Increased hepatic lipase activity and increased direct removal of very-low-density lipoprotein remnants in Watanabe heritable hyperlipidaemic (WHHL) rabbits treated with ethinyl oestradiol

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We studied the effects of ethinyl oestradiol on the serum concentrations and metabolism of very-low- and low-density lipoproteins (VLDL and LDL) in Watanabe heritable hyperlipidaemic (WHHL) homozygous rabbits, an animal model for familial hypercholesterolaemia. The results were compared with those in untreated homozygotes as well as in heterozygotes treated or not with ethinyl oestradiol. The gain in body weight was similar in all groups. Treatment with ethinyl oestradiol resulted in the homozygotes in an approx. 80 % decrease in the concentrations of lipids and apoprotein B in the $d < 1.019$ lipoprotein fraction; those in the LDL fraction did not change. In the heterozygotes, basal serum lipids and apoprotein B levels in the $d < 1.019$ fraction were low; ethinyl oestradiol treatment especially affected the LDL fraction (cholesterol –84 %, apoprotein B –64 %). Turnover experiments with ^{125}I -labelled VLDL revealed that, on treatment with ethinyl oestradiol, the fractional catabolic rate in homozygous rabbits increased 2-fold. The secretion rates of lipids and protein in the $d < 1.019$ fraction as estimated after injection of Triton WR-1339 was not decreased. In homozygotes and heterozygotes increases in post-heparin hepatic lipase activity of 62 and 80 % respectively were observed, with no changes in lipoprotein lipase activity. We conclude that ethinyl oestradiol induces in homozygous WHHL rabbits a direct removal of VLDL and VLDL remnants from the plasma, apparently due to an increase in hepatic lipase activity.

INTRODUCTION

The homozygous Watanabe heritable hyperlipidaemic (WHHL) rabbit lacks functional hepatic receptors for low-density lipoprotein (LDL) apoprotein B [1]. Consequently, the clearance of LDL in these rabbits is grossly impaired and the conversion of very-low-density lipoproteins (VLDL) to LDL is enhanced. Both factors contribute to the elevation of LDL levels. [1].

Ethinyl oestradiol has been shown to affect lipoprotein lipase and hepatic lipase activities [2–5], to modify VLDL metabolism [6] and to increase the levels of the LDL receptors [7–10]. By studying the effects of ethinyl oestradiol in LDL-receptor-defective rabbits, more insight can be gained about the role of lipoprotein lipase and hepatic lipase in lipoprotein metabolism. In the present study we treated WHHL rabbits with ethinyl oestradiol. It was found that, in contrast with the results in the literature [8], the drug influences mainly the concentrations of VLDL and intermediate-density lipoproteins (IDL) instead of that of LDL. The main purpose of the present study was therefore to elucidate the mechanism of action of ethinyl oestradiol.

MATERIALS AND METHODS

Preparation and implantation of the hormone depots

A piece of silastic tubing (length 5 cm, outer diameter 0.077 cm, Corning cat. no. 602-235) was filled under sterile conditions with 16 or 27 mg of ethinyl oestradiol (Sigma cat. no. E 4876) and was sealed at both ends. Control implants were prepared in the same way, but were empty. The hormone depots were immersed for

30 s in ethanol and were subsequently implanted subcutaneously into rabbits under general anaesthesia.

Experimental protocol

In the first part of the study, the effects of hormone release from the implants containing 16 or 27 mg of ethinyl oestradiol on serum lipids were studied in two female 3-year-old WHHL homozygous rabbits for 36 days. Blood samples were taken from fasting animals (20 h after last food supply) from ear veins after 4, 11, 19 and 36 days, and concentrations of serum lipids and of ethinyl oestradiol were measured.

In the second part of the study, one group of eight female homozygous WHHL and one group of eight female heterozygous WHHL rabbits, 4–5 months old, were studied. Half of the animals in both groups were given an implant containing 16 mg of ethinyl oestradiol and the remaining animals, matched for age and serum lipid concentrations, were given control implants. Blood was taken from fasted rabbits after 7, 14 and 28 days to determine the effect of the implants on serum lipids and on the activities of post-heparin lipoprotein lipase and hepatic lipase. At the end of the study a VLDL turnover study was performed.

In the third part of the study, the production rate of VLDL was measured in five control and five drug-treated WHHL homozygote rabbits after fasting for 20 h (28 days after implantation).

VLDL isolation and radiolabelling

Two homozygous WHHL rabbits, fasted for 16 h, were bled to deliver 75 ml of serum from which VLDL were isolated by

Abbreviations used: WHHL rabbits, Watanabe heritable hyperlipidaemic rabbits; VLDL, very-low-density (or pre- β) lipoproteins ($d < 1.006$); IDL, intermediate-density lipoproteins ($1.006 < d < 1.019$); LDL, low-density (or β) lipoproteins ($1.019 < d < 1.063$); VLDL remnants (β -VLDL), conversion products of VLDL with a density around 1.006 g/ml and maximally 1.025 g/ml, thought to be precursors for LDL; apo B, apoprotein B; FCR, fractional catabolic rate.

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centrifugation at 168 000 *g* for 18 h at 14 °C (density 1.006 g/ml) [11]. The VLDL were washed once using the same ultracentrifugation conditions, followed by dialysis overnight at 4 °C against 5 litres of dialysis buffer [0.15 M-NaCl, EDTA (0.5 mg/ml) and gentamycin (5 mg/l), pH 7.5]. The VLDL protein was radiolabelled with ¹²⁵I by a modification [12] of the method of McFarlane [13].

Unreacted iodine was removed by column chromatography on Sephadex G-25 M/PD-10 columns (Pharmacia) followed by dialysis overnight against 5 litres of dialysis buffer at 4 °C. The efficiency of labelling was 45%; more than 98% of the radioactivity was precipitated by 10% (w/v) trichloroacetic acid; 83.3% of the radioactivity was precipitated by 50% (v/v) propan-2-ol [14] and 10.6% was extracted into chloroform/methanol (2:1, v/v).

Studies with ¹²⁵I-labelled VLDL *in vivo*

A silicone catheter was injected into the ear vein of a sedated rabbit 2 days before the study. The length of the catheter was adjusted so that its tip was situated in the right atrium. A 9:1 (v/v) mixture of Dextran (100 g/l) and citrate (3.8 g/l) was instilled into the catheter to prevent clotting. Blood samples were obtained from fasted animals via the catheter, after which the radiolabelled VLDL was injected into a marginal ear vein. Each animal received approx. 10 µCi of the tracer, corresponding to 0.12 mg of VLDL protein. Rabbits were kept in restraining cages during the first 6 h of the experiment. Afterwards, they were transferred to their cages and were given free access to food and water. Blood samples were drawn into EDTA (1 mg/ml) at regular intervals up to 48 h. VLDL ($d < 1.006$), IDL ($1.006 < d < 1.019$) and LDL ($1.019 < d < 1.050$) were isolated by sequential ultracentrifugation for 18–24 at 168 000 *g* as described above. The LDL fractions were dialysed overnight. Apoprotein B (apo B) in each fraction was precipitated with 5% (v/v) propan-2-ol [14]. The ¹²⁵I-apo B-bound radioactivity was measured using a Philips PW 4800 γ -radiation counter. The radioactivity observed at the indicated times in the various fractions was related to that in the VLDL fraction measured 3 min after injection. The fractional catabolic rate (FCR) of VLDL apo B was obtained from bi-exponential analysis of the plasma radioactivity decay curves [15,16].

Measurement of the production of VLDL and IDL

Triton WR-1339 (Tyloxapol; Sigma T 8761) at 200 mg/ml in 0.15 M-NaCl was injected into an ear vein (at a dose of 400 mg/kg of body weight [8]) of five WHHL homozygous controls and five animals treated with ethinyl oestradiol. The body weight and serum lipid concentrations were similar to those in the rabbits studied above. Blood samples of 3 ml were obtained from the indwelling catheter before and at 1, 2.5, 4, 6 and 24 h after injection. Total plasma triacylglycerols as well as lipids and protein in the $d < 1.019$ fraction were measured. Production rates were calculated as the areas under the curves between $t = 0$ and 24 h, corrected for the area already present at $t = 0$.

Determination of lipoprotein lipase and hepatic lipase activities in post-heparin samples

Post-heparin serum samples were obtained 15 min after intravenous injection of 100 units of heparin/kg (Tromboliquine; Organon, Oss, The Netherlands). Lipoprotein lipase and hepatic lipase were measured selectively by an indirect method using a gum-arabic-stabilized emulsion of triolein (80 mg) and [³H]triolein (10 µCi; this substrate is sufficient for 50 determinations) [17]. Using the substrate containing 0.1 M-NaCl and an optimal amount of dialysed human serum (to provide activating apolipoprotein C-II) total triacylglycerol lipase activity

(lipoprotein lipase + hepatic lipase) was measured. Compared with this substrate, addition of NaCl to a concentration of 1 M and omitting the serum activator [17] resulted in > 95% inhibition of lipoprotein lipase (partially purified from rabbit post-heparin serum by heparin-Ultrogel A4-R by a discontinuous gradient between 0.72 and 1.5 M-NaCl) as also found by others [18]. The activity of partially purified hepatic lipase released from the gel at 0.72 M-NaCl was not influenced by addition of 1.0 M-NaCl and omission of the serum activation. The assays were performed by incubating 10 µl of post-heparin serum with 250 µl of either substrate, prepared as described [19], for 30 min at 30 °C. Released fatty acids were extracted according to Belfrage & Vaughan [20]. Lipoprotein lipase activity was calculated by subtracting the activity measured at 1.0 M-NaCl from that measured at 0.1 M-NaCl. The values obtained with this indirect method for rabbit post-heparin serum correlated well with those obtained by the method of Boberg *et al.* [18], with correlation coefficients of 0.78 and 0.90 for lipoprotein lipase and hepatic lipase respectively ($n = 15$). The possible interference by endogenous triacylglycerol in this assay was studied: increasing amounts (0–20 µl) of the $d < 1.019$ fraction from WHHL serum (VLDL triacylglycerol concentration of 6.3 mmol/l) was added to 250 µl of either substrate followed by either hepatic lipase or lipoprotein lipase which had been partially purified by heparin Ultrogel chromatography [18]. With increasing amounts of endogenous triacylglycerols no significant trends were observed in the activities of hepatic or lipoprotein lipases, as measured with the triolein-based substrate.

Analytical methods

To measure the concentrations of lipoproteins during the ethinyl oestradiol treatment period, VLDL + IDL fractions were isolated by sequential ultracentrifugation at $d < 1.019$ [11]. HDL fractions were isolated by precipitating the LDL from the LDL + HDL fraction using poly(ethylene glycol) 6000 [21].

Cholesterol was measured by the enzymic CHOD-PAP method (Boehringer, Mannheim, Germany). Triacylglycerols were determined using the sera-PAK kit of Miles. Phospholipid and free cholesterol were measured with enzymic kits (nos. 691844 and 310328 respectively; Boehringer). Protein was determined according to the method described by Lowry *et al.* [22]. Ethinyl oestradiol concentrations were assessed by radioimmunoassay using 17- α -[6,7-³H]ethinyl oestradiol as tracer and an antiserum raised in rabbits against ethinyl oestradiol-3-succinyl conjugated to BSA. The working dilution of the antiserum was 1:16000. Before the assay, the samples were extracted using Baker-10-SPE octadecyl (C₁₈) disposable columns. Solid phase anti-rabbit antibody coupled to cellulose was used to perform bound/free ligand separation. The sensitivity of the radioimmunoassay approximated to 200 fmol/ml at 50% relative binding of the ligand to the antibody.

Statistical analyses were performed with Student's *t* test for unpaired observations. A two-sided *P* value of less than 0.05 was considered to be significant. Unless indicated otherwise, results are expressed as means $\pm$ S.D.

RESULTS

Efficacy of the hormone implants in releasing ethinyl oestradiol

Basal serum ethinyl oestradiol concentrations in the two groups of female rabbits were 200 and 215 fmol/ml. In the animals with implants containing 16 and 27 mg of the drug the levels of ethinyl oestradiol were 18 and 35 times higher than the basal values after 11 days and 9 and 13 times higher after 36 days respectively. After 36 days the implants containing 16 mg were almost as

Table 1. Effects of treatment with ethinyl oestradiol on growth rate, serum lipids and lipoproteins and the activities of post-heparin lipoprotein lipase and hepatic lipase in WHHL homozygous and heterozygous rabbits

Measurements were made after 28 days of ethinyl oestradiol treatment. Values (means $\pm$ S.D., for four determinations) in mmol/l, unless indicated otherwise. The mean ($\pm$ S.D.) body weight before treatment in the WHHL homozygotes was 2.1 ± 0.1 kg ($n = 8$) and in the WHHL heterozygotes it was 2.1 ± 0.1 kg ($n = 8$). Significant differences compared with controls are indicated by * $P < 0.05$; ** $P < 0.01$. Abbreviation: NEFA, non-esterified fatty acids.

	WHHL homozygotes		WHHL heterozygotes	
	Control	Treated	Control	Treated
Body weight (kg)	2.3 ± 0.2	2.4 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
Serum cholesterol	19.7 ± 3.0	$13.6 \pm 1.1^{**}$	3.4 ± 0.6	$1.7 \pm 0.5^{**}$
Serum triacylglycerol	3.5 ± 1.7	$0.8 \pm 0.2^{**}$	0.7 ± 0.4	$0.6 \pm 0.1^*$
VLDL + IDL cholesterol	6.90 ± 2.10	$1.54 \pm 0.28^{**}$	0.50 ± 0.20	0.22 ± 0.12
VLDL + IDL triacylglycerol	1.62 ± 1.19	$0.19 \pm 0.06^{**}$	0.26 ± 0.06	0.20 ± 0.06
LDL cholesterol	12.34 ± 3.84	12.00 ± 0.94	2.06 ± 0.73	$0.33 \pm 0.18^{**}$
HDL cholesterol	0.37 ± 0.09	0.34 ± 0.19	0.78 ± 0.27	$0.91 \pm 0.26^{**}$
Hepatic lipase (μ mol NEFA/h per ml)	14.1 ± 2.1	$22.9 \pm 6.2^*$	6.5 ± 1.0	$11.7 \pm 3.1^*$
Lipoprotein lipase (μ mol NEFA/h per ml)	7.0 ± 2.8	10.6 ± 2.6	6.1 ± 1.4	8.6 ± 1.8
VLDL apo B (g/l)†	0.28 ± 0.15	$0.05 \pm 0.01^{**}$	0.04 ± 0.01	0.04 ± 0.01
IDL apo B (g/l)†	0.38 ± 0.11	$0.10 \pm 0.02^{**}$	0.08 ± 0.02	0.06 ± 0.02
LDL apo B (g/l)†	2.9 ± 0.5	2.8 ± 0.2	0.7 ± 0.2	$0.4 \pm 0.2^*$

† Corrected for the amount of non-apo B protein in the fractions.

effective in lowering the serum lipid concentrations as those of 27 mg. Therefore the 16 mg implants were chosen for further study.

Effect of treatment on serum lipoproteins in homozygous and heterozygous WHHL rabbits

Treatment with ethinyl oestradiol did not influence the growth of the rabbits under study (Table 1).

In the WHHL homozygotes and heterozygotes treated with ethinyl oestradiol, serum lipid concentrations decreased significantly due to a decrease in cholesterol and triacylglycerols in the VLDL + IDL fraction (Table 1). The decrease in these lipids in the $d < 1.019$ fraction was rapid: within 6–8 days at least 75% of the maximal decrease in these concentrations could be observed (results not shown). In the homozygotes, apo B in the VLDL + IDL fraction also decreased (Table 1), whereas LDL cholesterol and LDL apo B concentrations in the homozygotes were not affected, and did not show an upward trend at the time at which the VLDL + IDL lipids rapidly decreased. In the heterozygotes, the VLDL + IDL lipids did not change significantly, but LDL cholesterol concentrations decreased. The HDL cholesterol concentration did not change in the homozygotes, but increased significantly in the heterozygotes.

The chemical composition data revealed that, per mg of protein, VLDL and IDL fractions from the WHHL homozygotes contained relatively more cholesteryl esters and less triacylglycerols than the corresponding fractions from the heterozygotes. In fact, the chemical composition data of the VLDL fraction of untreated homozygotes gave similar values as in the IDL fraction from WHHL homozygotes and especially from WHHL heterozygotes, indicating gross accumulation of particles with IDL characteristics in the $d < 1.006$ fraction from the homozygotes. Treatment with ethinyl oestradiol resulted in relatively small changes in the chemical composition of VLDL and IDL in both groups of WHHL rabbits. The percentage of triacylglycerols in the VLDL and IDL fractions decreased slightly; nevertheless, the change in the cholesterol/triacylglycerol ratio was small, and the large difference in

comparison with that in the heterozygotes remained. During treatment with ethinyl oestradiol, the relative content of triacylglycerols in the LDL of WHHL homozygotes decreased and that of esterified cholesterol increased, resulting in an increase in the cholesterol/triacylglycerol ratio (Fig. 1).

Agarose gel electrophoresis of total serum and of the $d < 1.019$ and $d > 1.019$ fractions revealed that pre- β and β -VLDL bands had disappeared almost completely, whereas the β -band did not change (results not shown), indicating a decrease in the amounts of pre- β and especially of β -VLDL particles.

Post-heparin hepatic lipase activity after 28 days of treatment was increased in both the homozygotes and the heterozygotes (Table 1). Treatment with ethinyl oestradiol for 28 days had no significant effect on the activity of lipoprotein lipase in either the homozygotes or the heterozygotes.

Clearance of 125 I-labelled VLDL apo B and conversion into IDL apo B

125 I-labelled control VLDL from homozygous rabbits were removed more rapidly in the homozygotes treated with ethinyl oestradiol compared with homozygous controls (Fig. 2). The decay in radioactivity was biphasic. In the ethinyl oestradiol-treated homozygous animals the first phase accounted for the majority of the total decay of VLDL apo B activity. No precursor-product relationship could be observed with the radioactivity decay curves of VLDL and IDL. This indicates that a part of the VLDL is removed from the circulation directly, without conversion to IDL. The decay of particles in the second phase of the VLDL curve was similar to the decay of apo B observed in the IDL fraction. Apparently these particles have similar metabolic behaviour, which reflects the data on chemical composition. The second-phase decay of VLDL apo B and of IDL apo B in the ethinyl oestradiol-treated homozygotes was faster than in the controls (Fig. 2). In the one heterozygote rabbit treated with ethinyl oestradiol, the first phase of the VLDL apo B decay curve was considerably faster than, but the second phase decayed at a similar rate to, the control. In ethinyl

Fig. 1. Changes in chemical composition of lipoprotein fractions in serum from WHHL homozygotes and heterozygotes after treatment with ethinyl oestradiol

(a) VLDL ($d < 1.006$); (b) IDL ($1.006 < d < 1.019$); (c) LDL ($1.019 < d < 1.055$). Results are means $\pm$ S.E.M. of four rabbits in each group. $\square$, Homozygous controls; $\blacksquare$, homozygotes treated with ethinyl oestradiol; $\square$ (hatched), heterozygous controls; $\blacksquare$ (cross-hatched), heterozygotes treated with ethinyl oestradiol. Abbreviations: FC, free cholesterol; EC, esterified cholesterol; TG, triacylglycerol; PL, phospholipid.

oestradiol-treated animals, the IDL apo B bound radioactivity was considerably lower.

Analysis of the VLDL turnover data revealed FCRs of 1.14 and 1.03 pools/day in the untreated homozygotes and 2.2 and 2.7 pools/day in the treated homozygotes.

Measurement of VLDL production

Triton WR-1339 inhibits the degradation of VLDL [23]; therefore this method is used to estimate the production of VLDL. After injection of Triton WR-1339 a steady rise was observed in plasma triacylglycerols, in agreement with the literature [8,23]. There was also a steady rise in the concentration of cholesterol, triacylglycerols and protein in the $d < 1.019$ fraction. The maximum levels reached after 24 h of inhibition, corrected for the basal value, were comparable in controls and ethinyl oestradiol-treated rabbits (Table 2). The same was true for the areas under the curves, except for the triacylglycerol production in the $d < 1.019$ fraction which was lower in the treated group, in contrast with the values of plasma triacylglycerols (Table 2).

DISCUSSION

In the present study we show that treatment with ethinyl oestradiol dramatically decreased the lipid and apo B concentrations in the $d < 1.019$ fraction of serum from WHHL rabbits. Changes in the chemical composition after 28 days of treatment were slight in relation to the large differences in these values between the rabbits homozygous and heterozygous for this trait. This indicates that ethinyl oestradiol influences the number of VLDL and VLDL remnants rather than exerting a shift in the distribution of particles to higher densities. Indeed, on agarose gel electrophoresis lipoproteins with intermediary β -pre- β mobility had largely disappeared.

In turnover studies, we found that the FCR of VLDL apo B was increased 2-fold on ethinyl oestradiol treatment. A bi-exponential decay was observed. The first phase of the decay curve was particularly accelerated in both homozygotes and heterozygotes. However, the first phase of the VLDL apo B

Fig. 2. ^{125}I -apo B radioactivity decay curves in serum lipoprotein fractions of WHHL homozygous and heterozygous rabbits, with or without implants containing ethinyl oestradiol, after injection of ^{125}I -labelled VLDL apo B from a control homozygous WHHL rabbit

Two WHHL homozygous controls and two WHHL homozygotes treated with ethinyl oestradiol were studied; the results obtained agreed well and were averaged. In addition, experiments were performed in one WHHL heterozygous control and one WHHL heterozygote treated with ethinyl oestradiol. (a, b) WHHL homozygotes; (c, d) WHHL heterozygotes. (a, c) VLDL apo B decay in controls ($\bullet$) and ethinyl oestradiol-treated ($\circ$) rabbits; (b, d) IDL ($1.006 < d < 1.019$) apo B radioactivity decay in controls ($\bullet$) and in ethinyl oestradiol-treated ($\triangle$) rabbits. $\bullet$ --- $\bullet$, VLDL apo B decay in controls (shown to facilitate comparison with IDL apo B radioactivity decay curves).

Table 2. Production rates of VLDL lipids and protein

Values shown are maximal values at 24 h after Triton WR-1339 injection and are integrated 24 h concentrations of serum lipids and protein in the $d < 1.019$ fraction. Results are means $\pm$ S.D. ($n = 5$).

	Values after 24 h (mmol/l or *mg/l)		Integrated concentrations (mmol/24 h per l or *mg/24 h per l)	
	Controls	Treated	Controls	Treated
VLDL + IDL cholesterol	15.1 $\pm$ 2.4	9.11 $\pm$ 2.0	115 $\pm$ 22	101 $\pm$ 24
VLDL + IDL triacylglycerol	36.1 $\pm$ 4.6	22.1 $\pm$ 7.9	418 $\pm$ 51	273 $\pm$ 106
VLDL + IDL protein	2500 $\pm$ 340*	1485 $\pm$ 314*	13 500 $\pm$ 3800*	13 800 $\pm$ 3900*
Plasma triacylglycerols	39.0 $\pm$ 4.9	29.0 $\pm$ 16.5	458 $\pm$ 59	361 $\pm$ 213

curve, which was already more rapid in the heterozygotes than in the homozygotes, was stimulated in the heterozygotes to a much greater extent during ethinyl oestradiol administration. This indicates that the removal process is rate-limiting, and therefore more competition by unlabelled VLDL can be expected in the homozygotes compared with the heterozygotes for removal of the tracer from the circulation. Consequently it is primarily the increase in the FCR that is responsible for the decrease in the VLDL apo B pool during ethinyl oestradiol treatment. Indeed, by the Triton WR-1339 method no decreases were found in the VLDL and IDL production rates in ethinyl oestradiol-treated homozygotes, which confirms that treatment with ethinyl oestradiol influences the clearance of VLDL-IDL. From the literature it is clear that the production of VLDL in WHHL homozygotes is normal [8,23]. The increased VLDL + IDL concentrations in homozygotes, combined with the increased values for hepatic lipase, do not support the hypothesis that hepatic lipase has an important role in the removal of VLDL + IDL in these rabbits. It is more likely that in the controls the defective LDL receptor accounts for the increased VLDL + IDL concentrations. However, in the ethinyl oestradiol-treated rabbits a considerable increase in the activity of hepatic lipase was accompanied by a dramatic decrease in VLDL + IDL, despite the deficiency in LDL receptor activity. This suggests a causal relationship: hepatic lipase and the LDL receptor may act co-operatively, at least in the removal of VLDL + IDL in WHHL rabbits.

In agreement with our results in WHHL homozygotes conversion of VLDL radioactivity to IDL and LDL was low and did not change during treatment with ethinyl oestradiol in cholesterol-fed New Zealand white rabbits [2]. Hence the mechanism of VLDL apo B removal in both control and ethinyl oestradiol-treated cholesterol-fed animals did not require conversion of VLDL to LDL prior to its irreversible disappearance from the plasma compartment [2].

The findings of an increase in hepatic lipase are in contrast with the observations in human subjects, in which all oestrogenic steroids lower post-heparin hepatic lipase activity [3]. However, the dose used here is not physiological and the responses in different species cannot be compared. In addition, it is clear that modulations in the activity of hepatic lipase have a divergent impact on lipoprotein concentrations, obviously related to additional changes in the activity of the LDL receptor and the production rate of VLDL induced by ethinyl oestradiol. This may explain the deviation of our results from the hypothetical function of hepatic lipase, i.e. involvement in the conversion of small VLDL to LDL [24].

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