Very low density lipoprotein triglyceride kinetics during hepatic lipase suppression by estrogen

Studies on the physiological role of hepatic endothelial lipase

M.J. Tikkanen, T. Kuusi, E.A. Nikkilä and T. Sane

Third Department of Medicine, Central University Hospital, University of Helsinki, 00290 Helsinki, Finland

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The exact role of the heparin-releasable hepatic endothelial lipase has remained controversial. It has been suggested that it acts in concert with lipoprotein lipase in the step-wise delipidation of triglyceride-rich lipoproteins. On the other hand, there is evidence indicating that high density lipoprotein$_2$ is the preferred substrate for hepatic lipase. Here, it is shown that a moderate (27%) suppression of hepatic lipase activity by estrogen did not impair removal of ^{3}H-labeled very low density lipoproteins (VLDL) triglycerides, suggesting that this enzyme is not a major regulator of VLDL catabolism under physiological circumstances.

Lipoprotein *Hepatic lipase* *Lipoprotein lipase* *Very low density lipoprotein* *Estradiol valerate*

1. INTRODUCTION

Hepatic endothelial lipase (also called hepatic triglyceride lipase) is a lipolytic enzyme located in the endothelial cells of liver sinusoids [1]. It is released into the circulation by intravenous injection of heparin and its activity can be quantitated in postheparin plasma. It is generally accepted that lipoprotein lipase (LPL) is the major regulator of the step-wise catabolism of chylomicrons and very low density lipoproteins (VLDL), but the metabolic role of hepatic lipase has remained controversial. Recently, several investigators demonstrated an impaired removal of triglyceride(TG)-rich lipoproteins from the circulation of monkeys [2] and rats [3,4] by blocking hepatic lipase (HL) activity with specific anti-HL antibodies. They suggested that hepatic lipase has a physiological role in regulating the conversion of VLDL and intermediate density lipoproteins (IDL) to low density lipoproteins (LDL) [2–4]. On the other hand, evidence has been presented for a major role of hepatic lipase in the regulation of plasma high density lipoprotein$_2$ (HDL_2), and it has been suggested that sex hormone effects on HDL_2 are mediated by this enzyme [5].

Hepatic lipase activity is suppressed by exogenous estradiol [5] and its activity even follows fluctuations in endogenous estradiol [6]. In order to study the suggested regulatory role of hepatic lipase in VLDL-TG catabolism we suppressed this enzyme by estrogen administration in six postmenopausal women and investigated the kinetics of VLDL triglycerides labelled endogenously by injection of [^{3}H]glycerol. The removal rate of VLDL triglycerides from plasma was not reduced, suggesting that results obtained in previous animal studies are not necessarily applicable to physiological situations in man.

2. MATERIALS AND METHODS

2.1. *Subjects*

Six postmenopausal women aged 50–54 years volunteered for the study. Except for vasomotor climacteric complaints they were apparently healthy. Subject 1 had an elevated serum cholesterol level (8.48 mmol/l), whereas the others were

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normocholesterolemic (range: 4.74–5.80 mmol/l). All women had normal serum triglyceride levels (range: 0.69–1.70 mmol/l). Their relative body weights ranged between 102 and 158% (mean ± SD: 128.7 ± 21.7%). None of the women was on any medication, nor had they received hormonal or other treatment known to influence lipid metabolism during the three months preceding the initiation of estrogen.

2.2. *Estrogen treatment*

The VLDL triglyceride turnover studies as well as all laboratory examinations were carried out in the metabolic ward before starting treatment and after three months of oral treatment with estradiol valerate (Progynova®, Schering AG, obtained from Leiras Ltd, Turku, Finland), 2 mg daily. During treatment, all subjects were advised to continue their customary way of life including diet, drinking habits and exercise. Two subjects nevertheless lost weight (3.3 and 4.0 kg, see table 2).

2.3. *Determination of serum and VLDL lipids*

The VLDL fraction was isolated in a Sorvall OTD-2 ultracentrifuge at solvent density 1.006 g/ml (18 h) [7]. Cholesterol concentrations were determined according to the method of Röschlau et al. [8] using a commercial kit (no.187313, Boehringer, Mannheim) and triglyceride concentrations were analyzed in a Technicon Autoanalyzer II (Technicon Instruments, Tarrytown, NY, USA) [9].

2.4. *Determination of VLDL triglyceride turnover*

Turnover studies were carried out using endogenous labelling of VLDL triglycerides with [^{3}H]glycerol [10,11]. After i.v. injection of 150 μCi of [2-^{3}H]glycerol (The Radiochemical Centre, Amersham, England, spec. act. 200 mCi/mmol) specific activities of VLDL-TG were followed for 10 h. Fractional rate constants were calculated from the monoexponential part of the decay curves. Turnover (production) rates (TR) μmol/h expressed per kg of ideal body weight were calculated as the product of fractional catabolic rate (FCR) and estimated plasma VLDL-TG mass.

2.5. *Determination of postheparin plasma lipase activities*

Blood was drawn 5 and 15 min after an i.v. injection of 100 IU (1 mg) of sodium heparin (Leiras, Turku, Finland) per kg body weight. The postheparin plasma LPL and HL activities were measured by a specific immunochemical method [12]. The validity of the method has been discussed elsewhere [13,14].

3. RESULTS

3.1. *Changes in VLDL-TG kinetic parameters and lipase activities*

The effect of the estradiol valerate regimen on triglyceride concentrations and postheparin plasma lipase activities is shown in table 1. There were no significant changes in the average serum or VLDL-TG concentrations or LPL activity, but the mean HL activity was reduced from 14.0 to 10.3 $\mu mol \cdot h^{-1} \cdot ml^{-1}$ (−27%, $p < 0.01$). Individual changes in HL activity are illustrated in fig.1 together with the corresponding fractional

Table 1

Effect of estradiol valerate (E_2V, 2 mg/day) on triglyceride concentrations and lipase activities in postheparin plasma in six postmenopausal women

	Triglycerides (mmol/l)		PH-plasma lipase activity (μmol/h per ml)	
	Serum	VLDL	LPL	HL
Pretreatment	1.12 ± 0.39	0.69 ± 0.28	27.3 ± 4.2	14.0 ± 4.8
3 months on E_2V	1.32 ± 0.26	0.87 ± 0.50	28.0 ± 6.2	10.3 ± 4.3
	NS	NS	NS	$p < 0.01$

NS, not significant

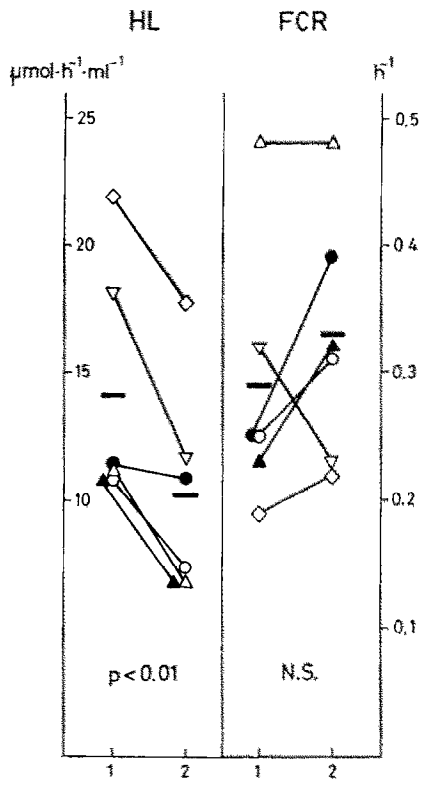

Fig.1. Changes in postheparin plasma hepatic lipase (HL) activities and fractional catabolic rates (FCR) of VLDL triglycerides in six postmenopausal women before (1) and after (2) three months treatment with estradiol valerate, 2 mg/day. (▲) Subject 1; (●) subject 2; (△) subject 3; (○) subject 4; (◇) subject 5; (▽) subject 6.

catabolic rate (FCR) values (see below) showing a decrease in enzyme activity (ranging from −4 to −38%) in each subject.

The individual changes in VLDL-TG concentrations and kinetic parameters are shown in table 2. VLDL-TG concentration and turnover (production) rate (TR) increased during estrogen treatment in all subjects except in subject 3 who had a reduction in both VLDL-TG concentration and TR. Significant (>2%) weight reductions occurred in two subjects, the greatest being a 4 kg reduction in subject 3 (table 2). The average fractional catabolic rate (FCR) did not change significantly, but there was some individual variation; four subjects exhibited an increase ranging from 16 to 56%, one (no.4) had no change, and one (no.6) showed a

Table 2

Changes in VLDL triglyceride concentrations, turnover rates and fractional catabolic rates (FCR) in six women after 3 months estrogen treatment (estradiol valerate, 2 mg/day)

	VLDL triglycerides						Weight (kg)	
	Concentration (mmol/l)		Turnover rate (μmol/kg IBW/h)		FCR (h^{-1})			
	Init.	Change	Init.	Change	Init.	Change	Init.	Change
1.	0.52	+0.18	5.82	+3.12	0.23	+0.09	74.6	−3.3
2.	0.46	+0.11	5.19	+4.93	0.25	+0.14	53.1	+0.7
3.	0.97	−0.40	11.50	−3.11	0.25	+0.06	74.4	−4.0
4.	0.35	+0.17	7.62	+3.72	0.48	+0.00	56.0	+0.5
5.	0.99	+0.03	9.89	+1.62	0.19	+0.03	85.5	+0.0
6.	0.86	+0.95	13.40	+6.80	0.32	−0.09	69.7	−1.2
$\bar{x}$	0.69	+0.17	8.90	+2.85	0.29	+0.07	68.9	−1.2

$r = 0.88, p < 0.05$

$\bar{x}$ = mean value

28% decrease. The same subject (no.6) also increased her VLDL-TG concentration by 110% due to development of VLDL-TG overproduction (20.2 μmol/kg per h) during estrogen therapy (fig.1; table 2).

3.2. *Correlations*

The changes in VLDL-TG concentrations correlated positively with those in turnover rates ($r = 0.88$, $p < 0.05$; table 2), but not with the changes in LPL or HL activity. The results were essentially similar when calculations were repeated using TR values expressed as mmol/h per kg body weight, or mmol/h. No significant correlations existed between FCR and postheparin plasma LPL or HL activities.

4. DISCUSSION

Some previous studies have suggested that hepatic lipase acts in concert with lipoprotein lipase in the catabolism of TG-rich lipoproteins along the VLDL ⟶ IDL ⟶ LDL pathway [2–4]. Stimulated by these reports we set out to test this hypothesis by investigating the turnover kinetics of VLDL-TG in the presence and absence of estrogen-induced suppression of hepatic lipase. The results indicate that suppression of HL activity in postheparin plasma was not accompanied by impaired clearance of VLDL-TG from plasma (in terms of a diminished FCR). Hence, the increments in VLDL-TG (table 2) were most likely caused by augmentation of VLDL-TG turnover (production) rates, as suggested by the positive correlation between the changes in VLDL-TG concentrations and turnover rates.

In calculating FCR we used single-exponential analysis of the 8-h decay curve. Thus, the late 'tail' of the decay curve caused by the slowly turning-over compartment postulated by Zech et al. [15] was not analyzed. This should not have altered our results significantly since values obtained by extended (multicompartmental) and by single-exponential analysis correlated well in normotriglyceridemic subjects [16]. The weight reduction in subjects 1 and 3 may have decreased the synthesis and concentrations of VLDL-TG in these two subjects but this should not affect FCR values [17,18].

The current results are in fact in accordance with many previous studies, indicating that the clearance of exogenous or endogenous fat was not impaired by administration of estrogen containing drugs [19–23]. On the other hand, under a variety of disease or experimental conditions suppression of HL activity has resulted in the accumulation of TG-rich lipoproteins in animals and man. Using a nonhuman primate model, Goldberg et al. [2] achieved complete inhibition of circulating HL activity and demonstrated that accumulation of VLDL, IDL and lesser density LDL in plasma occurred in association with an impaired conversion of radiolabeled VLDL to IDL and LDL. In some studies in the rat analogous results have been obtained [3,4]. Familial hepatic lipase deficiency is also characterized by accumulation of TG-rich lipoprotein fractions in plasma [24].

It is possible to reconcile these seemingly contradictory results. The present study shows that moderate reductions in HL activity resembling those brought about by cyclic fluctuations in estrogen production in normal females [6] do not impair the removal efficiency of TG in the VLDL density class. This does not rule out the possibility that a certain 'threshold' level of HL activity is required for the completion of the removal process. Thus, it is possible that hepatic lipase, although not a rate-limiting enzyme or even a major regulator in the catabolism of VLDL, is nevertheless necessary for the functioning of the VLDL ⟶ IDL ⟶ LDL pathway. Such a 'permissive' role of hepatic lipase would be in accordance with the findings that total or near-total inhibition of HL activity caused disturbances in the catabolism of TG-rich lipoproteins [2–4,24], whereas moderate changes in the physiological range did not. It is not clear whether this postulated 'permissive' effect of hepatic lipase is a direct effect on TG-rich lipoproteins, or an indirect effect caused by an impaired action on HDLZ.

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