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The Effect of Fasting on the Secretion of Lipoproteins and Two Forms of Apo B by Perfused Rat Liver (41415)

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Abstract. Rat livers from animals fed *ad lib* or fasted 24 hr were perfused by the single pass technique with ^{3}H-amino acids and label incorporation into lipoprotein apoproteins was measured. The lipoproteins were separated into very low density lipoproteins ($d < 1.006$), low-density lipoproteins ($1.006 < d < 1.06$), and high-density lipoproteins ($1.06 < d < 1.21$) by ultracentrifugation and the apoproteins of each density fraction were analyzed by gel filtration column chromatography in the presence of sodium dodecyl sulfate. The apoprotein fractions included apo B_h (higher-molecular-weight apo B), apo B_l (lower-molecular-weight apo B), apo E, and apo C. Fasting decreased total hepatic apoprotein secretion from 75 to 53 $\mu g \cdot g^{-1} \cdot hr^{-1}$ and label incorporation into the apoproteins of very low density lipoproteins was reduced by 48% with the greatest reduction in both apo B fractions. There was a redistribution of apo B_h label into the low-density lipoproteins with fasting resulting in secretion of lipoproteins enriched in apo B_h. The high-density lipoprotein fraction contained 27 and 41% of the apo B_l label isolated from liver perfusates of fasted and fed animals, respectively. The nascent high-density lipoprotein was enriched in labeled apo B_l compared to low-density lipoproteins indicating that apo B in this density range represents a distinct metabolic fraction. With fasting the apo B_h and apo B_l apoproteins behave independently in terms of the distribution of label incorporation into nascent hepatic lipoproteins.

Two forms of apo B have been reported in rat plasma by Krishnaiah *et al.* (1) and by Elovson *et al.* (2), and in human plasma by Kane *et al.* (3). We have separated the two forms of apo B in rat very low density lipoprotein (VLDL) using gel filtration column chromatography in the presence of SDS, into a higher-molecular-weight apo B_h and lower-molecular-weight apo B_l (4) which correspond to the apo B fractions of Krishnaiah *et al.* (1). The apo B_l was the dominant apo B protein in lymph (1). Incorporation of ^{3}H-amino acids into intestinal mesenteric lymph lipoproteins and nascent hepatic lipoproteins indicated that apo B_l was the only apo B protein synthesized by the intestine in contrast to the liver which has been shown to synthesize both proteins (5, 6). We have studied ^{125}I-VLDL turnover in rats and found metabolic differences between apo B_h and apo B_l with preferential hepatic clearance of apo B_l in contrast to preferential incorporation of apo B_h into low-density lipoproteins (LDL) (7).

The present experiments were undertaken to see if there were differences in labeled apo B_h and apo B_l distribution in hepatic secretory lipoprotein density classes and whether fasting could affect this distribution.

Materials and Methods. Livers of male rats of the Fischer 344 strain, 250–350 g, were perfused for 1 hr at 37° by the single-pass technique previously described (8). The perfusion medium was Krebs–Ringer–bicarbonate–0.1% glucose, pH 7.4, gas phase 95% O_2–5% CO_2, containing 0.1 μCi/ml of ^{3}H-amino acids (NET-250, New England Nuclear Co.). The perfusate was concentrated and lipoproteins were isolated sequentially at densities of 1.006, 1.06, and 1.21 as described (8). All calculations were based on total recovered radioactivity. After dialysis against 0.15 *M* NaCl–2 m*M* EDTA (pH 7.4), the lipoprotein fractions were delipidized by the method of Lux *et al.* (9) after addition of 1 mg of Dextran T-500 (Pharmacia Co., Piscataway, N.J.). The apoproteins were dissolved in a solution of 10% SDS, 10% glycerol, and 10% 2-mercaptoethanol containing bromophenol blue and applied to

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1.5 × 175-cm columns of Sepharose CL-6B (Pharmacia) in 1% SDS–0.1 *M* phosphate buffer, pH 7.0, for the isolation of labeled apoproteins (4). Fractions were counted in a liquid scintillation counter to a probable error of ±5%. Protein determinations were made by the Lowry method (10) and trichloroacetic acid-soluble glycogen measured by precipitation of 10% trichloroacetic acid extracts of liver with ethanol (1:2, v/v) followed by total carbohydrate determination by the phenol–sulfuric acid reagent (11). All values are expressed ±SEM and the significance of differences was calculated using Student's *t* test.

Results. *Label incorporation into nascent lipoproteins.* Total output of hepatic lipoprotein proteins was measured in VLDL, LDL, and HDL in fed and fasted animals. Fasting for 24 hr produced a marked loss of glycogen in all rats. There was a decline in VLDL output with fasting from 34 to 18 μg/g liver/hr without corresponding changes in LDL. The HDL output was reduced by 32%. The total apolipoprotein-specific activities were similar in VLDL, LDL, and HDL, comparing fasting to fed animals. The results are summarized in Table I.

Apolipoprotein distribution in lipoprotein fractions. [^{3}H]Apolipoproteins of VLDL, LDL, and HDL were separated by SDS column chromatography into apo B_h, apo B_l, apo E, and apo C and the radioactivity, expressed as a percentage of the total label for each apoprotein, is given in Table II. The total label incorporated into all apoproteins was 3930 ± 544 cpm·g^{-1}·hr^{-1} for the fed and 2597 ± 715 cpm·g^{-1} for the fasted rats. Representative chromatograms of VLDL apoproteins of fed and fasted animals are presented in Fig. 1. The major change in apoprotein distribution created by fasting was in the apo B distribution of VLDL and LDL. The total apo B label of VLDL declined, with a greater decline of apo B_l than apo B_h. The total apo B label of LDL increased, mainly resulting from an increased labeling of apo B_h. The label dis-

TABLE I. LIPOPROTEIN OUTPUT IN PERFUSATES OF RAT LIVER[a]

Type of rat	Lipoprotein output (μg protein·g liver^{-1}·hr^{-1}) VLDL	LDL	HDL
Fed	34 ± 3.53 (10)	16 ± 2.44 (10)	25 ± 2.97 (10)
Fasted	18* ± 4.64 (5)	18 ± 3.85 (5)	17 ± 3.95 (5)

[a] The liver total protein specific activity averaged 6.0 ± 0.85 cpm/μg (n = 10) in fed rats and 4.9 ± 1.06 cpm/μg in fasted rats (n = 5). The trichloroacetic acid-soluble glycogen content after 1 hr of perfusion was 17.5 ± 4.05 mg/g of liver in the fed and 0.6 ± 0.45 mg/g of liver in the fasted rats. The numbers in parentheses represent the number of observations. The protein-specific activities of the perfusate lipoprotein of fed animals averaged 51, 48, and 81 cpm/μg, respectively, compared with 54, 51, and 78 cpm/μg protein for VLDL, LDL, and HDL of fasted animals.

* Significantly different from the mean of the fed rats ($P < 0.05$).

TABLE II. PERCENTAGE OF TOTAL LABEL INCORPORATED INTO PROTEIN OF LIPOPROTEINS REPRESENTED BY INDIVIDUAL APOPROTEINS

Density class	Percentage of total labeled apolipoprotein: apo B_h	apo B_l	apo E[a]	apo C
	Fed rats			
VLDL	3.4 ± 0.89	2.7 ± 0.64	7.7 ± 1.3	25.1 ± 2.2
LDL	2.4 ± 0.49	1.4 ± 0.35	6.0 ± 0.85	8.5 ± 1.1
HDL	0.94 ± 0.20	1.3 ± 0.17	22.3 ± 2.2	18.2 ± 1.9
Total	6.8 ± 1.4	5.3 ± 0.81	36.1 ± 2.3	57.8 ± 3.5
	Fasted rats			
VLDL	1.8 ± 0.18	0.7* ± 0.073	8.9 ± 2.0	17.3 ± 2.7
LDL	6.2* ± 0.85	2.0 ± 0.23	9.4 ± 1.3	11.8 ± 0.70
HDL	1.7 ± 0.28	1.9 ± 0.27	21.4 ± 2.0	17.0 ± 1.3
Total	9.7 ± 0.84	4.5 ± 0.34	39.6 ± 2.4	46.2 ± 2.6

[a] In the HDL fraction, some of the label is present in apo A-I since the column chromatographic method does not completely resolve these two apoproteins. In two experiments, the apoproteins were separated by SDS–polyacrylamide gel electrophoresis as previously described (7) and the amount of label in apo E and apo A-I measured by slicing and counting the gels. The percentage of label in apo E expressed as a percentage of the total label in apo E plus apo A-I, was 84 and 83% in the HDL of perfusates from fed rats and 74 and 85% in those from fasted rats.

* Significantly different from the mean of the fed rats (n = 5, $P < 0.05$).

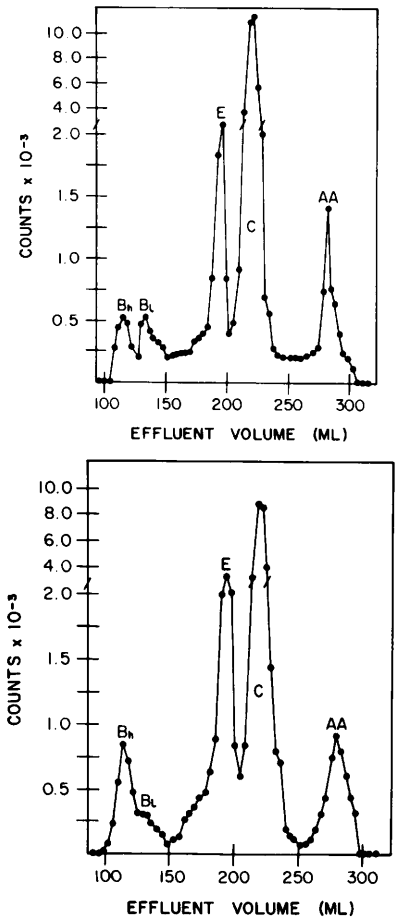

FIG. 1. SDS column chromatography of apolipoproteins from VLDL of perfused livers of fed and fasted rats. Representative chromatograms from a fed liver perfusate (A) and a fasted liver perfusate (B) are shown where labeled apoprotein distribution is plotted against effluent volume. The identity of each apoprotein peak is indicated as apo B_l, apo B_h, apo E, and apo C. The amino acid radioactivity peak is designated AA.

tribution of apo E and apo C remained essentially unchanged, though there was a trend toward increased labeling of all apoproteins in LDL. There was little difference between the HDL apoprotein distribution of fed compared to fasted rats. Nascent HDL ($1.06 < d < 1.21$ fraction) was relatively enriched in apo B_l in contrast to LDL, suggesting that this fraction contained a separate apo B_l-rich lipoprotein particle.

As can be calculated from the data in Table II, there was an increased relative amount of label in the LDL fraction as a result of fasting. It was accompanied by a greater degree of labeling in apo B_h compared with apo B_l as seen from the ratio apo B_h/apo B_l which increased from 1.46 to 3.06 (Table III).

Discussion. The current study compared fasted to fed rats in terms of labeled apolipoprotein distribution among lipoprotein fractions. Hepatic triglyceride output is decreased by fasting (12) but previous studies have not evaluated apo B synthesis into triglyceride-rich lipoproteins which contain apo B as their nonexchangeable "core" protein (13). In the present experiments we have evaluated apo B synthesis in nascent hepatic lipoproteins analyzing the apoprotein distribution by SDS columns which separate apo B into a higher-molecular-weight apo B_h and a lower-molecular-weight apo B_l (4). The apo B_h and apo B_l apparently are distinct protein fractions. They have similar but not identical amino acid compositions (1, 3, 4), and differences in sequence as indicated by peptide maps have been reported by Elovson *et al.* (2). The apo B_h and apo B_l also differ immunologically in terms of their binding to anti-apo B antibodies (1, 2).

The apo B fractions are metabolically distinct in the rat indicating that the structural differences are probably important to their metabolism. The apo B_l is the sole apo B protein synthesized and secreted as a component of intestinal lipoproteins in the rat (1, 5). In contrast the liver synthesizes both apo B_h and apo B_l as indicated by their

TABLE III. RATIO OF APO B_h TO APO B_l LABEL IN PERFUSATE LIPOPROTEINS

Density class	apo B_h/apo B_l fed	apo B_h/apo B_l fasted
VLDL	1.45 ± 0.197 (6)	2.51 ± 0.134* (5)
LDL	1.46 ± 0.242 (5)	3.06 ± 0.333* (5)
HDL	0.74 ± 0.140 (7)	0.94 ± 0.142 (5)

* Significantly different from the mean of the controls ($P < 0.05$). The numbers in parentheses represent the number of experiments.

presence in isolated hepatic Golgi vesicles (2) and incorporation of ^{3}H-amino acid label into apo B_h and apo B_l of nascent hepatic VLDL (5). Wu and Windmueller (6) have demonstrated secretion of both forms of apo B by the perfused liver and Bell-Quint and Forte (14) have shown that rat hepatocytes in culture synthesize both forms. Using ^{125}I-triglyceride-rich lipoproteins we have previously demonstrated preferential hepatic clearance of apo B_l compared to apo B_h with apo B_h preferentially entering the LDL fraction following *in vivo* injection (5) and Elovson *et al.* (2) and Wu and Windmueller (6) have found similar results with individual apo B proteins. Lipoprotein apoprotein label distribution was consistent with these findings 2 hr after *in vivo* ^{3}H-amino acid label injection (5). In these studies the VLDL was enriched in apo B_l and the LDL enriched in apo B_h label.

In the current study the effect of fasting was studied on the synthesis and distribution of hepatic apoprotein in VLDL, LDL, and HDL lipoprotein density fractions. Fasting decreased hepatic secretion of apoprotein mass and ^{3}H-amino acid incorporation into VLDL with the greatest decline in release of labeled apo B. There was no decline in LDL secretion and the LDL was enriched in total apo B primarily as the result of increased label in the apo B_h fraction. With the reduction of triglyceride output the liver apparently shifts secretion to an LDL density fraction which contains increased amounts of apo B_h. The LDL density fraction in rat liver perfusates is heterogeneous with respect to particle size and density (8), and the increased amounts of apo B_h may reflect the secretion of smaller particles which are normally enriched in apo B_h.

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