Effect of removal of lipoproteins of different composition on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and hepatic very low density lipoprotein secretion[1]

Paul E. A. Van Zuiden, Sandra K. Erickson, and Allen D. Cooper

Division of Gastroenterology, Stanford University Medical Center, Stanford, CA 94305

Abstract The effect of remnant lipoproteins on hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and hepatic very low density lipoprotein (VLDL) secretion was studied in the perfused rat liver and in vivo. As had been observed previously, when the liver was perfused with a lipid-free medium, HMG-CoA reductase activity increased about twofold after 150 min, and this increase could be prevented by the addition of chylomicron remnants to the medium. However, suppression below base line activity did not occur even with increasing amounts of remnant cholesterol. When chylomicron remnants prepared from triglyceride-rich particles were included in the medium, reductase activity was increased even above that in the control perfusions despite the fact that approximately the same amount of cholesterol was removed from these particles as from standard particles. In contrast, particles that were low in triglycerides and rich in cholesterol not only prevented the rise in reductase activity but inhibited it significantly below base line activity. Again, the total amount of cholesterol removed was the same as with the other types of particles. These results suggested that both the triglycerides and cholesterol exerted an effect on HMG-CoA reductase. Consistent with this hypothesis, a significant correlation was found between reductase activity and the ratio of triglycerides to cholesterol removed, but not to either alone. To explore the role of triglycerides further, the effect of these lipoprotein particles on VLDL secretion was determined. VLDL secretion was stimulated by both standard and triglyceride-rich remnants but not by triglyceride-poor remnants. The degree of stimulation with standard chylomicron was comparable to that induced by infusion of a comparable fatty acid load as oleic acid bound to albumin. In vivo a similar effect of these lipoproteins on HMG-CoA reductase activity was observed. Rats were injected with a lipoprotein bolus containing 7 mg of cholesterol, and reductase activity in the liver was measured 2 hr later. Standard chylomicrons and triglyceride-rich chylomicrons stimulated reductase to 157% and 187% of control activity, respectively, whereas cholesterol-rich VLDL suppressed reductase activity to 30% of control activity. These observations support the hypothesis that remnant lipoproteins have a dual effect on hepatic HMG-CoA reductase activity; the cholesterol in these lipoproteins suppresses hepatic reductase activity while the triglycerides concommitantly delivered stimulate reductase activity at least in part because they stimulate hepatic VLDL secretion. Therefore, the net response of hepatic HMG-CoA reductase to a particular dietary lipoprotein will depend upon the balance between the cholesterol and triglycerides carried to the liver.—**Van Zuiden, P. E. A., S. K. Erickson, and A. D. Cooper.** Effect of removal of lipoproteins of different composition on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and hepatic very low density lipoprotein secretion. *J. Lipid Res.* 1983. **24:** 418–428.

Supplementary key words cholesterol • triglycerides

It is well established that hepatic cholesterol synthesis is subject to inhibition by dietary cholesterol. It has also been demonstrated that chylomicrons, the lipoproteins of intestinal origin, when injected into rats in vivo also reduce the rate of cholesterol synthesis in liver in a dose- and time-dependent manner. Maximal inhibition (about 60%) is achieved 12 hr after a single bolus of 8 mg of lipoprotein cholesterol/100 g body weight (2).

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for cholesterol synthesis, is inhibited by about 80% after feeding cholesterol for 12–18 hr. The half-life for the enzyme activity has been estimated as 1–4 hr (3, 4). Therefore, one would expect that inhibition of enzyme activity by 50% should occur within 1–4 hr after administration of a bolus of lipoprotein cholesterol. To date this has not been demonstrated. When chylomicron remnants were added to liver perfusions the increase in HMG-CoA reductase normally induced by perfusion was prevented; however, HMG-CoA reductase was not inhibited below the initial value (5). Thus, it appeared that although HMG-CoA reductase could respond to a bolus of cho-

Abbreviations: VLDL, very low density lipoproteins; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; TCA, trichloroacetic acid; MVA, mevalonolactone.

[1] A portion of this work was presented at the 1981 meeting of the American Heart Association at Dallas, TX, and published in abstract form (1).

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lesterol in a lipoprotein, the degree of inhibition was not as substantial as might have been expected.

Chylomicron remnants also contain large amounts of triglyceride which is hydrolyzed to free fatty acids after uptake by the liver. Thus, in addition to cholesterol, chylomicron remnants may transport large amounts of fatty acids in the form of triglycerides to the liver. Goh and Heimberg (6) have established that when fatty acids are delivered to the liver there is both an increase in hepatic very low density lipoprotein (VLDL) triglyceride secretion and an increase in HMG-CoA reductase activity. Therefore, it is possible that two opposing regulatory events occur with HMG-CoA reductase and cholesterol synthesis when lipoproteins containing both cholesterol and triglycerides are taken up by the liver. The degree of inhibition of reductase observed could thus be the net result of the inhibitory effect of cholesterol and the stimulatory effect of fatty acids.

To test this hypothesis, the acute effects on hepatic HMG-CoA reductase of three different types of lipoprotein particles containing markedly different ratios of triglyceride and cholesterol were studied in the perfused liver system and in vivo. In addition, the effects of these lipoproteins on hepatic VLDL secretion by the perfused liver were investigated.

MATERIALS

Animals

Male Sprague-Dawley rats were used in all experiments. They were housed in a windowless room illuminated between 7 AM and 7 PM and fed a commercial rat chow for at least 7 days prior to use. Retired breeders, fed an artherogenic diet (7) for 3 to 4 weeks, were used as plasma donors for cholesterol-rich very low density lipoproteins. Liver donors weighed 120–160 g, lymph donors weighed 300–400 g, and the rats used in in vivo experiments weighed 180–220 g. Retired breeders were used for lipoprotein remnant preparation as described previously (8).

Chemicals

D,L 3-Hydroxy-3-methyl-[3-^{14}C]glutaryl coenzyme A (40–60 mCi/mmol), *R,S*[5-^{3}H]mevalonic acid (dibenzyldiethylenediamine salt, 1–5 Ci/mmol) and Aquasol were obtained from New England Nuclear; oleic acid, NADP, glucose-6-phosphate dehydrogenase, bovine serum albumin, glucose-6-phosphate (disodium salt), triolein (practical grade), and L-α-phosphatidylcholine (soybean, purity approx. 50%) were purchased from Sigma. Sodium taurocholate and dithiothreitol were from Calbiochem. 3-Hydroxy-3-methylglutaryl coenzyme A was from P.L. Biochemicals, and the triglyceride assay kit was from Boehringer. Eagle's basal medium containing Eagle's salts and L-glutamine was from Grand Island Biochemical Company. All other reagents were analytical grade.

METHODS

Lipoprotein preparation

Mesenteric lymph chylomicrons were prepared as described previously (9). A silastic catheter was inserted into the mesenteric lymph duct of a 300–400 g rat. Twenty-four hr later a solution of one whole egg dispersed in 125 ml of normal saline was continuously infused through an intragastric catheter at a rate of 1.8 ml/hr. The lymph was filtered through ten layers of gauze, layered under 0.9% NaCl, and centrifuged in a Beckman SW 41 rotor at 1.1×10^5 *g* for 45 min. The supernatant layer was separated with a tube slicer and resuspended in a small volume of buffered normal saline.

Chylomicrons rich in triglycerides were prepared by a slight modification of the method of Bennett-Clark (10). A sonicated emulsion containing 7 g of triolein, 4 g of L-α-phosphatidycholine, 0.1 g of sodium taurocholate, and 0.5 g of Tween 80 in 100 ml of water was infused intragastrically at a rate of 1.8 ml/hr. The collected lymph was processed for chylomicrons as described above.

Very low density lipoproteins rich in cholesterol were prepared as previously described (7). A group of 20–30 retired breeders was fed a diet containing 5% lard, 1% cholesterol, 0.1% propylthiouracil, and 3% taurocholic acid for a period of at least 3 weeks. The rats were killed between 1300 and 1500 hr by aortic puncture and the serum was separated from red blood cells by low speed centrifugation. Serum VLDL was collected by adjusting the density to 1.019 g/ml and centrifuging at 140,000 *g* for 16 hr in a Beckman SW 41 rotor. A tube slicer was used to collect the top layer containing the VLDL.

Lipoprotein remnants were prepared in eviscerated rats as previously described (8). Native (precursor) lipoproteins were injected at a rate of 0.6 ml/min through a femoral vein catheter. The injected lipoproteins contained no more than 8 mg of cholesterol in chylomicrons or cholesterol-rich VLDL and 2 mg of cholesterol in triglyceride-rich chylomicrons and were allowed to circulate for 3 hr. Cholesterol-rich VLDL was allowed to circulate for 30 min. The animals were exsanguinated by aortic puncture and blood was allowed to clot for 2 hr. Plasma was separated from red blood cells by low speed centrifugation. Serum contain-

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ing chylomicron remnants was centrifuged at 2.0×10^5 g for 120 min in a Beckman SW 41 rotor. The lipoprotein remnants were separated with a tube slicer and resuspended in 0.9% NaCl. Serum containing VLDL remnants was centrifuged at 2×10^5 g for 16 hr at density 1.019 g/ml and lipoprotein remnants were separated and resuspended. Triglyceride, cholesterol, and cholesteryl ester contents were determined on all lipoprotein preparations.

Lipoproteins that were to be radioiodinated were chromatographed on Bio-Gel A-50 M (BioRad Laboratories, Richmond, CA) to remove heme products, traces of albumin, and other impurities (11). The lipoproteins were resuspended in 0.15 M NaCl, 0.01% EDTA, pH 7.4, adjusted to the appropriate density with KBR, and recentrifuged. They were labeled with ^{125}I (sodium iodide, New England Nuclear, Boston, MA) as previously described (12) according to the method of McFarlane (13) as modified by Bilheimer, Eisenberg, and Levy (14). Not more than 2 ml of lipoproteins containing 1.0–8.0 mg of protein were dialyzed against a 1.0 M glycine buffer, pH 10.0, overnight. One to 3 mCi of carrier free Na-^{125}I was added to the lipoprotein followed by rapid injection of ICl solution (1:10, mol:mol, iodine:protein ratio assuming a molecular weight of 300,000 for the protein constituents). Unbound iodine was removed by passing the labeled lipoproteins through a small column of 2% agarose followed by dialysis at 4°C against the NaCl, EDTA buffer with at least four changes of buffer. The distribution of radioiodine was determined by measuring the percentage of ^{125}I that was trichloroacetic acid (TCA)-precipitable (12).

In vivo ^{125}I-labeled lipoprotein disappearance studies

^{125}I-Labeled lipoproteins of high specific activity were injected into a catheter that had been previously inserted into the inferior vena cava through the femoral vein. The total injected mass was less than 0.01 mg of protein. Control and experimental animals were of equivalent weight (300 ± 20 g). The catheter was rapidly flushed with 0.9% NaCl. Blood samples (0.1 ml) were drawn at 2-min intervals for 15 min then at 10-min intervals up to 45 min. Following the collection of each blood sample, the catheter was washed with 0.1 ml of 0.9% NaCl. Red blood cells were removed by centrifugation. A small sample was counted and, after addition of a carrier protein, a 10% TCA precipitation was performed on each time point. The kinetic parameters (slope, Y intercept, and $t_{1/2}$) were calculated using the MLAB curve fitting program (15).

Liver perfusion

After pentobarbital anesthesia, liver perfusion was performed in situ by the method of Mortimer (16) as described previously (5). Portal blood flow was interrupted at most for a few seconds during preparation of the organ. Oxygenation was accomplished with a silastic coil (Dow Corning Corp., Midland, MI) as described by Hamilton et al. (17). The entire perfusion system except the artificial lung was siliconized as described by Hamilton et al. (17). The plasma-free perfusate (40 ml, pH 7.4) contained 22% washed human red cells in Eagle's basal medium supplemented with 3 g of bovine serum albumin and 100 mg of glucose for each 100 ml of medium. Hemolysis during the perfusion was negligible. The perfusate circulated at 1.1 ml per min per g liver. Oxygenation prior to and during perfusion was maintained using a gas mixture containing 95% O_2 and 5% CO_2. Viability of the liver was judged at 5–10 min by color, O_2 extraction, and the absence of perfusate loss. This was monitored throughout the perfusion. Previously published data from this laboratory have shown excellent viability for 3 hr of perfusion (5) and unpublished evidence shows that rates of VLDL secretion and bile flow were linear for 4–5 hr. Immediately prior to establishing recirculation, a small lobe of liver weighing between 0.2 and 0.3 g was ligated, excised, and microsomes were prepared. Perfusions were carried out as outlined in **Fig. 1.** Lipoprotein remnants were added and allowed to circulate for 15–20 min. This lipoprotein-containing perfusate was then washed out over 5–10 min with approximately 60–80 ml of lipoprotein-free perfusate. When residual lipoproteins were all removed, recirculation was re-established. The effectiveness of the washout was determined by the absence of triglycerides in the perfusate. All three types of lipoprotein remnants were taken up with comparable efficiency. In most instances about 50% of the added material was removed in 15–20 min. The rates of removal were consistent with what was predicted by previous publications from this laboratory (18, 19). The perfusion was continued for an additional 2 hr and hourly samples were taken for triglyceride determination. At the end of the perfusion, the final perfusate was collected, its hematocrit and volume were recorded, and the red cells were removed by low speed centrifugation. The liver was removed, weighed, chilled in 0.9% NaCl, and a portion was taken for preparation of microsomes. A sample of the perfusate was taken for triglyceride determination and the remainder was used to estimate very low density lipoprotein secretion after isolation of VLDL by ultracentrifugation.

In one group of experiments instead of lipoproteins, oleic acid complexed to albumin was added to the perfusate at concentrations of 0.25 mM, 0.5 mM, or 1 mM. During the entire perfusion period oleic acid was added continuously to maintain a constant concentration (20). Free fatty acid uptake was equated with the amount infused (2.5, 5, and 10 mg/g liver per 2 hr). The re-

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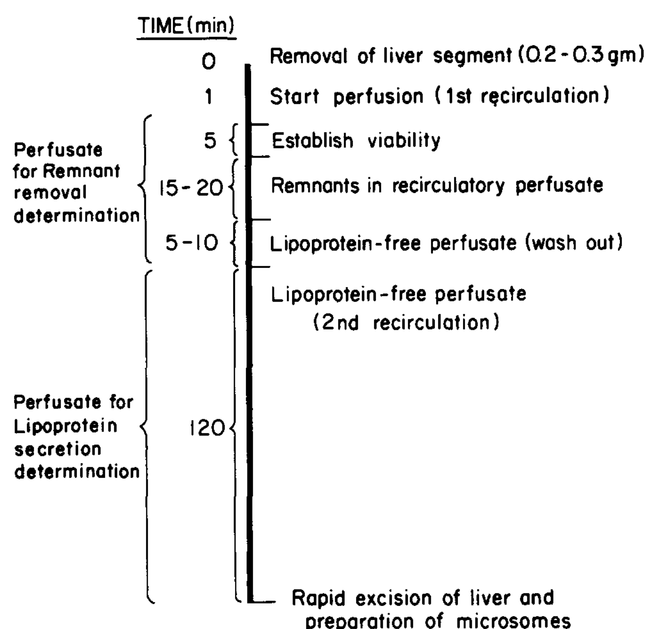

Fig. 1. Protocol for liver perfusion studies. Prior to starting a recirculating liver perfusion, a small lobe of the rat liver was resected, microsomes were prepared, and HMG-CoA reductase activity was determined. The perfusion was started by connecting inferior vena cava and portal veins to a recirculating system as described (5) previously. After viability was established lipoprotein remnants were added and allowed to circulate for 15–20 min. The remaining remnants were washed out with an excess of lipid-free perfusate consisting of Eagle's medium containing 3% fatty acid-poor bovine albumin, and 100 mg glucose/100 ml, pH 7.4. The efficiency of the washout was determined by measuring perfusate triglyceride. When all remnants were removed, recirculation with a lipid-free medium was continued for an additional 2 hr.

mainder of the perfusion protocol was performed as described.

In vivo experiments

The femoral vein of anesthetized rats weighing 160–220 g was cannulated with polyethylene tubing (PE10, Clay-Adams). A lipoprotein bolus containing 7 mg of cholesterol and various amounts of triglycerides was injected through the cannula into the experimental rats. Control rats were infused with an equal volume of normal saline (pH 7.4) The animals were killed 2 hr after injection and their livers were removed for analysis.

Preparation of microsomes

The liver samples were blotted dry, weighed, and homogenized in 5 volumes of Buffer A (0.1 M sucrose, 0.05 M KCl, 0.025 M KH_2PO_4, 0.03 M EDTA, pH 7.4) by three strokes at moderate speed in a glass-Teflon Potter-Elvejhem homogenizer. The homogenate was centrifuged for 15 min at 10,000 *g* to sediment unbroken cells, nuclei, mitochondria, and lysosomes. The supernatant was centrifuged for 60 min at 105,000 *g* to sediment microsomes. The 105,000 *g* supernatant was discarded and the pellet was resuspended in Buffer A. The 105,000 *g* centrifugation was repeated and the final pellet was resuspended in Buffer A. The microsomes were assayed for protein content and HMG-CoA reductase activity. In the in vivo experiments, livers were divided and microsomes were prepared in Buffer A and in Buffer A containing 50 mM sodium fluoride. The final resuspension was done in Buffer A alone.

Assay of HMG-CoA reductase activity

HMG-CoA reductase was assayed essentially as described previously (21), except that a total assay volume of 0.5 ml was used containing 0.25–1.0 mg of microsomal protein.

Chemical assays

Protein was determined by the method of Lowry et al. (22) or by the biuret method (23) using bovine serum albumin as a reference standard. Phospholipid phosphorus was determined colorimetrically by the method of Bartlett (24). Triglycerides were assayed enzymatically by the method of Eggstein and Kreutz (25) using the triglyceride assay kit from Boehringer. Total cholesterol was determined by gas–liquid chromatography as previously described (26).

RESULTS

Effect of lipoprotein composition on hepatic HMG-CoA reductase in liver perfusions

The livers of rats killed 3 hr (10 AM) after the onset of the light phase were perfused with lipid-free perfusate. HMG-CoA reductase activity was determined before and after 2.5 hr of perfusion. As had been reported previously (5), enzyme activity increased during the period of perfusion rising from 0.16 ± 0.03 to 0.28 ± 0.04 nmol MVA min^{-1} mg $protein^{-1}$ ($P < 0.005$, n = 12). It had been shown previously (5) that this increase was inhibited by the presence of cholesterol in the perfusate either in a phospholipid dispersion or in chylomicron remnants (5, 9). Despite addition of increasing amounts of cholesterol in chylomicron remnants, reductase activity remained near the initial level (not shown). However, in the intact animal, cholesterol feeding suppresses hepatic HMG-CoA reductase activity by greater than 50% within a few hours. Thus, it was surprising that cholesterol carried in chylomicron remnants was not effective in suppressing reductase activity below the initial value. To explain this observation it was hypothesized that the amount of concommitantly administered triglyceride might be a factor in determining the response of hepatic HMG-CoA reductase to a given amount of cholesterol in remnant lipoproteins.

To test this hypothesis, lipoprotein remnants of various triglyceride:cholesterol ratios were prepared. The

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TABLE 1. Composition of precursor lipoprotein remnants

	Triglyceride	Total Cholesterol	Free Cholesterol	Phospholipid	Protein[a]
			% dry weight		
Precursor lipoproteins					
Chylomicrons	84.3	2.4	0.7	12.2	0.8
Triglyceride-rich chylomicrons	90.2	0.4	0.2	8.1	1.1
Cholesterol-rich VLDL	22.5	35.1	7.3	31.2	9.7
Lipoprotein remnants					
Chylomicrons	67.2	7.4	ND[b]	17.6	6.6
Triglyceride-rich chylomicrons	83.5	0.9	ND	6.5	5.9
Cholesterol-rich VLDL	7.1	49.3	7.7	31.4	11.7

[a] Lipoprotein protein content was determined according to Lowry et al. (20) following Bio-Gel A50m chromatography.
[b] ND, not determined.

Chylomicrons were prepared from lymph obtained from lymph fistula rats that were fed a mixture of egg in 120 ml of 0.9% NaCl through an intragastric catheter (9). Triglyceride-rich chylomicrons were obtained similarly except that these rats were fed a mixture of triolein and lecithin (10). Cholesterol-rich VLDL was obtained from rats fed a standard atherogenic diet (7). Lipoprotein remnants were prepared in eviscerated rats as described in Methods. Results shown are the average of determinations on two separate preparations of lipoproteins in which the complete chemical composition was done. They are consistent with the triglyceride:cholesterol ratios that were determined on every lipoprotein batch used.

composition of the three types of remnants studied is given in **Table 1.** Cholesterol-rich VLDL remnants were used because a more extreme alteration of the triglyceride:cholesterol ratio could be obtained with these particles than with any chylomicron remnant preparation. The remnants were perfused for 15–20 min, the perfusate was removed, the liver was washed out with a lipoprotein-free perfusate, and then recirculation with a lipoprotein-free perfusate was established for 2 additional hours (see Fig. 1). The perfusates were collected to determine the amount of remnants removed. The cholesterol removed per g of liver in these experiments averaged 0.74 ± 0.05 μmol for normal chylomicron remnants, 0.88 ± 0.18 μmol for triglyceride-rich chylomicron remnants, and 0.96 ± 0.16 μmol for cholesterol-rich VLDL remnants. These amounts were not significantly different.

The three types of remnants had profoundly different effects on hepatic HMG-CoA reductase activity (**Table 2**). Remnants prepared from the standard chylomicrons prevented the increase in HMG-CoA reductase activity that occurs during lipid-free liver perfusions, but as noted before, they did not suppress reductase activity below the pre-perfusion level. In contrast, cholesterol-rich particles not only prevented the rise in reductase activity, but they decreased reductase activity to a level significantly below the initial value. The decrease of 25% over a 2.5-hr period is con-

TABLE 2. Effect of lipoprotein remnants on HMG-CoA reductase in the perfused liver

	Number	HMG-CoA Reductase Pre-perfusion	HMG-CoA Reductase Post-perfusion	Pre- vs Post-perfusion	Post-perfusion Control vs Lipoprotein Post-perfusion
		nmol MVA × mg prot^{-1} × min^{-1}			
Control (lipoprotein-free)	12	0.16 ± 0.03	0.28 ± 0.04	$P < 0.005$	
Chylomicron remnants	14	0.16 ± 0.03	0.17 ± 0.02	NS[a]	$P < 0.001$
Triglyceride-rich chylomicron remnants	9	0.16 ± 0.03	0.33 ± 0.06	$P < 0.005$	NS
Cholesterol-rich VLDL remnants	6	0.16 ± 0.03	0.12 ± 0.01	$P < 0.02$	$P < 0.001$

[a] NS, not statistically significant.

Livers were perfused as described in Methods. At the beginning of the perfusion, a lobe of the liver was removed and microsomal HMG-CoA reductase activity was determined. Chylomicron remnants, triglyceride-rich chylomicron remnants, and cholesterol-rich VLDL remnants were prepared as described. The amounts of cholesterol removed by the livers from these different lipoprotein remnants were comparable. After 15–20 min, lipoprotein remnants were washed out and the perfusion was continued for 2 hr. Hepatic HMG-CoA reductase activity was then determined in freshly prepared microsomes.

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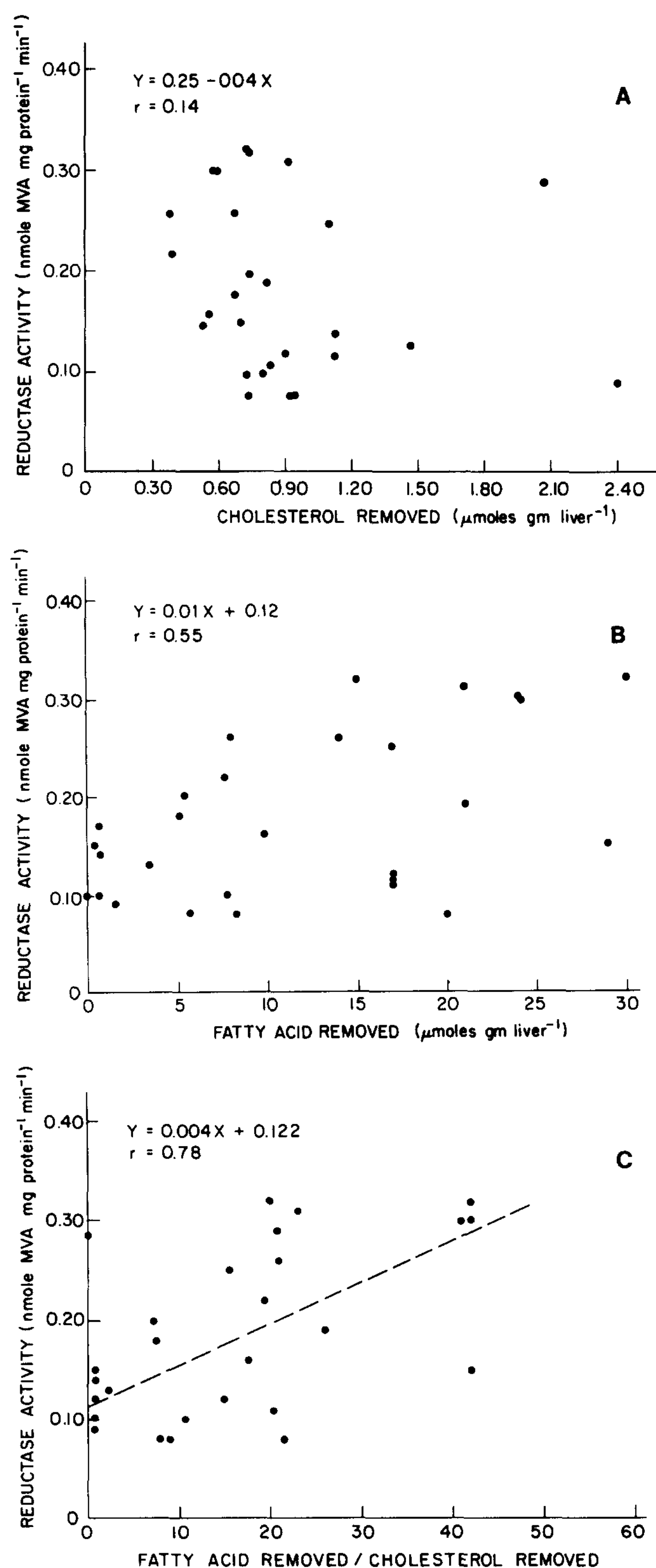

Fig. 2. Relationship between hepatic HMG-CoA reductase activity and lipid uptake from lipoprotein remnants by perfused rat livers. The techniques of liver perfusion and preparation of remnants were as described in the legend to Fig. 1 and in Methods. The removal of remnant cholesterol was calculated as the difference between the amount added and the amount of cholesterol recovered in the washout volume. Each point represents a separate experiment. Table I gives the relative weight composition of the lipoprotein remnants used. A: HMG-CoA reductase activity as a function of cholesterol removed. B: HMG-CoA reductase activity as a function of triglyceride removed. C: HMG-CoA reductase activity as a function of the ratio of the amount of fatty acid:cholesterol removed. Data from the same 29 perfusions were used to calculate the regressions although in some instances the points did not fit on the axis used for the illustration.

sistent with the rate of suppression expected if synthesis of HMG-CoA reductase had ceased. The triglyceride-rich chylomicron remnants, on the other hand, failed to prevent the increase in reductase activity and in fact actually enhanced it above the level achieved with control (lipid-free) perfusions, although this did not reach statistical significance. It must be stressed that these striking differences in reductase response were obtained after removal by the liver of very similar amounts of cholesterol.

When HMG-CoA reductase activity was plotted as a function of either the amount of cholesterol (**Fig. 2a**) or the amount of triglyceride removed (Fig. 2b), the correlations were poor. However, if reductase activity was plotted as a function of the molar ratio of the amount of fatty acids removed to that of cholesterol removed, a distinct relationship was observed (Fig. 2c). Thus, the more cholesterol delivered per unit of fatty acid the more suppressive the particle is likely to be. This demonstrates the importance of both lipids in the physiologic regulation of HMG-CoA reductase.

The lipid composition of the particles remaining in the perfusate after the initial perfusion period was the same as the particles added to the perfusate. This is consistent with previous observations suggesting that the particles are removed as a unit (9) and that neither the triglycerides nor the cholesterol were removed preferentially by the liver. Because cholesterol removal from the different remnant types was comparable, changes in the ratio of triglycerides:cholesterol determined the response of HMG-CoA reductase activity in confirmation of our initial hypothesis.

The effect of remnant lipoprotein composition on hepatic VLDL secretion by the perfused liver

It is well-established that free fatty acid influx is a principal determinant of the rate of VLDL secretion by the liver (18). VLDL secretion must result in cholesterol efflux. Thus, fatty acid infusion might be expected to stimulate the activity of HMG-CoA reductase to compensate for this loss. This sequence of events was in fact shown to occur by Goh and Heimberg (6). We hypothesized that the effects on HMG-CoA reductase observed in this study due to altering the lipoprotein triglyceride:cholesterol ratio might be explained by changes induced in VLDL secretion as a consequence of different fatty acid loads. Accordingly, the effects of comparable

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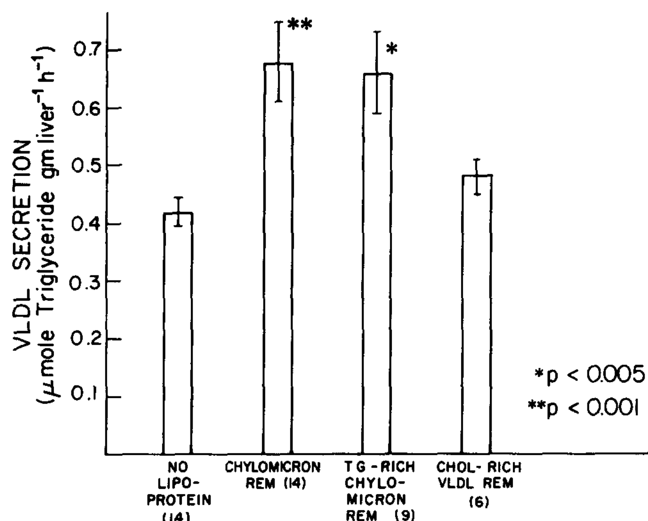

Fig. 3. Effect of lipoprotein remnants on hepatic very low density lipoprotein triglyceride secretion rate after comparable cholesterol uptake by the perfused rat liver. VLDL triglyceride secretion in the last 2 hr of recirculation with a lipid-free medium (see protocol, Fig. 1) was determined by enzymatic assay. Lipoprotein remnant removal was calculated as described in Fig. 2. The cholesterol removed per g liver averaged 0.74 ± 0.05 μmol for standard chylomicron remnants, 0.88 ± 0.18 μmol for triglyceride-rich chylomicron remnants, and 0.96 ± 0.16 μmol for cholesterol-rich VLDL remnants. These amounts were not significantly different. *, Different from control $P < 0.005$; **, different from control $P < 0.001$.

amounts of infused free fatty acids on this parameter were also studied. Chylomicron remnants stimulated the rate of VLDL secretion as compared to that observed with a lipid-free perfusion (**Fig. 3**). The degree of stimulation was proportional to the amount of triglyceride removed and the magnitude of the stimulation was similar to that induced by a comparable amount of free fatty acid (**Fig. 4**). Triglyceride-rich chylomicron remnants also stimulated VLDL secretion (Fig. 3), but the magnitude of the stimulation was not as great as would have been expected from the amount of fatty acid removed. As predicted by the hypothesis, the cholesterol-rich VLDL remnants did not stimulate VLDL secretion above the control level (Fig. 3).

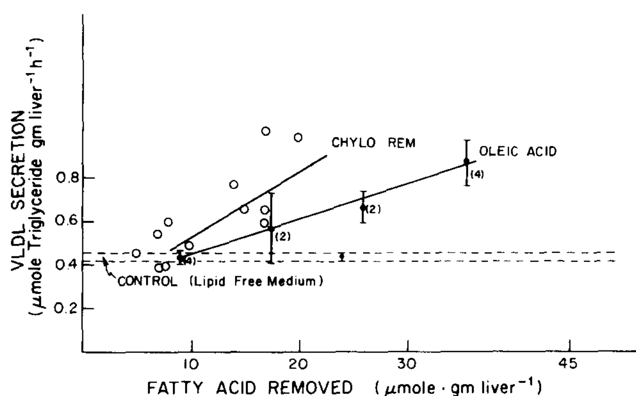

Fig. 4. The effect of fatty acids, either as chylomicron remnant triglycerides or oleic acid, on hepatic VLDL secretion in perfused rat livers. Oleic acid complexed to bovine serum albumin was infused continuously throughout the entire perfusion period. VLDL secretion and composition were determined on the last 2 hr of the recirculation perfusate. Data on VLDL composition of the high and low oleic acid infusion rates are given in Table 3. VLDL secretion following removal of normal chylomicron remnants was plotted as a function of the amount of fatty acid contained in remnants as triglycerides removed during the perfusion carried out as described in the legend to Fig. 1 and in Methods.

The compositions (triglyceride:cholesterol ratios) of the newly secreted VLDL particles isolated after perfusions with normal chylomicron remnants or cholesterol-rich VLDL were comparable to those secreted by livers perfused with lipid-free medium (**Table 3**). However, perfusion of triglyceride-rich chylomicron remnants or high dose oleic acid infusions resulted in secretion of VLDL particles with a significantly increased triglyceride:cholesterol ratio (Table 3).

Effects of native (precursor) lipoproteins on hepatic HMG-CoA reductase activity in vivo

In the intact animal a number of factors are involved in determining the amount and type of lipid reaching the liver. Therefore, it was important to ascertain whether the effects of various lipoproteins studied in the perfused liver system would be expressed in the regulation of hepatic HMG-CoA reductase activity in vivo.

First, it was necessary to show that the three different types of lipoproteins had similar disappearance rates in vivo. Trace amounts of ^{125}I-labeled normal chylomicrons, triglyceride-rich chylomicrons, or cholesterol-rich VLDL were injected into the femoral veins of rats and disappearance of the label from TCA-percipitable serum proteins followed. The $t_{1/2}$ for the first phase of disappearance was calculated by computer fit to the equation $D(t) = Ae^{Ct} + Be^{Dt}$. The initial $t_{1/2}$ calculated

TABLE 3. Effect of lipoprotein remnants and oleic acid infusions on the composition of hepatic VLDL secretion by perfused livers

		Triglyceride:Cholesterol Weight Ratio
Control (lipoprotein-free)	(10)	9.8 ± 0.6
Chylomicron remnants	(13)	10.0 ± 0.6
Triglyceride-rich chylomicron remnants	(7)	14.9 ± 1.2[a]
Cholesterol-rich VLDL remnants	(6)	8.9 ± 0.7
Oleic acid 0.5 mM	(4)	8.8 ± 1.0
Oleic acid 1.0 mM	(4)	14.8 ± 1.0[a]

[a] Different from control at $P < 0.001$.

Perfusions were performed as described in Table 2. VLDL (d < 1.006 g/ml) was separated from the collected final perfusate by ultracentrifugation and was resuspended in saline. Triglyceride and cholesterol contents were determined as described in Methods and the ratios were computed from the relative masses. Number of determinations in parentheses.

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TABLE 4. Effects of lipoproteins in vivo on hepatic HMG-CoA reductase

	Number	HMG-CoA Reductase − NaF	% Control	HMG-CoA Reductase + NaF	% Control
		nmol MVA × mg prot⁻¹ × min⁻¹		*nmol MVA × mg prot⁻¹ × min⁻¹*	
Control	18	0.18 ± 0.03	100	0.06 ± 0.01	100
Chylomicrons	9	0.28 ± 0.04	157[a]	0.05 ± 0.01	83
Triglyceride-rich chylomicrons	8	0.33 ± 0.06	187[b]	0.05 ± 0.01	83
Cholesterol-rich VLDL	9	0.05 ± 0.01	30[a]	0.02 ± 0.01	33

[a] Different from control at $P < 0.001$.
[b] Different from control at $P < 0.005$.
Rats were injected with different lipoproteins through a femoral vein catheter. Each experimental animal received a bolus of lipoproteins containing 7 mg of cholesterol and varying amounts of triglycerides. Control rats received comparable volumes of buffered saline (pH 7.4). After 2 hr, the animals were killed, the livers were removed, microsomes were prepared in buffer with or without 50 mM NaF, and HMG-CoA reductase activities were determined.

from C for normal chylomicrons was 2.4 min; for triglyceride-rich chylomicrons, 3.3 min; and for cholesterol-rich VLDL, 0.5 min. This initial disappearance has been shown previously to be due largely to hepatic removal for both chylomicrons and hypercholesterolemic VLDL (19) and the same is presumably true for the triglyceride-rich particles. Thus, virtually all of the lipoproteins should have been removed during the initial period of a 2-hr experiment.

To study the effects of the composition of these lipoproteins on hepatic HMG-CoA reductase, 7 mg of cholesterol as either normal chylomicrons or triglyceride-rich chylomicrons or cholesterol-rich VLDL was injected into the femoral vein. After 2 hr, the animals were killed and hepatic HMG-CoA reductase activity was determined. Both the standard and triglyceride-rich chylomicrons stimulated HMG-CoA reductase activity (**Table 4**). The triglyceride-rich chylomicrons had a more pronounced effect on HMG-CoA reductase activity which increased to 187% of control. The standard chylomicrons increased reductase activity to 157% of control. In contrast, the cholesterol-rich particles inhibited HMG-CoA reductase activity to 30% of control. These data are entirely compatible with the results obtained in the perfused liver studies and further support the hypothesis that the acute effect of lipoproteins on hepatic HMG-CoA reductase activity is a function of both the triglyceride and cholesterol content.

When microsomes were prepared in the presence of 50 mM NaF, HMG-CoA reductase activity was the same in control animals and in those receiving either normal or triglyceride-rich chylomicrons (Table 4). From these data it appeared that the total amount of enzyme activity had increased as well as the amount that could be activated. In contrast, hepatic microsomes that were prepared in 50 mM NaF from animals receiving cholesterol-rich VLDL had 33% (Table 4) of the control value suggesting that the total amount of functionally active enzyme had been decreased in these animals.

DISCUSSION

The decrease in cholesterol synthesis in the liver which occurs in vivo in response to cholesterol feeding (27) or intravenous injection of chylomicrons (2) has been well established. These phenomena generally have been examined only at relatively long intervals (6–48 hr) after administration of the stimulus. Because the half-life of HMG-CoA reductase activity, the rate-limiting enzyme for cholesterol biosynthesis, is 1–4 hr (3), and the activity can respond profoundly to changes in sterol content of the liver within 1 hr, (28) it should be possible to regulate hepatic reductase activity acutely with lipoprotein cholesterol. The observation (9) that liver perfusion with chylomicron remnants would suppress the rise in reductase activity ordinarily induced by perfusion with sterol-free medium but did not inhibit reductase activity below the baseline value as had been observed for 25-hydroxycholesterol, a cholesterol analogue (29), suggested that the regulation of HMG-CoA reductase by lipoproteins might be complex. Similar findings have been reported for cultured hepatocytes (30). The observations reported here, that increasing the amounts of cholesterol carried as chylomicron remnants did not result in any further inhibition of reductase activity, also supported this suggestion.

The results of Goh and Heimberg (6) showing that HMG-CoA reductase activity and VLDL secretion were increased by fatty acid infusions suggested to us that the response of HMG-CoA reductase to lipoproteins might be the net result of two opposing phenomena; one, the uptake of cholesterol and two, the uptake of triglycerides, which are a rich source of fatty acids. If this is

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correct, we reasoned that lipoproteins of different cholesterol and triglyceride compositions should have different effects on hepatic HMG-CoA reductase activity acutely, and that these effects should reflect the needs of the cell for cholesterol.

To study this hypothesis, we chose to prepare lipoproteins of three very different triglyceride:cholesterol ratios. These were the standard chylomicrons that have been previously used in this laboratory and by others (2, 9, 12, 18, 19, 31) with a triglyceride:cholesterol weight ratio of 35, triglyceride-rich chylomicrons with a ratio of 225, and cholesterol-rich VLDL with a ratio of 0.64. Despite the differences in cholesterol and triglyceride contents, the precursor lipoproteins were shown to be reasonably comparable in their disappearance rates in vivo, suggesting that it would be possible to interpret any differences in their effects as being due mainly to the different triglyceride:cholesterol ratios. Using remnants prepared from these lipoproteins and the perfused liver system, strong support for this hypothesis was obtained. Despite the delivery to the liver of comparable amounts of cholesterol, particles rich in triglycerides and poor in cholesterol stimulated HMG-CoA reductase while those poor in triglycerides and rich in cholesterol inhibited reductase below the pre-perfusion level. Finally, particles of intermediate composition were able to maintain HMG-CoA reductase at its base line level. The magnitude of the changes induced by the lipoproteins was modest but consistent with what would be expected over a 2.5-hr period for an enzyme with a half-life in the 1–4 hr range, especially considering the requirement for intracellular processing of cholesteryl ester which is slow relative to uptake (18).

Because in vivo there are a large number of other metabolic and hormonal factors that could affect HMG-CoA reductase activity, it was of interest to compare the acute effects of the various lipoproteins administered in vivo with those of their remnants in vitro. In fact there was good agreement. Triglyceride-rich lipoproteins stimulated and cholesterol-rich lipoproteins suppressed HMG-CoA reductase activity within 2 hr of administration. Interestingly, the standard chylomicrons stimulated reductase activity in vivo rather than leaving it unchanged as was observed in the perfused liver system. A possible explanation for this observation is that the large bolus of lipoprotein triglyceride administered might not be hydrolyzed in vivo as completely before uptake of the particle by the liver as it is with the 3 hr recirculation in an eviscerated rat used to prepare remnants in vitro. Thus in vivo these remnants could deliver more triglyceride per particle to the liver than was delivered by the standard chylomicron remnants prepared for use in the liver perfusion studies. This is consistent with the observation (31) that large chylomicrons are not as effective in suppressing cholesterol biosynthesis as are smaller chylomicrons.

Comparison of the reductase activities obtained when microsomes were prepared in the presence and absence of 50 mM NaF suggested that the rise induced in vivo of reductase activity in response to normal or triglyceride-rich chylomicrons was due in part to activation of the enzyme while inhibition of reductase by cholesterol-rich VLDL involved a decrease in the amount of net enzyme activity. Cholesterol-induced changes in the activation state of reductase have been observed by some but not all investigators (32, 33). The mechanism of these modulations remains to be elucidated.

Taken together these results suggest that HMG-CoA reductase regulation involves effects on regulators that are sensitive not only to cholesterol influx into the cell but also to fatty acid (triglyceride) influx. One straightforward explanation for the effects of the different lipoproteins observed here was suggested by the work of Goh and Heimberg (6) in which they showed that VLDL secretion increased with increasing amounts of fatty acid infused. In the present study it was shown that remnants of triglyceride-rich and standard chylomicrons stimulated VLDL secretion as compared to the rate in lipid-free (control) perfusions or perfusions containing cholesterol-rich, triglyceride-poor VLDL remnants. When chylomicron remnants were added to the perfusate, the rate of VLDL secretion observed was comparable with that induced by similar amounts of infused oleic acid. This suggested that over the 2-hr period, much of the triglyceride from remnants was hydrolyzed and that the free fatty acids were available for synthesis and secretion of lipoprotein triglycerides. The triglyceride-rich chylomicron remnants did not have as potent an effect on VLDL secretion as would have been predicted from the amount of fatty acids theoretically available. It is possible that either the triglycerides were not completely hydrolyzed during the period of perfusion or that some other factor such as cholesterol or apoprotein availability may have become rate-limiting. These effects of remnant triglyceride content on VLDL production provide a very plausible explanation for the response of HMG-CoA reductase because it has been established (6, 34) that concommitant with increased VLDL secretion, there is a stimulation of cholesterol biosynthesis. Presumably this occurs because of the need for cholesterol in the formation of lipoprotein particles. The same phenomenon may occur in vivo where a postprandial rise in VLDL has been reported (28).

The other components of the remnants may well also have effects on hepatic lipid metabolism. Thus, the particular phospholipid and glyceride composition of a particle may also affect metabolic responses of the liver. It is less likely that the apoprotein components will affect

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metabolism other than by determining the rate of particle uptake because most evidence suggests that this component is metabolized to free amino acids and small peptides. However, a direct effect of the apoproteins cannot be excluded. It is possible that the results reported here on the regulation of reductase may not be entirely applicable to the overall rate of cholesterol synthesis, which under some circumstances may be dissociated from the level of reductase activity (35).

The present results provide a simple, unifying mechanism to explain a number of discrepant observations in the literature. They explain how several dietary constituents can have prompt effects on hepatic cholesterol homeostasis via the remnant lipoprotein pathway and stress the importance of considering all dietary lipid components when trying to study or predict effects on hepatic, and thus whole body, cholesterol homeostasis. They also emphasize the fact that the hepatocyte has a substantial number of unique pathways of lipid metabolism that must be taken into account when studying regulation of cholesterol homeostasis in this organ.

In summary, we have demonstrated that a remnant lipoprotein can have two opposing effects on hepatic HMG-CoA reductase. The first, due to the cholesterol content, is inhibitory and the second, due to the triglyceride content, is stimulatory. Thus, the net effect on the short-term regulation of HMG-CoA reductase activity by a lipoprotein will depend upon the relative amounts of cholesterol and triglyceride present in the particles.

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