Lipid metabolic studies in oophorectomized women.
Effects induced by the addition of norethisterone acetate to two different oestrogens on serum individual phospholipids and serum lecithin fatty acid composition

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Abstract. Norethisterone acetate (NET) was administered to 11 oophorectomized women (mean age 34.5 ± 5.9 years) primed with the 17-C-alkylated ethinyl oestradiol (EOe) and the non-alkylated oestrogen, oestradiol valerate (OevV) in separate periods. Blood samples were drawn after 4 weeks without hormonal replacement therapy, after 6 weeks on each oestrogen and after 6 weeks on each oestrogen-progestogen combination. Routine liver function tests were carried out, assessment of individual phospholipids i.e. cephalin, lecithin, lysolecithin and sphingomyelin was done after thin layer chromatography. The relative fatty acid composition of serum and high density lipoprotein lecithin and serum cholesterol ester was determined by gas liquid chromatography. The redistribution of serum individual phospholipids (increase in lecithin and decrease in lysolecithin) induced by both oestrogens was reversed by the addition of NET. This lecithin-lysolecithin shift seen after NET administration could depend on a stimulation of the hepatic lipase with its phospholipase A1-activity. The 17-C-alkylated EOe, but not the non-alkylated OevV, caused an increase in palmitic and a decrease in stearic acid in the 1-position of serum lecithin. When NET (like EOe, 17-C-alkylated) was added to EOe, this palmitic-stearic acid shift was further accentuated, and it also appeared when NET was added to OevV. It is suggested that the shift in 1-position fatty acids of serum lecithin induced by EOe and NET is a non-hormonal 'drug-effect' linked to their 17-C-alkylation and liver toxicity. The reduction in arachidonic acid of serum lecithin and serum cholesterol ester seen after NET administration is interpreted as an anti-oestrogenic effect which could be mediated by a decreased transformation of linoleic to arachidonic acid in the liver.

The liver is a major extra genital target organ for endogenous and exogenous sex steroids and many of its essential functions are influenced by these substances (Song et al. 1969). Differences between 17-C-alkylated as compared to non-alkylated sex steroids have been demonstrated in their effects on liver function (Smith 1974). Accumulating data also indicate a sex steroid influence on the distribution of individual serum phospholipids (Svanborg 1968; Gustafson & Svanborg 1972). In rodents endogenous (Lyman et al. 1967) and exogenous oestrogens (Lyman et al. 1968) have been demon-

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strated to influence the major pathways for liver lecithin and cephalin synthesis and consequently also their relative fatty acid composition.

In a series of studies we have evaluated the relative fatty acid composition of serum lecithin in relation to the menstrual cycle (Gustafson et al. 1974) and during pregnancy (Johnson 1973; Samsie 1974). In the human there is however, only a limited knowledge as to the possible role of exogenous sex steroid effects on liver lecithin synthesis in the non-pregnant state. In a previous study we have found that the 'synthetic' i.e. 17-C-alkylated ethinyl oestradiol (EOe), and the so called 'natural' i.e. non-alkylated oestrogen oestradiol valerate (Oe2V) had similar effects on the relative distribution of individual phospholipids but that their influence on serum lecithin fatty acid composition differed. Norethisterone is the component to which all oestrane progestogens (more commonly known as 19-nortestosterone derivatives) are metabolized (Fortherby 1974). In order to evaluate how this synthetic progestogen (frequently used in combined oral contraceptives) modifies oestrogen influence on individual serum phospholipids and on serum lecithin fatty acid composition norethisterone acetate (NET) was administered to oophorectomized women who were oestrogen primed with EOe or Oe2V in separate periods.

None of the patients had any disease other than the cervical carcinoma except for one woman who had pharmacologically well controlled hypertension. The orally administered steroids were ethinyl oestradiol (EOe) 20 μg/day and oestradiol valerate (Oe2V) 2 mg/day. Each oestrogen was given alone for a 6 week period preceded by a least 4 weeks without hormonal replacement therapy. After 6 weeks on each oestrogen, norethisterone acetate (NET) 10 mg/day was added and the patients were given the combined oestrogen-progestogen regime for another 6 weeks. Hysterectomies according to Wertheim-Meigs had been performed 6 weeks prior to the EOe-period and within a timespan of 10 to 21 months. The patients were given Oe2V and Oe2V + NET as described above. Visits for blood sampling took place after at least 4 weeks without hormonal replacement therapy, after 6 weeks on each oestrogen and after 6 weeks on each oestrogen-progestogen combination. At each visit specific symptoms were recorded and the patients had a physical examination. Blood samples were drawn from an antecubital vein in the morning after an overnight fast.

Liver function tests
Determinations of serum bilirubin, alkaline phosphatase, S-ASAT and S-ALAT were made at the Department of Clinical Chemistry according to routine methods.

Individual phospholipids
Relative distribution of individual phospholipids was assessed according to the method of Vikrot (1965).

Gas-liquid chromatography (GLC) of methyl esters
Separation of lipids by thin layer chromatography (TLC) on silica gel with the isolation of lecithin and the preparation of fatty acid methyl esters from lecithin was carried out according to Olegård & Svennerholm (1970). Preparation of cholesterol esters and cholesterol fatty acid methyl esters was carried out as described by Skryten (1977). The extracts containing the fatty acid methyl esters were evaporated to a final volume of approximately 5 μl. One μl was analyzed in a Perkin Elmer Model 30

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**Table 1.**

<table>
<thead>
<tr>
<th>Cephalin</th>
<th>Lecithin</th>
<th>Lysolecithin</th>
<th>Sphingomyelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOe</td>
<td>2.6 ± 0.14</td>
<td>68.4 ± 0.59</td>
<td>7.0 ± 0.36</td>
</tr>
<tr>
<td>Oe2V</td>
<td>2.7 ± 0.15</td>
<td>68.7 ± 0.60</td>
<td>7.1 ± 0.96</td>
</tr>
</tbody>
</table>
apparatus, equipped with a flame ionization detector on a 200-cm glass column (3 mm in inner diameter), packed with 15% diethylene glucoluccinate (DEGS), and coated on Chromosorb W DMCs, 80–100 mesh. Nitrogen was used as carrier gas, and the column was operated at 193°C.

**Statistical methods**

Standard statistical methods were used to calculate means, standard deviations and correlations. Statistical significance was assessed using Student’s t-test.

**Results**

**Liver function tests**

Throughout the study all values were within normal range. The addition of NET to EOE caused a decrease in alkaline phosphatase (P < 0.05) while the addition of NET to Oe2V in addition reduced (P < 0.05) serum bilirubin and S-ASAT.

**Individual phospholipids** (Table 1, Fig. 1)

When NET was added to EOE the changes induced by the oestrogen were reversed i.e. there was a decrease (P < 0.001) in cephalin and lecithin concomitant with an increase (P < 0.001) both in lysolecithin and sphingomyelin. The addition of NET to Oe2V again caused a reduction (P < 0.05) in cephalin and lecithin concomitant with an increase in lysolecithin (P < 0.01).

**Serum lecithin relative fatty acid composition**

(Table 2, Fig. 2a, b)

The changes induced by EOE on the fatty acids in the 1-position of lecithin were accentuated by the addition of NET in that there was a further increase in palmitic (16:0) (P < 0.05) concomitant with a decrease in stearic (18:0) acid (P < 0.01). There was in addition an increase in linoleic (18:2) (P < 0.01) and a decrease in arachidonic (20:4) acid (P < 0.01) at the 2-position of lecithin. The addition of NET to Oe2V also caused a similar shift with an increase in palmitic (16:0) (P < 0.001) and a decrease in stearic (18:0) acid (P < 0.001). Again there was an increase (P < 0.01) in linoleic (18:2) acid.

**High density lipoprotein (HDL) lecithin relative fatty acid composition**

The addition of NET to EOE caused a further decrease in stearic (18:0) acid (P < 0.05) while when NET was added to Oe2V there was apart from this decrease in stearic (18:0) (P < 0.001) also an increase (P < 0.05) in palmitic (16:0) and linoleic (18:2) acids.
Table 2.
Relative fatty acid composition of serum lecithin (S-LEC) and serum cholesterol ester (S-CE) before the administration of ethinyl oestradiol (OEe) and oestradiol valerate (Oe2V) in 9 oophorecomized women. Figures given in mole per cent of methyl esters (mean ± SEM). 16:0 = palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 20:4 = arachidonic acid.

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-LEC EOe</td>
<td>30.8 ± 0.51</td>
<td>14.8 ± 0.51</td>
<td>12.9 ± 0.30</td>
<td>25.5 ± 0.54</td>
<td>6.4 ± 0.39</td>
</tr>
<tr>
<td>S-LEC Oe2V</td>
<td>29.5 ± 0.45</td>
<td>14.7 ± 0.45</td>
<td>12.5 ± 0.31</td>
<td>25.0 ± 0.83</td>
<td>7.1 ± 0.42</td>
</tr>
<tr>
<td>S-CE EOe</td>
<td>12.7 ± 0.56</td>
<td>1.3 ± 0.09</td>
<td>21.1 ± 1.60</td>
<td>54.4 ± 1.2</td>
<td>3.7 ± 0.31</td>
</tr>
<tr>
<td>S-CE Oe2V</td>
<td>12.2 ± 0.20</td>
<td>1.2 ± 0.07</td>
<td>21.7 ± 0.49</td>
<td>51.2 ± 1.0</td>
<td>3.9 ± 0.32</td>
</tr>
</tbody>
</table>

Fig. 2a.
Per cent change induced in relative fatty acid composition of serum lecithin by ethinyl oestradiol (EOe) 20 µg/day (unfilled columns) and by the addition of norethisterone acetate (NET) 10 mg/day (filled columns) in 9 oophorectomized women. Asterisks mark statistically significant changes induced by the addition of NET as compared to EOe induced values. * = 0.05 level, ** = 0.01 level, *** = 0.001 level. 16:0 = palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 20:4 = arachidonic acid.

Fig. 2b.
Per cent change induced in relative fatty acid composition of serum lecithin by oestradiol valerate (Oe2V) 2 mg/day (unfilled columns) and by the addition of norethisterone acetate (NET) 10 mg/day (filled columns) in 9 oophorectomized women. Asterisks mark statistically significant changes induced by the addition of NET as compared to Oe2V induced values. * = 0.05 level, ** = 0.01 level, *** = 0.001 level. 16:0 = palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 20:4 = arachidonic acid.
Per cent change induced in relative fatty acid composition of serum cholesterol by ethinyl oestradiol (EOe) 20 μg/day (unfilled columns) and by the addition of norethisterone acetate (NET) 10 mg/day (filled columns) in 9 oophorectomized women. Asterisks mark statistically significant changes induced by the addition of NET as compared to EOe induced values. * = 0.05 level, ** = 0.01 level, *** = 0.001 level. 16:0 = palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 20:4 = arachidonic acid.

Per cent change induced in relative fatty acid composition of serum cholesterol ester by oestradiol valerate (Oe2V) 2 mg/day (unfilled columns) and by the addition of norethisterone acetate (NET) 10 mg/day (filled columns) in 9 oophorectomized women. Asterisks mark statistically significant changes induced by the addition of NET as compared to Oe2V induced values. * = 0.05 level, ** = 0.01 level, *** = 0.001 level. 16:0 = palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 20:4 = arachidonic acid.

**Serum cholesterol ester relative fatty acid composition**

(Table 2, Fig. 3a,b)
NET added to either oestrogen decreased (P < 0.001) arachidonic (20:4) acid.

**Discussion**

In the present study, the increase in lecithin and decrease in lysolecithin induced by both oestrogens was reversed (concomitant with a reduction in cephalin) when NET was added. The lecithin-lysolecithin shift in serum appear to be an oestrogenic marker independent of type of oestrogen (Silfverstolpe, in press).

In a previous study in the same oophorectomized women levonorgestrel given alone caused a redistribution in individual phospholipids like the one induced by NET after oestrogen priming i.e. a decrease in lecithin and cephalin concomitant with an increase in lysolecithin (Silfverstolpe et al. 1979). This pattern in individual phospholipids was also induced by the administration of a synthetic androgen methylandrostanediol (Svanborg 1968). Synthetic progestogens which are 17-hydroxyprogesterone derivatives on the other hand do not induce similar changes in individual phospholipid distribution (Svanborg 1968; Silfverstolpe et al. 1979). It is thus tempting to suggest that the capacity of a sex steroid to induce this pattern in individual phospholipids is linked to its androgenic properties since methylandrostanediol and the nortestosterone derivatives NET and levonorgestrel are definitely more androgenic than the 17-hydroxyprogesterones. Although exogenous 17-C-alkylated steroids with androgenic properties induced
this shift in individual phospholipids, this finding does not allow conclusions as to endogenous androgens in this respect.

Progestogens with androgenic properties stimulate post-heparin lipolytic activity (PHLA) (Enholm et al. 1975a). PHLA consists of at least two activities: hepatic lipase activity (HLA) and lipoprotein lipase activity (LPLA). The increase in PHLA has for one steroid with androgen-progestogen properties (oxandrolone) been shown to depend on a selective increase in HLA (Enholm et al. 1975a). HLA (earlier considered a pure triglyceride lipase) has been shown also to have phospholipase A1 activity both with lecithin (phosphatidylcholine) and cephalin (phosphatidylethanolamine) as a substrate (Enholm et al. 1975b). Phospholipase A1 causes a deacylation of the lecithin 1-position fatty acid forming lysolceithin. Increased phospholipase A1 activity would thus decrease lecithin and increase lysolceithin in agreement with the pattern appearing when NET was added to either oestrogen. The lecithin-lysolceithin shift observed after NET administration might then indicate that the in vitro phospholipase A1 activity of HLA also may occur in vivo. Such a phospholipase activity would then also offer a possible explanation for the decrease in cephalin seen after the addition of NET to either oestrogen.

When NET was added to EOe in the present study a further increase in palmitic together with a decrease in stearic acid of serum lecithin was seen. In the 2-position there was an increase in linoleic and a decrease in arachidonic acid. The addition of NET to OeV induced the same shift in serum lecithin fatty acid composition. Serum lecithin is mainly synthesized in the liver and there is a rapid equilibration between liver and serum lecithin (Björnstad & Bremer 1966). The fastest and quantitatively most important pathway (pathway I) by which lecithin is synthesized in the liver preferably gives palmitic acid in 1-position and linoleic or oleic acid in 2-position. The pathway second in quantitatively important (pathway II) gives a lecithin with predominantly stearic acid in 1-position and arachidonic acid in 2-position. The lecithin from pathway II is formed by methylation of cephalin (phosphatidylethanolamine) (Gompertz 1973).

In both oestrogen-progestogen combinations it appears as if the addition of NET induced a stimulation of pathway I for the de novo synthesis of lecithin since the fatty acids characteristic of this pathway were increased concomitant with a decrease in the pathway II fatty acids. However, the lack of correlations between the fatty acids in 1- and 2-positions (for either pathway) together with the fact that lecithins are continuously catabolized or deacylated would indicate that a change in preference for lecithin synthesis pathways is probably only one factor in these sex steroid influences.

In a previous paper we have concluded that the stimulation observed in rodents of lecithin synthesis pathway II after oestrogen administration (Lyman et al. 1968) did not occur in man either on EOe or OeV (SiflVerstolpe et al., in press). The most striking effect in fatty acid composition of serum lecithin induced by exogenous sex steroids is the constant increase in palmitic and decrease in stearic acid whenever a 17-C-alkylated compound is given, regardless of its hormonal characteristics. When the present data are considered together with those of earlier studies in these women we thus find that the alkylated steroids EOe, NET and levonorgestrel induce this shift in the 1-position fatty acids of serum lecithin. When NET is added to EOe these changes are further accentuated. When the non-alkylated OeV or medroxyprogesterone acetate is administered the 1-position fatty acids are not influenced at all. Since it is well established that 17-C-alkylated steroids (regardless of hormonal characteristics) reduce liver excretory capacity (Smith 1974) it is suggested that this shift in palmitic-stearic acids should be considered an expression for liver toxicity or at least for a pharmacologic and not a hormonal effect. This interpretation is further supported by studies in alcoholics in which ethanol loading in amounts known to be toxic for the liver produced the same redistribution in serum lecithin fatty acids as did the 17-C-alkylated steroids (Alling et al. 1979).

As to the 2-position fatty acids of serum lecithin the addition of NET caused an increase in linoleic and a decrease in arachidonic acid. The reduction in arachidonic acid was also seen in cholesterol esters. In serum most of the cholesterol esters are formed by a transacylation whereby an unsaturated fatty acid in 2-position of lecithin is transferred to 3-position of cholesterol. Thus it is suggested that the reduction in cholesterol ester arachidonic acid might be secondary to the concomitant reduction in lecithin arachidonic acid. This selective reduction in arachidonic acid of cholesterol esters was also induced by NET and levonorgestrel when administered alone in previous studies in these women.
The relative increase in arachidonic acid content of serum lecithin induced by EOE and OeV is interpreted, in line with earlier suggestions (Ostwald et al. 1966) to be an oestrogenic hormonal effect. Furthermore, in the present study during NET administration there was an increase in linoleic acid concomitant with the decrease in arachidonic acid. Since arachidonic acid is formed by elongation and desaturation of linoleic acid, it is tempting to suggest that NET (and probably other nortestosterone derivatives with androgenic and/or anti-oestrogenic properties) interferes with the transformation of linoleic to arachidonic acid.

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**References**


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