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Estradiol Synthesis and Metabolism and Risk of Ovarian Cancer in Older Women Taking Prescribed or Plant-derived Estrogen Supplementation

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Abstract

Estradiol, the most potent of the biological estrogens, is implicated in the genesis of ovarian epithelial cancer, a heterogeneous cancer affecting mainly older women. The postmenopausal ovary traditionally has not been viewed as contributing significantly to estradiol synthesis, since this is thought to occur almost exclusively as the result of peripheral aromatization of adrenal androgens. Recent evidence supports a role for both normal and malignant ovarian tissue in *de novo* synthesis of estradiol using inactive biological precursors and available enzymatic pathways. The process is termed "intracrinology". The present paper reviews available evidence for the intracrinological synthesis of estradiol in ovarian surface epithelium. It further proposes how exogenous supplementation with synthetic hormone replacement may act to augment this process by increasing the risk of developing ovarian epithelial cancer in older women. Phytoestrogens are also examined for their role in regulating levels of estradiol metabolites with potent estrogenic and carcinogenic potential.

Keywords: Estradiol intracrinology synthesis/metabolism; Estrogen supplementation; Ovarian epithelial cancer; Older ovary; Ovarian surface epithelium

Abbreviations: CYP: Cytochrome P450 Enzyme; HSD: Hydroxysteroid Dehydrogenase Enzyme; E1S: Estrone Sulfate; DHEA-S: Dehydroepiandosterone – sulfate; STS: Steroid Sulfatase; HGSC: High Grade Serous Carcinoma; RT-PCR: Reverse Transcription Polymerase Chain Reaction; OSE: Ovarian Surface Epithelium; EST: Estrogen Sulfotransferase; SHBG: Sex Hormone Binding Globulin; COMT: Catechol-O-Methyl Transferase; OVCAR-3: Ovarian Cancer 3 Cell Line; P13: Phosphatidyl-inositol 3; AKT: Protein kinase B; NFκB: Nuclear Factor Kappa B; ER: Estrogen Receptor

Estradiol Synthesis in the Premenopausal Ovary

All steroid hormones, of which estradiol is an example, are lipids that have cholesterol as their common precursory substrate. The synthesis of sex steroid hormones from cholesterol involves a series of sequential steps that result in the cleavage of side-chains, reorganization of olefinic bonds, and the addition of hydroxyl groups. For estradiol synthesis, this pathway is from cholesterol to pregnanes, then on to androstanes and arriving finally at the estranes [1]. Estradiol synthesis requires the activity of members of the Cytochrome P450 enzyme family (gene constructs CYPs 11,17 and 19), and a second family of enzymes, the hydroxysteroid dehydrogenases (HSD), which catalyze bi-directional reactions involved in both the biosynthesis and inactivation of steroid hormones. HSD exist in several different isoforms [2], and cooperate with steroid sulfotransferases, reductases and steroid sulfatases to regulate the level of bioactive hormone in all target tissues [1].

Estradiol synthesis in the premenopausal ovary requires the synergistic efforts of at least two cell types that synthesize their product when stimulated by independent upstream mechanisms. In theca, pregnenalone undergoes enzymatic conversion to androstenedione in a sequential fashion. Since thecal tissue lacks P450 aromatase (CYP 19) the final conversion to estradiol occurs in the adjacent granulosa cells and requires the actions of 17β HSD1 in converting androstenedione to testosterone, after which aromatase completes the conversion through to estradiol 17β [1]. This pathway is subsequently termed "the aromatase pathway" for estradiol synthesis. In the luteal phase of the ovulatory cycle, mammalian theca and cells of the corpora lutea (theca lutein) act together with granulosa lutein cells of corpora lutea, to produce sizeable amounts of both estradiol and progesterone. Throughout the cycle, estrone-sulphate (E1S) and dehydroepiandosterone sulfate (DHEA-S - available in small amounts in the premenopausal ovary) may also undergo conversion to estrone and DHEA respectively via the sulfatases (STS). This is "the sulfatase pathway" for estradiol synthesis, since the actions of the sulfatases allows the final conversion through to estradiol to be completed by HSDs 3 and 17 and P450arom.

The localization and expression of the major enzymes required for ovarian steroidogenesis are shown in figure 1.

To summarize, the availability of cholesterol, the relative amounts and type of enzymes in each tissue or cell compartment, and the actions of FSH on granulosa cell [3] are the three variables upon which estradiol synthesis in the premenopausal mammalian ovary depends.

Estradiol and the Postmenopausal Ovary: Potential for Oncogenesis

Following menopause, peripheral estradiol levels in the blood are thought to be mainly due to contribution from the adrenals and peripheral aromatization of androgens to estrogen in adipose tissue and skin, where aromatase activity correlates with estradiol production. There is evidence however, that the postmenopausal ovary retains the ability to produce both androgens [4] and estrogen [5,6].

The degree to which ovarian surface epithelium (OSE) is capable of

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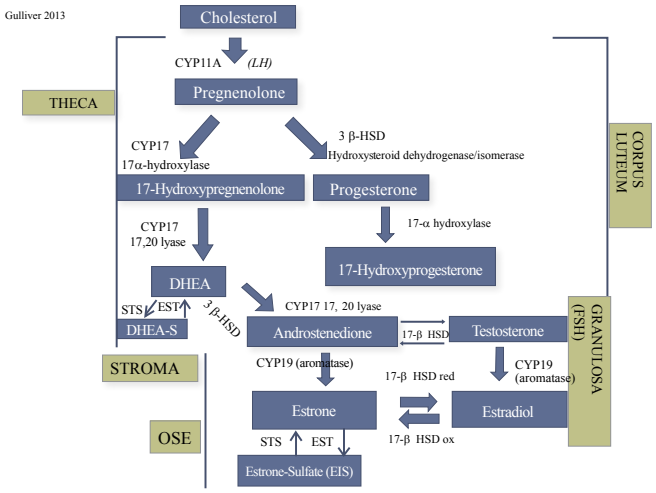

Figure 1: The ovary: Pathways of steroid hormone biosynthesis and the key enzymes involved.

steroidogenesis has only in recent years received attention, due largely to the OSE's unresolved role in ovarian epithelial cancer, a cancer of mainly older women. Although recent attention has turned to the adjacent fimbriated oviductal epithelium as being the tissue of origin for ovarian epithelial cancers (specifically high grade serous carcinomas (HGSC)) [7,8], the most up-to-date evidence retains a definitive oncogenic role for mammalian OSE cells near the hilus of the ovary, at the point of OSE-mesothelial transition [9]. Since epidemiological evidence increasingly links estrogen to ovarian epithelial cancer [10-18] and OSE has intracrinological capacity, the possibility of *in situ* estradiol-induced carcinogenesis in the postmenopausal ovary requires further investigation.

In vitro and *in vivo* evidence for aromatase activity in normal human OSE has been demonstrated using reverse transcription polymerase chain reaction (RT-PCR) [19,20] and immunohistochemistry [20,21]. Both benign and malignant ovarian epithelial tumors show immunohistochemical evidence of aromatase function in the cytoplasm of tumor cells [22], and aromatase is reported to be localized in stromal cells adjacent to ovarian carcinoma involving OSE [23]. In postmenopausal women, aromatase expression in normal OSE has been documented as six-fold higher than in stroma, and is also significantly greater in normal OSE when compared to malignant ovarian epithelial tumors [20]. This may indicate that when OSE undergoes malignant transformation, the aromatase pathway may not be the preferred pathway for the *de novo* synthesis of estradiol. Indeed the targeting of ovarian epithelial cancers with aromatase inhibitors has had mediocre success, perhaps not only due to the heterogeneity of ovarian cancers, but also because there is more than one player in localized estradiol synthesis. It has already been established that the sulfatase pathway is the dominant pathway for *in situ* estradiol synthesis in postmenopausal breast cancers, where it prevails over the aromatase pathway by some 50-200 fold [24].

Ovaries from older mice have been shown by aging-specific gene array to have increased not only mRNA levels of aromatase, but also progesterone receptor (PR) and estrogen sulfotransferase (EST) compared to younger mice. EST, an enzyme that catalyzes the conjugation of estrone to a sulphur group producing estrone sulfate (E1S, Figure 1), is the most abundant circulating estrogen conjugate and renders estrone inactive [25]. However, once the sulphur group is removed, estrone can bind to estrogen receptor but does so with low affinity. Interestingly, Zimon et al. [25] were not able to demonstrate increases in EST at protein level using Western Blot and immunofluorescence in older mouse ovary. Instead, they found levels of EST protein to be significantly reduced. EST mRNA expression has recently been reported to be markedly reduced in cultured primary ovarian epithelial cancer cells and all ovarian cancer cell lines when compared to normal OSE cells using quantitative RT-PCR [6].

Human OSE in cell culture additionally expresses steroid sulfatase (STS). STS works antagonistically to EST and converts E1S and dehydroepiandosterone-3 sulfate (DHEA-3-sulfate) to estrone and DHEA respectively (Figure 1). This raises the possibility that localized changes in the ratio of STS to EST in aging ovarian surface epithelium may boost levels of unconjugated estrone. Existing *in vitro* evidence using ovarian cancer cell lines, primary ovarian cancer cells and normal OSE, shows that activity of EST relative to STS is greater in normal OSE when compared to the cancerous state [6]. This finding may be important and invites more investigation into EST to STS ratios in OSE from postmenopausal women.

The significance of increased STS to EST ratio in OSE is that OSE also expresses mRNA for 17βHSD reductase required for the synthesis of estradiol from estrone [26]. In confluent culture, normal OSE from pre and postmenopausal women has been shown capable of *de novo* synthesis of both estrogen and progesterone using HSDs [27]. Thus, an increase in the STS to EST ratio may potentially boost local ovarian tissue levels of estradiol 17β, the far more potent biologically active estrogen that binds with strong affinity to its receptor. A current review targeting sulfatase activity for estrogen production in OSE supports this view [28] and underpins the emerging significance of the sulfatase pathway for generating potentially damaging levels of estradiol in OSE, conceivably predisposing it to oncogenesis.

It is possible that ovarian aging may have an effect on the stability of EST proteins. This may confer reduced ability to sulfonate estrone and estradiol obtained from peripheral blood via organic anion transporting polypeptides [28], which in turn may lead to increased levels of unconjugated estradiol in the ovary. Indeed in a recent study completed by our own lab, we found using radioimmunoassay, that some older mice had elevated levels of endogenous estradiol in ovarian tissue (>300 pg/mL; normal range 66.2-117 pg/mL). Furthermore, mice administered exogenous estradiol continued to show significantly elevated estradiol levels in ovarian tissue 2 weeks following cessation of estrogen treatment, indicating that unconjugated estradiol accumulates and is retained in ovarian tissue [29,30].

These findings may go some way to providing an explanation as to why older women taking exogenous estradiol in the form of hormonal replacement therapy carry greater risk for the development of ovarian epithelial cancer. Friel et al. [31] reported that an oral estradiol dose regimen of between 1-2 mg/day can lead to a condition of estradiol overdose - as evidenced by urinary excretion of estrone 5-10 times the upper limit of the reference range for premenopausal women. Of note is that the present recommended estradiol dose in the U.S remains anywhere between 0.45 mg/day–2 mg/day.

Phytoestrogen Intake and Risk of Ovarian Epithelial Cancer

While ample epidemiological evidence exists for hormone replacement therapies as having a role in the development of ovarian

cancers, some evidence suggests that phytoestrogens taken in the diet may actually have the opposite effect. Phytoestrogens which include isoflavones (mostly soy-derived) and lignans (derived from grains, seeds, vegetables, fruits and berries) are established as having both estrogenic and anti-estrogenic properties [32,33]. Phytoestrogen consumption has been reported to either confer significantly reduced risk of ovarian cancer [34-36] show a non-significant inverse association with ovarian epithelial cancer [37], or show no association with overall ovarian epithelial cancer risk [38]. Interestingly in the latter prospective cohort study, fiber intake was associated with a decreased risk of borderline, but not invasive ovarian epithelial cancer [38]. Re-uptake of estradiol metabolites found in bile is slowed by the presence of fiber in the intestine, lowering total estrogen. This mechanism for regulating estradiol may have little effect on established tumors capable of autonomous endocrine activity.

The differing results from the above studies may be a consequence of their differing methodologies (case-control based or prospective cohorts, dietary-derived only phytoestrogens versus dietary and supplement derived), different sample populations, age and menopausal status of the women. Moreover, ovarian cancers are highly heterogeneous and this has received little attention in the design and interpretation of these studies. Perhaps importantly, authors of the recent Swedish study [38] (where no association was seen between phytoestrogen intake and ovarian cancer risk) reported that overall bean/soy consumption in their cohort was low. Since other studies [34-37] were able to show a dose-response correlation, whereby subjects making up the highest quintile of phytoestrogen consumption showed reduced risk for ovarian cancer compared to those in the lowest quintile, the normally low intake of phytoestrogens in the Swedish cohort may have influenced results from that study. Studies which report changes to estradiol metabolism that reduce risk for developing ovarian cancer when women ingest higher rather than lower quantities of phytoestrogens in the form of flaxseed (a lignan) and isoflavones [39,40], lend further support for a protective role for phytoestrogens in the development of ovarian cancer.

There is an important emerging body of research investigating how ingested phytoestrogens act to control endogenous levels of estradiol by controlling the activity of estradiol's active metabolites. Circulating estradiol in human blood is controlled by regulatory mechanisms that act independently of the well-known endocrine feedback loops, including activin and inhibin. In humans, levels of Sex Hormone Binding Globulin (SHBG) act to bind and therefore control to some extent the actions of free unbound estradiol. Levels of SHBG increase with increasing levels of circulating estradiol and reflect the fact that more hormone is available for binding. The half-life of estradiol in the postmenopausal female is around 3 hours [41] with significant amounts of estradiol undergoing conversion to estriol and estrone followed by excretion of these well-known metabolites into the urine, bile and feces.

The formation of hydroxyl derivatives of estrogens (the catechol estrogens) during estradiol metabolism forms a potentially very important pathway for the control of endogenous estradiol levels. The principal hydroxylation products are 2-hydroxyestrone, 2-hydroxyestradiol, 4-hydroxyestrone, 4-hydroxyestradiol and 16-hydroxyestrone, 16-hydroxyestradiol [42]. Oxidation reactions allowing for the insertion of hydroxyl groups at positions 2-, 4-, or 16- are catalyzed by members of the Cytochrome P450 (CYP) family of enzymes. CYP1A1 catalyzes the 2-hydroxylation of estradiol and can be induced by dietary constituents [42]. CYP1B1 catalyzes both 16α- and 4-hydroxylation [42,43], and it has been reported that 16-hydroxyestradiol may be induced by pesticides and other xenobiotic carcinogens [42]. Estradiol catabolism continues when poly-hydroxyl entities undergo conjugation with sulfates and gluconarates, or are methylated via catechol-O-methyl transferase (COMT) before being excreted in the urine. However, 4-hydroxyestrogens are unstable, and can be oxidized by peroxidases to form highly reactive semi-quinone and quinone metabolites [42,43].

Metabolite activity can be measured from samples of blood, tissue and urine. It is known that 2-hydroxyestrones are rapidly methylated in the blood by COMT and have anti-estrogenic (anti-proliferative) effects [43]. Although 4-hydroxyestrone and 4-hydroxyestradiol are found only in small amounts in the blood, they none-the-less have estrogenic action (hyperplasia, hypertrophy) and carcinogenic potential via the generation of free radicals [43-45]. The metabolite 16α-hydroxyesterone has been found to have potent estrogenic effects equivalent to, or stronger than, 17β-estradiol. These include DNA synthesis, persistent proliferation and anchorage-dependent growth [42,46].

A diet that is high in soy isoflavones increases the 2α-hydroxyestrone to 16α-hydroxyestrone ratio and lowers mid-cycle gonadotropin levels, leading to decreases in circulating estradiol, progesterone and SHBG in premenopausal women [47]. Decreases in 16α-hydroxyestrone, 4-hydroxyestrone and 4-hydroxyestradiol have also been shown in premenopausal women with high soy isoflavone intakes of 130 mg/day compared to those with low intakes of 7-10 mg/day [40]. Moreover, the daily ingestion of large amounts (10 g) of ground flaxseed over a period of seven weeks in postmenopausal women has been reported to dramatically induce 2-hydroxylation of estrone and improve the ratio of 2/16α-hydroxyestrone, whereas only moderate effects were observed with reduced intakes of 5 g flaxseed per day [39]. Interestingly, supplementation with flaxseed lignans appears superior to soy in altering estrogen metabolism in postmenopausal women with respect to increasing 2α to 16α- hydroxylation. Flaxseed also moderately inhibits Cytochrome P450arom [48,49] and modulates the activity of the 17-HSD [50].

Some studies investigating estrogen metabolism and breast cancer have shown that estradiol metabolism favoring formation of 2-α hydroxylation over 16α-hydroxylation decreases the risk for developing breast cancer [51,52], although other studies show mixed results [53]. The differences in results may relate to the varying methodologies employed by researchers, but may also relate to menopausal status in the women studied. Recently, in an *in vitro* study using ovarian cancer cell line OVCAR-3 to examine the effects of the metabolites of estradiol on proliferation and apoptosis in comparison to estradiol itself, the 17β proliferative and anti-apoptotic activity of the 16α-hydroxylated estrone was shown to outstrip that of estradiol. Surprisingly 4α-hydroxyestrone gave similar results to 17β estradiol at physiologic concentrations [54], and may exert its effects through the PI3K/Akt signaling pathway to promote ovarian carcinogenesis. Importantly, the 2α-hydroxyestrone metabolite was shown to have little activity. Although there is more research needed, these results indicate that the maintenance of pro-oncogenic to anti-oncogenic estradiol metabolites in the ovary may prove to be a very important factor in the genesis of ovarian epithelial cancer.

Finally, many phytoestrogens are known to bind both functional estrogen receptor subtypes (ERα and ERβ), and are capable of inducing transcription of estrogen responsive target genes in a dose-dependent manner [55-58]. Whether phytoestrogen binding to ER produces

the same or opposite effect to estradiol appears to depend on several factors: the type and amount of phytoestrogen [55-60], its relative binding affinity for the receptor subtype [56,57,61], the abundance from tissue to tissue of one ER subtype relative to the other [62], the presence of low-affinity (type II) nuclear binding sites [63], the ability of the phytoestrogen to utilize other non-genomic modalities (such as akt phosphorylation and NFκB) to modulate estrogenic and carcinogenic effects [64,65], and the presence of endogenous estrogen [66]. Activation of ERα by estradiol induces marked proliferation in normal and cancerous ovarian epithelial cells *in vitro* and *in vivo* [29,67-69], whereas activation of ERβ opposes the proliferative effects of ERα and has pro-apoptotic and anti-tumoral effects [69-71].

Phytoestrogens are known to bind ER with much lower affinity than estradiol [60,61], and preferentially bind ERβ [72]. Moreover, they induce the transcription of estrogen-responsive target genes to a much greater degree when bound to ERβ, rather than when bound to ERα [66]. Phytoestrogens are also capable of inducing ER-mediated gene transcription to higher levels than estradiol itself [66]. Taken together, it may be proposed that in tissues such as ovary where ERβ is abundantly expressed, phytoestrogens may act to augment the anti-carcinogenic effects of that receptor subtype. However, it should be noted that some phytoestrogens (e.g. genistein and resveratrol) have been known to act synergistically with estradiol in MCF-7 breast cancer cells [66,73], and can act as 'super agonists' that bind ERα as well as ERβ. It is therefore important that further research defines the effects of different phytoestrogens on the ovary, and elucidates the cellular and molecular basis for their action.

Conclusion

At this time there appears to be a paucity of both *in vitro* and *in vivo* data for estradiol intracrinology and metabolite activity in the older female, and in the development of ovarian epithelial cancer. Since long-term exposure to estradiol is an established risk factor for ovarian cancer, this is an area of research that requires much more attention.

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