Mechanism of Triglyceridemia in Hypercholesterolemic Rabbits

By C. C. Huang, M.D., and K. J. Kako, M.D.

ABSTRACT
To elucidate mechanisms responsible for the increased plasma triglyceride (TG) which occurs in diet-induced hypercholesterolemia in rabbits, albino rabbits were fed a 3% cholesterol diet for various durations, and the following determinations were carried out: plasma lipid levels; turnover rate of plasma TG (labeled very low density lipoprotein, or glycerol-2-3H method); lipoprotein lipase activity of the heart and plasma; fatty acid and acetate incorporation into TG in the liver (homogenates and slices). Plasma levels of both free and esterified cholesterol and phospholipid increased rapidly, while TG increased relatively slowly during cholesterol feeding without change in hepatic TG synthesis. The fractional turnover was, on the other hand, depressed within a week. The decrease in plasma TG in response to a heparin injection was less in hypercholesterolemic rabbits than in normal animals. However, the measured activity of heart lipoprotein lipase of cholesterol-fed rabbits was higher than that of the control group. When the effects of two substrates (very low density lipoprotein from normo- and from hypercholesterolemic plasma) were compared, the apparent activity was lower with the latter. The degree of this inhibition was proportional to the amount of cholesterol in the lipoproteins. The inhibition of lipoprotein lipase activity was also observed with the addition of cholesterol to activated Ediol, but not with addition of esterified cholesterol. Lineweaver-Burk plots were constructed using partially purified plasma lipoprotein lipase, and Km values for Ediol, Ediol plus protamine sulphate, and Ediol plus cholesterol were calculated. The presence of cholesterol in the substrate of lipoprotein lipase competitively inhibits the enzyme activity, and this is the mechanism of hyperglyceridemia observed in diet-induced hypercholesterolemic rabbits.

ADDITIONAL KEY WORDS
- plasma lipoprotein lipase
- very low density lipoprotein
- plasma triglyceride turnover
- heart lipoprotein lipase
- cholesterol inhibition of lipoprotein lipase
- hepatic triglyceride synthesis

There are a number of clinical entities which accompany hypercholesterolemia, hyperglyceridemia, and defective postheparin lipolytic activity (1-4). Experimentally, when rabbits are fed a diet containing a high cholesterol content, not only does the plasma concentration of cholesterol increase, but an increase in plasma phospholipid and triglyceride (TG) levels also occurs (5). Although the interaction between plasma cholesterol and phospholipid has attracted much attention (6, 7), the mechanisms of hyperglyceridemia observed in these rabbits have not been elucidated. This study was carried out to investigate changes in TG metabolism of hypercholesterolemic rabbits. It was soon found that lipoprotein lipase (LPL) hydrolyzes the TG of the very low density lipoprotein (VLDL) sampled from these rabbits to a lesser extent than that sampled from normal rabbits. This was proved to be due to the presence of an abnormal quantity of cholesterol in the lipoprotein. Cholesterol...
added to an artificial substrate of LPL, activated TG emulsion, similarly inhibited enzyme activity.

Materials and Methods

Albino rabbits, 2.1 to 2.8 kg, were fed rabbit pellets containing 1% cholesterol-fed and non-treated (control) rabbits. Ediol was activated by incubating it together with the serum of a fasted dog. The activated emulsion was washed twice with physiological saline and adjusted to the original volume. TG content of the activated Ediol was 91.5% of the original. VLDL was prepared by the method of Havel et al. Serum of both the control and cholesterol-fed rabbits was centrifuged at 115,000 X g for 1 hour at 10°C. The oily top layer, about 1 cm, was aspirated and used as VLDL. Lipid composition of this lipoprotein was determined as described above. The lipoprotein was not washed with a salt solution of known density; although the densities of sera and normo- and hypercholesterolemia may not be identical.

The assay method of LPL was similar to that described by Akoosi and Mallow. The weighed left ventricle (after removal of visible fat) was rinsed, cut into fine pieces and homogenized. The homogenate (100 mg) was incubated in 6.5 ml of Krebs phosphate buffer (pH was adjusted to 8.5 with NH₄OH). Phospholipid phosphorus was determined by the method of Hurst. Total and free cholesterol were determined as reported previously. In brief, lipids were extracted with chloroform-methanol (2:1, v/v). Phospholipid phosphorus was determined by the method of Jagannathan after adsorbing phospholipids with Florisil.

Heart LPL Activity

Both Ediol and VLDL were used as substances of the enzyme of heart homogenates prepared from cholesterol-fed and non-treated (control) rabbits. Ediol was activated by incubating it together with the serum of a fasted dog. The activated emulsion was washed twice with physiological saline and adjusted to the original volume. TG content of the activated Ediol was 91.5% of the original. VLDL was prepared by the method of Havel et al. Serum of both the control and cholesterol-fed rabbits was centrifuged at 115,000 X g at 10°C. The oily top layer, about 1 cm, was aspirated and used as VLDL. Lipid composition of this lipoprotein was determined as described above. The lipoprotein was not washed with a salt solution of known density; although the densities of sera and normo- and hypercholesterolemia may not be identical.

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The assay method of LPL was similar to that described by Akoosi and Mallow. The weighed left ventricle (after removal of visible fat) was rinsed, cut into fine pieces and homogenized. The homogenate (100 mg) was incubated in 6.5 ml of medium composed of either 0.6 ml of 5% Ediol (30 mg of coconut oil) or 0.6 ml of the VLDL fraction of rabbit plasma, 210 µg of heparin, 2.7 ml of serum, 135 mg of bovine serum albumin and Krebs phosphate buffer (pH 8.5, adjusted with NH₄OH). Free fatty acids (FFA) were titrated before and after 1 hour's incubation at 37°C. The rate of hydrolysis was constant over this incubation period. Extraction and titration of fatty acid (FA) were carried out according to Goss and Lein's modification of Dole's method. The activity was expressed as milliequivalents of FA released per gram of tissue per hour.

For an inhibition study, 27 mg of cholesterol, cholesterol acetate, and cholesterol stearate were separately dissolved in 1 ml of acetone, and then mixed with 3 ml of 5% Ediol (pH 8.5). The aceton was evaporated by shaking the mixture at 37°C for 2 hours. The same procedure carried out with acetone but without cholesterol did not influence the activity of LPL. The final volume of this mixture was 5 ml. An aliquot of 1 ml was taken before and after 1 hour's incubation at 37°C and FA titrated.

Plasma LPL Activity

Plasma LPL activity was determined in three ways: (1) by using postheparin plasma, (2) by measuring TG change in response to heparin injection, and (3) by using partially purified enzyme.

(1) Plasma was obtained from the rabbit 10 minutes after an iv injection of 1 mg/kg of heparin. One milliliter of the plasma was incubated in the medium containing 100 mg of bovine serum albumin, 50.0 mg of Ediol TG, 1 ml of 0.1M Tris (pH 8.5) and Krebs phosphate buffer (pH was adjusted to 8.5 with NH₄OH). The final volume of this mixture was 5 ml. An aliquot of 1 ml was taken before and after 1 hour's incubation at 37°C and FA titrated.

(2) The in-vivo LPL activity was measured by comparing plasma TG levels before and 10 minutes after the intravenous injection of 1 mg/kg heparin. TC was quantified by the method of Jagannathan after adsorbing phospholipids with Florisil.

(3) Partial purification of plasma LPL was carried out according to the method described by Fielding. Two normally fed rabbits received 1 mg/kg of heparin and were bled. One-fiftieth volume of 5% Ediol was mixed with approximately 60 ml of plasma and the mixture shaken at 37°C for 7 minutes to allow the formation of an enzyme-substrate complex. The latter was harvested at centrifugation at 7,480 X g for 1 hour at 5°C and washed three times with NH₄OH-NH₄Cl buffer (0.05M, pH 8.5) containing sucrose and heparin, then centrifuged. All further steps were carried out at 5°C. The enzyme-
substrate complex was made soluble by mixing with the ammonium buffer containing 0.5% sodium deoxycholate, 0.5 mM potassium oleate, and 30 μg/ml heparin. After centrifugation (99, 972 x g for 1 hour), the enzyme in the aqueous phase was purified by adsorbing it on calcium phosphate gel. LPL was eluted with the above buffer containing 0.05M sodium citrate. Protein content was determined by the method of Lowry et al. (19). By this procedure the enzyme was approximately 800-fold purified or, in other words, the activity was increased from 0.21 μEq FA released/hr/mg protein found in the plasma to 171 μEq/hr/mg in the final suspension.

For a kinetic study, 5.4 mg of cholesterol or 4 mg of acetate were added to various amounts of activated Ediol (14 to 55 mg of coconut oil). Cholesterol was dissolved by using 0.8 ml of acetone which was subsequently evaporated by shaking this buffer mixture at 37°C for 2 hours. For the control, cholesterol and protamine were omitted. Incubation and FA determination were similar to above. The medium contained 100 mg bovine serum albumin fraction V, 0.1 ml of 1.35M Tris (pH 8.1), 168 μg heparin (omitted when protamine was used), and Krebs phosphate buffer (pH 8.5) in a final volume of 5.0 ml (17). The results of this experiment were plotted by following the formula proposed by Lineweaver and Burk (21).

TRIGLYCERIDE SYNTHESIS

Incorporation of palmitate and of acetate into hepatic TG was studied as follows: For palmitate incorporation, the method of Tietz and Shapiro was adapted (22). The liver was homogenized in 3 vol of KCl (0.139M), Tris (0.05M, pH 7.5) and centrifuged (700 x g, 10 min) to remove cell debris and nuclei. The incubation was carried out at 37°C for 1.5 hours in a medium composed of potassium phosphate buffer (6.7 mM, pH 7.5), MgCl₂ (3.3mM), ATP (3.3 mM), and 1.0 μc of potassium palmitate-l-14C (specific activity: 27.65 μc/μmole). The final volume of the incubation mixture was 3 ml.

For the determination of TG synthesis from acetate, the method of Longmore et al. was used (23). Liver slices, 0.5 g, were incubated at 37°C for 2 hours. The medium contained 20 mM KHCO₃, 20 mM MgCl₂, 2.5 mM CaCl₂, 100 mM KCl, 30 mM glucose, and 10 mM sodium acetate-1, 2,14C (5 μc). The medium was gassed with 95% oxygen and 5% CO₂ before each experiment.

In both series of experiments, lipids were extracted according to Folch et al. (24), and separated by thin layer chromatography on a plate coated with 0.25 mm silica gel G. Chromatograms were developed with petroleum ether-ether-acetic acid (80:20:2, v/v/v) (25). To prevent tailing of FFA, the proportion of acetic acid was increased from one part prescribed in the original paper (25) to two parts. The spots were verified by iodine vapour, scraped and counted in 10 ml of 4% Cab-O-Sil® and 0.4% of Omnifluor® in toluene. An aliquot of the chloroform extract was counted in 0.4% Omnifluor® in toluene for the total activity determination, which was equal to 31 to 40% of the activity of the palmitate added.

The FFA was extracted from liver homogenates and titrated by the method of Coss and Lein (16). Since the heptane extract was not washed (16), some acidic phospholipid may have existed in the extract, although the effect of this contamination should not be great, because the hepatic phospholipid concentration was unchanged by cholesterol feeding. The amount of FA esterified to TG and the amount of acetate converted to TG were calculated, and were expressed both per gram of liver and per kilogram of body weight.

TURNOVER OF TRIGLYCERIDE

The turnover rate of plasma TG was measured by the following two methods.

1) Seventeen rabbits on a high cholesterol diet for a period of 0 to 49 days were fasted overnight. Four rabbits were fed on regular diet for various periods (0 to 28 days) to see incidental changes in turnover rate and they served as controls. The labeled VLDL (d < 1.006) was injected into each rabbit and the disappearance of radioactivity of the TG fraction followed (26). The VLDL was prepared from the plasma of a donor rabbit which had received 500 μc of glycerol-2-1H 1 hour before exsanguination. Timed plasma samples (0 to 270 minutes) were analyzed for lipid content and radioactivity. For the latter, TG was separated by thin-layer chromatography as described above. Activities in the plasma TG fraction were plotted on semilog paper in order to obtain the half-turnover time. From this value the fractional turnover rate was calculated by the standard formula (27). The turnover rate was derived by assuming that the size of the TG pool is equal to the plasma volume (38.8 ml/kg) (28) of a rabbit.

2) To verify the results obtained with the above measurement a second method (29) was set up. Rabbits received an iv injection of 80 μc of glycerol-2-3H. The specific activity in the plasma TG fraction was measured for a period of

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Time course of changes in plasma lipids and turnover of triglyceride. The abscissa indicates a duration of feeding of a high cholesterol diet in days. A illustrates a change in plasma total cholesterol level (circles) and free cholesterol (squares). B illustrates a change in plasma phospholipid (triangles) and triglyceride (dots). In both, the ordinate indicates milligrams of lipid/100 ml of plasma, and the average number of experiments was five, except that of the controls (N = 30). C illustrates the fractional turnover of the plasma triglyceride pool (hour⁻¹) and D the turnover rate (milligrams of triglyceride/hour). Values were obtained by utilizing the disappearance of labeled very low density lipoprotein-triglyceride. Dots in C and D represent mean values of three (0 to 14 days) or two (21 to 49 days) experiments each. Values of parallel-fed control rabbits are also shown as circles and broken lines.

Results

The plasma levels of cholesterol, cholesterol esters and phospholipid rapidly elevated as the high cholesterol diet continued (Fig. 1). The rate of increase in TG level was much slower, resulting in a level approximately threefold the control value in 7 weeks, while cholesterol ester, free cholesterol and phospholipid increased 25-, 10- and 5-fold the control, respectively. Furthermore, Figure 1 illustrates the changes in the rate of TG turnover measured by the VLDL method during cholesterol feeding. The fractional turnover rate decreased immediately and remained low during the experimental period, whereas the turnover rate decreased for a while, then gradually increased. A similar time course was observed by the measurement of the turnover rate using labeled glycerol. With the latter method, the turnover rate was depressed throughout the period studied (2 to 70 days) and did not exceed the control value. The control turnover rate measured by the latter method was somewhat higher (36.2 ± 7.2 mg/hr; body weight = 2.8 kg; N = 4) than that measured by the VLDL method (22.6 mg/hr, N = 3), in agreement with the report by Gross et al. (30). Rabbits fed regular diet did not show any change in plasma lipids and TG turnover. Control values of the fractional time (0 to 330 minutes). In some experiments, the activity in the aqueous phase was also determined as an indication of the fate of glycerol-¹⁴C. The turnover rate was calculated as above. The TG pool defined by these two methods may not have been identical, however.
MECHANISM OF TRIGLYCERIDEMIA

The decrease in plasma TG content in response to a heparin injection was not significant in the rabbit with the high plasma cholesterol level, but there was a significant decrease in control (Fig. 2), although the basal levels of TG were unequal due to the presence of lipemia in the former case. It was postulated therefore that the LPL activity might have been depressed. Consequently, the lipase activity of heart homogenate was assayed by the standard procedure with Ediol. Contrary to expectation, it was found that the LPL activity in the hypercholesterolemic rabbit was significantly higher than that in the normocholesterolemic rabbit (Fig. 3). These hearts were taken after 1 to 13 weeks (average 3 weeks) of high cholesterol regimen. No definite relationship was observed between the activity of the enzyme and the duration of the regimen, except during the very early period.

These results suggest that inhibition might result from some factors existing together with the lipoprotein TG, natural substrate of the LPL. Hence, VLDL was isolated with an ultracentrifuge and used as a substrate of the lipase. The composition of VLDL is shown in Table 1, which indicates a striking difference in lipid contents of the normo- and hypercholesterolemic VLDL. Since the density of TG and cholesterol is 0.92 and 1.06 (g/ml), respectively, the lipoprotein fraction obtained from hypercholesterolemic rabbits may have a heavier density than that from normocholesterolemic rabbits. Figure 4 illustrates an apparent decrease in LPL activity as the proportion of VLDL obtained from the hypercholesterolemic rabbit was raised and that of VLDL from the normocholesterolemic

### TABLE 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>N</th>
<th>Free cholesterol</th>
<th>Esterified cholesterol*</th>
<th>Phospholipid</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.24 ± 0.05</td>
<td>0.51 ± 0.06</td>
<td>1.53 ± 0.28</td>
<td>4.42 ± 0.66</td>
</tr>
<tr>
<td>3% cholesterol</td>
<td>5</td>
<td>2.51 ± 0.71</td>
<td>4.71 ± 1.26</td>
<td>5.46 ± 1.45</td>
<td>1.99 ± 0.33</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&gt; 0.01</td>
</tr>
</tbody>
</table>

VLDL = very low density lipoprotein. Values are means ± se. *Expressed as weight of cholesterol, i.e., difference of the total minus free cholesterol.
rabbit was lowered. As a result of the experimental design, TG content could not be kept constant in this experiment. However, a comparison of the first and second columns immediately reveals that it is an increase in cholesterol and not the level of TG which influences the apparent enzyme activity to a great extent in these instances. The results of this experiment also suggest that the ratio of TG to cholesterol of less than three would appreciably decrease the activity of LPL.

The inhibition by VLDL of hypercholesterolemia was observed regardless of the source of enzyme; namely, using heart homogenates of normal rabbits (Fig. 4) as well as those of rabbits fed on a cholesterol diet (Fig. 5). Similarly, by using plasma LPL, there was an apparent decrease in the release of FA during incubation (Fig. 5). Therefore, this phenomenon was indeed due to a factor in the substrate of the LPL and not related to the enzyme or to sources (hearts of normo- and hypercholesterolemic rabbits and postheparin plasma) of the enzyme.

To identify this factor conclusively, various constituents of plasma lipoprotein were added to a relatively pure substrate of the lipase, Ediol (Fig. 6). The results clearly demonstrate that the inhibitor is cholesterol, and not cholesterol acetate or stearate, or lecithin. Although acetone, which was used as a solvent for cholesterol and its esters, is known not to influence the properties of LPL (12, also see Materials and Methods) or Ediol (31), it was evaporated before LPL assay in this study.
MECHANISM OF TRIGLYCERIDEMIA

FA RELEASED
\[ \text{\( \mu Eq/hr \cdot g \)} \]

\[ \text{160} \]
\[ \text{120} \]
\[ \text{80} \]
\[ \text{40} \]
\[ \text{0} \]

\[ \text{EDIOI} \]
\[ \text{PH CH, CHOL, CHOL ACET, CHOL STEAR, CHOL} \]

**FIGURE 6**
Effect of addition of various lipids upon apparent lipoprotein lipase activities. Lipoprotein lipase activity was determined by incubating normal heart homogenates in a medium containing activated triglyceride emulsion (Ediol) and by titrating fatty acid released (ordinate). The results are shown from left to right as effect of lecithin addition (PH CH), cholesterol acetate addition (CHOL ACET), cholesterol stearate addition (CHOL STEAR), no addition and free cholesterol addition (CHOL). Numbers in columns are number of experiments; values are means \( \pm SE \). Final concentration of the triglyceride added was 2.4 mg/ml and that of other substances 0.77 mg/ml.

Similar inhibition was also observed by using, instead of Ediol, VLDL to which an extra amount of cholesterol had been added. The magnitude of inhibition was directly proportional to the amount of cholesterol added (Fig. 4). In other words, cholesterol inhibition is a relative one: the greater the amount of cholesterol, the greater the degree of inhibition (Fig. 4). With a greater amount of LPL (e.g., in hypercholesterolemic hearts), relatively less cholesterol inhibition occurred (Fig. 5). Cholesterol, 5.4 mg, in the presence of 14 to 55 mg of TG of Ediol enabled us to construct a Lineweaver-Burk’s graph (21) (Fig. 7). Cholesterol inhibition under the conditions studied appears greater than that by a well-known inhibitor, protamine sulphate, 4 mg (Fig. 7). The 50% inhibition observed with protamine is comparable to that reported by other investigators (20). This inhibition is competitive in nature with a \( K_m \) of 0.248 mM for Ediol, 0.412 mM for Ediol plus protamine and 0.825 mM for Ediol plus cholesterol.

The recovery of FA (6 \( \mu Eq \)) added to the substrate of LPL during the assay was 92.1% after 1 hour’s incubation indicating that FA released from TG is not adsorbed onto cholesterol, nor esterified to form cholesterol ester. The latter point was verified further by a direct determination of free and esterified cholesterol before and after the incubation under conditions similar to those of the LPL assay. There was no change in the amount of cholesterol ester.

FA esterification to TG by hepatic homogenates was unchanged in hypercholesterolemic rabbits (85.6 ± 13.1 in control, \( N = 4 \), and 81.2 ± 9.3 in hypercholesterolemic, \( N = 8 \), \( \mu Eq/kg \) body weight during 90 minutes of incubation). Livers obtained from rabbits which received a high cholesterol diet for only 1 week behaved similarly to those of rabbits which received it for 3 to 17 weeks. Likewise, the magnitude of TG synthesis from labeled acetate was similar in liver slices of normo- and hypercholesterolemic animals. These results support the hypothesis that the mechanism of hyperglyceridemia in these rabbits is an inhibition of LPL activity rather than an acceleration of TG synthesis.

**Discussion**

This study demonstrates that the presence of cholesterol in the substrate of LPL inhibits competitively the activity of this enzyme, the inhibition being observed using both VLDL and activated TG emulsion as substrates. On the other hand, esterified cholesterol or lecithin did not influence the activity of LPL. These observations, supported by the findings of the TG turnover study and hepatic TG synthesis, strongly suggest that the mechanism of triglyceridemia in hypercholesterolemic rabbits is an increased proportion of cholesterol in VLDL which results in a less suitable substrate for LPL.
Heart homogenates were used as sources of LPL in most of our experiments, because the LPL activity in the heart is high (20, 32) and hence its depression was convenient to assess. Inhibition similar to that found by heart LPL was also observed by plasma lipase before and after partial purification. However, a depression of lipase activity of other organs was difficult to prove, since the activity of adipose tissue LPL of an overnight-fasted animal is quite low (32) and striated muscle, with the exception of diaphragm (33), does not possess long-chain TG lipase (34). The LPL was assayed by using mixed substrates, i.e., a commercial TG emulsion containing monoglyceride, or VLDL. As a result, the activity expressed in this paper is the activity of "LPL" composed of tri-, di- and monoglyceridases (35). The activity of other lipases reported to exist in heart tissue should not influence the results greatly because of the unfavorable assay condition (pH = 8.5) for them (33).

The cholesterol content of VLDL increased, together with that of phospholipid in hypercholesterolemic rabbits, as compared to values from controls (Table 1). French et al. (36), Garlick et al. (37) and Spitz (38) all observed a similar increase of cholesterol content and a relative decrease in TG in the VLDL of rabbit during cholesterol feeding. VLDL (prebeta lipoprotein) containing a large amount of cholesterol may possess characteristics similar to those of low density lipoprotein (LDL) (beta lipoprotein), i.e., it may not be a very suitable substrate for LPL (39, 40). Its density may change since the density of cholesterol is relatively high compared to that of TG. Some workers postulate that VLDL may transform to another class of lipoprotein after hydrolysis of its TG (40, 41). Albrink
described redistribution of cholesterol to lower density fractions of plasma lipoprotein with increasing TG concentrations, probably because of an interaction between cholesterol and TG (42). The participation of cholesterol in the VLDL may further be influenced by the extent of unsaturation of component FA (43). Frederickson et al. believe that subfractionation of the lipoprotein spectrum on the basis of density may not always be ideal (41); for instance, in the case of Type III hyperlipoproteinemia, the VLDL fraction (d < 1.006) contains lipoprotein having a high cholesterol content and characteristics of beta mobility on electrophoresis (1, 41). Our study now indicates that excess cholesterol in the VLDL inhibits the activity of LPL.

The quantitative relationship among the enzyme (LPL), substrate (lipoprotein TG), and inhibitor (lipoprotein cholesterol) appears complex and interdependent. Apparent activity measured with a substrate containing very little cholesterol (activated Ediol) is higher than that measured by using normal VLDL which contains more cholesterol (Figs. 3, 5), although this difference could be partly due to their differing monoglyceride contents. While the LPL of the hearts of hypercholesterolemic rabbits shows a higher activity, measured by using Ediol (Fig. 3) or VLDL (Fig. 5), than does the LPL of the control animals, the difference is not significant when these activities are compared using the corresponding lipoproteins, i.e., the LPL of the hypercholesterolemic with the hypercholesterolemic lipoprotein, and the LPL of the normocholesterolemic with the normocholesterolemic lipoprotein (Fig. 5). When the activity is measured by using the two types of lipoproteins, one with and one without high cholesterol, a depression of activity is observed regardless of the source of the enzyme (Figs. 4, 5).

French et al. (36) in 1955 found that the presence of an excess of cholesterol in plasma has an inhibitory effect on the clearing reaction by added heparin. This was attributed to cholesterol containing lipoproteins rather than to cholesterol itself, since the rate of clearing was uninfluenced by the chyle to which a cholesterol suspension was added or the chyle obtained from the cholesterol-fed rabbit. This discordant result may be due to the relatively small amount of cholesterol which they used. Furthermore, the lipemic plasma of a rabbit may not be suitable for the turbidimetric estimation of the clearing effects of heparin (44).

Brown et al. (45) observed an inhibition of the clearing reaction by LDL in dog serum. Likewise, Klein et al. (46) found a relatively high level of antilipolytic activity in hyperlipemic serum. The cholesterol concentration of these materials is high and, therefore, the results give support to our conclusion that high cholesterol is the antilipolytic factor. However, the TG metabolism of cholesterol-fed rabbits and human patients may not be identical. Consequently, it is difficult to explain solely with the proposed hypothesis of TG disposal the fact that the plasma TG level rises concomitantly to a fall in plasma cholesterol in response to the administration of cholestyramine (47), while ethyl chlorophenoxyisobutyrate (Atromid-S) decreases both lipid levels (48). The mechanism responsible for clinical cases of Type II hyperlipemia (1), in which hypercholesterolemia does not necessarily accompany hyperglyceridemia, must again be dissimilar to the proposed mechanism in cholesterol-fed rabbits.

Although numerous inhibitors of LPL have been described, most inhibitors are not physiological substances. Furthermore, the mechanisms of their action have not been completely clarified. Inhibitors may act as surface active agents (49) through their interaction with anionic groups of LPL (49) or through their binding with heparin (20, 50). The mechanism of cholesterol-lipase interaction may be adsorption of the lipase at the surface of lipid particles (51), or a change in size of lipoprotein particles. Robinson et al. (51) observed that the turbidity of a cholesterol suspension is cleared by sodium oleate, suggesting that the FFA released during hydrolysis by clearing-factor lipase may interact with cholesterol or may make chole-
terol soluble. Indeed, emulsifying action of cholesterol on fats had been reported (52). A weak binding force between cholesterol and phospholipid (53, 54) and between cholesterol and TG (55) have also been investigated recently.

The results of the TG turnover measurements give additional support to the hypothesis proposed. The fractional turnover decreased in the beginning and remained low as the high cholesterol regimen continued (Fig. 1). This initial drop, which precedes the change in the plasma TG level, must be a result of the inhibition of LPL activity by cholesterol that was then rapidly increasing (Fig. 1). This is followed by a compensatory increase in enzyme activity (see also Fig. 3) in response to this inhibition, resulting in a gradual rise in turnover rate of TG. It is also possible that the increasing availability of substrate for LPL is at least partly responsible for this rise in turnover. Whereas a time course of changes in turnover rates measured by the two methods (labeled VLDL and glycerol) showed some difference (see Results), the fractional turnover values were consistently low during cholesterol feeding, suggesting that an increased entry of TG into the plasma compartment is unlikely. Indeed, the rate of hepatic FA esterification and TG synthesis in these rabbits was determined by the techniques utilizing labeled palmitate and acetate and found not to alter significantly. There was also no change in plasma FFA, one of the factors controlling hepatic TG output.

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References


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