Vol. 81, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1978 Pages 82-88

MONOACYLGLYCEROL ACCUMULATION IN LOW AND HIGH DENSITY LIPOPROTEINS DURING THE HYDROLYSIS OF VERY LOW DENSITY LIPOPROTEIN TRIACYLGLYCEROL BY LIPOPROTEIN LIPASE

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Received January 9,1978

Summary. We have demonstrated that low and high density lipoproteins from monkey plasma are capable of accepting and accumulating monoacylglycerol that is formed by the action of lipoprotein lipase on monkey lymph very low density lipoproteins. Furthermore, the monoacylglycerol that accumulates in both low and high density lipoproteins is not susceptible to further hydrolysis by lipoprotein lipase but is readily degraded by the monoacylglycerol acyltransferase of monkey liver plasma membranes. These observations suggest a new mechanism for monoacylglycerol transfer from triacylglycerol rich lipoproteins to other lipoproteins. In addition, the finding that monoacylglycerol bound to low and high density lipoprotein is degraded by the liver enzyme but not lipoprotein lipase lends support to the hypothesis that there are distinct and consecutive extrahepatic and hepatic stages in the metabolism of triacylglycerol in plasma lipoproteins.

Introduction. In a previous publication (1) we demonstrated that monoacylglycerol is the major acylglycerol produced by the lipoprotein lipase catalyzed hydrolysis of triacylglycerol in chylomicron and VLDL. In addition, with increasing concentrations of albumin in the incubation mixtures (hence the number of binding sites available), increasing amounts of monoacylglycerol accumulated. This was due to the transfer of monoacylglycerol from the remnant lipoprotein to albumin. In that study we found that monoacylglycerol bound to both remnant lipoprotein and albumin was degraded by MGAT in the liver plasma membrane. On the other hand, lipoprotein lipase degraded monoacylglycerol in remnant lipoprotein but not that monoacylglycerol bound to albumin. When plasma was included in the incubation of chylomicra, albumin, and lipoprotein lipase, there was an additional increase in the accumulation of monoacylglycerol. This observation could not be explained by the small amount of added albumin present in the plasma or by the inhibition of lipoprotein lipase. It appeared, therefore,

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Abbreviations used are: MGAT, monoacylglycerol acyltransferase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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that a plasma factor(s) was present that either altered the specificity of the lipoprotein lipase or sequestered the product monoacylglycerol from further lipoprotein lipase activity. In this paper we demonstrate that the plasma factor(s) responsible for the accumulation of monoacylglycerol are LDL and HDL, at least in part.

Materials and Methods

Lipoproteins. VLDL radiolabeled in vivo with trioleoyl[2-^{3}H]glycerol and tri[1-^{14}C]oleoyl glycerol were obtained from monkey lymph as previously described (1). Plasma was obtained from monkeys maintained on a control low cholesterol diet and collected after an overnight fast. 5,5'Dithio(bis)-2-nitrobenzoic acid was added, final concentration 10^{-7} M, to inhibit any lecithin:cholesterol acyltransferase activity. The lipoproteins present in fasting plasma were floated as described by Rudel (2) (fasting plasma lipoproteins consist almost entirely of about 70% HDL and 25% LDL) and dialyzed against 0.15 M NaCl-0.01% w/v EDTA pH 7.4. The lipoproteins were then concentrated tenfold using an Amicon PM-10 Diaflo-Ultrafiltrator.

Enzymes and other materials. Lipoprotein lipase was purified from bovine milk according to the method of Olivecrona (3) and Kinnunen (4). Liver plasma membrane fragments, the source of MGAT, were isolated from African green monkeys by the method of Neville (5) and characterized by their marker content (6). Bovine serum albumin was defatted by the method of Chen (7).

Incubations and column chromatography. Incubation mixtures consisted of 80 μmol VLDL triacylglycerol (specific radioactivity $5X10^{5}$ cpm[^{3}H]-$2.5X10^{5}$ cpm[^{14}C]/μmol triacylglycerol, 380 mg albumin, dialyzed and concentrated lipoproteins from 20 ml plasma, 100 mM Tris-HCl pH 7.5, 18 μg lipoprotein lipase and water to bring the NaCl in the lipase preparation to a final concentration of 0.15 M. The reaction was started by the addition of the enzyme; incubations were for fifteen minutes at 37°C, and were stopped by the addition of 5 M NaCl to bring the final NaCl concentration to 1.0 M. Incubation mixtures were then concentrated on PM-10 Diaflo-Ultrafilters and separation of the lipoprotein and protein constituents were carried out on Bio-Gel A15 m columns (1.5X90 cm) (2). Lipoprotein lipid and protein analyses and radioactivity measurements were as previously described (1).

Results. The elution profile and the distribution of the radiolabeled products from the incubation of VLDL labeled with tri[^{14}C]oleoyl[^{3}H]glycerol, plasma LDL, HDL, albumin, and lipoprotein lipase are shown in Figure 1. For the sake of simplicity, only [^{3}H]acylglycerol and free [^{14}C]oleic acid values are shown; the distribution of [^{14}C]acylglycerol was proportionate to the [^{3}H]acylglycerol. The order of elution was first a spectrum of remnant lipoproteins (I and II, decreasing in size) followed by LDL (III) and HDL (IV) that just preceded the albumin peak (fractions 80-90). Most unhydrolyzed

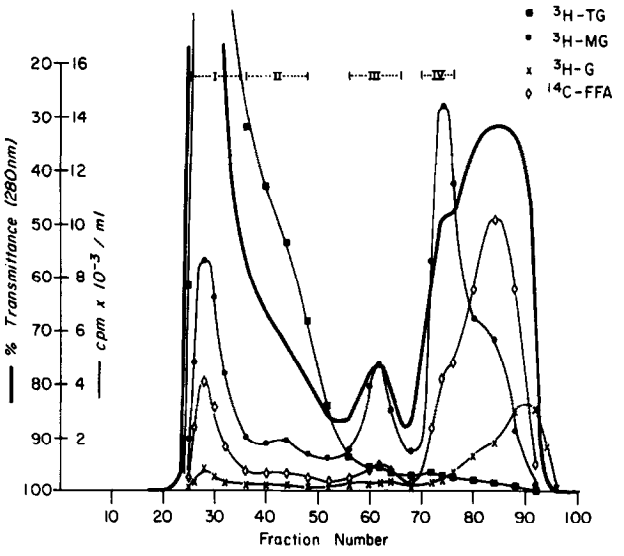

Figure 1. The elution profile and distribution of radioactivity in VLDL, LDL, HDL and albumin separated by column chromatography. (See Materials and Methods for details.) Symbols are: I, large very low density lipoprotein remnants; II, small very low density lipoprotein remnants; III, low density lipoproteins; IV, high density lipoproteins; TG, triacylglycerols; MG, monoacylglycerols; G, glycerol; FFA, free fatty acids.

triacylglycerol remained with the remnant lipoproteins whereas nearly seventy-five percent of the monoacylglycerol was transferred to LDL, HDL, and albumin. The small amount of triacylglycerol in the LDL pool probably represents contamination by small particles of remnant lipoprotein. In control incubations without added LDL and HDL all monoacylglycerol was retained in the remnant lipoprotein. These results demonstrate that, in the presence of the LDL and HDL from the plasma, monoacylglycerol was absorbed from the VLDL as it was produced by the action of the lipoprotein lipase.

The various lipoprotein fractions were combined into four pools (see top of Figure 1), large and small remnant lipoprotein, LDL, and HDL. The HDL contained a small amount of the overlapping albumin peak (about ten to fifteen percent). These were then reincubated in the presence of combinations of albumin, lipoprotein lipase, or plasma membrane MGAT (Table 1). Since both large and small remnant lipoproteins gave the same results, only those of the

Table 1

Percent Distribution of [^{3}H]Glyceryl and [^{14}C]Oleoyl Radiolabels

Additions		TG	DG	MG	FFA	G
A. Small VLDL remnant (pool II)						
None	[^{3}H]	70.7	6.5	13.7	—	4.5
	[^{14}C]	79.1	2.6	4.9	11.2	—
Albumin	[^{3}H]	53.5	7.7	21.0	—	13.2
	[^{14}C]	59.3	3.6	7.7	27.1	—
LPL + Albumin	[^{3}H]	24.7	5.9	32.9	—	32.3
	[^{14}C]	26.1	3.0	12.7	56.6	—
MGAT	[^{3}H]	69.0	6.0	1.9	—	18.7
	[^{14}C]	78.1	2.7	0.3	16.9	—
B. LDL (pool III)						
None	[^{3}H]	18.1	9.1	54.2	—	11.9
	[^{14}C]	25.7	2.6	27.0	41.8	—
Albumin	[^{3}H]	16.1	2.6	57.2	—	17.0
	[^{14}C]	20.8	4.2	26.4	46.1	—
LPL + Albumin	[^{3}H]	9.2	8.4	48.4	—	27.8
	[^{14}C]	12.2	4.8	24.0	55.9	—
MGAT	[^{3}H]	16.5	7.4	5.3	—	64.5
	[^{14}C]	25.0	5.4	1.3	66.0	—
C. HDL (pool IV)						
None	[^{3}H]	5.1	4.3	69.3	—	15.5
	[^{14}C]	6.1	2.6	31.7	56.3	—
Albumin	[^{3}H]	4.8	4.9	63.9	—	19.1
	[^{14}C]	5.7	3.3	31.5	56.1	—
LPL + Albumin	[^{3}H]	4.6	4.9	57.4	—	27.5
	[^{14}C]	5.0	2.1	27.6	62.3	—
MGAT	[^{3}H]	5.1	5.1	10.8	—	74.3
	[^{14}C]	6.1	2.3	5.3	83.3	—

Aliquots of pools II, III, and IV were incubated for thirty minutes where indicated with 40 mg albumin, 6 μg LPL and 40 mg albumin or 400 μg liver plasma membrane protein.

Abbreviations are: TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; FFA, free fatty acid; G, glycerol.

small remnant lipoprotein are shown (Table 1, panel A).

Some lipoprotein lipase remains bound to the remnant lipoprotein as evidenced by the continued hydrolysis of triacylglycerol upon addition of albumin (part A). In control experiments albumin was shown to have no lipolytic activity. Conversely, MGAT hydrolyzed monoacylglycerol but had no activity on triacylglycerol (part A). There appears to be little lipoprotein lipase bound to LDL and HDL (parts B and C) since the addition of albumin did not promote significant hydrolysis unless more lipoprotein lipase was added. On the other hand, MGAT almost completely degrades the monoacylglycerol in both LDL and HDL. Under all conditions attempted, MGAT did not degrade triacylglycerol (parts A, B, and C).

Discussion. The results reported here demonstrate the reason for the observed accumulation of monoacylglycerol during the lipoprotein lipase catalyzed hydrolysis of triacylglycerol in VLDL in the presence of plasma (1); namely, monoacylglycerol is transferred to LDL and HDL that are not substrates for lipoprotein lipase. The failure of lipoprotein lipase to degrade monoacylglycerol once it is transferred to LDL, HDL, or albumin could be due to the lack of an activator apoprotein or to the presence of some type of barrier that prevents interaction between the lipoprotein lipase and monoacylglycerol. Even though added lipoprotein lipase is capable of degrading triacylglycerol in our LDL preparation, we cannot be sure that lipoprotein lipase is active on the LDL rather than contaminating remnant lipoprotein. Complete separation of these two must be achieved before we can answer this question. Earlier, we found that liposomes of monoacylglycerol and phosphoglyceride were absorbed by lipoproteins [mainly LDL and HDL (8)]. This plus our finding that monoacylglycerol will accumulate in remnant lipoprotein (1) indicates that monoacylglycerol need not be transferred directly to an absorbent, unlike the fate of free fatty acid that must be transferred directly to albumin (9). At this time we cannot rule out the possibility that an exchange protein is functioning in this system. However, this would have to be of rather low density ($d < 1.225$)

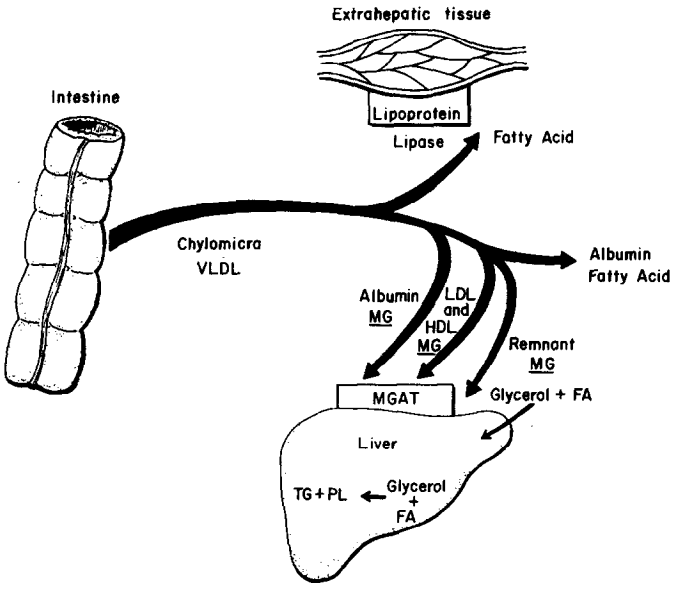

Figure 2

since floated lipoproteins were used.

These findings suggest that LDL and HDL, as well as albumin, play an important role in the clearance of triacylglycerol rich lipoproteins. Figure 2 depicts a proposed sequence of events. As the lipoproteins from the intestinal lymph enter circulation, they are acted upon by the extrahepatic lipoprotein lipase. The free fatty acid becomes attached to albumin or is absorbed directly by the tissue while the monoacylglycerol can either be absorbed by the extrahepatic tissue, absorbed to albumin (1), or LDL and HDL, as shown here. The monoacylglycerol absorbed to albumin or LDL and HDL, as well as that remaining with remnant lipoprotein, then circulates to the liver where it is degraded by MGAT on the surface of the hepatocyte. The products of degradation are then rapidly taken into the hepatocyte and incorporated into triacylglycerol as well as phosphoglyceride (manuscript in preparation).

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