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Lipid and Carbohydrate Metabolism Studies in Oophorectomized Women: Effects Produced by the Addition of Norethisterone Acetate to Two Estrogen Preparations*

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Summary. Norethisterone acetate (NET) was administered to 11 oophorectomized women, primed with either 17-C-alkylated ethinylestradiol (EE) or the non-alkylated estrogen, estradiol valerate (E_2V), to evaluate the effects on lipid metabolism. Blood samples were drawn after a period without hormonel replacement therapy and after 6 weeks on each estrogen and estrogen-progestogen combination. Serum and lipoprotein lipids were followed and an oral glucose tolerance test was performed with blood glucose and plasma insulin determinations.

NET reversed the increase in serum triglycerides induced by EE and, when added to either estrogen, increased low density lipoproteins and reversed the high density lipoprotein lipid increase induced by both estrogens. The NET + EE, but not the NET + E_2V combination, impaired glucose tolerance.

Key words: Estrogens – Progestogens – Menopause – Oophorectomy – Serum lipoproteins

Introduction

In postmenopausal hormone replacement therapy combined estrogen-progestogen regimens are nowadays frequently used. The metabolic effects of such therapy are only partly known. Disturbance of lipoprotein metabolism is one factor contributing to the early development of coronary heart disease (CHD). A low content of serum high density lipoproteins (HDL) is a risk factor which has attracted particular interest during recent years (Witztum and Schonfeld

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1979). As case control (Mann and Inman 1975) and large-scale prospective studies (Beral 1977) have shown that myocardial infarction may be associated with the use of oral contraceptives interest has been focused on the effects of oral contraceptives and their components on lipid metabolism. In general, oral contraceptives decrease HDL cholesterol (Arntzenius et al. 1978) and several studies (Bradley et al. 1978; Larsson-Cohn et al. 1979) indicate that the progestogen component is responsible for this effect.

Because lipoprotein lipid composition changes during the menstrual cycle, oophorectomized women were recruited to a trial aimed at studying the effects of norethisterone acetate (NET), the component to which 19-nor-testosterone derivatives of the estrane series are metabolized (Fotherby 1974), on women primed in separate periods with the 17-C-alkylated ethinylestradiol (EE) and then the non-alkylated estrogen estradiol valerate (E_2V).

Patients and Methods

Patients

Eleven women treated by radiotherapy and Wertheim's hysterectomy for stage IB or IIA carcinoma of the cervix participated in the study (which had been approved by the local Ethics Committee) after giving their informed consent. Their mean age at the time of operation was 34.5 years (range 27–45 years). None of the patients had any disease other than the cervical carcinoma except for one woman who had hypertension (well controlled on hydralazine and propranolol in constant doses).

Six weeks after Wertheim's hysterectomy, ethinylestradiol (EE) (20 μg/day) was administered and after 6 weeks on EE norethisterone acetate (NET) (10 mg/day) was added for another 6 weeks. From 10–21 months later, and after at least 4 weeks without hormone replacement therapy, estradiol valerate (E_2V) (2 mg/day) was administered for 6 weeks followed by 6 weeks on the combination E_2V (2 mg/day) and NET (10 mg/day).

Physical examination was done and blood samples were taken before treatment for carcinoma, as well as before and after each course of estrogen and estrogen-progestogen therapy.

Serum Lipid and Lipoprotein Analysis

Lipid extraction and triglyceride analysis were performed according to Carlsson (1963), total cholesterol according to the modification of Zlatkis et al. (1953) as described by Crawford (1958), and phospholipids according to Svanborg and Svennerholm (1961). Preparative ultracentrifugation of serum was performed at 4° C and 40,000 *g* for 22 h in one step with a modification of a gradient method described by Walton et al. (1965) using 4 ml of serum. Three fractions were isolated: very low density lipoproteins (VLDL) $d < 1.006$ g/ml, low density lipoproteins (LDL) $d = 1.006–1.063$ g/ml and high density lipoproteins (HDL) $d > 1.063$ g/ml. Within each lipoprotein fraction, triglycerides, total cholesterol and phospholipids were assessed using the methods described above. "Total lipids" in lipoprotein fractions refers to the sum of phospholipids, total cholesterol and triglycerides values within each fraction without correction for the fatty acid content of cholesterol esters.

α-Lipoprotein Cholesterol (α-Lp-CH)

α-Lipoprotein was isolated from whole serum after the precipitation of VLDL and LDL with heparin and manganese chloride (Burstein et al. 1970). The cholesterol content of α-lipoprotein was determined according to the method of Leppänen (1956).

Oral Glucose Tolerance Test (OGTT)
with Concomitant Heparin Plasma Insulin Determinations

Onehundred gram of glucose in 200 ml of water was administered orally. Blood samples were drawn before and 30, 60, 90, and 120 min after the glucose load. The blood glucose concentration was determined by a glucose oxidase method (Levin and Linde 1962) and plasma insulin by the method of Hales and Randle (1963).

Statistical Methods

Standard statistical methods were used to calculate means, standard deviations and standard errors of the mean. Statistical significance was determined using Student's *t*-test.

Conversion to SI Units

Serum and lipoprotein lipids as well as blood glucose values were originally calculated in mg/100 ml. Conversion to mmol/l was done using the following conversion factors: phospholipids f= 0.013, total cholesterol f = 0.026, triglycerides f = 0.012 and blood glucose f = 0.056.

Results

Clinical Observations

Before treatment with either EE or E_2V, eight patients had typical estrogen deficiency symptoms such as hot flushes and perspiration. On replacement therapy with either of the two estrogens seven of these eight women were completely relieved of estrogen deficiency symptoms and the remaining woman stated that her symptoms were improved.

The addition of NET to either estrogen caused recurrence of hot flushes and perspiration in five women. NET added to either of the estrogen preparations induced a modest weight gain but had no effect on blood pressure.

Effects of Treatment for Cervical Carcinoma

Comparison before and after the combined radiological and surgical treatment only revealed an increase ($p < 0.05$) in serum triglycerides.

Pretreatment Levels (Table 1, Fig. 2)

The total VLDL lipids ($p < 0.05$), the VLDL-cholesterol ($p < 0.01$) and the sum of glucose values during OGTT ($p < 0.05$) were lower before the administration of E_2V than before EE.

Table 1. Pretreatment levels of lipoprotein lipids before the administration of ethinylestradiol 20 μg/day (OEE) ($n = 9$) and estradiol valerate 2 mg/day (OE_2V) ($n = 11$). Means ± SEM mmol/l. Statistics based on nine paired observations

	VLDL			LDL			HDL		
	PL	CH	TG	PL	CH	TG	PL	CH	TG
OEE	0.53 ± 0.08	1.13 ± 0.17	1.01 ± 0.16	1.36 ± 0.16	4.45 ± 0.48	0.27 ± 0.03	1.53 ± 0.08	1.80 ± 0.18	0.17 ± 0.02
OE_2V	0.48 ± 0.06	0.87 ± 0.12	0.96 ± 0.16	1.6 ± 0.07	4.91 ± 0.27	0.30 ± 0.02	1.48 ± 0.01	1.75 ± 0.11	0.13 ± 0.01
OEE vs OE_2V	NS	$p < 0.01$	NS	NS	NS	NS	NS	NS	NS

VLDL = very low density lipoproteins, PL = phospholipids, LDL = low density lipoproteins, CH = total cholesterol, HDL = high density lipoproteins, TG = triglycerides, NS = not significant

Table 2. Changes in percent in lipid composition of lipoprotein fractions induced by ethinylestradiol (EE), 20 μg/day and estradiol valerate (E_2V) 2 mg/day, and by the addition of norethisterone acetate (NET), 10 mg/day, to either estrogen in oophorectomized women. Figures in brackets denote numbers of paired observations

	VLDL			LDL			HDL		
	PL	CH	TG	PL	CH	TG	PL	CH	TG
EE vs. O	+28 (8)	+ 8 (8)	+27 (8)*	+30 (8)	− 2 (8)	+70 (8)**	+25 (8)	+ 9 (8)	+48 (8)
E_2V vs. O	− 8 (11)	− 7 (11)*	−13 (11)*	−11	−15 (11)*	−13 (11)*	+16 (11)*	+15 (11)*	+11 (11)
EE+NET vs. EE	−30 (9)*	−18 (9)	−38 (9)**	+13 (9)	+39 (9)**	−16 (9)	−43 (9)***	−37 (9)**	−36 (9)
E_2V+NET vs. E_2V	− 3 (11)	− 6 (11)	−11 (11)	+25 (11)**	+30 (11)**	+27 (11)**	−29 (11)**	−31 (11)**	−21 (11)*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

VLDL = very low density lipoproteins, PL = phospholipids, LDL = low density lipoproteins, CH = total cholesterol, HDL = high density lipoproteins, TG = triglycerides

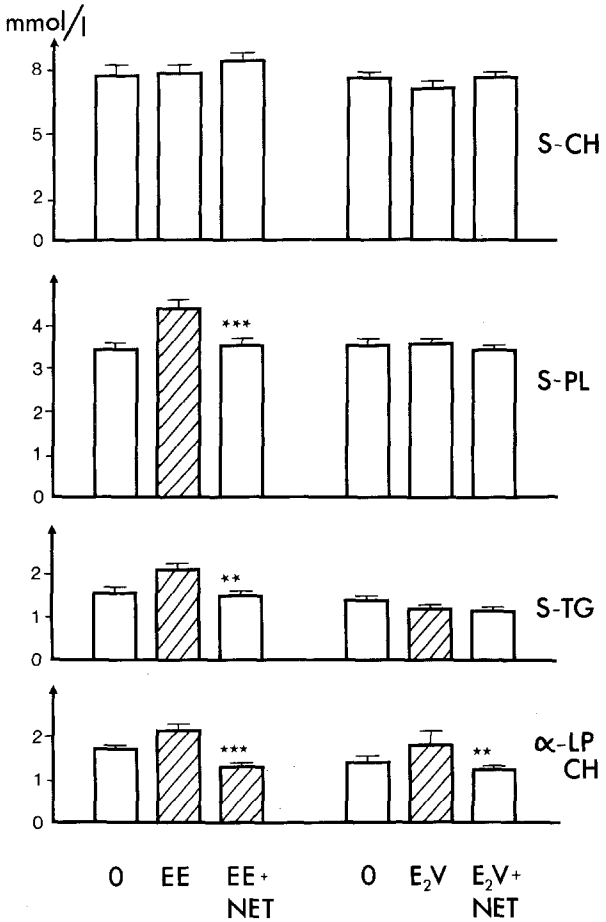

Fig. 1. Changes in serum total cholesterol (S-CH) ($n = 11$), serum phospholipids (S-PL) ($n = 11$), serum triglycerides (S-TG) ($n = 11$) and α-lipoprotein cholesterol (α-Lp-CH) ($n = 8$) induced by the addition of norethisterone acetate (NET), 10 mg/day, to ethinylestradiol (EE), 20 μg/day, and estradiol valerate (E_2V), 2 mg/day, in oophorectomized women. Means ± SEM. *Hatched columns* indicate significant differences compared to pretreatment levels (0) ($p < 0.05$) and *asterisks* indicate changes induced by the addition of NET, as compared to values induced by EE and E_2V, respectively. ** 0.01 level, *** 0.001 level

Effects of the Addition of NET to EE and E_2V

Serum and Lipoprotein Lipids (Fig. 1, Table 2). EE induced an increase in serum and VLDL triglycerides which was reversed by the addition of NET ($p < 0.01$). NET added to either estrogen increased LDL cholesterol ($p < 0.01$) as well as LDL total lipids ($p < 0.05$). NET also decreased HDL cholesterol as well as HDL total lipids ($p < 0.01$) when added to either EE or E_2V. NET administration also reversed the increase in α-Lp-CH induced by both estrogens ($p < 0.001$ when added to EE and $p < 0.01$ when added to E_2V).

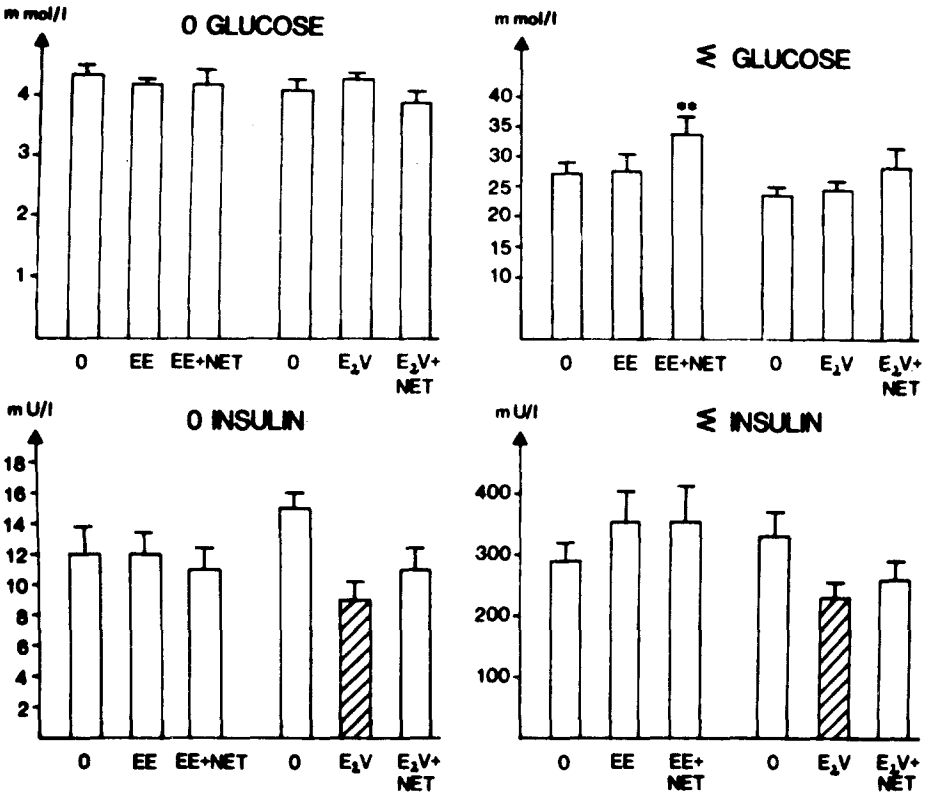

Fig. 2. Changes in blood glucose and plasma insulin induced by ethinylestradiol (EE), 20 μg/day, and estradiol valerate (E_2V), 2 mg/day, and by the addition of norethisterone acetate (NET), 10 mg/day, to either estrogen in oophorectomized women. Means ± SEM. 0 Glucose = fasting blood glucose ($n = 11$), $\lessgtr$ Glucose = sum of glucose values during oral glucose tolerance test (OGTT) ($n = 10$), 0 Insulin = fasting plasma insulin ($n = 11$), $\lessgtr$ Insulin = sum of insulin values during OGTT ($n = 11$). *Hatched columns* indicate significant changes induced by E_2V ($p < 0.01$) and *asterisks* (** $p < 0.01$) indicate changes induced by the addition of NET

Blood Glucose and Plasma Insulin (Fig. 2). E_2V decreased ($p < 0.01$) fasting plasma insulin and the sum of insulin values during OGTT, while NET added to EE increased ($p < 0.01$) the sum of glucose values.

Discussion

The only significant effect of the combined radiotherapy and surgery was a moderate increase in serum triglycerides without any concomitant change in carbohydrate metabolism. In the comparison between pretreatment levels the only differences noted were lower VLDL lipid and sum of glucose values (during OGTT) prior to E_2V administration. This has been interpreted as an acceptable stability in lipid and carbohydrate variables, postoperatively and between consecutive investigation periods.

Impaired glucose tolerance was observed when NET was added to EE but not when it was added to E_2V. Impaired glucose tolerance was also seen in another study in these women when NET was administered alone (Silfverstolpe et al. 1979). These findings are consistent with earlier proposals (Beck 1977) that alkylated EE accentuates the glucose impairment induced by nortestosterone derivatives like NET.

There is general agreement that ethinyl estrogens increase serum triglycerides by raising the VLDL triglyceride production rate (Glueck and Fallat 1975). In this study NET reversed the increase in serum and VLDL triglycerides induced by EE, while it did not accentuate the reduction caused by E_2V. These data on the varying influence of NET on serum and VLDL triglycerides may be compared with earlier findings indicating that NET reduces serum triglycerides more markedly in patients with hypertriglyceridemia than in normolipidemic subjects (Glueck et al. 1971). The reduction in VLDL triglycerides induced by NET may be due to an increased VLDL catabolism. Oxandrolone, another steroid with androgenic properties, has been shown to increase the VLDL turnover rate (Glueck et al. 1973). An increased VLDL catabolism would cause an enhanced transfer to LDL and thereby increased LDL lipids, as seen when NET was added to either estrogen.

EE and E_2V increased α-Lp-CH and E_2V HDL total lipids as well. In general, estrogens increase HDL lipids. This has been shown both with alkylated estrogens (Gustafson and Svanborg 1972; Wallentin and Larsson-Cohn 1977) and with the non-alkylated E_2V (Tikkanen et al. 1979). NET added to either estrogen decreased α-Lp-CH and HDL total lipids. Findings from earlier studies in these women (Silfverstolpe et al. 1979) suggest that the HDL-lowering effect of a synthetic progestogen might be linked to its androgenic properties. Synthetic androgens have also been shown to decrease HDL (Solyom 1971). In one recent study HDL cholesterol has been positively correlated to endogenous androgen levels (Nordøy 1979). Thus, the HDL-reducing effect of synthetic steroids with androgenic properties might be a drug effect rather than a physiological hormonal influence. The HDL reduction might be induced by an increased removal due to enhanced hepatic lipase activity, as recently suggested (Nikkillä et al. 1980).

An antagonistic influence between ethinyl estrogens and synthetic progestogens on various aspects of lipid metabolism has been suggested (Brody et al. 1968; Larsson-Cohn et al. 1970; Gustafson and Svanborg 1972). To our knowledge however, there are few studies in which progestogens have been administered separately to estrogen-primed women. In one recent study in young fertile women, however, synthetic progestogens prevented the increase in VLDL and HDL lipids induced by EE (Goldzieher et al. 1978), in agreement with the present findings in oophorectomized women. It appears therefore that when ethinyl estrogens and 19-nor-testosterone derivatives are combined, as in all currently available oral contraceptives, the two components tend to oppose each other in their effects on serum lipoprotein content and distribution. This may explain the varying effects of oral contraceptives with different ratios between EE and levonorgestrel on lipoprotein lipids (Larsson-Cohn et al. 1979).

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