediated effects of estrogens. It is reasonable, in fact, that the hormones that were assumed in more juvenile age have both a direct and an indirect effect, this last mediated from the vascular receptor. This is the reason why they keep on protecting the vessel in a continuum with the fertile period. When the estrogens are administered far after the onset of the menopause, they have only a partial, direct effect. The receptor-mediated effect lacks, since the estrogen receptors tend to diminish in number with aging. From this point of view, the above cited trials (HERS and WEST) dealt with old populations, not taking in any consideration this important physiopathological element.

3. Routes of administration of estradiol, and cardiovascular risk markers

A clinically more relevant difference in cardiovascular risk associated with HRT use may be related to the mode of treatment, i.e. transdermal (gels or patches) versus oral treatments. The administration of 17β-estradiol transdermally (gels or patches), as opposed to orally has a significantly different effect on the immediate markers of cardiovascular risk factors.

For the past 30 years, two conflicting theories have developed on the best way of obtaining cardiovascular benefits by administering estrogens to postmenopausal women.

Up until 2003, the majority of investigators believed that these vascular benefits were specifically linked to the pharmacological effects on the HDL and LDL-cholesterol levels provoked by the first pass effect of estrogens in the liver. This can only be achieved by oral administration [14,15]. The known secondary effects of this method of administration, such as increased triglycerides or haemostasis modifications [16,17], were considered to be clinically irrelevant for the

vast majority of investigators. The possible association of synthetic progestin was expected to have only a minor effect on lipid modifications which remained globally favourable, although the co-prescription of MPA, instead of progesterone, significantly reduced the apparent advantages in the PEPI randomised study [18]. In USA, since the 1960s, some 90% of HTs prescribed for menopausal women were in fact oral administration of a pharmacological estrogen therapy [15] and the results of HERS, WEST and WHI were largely unexpected [19,20].

In some European countries, since the 1970s, the non-oral administration of an estradiol replacement was recommended [21,22]. For the opponents of oral estrogen therapy, the pharmacological accumulation of estrogens in the liver induces metabolic modifications comparable to those during pregnancy [22,23]. These modifications are very different from those produced by lipid-lowering medicines and are, for the main part, unfavourable for menopausal women. These include increased triglycerides, linked to a decrease in the size of LDL particles, higher levels of C-reactive protein, and activation of coagulation [21–28]. There is a danger therefore, that this pharmacological method of administration, reduces—and not increases-the anti-atherogenic effects of estradiol, and therefore increases the risk of venous and arterial thromboembolism. According to this hypothesis, the main source of hormone dependent cardio-vascular benefits is not the hepatocyte but the arterial endothelium which is estradiol dependent in women [29–35].

The choice of the progestin could therefore be important, not so much for the control of the hepatic metabolism of cholesterol but for the progestin-estradiol agonistic or antagonistic effects on endothelial function that are also progesterone-dependent [36–42].

Randomised comparative studies have consistently confirmed the superiority of transdermal estradiol replacement to oral formulations on the main intermediates risk markers (triglycerides, size of LDL particles, coagulation, C-reactive-protein) (Table 1). This provides a plausible argument in favour of a real difference in the benefit/risk ratio between the two routes of administration [24–28,43–46].

One small randomised (PHASE) study failed to demonstrate a potential safety improvement with the use of transdermal estradiol [47]. This study recruited

Table 1
Transdermal estradiol (E2) replacement and oral formulations on the main intermediates risk markers of CHD in comparative randomized trials

	OralE2/CEE	TransdermalE2	
Triglycerides	↗	↘	[24,27]
LDL particle size	↘	↗	[25,28]
Frag 1 + 2 prothrombin	↗	=	[26,43]
Von Willebrand F	↗	=	[99]
C-Reactive Protein	↗	=or ↘	[43–45]

255 postmenopausal women with angiographically-proven ischaemic heart disease. Those women were randomly assigned to no treatment ($n = 121$) or transdermal patches delivering either estradiol alone ($n = 58$) or estradiol + norethisterone ($n = 76$). After an average of 31 months, the CHD event rate for the HT group was 15.6% compared with 12.6% in the control group. Per-protocol analysis based on only 81 HT women showed a non-significant increase in the event rate ratio for HT group compared with control group (1.49; 95% confidence interval: 0.93–2.36; $p = 0.11$). However, this study had important limitations including the small sample size and high dropout rate (40% in the HT arm). Another problem was related to the choice of the transdermal patches delivered by a pharmaceutical company which is no longer commercially active (Ethical Pharmaceutical, Ely, UK) and with uncertain pharmacokinetic characteristics.

Last but not least, none of the CHD secondary prevention trials have shown up to now any role of oral estrogen administration in reducing recurrent events. This provides the whole rationale behind the fact that oral estrogens do not improve the function of diseased vessels, and a large amount of available evidence shows that they indeed do not [48].

3.1. Venous thromboembolism

Oral estrogen therapy activates blood coagulation [16,17,24,26] and increases the risk of venous thromboembolism (VTE) in postmenopausal women [1,7,9,15]. Transdermal estrogen has little or no effect on haemostasis [24,26,49], but clinical data evaluating its effect on the thrombotic process are scarce. A multicentre hospital-based case–control study of postmenopausal women conducted in France during

1999–2002 [50], recruited 155 consecutive cases with a first documented episode of idiopathic VTE (92 with pulmonary embolisms and 63 with deep venous thrombosis), and 381 controls matched for centre, age, and time of recruitment. Overall, 32 (21%) cases and 27 (7%) controls were current users of oral estrogens, whereas 30 (19%) cases and 93 (24%) controls were current users of transdermal estradiol. After adjustment for potential confounding variables, the odds ratio for VTE in current users of oral and transdermal estrogens compared with non-users was 3.5 (95% CI: 1.8–6.8) and 0.9 (0.5–1.6), respectively. Estimated risk for VTE in current users of oral compared with transdermal estrogen users was 4.0 (1.9–8.3). Oral estrogen therapy is associated with risk of VTE in postmenopausal women whereas transdermal estradiol replacement is not. These data provide epidemiological evidence that transdermal ERT may be safer than oral ERT with respect to thrombotic risk.

Biological evidence supports the difference in the risk of VTE between oral and transdermal routes of administration. Studies of ET and haemostatic variables are scarce but randomised trials have shown that oral ET increased plasma level of prothrombin fragment F1 + 2, which is a marker for in vivo thrombin generation and a predictor of VTE risk [49]. Two trials provided evidence that oral, but not transdermal, ET raised fragment F1 + 2 levels [26,43]. A lower antithrombin activity has also been reported in users of oral ERT compared with users of transdermal ERT [51]. Thus, oral ERT may impair the balance between procoagulant factors and antithrombotic mechanisms, whereas transdermal ET appears to have little or no effect on haemostasis.

Activated protein C (APC) resistance has recently emerged as a risk factor for VTE [52,53]. APC resistance has been demonstrated in women using oral contraceptive [54] but also in those using oral ET [55]. However, two randomised trials recently showed that these results did not apply to users of transdermal ET [56,57]. Taken together, these findings provide a plausible biological mechanism for the increased risk of VTE among users of oral ET and they add to the current evidence that transdermal ET is not involved in the thrombotic process.

A meta-analysis of the main findings from randomised trials on the long-term effects of ET showed that pulmonary embolism accounted for about one third of excess incidence of potentially fatal events in healthy postmenopausal women using ET over 5-year period [11]. Therefore, use of transdermal ERT may substantially improve the benefit-risk profile among ERT users. This is even more relevant in the light of the announced results of the CEE-only arm of the WHI, that report the presence of increased VTE and stroke incidence in women receiving oral estrogens that is similar to what was found in the association arm CEE + MPA, confirming the indication that it is the oral administration of estrogen which is specifically linked to increased VTE risk. The clinical relevance of those findings might be even more important for women at high risk for VTE who require ET for severe menopausal symptoms.

3.2. CHD and stroke

The first randomised trials on cardiovascular prevention resulted in failure to confirm any cardiovascular benefit related to oral ET, with a homogeneous trend of the appearance of more frequent, more serious and earlier cardiovascular accidents in women taking oral formulations compared to those taking a placebo [1,6–8,11]. Although laboratory and animal research conducted on the activity of estrogens of vessels showed that estrogen therapy induced an increase in circulating HDL-c and a decrease in LDL-c, the clinical use of ET does not translate into a slowing of atherosclerosis progression [58–61]. The most convincing explanation is that a parallel increase in HDL-c and triglycerides and parallel decrease in LDL-c and LDL particle size is due to a pharmacological effect on liver metabolism which is quite different from the beneficial changes induced by normolipemic drugs [23,61]. Although there is a potential for beneficial cardiovascular effect of the endogenous estradiol (and progesterone), the oral administration of estrogens causes unwanted side effects instead of the expected pharmacological benefits on cholesterol metabolism. These unanimously unwanted side effects include a rise in triglycerides, a drop in LDL particle size, an activation of coagulation and an increase in C-reactive protein [28,43–46,49–52,56,57]. With the non-oral route of administration, the observed effects are quite different with a decrease of triglycerides and LDL and no effect mediated by the liver, but definitive evidence-based data showing a clinical significance is lacking. Consequently, non-oral estradiol could be the first choice

for symptomatic postmenopausal women until new evidence would prove otherwise.

There is no apparent negative effect of non-oral estradiol replacement in France on CHD where the female cardiovascular mortality before the age of 74 is among the lowest in the world. The use of HTs (all types) by French postmenopausal women soared at the start of the 1990s reaching, in 1999, more than 5 million prescriptions each year with more than 21 million units of HTs (estrogen or estro-progestins) and more than 2 million users [62].

The transdermal formulations represented 56% of HT prescriptions for users between 45 and 54 years of age, 70% for users between 55 and 64 years and 74% for users between 65 and 74 years.

The evolution of female mortality by CHD in France is not, overall, related to a clear increase in cardiovascular risk due to HT. The relative increase in CHD incidence expected in the 55–64 age group did not occur despite a rapidly increasing exposure to transdermal formulations of HRT since 1990 [63].

For symptomatic postmenopausal women willing to start or continue an HT, non-oral estradiol could be the considered for first-line prescription, especially in women at high risk of cardiovascular disease.

4. Effects of HRTs on breast cancer risk

Despite the fact that natural progesterone has been proved to have beneficial effects on several major targets such as the endometrium, the brain, macrophages, and the arterial wall [36–41], the potential for an increased risk in breast cancer linked to the use of some synthetic progestins [1,2,11,64] restricts the prescription of any type of progestogens and therefore tends to inflate the rate of hysterectomies in peri and postmenopausal women. However, the available evidence of risk for breast cancer is limited to only three synthetic progestins suspected for 10 years to be inadequate for the control of estrogen effects in breast [65,66], and better benefits/risks ratios with other steroids remain plausible.

4.1. Effects of estrogens on the breast

Estrogens are clearly the main hormones to trigger both proliferation of normal breast epithelial cells and progression of breast cancer cells [67,68]. One of the prevailing theory concerning the mechanism of estrogen-induced increase of breast cancer risk postulates that estrogens increase the proliferation of breast cells and thereby the number of errors occurring during cell replication, but notwithstanding the large research efforts devoted in this area in the last years, this has not been clearly demonstrated, and many experts believe now that estrogens may only act as promoting agents of already existing tumors, being therefore devoid of oncogenic power. Other mechanisms may be responsible, i.e. the formation of estradiol and estrone metabolites reacting with DNA. Evidence is growing that both mechanisms may be operative in an additive or synergistic fashion.

It was demonstrated that certain estradiol metabolites have antiproliferative and antiangiogenetic properties [69,70]. The distribution of primary reactive estradiol metabolites was investigated in 37 postmenopausal women treated either with 2 mg oral or 0.05 mg transdermal estradiol [71]. No significant changes were found with the patch. In contrast, under oral estradiol, an almost 10-fold increase was observed, not only in 2α-hydroxyestrone, but also in 16α-hydroxyestrone, a D-ring metabolite with high biological activity [72]. Particularly women smokers under treatment with oral estradiol produced significantly more 4-hydroxyestradiol, which (via quinone formation) can have DNA-toxic effects increasing the risk of developing breast cancer [73]. However, the formation of potentially toxic metabolites was avoided if estradiol was used transdermally.

Before menopause, the main source of estrogens for all target tissues including breast is the cyclic secretion of estradiol by ovaries in the systemic circulation.

After menopause, despite the ovarian estradiol secretion drop, there is only a partial regression of the normal breast tissue and breast cancer incidence continues to increase with age that remains the major risk factor for cancer. Most breast cancers are diagnosed after the menopause [74] when serum estradiol levels are very low, but, even in these circumstances, an important role of this hormone in the development of the disease remains pivotal.

Breast adipose tissue and most breast carcinomas acquire aromatase (metabolising androstenedione to estrone) and 17ß-hydroxysteroid dehydrogenase type 1 (metabolising estrone to estradiol) activities efficient

enough to locally synthesize estradiol from androgenic substrates. This pivotal mechanism maintains the growth of estrogen-dependent cancers independently of estrogen serum levels [75]. However, during the menopausal transition, the main source of estrogen for the breast switches from the premenopausal cyclic serum production of estradiol by the ovaries to a postmenopausal in situ continuous synthesis of estrogens from circulating androgens, their local aromatisation to estrone, and then, reduction of estrone to estradiol.

Therefore, the overall risk of again raising serum estradiol or estrone in postmenopausal women is expected to be relatively low [76,77]. It concerns mainly women with a lean body weight and symptoms of estrogen deprivation, likely to have low aromatase and 17ß-hydroxysteroid dehydrogenase type 1 activities. If these women use estrogen replacement therapy, their breast cancer risk increases to the level observed in untreated postmenopausal women who are slightly overweight and do not experience climacteric symptoms. This risk does not appear to be dose-related. The use of an estradiol or estrone replacement therapy does not alter the breast cancer risk in women who are slightly overweight, have no symptoms of estrogen deprivation and then are likely to have relatively high aromatase and 17ß-hydroxysteroid dehydrogenase type 1 activities. The positive correlation between BMI and risk of breast cancer in postmenopausal women is clearly linked to the ability of adipose tissue to synthesise estradiol continuously [78]. The reanalysis of 51 epidemiologic studies showed that estrogen therapy increases the risk of breast cancer only in postmenopausal women with a BMI <25, which is consistent with the observed lack of estrogen-dose related effect [75]. This hypothesis should be accepted according also with the results of the WHI estrogen-only arm (3). In this last trial, in fact, there was a largely surprising significant risk reduction for breast cancer of 33%. This was due to the specific characteristic of the selected study population: women were, on average, obese (mean BMI: 30.1 kg/m^2; 45% of the population had a BMI >45 kg/m^2), and It is well known that hormone therapy does not increase breast cancer risk in obese women. Currently, the question why breast cancer risk was reduced in the trial remains opened, as also concluded by the authors.

By contrast, breast tenderness is clearly estrogen-dose related [5].

4.2. Estrogen agonist and antagonist effects of progestogens on breast

Progestogens have different influences on the main estrogen activities in pre- or postmenopausal women, when breast is mostly stimulated by cyclic fluctuations in serum estradiol or by continuous in-situ synthesis through aromatase and 17ß-hydroxysteroid dehydrogenase type 2 activities.

4.3. Progestogens in premenopausal women

The main estrogen antagonist effect of progestogens is primarily linked to their ability to decrease estradiol ovarian production through their anti-gonadotropic effect. A second effect is related to a local impact of progesterone and some synthetic progestins able to down-regulate estradiol receptors within normal breast epithelial cells [67] and to stimulate 17ß-hydroxysteroid dehydrogenase type 2 activity (metabolizing estradiol to estrone) [79,80]. Applying a natural progesterone gel on breast skin decreases the mitotic activity stimulated by estradiol [81,82]. Also, a combination of topical progesterone and synthetic oral progestins has been successfully used to treat mastodynia and to decrease the risk of breast cancer in women with benign breast diseases [83].

In women using oral contraceptives both the anti-gonadotropic effect and the 17ß-hydroxysteroid dehydrogenase type 2 stimulation of the progestin are ineffective on co-administered exogenous ethinyl-estradiol, and the incidence of benign diseases of the breast is related to the individual metabolism of both steroids with a tendency to decrease with lower dose of estradiol and higher dose of progestin [84]. However, some synthetic progestins with androgenic properties may become mostly estrogen agonists in some individuals by directly binding to estrogen receptors [65].

Oral contraceptives do not seem to have significant influence on breast cancer risk [85], although contraceptives containing high doses of ethinyl-estradiol and or potentially androgenic progestins such as norgestrel remain suspected to slightly increase the risk [86].

4.4. Progestogens in postmenopausal women

The anti-gonadotropic effect of progestogens is not useful in postmenopausal women and the use of andro-

genic progestins may upregulate estradiol receptors. Moreover, the effects on 17ß-hydroxysteroid dehydrogenase activities are quite different from one progestin to one other, and medroxyprogesterone acetate, norethisterone and norgestrel have been shown to preferentially stimulate the *iso*-enzyme 1 metabolizing estrone to estradiol, while this is not the case with progesterone [66]. This is specifically worrying when the estrogen is co-administered by the oral route and induces a striking accumulation of estrone in the breast tissue [87]. The WHI and MWS studies reporting alarming results on breast cancer incidence and possibly on mortality have analyzed exclusively the effects of these three synthetic progestins [2,64] but other studies on treatments using more physiological combinations deliver a better message [88,89].

It was demonstrated that equine estrogens have a proliferative action similar to 17ß-estradiol and that the continuous addition of progestins did not result in any major reduction of proliferative potency [90]. Some progestins may even enhance the estrogen-induced proliferation of pre-existing breast cancer cells in vitro, particularly when combined with certain equine estrogens. However, in none of the tested circumstances progestogens increase the proliferative effect of estradiol, and progesterone has no deleterious effect even at pharmacological levels, by contrast with progestins.

In in vitro studies, natural progesterone down-regulates estradiol receptors and inhibits the cell proliferation stimulated by estradiol in normal human breast epithelial cells [67]. Similarly, progesterone arrests human breast cancer cells in G-1 phase on the second cycle by up-regulating cyclin dependent kinase inhibitors (p 21–p 27) and down regulating cyclin D1 [91,92]. Some studies performed on breast tumor cell lines show a pro-apoptotic effect of progesterone [93], reproduced by some non androgenic progestin [94] while another study shows an anti-apoptotic effect of medroxyprogesterone acetate (MPA) [95]. Therefore, in vitro studies show dominant estradiol antagonist activities from progesterone, decreasing the growth of human normal and malignant breast epithelial cells, but also show large discrepancies in the effects of various synthetic progestins. Based on the analysis of proliferation markers in surgical biopsies from normal human postmenopausal breast tissue, mitogenic activity appears to be higher during HRT which combines oral conjugated equine estrogens with medroxyprogesterone acetate [96] than during HRT which combines transdermal estradiol and progesterone [97]. In two cohorts of long-term users of this last combination, no increase in breast cancer incidence has been detected [88,98].

In conclusion, it is misleading to put all progestogens in the same bag, irrespective of their chemical structure and specifically of their ability to stimulate estradiol synthesis and activities in the postmenopausal breast. Also, their effect may vary whether it is estrone or estradiol that is mainly accumulated in the breast tissue. The hypothesis of progesterone and some progesterone-like progestins decreasing the proliferative effect of estradiol in the postmenopausal breast remains highly plausible and should be, until the coming of new evidences, the first choice for symptomatic postmenopausal women.

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