A single high-cholesterol, high-fat meal preferentially increases low molecular weight apolipoprotein B concentration in rat plasma

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Abstract Rats were fed either rat chow (control), chow + 20% olive oil (olive oil), or chow + 20% olive oil + 2% cholesterol (olive oil/cholesterol) as a single meal to study the short-term effects of fat and the above combination of fat/cholesterol-containing diets on plasma apoB concentration and its influence on the distribution of apoB subspecies. Rats were given their meals and allowed to consume them ad libitum until they were killed, 3 hr or 9 hr afterwards. Three hours after feeding, serum triglyceride concentrations were increased to the same extent in both the olive oil and olive oil/cholesterol-fed rats as compared with concentrations in control rats, but serum apoB concentrations did not differ among the groups. Nine hours after feeding, serum triglyceride concentrations were still equally elevated in both experimental groups; however, in the olive oil/cholesterol-fed rats, total serum apoB as well as total serum cholesterol were increased above both the control and olive oil groups. In addition, the d < 1.21 g/ml lipoprotein $apoB_l/apoB_h$ ratio of the olive oil/cholesterol-fed rats was greatly increased at 9 hr, whereas $apoB_l/apoB_h$ ratio in the d < 1.21 g/ml fraction of the olive oil group was unchanged, despite the increase in plasma triglyceride concentration. In the olive oil/cholesterol-fed rats at 9 hr, cholesterol, total apoB, $apoB_l$, and $apoB_h$ of both VLDL and IDL were greater than in the control or olive oil rats. In d < 1.21 g/ml lipoproteins, VLDL, and IDL, the increases in $apoB_l$ concentrations were of a greater magnitude than the increases in $apoB_h$. Although d < 1.21 g/ml lipoprotein $apoB_l/apoB_h$ ratio in the olive oil/cholesterol-fed rats was not increased at 3 hr, IDL $apoB_l$ concentration was higher than in either the control or olive oil groups; since this represented only a small fraction of the total apoB, it was not reflected in the d < 1.21 g/ml lipoprotein values. These observations indicate that dietary cholesterol affects $apoB_l$ metabolism differently than $apoB_h$ metabolism. —**DeLamatre, J. G., B. R. Krause, L. Wong, C. A. Hoffmeier, and P. S. Roheim.** A single high-cholesterol, high-fat meal preferentially increases low molecular weight apolipoprotein B concentration in rat plasma. *J. Lipid Res.* 1985. **26:** 924–929.

Supplementary key words apoB distribution • apoB subspecies • cholesterol feeding

Two separate subspecies of apolipoprotein B have been described for the rat (1) and four subspecies for the human (2, 3). These can be separated using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) with low concentrations of acrylamide (1, 2, 4–6) or by SDS-gel filtration column chromatography (7). The two rat subspecies appear as a large 335,000 molecular weight doublet and as a smaller 240,000 molecular weight band; they are referred to as high ($apoB_h$) and low ($apoB_l$) molecular weight apoB (7). The rat liver is capable of synthesizing both $apoB_h$ and $apoB_l$ (8–10), whereas the intestine synthesizes only $apoB_l$ (5, 6, 10). Tracer studies indicate that hepatic VLDL $apoB_l$ has a shorter half-life in the plasma than does hepatic VLDL $apoB_h$ (4, 5, 11). Thus, it appears likely that at least two types of VLDL particles could be produced by the liver, one that contains either $apoB_l$ alone or $apoB_l$ with $apoB_h$, and one that contains only $apoB_h$. Furthermore, the apoB heterogeneity of the VLDL may determine the metabolic heterogeneity of these lipoprotein particles (4, 7, 11).

Previously we have shown that serum apoB concentrations increase after 4 weeks in rats fed chow supplemented with olive oil and cholesterol (12). This increase in whole serum apoB is due to greatly increased VLDL- and IDL-apoB concentrations (12). In the present study, we determined that these alterations in apoB occurred acutely (after a single meal) and further extended our observations to include quantification of the high- and low-molecular weight apoB subspecies. Preliminary results have been reported (13).

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; B_h, high molecular weight apolipoprotein B (335,000); B_l, low molecular weight apolipoprotein B (240,000); SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; EDTA, ethylenediaminetetraacetic acid; EIA, electroimmunoassay.

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METHODS AND MATERIALS

Animals and diets

Male Sprague-Dawley rats (275–300 g) were maintained on laboratory chow (#5001, Ralston Purina Co., St. Louis, MO) using a regular 12-hr, light/dark cycle (6 AM/6 PM). On the day before a feeding experiment, the chow was taken from the animals at 3 PM and the rats were given their appropriate meals at 12 midnight to ensure that food consumption began simultaneously in all rats. The animals were then allowed free access to their food until they were killed. The following meals were given: ground laboratory rat chow (control); 20% olive oil in laboratory rat chow (olive oil-fed); and 20% olive oil/2% cholesterol in laboratory rat chow (olive oil/cholesterol-fed). The rats were then bled via the abdominal aorta at 3 AM or 9 AM while under light ether anesthesia. All blood samples, which were kept on ice during the bleedings, were centrifuged immediately at 12°C to obtain serum. Sodium azide and EDTA were then added at a final concentration of 0.1% and were present in all solutions used for lipoprotein isolation.

Lipoprotein isolation

On the same day that the animals were killed, lipoproteins were separated according to the method of Havel, Eder, and Bragdon (14), using an SW-41 rotor in a Beckman L5-50 ultracentrifuge. Very low density lipoproteins (VLDL) of d $<$ 1.006 g/ml, intermediate density lipoproteins (IDL) of d 1.006–1.03 g/ml, and low density lipoproteins (LDL) of d 1.03–1.063 g/ml were separated by a single 24-hr ultracentrifugation at 265,000 *g*. A layer of saline of the appropriate density was placed above all samples. Lipoproteins of d $<$ 1.21 g/ml were obtained by subjecting serum to a single spin in a Beckman L3-40 centrifuge using a fixed angle 40.3 rotor with 2-ml adaptors. A 1.5-ml aliquot of serum was adjusted to 1.24 g/ml by the addition of KBr, and a solution of 1.21 g/ml was layered over the serum sample. The lipoproteins were then spun for 48 hr at 114,000 *g*.

Apolipoprotein quantification

ApoB was quantified by electroimmunoassay (EIA) (15) as modified by Bar-On, Roheim, and Eder (16) and previously described (12). The arbitrary units by EIA represent a percentage of a standard serum pool. ApoB subspecies ($apoB_h$ and $apoB_l$) were separated by SDS-PAGE (17) using 3.5% acrylamide (6). Gels were stained with 0.13% Coomassie blue R-250 (50% methanol–10% acetic acid) overnight and destained against four changes of 10% acetic acid. Gels were run using 5–25 μg of total apoB protein; in this range we observed linearity for $apoB_h$ and $apoB_l$. Based on five points with three samples at each point, the *r* and intra-assay coefficients of variation, respectively, were 0.89 and 9.1% for $apoB_h$; 0.91 and 6.4% for $apoB_l$; and 0.89 and 9.4% for total apoB.

The validity of the scanning assay was established by comparing the scanning results to results obtained by the rocket immunoassay and the pyridine dye elution technique (18). From the data in Table 2, it can be seen that densitometric determinations for total apoB agree quite well with the immunoassay determinations, even when the proportion of $apoB_l$ is relatively increased, as in the case of the olive oil/cholesterol-fed rats. Therefore, the polyclonal antibody used in this study is probably equally reactive to $apoB_h$ and $apoB_l$. This has been confirmed using the polyclonal antibody in an enzyme-linked immunosorbent assay system (Laurence Wong, unpublished observations). Here standard curves for VLDL, IDL, LDL, and d $<$ 1.21 g/ml lipoproteins, all of which have different $apoB_l/B_h$ ratios, were the same. Finally, when $apoB_h$ and $apoB_l$ were determined by the pyridine dye elution technique, the results obtained were in good agreement with those obtained by the ELISA assay.

Analytical and statistical methods

Cholesterol and triacylglycerol concentrations of whole serum were determined enzymatically by the methods of Allain et al. (19) and Bucolo and Davis (20), respectively. Protein was determined by the method of Lowry et al. (21) as modified by Sata, Havel, and Jones (22), using bovine serum albumin as a standard. For statistical analysis, one-way analysis of variance was used followed by the Student-Neuman-Kuels multiple range test (23). A P value $<$ 0.05 was considered significant.

RESULTS

Three hours after the animals were given their meals, serum cholesterol concentrations increased by 20% in the olive oil-fed rats and by 36% in the olive oil/cholesterol-fed rats (**Table 1**). The serum triacylglycerol concentrations in both experimental groups were greatly increased over control, indicative of active fat absorption and chylomicron synthesis and secretion. Serum apoB concentrations, however, were not different in any groups 3 hr after feeding, as determined either by EIA or densitometry (Table 1). Thus, although serum triglyceride concentration indicated chylomicron production was taking place at 3 hr, this did not result in a detectable change in serum apoB concentrations. Nine hours after the rats consumed their meals ad libitum, triglyceride concentrations in both the olive oil and olive oil/cholesterol-fed groups were still increased over control values (**Table 2**). Although serum apoB concentrations in the olive oil-fed rats did not significantly differ from those of the control rats, the serum apoB concentrations in the olive oil/cholesterol-fed rats

TABLE 1. Serum concentrations of cholesterol, triacylglycerol, and apolipoprotein B of rats 3 hr after feeding

	Control	Olive Oil	Olive Oil/ Cholesterol
Number	11[a]	11	11
Cholesterol (mg/dl)	56 ± 2[b]	67 ± 4[c,d]	76 ± 2[c,d]
Triacylglycerol (mg/dl)	93 ± 10	146 ± 10[c]	179 ± 24[c]
ApoB			
A.U. by EIA	84 ± 5	90 ± 7	90 ± 6
A.U. by densitometry[e]	780 ± 20[f]	902 ± 100	838 ± 48
Ratio $\frac{apoB_l}{apoB_h}$ (d < 1.21 g/ml lipoprotein)	0.43	0.39	0.49

[a] Number of samples: eleven individual rats.
[b] Value ± SEM.
[c] Significantly different from control, $P < 0.05$.
[d] Significantly different from other experimental group, $P < 0.05$.
[e] Refers to total apoB (i.e., $apoB_h$ and $apoB_l$).
[f] Number of samples: three pools of four rats/pool.

were significantly greater (>50%) than those of both the olive oil and control groups. This difference in the serum apoB concentrations among the groups corresponded with differences in both serum and liver cholesterol concentrations; the serum and liver cholesterol concentrations of the olive oil/cholesterol-fed rats were greater than those of both the control and olive oil groups. Examination of the serum apoB concentrations shows that apoB increased above control values only in the olive oil/cholesterol-fed group, and then only at 9 hr, when the plasma cholesterol concentrations were greatly increased (at least 50%) above control values.

In these experiments, $apoB_l$ and $apoB_h$ concentrations were determined in total d < 1.21 g/ml fractions as well as in VLDL, IDL, and LDL. The d < 1.21 g/ml fraction was used to observe the $apoB_l/apoB_h$ ratio of total serum lipoproteins. At 3 hr, the $apoB_l/apoB_h$ ratio in the d < 1.21 g/ml fraction was not different among the dietary groups (Table 1). The IDL-$apoB_l$ concentration in the olive oil/cholesterol-fed rats, however, was found to be 8.5-fold higher than in the control rats and 3-fold higher than in the olive oil-fed rats (**Fig. 1**). $ApoB_h$ concentrations in IDL increased to a similar extent in both groups receiving olive oil and was, therefore, not altered significantly by the presence of dietary cholesterol. The increased IDL apoB did not result in a significant increase in the total apoB, since it represents a small fraction of the total. The increase in serum IDL-$apoB_l$ concentrations of the olive oil/cholesterol-fed rat was associated with an increase in IDL cholesterol concentration that was greater than in both the control and the olive oil-fed rats (**Fig. 2**).

At 9 hr, the $apoB_l/apoB_h$ ratio of d < 1.21 g/ml lipoproteins, which was 0.33 in the control and 0.37 in the olive oil-fed rats, increased to 0.92 in animals receiving both olive oil and cholesterol (Table 2). SDS-PAGE gel patterns of the d < 1.21 g/ml fractions illustrate these changes in the apoB subspecies of the olive oil/cholesterol-fed rats (**Fig. 3**). When the apoB subspecies concentrations in the individual fractions were determined, both VLDL and IDL showed alterations in both total apoB

TABLE 2. Serum concentrations of cholesterol, triacylglycerol, and apolipoprotein B of rats 9 hr after feeding

	Control	Olive Oil	Olive Oil/ Cholesterol
Number	4[a]	4	4
Cholesterol (mg/dl)	61 ± 2[b]	69 ± 1[c,d]	102 ± 2[c,d]
Triacylglycerol (mg/dl)	102 ± 8	210 ± 16[c]	212 ± 30[c]
ApoB			
A.U. by EIA	143 ± 5	145 ± 3[d]	236 ± 7[c,d]
A.U. by densitometry[e]	1341 ± 188	1464 ± 88	2164 ± 260
Ratio $\frac{apoB_l}{apoB_h}$ (d < 1.21 g/ml lipoprotein)	0.33	0.37	0.92
Liver cholesterol (mg/g wet weight)	2.85 ± 0.07	3.46 ± 0.12[c,d]	5.35 ± 0.08[c,d]

[a] Number of samples: four pools of four rats/pool.
[b] Value ± SEM.
[c] Significantly different from control, $P < 0.05$.
[d] Significantly different from other experimental group, $P < 0.05$.
[e] Refers to total apoB (i.e., $apoB_h$ and $apoB_l$).

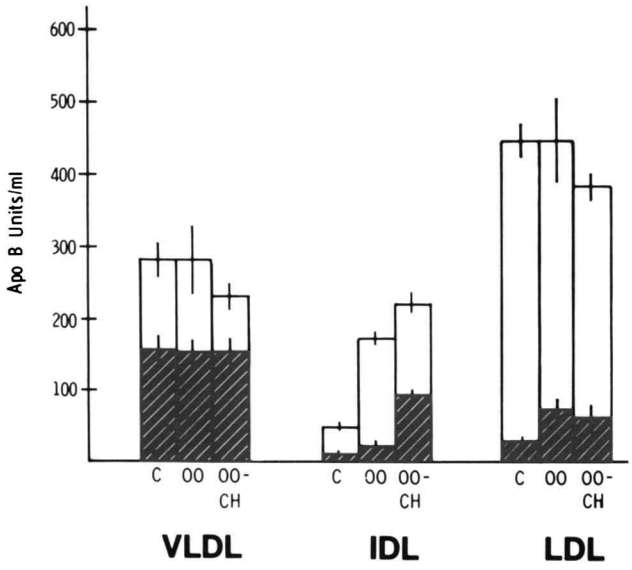

Fig. 1. Concentration of $apoB_h$ and $apoB_l$ in VLDL, IDL, and LDL 3 hr after feeding control (C), olive oil (OO), and olive oil/cholesterol (OOCH) meals; □ $apoB_h$; ▩ $apoB_l$. Values ± SEM based on three pools of four rats per pool.

concentrations and in apoB subspecies distribution (**Fig. 4**). No change in apoB subspecies concentration was observed in the olive oil-fed group except a small increase in IDL $apoB_h$. The VLDL and IDL fractions of olive oil/cholesterol-fed rats contained more total apoB, $apoB_l$, and $apoB_h$. Compared with control values, $apoB_l$ of the olive oil/cholesterol-fed rats was increased two-fold in VLDL and ten-fold in IDL. Increments in $apoB_h$ concentrations in the olive oil/cholesterol group were of lesser magnitude in both VLDL and IDL. No changes in the apoB subspecies were observed in LDL. The cholesterol content of all apoB-containing lipoprotein fractions increased with the olive oil/cholesterol diet, but not in olive oil-fed rats (**Fig. 5**). At 9 hr, the increases in total apoB, $apoB_l$, and $apoB_h$ concentrations seen in both the VLDL and IDL fractions of the olive oil/cholesterol-fed rats were correlated with increases in the cholesterol concentration of those fractions.

DISCUSSION

In this study, total serum apoB concentrations were determined by both EIA and by linear scanning of SDS-PAGE gels. The results obtained at both 3 hr (Table 1) and 9 hr (Table 2) demonstrate that the methods were in good agreement. Since immunological methods for assaying $apoB_l$ and $apoB_h$ concentrations are not available, we used densitometric scanning of polyacrylamide gels to determine $apoB_l$ and $apoB_h$ concentrations. However, the slopes of the standard curves for $apoB_h$ and $apoB_l$ were 0.07 and 0.02, respectively, indicating that the chromogenicities of $apoB_h$ and $apoB_l$ were not identical. This difference in the slopes tended to minimize the increases in $apoB_l$, since the amount of sample applied to the gels for control and experimental groups represented equivalent starting plasma volumes. Therefore, the entire lipoprotein fraction distribution of $apoB_l$ and $apoB_h$ was repeated using the pyridine dye elution technique (18). In these experiments, the quantities of protein applied were adjusted so that they were nearly equivalent. The results obtained in this case agreed quite well with those shown with densitometric scanning, and the increases in $apoB_l$ and $apoB_h$ were quantitatively similar.

Schonfeld, Bell, and Alpers (24) did not observe a change in plasma apoB concentration when rats were given corn oil by a gastric tube. In agreement with those findings, we did not see a change in total apoB concentration in rats fed the olive oil diets. However, when cholesterol was added to the same diet, we observed a 63% increase in plasma apoB at 9 hr. This observation suggests that cholesterol of dietary origin can profoundly affect apoB metabolism in the rat. The observation that an increase in $apoB_l$ concentration was responsible for a large portion of the increase in total serum apoB in the cholesterol-fed rats suggests that $apoB_l$ may have an important role in the metabolism of dietary cholesterol in the rat.

The increased $apoB_l$ found in the plasma of cholesterol-fed rats may have been derived from the intestine, since

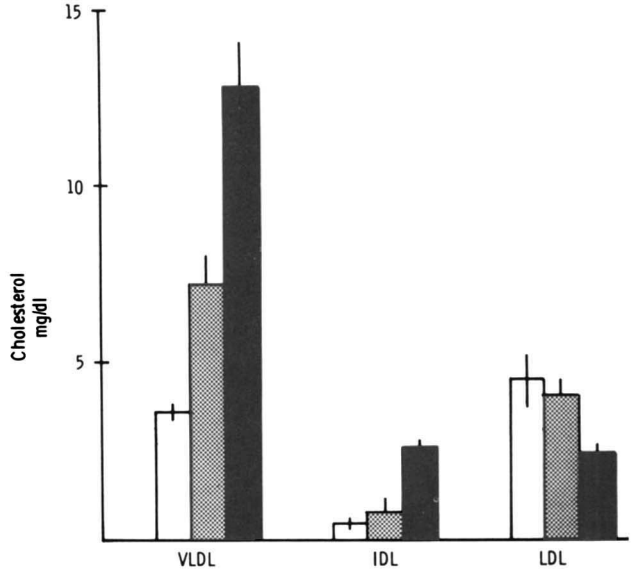

Fig. 2. Concentration of cholesterol in VLDL, IDL, and LDL 3 hr after feeding control, olive oil, and olive oil/cholesterol meals; □ control; ▩ olive oil; ■ olive oil/cholesterol. Values ± SEM based on three pools of four rats per pool.

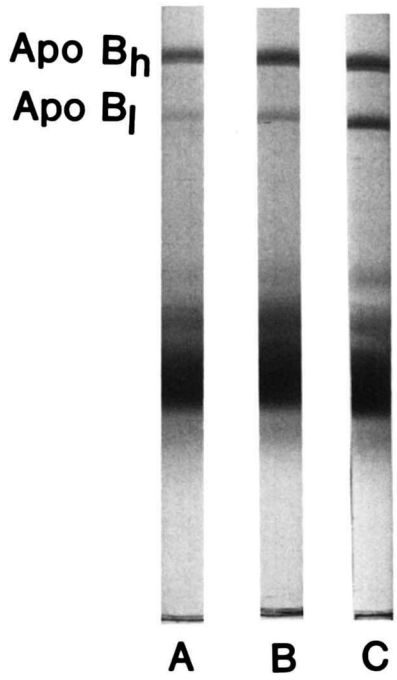

Fig. 3. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis showing $apoB_h$ and $apoB_l$ of d < 1.21 g/ml lipoproteins 9 hr after feeding control, olive oil, and olive oil/cholesterol meals. (A) control; (B) olive oil; (C) olive oil/cholesterol. Samples applied to the gels represented equal plasma volumes for all groups.

$apoB_l$ is synthesized in the intestine (5, 6, 10). If the increased apoB in plasma of cholesterol-fed rats is from the intestine, then the question is whether this is due to an increased synthesis and secretion or a decreased plasma clearance. Although fat feeding is known to stimulate intestinal $apoB_l$ production (5, 6), it is not known whether added dietary cholesterol will increase intestinal apoB production above that of the same high-fat diet without the cholesterol. Riley et al. (25) found an increased concentration of IDL in mesenteric lymph from olive oil/cholesterol-fed rats. Retinol incorporation into LDL indicated that it was of intestinal origin. This would suggest that the IDL-$apoB_l$ found in plasma of the olive oil/cholesterol-fed rats may be derived from the intestine.

Rat liver synthesizes and secretes both $apoB_h$ and $apoB_l$ (8–10). The increased plasma $apoB_h$ seen in the cholesterol-fed rat had to be derived from the liver, since in the rat the liver is the only organ known to produce $apoB_h$ in a significant quantity. An interesting aspect of the cholesterol-fed rat is whether the increased plasma $apoB_l$ seen in this animal is also derived from the liver. Related to both of the above considerations is the question of whether the increased plasma $apoB_h$ and $apoB_l$ of the cholesterol-fed rat are due to an increased synthesis, decreased plasma clearance, or both. Because plasma $apoB_h$ concentrations are increased, it is tempting to speculate that the increased plasma $apoB_l$ is derived from the liver. Others have found that livers from hypothyroid cholesterol-fed rats contain cholesterol-enriched lipoproteins of IDL density and size in liver cells (26, 27). In this study, there was a ten-fold increase in IDL-$apoB_l$ concentrations in the olive oil/cholesterol-fed rat at 9 hr. If this IDL-$apoB_l$ is derived from the liver, it could be the result of increased synthesis and secretion due to a large influx of cholesterol into the liver or the result of decreased catabolism because of an altered lipid composition of the lipoprotein. Davis and McNeal (28) found increases in plasma $apoB_h$ and $apoB_l$ from rats given an olive oil/cholesterol diet for 4 weeks that were similar to the increases in plasma $apoB_h$ and $apoB_l$ we observed with a single feeding. However, $apoB_h$ and $apoB_l$ synthesis was unchanged in hepatocyte preparations from rats fed the olive oil/cholesterol diets. This result suggests that the olive oil/cholesterol diet results in a decreased catabolism of $apoB_h$ and $apoB_l$.

Taken together, these observations indicate that cholesterol of dietary origin is able to affect apoB metabolism. The effect of dietary cholesterol on $apoB_h$ metabolism probably differs from the effect on $apoB_l$ metabolism, as evidenced by the differential effects on plasma concentrations and lipoprotein distributions.

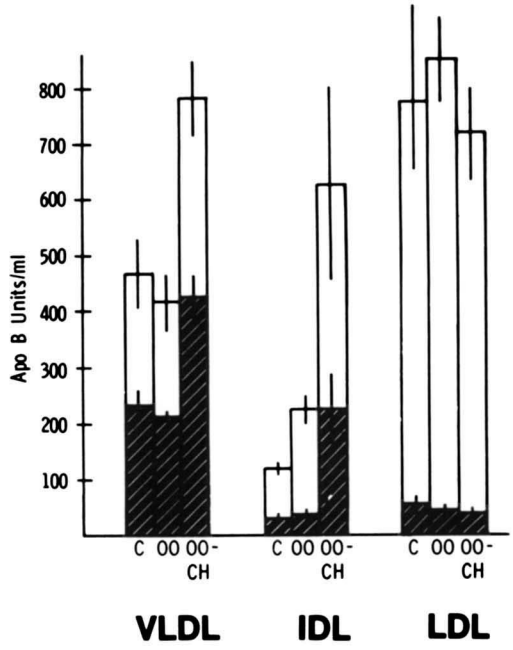

Fig. 4. Concentration of $apoB_h$ and $apoB_l$ in VLDL, IDL, and LDL nine hours after feeding control (C), olive oil (OO), and olive oil/cholesterol (OOCH) meals. □ $apoB_h$; ■ $apoB_l$. Values ± SEM based on four pools of four rats per pool.

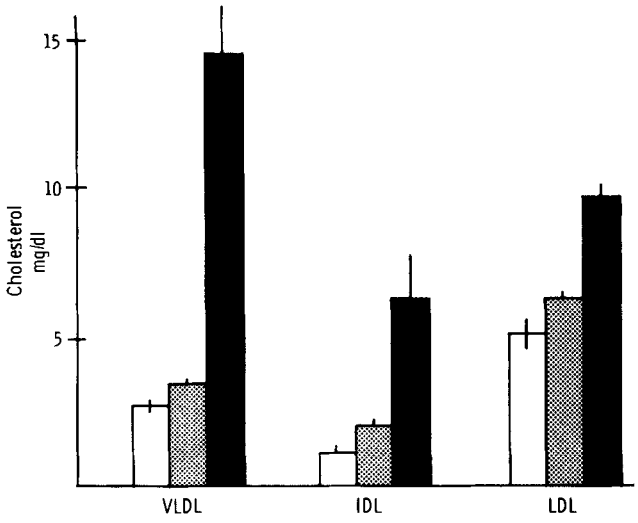

Fig. 5. Concentration of cholesterol in VLDL, IDL, and LDL nine hours after feeding control, olive oil, and olive oil/cholesterol meals. □ control; ▨ olive oil; ■ olive oil/cholesterol. Values ± SEM based on four pools of four rats per pool.

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