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Controlling the Levels of Estrogen Receptor α and β Activation in Postmenopausal Women During Hormone Replacement Therapy: A Novel Strategy for Achieving Optimal Health Outcomes

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Abstract. Recent studies from our laboratory have lead to the suggestion that the human ER α and ER β signaling systems are differentially activated under different physiological conditions. In a non-pregnant young woman, the ER α system is preferentially activated over the ER β system, mainly by estrone and its major oxidative metabolite, 2-hydroxyestrone. These two estrogens are among the quantitatively major estrogens present in a young woman, and they have approximately 4-fold preferential activity for ER α over ER β . During pregnancy, however, ER β is preferentially activated over ER α , which is caused by various pregnancy estrogens, mainly estriol and other *D*-ring derivatives of 17 β -estradiol. These estrogens have preference for binding to ER β over ER α , and some of them are produced in unusually large quantities. In light of this new finding, it is hypothesized that estrogens ideal for female hormone replacement therapy should be those that will produce a hormonal condition mirroring what is found in a non-pregnant young woman rather than in a pregnant woman. The endogenous estrogen derivatives, such as the sulfated conjugates of estrone, are among the ideal candidates for achieving this clinical purpose. In comparison, Premarin, the most commonly used hormone replacement therapy that contains 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 over-stimulation of the human ER β signaling system.

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Abbreviations used: ER, estrogen receptor; ER α and ER β , the ER α and β subtype, respectively; E₂, 17 β -estradiol; E₁, estrone; OH, hydroxy; RBA, relative binding affinity.

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Introduction

At present, the most commonly-used estrogen for HRT is Premarin, consisting 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 to release biologically-active estrogens. For decades, the general scientific belief has always been that "an estrogen is an estrogen", *i.e.*, all estrogens were assumed to exert similar pharmacological actions in the body. However, this dogmatic view has been gradually changed in the past decade. A recent study from our laboratory compared the activity of a number of endogenous estrogen metabolites, including many of those contained in Premarin, for human ER α and ER β [5]. It was found that while E₂ (perhaps the best-known endogenous estrogen) has nearly the highest and almost identical binding affinity for human ER α and ER β , many of its metabolites have widely different preference for the activation of human ER α and ER β [5]. In addition, the predominant estrogens that are present in a pregnant woman are very different from those present in a non-pregnant woman. Furthermore, these estrogens have different preference for activation of human ER α and ER β . Based on these lines of information, it is proposed that differential activation of ER α over ER β is an important factor for achieving optimal clinical outcomes in postmenopausal HRT. In this article, I also briefly discussed the types of estrogens that would be more ideal for human use as menopausal HRT.

Different endogenous estrogens are 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 human urinary excretion of various estrogens (mostly as conjugates) as a global indicator of the biosynthesis and metabolism of endogenous estrogens *in vivo* [9,10]. Based on recent data from our laboratory, it is estimated that the total daily amount of various urinary estro-

gens excreted from a late pregnant woman is 2 to 3 orders of magnitude higher than the amount excreted by a non-pregnant woman of the same age group. In addition, the composition of the urinary estrogens in pregnant and non-pregnant women is also widely different. Representative profiles of various endogenous estrogens found in the urine of pregnant and non-pregnant young women are summarized in Table 1.

In the urine samples obtained from non-pregnant young women, the conjugated forms of 2-OH-E₁, followed by 16 α -OH-E₂ (E₃), 16 α -OH-E₁ and E₁, are the predominant estrogens. The amount of E₂ and its major metabolites 2-OH-E₂ and 2-methoxy-E₂ was much less than that of E₁ and its corresponding metabolites. The relative composition of various estrogens in circulation is believed to be largely comparable to what is seen in the urine. The presence of higher levels of E₁ over E₂ in a non-pregnant woman is largely attributable to the high levels of the oxidative 17 β -hydroxysteroid dehydrogenase (17 β -HSD), which catalyzes the facile conversion of E₂ to E₁. The conversion of E₁ to 2-hydroxy-E₁ or E₂ to 2-OH-E₂ is catalyzed by various cytochrome P450 enzymes [11–14], and the subsequent *O*-methylation to form 2-methoxy-E₁/E₂ is catalyzed by catechol-*O*-methyltransferase (COMT) [7,15].

There is a drastic change in the endogenous estrogen composition during pregnancy. E₃ becomes the predominant estrogen and is produced in unusually large quantities. The daily amount of this estrogen (in its conjugated forms) released into the urine of a late pregnant woman is 200–1000 times higher than any of the quantitatively-major estrogens produced in a non-pregnant woman. Notably, several other *D*-ring estrogen derivatives, such as 17-epi-E₃, 16-epi-E₃, 16,17-epi-E₃ and estetrol (15 α -OH-E₃), are also produced in readily detectable quantities at late stages of pregnancy. These *D*-ring derivatives are usually only present at low or undetectable levels in non-pregnant young women. Similar results were also reported in earlier studies [11,12].

In summary, although E₂, the best-known endogenous estrogen, is not the predominant estrogen produced in the body of a pregnant

TABLE 1. Comparison of daily urinary secretion (mean \pm S.D.) of endogenous estrogen metabolites during the pre-ovulatory phase (days -6 to -10), ovulation (day 0), and post-ovulatory phase (days 6 to 10) of a normal non-pregnant woman with that of five pregnant women.

Estrogen	Non-pregnant woman ($\mu\text{g}/24$ hour urine)			Pregnant woman ($\mu\text{g}/24$ hour urine)
	Days -6 to -10 (pre-ovulatory phase)	Day 0 (ovulation)	Days 6 to 10 (post-ovulatory phase)	
Estrone (E ₁)	6.2 \pm 3.9	30.8 \pm 15.6	16.2 \pm 12.4	49.1 \pm 37.8
17 β -Estradiol (E ₂)	0.9 \pm 0.8	3.8 \pm 1.9	1.6 \pm 0.2	26.5 \pm 11.3
2-Hydroxyestrone (2-OH-E ₁)	5.7 \pm 4.0	22.5 \pm 12.0	8.8 \pm 1.8	22.1 \pm 11.4
4-Hydroxyestrone (4-OH-E ₁)	0.7 \pm 0.4	2.2 \pm 0.2	1.1 \pm 0.3	2.4 \pm 0.8
16 α -Hydroxyestrone (16 α -OH-E ₁)	2.4 \pm 1.5	13.6 \pm 6.4	5.0 \pm 3.3	532.4 \pm 948.8
2-Methoxyestrone (2-MeO-E ₁)	2.4 \pm 0.2	0.5 \pm 0.8	0.8 \pm 0.4	6.9 \pm 3.4
2-Hydroxyestradiol (2-OH-E ₂)	0.8 \pm 0.4	1.8 \pm 1.2	1.2 \pm 0.3	3.4 \pm 3.6
4-Hydroxyestradiol (4-OH-E ₂)	ND	ND	ND	0.5 \pm 0.1
2-Methoxyestradiol (2-MeO-E ₂)	ND	ND	ND	11.5 \pm 14.5
Estriol (E ₃)	6.9 \pm 2.3	28.8 \pm 12.2	15.7 \pm 5.8	11174.8 \pm 9304.3
16-Epiestriol (16-EpiE ₃)	ND	ND	ND	562.3 \pm 626.3
17-Epiestriol (17-EpiE ₃)	ND	ND	ND	ND
16,17-Epiestriol (16,17-EpiE ₃)	ND	ND	ND	176.7 \pm 72.2
2-Hydroxyestriol (2-OH-E ₃)	ND	ND	ND	86.8 \pm 73.7
Estetrol (15 α -OH-E ₃)	ND	ND	ND	302.0 \pm 273.3

Note: The collection of human urine samples was approved by the Institutional Review Board. The urinary estrogens were determined by 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 Na₂AC 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 ng/ μL E₂-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 hours. 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 of 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 hour. The TMS derivatives of estrogen metabolites were detected by using the GC/MS. The GC/MS apparatus consisted of an Agilent 6890N GC with 7683 auto-sampler, 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. The oven temperature was as follows: The starting was set at 180°C, then increased at 4°C/min to 260°C, hold at this temperature for 5 min, then further increased at 5°C/min to 300°C, and hold for 5 min at 300°C (with the AUX temperature at 280°C). "ND" denotes that the estrogen metabolite of interest was not detected.

woman or of a non-pregnant woman. The major endogenous estrogens that are produced in a non-pregnant woman are vastly different in quantity and also in composition from those produced in a pregnant woman.

The biological activity of pregnancy and non-pregnancy estrogens is different

As discussed above, the endogenous estrogens formed in a non-pregnant woman are

vastly different in quantity and composition from those produced in a pregnant woman. There is also evidence showing that the endogenous estrogens produced in a non-pregnant young woman will exert very different physiological functions from the estrogens produced during pregnancy.

First, studies in recent years by us and also by others have shown that some of the E₂ derivatives can exert unique biological functions that are not shared by the parent hormone E₂ (reviewed in ref. [6–8,14–16]). For instance, an earlier study showed that 4-OH-E₂, a well-known hydroxylated metabolite of E₂, has a far stronger blood cholesterol-lowering effect in rats than E₂ [16], although its uterotrophic activity [16,17] and ER-binding affinity are slightly lower than E₂ [5,17]. Also, it is well documented that the catechol estrogens are chemically-reactive and potentially genotoxic/mutagenic, which have been suggested to play an important role in mediating hormonal carcinogenesis [18–20]. In contrast, 2-methoxyestradiol, a nonpolar endogenous E₂ metabolite with little binding affinity for human ER α and ER β , has a strong anti-proliferative, antiangiogenic and apoptotic actions [7,21]. It has been suggested that increased biosynthesis of this nonpolar estrogen metabolite is highly beneficial for the protection against estrogen-induced hormonal cancers [7,15].

Interestingly, an earlier study showed that the E₂ 15 α -hydroxylase activity which catalyzed the formation of 15 α -OH-E₂ and 15 α -OH-E₃ (estetrol) is selectively elevated by 50 to 70-fold in a localized area of the uterine endometrium where the imbedding of the fertilized ovum took place [22]. Although the exact biological functions of the 15 α -hydroxylated estrogens are not clear at present, it is likely that the formation of 15 α -OH-E₂ and 15 α -OH-E₃ (estetrol) may serve important physiological functions, such as facilitating the imbedding process. Similarly, it is of note that the amount of 15 α -hydroxylated estrogens present in the urine of a late-stage pregnant woman can be used as a reliable indicator of fetal well-being, in particular of fetal lung functions [23–27].

Second, in a recent study, our laboratory studied a large number of endogenous estrogen

derivatives for their binding affinity for human ER α and ER β [5]. We found that the major estrogens present in a non-pregnant young woman have clearly different preference for the activation of ER α *vs* ER β compared to the estrogens present in a pregnant woman. Some of the relevant data are briefly discussed below.

It was observed that E₁ and 2-OH-E₁, two of the quantitatively-major estrogens present in a non-pregnant woman, have a modest but significant preference for binding to human ER α over ER β [5]. E₁ had 3- to 4-fold higher preference for binding to human ER α than for ER β . Similarly, 2-OH-E₁ (the 2-hydroxylated metabolite of E₁) also has approximately 4-fold preference for activation of ER α over ER β . Notably, E₁ and 2-OH-E₁ have markedly lower binding affinity for human ER α and ER β compared to E₂. I believe the relatively lower binding affinity of E₁ and 2-OH-E₁ actually is an advantage rather than a disadvantage, because they would pose a lower risk for causing over-stimulation of the ER α and ER β signaling systems *in vivo*.

Very differently, E₃, the quantitatively-predominant estrogen produced during human pregnancy, has a significant preference for binding to ER β over ER α [5]. Although E₃ had a rather low binding affinity for human ER α compared to E₂ (RBA 11% of E₂), it retained a relatively high binding affinity for ER β (RBA 35% of E₂). Therefore, E₃ has an approximately 3 to 1 preference for binding to ER β than for ER α . Similarly, 16 α -OH-E₁, another well-known hydroxylated metabolite of E₁ that is also formed in very large quantity during pregnancy, has a higher binding preference for ER β than ER α when compared to E₁.

16,17-Epiestriol had a very low binding affinity for human ER α , but it had a preferential affinity for ER β ; the difference of its binding affinity for ER β over ER α is 18-fold. Notably, this unique endogenous estrogen metabolite is usually undetectable in a non-pregnant woman, but it is present at considerable levels during pregnancy (Table 1).

Taken together, it is clear that there is a distinct difference in the ratio and also intensity of ER α and ER β activation in a non-pregnant young woman compared to a pregnant woman.

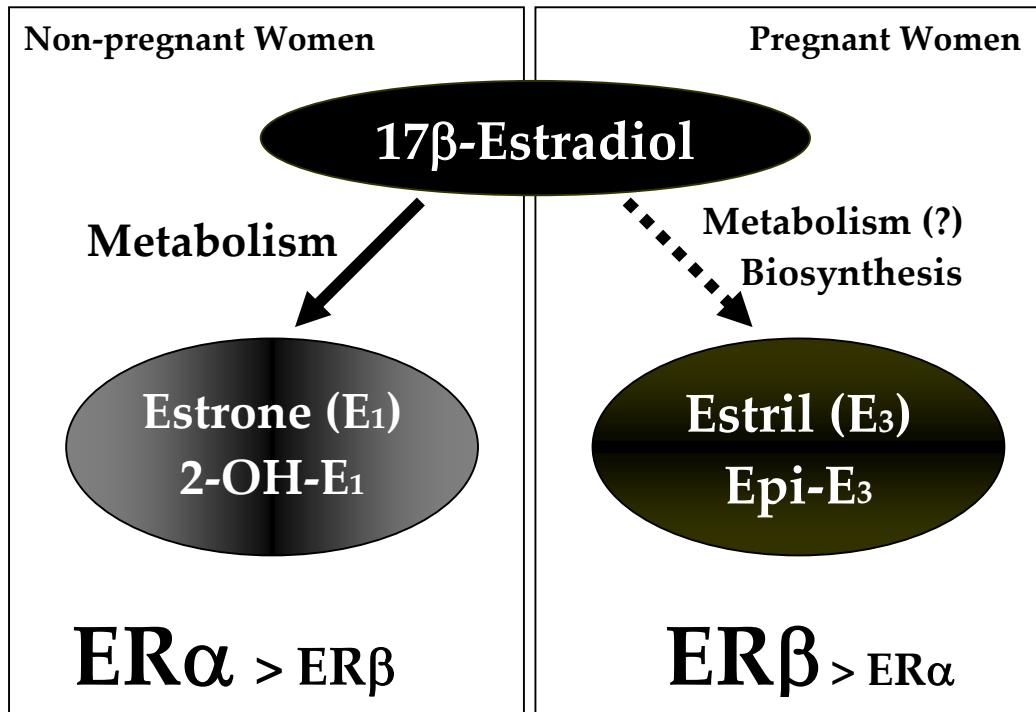
Differential Activation of the Human ER α and ER β 

Figure 1. Differential activation of the human ER α and ER β in non-pregnant women *vs.* in pregnant women. In a non-pregnant woman, estrone (E₁) is believed to be the major circulating estrogen. 17 β -Estradiol (E₂) biosynthesized in the body is quickly converted to E₁ by the 17 β -hydroxysteroid dehydrogenase (17 β -HSD) present in various non-gonadal tissues, such as the liver. E₁ has rather low binding affinity for ER α and ER β compared to E₂, but it has a preferential binding affinity for ER α over ER β . 2-OH-E₁ is the major metabolite of E₁ *in vivo*, and it is a very weak estrogen also with a preference for ER α over ER β . Therefore, it is apparent that there is a preferential activation of the ER α system over the ER β system in a non-pregnant woman. In comparison, during pregnancy, the quantitatively-predominant estrogens present in the circulation are estriol (E₃) or 16 α -OH-E₁, which are either metabolically formed from 16 α -hydroxylation of the endogenous estrogens (such as E₂ or E₁), or are formed via other biosynthetic pathways in the fetoplacental unit. Note that both of these two estrogens, which are formed in relatively small quantities in a non-pregnant woman, have a preferential binding affinity for ER β over ER α . Therefore, there is a clear difference in the ER activation profiles in a non-pregnant woman *vs.* that in a pregnant woman. For hormone replacement therapy (HRT), the ideal situation is to mimic the hormonal status in a non-pregnant healthy young woman instead of mimicking the hormonal condition in a pregnant woman. Notably, the current hormone replacement therapy (HRT) with the use of Premarin would largely mimic the hormonal conditions of a pregnant woman because our recent study (5) showed that many of the estrogens contained in Premarin (isolated from pregnant mare's urine) would also produce a preferential stimulation of the human ER α over ER β .

The major estrogens produced in a non-pregnant woman would modestly favor the activation of ER α over ER β . However, during pregnancy, there is a preponderance of activation of ER β over ER α , which is exerted by various pregnancy estrogens, mainly E₃, which is produced in unusually large quantities. Such a preferential activation of ER β is believed to play an indispensable role in mediating the various actions of en-

dogenous estrogens that likely are 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 the observations that ER β has a wide distribution in maternal reproductive and lymphatic organs in rats as well as in various tissues in the fetus [28–30].

Selection of estrogens for postmenopausal hormone replacement therapy (HRT)

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 they do not contain E₃. However, they contain a number of unique equine estrogens, many of which are basically not produced in humans. Interestingly, our recent analysis showed that several of the equine estrogens contained in Premarin are functionally similar to the human pregnancy estrogen E₃ with respect to their preferential binding ability for human ER β compared to ER α [5].

Let me give a few examples here. Our recent study showed that while 17 β -dihydroequilenin has a low binding affinity for ER α (35% of E₂), it has a high binding affinity for ER β (RBA 100% of E₂). Equilin (*i.e.*, 7-dehydro-E₁) has a decreased binding affinity for ER α compared to E₁ (its RBA 40% of E₁), but it has drastically increased binding affinity for ER β (its RBA 631% of E₁). Similarly, D-equilenin has a much weaker binding affinity than E₁ for human ER α (RBA 20% of E₁), but its binding affinity for ER β is >3 times higher than that of E₁.

Taken together, it is evident that many of the equine estrogens contained in Premarin have a strong, differential binding affinity for human ER β over ER α , which is very similar to the human pregnancy estrogen E₃.

Ideally, when an estrogen or a combination of estrogens is being evaluated for use in postmenopausal HRT, its ability to more closely mimic the hormonal environment found in a normal non-pregnant young woman but not one found in a pregnant woman is a crucial pharmacological property. Since very different types of estrogens are produced in pregnant *vs* non-pregnant women and they serve widely different physiological purposes, it is logical that the use of endogenous estrogens found in a non-pregnant young woman would be better for HRT than the use of estrogens predominantly produced during pregnancy. The former may include a combination of the sulfates of E₁ and 2-

OH-E₁ and possibly other endogenous estrogens (such as the conjugates of 2-methoxyestrogens). The inclusion of methoxyestrogen sulfates as part of the HRT may be beneficial because of the strong antitumorigenic activity of 2-methoxyestradiol [7,15]). Given that many of the endogenous estrogens may have a rather rapid metabolic disposition in the body, some other naturally-occurring or synthetic estrogens that have longer half-lives and can also provide a similar preferential activation of the ER α system as E₁ may also be useful as alternatives. For instance, since 17 α -E₂ has similar ER-binding preference as E₁ but it cannot be converted to E₂ by 17 β -hydroxysteroid dehydrogenase (17 β -HSD), the sulfate conjugates of 17 α -E₂ may serve as alternatives to E₁-3-sulfate to achieve similar biological functions.

Here I also like to suggest that using sulfated estrogens for human HRT would be better than using the corresponding parent estrogens. The main reasons are: (i) The sulfated estrogens are inactive themselves (with little or no binding affinity for human ER α and ER β [5]), but they can be enzymatically hydrolyzed to release bioactive estrogens in a variety of tissues in the body. Earlier studies showed that the estrogen target organs, such as the breast and uterus, contained much higher levels of estrogen sulfatase activity than other tissues [34–37]. As such, oral administration of estrogen sulfates would have the natural cushion effect which would avoid causing unwanted over-stimulation of the ER systems throughout the body. Instead, they usually would only activate those target tissues or cells that are most in need of estrogenic stimulation. Here it is also of note that several recent studies have shown that the estrogen target cells can actively transport E₁-3-sulfate into the cells [38,39]. Moreover, these cells may selectively adjust their ability to actively transport E₁-3-sulfate into the cells to release the biologically-active estrogens, depending on their hormonal needs. Theoretically, such a mechanism would offer certain degrees of target organ selectivity of estrogenic stimulation. (ii) Compared to estrogen glucuronides, estrogen sulfates are probably better because they usually have a lower clearance rate and a longer half-lives (T_{1/2}) in human, thereby

making them pharmacologically more useful [40, 41].

Based on the discussion given above, it is suggested that the use of estrogens to produce a modest level of stimulation of both ER α and ER β systems with a slight preference for the ER α system would be better for postmenopausal HRT than the use of estrogens that confer a predominant activation of the ER β 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 considerable amount of E₁-3-sulfate contained in Premarin, which presumably is good for its intended purpose as a HRT, the fact is that it also contains many other very potent equine pregnancy estrogens which would jointly produce a strong over-stimulation of the ER β system. Similarly, genistein, a potent and preferential partial agonist of human ER β , would be even less suitable than Premarin for use as postmenopausal HRT because it will essentially produce a near selective ER β stimulation. This suggestion is in agreement with recent clinical observations showing that the singular use of genistein is mostly ineffective as a HRT in postmenopausal woman [42,43].

Conclusions

For decades, the general scientific belief had been that all estrogens would exert the same or highly similar pharmacological actions in a woman's body. In fact, 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 E₁-3-sulfate. This bioassay mostly measured the ER α -mediated uterotrophic activity. Even to this day, little is known about the precise hormonal strength of Premarin and each of the bioactive components for human ER α and ER β systems.

E₂ is among the most potent endogenous estrogens and has almost equal binding affinity for human ER α and ER β , but it is not a major estrogen present in a woman. In fact, it is E₁ or E₃ that

is the quantitatively major estrogen present in a woman under different physiological conditions. Although their binding affinities for ER α and ER β are lower than E₂, they provide a differential activation of the ER α or ER β signaling system. Our recent study showed that the endogenous estrogens (such as E₁ and 2-OH-E₁) that are present in a non-pregnant woman would mainly activate the ER α system, whereas the estrogens (such as E₃ and epi-E₃) that are present in pregnant woman would provide a predominant activation of the ER β system. Therefore, the facile metabolic conversion of E₂ to E₁ or of E₂ to E₃ in a woman provides an important means for achieving differential activation of the ER α or ER β signaling system under different physiological conditions.

It is suggested that the more suitable estrogens for human HRT would be those that can mimic the physiological estrogenic stimulation in a premenopausal non-pregnant woman, but not that in a pregnant woman. Based on this new concept, it appears that the naturally-occurring estrogens like estrone (E₁) and estrone-3-sulfate (E₁-3-sulfate) would be more suitable for use as postmenopausal HRT than Premarin, which are essentially composed of pregnancy estrogens (with a strong ER β preference). It is apparent that a balanced activation of the ER α and ER β systems with a modest preference toward the ER α system would be better for HRT compared to estrogens that would predominantly activate the ER β system. An optimally-adjusted activation of the ER α and ER β signaling systems is believed to help maximize the beneficial effects of HRT, in addition to minimizing its untoward effects.

References

1. Nilsson S, Gustafsson JA [2002] Estrogen receptor action. *Crit Rev Eukaryot Gene Exp* 12: 237-257.
2. Carpenter KD, Korach KS [2006] Potential biological functions emerging from the different estrogen receptors. *Ann NY Acad Sci* 1092: 361-373.
3. Jordan VC [2007] SERMs: Meeting the promise of multi-functional medicines. *J Natl Cancer Inst* 99: 350-356.
4. Jordan VC [2007] Chemoprevention of breast cancer with selective oestrogen-receptor modulators. *Nat Rev Cancer* 7: 46-53.
5. Zhu BT, Han GZ, Shim JY, Wen Y, Jiang XR [2006]

- Quantitative structure-activity relationship (QSAR) of various endogenous estrogen metabolites for human estrogen receptor α and β subtypes: Insights into the structural determinants favoring a differential subtype binding. *Endocrinology* 147: 4132–4150.
6. **Zhu BT, Conney AH** [1998] Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis* 19: 1–27.
 7. **Zhu BT, Conney AH** [1998] Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? *Cancer Res* 58: 2269–2277.
 8. **Weisz J** [1991] Metabolism of estrogens by target cells: Diversification and amplification of hormone action and the catechol estrogen hypothesis. In: *New Biology of Steroid Hormones* (Hochberg R.B. and Naftolin, F. Eds), pp 101–112, Raven Press, New York.
 9. **Fotsis T, Adlercreutz H** [1987] The multicomponent analysis of estrogens in urine by ion exchange chromatography and GC-MS—I. Quantitation of estrogens after initial hydrolysis of conjugates. *J Steroid Biochem* 28: 203–213.
 10. **Fotsis T** [1987] The multicomponent analysis of estrogens in urine by ion exchange chromatography and GC-MS—II. Fractionation and quantitation of the main groups of estrogen conjugates. *J Steroid Biochem* 28: 215–226.
 11. **Lee AJ, Cai MX, Thomas PE, Conney AH, Zhu BT** [2003] Characterization of the oxidative metabolites of 17 β -estradiol and estrone formed by fifteen selectively-expressed human cytochrome P450 isoforms. *Endocrinology* 144: 3382–3398.
 12. **Lee AJ, Kosh JW, Conney AH, Zhu BT** [2001] Characterization of the NADPH-dependent metabolism of 17 β -estradiol to multiple metabolites by human liver microsomes and selectively-expressed human cytochrome P450 3A4 and 3A5. *J Pharmacol Exp Ther* 298: 420–432.
 13. **Lee AJ, Mills LH, Kosh JW, Conney AH, Zhu BT** [2002] NADPH-dependent metabolism of estrone by human liver microsomes. *J Pharmacol Exp Ther* 300: 838–849.
 14. **Zhu BT, Lee AJ** [2005] NADPH-dependent metabolism of 17 β -estradiol and estrone to polar and nonpolar metabolites by human tissues and cytochrome P450 isoforms. *Steroids* 70: 225–244.
 15. **Zhu BT** [2002] Catechol-O-methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: Importance in pathophysiology and pathogenesis. *Curr Drug Metab* 3: 321–349.
 16. **Liu D, Bachmann KA** [1998] An investigation of the relationship between estrogen, estrogen metabolites and blood cholesterol levels in ovariectomized rats. *J Pharmacol Exp Ther* 286: 561–568.
 17. **Ball P, Knuppen R** [1980] Catecholestrogens (2- and 4-hydroxyestrogens): Chemistry, biogenesis, metabolism, occurrence and physiological significance. *Acta Endocrinol* 232: 1–127.
 18. **Liehr JG** [2000] Is estradiol a genotoxic mutagenic carcinogen? *Endocr Rev* 21: 40–54.
 19. **Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D** [2000] Estrogens as endogenous genotoxic agents—DNA adducts and mutations. *J Natl Cancer Inst Monogr* 27: 75–93.
 20. **Yager JD, Davidson NE** [2006] Estrogen carcinogenesis in breast cancer. *N Engl J Med* 354: 270–282.
 21. **Sutherland TE, Anderson RL, Hughes RA, Altmann E, Schuliga M, Ziogas J, Stewart AG** [2007] 2-Methoxyestradiol — A unique blend of activities generating a new class of anti-tumour/anti-inflammatory agents. *Drug Discov Today* 12: 577–584.
 22. **Chakraborty C, Davis DL, Dey SK** [1990] Estradiol-15 α -hydroxylation: A new avenue of estrogen metabolism in peri-implantation pig blastocysts. *J Steroid Biochem* 35: 209–218.
 23. **Tulchinsky D, Frigoletto FD Jr, Ryan KJ, Fishman J** [1975] Plasma estetrol as an index of fetal well-being. *J Clin Endocrinol Metab* 40: 560–567.
 24. **Kundu N, Grant M** [1976] Radioimmunoassay of 15 α -hydroxyestriol (estetrol) in pregnancy serum. *Steroids* 27: 785–796.
 25. **Notation AD, Tagatz GE** [1977] Unconjugated estriol and 15 α -hydroxyestriol in complicated pregnancies. *Am J Obstet Gynecol* 128: 747–756.
 26. **Taylor NF, Schackleton CHL** [1978] 15 α -Hydroxyestriol and other polar oestrogens in pregnancy monitoring — A review. *Ann Clin Biochem* 15: 1–11.
 27. **Kunda N, Wachs M, Iverson G, Petersen LP** [1981] Comparison of serum unconjugated estriol and estetrol in normal and complicated pregnancies. *Obstet Gynec* 58: 276–281.
 28. **Saunders FJ** [1968] Effects of sex steroids and related compounds on pregnancy and on development of the young. *Physiol Rev* 48: 601–643.
 29. **Hewitt SC, Korach KS** [2003] Oestrogen receptor knockout mice: Roles for oestrogen receptors α and β in reproductive tissues. *Reproduction* 125: 143–149.
 30. **Lemmen JG, Broekhof JL, Kuiper GG, Gustafsson J-Å, van der Saag PT, van der Burg B** [1999] Expression of estrogen receptor α and β during mouse embryogenesis. *Mech Dev* 81: 163–167.
 31. **Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators** [2002] Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 288: 321–333.
 32. **Scarabin PY, Oger E, Plu-Bureau G, Estrogen and Thromboembolism Risk Study Group** [2003] Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. *Lancet* 362: 428–432.
 33. **Smith NL, Heckbert SR, Lemaitre RN, Reiner AP, Lumley T, Weiss NS, Larson EB, Rosendaal FR, Psaty BM** [2004] Esterified estrogens and conjugated equine estrogens and the risk of venous thrombosis. *JAMA* 292: 1581–1587, 2004.
 34. **Hobbirk R** [1985] Steroid sulfotransferases and steroid sulfate sulfatases: Characteristics and biological roles. *Can J Biochem Cell Biol* 63: 1127–1144.

35. **Santner SJ, Feil PD, Santen RJ** [1984] In situ estrogen production via the estrone sulfatase pathway in breast tumors: relative importance versus the aromatase pathway. *J Clin Endocrinol Metab* 59: 29–33.
36. **MacIndoe J H, Woods G, Jeffries L, Hinkhouse M** [1988] The hydrolysis of estrone sulfate and dehydroepiandrosterone sulfate by MCF-7 human breast cancer cells. *Endocrinology* 123: 1281–1287.
37. **Pasqualini J R, Gelly C, Nguyen BL, Vella C** [1989] Importance of estrogen sulfates in breast cancer. *J Steroid Biochem* 34: 155–163.
38. **Miki Y, Suzuki T, Kitada K, Yabuki N, Shibuya R, Moriya T, Ishida T, Ohuchi N, Blumberg B, Sasano H** [2006] Expression of the steroid and xenobiotic receptor and its possible target gene, organic anion transporting polypeptide-A, in human breast carcinoma. *Cancer Res* 66: 535–542.
39. **Nozawa T, Suzuki M, Takahashi K, Yabuuchi H, Maeda T, Tsuji A, Tamai I** [2004] Involvement of estrone-3-sulfate transporters in proliferation of hormone-dependent breast cancer cells. *J Pharmacol Exp Ther* 311: 1032–1037.
40. **Twombly GH, Levitz M** [1960] Metabolism of estrone-C¹⁴-16 sulfate in women. *Am J Obstet Gynecol* 80: 889–326.
41. **Ruder HJ, Loriaux L, Lipssett MB** [1972] Estrone sulfate: Production rate and metabolism in man. *J Clin Invest* 51: 1020–1033.
42. **Fitzpatrick LA** [2003] Soy isoflavones: Hope or hype? *Maturitas* 44 (Suppl 1): S21-S29.
43. **Rozman KK, Bhatia J, Calafat AM, Chambers C, Culty M, Etzel RA, Flaws JA, Hansen DK, Hoyer PB, Jeffery EH, Kesner JS, Marty S, Thomas JA, Umbach D** [2006] NTP-CERHR expert panel report on the reproductive and developmental toxicity of genistein. *Birth Defects Res B Dev Reprod Toxicol* 77: 485–638.