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Oral estradiol-17β raises the level of plasma high-density lipoprotein in menopausal women by slowing down its clearance rate

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Abstract. Plasma lipoprotein composition, plasma kinetics of autologous [^{125}I]HDL and the metabolism of iv administered radioactively labelled artificial chylomicrons were studied in postmenopausal women during a control period and after 4 months of oral estradiol-17β treatment (1 mg/m^2 body surface per day). Drug treatment significantly raised plasma HDL-cholesterol levels (19%) in the fasting state and total apolipoprotein A-I (16%), but did not interfere with triglyceride, VLDL, LDL or apolipoprotein-B values. As compared with the control period, estradiol-17β administration significantly slowed down plasma [^{125}I]HDL clearance by about 82% and reduced the delipidation index of the injected artificial chylomicrons by 47% as a consequence of impaired plasma lipolytic activity.

HDL-cholesterol is a powerful predictor of coronary heart disease (CHD) in middle-aged men as well as in elderly women. As shown in the Lipid Research Clinics Follow-up Study (1), estrogen therapy protects postmenopausal women against CHD seemingly by raising the HDL-cholesterol level.

The magnitude of the effects of estrogens on plasma lipoproteins may vary according to the type and dose of the estrogen derivative utilized, but in general, estrogens markedly increase total plasma HDL, particularly the HDL-2 fraction, with little effect on the other lipoprotein fractions (reviewed in 2,3). However, the mechanisms of action of these hormones on plasma lipoprotein are not fully understood. For instance, the increase in total HDL-2 levels after ethynyl-estradiol treatment has been attributed to a simultaneous lowering of the post-heparin hepatic lipase activity and to a faster apolipoprotein A-I synthesis rate (4), or to a slower HDL catabolism rate (5), although the latter was demonstrated in only one subject.

In the present report, the plasma clearance rates of autologous total HDL and of synthetic chylomicron remnant-like particles were investigated in postmenopausal women prior to and after administration of the natural estrogen estradiol-17β.

Patients and Methods

Estradiol-17β (Estrace®, Bristol-Myers) was administered orally (1 mg/m^2 body surface) to 19 postmenopausal non-smoking, healthy women for 4 months, as a single daily dose. Hot flashes were the sole indication for the drug. All subjects were maintained on their regular diets; their liver and renal function tests were within the normal range throughout the study. Four of them were black, 1 oriental and 14 white; their mean ±SD age was 50±4.8 years, and they were studied 15-23 months after cessation of menses (18±3.5); mean body mass index was 23.83±1.23 kg/m^2.

Patient compliance was checked by radioimmunoassay plasma hormonal measurements (LH, FSH and estradiol-17β) performed at the same time as the lipid analyses.

Blood was drawn after a 12-h fasting period into 0.1 ml 8% EDTA, 5% sodium azide and 0.1% chloramphenicol. The protocol was approved by the Hospital Ethical Committee and participants signed a formal written consent.

Lipid and lipoprotein analysis

Plasma lipid values were measured by enzymatic procedures: cholesterol measured by the Chod-Pap method (Boehringer Mannheim, Merck SA, R. J., Brazil), triglycerides by the Enz-color method (Bio-Diagnostica, SP, Brazil), and plasma lipoprotein fractions (VLDL and LDL) were indirectly estimated using the Friedewald formula (6) after precipitation with dextran sulphate and magnesium chloride (Wiener Lab, Argentina); HDL-cholesterol was measured in the supernatant.

Total plasma apolipoprotein B (apo B) and A-I (apo A-I) were measured by radial immunodiffusion on agarose gel plates (Daiichi Pure Chemicals, Tokio, Japan).

HDL kinetic study

HDL was isolated from plasma in three sequential ultracentrifugations using a 50 Ti rotor in a L7-55 ultracentrifuge (Beckman Instruments, Palo Alto, California): after separating HDL from the lighter lipoprotein, the HDL fraction was re-spun twice in order to eliminate any albumin contaminant (7). Bulk HDL protein was then labelled with ^{125}I by the iodogen method (Pierce Chemical Co, Illinois) (8). After extensive dialysis and filtration through a Millipore filter (0.22 μm), 50-70 μCi of [^{125}I]HDL were pulse infused iv into each subject prior to (control period) and after 4 months of estradiol-17β treatment. Potassium iodide solution was administered orally on the day preceding and throughout the 9 days of the blood radioactivity analysis. The initial blood sample collected 1 h after isotope infusion represents the 100% radioactivity value in plasma (cpm/ml). Plasma aliquots were extensively dialysed against saline and radioactivity counts were obtained with an Abbott gamma counter. The residence time of [^{125}I]HDL in plasma was calculated as the area under the plasma radioactivity decay curve (cpm/ml) (9). Statistical differences between the control and treatment periods were compared by the Student's paired t-test (significance levels stated in tables and figures).

Artificial chylomicron remnant kinetic study

An artificial chylomicron remnant-like lipid emulsion was prepared according to previously described methods (10). The emulsion contained triolein (78.9%), L-α-phosphatidylcholine (11.0%), cholesteryl-oleate (8.7%), and free cholesterol (2%), (Nu-check Prep, Elysian, MN), together with glyceryl-tri-[9,10(n)-^{3}H]-oleate ([^{3}H]TG) and cholesteryl-[1-^{14}C]-oleate ([^{14}C]CE) (Amersham International, Buckinghamshire, UK). The pyrogen-free emulsion was filtered through Millipore disks (0.22 μm) and rapidly infused iv. Thereafter blood samples were drawn at regular intervals during 30 min into EDTA-sodium azide-containing tubes. ^{3}H and ^{14}C radioactivity was measured in the triglyceride and cholesteryl-ester bands eluted from silica gel thin-layer chromatography after extraction with Folch solution (chloroform 2: math and 1 v/v). Data were plotted (cpm/ml of plasma) as per cent of the initial value and the residence time (RT) was calculated as mentioned above for [^{125}I]HDL. The residence time of [^{3}H]TG measures two simultaneous processes, i.e. the rates of chylomicron particle removal and the shedding-off of particle triglycerides as fatty acids owing to the action of the enzyme lipoprotein lipase. The residence time of [^{14}C]CE determines splanchnic organ particle uptake rate because [^{14}C]CE is not appreciably exchanged with other plasma lipoproteins during the short course of the experiment. Therefore, the fraction of chylomicron triglycerides that leaves the particles in the form of fatty acids can be estimated as a delipidation index:

DI = 100 (1-RT[^{3}H]TG/RT[^{14}C]CE) (11).

Results

Plasma hormonal measurements by RIA differed significantly between the control and treatment periods, with respective mean (±SD) values of 170±37 and 94±36 IU/l for FSH, 71±24 and 47±18 IU/l for LH, and less than 20 and 315±198 ng/l for estradiol-17β (all differences significant at $p<0.05$).

Plasma lipids, lipoprotein fractions determined by the Friedewald formula, and apolipoprotein are presented in Table 1. Estradiol-17β treatment significantly raised plasma HDL cholesterol concentration by 19% and apo A-I by 16%, a result compatible with an increased number of HDL particles. Other plasma lipids and apo B levels were not significantly modified by the hormone.

Table 1.
Postmenopausal plasma lipids, lipoprotein fractions (N=19) and apolipoproteins (N=8) during a control period and after 4 months of estradiol-17β treatment.

	Control	Treatment
	mmol/l	
Cholesterol	5.40±1.35	5.32±1.30
Triglycerides	1.41±0.51	1.42±0.64
VLDL-cholesterol	0.65±0.25	0.65±0.30
LDL-cholesterol	3.26±1.18	3.12±1.23
HDL-cholesterol	1.48±0.57	1.78±0.65[a]
	g/l	
apo B	1.22±0.26	0.74±0.20
apo A-I	1.22±0.26	1.42±0.60[a]

Results are expressed as mean ± SD.
a: significantly different from control at $p<0.001$

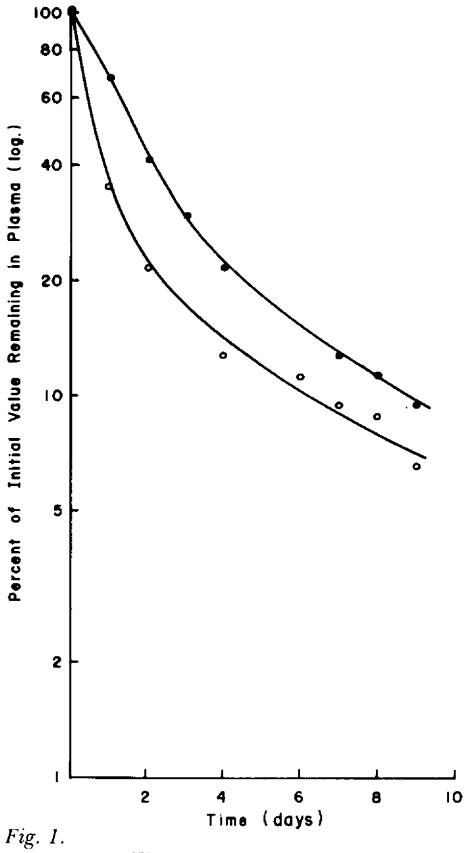

Fig. 1.
Autologous [^{125}I]HDL was pulse infused iv once in each subject (N=7) during the control period (○-○) and after 4 months of oral treatment with estradiol-17β (●-●). The 100% value on the ordinate (cpm/ml) corresponds to the blood sample drawn 1 h after infusion. Data are geometric means of all plasma values expressed as per cent of the initial result.

Fig. 1 represents the geometric means of the pooled plasma [^{125}I]HDL data expressed as cpm/ml in 7 women prior to and after the 4-month period of estradiol-17β treatment. [^{125}I]HDL residence time, expressed as mean days ± SD, was 1.72±0.55 in the control period as compared with 3.13±0.99 after estradiol-17β (a significant difference at $0.025<p<0.05$). The 82% longer [^{125}I]HDL plasma residence time in response to the drug clearly indicates that total HDL clearance slowed down considerably. Estrogens in general are known to reduce the activity of hepatic lipase while not interfering with that of lipoprotein lipase (12), although it is well known that estrogens considerably worsen primary hypertriglyceridemia (4). Synthetic estrogens bring about a rise in plasma TG levels which has been attributed to stimulation of hepatic VLDL production (4). However, natural estrogens have been reported either to leave plasma TG values unchanged (2), or increased (13). In the present report, we investigated whether the chylomicron remnant-like particle metabolism might be influenced by hormone administration considering that: 1. hepatic lipase directly metabolizes HDL components (14), 2. plasma HDL level appears to be inversely correlated with hepatic lipase activity, and 3. both lipase species (hepatic and lipoprotein lipase) metabolize triglyceride-rich particles, namely, chylomicrons and VLDL, but hepatic lipase is particularly effective in the metabo-

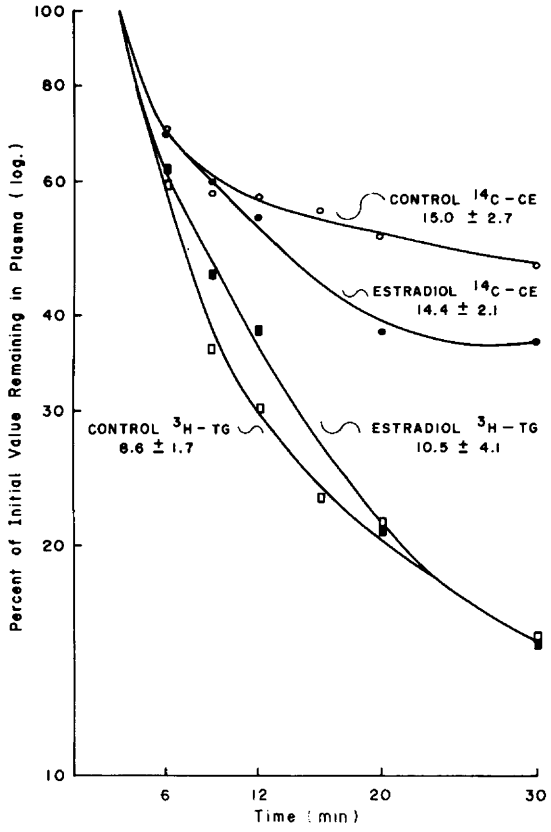

Fig. 2.
Artificial chylomicron remnant-like particles doubly labelled with glycerol-tri-[9,10(n)-^{3}H]-oleate ([^{3}H]TG) and cholesteryl-[1-^{14}C]-oleate ([^{14}C]CE) were pulse infused iv once during the control period (○, □) and after 4 months of oral treatment with estradiol-17β (●; ■). The initial 3-min blood sample expresses the 100% value (cpm/ml of plasma) on the ordinate. Data are geometric means of plasma residence time values.

lism of intermediate-density lipoprotein (IDL) and HDL (15).

For this purpose, we utilized an artificial emulsion that simulates the metabolism of natural chylomicrons (10). This emulsion made is richer in total cholesterol than the natural lymph chylomicrons, its composition being closer to that of a chylomicron remnant, i.e. resembling the chylomicron immediately after it enters the plasma, having lost some of its triglyceride content, and having gained all plasma apolipoproteins except apo B (10). When the delipidation index of this artificially labelled particle is measured, the activity of the lipoprotein-lipase enzymes is evaluated under physiological conditions, as opposed to the indirect in vitro measurement made by iv heparin infusion.

As shown in Fig. 2, estrogen therapy significantly reduced the chylomicron delipidation index of 5 women by about 47%, with mean delipidation index values of 28.8 ± 19.5 as opposed to $42.2 \pm 13.8\%$ for the controls (differences significant at $p<0.025$).

Discussion

Schaefer et al. (4) showed that ethynyl-estradiol does not slow down HDL residence time, but increases plasma apo A-I concentration in the HDL fraction, which must be due to an increased rate of apo HDL synthesis. In contrast, in one case reported by Hazzard et al. (5) ethynyl-estradiol selectively prolonged the residence time of apo A-I and moderately depressed its rate of synthesis.

In the present study, estradiol-17β treatment elicited a much higher [^{125}I]HDL residence time (82%) in relation to the modest increase in HDL level either as HDL-cholesterol (19%) or as apo A-I (16%). Elevation of the latter value is known to correlate with a decreased apo A-I fractional catabolic rate in women (16), but does not fully explain the mechanisms involved in the hormonal action. Thus, it is likely that structural differences in the estrogen molecule cause different metabolic activities, i.e. ethynyl estradiol induces a higher rate of apo A-I synthesis, whereas estradiol-17β may simultaneously slow down both apo A-I removal and synthesis rates. In fact, alterations in the plasma lipoprotein profile vary according to the estrogen drug utilized. For instance, estradiol-17β has recently been claimed to raise total cholesterol, HDL and triglycerides without influencing the LDL-cholesterol level, whereas estrone sulphate lowers total cholesterol and LDL-cholesterol, raises the HDL level and does not interfere with the triglyceride concentration (13). Also, the response to the hormones are dose-related (13).

Hormonal treatment caused a lower delipidation index as that determined in the fasting state. This change bore no relationship to the fasting plasma triglyceride concentration since the latter did not increase in the present study, although it was reported to rise in another investigation (13). It is known that hepatic lipase preferentially hydrolyses HDL lipids, whereas lipoprotein lipase predominantly hydrolyses chylomicrons and VLDL-triglycerides (16). Clinical and experimental studies have shown that decreased lipoprotein lipase activity causes a rise in plasma triglycerides and a fall in HDL concentration, whereas impaired hepatic lipase activity simultaneously increases plasma triglycerides (as represented by VLDL-TG) and HDL levels (17,18). In this regard, a high HDL concentration and a dimished lipolysis rate in plasma after estradiol-17β are compatible with a preferential impairment of hepatic lipase which, although not capable of influencing the plasma triglyceride level as measured in the fasting state, did elicit a defect in the lipolysis of large particles as disclosed by the more sensitive chylomicron infusion test. This lipolysis deficiency may also be detected by measuring plasma triglyceride concentrations when an oral fat tolerance test is performed. In the present study it was not possible to determine to which enzyme (hepatic or lipoprotein lipase) this mild lipolysis deficiency could be attributed. However, hepatic lipase deficiency is the most likely candidate since clinical conditions of lipoprotein lipase deficiency are known markedly to raise plasma triglyceride concentration.

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