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Evidence that reverse cholesterol transport is stimulated by lipolysis of triglyceride-rich lipoproteins

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The hypothesis that reverse cholesterol transport by high density lipoprotein (HDL) is augmented by lipolysis of triglyceride-rich lipoproteins received support from experiments in rabbits whose tissue cholesterol had been pre-labeled with [^{3}H]cholesterol several weeks earlier. When lipolysis was stimulated by intravenous heparin (which releases lipoprotein lipase from vascular endothelium), reciprocal changes in plasma triglyceride and HDL cholesterol concentrations were accompanied by a rise in the specific radioactivity of HDL cholesterol, indicative of increased transfer of cholesterol into HDL from slowly exchanging cholesterol pools in extra-hepatic tissues.

Cholesterol; Triglyceride; High density lipoprotein; Heparin; Lipoprotein lipase; Rabbit

1. INTRODUCTION

Triglyceride fatty acids are transported from ileum and liver to peripheral cells as components of chylomicrons and very low density lipoproteins (VLDL) [1]. Hydrolysis of the triglyceride in these particles by lipoprotein lipase (LPL), bound to the luminal surface of vascular endothelium, produces smaller, denser particles (chylomicron remnants and low density lipoproteins; LDL), which are rich in cholesteryl esters and are cleared from the circulation in many tissues by receptor-mediated endocytosis [2]. Cholesterol delivered to peripheral cells in this way is returned to the liver via high density lipoproteins (HDL), a process referred to as reverse cholesterol transport [3]. The mechanism by which reverse cholesterol transport is regulated is not understood. It has been hypothesized that it may be augmented by the lipolysis of chylomicrons and VLDL [4], thereby mitigating the deposition of excess cholesterol in peripheral cells during plasma triglyceride transport. In this report we present evidence which supports this hypothesis from experiments in rabbits.

2. MATERIALS AND METHODS

2.1. *Experimental protocol*

Six experiments were carried out in 28 non-fasted male New Zealand White rabbits (3.5–4.0 kg; 4 or 5 animals per experiment). Each was given an intravenous (i.v.) injection of 1.0 ml of rabbit serum containing 0.45–1.0 mCi of 1α,2α-[^{3}H]cholesterol (Amersham Corp., USA) to label tissue cholesterol pools [5]. 28–112 days later (mean: 69 days) lipolysis of triglyceride-rich lipoproteins was stimulated by i.v. injection of 1000 units of bovine lung heparin (Upjohn, MI, USA) in 1.0 ml sterile 0.15 mol/l NaCl. At timed intervals blood samples (2–5 ml) were collected from an ear vein into Na_2EDTA (1 mg/ml), and centrifuged for 15 min at 4°C.

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2.2. *Laboratory procedures*

Plasma HDL particles were isolated by several different methods: precipitation of other lipoproteins with heparin and $MnCl_2$ (final conc: 183 u/ml and 92 mmol/l [6], sodium phosphotungstate and $MgCl_2$ (3.56 g/l and 44 mmol/l) [7], or polyethylene glycol 8000 (80 g/l) [8]; high performance liquid chromatography (HPLC) [9]; and ultracentrifugation in a discontinuous NaBr gradient [10]. Triglyceride and total cholesterol (i.e. esterified plus unesterified cholesterol) were assayed by enzymic colorimetric procedures (Boehringer-Mannheim, cat. nos. 877557 and 236691) in a microtiter spectrophotometer. Cholesterol radioactivity was assayed using a PPO/POPOP/Triton X-100/toluene scintillant.

3. RESULTS

Induction of lipolysis was confirmed by rapid decreases ($P<0.02$) in plasma triglyceride concentration (Fig. 1). Plasma total cholesterol concentration also decreased, presumably reflecting tissue uptake of newly formed chylomicron remnants and LDL. Plasma HDL cholesterol concentration increased by 20–60% ($P<0.02$; Fig. 1).

During the 7 days before heparin, plasma cholesterol specific radioactivity decreased on average by 2.7% per day, consistent with the known rate of cholesterol turnover in rabbits [11]. During the 96 h after heparin the rate of decay of specific radioactivity decreased in most animals. In 17 of 20 animals in which measurements

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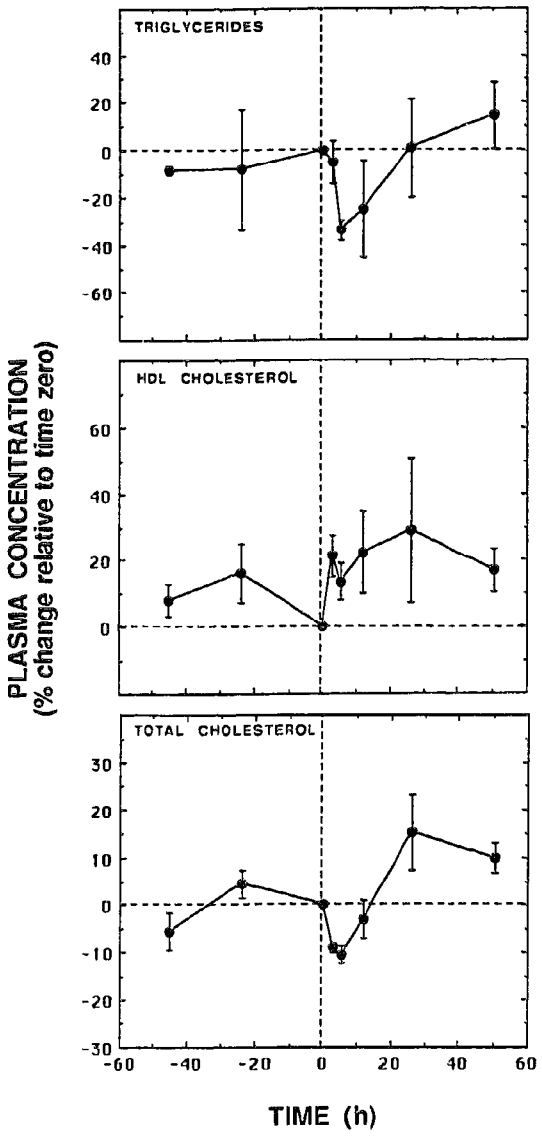

Fig. 1. Effects of heparin-induced lipolysis on plasma triglyceride, HDL cholesterol and total cholesterol concentrations (one experiment). Four rabbits were each given 1000 units of heparin i.v. Plasma HDL was separated from other lipoproteins by heparin/$MnCl_2$ precipitation [6]. Results are expressed as percentages (means and SE) of the time 0 values.

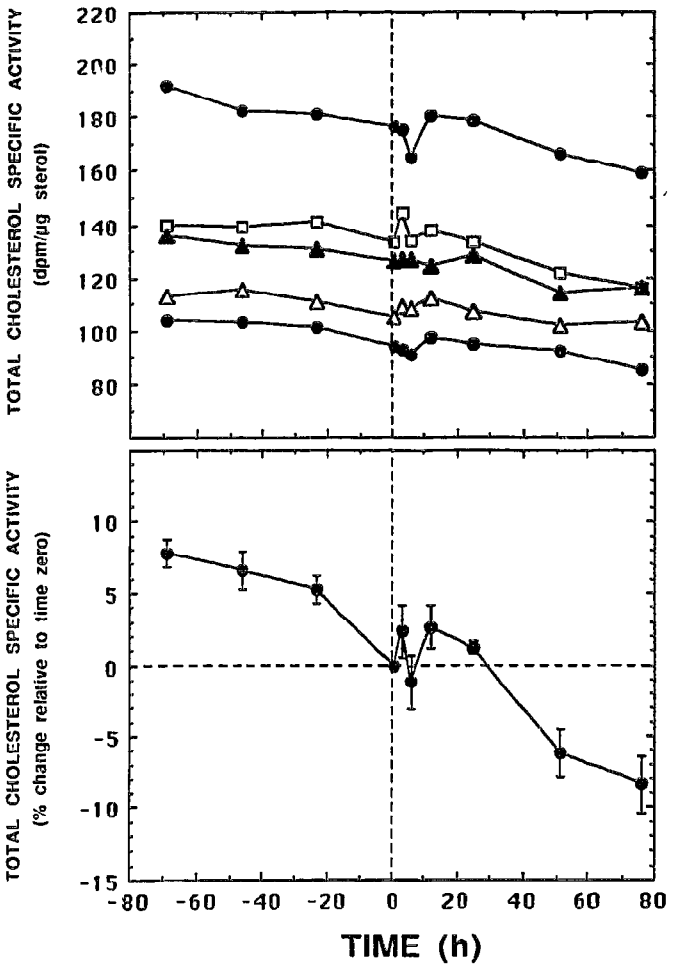

Fig. 2. Effects of heparin-induced lipolysis on plasma cholesterol specific radioactivity in 5 rabbits (one experiment). (Upper panel) Individual results. (Lower panel) Means and SE of percentage changes relative to time 0. 4–8 weeks before the experiment each animal was given 0.45 or 1.0 mCi of 1α,2α-[^{3}H]cholesterol i.v. in 1 ml of rabbit serum. Each was given 1000 units of heparin i.v. at time 0. Results at 12 and 24 h after heparin were significantly greater than the time 0 values ($P<0.05$).

were made at 3, 6, 12, 24 and 48 h, specific radioactivity increased during this period ($P<0.002$), reaching a peak value at 12 or 24 h (Fig. 2). Lipoprotein fractionation revealed that this was owing largely or entirely to a rise in that of HDL cholesterol (Fig. 3). In contrast, the specific radioactivity of non-HDL cholesterol sometimes decreased after heparin. The differing behavior of these two fractions was more evident when separated by precipitation than when ultracentrifugation or HPLC were used, presumably reflecting exchange of cholesterol between HDL and other lipoproteins in vitro [12].

4. DISCUSSION

In these experiments lipolysis of triglyceride-rich lipoproteins was stimulated by heparin, which releases LPL from vascular endothelium [1]. We studied rabbits, because they are deficient in a second heparin-releasable enzyme, hepatic endothelial lipase, which hydrolyzes HDL triglyceride and phospholipid [13]. In this way we were able to investigate the effect of stimulating the hydrolysis of chylomicron and VLDL triglycerides in the absence of increased hydrolysis of HDL lipids.

Tissue cholesterol was labeled by i.v. infusion of [^{3}H]cholesterol. Injection of heparin was then delayed several weeks to ensure that the specific radioactivity of cholesterol in slowly exchanging pools, which are

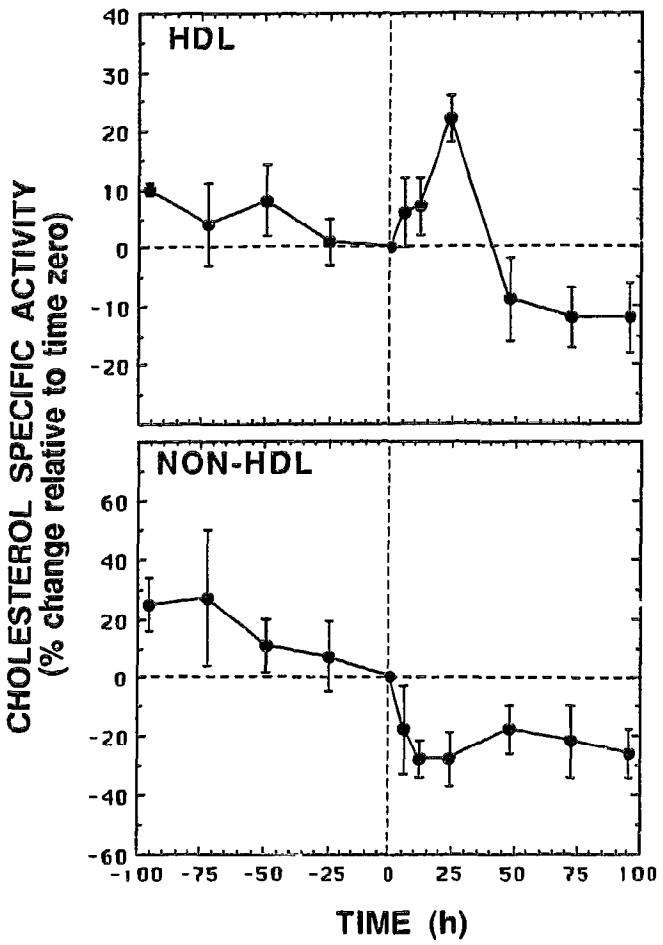

Fig. 3. Effects of heparin-induced lipolysis on the specific radioactivities of HDL and non-HDL cholesterol in 5 rabbits (one experiment). 12–16 weeks before the experiment each animal was given 1.0 mCi of 1α,2α-[^{3}H]cholesterol. Each was given 1000 units of heparin i.v. at time 0. HDL was separated from other lipoproteins by $MgCl_2$/sodium phosphotungstate precipitation [7]. Results are presented as changes (means and SE) relative to time 0 values. Specific radioactivities of both fractions 24 h after heparin were significantly different ($P<0.01$) from their values at time 0.

known to be confined to extra-hepatic tissues [5], would exceed that of plasma cholesterol at the time of the experiment [11]. Under such conditions the acute rise in plasma cholesterol specific radioactivity which we observed is indicative of an increased transfer of cholesterol from peripheral tissues into plasma [11]. Theoretically, this could have been due either to a net efflux of cholesterol mass from intracellular pools (reverse cholesterol transport), or to an increase in the bi-directional exchange of unesterified cholesterol (UC) that occurs between lipoproteins and cell membranes [12]. The latter explanation is unlikely, however, as the UC of cell surface membranes belongs largely to the same kinetic pool as plasma UC [14]. The probability that lipolysis augmented reverse cholesterol transport is strengthened by the fact that the increase in specific radioactivity was often confined to HDL cholesterol.

Increases in HDL cholesterol concentration have been well documented during lipolysis, and have been attributed to two processes: the transfer of surface remnants (composed of UC, phospholipid and C-apolipoproteins) from triglyceride-rich lipoproteins to HDL; and a decrease in the transfer of cholesteryl esters in the opposite direction [15]. Our present observations now provide evidence that mobilization of tissue cholesterol also contributes.

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