# Studies on the etiology of the hyperlipemia in rats fed an atherogenic diet[1]

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**Abstract** The etiology of the hyperlipemia which occurs in cholesterol- and fat-fed hypothyroid rats was investigated. In hyperlipemic rats the disappearance rate of $^{125}$I-labeled chylomicron remnants was markedly prolonged ($t_{1/2}$ of 13.1 ± 0.9 min versus $t_{1/2}$ of 2.1 ± 0.5 min in controls). However, the ability of isolated livers from these rats to remove remnants was not significantly different from that of control livers. This suggested that the delay in removal was due to an increase in the circulating remnant concentration without a removal defect. $^{125}$I-labeled VLDL from hyperlipemic rats was removed by isolated livers from normal rats at a rate similar to normal chylomicrons or hepatic VLDL and more slowly than normal remnants. This suggested that remnants were not the principal constituents of the VLDL in these animals. Moreover when these VLDL were injected into intact normal rats they were removed with a $t_{1/2}$ (5.5 ± 1.2 min) comparable to normal VLDL rather than remnants. Finally, livers from hyperlipemic rats were perfused and the composition of the VLDL secreted was examined. Compared to controls or animals fed propylthiouracil, these livers secreted a particle which was triglyceride-poor and cholesteryl ester-rich. Thus, the etiology of the hyperlipemia has several components. There is both expansion of the remnant pool and secretion of an abnormal lipoprotein.—**Kris-Etherton, P. M., and A. D. Cooper.** Studies on the etiology of the hyperlipemia in rats fed an atherogenic diet. *J. Lipid Res.* 180. **21:** 435–442.

**Supplementary key words** very low density lipoproteins · chylomicron remnants · lipoproteins · hypercholesterolemia

Lipoproteins which are isolated by ultracentrifugation at d < 1.006 g/ml are termed very low density lipoproteins and constitute a heterogeneous group of particles. In addition to particles of hepatic origin (1) there are similar lipoproteins of intestinal origin (2) referred to as small chylomicrons or intestinal VLDL. Recently it has been found that during the catabolism of chylomicrons and VLDL, triglyceride-depleted lipoproteins, designated remnants, are formed and they have a size, composition and density that place them in this ultracentrifugal fraction (3, 4). Hence an accumulation of lipoproteins with a d < 1.006 g/ml could occur by overproduction or delayed removal of any or all of these lipoprotein species. Moreover since both the conversion of VLDL and chylomicrons to remnants by lipoprotein lipase (5) and the hepatic removal of remnants are saturable processes (6–8) that occur at sites which could be shared by more than one particle type (9), an alteration in the metabolism of one particle can promptly have an effect on the metabolism of other particles.

Feeding a high fat, high cholesterol diet to hypothyroid rats results in profound hypercholesterolemia with a variable increase in plasma triglyceride (10). The bulk of the lipid accumulates in the d < 1.006 g/ml range (11). These lipoproteins have a mobility between β and pre β and have a very high cholesterol to triglyceride ratio (11). Previous studies of the etiology of this abnormality in rats have led to the suggestion that there is accumulation of chylomicron remnants (12). A similar conclusion was reached in a study of rabbits with diet-induced hyperlipemia (13). In both reports the possibility that there was a defect in the hepatic removal of chylomicron remnants was suggested. However, these studies used indirect techniques to assess remnant metabolism.

The present study further elucidates chylomicron remnant metabolism in the hyperlipidemic rat. These studies led us to conclude that although there was a delay in remnant disappearance there was not a primary defect in hepatic remnant removal. Moreover, the abnormal cholesterol content of the d < 1.006 g/ml particles was partially accounted for by the hepatic secretion of a triglyceride-poor, cholesterol-rich VLDL.

Abbreviations: VLDL, very low density lipoproteins; TCA, trichloroacetic acid; TMU, tetramethyl urea; PTU, propylthiouracil.

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## MATERIALS AND METHODS

### Animals

Sprague-Dawley strain rats (Simonson Laboratories, Gilroy, CA) were housed in a windowless room illuminated from 700 to 1900 hr and fed standard laboratory rat chow or an atherogenic diet for 3–4 weeks. The experimental diet containing chow, 5% lard, 1% cholesterol, 0.1% propylthiouracil (PTU) and 3% taurocholic acid was prepared according to Mahley and Holcombe (11). Retired breeders were used as plasma donors. They either maintained or lost 5–10% of their body weight on the experimental diet while controls increased their body weight by approximately 10%. Male weanlings were used as liver donors. They demonstrated considerable growth retardation on the experimental diet (105–135 g versus approximately 200–220 g for controls). Ether anesthesia was used for operative procedures and pentobarbital was used for liver perfusion.

### Preparation of chylomicrons, chylomicron remnants and VLDL

Mesenteric lymph was collected as previously described (14). An egg and saline solution (1 egg per 125 ml 0.9% NaCl) was continuously infused (2 ml/hr) intragastrically via a stomach catheter. Rats were placed in restraining cages and allowed free access to 0.45% NaCl solution and laboratory rat chow. The lymph was collected at room temperature in 125-ml flasks to which 20 mg of dipotassium EDTA and 4 mg of gentamycin (Schering Corporation, Kenilworth, NJ) had been added.

Chylomicrons were prepared as previously described (7); lymph was layered under 0.9% NaCl and spun in a Beckman model L2-65 ultracentrifuge in a SW 41 rotor at $1.1 \times 10^5 g$ for 45 min and the supernatant layer was separated using a tube slicer.

Chylomicron remnants were prepared in vivo using a modification of the Redgrave technique (3) as previously described (7). Chylomicrons containing 8 mg or less of cholesterol were injected into the femoral vein of eviscerated retired breeders and allowed to circulate for 3 hr, after which time blood was collected from the abdominal aorta. The red blood cells were separated, washed with 0.9% NaCl, and the plasma and saline washings were combined and centrifuged at $2.0 \times 10^5 g$ for 120 min in a SW 41 rotor. The lipoproteins were separated with a tube slicer and resuspended in 0.9% NaCl.

Plasma was obtained from non-fasted animals between 1400 and 1800 hr and VLDL was isolated using the technique of Havel, Eder, and Bragdon (15). Intestinal VLDL was obtained by adjusting the density of lymph to 1.006 g/ml after removing the chylomicrons and centrifuging as above. Hepatic VLDL was obtained from liver perfusions (see below).

### Radioiodination

All lipoproteins were chromatographed on Bio-Gel A-50m (BioRad Laboratories, Richmond, CA) to remove heme products, traces of albumin and other impurities (16). 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) according to the method of McFarlane (17) as modified by Bilheimer, Eisenberg, and Levy (18). 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 a rapid injection of ICl solution (1:1, mol:mol, iodine:protein ratio assuming a molecular weight of 300,000 for the protein constituents). Unbound iodide 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 the radioiodine was determined by measuring the percentage of $^{125}I$ which was extractable (lipid) by the method of Folch, Lees, and Sloane Stanley (19), TCA-precipitable (total protein), and TMU-precipitable (20) (apo B). In general, unlabeled lipoproteins were added first to act as carriers.

### Liver perfusion

Liver perfusions were performed as previously described (21). The perfusion was started with 80 ml of a lipoprotein-free perfusate. When liver viability was established, the perfusate was changed to one containing a radioiodinated lipoprotein. Each liver was perfused for 5 min with a single pass of perfusate. Following the perfusion, the liver was flushed with ice-cold 0.9% NaCl, removed, and weighed.

Perfusate samples were removed from the tube leading to the liver at the start and at the end of the experimental perfusion. Portions of liver and aliquots of perfusate were used to determine the amount of $^{125}I$ present.

For hepatic VLDL production studies, each liver was perfused with a protein-free recirculating perfusate for 2 hr. The liver was flushed, extirpated, and weighed. The hematocrit was measured and the red blood cells were removed by centrifugation. VLDL was isolated as described above, and analyzed for total protein, cholesterol (total and free), triglyceride, and phospholipid.

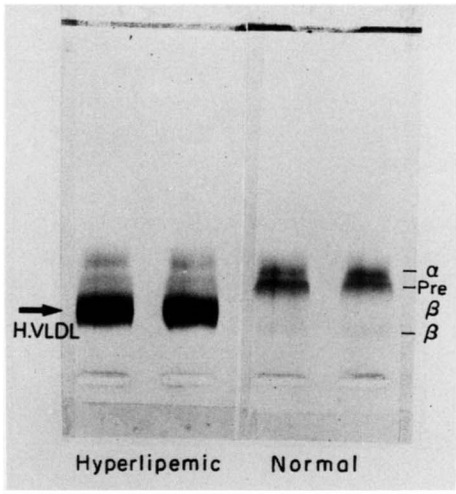


**Fig. 1.** Serum lipoprotein electrophoresis. Electrophoresis was performed on serum from normal and hyperlipemic rats by the method of Noble (21).

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

$^{125}$I-labeled lipoproteins of high specific activity were injected into a catheter which had been previously inserted into the inferior vena cava through the femoral vein. The total injected mass was less than 0.01 mg protein. Control and experimental animals were of equivalent weight (315 ± 10 g).

The catheter was rapidly flushed with 0.9% NaCl. Blood samples (0.1 ml) were drawn at 1-min intervals for approximately 15 min. Following the collection of each blood sample, the catheter was washed with 0.1 ml 0.9% NaCl. Red blood cells were removed by centrifugation and the radioactivity in the plasma was measured. After addition of unlabeled lipoprotein to serve as a carrier, the apoprotein B moiety of each plasma sample was precipitated with tetramethyl urea (20) and counted. Livers were removed from the rats given labeled VLDL, weighed, and the $^{125}$I radioactivity was determined. The kinetic parameters (slope, Y intercept and t½) were calculated using the MLAB curve fitting program (22).

TABLE 1. Composition of D < 1.006 lipoproteins from normal and hyperlipemic rats

| | Triglyceride | Cholesterol | Phospholipid | Protein |
|---|---|---|---|---|
| | *% by weight* | | | |
| Normal | 45 | 7 | 21 | 27 |
| Hyperlipemic | 5 | 48 | 25 | 21 |

Serum from six rats was pooled and the lipoproteins of d < 1.006 g/ml were isolated as described in Methods. Composition of this fraction was determined as described in Methods. Each number is the mean of two determinations.

### Chemical methods

Lipoprotein electrophoresis was done by the method of Noble (23). Plasma cholesterol was determined colorimetrically by the method of Leffler (24), perfusate cholesterol was determined by gas–liquid chromatography (25), and plasma triglyceride was assayed enzymatically (26). Protein was measured by the method of Lowry et al. (27). For triglyceride-rich samples 0.2 ml of a 5% deoxycholate solution was added to clarify the solution and the same amount was added to the standards. The total volume was 6.5 ml.

## RESULTS

### Serum lipoprotein composition

In animals fed the atherogenic diet there was a marked increase in serum cholesterol (hyperlipemic, 859 mg/dl versus control, 37 mg/dl). Lipoprotein electrophoresis is shown in **Fig. 1.** The predominant band migrated more slowly than normal pre β but did not quite have β mobility. The α band was unchanged. **Table 1** gives the composition of the particles isolated at d < 1.006 g/ml. There was a marked increase in the cholesterol content with a reciprocal decrease in the triglyceride content and little change in protein or phospholipid. **Table 2** shows the results of radioiodination of each of the lipoprotein types. In general these results are similar to those reported by others (10–12, 18).

TABLE 2. Distribution of $^{125}$I in lipoproteins

| | TCA-Precipitable | TMU-Precipitable | Lipid Soluble |
|---|---|---|---|
| | *% total counts* | | |
| Remnant | 82 | 54 | 18 |
| VLDL—normal serum | 90 | 26 | 10 |
| VLDL—hyperlipemic serum | 85 | 55 | 15 |
| VLDL—hepatic | 80 | 75 | 20 |

The distribution of $^{125}$I was determined following radiolabeling of various lipoproteins. The results shown are for representative experiments.

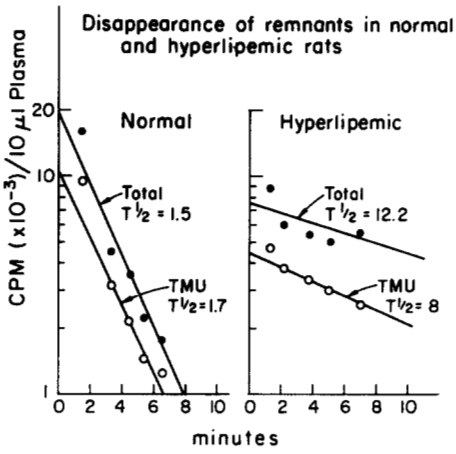


**Fig. 2.** Disappearance of remnants in normal and hyperlipemic rats. Radioiodinated chylomicron remnants were injected into normal or hyperlipemic rats. One minute was allowed for equilibration and then samples were taken at frequent intervals. The radioactivity in 10 µl of plasma was determined. The disappearance kinetics were estimated by computer (20). The line is plotted on a semi-log scale. A representative experiment is shown.

## Disappearance of remnants in normal and hyperlipidemic rats

Redgrave (12) reported previously that when chylomicrons were injected into rats and the disappearance of radiolabeled triglyceride and cholesterol followed the rate of removal of chylomicron remnants was markedly decreased in hyperlipemic rats. In the present investigation remnant removal was studied directly. A trace quantity of $^{125}$I-labeled chylomicron remnants was injected into normal or hyperlipemic rats and the disappearance of the label during the following 15 min was measured. Both total $^{125}$I and TMU-insoluble $^{125}$I disappearance was determined. Remnants always disappeared significantly more slowly in hyperlipidemic rats than in controls (**Fig. 2**).

By use of a curve fitting program an average t½ of 2.1 ± 0.5 min for control rats and 13.1 ± 0.9 min for the hyperlipemic rats was obtained. In this experiment it was assumed that the particles are removed only by the liver, that they are distributed only in the plasma space, and that equilibration is complete in one minute. These assumptions appear to be reasonable. Very similar results for t½ (2.2 and 9.9 min) were obtained for the disappearance of TMU-precipitable counts suggesting that apo B was removed at a similar rate to the other apoproteins. This is consistent with the concept that the particle is removed as a unit (6, 8). These results provide a direct measurement of the rate of removal of remnants during the postprandial period and demonstrate directly that there is delayed remnant removal in the hyperlipemic rat.

It has been suggested that the delay in remnant removal may be the result of an hepatic removal defect (13). However, since the removal process is saturable (7, 8) similar results would be obtained if there were an increased remnant concentration without a change in the removal mechanism.

## Removal of chylomicron remnants by livers of normal and hyperlipemic rats

To assess directly the hypothesis that there is a "remnant removal defect" in hyperlipemic rats, the ability of their livers to remove remnants was compared with that of normal rat livers. $^{125}$I-labeled remnants were perfused through livers for 5 min without recirculation. The amount removed was measured by counting a weighed liver slice. In each experiment livers from normal and hyperlipemic rats were perfused with portions of the same batch of lipoproteins on the same day. Although there was significant variability between batches of lipoproteins, results with a single batch of remnants were consistent. There was no discernible difference between the rate of uptake of remnants by livers from normal or from hyperlipemic rats. In **Fig. 3,** two experiments which are representative of the extremes of uptake rates are shown. The lack of difference was apparent whether the data were analyzed as a group or if the experiments were analyzed as pairs. As expected, the rate of uptake of remnants was always greater than the rate of uptake of chylomicrons at a comparable concentration (**Fig. 4**). Moreover, when liver sizes were compared, the hyperlipemic rats had significantly larger livers (6.9% versus 4.5% of body weight). Hence their total capacity to remove remnants was at least as great as that of normal

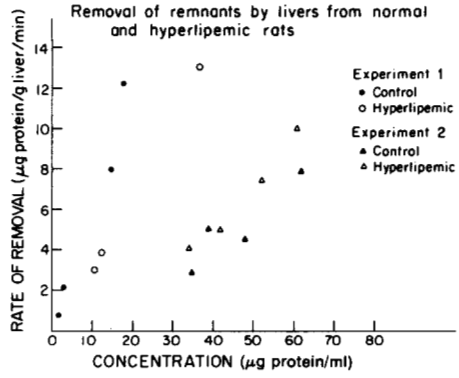


**Fig. 3.** Removal of remnants by livers from normal and hyperlipemic rats. Livers from normal or hyperlipemic rats were perfused in a single pass with a perfusate containing $^{125}$I-labeled chylomicron remnants and the rate of uptake by the liver was determined. Control and experimental livers were perfused on the same day with aliquots of the same lipoproteins. Two experiments representing the extremes of uptake rates are shown. Each point is the mean of two determinations from one liver.

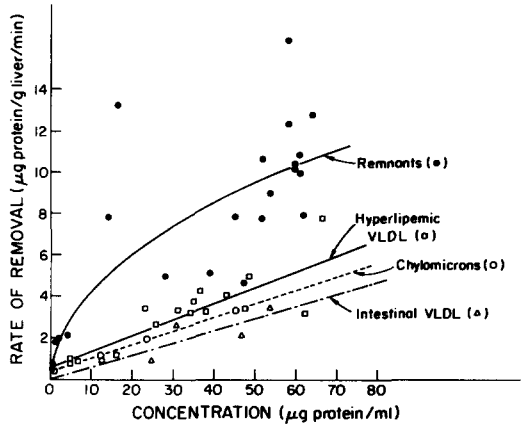


**Fig. 4.** Removal of lipoproteins by normal liver. Livers were perfused with various classes of $^{125}$I-labeled lipoproteins. One batch of chylomicrons and of hepatic VLDL were tested. Seven different batches of remnants and four different batches of VLDL from hyperlipemic rats were tested. Each point is the mean of two samples taken from one liver. The lines were fitted by least squares regression and the curve by hand.

rats of comparable size. As a corollary, the transport rates of other lipoprotein classes by liver from normal and hyperlipemic rats were compared. Again no differences were observed in the ability of liver from the two types of animals to remove either unmetabolized chylomicrons or hyperlipemic VLDL (data not shown). Taken together these data fail to support the hypothesis that the regimen used to induce hyperlipidemia caused a remnant removal defect. Thus it is concluded that the delay of remnant removal is primarily a consequence of an increased serum remnant concentration.

### Removal of normal and hypercholesterolemic VLDL by normal liver

Ross and Zilversmit (13) concluded that in rabbits fed a similar atherogenic diet the d < 1.006 g/ml lipoproteins which accumulated were primarily remnants. They based this conclusion on the fact that labeled dietary retinol accumulated in the plasma of the hyperlipemic rabbit. To assess whether remnants constituted the bulk of the VLDL in hyperlipemic rats, the rate of removal of this lipoprotein fraction by normal liver was measured. The rate of removal of VLDL from hyperlipemic serum was compared to the rate of removal of remnants from normal animals, unmetabolized chylomicrons, intestinal VLDL, hepatic VLDL, and normal serum VLDL. The results are shown in Fig. 4. VLDL from hyperlipemic rats had a removal rate similar to normal chylomicrons, and intestinal and hepatic (not shown) VLDL. In contrast, remnants had a significantly higher uptake rate. Serum VLDL appeared to have a high uptake rate as well (not shown). However, since this fraction contains a lower percentage of protein as apo B than the other VLDL (Table 2), a direct comparison with the other lipoproteins is difficult.

These results were somewhat surprising. They suggested that, although the remnant concentration is elevated in these animals, there must also be an increase in the concentration of other constituents of the VLDL fraction so that chylomicron remnants are still not the primary component of the abnormal VLDL.

### VLDL production by livers of hypercholesterolemic rats

In the hyperlipemic animals, the serum triglyceride concentration was only moderately elevated (11) despite the evidence from the preceding experiments that there was accumulation of "VLDL" and not just remnants. This suggested that there might be an increased hepatic or intestinal production of VLDL or a delay in VLDL clearance. To investigate the first possibility the livers from normal and hyperlipemic rats were isolated, perfused, and the composition and rates of VLDL secretion were compared. The rate of protein and phospholipid secretion into the d < 1.006 g/ml fraction was comparable in control and experimental animals (**Table 3**). However, dramatic differences in cholesterol and triglyceride secretion rates were observed. Livers from hyperlipemic rats secreted

TABLE 3. VLDL output by perfused livers from rats fed experimental diets[a]

| | Total Lipid Secretion | Composition | | | | |
|---|---|---|---|---|---|---|
| | | Triglyceride | Phospholipid | Free Cholesterol | Cholesteryl Ester | Protein |
| | *mg/g/hr* | *% dry weight* | | | | |
| Control | 0.30 | 67 | 17 | 5 | 2 | 9 |
| Hypothyroid | 0.16 | 70 | 15 | 5 | 2 | 7 |
| Fat fed | 0.34 | 40 | 20 | 7 | 26 | 7 |
| Fat fed hypothyroid | 0.26 | 46 | 18 | 8 | 19 | 8 |

[a] Values are the means of three experiments in each group.
Livers from rats fed control or experimental diets were removed and perfused for 3 hr. The perfusate was collected, the d < 1.006 g/ml lipoproteins isolated and their composition determined.

significantly less triglyceride than normal while they secreted almost five times more cholesterol than control livers. Thus, it appears that hypercholesterolemic rats substituted cholesterol for triglyceride in their VLDL.

To investigate whether the high fat content and the hypothyroidism were both needed to induce the abnormality, animals fed the fat alone or PTU alone were also studied. The fat feeding alone (Table 3) was sufficient to induce the secretion of cholesterol-rich lipoproteins while PTU alone did not alter the gross composition appreciably. It appears that there is virtually a one for one substitution of cholesteryl ester for triglyceride in the particles from the fat-fed animals.

Although it is not entirely clear from these data whether the secretion rate of $d < 1.006$ g/ml particles is elevated in vivo, the secretion of particles of such unusual composition helps to explain the presence of massive hypercholesterolemia with only a modest elevation of serum triglyceride concentration.

### Disappearance of hypercholesterolemic rat VLDL in normal rats

It is possible that VLDL accumulates in the serum of hypercholesterolemic rats because they cannot be activated to form remnants. This hypothesis would also explain the failure of the accumulated material to behave like remnants. In an effort to exclude this possibility, VLDL from hyperlipemic rats was radioiodinated and injected into normal rats. The disappearance of the radioactivity was followed for 15 min. There was a rapid disappearance of $^{125}I$ from the plasma. The t½ of 5.5 ± 1.2 min was similar to that previously reported for normal VLDL in control rats (28). Interestingly, the t½ was substantially longer than the t½ of remnants (2.2 ± .5 min, Fig. 2), further supporting the contention that this VLDL fraction is not composed primarily of chylomicron remnant. After 15 min, 70% of the radioactivity was recovered in the liver. This is comparable to the rate reported for removal of VLDL cholesteryl ester by normal liver (28). Thus these particles are capable of being activated and removed in a manner analogous to normal VLDL.

These results suggest that the VLDL of hypercholesterolemic rats can be activated normally and rapidly removed by liver, although they do not exclude the possibility that this process is abnormal in the hypercholesterolemic animal. Along with the finding of an increased remnant concentration, these results make a "remnant formation" defect unlikely, although it is reasonable to expect that the conversion of VLDL to remnants may be slowed because this process is saturable (5, 9).

## DISCUSSION

In the present study, radioiodinated lipoproteins were used to study the etiology of the hyperlipemia in the cholesterol- and fat-fed, hypothyroid rat. This approach enabled us to study the process with respect to changes in particle numbers because the difference in the amount of protein per particle is less marked between the various triglyceride rich lipoprotein types than are differences in the lipid composition. This approach was especially important for the studies of this hyperlipemia because the dramatic alterations of cholesterol and triglyceride content found in the primary particles would have rendered comparison in terms of these constituents useless.

Redgrave and Snibson (12) investigated chylomicron metabolism in fat-fed hypothyroid rats. They found that the triglyceride disappeared rapidly from the circulation but that its cholesterol remained in the circulation for a prolonged period. This led them to speculate that chylomicron remnants accumulated in the serum of these animals. A similar conclusion was reached by Ross and Zilversmit (13) who used retinol as a marker for remnants in the fat-fed rabbit. In our study the hypothesis that remnant removal was delayed in the fat-fed, hypothyroid rat was proven directly by following the disappearance of a trace of radiolabeled remnants. The t½ of 2.1 min for remnants gives a reliable direct estimate for the normal rate of this process in postprandial rats. This rapid removal implies that normally the concentration of remnants in the circulation is very low. If one assumes that the disappearance of remnants from the circulation is the result of hepatic uptake then an attempt to correlate the in vivo and in vitro data can be made. Although this assumption is an oversimplification, some insight into the process was gained. Based on the kinetic parameters previously published (7, 8) for chylomicron remnant removal by liver, the four-fold increase in the half life found in the animals on the dietary regimen in vivo would require approximately a twelve-fold increase in circulating remnant concentration. This estimate assumes that the regimen did not affect the removal process directly. The assumption is justified by the fact that the removal process was found to be normal in the hyperlipemic rat. This latter finding was somewhat at variance with the speculation that hypothyroidism might affect the removal process (13). Floren (29) reported that there was an alteration in the Km for remnant removal by hepatocytes derived from such animals. However, he concluded that this change was not adequate to explain the delayed remnant removal. Moreover, in a previous study from this laboratory (7) and in the studies of

Sherill and Dietschy (8) and Andersen, Nervi, and Dietschy (30), neither cholesterol feeding nor fasting significantly affected the removal process. Thus it appears that the delayed removal of remnants is the result of an increased production of chylomicrons induced by the diet. We suggest that this leads to an increase in remnant formation causing partial saturation of the removal mechanism.

In the study of hyperlipemic rabbits (13) it was suggested that remnants were the principal components of this abnormal VLDL. In this study in the rat it was found that the VLDL from the hyperlipemic animals did not behave like remnants. This suggested that while remnants were accumulating in this density range other non-remnant particles must also be accumulating, expanding the non-remnant VLDL concentration as well and thus keeping the proportion of remnants to non-remnants in the VLDL range relatively normal. The discrepancy in the reports may be due to species difference or to differences in methodologies. In the rabbit, retinol was used as a marker of alimentary lipoprotein accumulation. In the present study the behavior of the lipoproteins in a perfused liver was used to test for the presence of remnants. Although serum VLDL from hyperlipemic rats was removed by liver slightly more avidly than chylomicrons or hepatic or intestinal VLDL, its removal rate was substantially below that of normal chylomicron remnants. Thus either remnants were not the principal component of the VLDL, or if they were, they were abnormal and lacked some component, perhaps an apoprotein, which is essential for rapid removal. However, if this were the case they would not have competed with normal remnants and the delayed removal of normal remnants would not have been found. Moreover, when these VLDL particles were injected into normal rats they could be rapidly activated since they could be promptly removed by the liver. However, in the intact animal as well, the rate at which they were cleared resembled that of normal VLDL rather than that of remnants.

Because lipoprotein lipase is saturable and both chylomicrons and VLDL compete for this enzyme (5), it is relatively easy to envision how increased dietary fat would lead to a decreased rate of VLDL clearance. This would help to explain why remnants were not the only component of the $d < 1.006$ g/ml class to increase in concentration in the serum and thus were not the principal components of the $d < 1.006$ g/ml fraction in these rats. However, if normal VLDL were accumulating, a more substantial increase in the serum triglyceride concentration would have been anticipated. This apparent contradiction was resolved by the finding that the liver of these animals secreted a dramatically altered lipoprotein. The lipoproteins which floated at $d < 1.006$ g/ml had a very low triglyceride content and a high cholesterol content. Recently similar findings have been reported by Noel et al. (31) and Swift et al. (32) in a preliminary communication. Both of these groups have suggested that there may also be a substantial increase in apo E synthesis and secretion. In considering the relative contributions of the hypothyroidism and the fat feeding in the genesis of the abnormality, it appeared that hypothyroidism was not necessary for the abnormal cholesterol to triglyceride ratio although it may contribute to the apoprotein abnormalities (33).

One other consideration in the genesis of the abnormality in these animals is the nature of the alimentary lipoprotein which they secrete. Although we did not study chylomicrons or chylomicron remnants from the rats fed an atherogenic diet, Riley et al. recently have (34). They describe an alteration in lymph lipoprotein, with the appearance of a $\beta$ VLDL. Such particles could of course contribute to the non-remnant $d < 1.006$ g/ml pool.

Taken together, the results from these experiments suggest that the dietary regimen which induces atherosclerosis in the rat causes a hyperlipemia by affecting several aspects of lipoprotein metabolism. There is a marked increase of the circulating remnant concentration without a hepatic removal defect. In addition, other components of the $d < 1.006$ g/ml class appear to accumulate. Finally, the unusual cholesterol to triglyceride ratio is contributed to by the hepatic secretion of a lipoprotein of dramatically altered composition.

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## REFERENCES

1. Heimberg, M., I. Weinstein, G. Dishman, and M. Fried. 1965. Lipoprotein transport by livers from normal and $CCl_4$ poisoned animals. *Amer. J. Physiol.* **209:** 1053–1060.
2. Ockner, R. K., F. B. Hughes, and K. J. Isselbacher. 1969. Very low density lipoproteins in intestinal lymph: origin, composition, and role in lipid transport in the fasting state. *J. Clin. Invest.* **48:** 2079–2088.
3. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–471.
4. Mjøs, O. D., O. Faergeman, R. L. Hamilton, and R. J.

Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
5. Higgins, J. M., and C. J. Fielding. 1975. Lipoprotein lipase. Mechanism of formation of triglyceride-rich remnant particles from very low density lipoproteins and chylomicrons. *Biochemistry.* **14:** 2288–2293.
6. Carrella, M., and A. D. Cooper. 1979. High affinity binding of chylomicron remnants to rat liver plasma membranes. *Proc. Natl. Acad. Sci.* **76:** 338–342.
7. Cooper, A. D., and P. Y. S. Yu. 1978. Rates of removal and degradation of chylomicron remnants by isolated perfused rat liver. *J. Lipid Res.* **19:** 635–643.
8. Sherill, B. C., and J. M. Dietschy. 1977. Characterization of the sinusoidal transport process responsible for uptake of chylomicrons by the liver. *J. Biol. Chem.* **253:** 1859–1867.
9. Brunzell, J. D., W. R. Hazzard, D. Porte, Jr., and E. L. Bierman. 1973. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J. Clin. Invest.* **52:** 1578–1585.
10. Whissler, R. W., M. L. Eilert, M. A. Schroeder, and L. Cohen. 1954. Production of lipomatous and atheromatous arterial lesions in the albino rat. *AMA Arch. Pathol.* **57:** 333–351.
11. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of the plasma lipoproteins and apoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18:** 314–324.
12. Redgrave, T. G., and D. A. Snibson. 1977. Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. *Metabolism.* **26:** 493–503.
13. Ross, C. A., and D. B. Zilversmit. 1977. Chylomicron remnant cholesteryl esters as the major constituent of very low density lipoproteins in plasma of cholesterol-fed rabbits. *J. Lipid Res.* **18:** 169–181.
14. Cooper, A. D., and R. K. Ockner. 1974. Studies of hepatic cholesterol synthesis in experimental acute biliary obstruction. *Gastroenterology.* **66:** 586–595.
15. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
16. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757–768.
17. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53.
18. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
19. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
20. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
21. Cooper, A. D. 1976. The regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the isolated perfused rat liver. *J. Clin. Invest.* **57:** 1461–1470.
22. Knott, G. D., and D. K. Reece. 1972. MLAB: a civilized curve fitting system. *In* Proceedings of the ONLINE 1972 International Conference. Brunel University, England. **1:** 497–526.
23. Nobel, R. E. 1968. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* **9:** 693–700.
24. Leffler, H. H. 1959. Estimation of cholesterol in serum. *Amer. J. Clin. Pathol.* **31:** 310–313.
25. Cooper, A. D. 1977. The metabolism of chylomicron remnants by isolated perfused rat liver. *Biochim. Biophys. Acta.* **488:** 464–474.
26. Eggstein, M., and F. H. Kreutz. 1966. Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe. I. Mitteilung. Prinzip, Durchführung und Besprechung der Methode. Klin. Wschr. **44:** 262–267.
27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
28. Faergeman, O., and R. J. Havel. 1975. Metabolism of the cholesteryl esters of rat very low density lipoproteins. *J. Clin. Invest.* **55:** 1210–1218.
29. Floren, C. Effects of hypothyroesis and cholesterol feeding on the clearance of chylomicron remnants in vivo and by rat hepatocyte monolayers. Ph.D. Thesis, Department of Physiological Chemistry, University of Lund, Lund, Sweden.
30. Andersen, J. M., F. O. Nervi, and J. M. Dietschy. 1977. Rate constants for the uptake of cholesterol from various intestinal and serum lipoprotein fractions by the liver of the rat in vivo. *Biochim. Biophys. Acta.* **486:** 298–307.
31. Noel, S-P., L. Wong, P. J. Dolphin, L. Dory, and D. Rubenstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64:** 674–683.
32. Swift, L. L., N. R. Manowitz, G. D. Dunn, and V. S. LeQuire. 1979. Cholesterol (CH) and saturated fat diet induces hepatic synthesis of CH-rich lipoproteins (LP). *Clin. Res.* **27:** 378A.
33. Dory, L., J. Delamatre, and P. S. Roheim. 1979. Apolipoprotein profile in hypothyroid rats. Abstracts of the 33rd Annual Meeting of the Council on Arteriosclerosis, p. 9.
34. Riley, J. W., R. M. Glickman, A. R. Tall, and P. H. R. Green. 1979. Abnormal lipoproteins in response to chronic cholesterol feeding. *Circulation.* **60:** 11–32.