Is it necessary to control the level of estrogen receptor α and ß activation in postmenopausal hormone replacement therapy in order to achieve the optimal outcome? (Review)

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Abstract. Endogenous estrogens exert an array of biological actions on women, many of which are mediated by the estrogen receptors (ERs) α and ß. Results from our recent studies suggest that the human ERα and ERß systems are differentially activated under different physiological conditions. In non-pregnant young women, the ERα system is preferentially activated over the ERß system, mainly by estrone (E_1) and its major oxidative metabolite, 2-hydroxy-E_1. These two estrogens are among the quantitatively major estrogens present in young women, and have approximately 4-fold preferential activity for ERα over ERß. During pregnancy, however, there is a preponderance of activation of ERß over ERα conferred by various pregnancy estrogens such as estriol and other *D*-ring derivatives of 17ß-estradiol (E_2). These estrogens have an up to 18-fold preference for binding to ERß than for ERα, and some of them are produced in unusually large quantities. Given this new information, it is hypothesized that the estrogens ideal for female hormone replacement therapy (HRT) would be those which produce a hormonal condition mirroring that found in non-pregnant young women rather than in pregnant women. Endogenous estrogen derivatives, such as the sulfated conjugates of E_1, may be among the ideal candidates for achieving this clinical purpose. In comparison, Premarin, the most commonly-used HRT containing a mixture of conjugated estrogens isolated from pregnant mare's urine, is less suitable because several of its estrogenic components can produce a strong preferential overstimulation of the human ERß signaling system.

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Abbreviations: ER, estrogen receptor; ERα and ERß, estrogen receptor α and ß subtypes; E_1, estrone; E_2, 17ß-estradiol; OH, hydroxy; RBA, relative binding affinity

Key words: hormone replacement therapy, estrogen receptor α and ß subtypes, Premarin, estrogens

Contents

1. Introduction

Female hormone replacement therapy (HRT), also commonly called menopausal hormone therapy, is a hormonal treatment for peri- or post-menopausal women undertaken to reduce the discomfort and health problems associated with diminished circulating ovarian hormones (namely, estrogens and progesterone). HRT usually provides a low dose of an estrogen (or a mixture of estrogens), often in combination with a progestin. In the past few decades, the most commonly-used estrogen treatment for HRT has been Premarin, which consists of a mixture of mostly sulfated estrogens isolated from pregnant mare's urine. The hormonal activity of these conjugated estrogens *in vivo* results from their enzymatic hydrolysis and releases biologically active estrogens.

Until a few years ago, the generally-held scientific belief was that 'an estrogen is an estrogen', i.e., that all estrogens exert similar pharmacological actions on the body. However, this dogmatic view has gradually changed over the past decade due, in large part, to the emergence of the following body of new knowledge.

First, it has become known that multiple subtypes of estrogen receptor (ER) exist (1,2). These have very different tissue and cell distribution in the body, and both overlapping and completely different biological functions in different target tissues or cells (1,2).

Second, studies have shown that certain ER agents, such as tamoxifen and raloxifene, can selectively modulate the function of ERs in different target tissues/cells in different ways, serving as ER antagonists in one tissue (such as the breast) but as weak agonists in another (such as bone) (3,4).

Third, certain endogenous estrogens and their metabolic derivatives have very different binding affinities for human ERα and ERß (discussed below) (5). Furthermore, some of the estrogen metabolites that are often selectively formed in certain target cells or formed under unique physiological or pathological conditions can exert very different biological functions that are not necessarily shared by their parent hormone 17ß-estradiol (E_2) (6-8).

Recently, we systematically compared the activity of a large number of endogenous estrogen metabolites, including many of those contained in Premarin, relating to human ERα and ERß (5). We found that while E_2 (perhaps the best-known endogenous estrogen) has nearly the highest, and almost identical, binding affinity for human ERα and ERß, many of its metabolites have widely different preferences for human ERα and ERß activation (5). In addition, it should be noted that the predominant estrogens in pregnant women are very different from those present in non-pregnant ones, and that these estrogens have widely different preferences when it comes to the activation of human ERα and ERß. Based on this information, a new concept is proposed here, one which suggests that differential activation of ERα over ERß may be a crucial factor in achieving optimal clinical outcome in postmenopausal HRT. Recommendations are made as to which types of estrogens would be ideal for human use in menopausal HRT.

2. Differences in the composition and quantity of endogenous estrogens produced in pregnant and non-pregnant women

A large number of endogenous estrogen derivatives are known to be present in humans. Studies have been conducted in the past to determine the human urinary excretion of various estrogens (mostly as conjugates) as a global indicator of the bio-synthesis and metabolism of endogenous estrogens *in vivo* (9,10). Based on our recent data (Table I), it is estimated that the total daily amount of various urinary estrogens excreted from a late-stage pregnant woman is 2-3 orders of magnitude higher than the amount excreted by a non-pregnant woman of the same age group. In addition, the composition of urinary estrogens in pregnant and non-pregnant women is widely different. Representative profiles of various endogenous estrogens found in the urine of pregnant and non-pregnant young women are summarized in Table I.

In the urine samples obtained from non-pregnant young women, the conjugated forms of 2-OH-E_1, followed by 16α-OH-E_2 (E_3), 16α-OH-E_1 and E_1 (estrone), are the predominant estrogens. The amount of E_2 and its major metabolites 2-OH-E_2 and 2-methoxy-E_2 was much less than that of E_1 and its corresponding metabolites. The relative composition of the various estrogens in circulation is believed to be comparable to that of urine. The presence of higher levels of E_1 than of E_2 in non-pregnant women is largely attributable to high levels of oxidative 17ß-hydroxysteroid dehydrogenase (17ß-HSD), which catalyzes the facile conversion of E_2 to E_1. The conversion of E_1 to 2-hydroxy-E_1 or E_2 to 2-OH-E_2 is catalyzed by cytochrome P450 enzymes (11-14) and by subsequent *O*-methylation, which forms 2-methoxy-E_1/E_2 is catalyzed by catechol-*O*-methyltransferase (COMT) (7,15).

There is a drastic change in endogenous estrogen composition during pregnancy. E_3 becomes the predominant estrogen and is produced in unusually large quantities. The daily amount of this estrogen (in its conjugated form) released into the urine of late-stage pregnant women is 200-1000 times higher than that of any of the quantitatively major estrogens produced in non-pregnant women. Notably, several other *D*-ring estrogen derivatives, such as 17-epi-E_3, 16-epi-E_3, 16,17-epi-E_3 and estetrol (15α-OH-E_3), are also produced in readily-detectable quantities during the late stages of pregnancy. These *D*-ring derivatives are usually only present in low or undetectable levels in non-pregnant young women. Similar results have been reported in previous studies (11,12).

In summary, although E_2 is perhaps the best-known endogenous estrogen in humans, it is not the predominant estrogen produced in the body of pregnant women or of non-pregnant women. The major endogenous estrogens that are produced in non-pregnant women are vastly different in quantity and also in composition from those produced in pregnant women.

3. Differences in the biological activity of pregnancy and non-pregnancy estrogens

It is hypothesized that the endogenous estrogens produced in a non-pregnant young woman will exert very different physiological functions than those produced during pregnancy. This hypothesis is supported by the following evidence.

First, as discussed above, the endogenous estrogens formed in non-pregnant women are vastly different in quantity and composition from those produced in pregnant women.

Second, studies in recent years by us and by others have shown that some E_2 derivatives can exert unique biological functions that are not shared by their parent hormone E_2 (reviewed in refs. 6-8,14-16). For instance, a previous study showed that 4-OH-E_2, a well-known hydroxylated metabolite of E_2, has a far stronger blood cholesterol-lowering effect on rats than does E_2 (16), although its uterotropic activity (16,17) and ER-binding affinity are slightly lower than that of E_2 (5,17). Also, it is well documented that catechol estrogens are chemically reactive and potentially genotoxic/mutagenic, and it has been suggested that they play an important role in mediating hormonal carcinogenesis (18-20). In contrast, 2-methoxyestradiol, a non-polar endogenous E_2 metabolite with little binding affinity for human ERα and ERß, has a strong anti-proliferative, anti-angiogenic and apoptotic effect (7,21). It has been suggested that increased biosynthesis of this non-polar estrogen metabolite is highly beneficial for protection against estrogen-induced hormonal cancers (7,15).

A previous study showed that E_2 15α-hydroxylase activity, which catalyzes the formation of 15α-OH-E_2 and 15α-OH-E_3 (estetrol), was selectively elevated by 50- to 70-fold in a localized area of the uterine endometrium, where the imbedding of the fertilized ovum had taken place (22). Although the exact biological functions of the 15α-hydroxylated estrogens are not clear at present, it is likely that the formation of 15α-OH-E_2 and 15α-OH-E_3 (estetrol) may be involved in the imbedding process. Similarly, the amount of 15α-hydroxylated estrogens present in the urine of a late-stage pregnant woman can be used as a reliable

Table I. Comparison of daily urinary secretion (mean ± SD) of endogenous estrogen metabolites during the pre-ovulatory phase, ovulation, and post-ovulatory phase of a normal non-pregnant woman with that of five pregnant women.

Estrogen	Non-pregnant woman (μg/24-h urine)			Pregnant women (μg/24-h urine)
	Pre-ovulatory phase (days 6-10)	Ovulation (day 0)	Post-ovulatory phase (days 6-10)	
Estrone (E_1)	6.2±3.9	30.8±15.6	16.2±12.4	49.1±37.8
17ß-Estradiol (E_2)	0.9±0.8	3.8±1.9	1.6±0.2	26.5±11.3
2-Hydroxyestrone (2-OH-E_1)	5.7±4.0	22.5±12.0	8.8±1.8	22.1±11.4
4-Hydroxyestrone (4-OH-E_1)	0.7±0.4	2.2±0.2	1.1±0.3	2.4±0.8
16α-Hydroxyestrone (16α-OH-E_1)	2.4±1.5	13.6±6.4	5.0±3.3	532.4±948.8
2-Methoxyestrone (2-MeO-E_1)	2.4±0.2	0.5±0.8	0.8±0.4	6.9±3.4
2-Hydroxyestradiol (2-OH-E_2)	0.8±0.4	1.8±1.2	1.2±0.3	3.4±3.6
4-Hydroxyestradiol (4-OH-E_2)	ND	ND	ND	0.5±0.1
2-Methoxyestradiol (2-MeO-E_2)	ND	ND	ND	11.5±14.5
Estriol (E_3)	6.9±2.3	28.8±12.2	15.7±5.8	11,174.8±9,304.3
16-Epiestriol (16-epi-E_3)	ND	ND	ND	562.3±626.3
17-Epiestriol (17-epi-E_3)	ND	ND	ND	ND
16,17-Epiestriol (16,17-epi-E_3)	ND	ND	ND	176.7±72.2
2-Hydroxyestriol (2-OH-E_3)	ND	ND	ND	86.8±73.7
Estetrol (15α-OH-E_3)	ND	ND	ND	302.0±273.3

The collection of human urine samples was approved by the Institutional Review Board. Urinary estrogens were determined using the GC/MS method, as described below. An aliquot (1 ml) of the urine sample was transferred to a 1.5-ml microcentrifuge tube containing 200 μl 2 M Na_2AC buffer (pH 5.0), and the mixture was centrifuged at 14,000 rpm for 5 min. The supernatant (1 ml) was transferred to a small glass tube containing 20 μl of 0.5 μg/μl E_2-D2 (in pure ethanol) as the internal standard, and 75 μl of H-2 sulfatase as the enzyme for hydrolysis of estrogen conjugates. The reaction mixture was incubated at 37˚C for 12 h. After incubation, the tubes were centrifuged at 4,000 rpm for 10 min, and the supernatants were transferred to another set of test tubes and extracted with 5 ml hexane/ethyl acetate (v:v, 3:2). The organic extracts were removed and dried under a stream of nitrogen gas. BSTFA (100 μl) was added for derivatization at 65˚C for 1 h. The TMS derivatives of estrogen metabolites were detected using GC/MS. The GC/MS apparatus consisted of an Agilent 6890N GC with 7683 auto-sampler and an Agilent 5973 MS network, coupled with a HP-5MS capillary column. The front inlet temperature was 260˚C, and the column flow rate was 1.0 ml/min. Oven temperatures were as follows: initial temperature was set at 180˚C, then increased by 4˚C/min to 260˚C. This temperature was kept constant for 5 min, then further increased by 5˚C/min to 300˚C and kept constant for 5 min at 300˚C (with the AUX temperature at 280˚C). ND, the estrogen metabolite of interest was not detected.

indicator of fetal well-being, and in particular of fetal lung functions (23-27).

Third, we recently studied the binding affinity of a large number of endogenous estrogen derivatives for human ERα and ERß (5). We found that the major estrogens present in non-pregnant young women had a clearly different preference for the activation of ERα over ERß, compared to the estrogens present in pregnant women. Some of the relevant data are briefly discussed below.

We found that E_1 and 2-OH-E_1, two of the quantitatively major estrogens present in non-pregnant women, had a modest but significant preference for binding to human ERα over ERß (5). E_1 had a 3- to 4-fold higher preference for binding to human ERα than to ERß. Similarly, 2-OH-E_1 (the 2-hydroxylated metabolite of E_1) also had an ~4-fold preference for the activation of ERα over ERß. Notably, E_1 and 2-OH-E_1 had markedly lower binding affinity for human ERα and ERß compared to E_2. It is reasonable to believe that the relatively lower binding affinity of E_1 and 2-OH-E_1 is actually an advantage rather than a disadvantage, because they pose a lower risk of causing over-stimulation of the ERα and ERß signaling systems *in vivo*.

On the other hand, E_3, the quantitatively predominant estrogen produced during human pregnancy, had a significant preference for binding to ERß over ERα (5). Although E_3 had a rather low binding affinity for human ERα compared to E_2 (RBA 11% of E_2), it retained a relatively high binding affinity for ERß (RBA 35% of E_2). Therefore, E_3 had an ~3 to 1 preference for binding to ERß over ERα. Similarly, 16α-OH-E_1, another well-known hydroxylated metabolite of E_1 that is formed in very large quantities during pregnancy, had a higher binding preference for ERß than ERα when compared to E_1.

16,17-Epiestriol had a very low binding affinity for human ERα, but a preferential affinity for ERß; the difference in binding affinity for ERß over ERα was 18-fold. Notably, this unique endogenous estrogen metabolite is usually undetectable in non-pregnant women, but is present at considerable levels during pregnancy (Table I).

In summary, it is evident that there is a distinct difference in the ratio and also intensity of ERα and ERß activation in

non-pregnant young women compared to pregnant ones. The major estrogens produced in non-pregnant women modestly favor the activation of ERα over ERß. However, during pregnancy there is a preponderance of activation of ERß over ERα exerted by various pregnancy estrogens, in particular by E_3, which is produced in unusually large quantities. This preferential activation of ERß is believed to play an indispensable role in the mediation of the various actions of the endogenous estrogens that are likely required for the development of the fetus, as well as for fulfilling other physiological functions related to pregnancy such as the suppression of autoimmune response against the fetus. These suggestions are in line with observations that ERß has a wide distribution in maternal reproductive and lymphatic organs in rats, as well as in various tissues in the fetus (28-30).

4. Biological activity of estrogens contained in Premarin

Premarin, the most commonly used HRT, contains a mixture of conjugated estrogens isolated from pregnant mare's urine. The major estrogens produced in a pregnant mare are quite different from those produced in a pregnant woman and do not contain E_3. However, they do contain a number of unique equine estrogens, many of which are basically not produced in humans. Our recent analysis showed that several of the equine estrogens contained in Premarin are functionally similar to the human pregnancy estrogen E_3 with respect to their preferential binding affinity for human ERß over ERα (5).

For example, while 17ß-dihydroequilenin had a low binding affinity for ERα (35% of E_2), it had a high binding affinity for ERß (RBA 100% of E_2). Equilin (i.e., 7-dehyro-E_1) had a decreased binding affinity for ERα compared to E_1 (RBA 40% of E_1) and a drastically increased affinity for ERß (RBA 631% of E_1). Similarly, *D*-equilenin had a much weaker binding affinity than E_1 for human ERα (RBA 20% of E_1), but its binding affinity for ERß was >3 times higher than that of E_1.

All together, it is evident that many of the equine estrogens contained in Premarin have a strong differential binding affinity for human ERß over ERα, which is very similar to the human pregnancy estrogen E_3.

5. Which estrogens are ideal for postmenopausal hormone replacement therapy?

The risks and benefits of Prempro (Premarin + progestin) in healthy postmenopausal women were evaluated by following a total of 16,608 women, aged 50-79 years (average age, 63 at study intake) (31). In this study, one branch followed patients who received either a combination of equine estrogens plus a progestin (8,506 women) or a placebo (8,102 women) for 5.2 years. It was found that there was an increased risk of breast cancer with the use of Prempro. The risk of coronary artery disease, strokes and pulmonary embolism was increased as well. The study found that the measured risks of this combination outweighed its measured benefits. For women aged 50-59, there was an observed trend towards a reduced risk of cardiovascular disease (relative risk, 0.56; 95% confidence interval, 0.30-1.03). Similarly, results from other studies suggested that when equine estrogens were administered orally, liver functions were altered and the risk of blood clots was increased (32).

A previous study indicated that the adverse effects of oral conjugated equine estrogens may not be generalized to other estrogens (33). It appeared that while the conjugated equine estrogens were found to be associated with an increased risk of venous thrombosis, this risk was not associated with the use of esterified estrogen. Similarly, previous reports have suggested that the use of 17α-ethynyl-estradiol contained in birth control pills appears to have different health effects on young women than does the use of equine estrogen-based HRT in postmenopausal women. It is not known whether the beneficial effect of 17α-ethynyl-estradiol is due partly to its relatively higher preference for the human ERα over ERß as compared to equine estrogens.

It seems reasonable to suggest that an important empirical criterion that should be considered when an estrogen or a combination of estrogens is being evaluated for use in postmenopausal HRT is their ability to restore the hormonal environment to one found in normal non-pregnant young women, and not to one found in pregnant women. Since very different types of estrogens are produced in pregnant vs non-pregnant women and serve widely different physiological purposes, it is suggested that the use of endogenous estrogens found in non-pregnant young women will be more ideal for HRT than the use of estrogens predominantly produced during pregnancy. The former may include a combination of the sulfates of E_1 and 2-OH-E_1, and possibly other endogenous estrogens (such as the conjugates of 2-methoxyestrogens). The inclusion of methoxyestrogen sulfates in HRT may be beneficial because of 2-methoxyestradiol's strong anti-tumorigenic activity of (7,15). Given that many endogenous estrogens may have rather rapid metabolic disposition in the body, some other naturally-occurring or synthetic estrogens with longer half-lives can also provide a similar preferential activation of the ERα system as E_1 and may be useful as alternatives. For instance, since 17α-E_2 has a similar ER-binding preference as E_1 but cannot be converted to E_2 by 17ß-hydroxysteroid dehydrogenase (17ß-HSD), the sulfate conjugates of 17α-E_2 may serve as alternatives to E_1-3-sulfate in order to achieve similar biological functions. In addition, our recent studies have shown that 17α-E_2 has a strong protective effect against neuronal cell death both *in vitro* and *in vivo* (unpublished data).

This would be a good time to suggest that using sulfated estrogens for human HRT would be better than using their corresponding parent estrogens. The main reasons are, first, the sulfated estrogens are themselves inactive (with little or no binding affinity for human ERα and ERß) (5), but can be enzymatically hydrolyzed to release bioactive estrogens in a variety of tissues in the body. Previous studies have shown that the estrogen target organs, such as the breast and uterus, contain much higher levels of estrogen sulfatase activity than other tissues (34-37). Second, oral administration of estrogen sulfates would provide a natural cushion effect by avoiding unwanted over-stimulation of the ER systems throughout the body. Instead, they would usually only activate those target tissues or cells most in need of estrogenic stimulation. Here, it is also worth noting that several recent studies have shown that estrogen target cells can actively transport E_1-3-sulfate

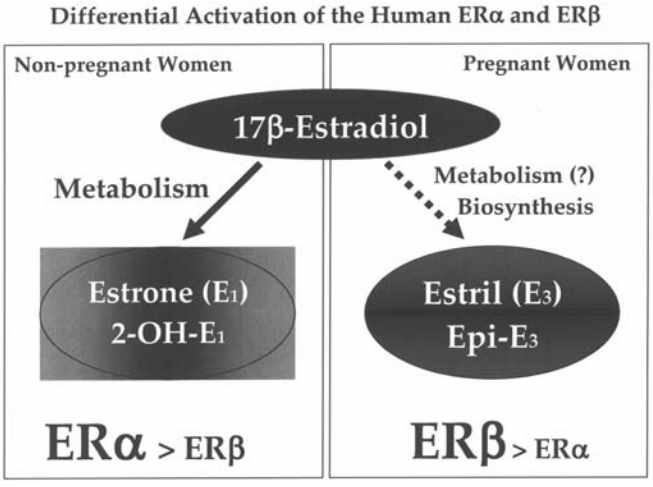

Figure 1. Differential activation of the human ERα and ERß in non-pregnant vs. pregnant women. In non-pregnant women, estrone (E_1) is believed to be the major circulating estrogen. 17ß-Estradiol (E_2) biosynthesized in the body is quickly converted to E_1 by the 17ß-hydroxysteroid dehydrogenase (17ß-HSD) present in various non-gonadal tissues, such as the liver. E_1 has rather low binding affinity for ERα and ERß compared to E_2, but has a preferential binding affinity for ERα over ERß. 2-OH-E_1 is the major metabolite of E_1 *in vivo*, and is also a very weak estrogen with a preference for ERα over ERß. It is therefore apparent that there is a preferential activation of the ERα system over the ERß system in non-pregnant women. In comparison, during pregnancy, the quantitatively predominant estrogens present in the circulation are estriol (E_3) or 16α-OH-E1, which are either metabolically formed from 16α-hydroxylation of endogenous estrogens (such as E_2 or E_1), or via other bio-synthetic pathways in the feto-placental unit. Both of these estrogens, which are formed in relatively small quantities in non-pregnant women, have a preferential binding affinity for ERß over ERα. Therefore, there is a clear difference in the ER activation profiles of non-pregnant vs. pregnant women. For hormone replacement therapy (HRT), the ideal situation is to mimic the hormonal status of non-pregnant healthy young women instead of that of pregnant ones. The current HRT, with its use of Premarin, largely mimics the hormonal conditions of a pregnant woman because, as our recent study (5) showed, many of the estrogens contained in Premarin (isolated from pregnant mare's urine) produce a preferential stimulation of human ERß over ERα.

into the cells (38,39). Moreover, these cells may selectively adjust their ability to actively transport E_1-3-sulfate into the cells to release biologically active estrogens depending on their hormonal needs. Theoretically, such a mechanism would offer certain degrees of target organ selectivity of estrogenic stimulation. Third, compared to estrogen glucuronides, estrogen sulfates are probably better because they usually have a lower clearance rate and a longer half-life ($T_{1/2}$) in humans, thereby making them pharmacologically more useful (40,41).

Based on the above discussion, it is suggested that the use of estrogens to produce a modest level of stimulation of both the ERα and ERß systems, with a slight preference for the ERα system, would be better for postmenopausal HRT than the use of estrogens that confer a predominant activation of the ERß system. It is apparent that Premarin, the most widely prescribed HRT, may not be the most suitable combination of estrogens for achieving this clinical purpose. Notably, while there is a considerable amount of E_1-3-sulfate contained in Premarin, which presumably is good for its intended purpose as an HRT, the fact is that it also contains many other very potent equine pregnancy estrogens which, jointly, result in a strong over-stimulation of the ERß system. Similarly, genistein, a potent and preferential partial agonist of human ERß, would be even less suitable than Premarin for use as postmenopausal HRT because it would essentially produce a near selective ERß stimulation. This is in agreement with recent clinical observations, showing that the singular use of genistein is mostly ineffective as an HRT in postmenopausal women (42,43).

6. Concluding remarks

Until recently, the general scientific belief has been that all estrogens exert the same or highly similar pharmacological actions on a woman's body. When the oral tablet of Premarin was first approved by the U.S. Food and Drug Administration (FDA) for human use in 1942, its 0.625 mg dosage (still in use today) was actually assigned solely on the basis of its estrogenic potency in a rat bioassay that was found to be equivalent to 0.625 mg of sodium E_1-3-sulfate. This bioassay mostly measured the ERα-mediated uterotropic activity. Even to this day, little is known about the precise hormonal strength of Premarin and each of the bioactive components for human ERα and ERß systems.

As discussed above, although E_2 is among the most potent endogenous estrogens and has almost equal binding affinity for human ERα and ERß, it is not one of the major estrogens present in women. In fact, E_1 or E_3, depending on the physiological conditions, are the quantitatively major estrogens present. Although their binding affinities for ERα and ERß are lower than those of E_2, they provide a differential activation of the ERα or ERß signaling system. Our recent study showed that endogenous estrogens (such as E_1 and 2-OH-E_1) present in non-pregnant women mainly activate the ERα system, whereas estrogens (such as E_3 and epi-E_3) present in pregnant women predominantly activate the ERß system. Therefore, the facile metabolic conversion of E_2 to E_1 or of E_2 to E_3 in women provides an important means of achieving differential activation of the ERα or ERß signaling system under different physiological conditions. This concept is summarized in Fig. 1.

It is reasonable to suggest that the estrogens most suitable for human HRT would be those that can mimic the physiological estrogenic stimulation of premenopausal non-pregnant women, and not that of pregnant women. Based on this new concept, it appears that naturally-occurring estrogens like E_1 and E_1-3-sulfate would be more suitable for use as postmenopausal HRT than Premarin, essentially composed of pregnancy estrogens (with a strong preference for ERß). It is apparent that a balanced activation of the ERα and ERß systems, with a modest preference for the ERα system, would be better for HRT, compared to estrogens that predominantly activate the ERß system. It is believed that an optimally-adjusted activation of the ERα and ERß signaling systems would help maximize the beneficial effects of HRT, and additionally minimize its untoward effects.

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