Mechanisms of Hypertriglyceridemia in the Coconut Oil/Cholesterol-Fed Rabbit

Increased Secretion and Decreased Catabolism of Very Low Density Lipoprotein

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Rabbits fed a 14% coconut oil/0.5% cholesterol (CNO/Chol) diet develop mild to severe hypertriglyceridemia compared with rabbits fed a 14% olive oil/0.5% cholesterol (OO/Chol) diet. Lipids and apolipoprotein (apo) B were significantly higher in the very low density lipoprotein (VLDL) and intermediate density lipoprotein fractions from CNO/Chol than from OO/Chol rabbits. Yet, the particle diameters of these lipoproteins were similar in both diet groups, indicating that CNO/Chol rabbits had a much larger number of VLDL and intermediate density lipoprotein particles in plasma. Although the composition of CNO/Chol VLDL differed from that of OO/Chol VLDL, the rates of triglyceride hydrolysis of CNO/Chol VLDL and OO/Chol VLDL by postheparin lipoprotein lipase in vitro were the same, suggesting that VLDLs from the two diet groups were equally good substrates for lipoprotein lipase. To determine the mechanisms of hypertriglyceridemia in the CNO/Chol rabbit, triglyceride and apo B of CNO/Chol VLDL and OO/Chol VLDL were labeled with tritium-containing triolein and iodine-131 and injected intravenously into CNO/Chol and OO/Chol rabbits. The fractional clearance rate for triglyceride in OO/Chol rabbits was twice that of CNO/Chol rabbits, which parallels the previously observed differences in postheparin lipoprotein lipase activity. Although the average fractional removal of apo B did not differ between diet groups, there was a significant inverse relation between plasma cholesterol and apo B fractional clearance rate. We conclude that the hypertriglyceridemia and the enhanced hypercholesterolemia in the CNO/Chol rabbit results primarily from increased hepatic secretion of VLDL and a modest decrease in VLDL triglyceride clearance capacity. (Arteriosclerosis and Thrombosis 1991;11:918–927)
particles, pools of very low density lipoprotein (VLDL) from CNO/Chol and OO/Chol rabbits were exposed in vitro to normal rabbit postheparin LPL. Previous studies had shown that postheparin plasma LPL activity was significantly higher in chronically fed OO/Chol than in CNO/Chol rabbits. To determine whether this difference in LPL would be reflected in differential removal of plasma VLDL, the disappearance of tritiated triolein- and iodine-131 apo B-labeled VLDL from plasma was investigated.

**Methods**

**Animals and Diets**

Female New Zealand White rabbits (Becken Farm, Sanborn, N.Y.) weighing between 2.5 and 3.5 kg were used in all experiments. Rabbits were caged individually and had free access to water. Rabbits were offered daily 100 g of a diet containing 85.5 g commercial chow (Purina Lab Rabbit Chow 5321,Ralston Purina, St. Louis, Mo.), 0.5 g cholesterol USP (ICN Biochemicals, Cleveland, Ohio), and 14 g of either hydrogenated coconut oil (CNO/Chol, ICN Biochemicals Inc.) or olive oil (OO/Chol, Felippo Berio and Co., Lucca, Italy). Cholesterol was dissolved in the oil at 120°C, added to chow, and mixed thoroughly.

In general, the following experiments had more rabbits in the CNO/Chol groups; we had observed in previous experiments that variability among rabbits fed CNO/Chol is greater than that in rabbits fed OO/Chol.

All animal protocols were in accordance with Cornell University guidelines.

**Apolipoprotein B**

Plasma samples used for this experiment were taken from rabbits fed either CNO/Chol (n=14) or OO/Chol (n=7) for 27 days. Total apo B and the ratio of apo B-48 to apo B-100 were determined.

One-milliliter aliquots of plasma taken before a high-fat/cholesterol meal (0 hour) and 12 and 18 hours after the meal were ultracentrifuged (Beckman 50.3 rotor, 4°C, 1.51x10^8 g x min for HDL, 2.27x10^8 g x min for LDL) as described by Havel et al. Total cholesterol, EC, and UC were analyzed after saponification by the method of Zak et al. EC is expressed as cholesterol mass times 1.7. Phospholipid phosphorus was determined by the method of Bartlett with a conversion factor of 25 to calculate phospholipid mass.

Particle diameters of VLDL, IDL, and LDL were determined by the method of Lowry et al with bovine serum albumin as the standard. For the other lipid determinations, an aliquot of each fraction was extracted with 2:1 (vol/vol) CHCl3/CH3OH. Esterified (EC) and unesterified (UC) cholesterols were separated on precoated thin-layer chromatography plates (Silica Gel 60, E. Merck, Darmstadt, FRG) with hexane/diethyl ether/acetic acid (80:20:1, vol/vol/vol). The lipid fractions were eluted from silica gel with CHCl3/CH3OH (9:1, vol/vol) and dried under N2.

Total cholesterol, EC, and UC were analyzed after saponification by the method of Zak et al. Total cholesterol mass times 1.7. Phospholipid phosphorus was determined by the method of Bartlett with a conversion factor of 25 to calculate phospholipid mass.
in which \( r \) is the core radius; \( d \) is the particle diameter; PL is phospholipid mass; PRO is protein; and 1.093, 1.044, 0.968, 0.97, and 0.705 are partial specific volumes. The thickness of the surface layer of the lipoprotein was assumed to be 21.5 Å.12,13

In Vitro Hydrolysis of Coconut Oil/Cholesterol and Olive Oil/Cholesterol Very Low Density Lipoprotein

Pools of 40 ml plasma from OO/Chol rabbits (n=7) and 20 ml from CNO/Chol rabbits (n=7) were ultracentrifuged (Beckman 60Ti rotor, 10°C, 2.15×10^9 g×min) to isolate the d<1.006 g/ml lipoproteins. The top 6 ml was ultracentrifuged again (Beckman 50.3 rotor, 10°C, 1.30×10^9 g×min) to concentrate the d<1.006 g/ml further. TG concentrations of the pools were determined with an enzymatic kit (reagent set triglycerides GPO [701912], Boehringer Mannheim Biochemicals); the CNO/Chol VLDL pool was diluted with a d=1.006 NaCl solution to equalize the TG concentrations of the two pools (3.42 mg/ml). Activator plasma, tris(hydroxymethyl)aminomethane (Tris), and bovine serum albumin (1.235 M Tris, 1.46% bovine serum albumin, pH 8.4) were added to each pool.

For each pool, in vitro lipolysis was determined after incubation at 37°C for 0.5, 1, or 6 hours. The following were added to each tube: 0.068 ml of either a 0.12 M NaCl (low-salt) or a 5.16 M NaCl (high-salt) solution, 1.49 mg TG (from CNO/Chol or OO/Chol), and 0.02 ml postheparin plasma from a control rabbit as the source of lipase.2 A blank containing 0.02 ml saline in place of the postheparin plasma was included for each sample tube to account for any free fatty acids already present or released by lipase existing in the VLDL. We have previously shown that there is very little or no LPL activity in plasma in the absence of a heparin injection (0.006 μmol fatty acid released/ml plasma/hr; M. Van Heek and D.B. Zilversmit, unpublished data).

After the samples and blanks were incubated for 0.5, 1, or 6 hours at 37°C, the reaction was stopped with 1.4:1.2:1.0 (vol:vol:vol) CH₃OH/CHCl₃/heptane.14 To correct for free fatty acids in the source of lipase (postheparin plasma), 0.02 ml postheparin plasma was added to all blanks after the reaction was stopped but before the extraction was performed.

All samples and blanks were extracted by a modification of the method of Bloom.15 Free fatty acids were separated from other lipid components by thin-layer chromatography and were transmethylated, and free fatty acid masses were determined by gas-liquid chromatography with 17:0 free fatty acid (Alltech, Deerfield, Ill.) as an internal standard. Fatty acid methyl esters RM 4 (olive oil standard, Supelco, Bellefonte, Pa.), RM 5 (coconut oil standard, Supelco), and FAME E (C8–C16, Alltech) were used as compositional standards. The amount of fatty acids released by LPL was calculated by subtracting fatty acids released by hepatic lipase (high salt) from those released by total lipase (low salt).

Preparation of Tritium- and Iodium-131-Labeled Very Low Density Lipoprotein

Ten milliliters whole blood was obtained from CNO/Chol (n=9) and OO/Chol (n=9) rabbits that had been fed their respective diets for 14 days. Plasma for each group was pooled, and VLDLs (d<1.006 g/ml) from CNO/Chol (VLDLCNO) and OO/Chol (VLDLOO) pools were separated by ultracentrifugation (Beckman 60Ti rotor, 4°C, 2.00×10^8 g×min). A single ultracentrifugation was chosen to minimize loss of apolipoproteins and/or denaturation (aggregation) of the lipoprotein complex. VLDLCNO and VLDLOO were labeled with [¹³¹I]triolein (glycerol tri[9,10(n)-³H]oleate; Amersham Corp., Arlington Heights, Ill.) as previously described.16,17 Briefly, to obtain [¹³¹I]triolein-labeled VLDL pools free of liposomes, HDL was initially incubated with H-labeled lipoproteins in the presence of d>1.21 g/ml rabbit lipid transfer activity; the H-labeled HDLs thus produced were separated from the liposomes by ultracentrifugation (d=1.063 g/ml, Beckman 60Ti rotor, 4°C, 3.4×10^8 g×min) and incubated with VLDLCNO or VLDLOO. The resulting H-labeled VLDL pools were then separated from HDL and lipid transfer activity by ultracentrifugation (d=1.006 g/ml, Beckman 60Ti rotor, 4°C, 2.00×10^8 g×min).

Other aliquots of the VLDLCNO and VLDLOO pools were iodinated by the iodine monochloride method of McFarlane18 at pH 10 to minimize lipid labeling.¹⁹ [¹³¹I] (2.5 mCi) was used to label 6.33 mg VLDLCNO and VLDLOO protein, which was determined by the method of Lowry et al.¹⁶ Unreacted iodine was removed by PD-10 gel filtration and extensive dialysis against phosphate-buffered saline.

Injection Experiments

The [¹³¹I]- and [¹³¹I]triolein-labeled VLDLCNO were mixed before injection. After 21 days of high-fat/cholesterol feeding, 1 ml of either the VLDLCNO or the VLDLOO dose was injected into the marginal ear vein of CNO/Chol (n=8) or OO/Chol (n=5) rabbits, respectively. Blood samples were collected from the other marginal ear vein (into tubes containing 4% NaCit, 0.4 M EDTA, pH 7.4, 0.01 ml/ml blood) at 5, 15, 30, 60, 120, and 180 minutes after injection.

Tritium and Iodine-131 Analyses

One milliliter of each dose was diluted to 100 ml with phosphate-buffered saline to approximately mimic the dilution in plasma. After the plasma was isolated, 0.050 ml of each plasma sample as well as 0.050 ml of each diluted dose was counted directly for [¹³¹I] in a Beckman Gamma 8000 counter. A 1.0-ml aliquot of the diluted doses and all plasma samples were ultracentrifuged at d=1.006 g/ml (Beckman 50.4, 50.3, and 40.3 rotors; 4°C, 1.6×10^8 g×min). Tubes were sliced to separate the d<1.006 and d>1.006 g/ml fractions, and both fractions were counted directly for [¹³¹I]. VLDL TG was determined by enzymatic assay (Boehringer Mannheim).
To determine the amount of $^{131}$I contained in the apo B portion of the $d<1.006$ g/ml lipoproteins and the diluted doses, apo B was precipitated. Briefly, an aliquot of each sample was made to 50 mM NaH$_2$PO$_4$, 1% SDS, pH 6.8; heated to 80°C for 5 minutes; and allowed to cool. Lipoproteins were delipidated with a 45:55 mixture of butanol/isopropl ether (vol/vol) and centrifuged. The supernatant and the apo B pellet were separated, and the $^{131}$I was counted.

Lipids were extracted from the $d<1.006$ and $d>1.006$ g/ml fractions with 10 ml hexane after addition of 10 ml each of ethanol and H$_2$O. Lipids were dried under N$_2$, 10 ml ACS scintillant (Amer- sham) was added to each sample, and $^3$H was counted. Correction was made for the $^{131}$I present in the lipid fraction.

Analysis of the dose showed that 85% of the $^3$H was located in the $d<1.006$ g/ml fraction; the remainder was found in the $1.006<d<1.019$ g/ml fraction. $^3$H data are therefore presented as a percentage of the total $^3$H in the VLDL fraction of the dose. SDS–polyacrylamide gel electrophoresis (PAGE) revealed that 80% of the $^{131}$I was located in albumin. In the preparation of each dose, 40 ml undiluted lipidd-rich plasma was ultracentrifuged in a single tube. The albumin had probably been “trapped” in the large amount of the $d<1.006$ g/ml lipid-rich supernate and thereby became part of the labeled dose. When much smaller aliquots were diluted and ultracentrifuged as was done with the postinjection plasma samples in this experiment and others described in these “Methods,” little (<5%) or no albumin contamination occurred (as determined by SDS-PAGE). A subset of these $d>1.006$ g/ml fractions was further separated at $d=1.019, 1.063$, and 1.21 g/ml. Virtually all of the $^{131}$I in the $d<1.006$ g/ml fraction of the plasma samples was present in albumin. The labeled albumin in the samples served to determine the plasma volume of the recipient animals. Plasma volume was determined by extrapolating the logarithmic values of plasma radioactivities at 5, 15, 30, and 60 minutes to $t=0$. Plasma volumes were $3.70\pm0.13$ (mean±SEM) and $3.60\pm0.08$% of body weight for CNO/Chol and OO/Chol rabbits, respectively; these values agree with previously published values for cholesterol-fed rabbits. The percentage of $^{131}$I-VLDL apo B remaining in plasma was calculated by the following equation

$$^{131}I\text{-VLDL apo } B_{\text{plasma}} = \frac{^{131}I\text{-VLDL apo } B_{\text{sample}}}{^{131}I\text{-VLDL apo } B_{\text{dose}}} \times 100$$

**Data Analyses**

Data were analyzed for statistical significance by Student’s $t$ test and fitted by linear and nonlinear least-squares regression analyses (Systat Inc., Evans- ton, Ill.). To determine the best models for the data in linear regression, residual plots were analyzed and data were transformed where appropriate. The $^3$H data were analyzed by either the Levenberg-Marquardt or the Quasi-Newton method (Systat) of nonlinear weighted least-squares fitting, assuming a two-pool model for all curves between 0 and 3 hours, and fractional clearance rate was calculated. Fractional clearance rate for the $^{131}$I data could not be calculated as described above because none of the curves described a conventional disappearance process (see Figure 7 in “Results”). Fractional clearance rate was therefore calculated by a different method. The area under the curve from $t=0$ to $t=60$ minutes was determined by the trapezoidal method; the area under the remainder of the curve was analyzed by the Quasi-Newton method, assuming a single monoexponential (Systat). Production rate for TG was calculated by multiplying the fractional clearance rate by the VLDL TG pool size.

**Results**

We had previously observed that feeding a single OO/Chol meal to rabbits fed CNO/Chol resulted in an increase in LPL and a concomitant decrease in plasma TG levels after 24 hours. In the present study we sought to determine whether OO/Chol particles might be more readily hydrolyzed than CNO/Chol particles because of differences in particle size or composition.

**Lipoprotein Size, Composition, and Hydrolysis**

*Apollipoprotein B.* Figure 1 shows the mean of the $d<1.019$ g/ml plasma TG and apo B concentrations for CNO/Chol and OO/Chol rabbits before and after a high-fat/cholesterol meal. Although plasma TG increased during the postprandial period in both CNO/ Chol and OO/Chol rabbits, apo B in the $d<1.019$ g/ml fraction did not change in either group. At all time points, there was three times as much $d<1.019$ g/ml apo B in CNO/Chol rabbits as in OO/Chol rabbits. As determined by scanning densitometry, apo B-48 comprised less than 4% of the total apo B at all time points (data not shown), indicating that nearly all of the apo B was of hepatic origin.

*Lipids and protein.* In different groups of rabbits the amount of TG, protein, phospholipid, EC, and UC was measured in lipoproteins from CNO/Chol and OO/Chol rabbits. Figure 2 shows that the amounts of lipid and protein in the VLDL, IDL, and LDL fractions were significantly ($p<0.001$) higher in CNO/Chol rabbits than in OO/Chol rabbits. In HDL, total cholesterol, TG, protein, and phospholipid were nearly the same in both groups. The small quantity of cholesterol did not allow reliable determination of EC and UC.

*Particle diameter.* Figure 3 shows that the particle diameters (see calculation in “Methods”) were similar in VLDL, IDL, and LDL obtained from CNO/ Chol and OO/Chol rabbits. That CNO/Chol rabbits have much higher levels of $d<1.019$ g/ml lipids and apo B-100 than do OO/Chol rabbits yet have particle diameters similar to those of OO/Chol rabbits indi-
cates that CNO/Chol rabbits have many more 
$d<1.019 \text{ g/ml lipoprotein particles. Because the apo} 
B-48 \text{ to apo B-100 ratio was less than } 0.04, \text{ one can} 
\text{conclude that these VLDL and IDL particles are} 
\text{mostly of hepatic origin.}

**Lipoprotein composition.** The percentage distributions of the lipid and protein of VLDL, IDL, LDL, 
and HDL are shown in Figure 4. Each percentage 
was calculated as the weight of the lipid or protein 
divided by the sum of all lipids plus protein in each 
fraction multiplied by 100. All CNO/Chol lipoproteins 
contained significantly ($p<0.01$) more TG and 
phospholipid than did OO/Chol, whereas OO/Chol 
lipoproteins were significantly ($p<0.01$) more en-
riched in protein and cholesterol, particularly EC 
(not determined in HDL). Also, in the two diet 
groups the fatty acids of the whole plasma TG closely 
resembled the fats that were fed (data not shown).

**In vitro hydrolysis of coconut oil/cholesterol and olive oil/cholesterol very low density lipoprotein.** To determine 
if hydrolysis of TG in VLDL from CNO/Chol rabbits 
was slower than that for VLDL from rabbits fed 
OO/Chol, pools of VLDL from CNO/Chol and OO/ 
Chol rabbits were exposed to normal rabbit posthep-
arin plasma. Figure 5 shows that hydrolysis of CNO/ 
Chol and OO/Chol TG by LPL was essentially the same 
in the diet groups despite differences in fatty acid 
composition. This was the case during 0.5, 1, and 6

**Figure 1.** Bar graph showing concentration 
(mg/dl) of plasma $d<1.019 \text{ g/ml triglyceride (})$ 
and apolipoprotein B ($\square$) before a high-fat/ 
cholesterol meal (0 hour) and 12 and 18 hours 
after the meal. CNO/chol, n=14; OO/chol, n=7. 
Values are mean±SEM. CNO, coconut oil; chol, 
cholesterol; OO, olive oil.

**Figure 2.** Bar graphs showing 
plasma triglyceride (TG), protein 
(PRO), phospholipid (PL), unester-
ified cholesterol (UC), and esterified 
cholesterol (EC) in the form of very 
low density lipoprotein (VLDL), in-
termediate density lipoprotein (IDL), 
low density lipoprotein (LDL), and 
high density lipoprotein (HDL) in 
CNO/Chol (■, n=7) and OO/Chol 
(□, n=7) rabbits. (UC and EC were 
not analyzed in HDL.) Concentra-
tion is expressed as milligrams in a 
given lipoprotein fraction per 100 
milliliters of plasma, mean±SEM. 
CNO, coconut oil; Chol, cholesterol; 
OO, olive oil.
Injection Experiments: Disappearance of Tritium-Labeled Triglyceride and Iodine-131 Apolipoprotein B From d<1.006 Plasma

To determine the relative disappearance of VLDL TG and apo B from the plasma of CNO/Chol versus OO/Chol rabbits, VLDL pools labeled with both $^3$Htriolein and $^{131}$I were injected into rabbits. The labeled pool comprising CNO/Chol VLDL was injected into CNO/Chol rabbits; the labeled OO/Chol VLDL pool was injected into OO/Chol rabbits. The
disappearance of the labels was monitored for a 3-hour period. Figure 6 shows the disappearance of labeled TG from the plasma $d<1.006$ g/ml fraction. Three hours after injection, 17% and 8% of the $^3$H remained in the plasma of CNO/Chol and OO/Chol rabbits, respectively. In seven of eight CNO/Chol rabbits, the fractional removal of labeled TG was slower than the fractional removal in all OO/Chol rabbits. The one CNO/Chol rabbit with a much higher fractional TG removal (fractional clearance rate $=1.92 \cdot hr^{-1}$) was the only CNO/Chol rabbit with a normal VLDL TG level (VLDL TG $=67$ mg/dl). Table 1 shows the mean VLDL TG values during the 3-hour injection study, the fractional clearance rates, and the TG production rates for OO/Chol and hypertriglyceridemic CNO/Chol rabbits. The fractional clearance rate in OO/Chol rabbits was approximately double that of CNO/Chol rabbits; the production rate was five times as high in CNO/Chol rabbits as in OO/Chol rabbits.

Table 1. Very Low Density Lipoprotein Concentrations, Fractional Clearance Rates, and Production Rates of Plasma Triglyceride in Coconut Oil/Cholesterol- and Olive Oil/Cholesterol-Fed Rabbits

<table>
<thead>
<tr>
<th>Variable</th>
<th>CNO/Chol*</th>
<th>OO/Chol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>VLDL TG (mg/dl)</td>
<td>232±26†</td>
<td>27±4</td>
</tr>
<tr>
<td>Fractional clearance rate (hr$^{-1}$)</td>
<td>0.69±0.06†</td>
<td>1.13±0.09</td>
</tr>
<tr>
<td>Production rate (mg/hr)</td>
<td>161±32†</td>
<td>31±8</td>
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</table>

Values are mean±SEM. CNO, coconut oil; Chol, cholesterol; OO, olive oil; VLDL TG, very low density lipoprotein triglyceride. *Mean±SEM without the values for one normotriglyceridemic CNO/Chol rabbit. †Significantly different from OO/Chol group at $p<0.001$.

Figure 7 shows the disappearance of $^{131}$I-labeled apo B from the $d<1.006$ g/ml plasma of CNO/Chol and OO/Chol rabbits. After 3 hours, 60% and 48% of the $^{131}$I-VLDL apo B still remained in the plasma of CNO/Chol and OO/Chol rabbits, respectively, compared with 17% and 8% remaining for $^3$H-VLDL TG (Figure 6). Although disappearance was somewhat faster in OO/Chol rabbits, the 3-hour fractional clearance rate for apo B did not differ significantly between the two groups (0.198±0.022 versus 0.210±0.042 hr$^{-1}$ for CNO/Chol and OO/Chol rabbits, respectively, mean±SEM). Figure 8 shows that for the combined data of CNO/Chol and OO/Chol rabbits, plasma cholesterol and the fractional clearance rate of apo B were inversely correlated ($r=-0.74, p<0.006$); this relation was also present for the CNO/Chol group by itself.

**Discussion**

The present experiments addressed possible mechanisms of hypertriglyceridemia in the CNO/Chol-fed rabbit. We found that the amounts of lipid and protein, specifically apo B-100, were significantly higher in the VLDL and IDL fractions of plasma from CNO/Chol rabbits compared with those from OO/Chol rabbits but that the particle diameters of these lipoproteins in the two groups did not differ. These results suggest that the plasma of the CNO/Chol rabbits contained many more lipoprotein particles and that these were largely of hepatic origin. Furthermore, although the composition of VLDL in the two diet groups differed, in vitro rates of VLDL TG hydrolysis were the same, indicating that CNO/Chol VLDL particles in the two groups were probably an equally good substrate for LPL. In both diet groups, less than 4% of apo B in VLDL and IDL was apo B-48; the concentration of...
apo B-100 and apo B-48 did not change in either group during the 18-hour period after ingestion of a high-fat/cholesterol meal despite increases in postprandial TG ranging from 40% to 140%. These results extend those of Kroon et al., who found that at a single time point in the nonfasted state, 90% of total apo B was apo B-100 in rabbits fed a 10% corn oil/0.5% cholesterol diet for 1–2 months; both studies suggest that in the preprandial and postprandial state, very little apo B of intestinal origin was retained in plasma. Apparently, the increase in postprandial TG was not large enough to result in an observable increase in either apo B-48 or apo B-100.

Previous studies have shown that after 20 days of high-fat/cholesterol feeding, postheparin LPL activity in CNO/Chol rabbits was 35% lower than that of OO/Chol rabbits. The present experiments showed that the production rate of VLDL TG was five times higher in the CNO/Chol than in the OO/Chol group. Although the lower LPL activity in CNO/Chol rabbits may account for some of the hypertriglyceridemia, it seems likely that overproduction of hepatic TG is the major determinant. An exception to this was one CNO/Chol rabbit with a low TG level despite the fact that the TG production rate of this animal was as elevated as that of the other CNO/Chol rabbits.

**Figure 7.** Plot and curves showing removal of iodine-131-labeled apolipoprotein B very low density lipoprotein (% I-VLDL apo B) from the VLDL fraction (d<1.006) of plasma in CNO/Chol (○, n=8) and OO/Chol (●, n=5) fed rabbits. 131I radioactivity in apo B in VLDL of the doses was 12,000 cpm. All rabbits were fed a chow diet containing 0.5% cholesterol (Chol) and either 14% coconut oil (CNO) or 14% olive oil (OO) for 21 days before injection.

**Figure 8.** Scatterplot showing plasma cholesterol (mg/dl) versus fractional clearance rate (l/h) of iodine-131-labeled apolipoprotein B in CNO/Chol (●, n=8) and OO/Chol (○, n=5) fed rabbits after 21 days of 14% high-fat/0.5% cholesterol (Chol) (wt/wt) feeding. The equation of the function for all rabbits is $y=2636e^{-2.14t}$. Logarithmic transformation of the data for all animals is log $y=3.42-2.14t$ ($r=-0.74$, $p<0.000$). For CNO/Chol rabbits, the equation is log $y=3.40-1.79t$ ($r=-0.69$, $p<0.05$). CNO, coconut oil; OO, olive oil.
However, in this rabbit the fractional clearance rate was substantially higher than that of all other rabbits, thereby counteracting the increased production rate and thus maintaining normal TG.

In the 3-hour period after injection, no significant difference in the fractional removal of apo B was found between the two groups. Spady and Dietschy\(^{27}\) found that in the hamster, CNO/Chol feeding caused a far greater downregulation of apo B/E receptors than did either cholesterol plus safflower oil or cholesterol feeding alone. In the present study, regression analysis of the data obtained on all rabbits did show an inverse relation between plasma cholesterol and fractional apo B removal rate. The animals with the highest plasma cholesterol levels had lower apo B fractional clearance rates, and these rabbits were largely CNO/Chol fed. Possibly after a much longer feeding period when plasma cholesterol levels would be expected to differ more, the fractional removal rate of apo B in CNO/Chol and OO/Chol rabbits might well differ.

In the \(^{31}\)I-apo B clearance studies, the plasma VLDL apo B showed an increase in radioactivity during the first hour in both OO/Chol and CNO/Chol rabbits. Although there is no explanation for this phenomenon, it is significant that it occurred in all 13 animals and therefore may be physiological rather than due to random variation. We have observed similar “bumps” in the disappearance curves of VLDL or chylomicrons labeled with \(^{131}\)I, \(^{14}\)C, \(^{23}\)H, \(^{125}\)I, \(^{24}\)C, \(^{14}\)C-1 labeled cholesteryl ester, \(^{32}\)P, or retinyl palmitate.\(^{30}\) The disappearance and reappearance of radioactivity could represent temporary adherence of VLDL to the endothelium followed by release from this site, or it could represent uptake and resecretion from the liver.

In summary, the present studies have shown that after 19 days of feeding CNO/Chol or OO/Chol diets, particle sizes of VLDL and IDL did not differ; despite compositional differences, in vitro hydrolysis of VLDL from CNO/Chol and OO/Chol rabbits was similar, suggesting that the VLDLs from the two groups were equally suitable substrates for LPL. Production rates of TG and apo B-100 were substantially higher in CNO/Chol rabbits than in OO/Chol rabbits, yet apo B fractional removal was the same, indicating that hypersecretion of TG-rich VLDL contributes to hypertriglyceridemia as well as to the higher plasma cholesterol in CNO/Chol rabbits. These results differ from those of Groot et al.,\(^{31}\) who found that the relative hypertriglyceridemia in rats fed palm oil was due to decreased TG catabolism and not to increased TG synthesis. Fractional removal of labeled plasma TG in CNO/Chol rabbits was only one half that of OO/Chol rabbits, and heparin-releasable LPL\(^{2}\) was significantly but not greatly lower in CNO/Chol rabbits. These findings support the previous hypothesis\(^{2}\) that the hypertriglyceridemia also results in part from a lower clearance capacity of plasma TG in CNO/Chol compared with that of OO/Chol rabbits.

References


KEY WORDS • hypercholesterolemia • olive oil • apolipoprotein B • very low density lipoprotein turnover • lipoproteins
Mechanisms of hypertriglyceridemia in the coconut oil/cholesterol-fed rabbit. Increased secretion and decreased catabolism of very low density lipoprotein.

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