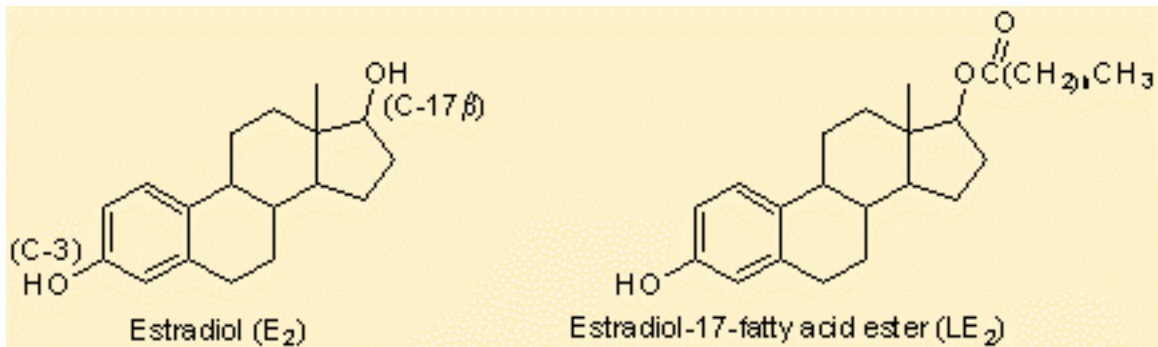


The Physiology of the Steroid Fatty Acid Esters

Fatty acid esters of steroids were discovered only recently when compared to all other steroid metabolites. In 1979 we discovered in the adrenal gland, non-polar metabolites of the 5- β -hydroxysteroids, pregnenolone, 17-hydroxypregnenolone and dehydroepiandrosterone. We subsequently identified these metabolites as fatty acid esters. Subsequently, we and others found that all of the families of steroids can be esterified with fatty acids. Our interest has been focused recently on the esters of estrogens and androgens because these compounds are the naturally occurring metabolites of similar synthetic sex steroid esters that have been used pharmacologically for decades. Our studies are concerned with the physiological role played by these potent hormones as androgens and estrogens. We are also deeply involved in studies of the blood borne esters of estradiol in inhibiting heart disease.

Women are far less susceptible to coronary heart disease than men until the onset of menopause, then its incidence in women increases dramatically. Since the increased risk of arteriosclerosis can be prevented by estrogen replacement, it is thought that ovarian estrogens cause this gender difference. Estrogens have several different cardio protective actions on lipids, including increasing HDL, decreasing LDL and total cholesterol. But, these effects on plasma lipids are thought to account for less than one half of the protective action of estrogens on coronary artery disease. Another action that might account for some of the protective effect of estrogens is the inhibition of the oxidation of LDL. The modification of LDL by oxidation is widely recognized as a critical element in the initiation of coronary artery disease. Oxidized LDL is rapidly taken up by macrophages through "scavenger pathways" that internalize the modified LDL through receptors other than LDL receptors. Macrophages degrade the lipoprotein and, in the process, are converted into lipid rich foam cells that are characteristically found in early arteriosclerotic plaques. Antioxidants that inhibit LDL oxidation *in vitro* have also been shown both to protect LDL from oxidation *in vivo* and to inhibit the formation of atherosclerotic lesions. Estrogens administered *in vivo* as well as *in vitro* can inhibit LDL oxidation.

LDL protection has been demonstrated in menopausal women given therapeutic doses of equine estrogens or E2 to attain premenopausal blood levels of E2. In contrast to the *in vivo* effect of estrogens, *in vitro* protection by the direct addition of estrogens to isolated LDL requires μM concentrations of estradiol, amounts that are far in excess of the concentration in blood. The *in vitro* anti-oxidative action of estradiol, and many of its metabolites, is an unusual non-classical estrogenic action that is not mediated through the estrogen receptor and is usually thought not to be of biological significance because of the large concentration of phenolic steroid required.



Recently we and others have shown that when E2 is incubated in plasma at physiological concentrations, the LDL isolated from this plasma is protected from oxidation. During the incubation with plasma, E2 is enzymatically esterified (Figure above). We have shown that while E2 is esterified in tissue by a specific estradiol acyltransferase, in blood it is esterified by the same enzyme that esterifies cholesterol, lecithin:cholesterol acyltransferase (LCAT). However, the esterification of E2 by LCAT is unusual since the 17β-alcohol at which E2 is esterified is sterically hindered compared to the 3β-hydroxyl group esterified in other LCAT substrates, including cholesterol. Furthermore, The LCAT esterification of the 17-hydroxyl group of E2 is specific to the estrogens since several androgens, testosterone and 5α-dihydrotestosterone, that possess the same 17-hydroxyl group are not substrates for this enzyme. Thus, the esterification of the 17-hydroxyl group of E2 in blood is a specific process that does not occur for any steroid other than estrogens and we believe it points to the importance of this process in the protection of women from heart disease.

Current Studies

LDL oxidation

We are investigating the relationship of estradiol (and other estrogens) esterification to the protection of LDL from oxidation. We are studying the ability of the various E2-esters to protect LDL from oxidation in several model systems, including oxidation by several different cells. Further, we are investigating various pharmacological estrogens for their ability to be esterified by LCAT. Some of these commonly used estrogens are not esterified at all, while others are esterified at rates more rapid than E2. Obviously, if our hypothesis linking LCAT esterification to protection against atherosclerosis is correct, these differences can have important ramifications in women's health. Other studies are investigating whether E2, in contrast to potent estrogens that we have found, are not esterified can protect against atherosclerosis *in vivo*. For this study we are using the LDL-receptor knockout mouse, an animal that has high levels of LDL, and develops heart disease rapidly. Other studies are investigating the mechanism by which tiny amounts of the E2-esters interferes with LDL oxidation.

Estradiol-esters in the ovary

In addition to these studies, we are investigating the role of the estradiol esters in cell growth and proliferation in the ovary. It is known that oxidative stimulation by small amounts of various oxidants cause cell proliferation. We have found that ovarian follicular fluid contains very large amounts of LCAT synthesized E2-esters. The hypothesis that we are investigating is these estrogen esters control the growth of ovarian thecal cells.

Androgen esters

We have shown that relatively large amounts of testosterone fatty acid esters are present in male fat. These esters are highly androgenic and have a very long biological half-life because they are protected from metabolic catabolism. We hypothesize that these esters are capable of paracrine stimulation of the growth of neighboring androgen target organs and thus, because of their proximity, can maintain these organs without a requirement for

blood-borne testosterone. We are investigating the effect of various trophic factors and hormones on the synthesis and hydrolysis of androgen esters, and the correlation of these stimuli in the growth or maintenance of various androgen dependent organs.