Role of the liver in low-density-lipoprotein catabolism in the rat

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Plasma low-density-lipoprotein (LDL) kinetics and hepatic LDL uptake were studied in the rat after an intravenous pulse injection of [^{14}C]sucrose-labelled LDL. Some 96% of injected radioactivity was associated with apoprotein B of LDL (d 1.020–1.050). The disappearance of labelled LDL from plasma was accompanied by a linear increase in the hepatic uptake of LDL, up to 12 h after injection. Oestradiol treatment lowered plasma cholesterol concentration by 58% and the intravascular pool of LDL by 78%. This was associated with a 4-fold increase in the fractional catabolic rate of LDL and a 2-fold increase in the hepatic uptake of LDL. Oestradiol treatment did not significantly change the synthesis rate of LDL; it decreased the skin and lung uptake of LDL, but increased adrenal uptake. These results suggest that the liver plays an important role in the regulation of plasma LDL concentration.

LDL represents an endpoint in the intravascular catabolism of lipoproteins. Its subsequent fate is largely dependent on tissue uptake and catabolic processes. In man, LDL is the major cholesterol-transporting lipoprotein, and its concentration in plasma is regulated by rates of synthesis of LDL and by the efficiency of catabolism via high-affinity receptor mechanisms or receptor-independent pathways.

Various experiments have shown that the rate of removal of LDL from plasma is accelerated by certain drugs which have been shown *in vitro* to increase LDL receptor activity in the liver and other tissues (Chao *et al.*, 1979; Windler *et al.*, 1980). Until recently it has not been possible to measure the catabolic capacities of various tissues *in vivo*, because radioactive tracers are rapidly lost from cells after hydrolysis of protein or cholesteryl ester. Newer labelling techniques for LDL (Pittman *et al.*, 1979; Stein *et al.*, 1981) have provided tracers that are retained within the cells, and hence are able to provide a cumulative measure of the amount of LDL degraded in various tissues *in vivo*.

In several species, including the rat, these new techniques have shown that more than one-third of plasma LDL is degraded in the liver (Stein *et al.*, 1981; Pittman *et al.*, 1982). These and other observations raise the possibility that changes in

the capacity of the liver to take up and degrade LDL may regulate plasma LDL concentration. To investigate this question, we have decreased LDL in the rat by treatment with oestradiol and have measured LDL kinetics and hepatic LDL uptake by some of the new techniques mentioned above.

Methods

Treatment of animals

Male Wistar rats (200–250 g) were fed on standard commercial diet, with free access to food and water. 17α -Ethinyloestradiol (0.25 mg/kg) in propylene glycol (0.2 ml) was subcutaneously injected daily for 7 days. Control rats received equal volumes of propylene glycol.

Preparation and labelling of LDL

Rat LDL (d 1.020–1.050 g/ml) was isolated from plasma by sequential ultracentrifugation (Havel *et al.*, 1955). LDL was labelled with [$\text{U-}^{14}\text{C}$]sucrose, as described by Pittman *et al.* (1979). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Weber & Osborn, 1969) showed that 96% of ^{14}C radioactivity was associated with apoprotein B, 0.85% with apoprotein AIV, 1.0% with apoprotein E and 2.4% with apoprotein AI. The [^{14}C]Suc-LDL was biologically screened through a rat to remove any unbound tracers. The purity and stability of labelled LDL were checked by gel

Abbreviations used: LDL, low-density lipoprotein; HDL, high-density lipoprotein; [^{14}C]Suc-LDL, [$\text{U-}^{14}\text{C}$]sucrose-labelled LDL.

filtration on 6%-agarose columns (Fainaru *et al.*, 1977).

LDL kinetic studies

The screened LDL preparation was injected into the tail vein of rats ($0.5 \mu\text{Ci}$ in $50 \mu\text{g}$ of protein). All operative procedures were performed under light diethyl ether anaesthesia. Plasma decay curves were constructed by periodic blood sampling over 12–24 h. Fractional catabolic rates were calculated as described by Matthews (1957), by using a two-compartment model for the disappearance of LDL. The LDL protein concentration could not be directly assessed because of losses associated with repeated washing of lipoproteins by ultracentrifugation. It was derived indirectly from the cholesterol concentration in unwashed LDL and the ratio (0.67) of cholesterol to protein previously determined in a washed LDL pool. Protein was measured as described by Lowry *et al.* (1951), with bovine serum albumin as standard. Cholesterol was measured by g.l.c., after preliminary extraction with chloroform/methanol (2:1, v/v). The intravascular pool of LDL protein was calculated from the plasma concentration and the plasma volume (4.05% of body weight). Absolute catabolic rate was the product of the intravascular pool and the fractional catabolic rate in each case.

Measurement of tissue accumulation of [^{14}C]sucrose

To measure the time course of accumulation of [^{14}C]sucrose in the liver, groups of three animals

Fig. 1. Gel-chromatography pattern of rat plasma lipoproteins and [^{14}C]Suc-LDL

A sample (1.5 ml) of either rat plasma or [^{14}C]Suc-LDL was applied on a column ($0.9 \text{ cm} \times 90 \text{ cm}$) of 6% agarose gel. The column was operated at 23°C with a constant flow rate of 7 ml/h , and 1.2 ml fractions were collected. —, Elution pattern of unlabelled native lipoproteins. The identities of various lipoprotein classes were confirmed by using pure standards., ^{14}C -radioactivity peak of [^{14}C]Suc-LDL, with material immediately after screening (30 min) or 24 h after administration to a rat. Abbreviation: VLDL, very-low-density lipoprotein.

were exsanguinated through the abdominal aorta every 3 h over a 24 h period. The livers were removed and perfused with $0.15 \text{ M-NaCl}/0.01\%$ EDTA. From each animal 1 g of liver was homogenized, and samples were taken for radioactivity counting and for trichloroacetic acid precipitation. Acid-soluble material represented degraded LDL.

In other studies, 12 h after administration of [^{14}C]Suc-LDL, animals were exsanguinated through the abdominal aorta and various organs were perfused *in situ* with NaCl/EDTA. The liver, kidneys, lungs, skin, muscle etc. were removed and weighed. Samples of all tissues (approx. 0.2 g , except liver, which was homogenized) were digested overnight in NCS tissue solubilizer (The Radiochemical Centre, Amersham, Bucks., U.K.) at 50°C . After cooling, they were neutralized with acetic acid, and 0.1 ml samples were taken for radioactivity counting. Literature values relating tissue masses to total body weight were used to determine masses of diffuse organs such as muscle, skin etc. (Caster *et al.*, 1956).

Results

The purity of radioactive lipoproteins was confirmed by gel filtration. Fig. 1 shows the elution pattern of unlabelled rat plasma lipoproteins and of screened [^{14}C]Suc-LDL. The screened [^{14}C]Suc-LDL was eluted as a single peak with the same elution volume as unlabelled native LDL. A labelled LDL preparation obtained 24 h after administration of screened [^{14}C]Suc-LDL into a rat also showed a similar elution pattern, confirming the stability of the labelled product *in vivo*.

The time courses of disappearance of labelled LDL from plasma after injection of [^{14}C]Suc-LDL and its appearance in the liver are shown in Fig. 2. The uptake of labelled LDL in the liver increased linearly with time up to 12 h. The net accumulation began to decline after 18 h, suggesting leakage of the [^{14}C]sucrose from the liver cells. Trichloroacetic acid precipitation of liver and various tissue homogenates indicated that 95% of radioactivity was in the acid-soluble fraction.

Fig. 3 shows plasma decay curves of [^{14}C]Suc-LDL in a control and in an oestradiol-treated rat. Decay in the treated animal was significantly faster than in the control. LDL kinetic parameters and the proportion of accumulation in the liver are shown in Table 1. Oestradiol treatment decreased plasma cholesterol by 58% and LDL protein by 78%. There was also a decrease in HDL cholesterol, but this has not been systematically documented in the present study. Treatment led to a 4-fold increase in the fractional catabolic rate of LDL, and this was accompanied by a 2-fold increase in

Table 1. *Metabolic parameters for control and oestradiol-treated rats*

All values are means $\pm$ s.d. ($n = 6$). Statistical analyses were performed by Student's t test: P , probability of significance; ns, not significant.

	Plasma cholesterol (mg/dl)	Intravascular pool of LDL (μ g)	Fractional catabolic rate (h^{-1})	Absolute catabolic rate ($mg \cdot kg^{-1} \cdot day^{-1}$)	Percentage of injected dose accumulated in the liver at 12 h
Control	58.18 ± 3.94	1314 ± 282	0.136 ± 0.008	19.76 ± 3.96	24.33 ± 2.58
Oestradiol-treated	24.53 ± 2.81	286 ± 130	0.533 ± 0.096	21.37 ± 9.09	45.50 ± 5.65
Control versus treated	$P < 0.001$	$P < 0.001$	$P < 0.001$	ns	$P < 0.001$

Fig. 2. Plasma disappearance and liver accumulation of $[^{14}C]$ Suc-LDL after intravenous injection

▲, Time course of disappearance from plasma; ●, time course of accumulation in the liver.

the proportion of LDL degraded in the liver. The absolute catabolic rates in the control and oestradiol-treated rats did not differ significantly.

The proportions of plasma LDL degraded in various tissues are shown in Table 2. In oestradiol-treated rats there was a significant decrease in LDL accumulation in the lungs and skin, and there was a significant increase in the adrenals.

Discussion

Conversion of cholesterol into bile acids in the liver is quantitatively the most important route by

Fig. 3. Plasma disappearance of $[^{14}C]$ Suc-LDL after intravenous injection in a control (●) and an oestradiol-treated rat (■)

The plasma decay study was performed as outlined in the Methods section. The percentage of the total injected dose remaining in the plasma at each point was plotted against time.

which cholesterol is removed from the body. Therefore any decrease in plasma cholesterol might be expected to involve increased catabolism in the liver of lipoproteins that transport cholesterol. Although HDL is the dominant cholesterol-transporting class in the rat, we have focused attention on LDL catabolism because of its putative role in atherogenesis.

Our findings indicate that liver in the rat makes

Table 2. Tissue accumulation of [^{14}C]Suc-LDL in control and oestradiol-treated rats 12 h after injection. Results are means $\pm$ S.D. ($n=6$). Statistical analyses were performed by Student's t test: * $P<0.001$; † $P<0.05$.

Tissue	[^{14}C]Suc-LDL (% of injected dose)	
	Control rats	Oestradiol-treated rats
Liver	24.33 $\pm$ 2.58	45.50 $\pm$ 5.65*
Kidney	1.80 $\pm$ 0.95	0.98 $\pm$ 0.53
Lung	0.51 $\pm$ 0.12	0.32 $\pm$ 0.07†
Adrenal	0.25 $\pm$ 0.16	2.85 $\pm$ 1.08*
Skin	5.93 $\pm$ 1.10	3.63 $\pm$ 0.32*
Muscle	4.86 $\pm$ 1.35	3.70 $\pm$ 2.71
Plasma, faeces, intestine and other tissues	49.80 $\pm$ 10.30	35.75 $\pm$ 9.40

the largest single organ contribution to the catabolism of LDL, in agreement with findings by Carew *et al.* (1982). But this does not automatically suggest a major role for the liver in the regulation of plasma LDL concentrations, as a substantial proportion of LDL is still catabolized in the extra-hepatic tissues. The disappearance of [^{14}C]Suc-LDL from plasma was accompanied by a linear increase in the accumulation of [^{14}C]sucrose in the liver (Fig. 2), and this is supportive evidence for a regulatory role of the liver in LDL catabolism. This experiment also indicated that measurement of [^{14}C]sucrose accumulation in the liver beyond 12–18 h did not reflect quantitatively the catabolism of LDL in that organ, owing to slow leakage of [^{14}C]sucrose from the cells.

In the present study, the absolute catabolic rate did not change with oestradiol treatment, despite significant changes in plasma cholesterol, intravascular pool, fractional catabolic rate and the proportion of LDL degraded in the liver (Table 1). Since in the steady state the absolute catabolic rate is equivalent to the synthesis rate, we infer that there is no change in the rate of LDL synthesis. The marked changes in plasma LDL concentration with oestradiol treatment are therefore due to changes in LDL removal rate alone. We have further observed that the increased removal of LDL is associated with an increased accumulation of [^{14}C]Suc-LDL-degradation products in the liver, as opposed to an increased accumulation in extra-hepatic tissues. Tissues such as the lung and skin manifested a significant decrease in LDL degradation. Taken in conjunction with the time-course experiment, this provides for the first time direct support *in vivo* for our suggestion that catabolism of LDL in the liver regulates plasma LDL concentration. Other evidence from experiments in dogs further supports this concept (Kovanen *et al.*, 1981).

It is now well established that under normal

circumstances about 67% of the LDL uptake in the liver is receptor-mediated (Carew *et al.*, 1982). Since it has been shown that oestradiol treatment results in an increase in the number of hepatic LDL receptors (Windler *et al.*, 1980), it is reasonable to conclude that the decrease in plasma LDL in these animals is due to the increased uptake of LDL in the liver via the receptor pathway. It therefore appears that receptor-mediated LDL uptake in the liver plays a major role in the regulation of plasma LDL concentration.

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