# Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid[1]

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**Abstract** Apolipoprotein B (apoB) synthesis rates have been determined, in vivo, in rat enterocytes. Following intralumenal administration of a pulse of [$^3$H]leucine, newly synthesized apoB was quantitated by specific immunoprecipitation and compared to [$^3$H]leucine incorporation into total, trichloroacetic acid-insoluble protein. ApoB synthesis rates were determined after acute administration of either 0.1 or 1 g of triglyceride to fasting animals. No differences were found at any time from 90 min to 6 hr after challenge and values were not different from the basal values established in fasted controls. Animals rechallenged with triglyceride after 8 days' intake of fat-free chow also failed to demonstrate a change in intestinal apoB synthesis rate. By contrast, enterocyte content of apoB appeared to fall, temporarily, with the onset of active triglyceride flux. Groups of animals were then subjected to external bile diversion for 48 hr, a maneuver designed to remove all lumenal sources of lipid. Jejunal apoB synthesis rates fell by 43% (from 0.76% ± 0.14 to 0.43% ± 0.12, $P < 0.001$), a change that was completely prevented by continuous replacement with 10 mM Na taurocholate. The suppression of jejunal apoB synthesis, induced by prolonged bile diversion, was reversed after 14 hr, but not 8 hr, of intralumenal perfusion with 10 mM Na taurocholate. The addition of micellar fatty acid-monoolein to the perfusate for 4 hr produced no further change in apoB synthesis. Ileal apoB synthesis rates fell by 70% (from 0.61% ± 0.15 to 0.18% ± 0.10, $P < 0.001$) following 48 hr external bile diversion, a change that was only partially prevented by continuous bile salt replacement. These results suggest that jejunal apoB synthesis demonstrates bile salt dependence but not regulation by acute triglyceride flux. The data further suggest that key aspects of the regulation of apoB synthesis by cellular lipid flux may be mediated independently in jejunal and ileal enterocytes. —**Davidson, N. O., M. E. Kollmer, and R. M. Glickman.** Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* 1986. **27:** 30–39.

**Supplementary key words** intestinal lipoproteins • chylomicron formation • enterohepatic circulation • bile salts • phospholipid • jejunum • ileum

Apolipoprotein B (apoB) is a large hydrophobic protein synthesized in mammalian liver and intestine as a surface component of both triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins, VLDL) and low density lipoproteins, LDL (1–3). Important differences exist between the native forms synthesized by liver and intestine, particularly in regard to the size (4) and metabolic fate (5) of each. Little work has been forthcoming, however, as to the regulation of apoB synthesis in vivo. Although studies of intestinal apoB content in response to triglyceride administration have produced conflicting results in rats (6) and humans (7), it was recently proposed that intestinal secretion of apoB in the rat was strongly linked to active triglyceride flux (8). In that report, a combination of differential labeling with [$^3$H]- and [$^{14}$C]-leucine was employed to illustrate that the intestinal contribution to circulating apoB was reduced from 16% to 5% following the elimination of dietary triglyceride (8).

We now report the development and characterization of techniques that allow direct quantitation of apoB synthesis in rat enterocytes in vivo. We further report the results of studies conducted to determine the role of triglyceride and biliary lipid flux as stimuli to intestinal apoB synthesis. These studies suggest that acute triglyceride flux is not a stimulus to intestinal apoB synthesis. By contrast, biliary lipid flux appears to play an important role in the regulation of intestinal apoB metabolism, aspects of which appear to be regulated independently in jejunal and ileal enterocytes.

## MATERIALS AND METHODS

### Animals and dietary protocol

Male Sprague-Dawley rats (200–300 g) from Charles River, Wilmington, MA were used throughout. Except where indicated, all animals had free access to water and

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; PBSA, phosphate-buffered saline plus 1% (w/v) bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TCA, trichloroacetic acid.

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standard pelleted rat chow (Ralston-Purina, St. Louis, MO) containing a minimum of 4.5% fat by weight as determined by the supplier. Where indicated, animals were fed a fat-free pelleted chow (diet #0848K, Bio-Serv, Frenchtown, NJ) containing 69% carbohydrate, 18% protein, and 5% fiber by weight. Minerals and vitamins were supplemented by the manufacturer to ensure nutritional adequacy. These diets were administered ad libitum for 8 days prior to study.

### Acute triglyceride flux: experimental protocol

Animals were fasted for 12–14 hr and were anesthetized with diethyl ether. A small laparotomy incision was made and 2 ml of 10% Intralipid (Cutter, Berkeley, CA) diluted 1:1 with isotonic saline was instilled into the duodenum using a transpyloric approach. Control animals received 10% dextrose–saline. The incision was closed with skin clips and the animals were returned to their cages, the entire procedure taking less than 2 min. Animals studied in this fashion received approximately 100 mg of triglyceride. Where indicated, other groups of animals received 5 ml of 20% Intralipid by intragastric gavage, thereby receiving 1.0 g of triglyceride. Control animals received an isocaloric bolus of a high nitrogen, fat-free elemental formula (CRITICARE HN, Mead-Johnson, Evansville, IN). At appropriate time intervals following bolus administration, animals were re-anesthetized and enterocytes were pulse-labeled as described below.

### External bile diversion: surgical procedures and infusion protocol

Animals were anesthetized with sodium pentobarbital and the common bile duct was cannulated with polyethylene tubing (i.d. 0.58 mm, o.d. 0.965 mm) at a point just distal to the bifurcation of the common hepatic ducts. The distal end of the cannula was exteriorized and the bile duct was doubly ligated with silk sutures. The duodenum was cannulated with polyethylene tubing (i.d. 0.86 mm, o.d. 1.27 mm) to allow constant perfusion for the duration of the study. Animals were transferred to restraining cages and received a constant infusion of 10% glucose, 0.9% NaCl, 0.04% KCl (D10-saline) at a rate of 2.4 ml/hr for 48 hr. Where indicated, Na taurocholate was added to the infusate at a concentration of 10 mM and the pH was adjusted to 7.0. In some studies, where indicated, animals were infused with D10-saline–10 mM Na taurocholate for 44 hr, and they then received a micellar lipid load composed of 10 mM Na taurocholate–10 mM monoolein–18 mM oleic acid, pH 7.6. This micellar lipid mixture was in all cases observed to be crystal clear. The infusate was administered at 2.4 ml/hr for 4 hr and provided approximately 80 mg of lipid. Sham-operated controls underwent laparotomy and exposure, but not cannulation, of the common bile duct. These animals were infused via a duodenal cannula with D10-saline, as described above.

After 48 hr of infusion, animals were removed from their cages and re-anesthetized. In each animal a proximal loop of jejunum and a 10-cm loop of distal ileum (distal end 5 cm proximal to the ileocecal valve) were prepared as described below.

### Pulse-labeling of enterocytes and immunoprecipitation protocol

Loops of jejunum (10 cm from ligament of Treitz) or ileum were pulse-labeled with 500 $\mu$Ci of L [4,5-$^3$H]leucine (>120 Ci/mmol, Amersham) for 9 min, whereupon the animal was killed by exsanguination (9). The loop was removed and flushed with iced PBS–20 mM leucine–1 mM DTT and subsequently submitted to enterocyte isolation as previously described (9, 10). The cell pellet was washed extensively and homogenized in PBS–1% Triton–2 mM leucine–1 mM PMSF–1 mM benzamidine, pH 7.4. After removal of an aliquot for measurement of total protein concentration (11) and trichloroacetic acid (TCA)-insoluble radioactivity, the remaining homogenate was centrifuged at 105,000 *g* for 60 min. All procedures were performed at 0–4°C and the resulting supernatant samples were stored at −75°C until analysis. Aliquots of cytosolic supernatant were mixed with washed Pansorbin (Calbiochem, CA) (12) and subsequently reacted with excess anti-apoB-100 antiserum for 18 hr at 4°C. Antiserum was raised in New Zealand White rabbits following injection of gel slices of apoB-100 from rat serum LDL. Rabbits were boosted at 3 months and bled periodically thereafter from a marginal ear vein. Following a second addition of Pansorbin and extensive washing (9), the liberated immune complex was applied to 5.6% polyacrylamide disc gels (13) and, following electrophoresis, sliced into 1–2-mm slices and subjected to liquid scintillation spectrometry as described (9). Antiserum excess was established by reacting aliquots of supernatant containing $^{125}$I-labeled LDL (see below) with anti-apoB-100 antiserum and comparing the recovery of $^{125}$I in the final pellet to that obtained in parallel incubations run with $^{125}$I-labeled LDL and antiserum alone. Recovery of $^{125}$I-labeled LDL when subjected to the entire procedure consistently exceeded 95%.

### Radioimmunoassay of apoB

Rat serum LDL (d 1.022–1.050 g/ml) was prepared by sequential ultracentrifugation and was routinely found to contain >85% apoB-100 by scanning densitometry of SDS disc gels. The LDL preparation was dialyzed into 50 mM phosphate buffer, pH 7.5, and 10–20 $\mu$g was iodinated for 10 sec using the chloramine-T procedure (14). Separation of protein-bound from free iodine was achieved by passage of the iodination mixture through a 30 × 1 cm

column of Sephadex G75 medium (Pharmacia, Piscataway, NJ). Material eluting in the void volume, referred to hereafter as $^{125}$I-labeled LDL, was found to be 40–50% immunoprecipitable with approximately 80% of the counts coprecipitating with carrier BSA upon addition of 20% TCA.

The conditions for RIA were standardized using rat serum LDL prepared by sequential ultracentrifugation with repeated washing at the upper density limits to ensure a final preparation containing essentially only apoB-100. LDL protein concentration was determined by the procedure of Lowry et al. (11) and known quantities (from 938 to 4 ng in 100 μl of PBSA) were added to 10 × 75 mm silanized glass test tubes containing 20 μl of PBSA–25% Triton. Thereafter, each tube received 300 μl of a solution containing approximately 30,000 cpm of $^{125}$I-labeled LDL and 1:2000 preimmune rabbit serum, followed by 100 μl of a 1:5000 dilution of rabbit anti-apoB-100 antiserum. The tubes were vortexed following each addition and incubated at 4°C for 48 hr. Following addition of goat anti-rabbit IgG antiserum and incubation at 4°C for a further 24 hr, the assay was harvested and counted in an LKB gamma counter (Rackgamma II, LKB Instruments, Gaithersburg, MD) with data reduction performed by standard software.

A large pool of rat serum was obtained by exsanguinating ten nonfasting rats. This pool of rat serum was aliquoted and frozen at −75°C. Having constructed a standard curve using serum LDL, aliquots of rat serum were assayed against this primary standard and found to contain approximately 30 mg/dl apoB. This pool of serum was thenceforth used as a secondary standard and found to give displacement curves superimposable with serum LDL. Frozen at −75°C, this pool has given reliable displacement curves for over 2 years.

### Standardization of conditions for quantitative assay of enterocyte apoB

Having established the conditions for RIA of serum apoB to include incubation with a final Triton concentration of 1%, it was necessary to validate that these same conditions would reliably liberate enterocyte apoB. This issue was addressed in several ways.

First, preparations of enterocytes from animals given 1 g of triglyceride by gavage 2-3 hr prior to killing were assayed under the conditions outlined above to determine reproducibility of apoB mass determination. Four separate pools of enterocytes were assayed three times each with a mean coefficient of variation of 5%. Next, to aliquots of enterocyte supernatants with known contents of apoB was added serum LDL containing known amounts of both total protein [Lowry et al. assay (11)] and apoB-100 mass, as determined by RIA. The mean incremental recovery of apoB, from 16 such experiments, was 105% of predicted.

Secondly, the recovery of apoB from a triglyceride-rich lipoprotein sample was determined. Rat serum d < 1.022 g/ml protein was assayed by a modification (15) of the Lowry et al. assay (1) before and after isopropanol precipitation (16) of the apoB-containing lipoproteins. Values for apoB mass were deduced by subtraction and compared to values for apoB mass by RIA of the starting material. A series of 12 determinations over an eightfold range yielded apoB mass values by RIA of 91 ± 15% those by total protein assay.

Thirdly, the question was addressed as to whether the extractability of enterocyte apoB was influenced by variations in mucosal triglyceride content. Groups of rats were therefore subjected to external bile diversion for 48 hr to eliminate mucosal triglyceride, while other animals received 1 g of triglyceride by gavage 2–3 hr prior to study to produce maximal mucosal triglyceride flux. Loops of jejunum were prepared as detailed above and enterocytes were harvested. Eight separate pools of enterocytes were studied from fat-fed rats and eight pools from bile-diverted rats. Each pool of enterocytes was homogenized in PBS–1% Triton and 105,000 *g* supernatants were prepared. Homogenate and supernatant were assayed for recovery of apoB mass (by RIA) and recovery of newly synthesized apoB (by quantitative immunoprecipitation).

Mass recovery in the 105,000 *g* supernatants averaged 86% and 81% for bile-diverted and fat-fed animals, respectively, values representing the mean for eight assays each. Reextraction of the 105,000 *g* pellet with PBS–1% Triton produced an additional 12–15% of the total apoB mass in bile-diverted animals and 5–10% in fat-fed animals. In four experiments, reextraction of the 105,000 *g* pellet was performed with either PBS–1% SDS or PBS–1% decyl sulfate to determine whether further solubilization could be achieved with ionic detergents. Comparable recoveries were obtained with both agents, yielding an additional 3–6% apoB, indicating that 1% Triton was the superior choice of detergent. A third extraction of the sample with 1% Triton yielded negligible apoB mass (0.5–1% total). In conclusion, recovery of apoB in the *first 105,000 g supernatant*, following PBS–1% Triton homogenization, averaged 84–94% *of the total recoverable apoprotein mass*, with no discernible differences attributable to the state of mucosal triglyceride flux.

Recovery of newly synthesized apoB was quantitated by comparing immunoprecipitable apoB counts in homogenate and 105,000 *g* supernatant. Values were again comparable for bile-diverted (89%) and fat-fed animals (93%). Reextraction of the 105,000 *g* pellets with 1% SDS resulted in no detectable immunoprecipitable apoB counts, while reextraction with 1% decyl sulfate gave consistently high background counts (200–300 cpm/slice—compare with Fig. 1) throughout the gel. These results again confirm the superiority of extraction with PBS–1% Triton.

Thus, these control studies directly validate the extraction and assay protocols used throughout the experiments to be described below.

### Chemicals and reagents

The sources and purity of all materials have been previously described (9).

### Data expression and statistical comparison

ApoB synthesis is expressed as a relative rate, % total protein synthesis. The value is derived by expressing [$^3$H]-leucine incorporation into immunoprecipitable apoB, per volume of supernatant, as a fraction of its incorporation into the same volume of homogenate, following precipitation with hot 10% trichloroacetic acid. Each value represents the mean of duplicate assays and reflects the subtraction of nonspecific and background radioactivity. ApoB content is expressed as ng per mg of homogenate protein (11). Comparisons between groups were made by paired and unpaired *t*-test.

## RESULTS

### Characterization of immunoprecipitation procedure for determining apoB synthesis rate

As illustrated in **Fig. 1**, apoB immunoprecipitated from supernatants of jejunal enterocytes revealed a single monomorphic peak with an apparent $M_r$ of approximately 230,000. This is similar to previous reports (3, 4) and suggests that low molecular weight apoB or apoB-48 is the exclusive product of rat enterocytes. Extensive homology between intestinal apoB and circulating plasma LDL apoB is suggested by the competitive displacement of newly synthesized apoB from the immune complex following addition of 70 $\mu$g of autologous serum LDL (>85% apoB-100, Fig. 1). Samples of supernatant reacted with anti-apoB-100 antiserum contained no further immunoreactive apoB as judged by repeat immunoprecipitation and no detectable peak of apoB radioactivity following incubation with pre-immune serum (data not shown).

### ApoB synthesis in jejunal enterocytes: regulation by triglyceride flux

Groups of animals subjected to either a 14- or 30-hr fast were studied to determine basal intestinal apoB synthesis. Synthesis rates (% total protein) in jejunal enterocytes [0.74 ± 0.16 (SD) n = 6, 14-hr fast or 0.775 ± 0.08 (SD) n = 4, 30 hr fast] were not significantly different, despite a trend, from the respective rates in ileal enterocytes [0.54 ± 0.12 (SD) n = 6 and 0.58 ± 0.23 (SD) n = 4], $P > 0.05$. This contrasts with the significant regional differences previously demonstrated (9) for apoA-I synthesis. To determine the role of acute triglyceride flux, animals were fasted for 14 hr and challenged either with triglyceride or an isocaloric, fat-free bolus. As illustrated in **Fig. 2A**, there was no effect of intraduodenal triglyceride, at 90 min or 4 hr, upon jejunal apoB synthesis. Intragastric administration of 1.0 g of triglyceride (Fig. 2A) failed to alter jejunal apoB synthesis at either 4 or 6 hr. Enterocyte content of apoB appeared to fall with active triglyceride flux, although the appearance of this event was somewhat more delayed following intragastric as compared to intraduodenal administration of triglyceride (Fig. 2B).

Prolonged intake of a fat-free diet (for 8 days) followed by intraduodenal triglyceride administration was not accompanied by a change in jejunal apoB synthesis. By contrast, enterocyte apoB content again demonstrated a significant fall (Fig. 2 A, B) suggesting that the response to active chylomicron secretion involves mobilization and temporary depletion of an intracellular pool of apoB.

Total protein synthesis, as judged by [$^3$H]leucine incorporation into trichloroacetic acid-insoluble radioactivity per mg of protein, was no different at any time between control and triglyceride-exposed animals (**Table 1**). Furthermore, despite the fall in apoB content, specific activity

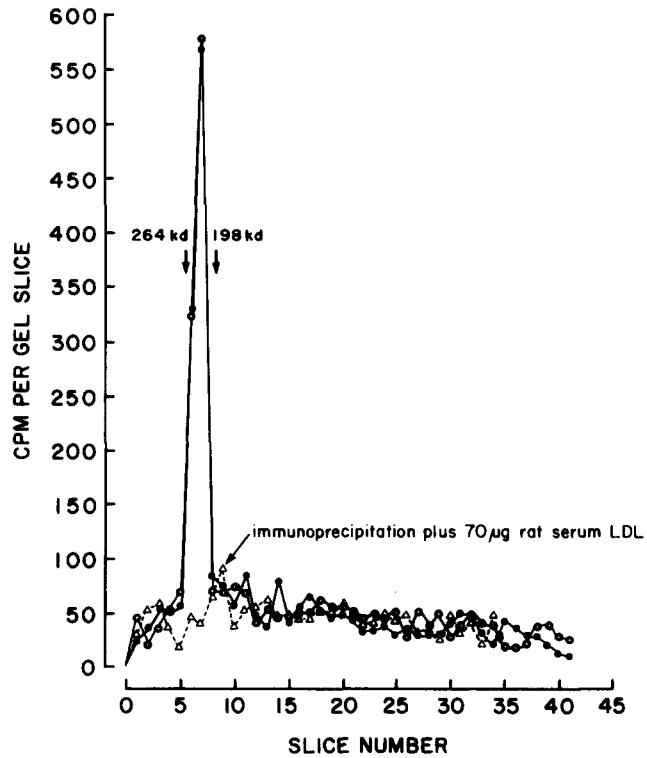


**Fig. 1.** Immunoprecipitable apoB from jejunal enterocytes. Duplicate samples of cytosolic supernatant (○, ●) were reacted with excess anti-apoB-100 antiserum and the immune complex was subjected to SDS-PAGE (Methods). A further aliquot was similarly incubated along with 70 $\mu$g of autologous serum LDL (△). Molecular weight markers (cross-linked bovine serum albumin) are indicated by the arrows.

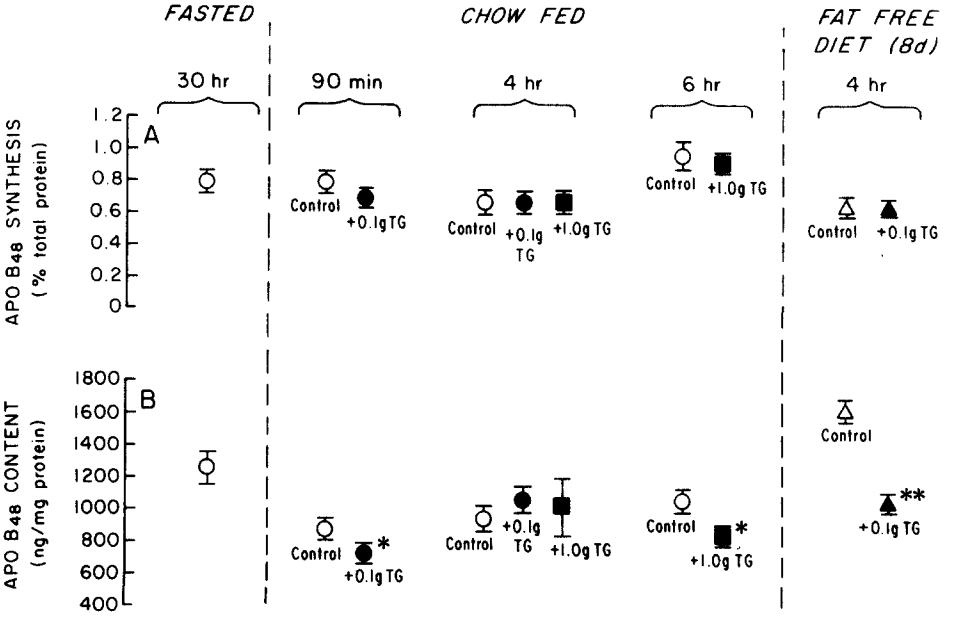


**Fig. 2.** ApoB synthesis in jejunal enterocytes: effects of acute triglyceride flux. A: Animals (4–6 per group) were allowed ad libitum access to standard or fat-free chow for 8 days prior to study. After a 14-hr fast, animals received a triglyceride challenge with either 0.1 g of triglyceride, administered intraduodenally (●) as 2 ml of 10% Intralipid–0.9% NaCl 1:1 (v/v), or 1.0 g (■) by intragastric gavage of 5 ml of 20% Intralipid. Control animals received an isocaloric, fat-free bolus (○). ApoB synthesis rates were determined at the indicated times. Data are expressed as mean ± SE. B: ApoB content was determined by RIA and represents mean ± SE. *, $P < 0.05$; **, $P < 0.01$.

of enterocyte apoB ranged from 5.4 to 12.2 cpm per ng with no discernible differences between triglyceride-exposed and control animals (Table 1).

### Intestinal apoB synthesis: regulation by biliary lipid flux

Having determined that jejunal apoB synthesis exhibited no short term regulation in response to acute triglyceride flux, studies were designed to test the hypothesis that apoB synthesis in the fasting state may be maximally expressed. In order to assess the contribution of biliary lipid flux to this expression, animals were surgically bile-diverted for 48 hr and perfused with physiological buffers as outlined above (Methods).

External bile diversion for 48 hr produced a significant reduction in jejunal apoB synthesis [sham-operated 0.76% ± 0.14 (SD) vs 0.43% ± 0.12 (SD) following external bile diversion, $P < 0.001$]. This effect was completely prevented by the continuous administration of 10 mM Na taurocholate (**Fig. 3A**). Addition of a fatty acid–monoolein micellar infusion (80 mg of lipid supplied over 4 hr) failed to produce an additional effect on apoB synthesis (Fig. 3A), thereby confirming the earlier observations in regard to triglyceride flux (Fig. 2). Enterocyte apoB content appeared to increase when 10 mM Na taurocholate was infused into bile-diverted rats (Fig. 3B) and a small but insignificant fall was encountered upon the addition of micellar lipid (Fig. 3B).

The time course of suppression and reinduction of jejunal apoB synthesis was further characterized. Twelve hr bile diversion was found to produce 84% of the achievable suppression and only small decremental changes were encountered at 24 and 48 hr (**Fig. 4**). Using a time of 30 hr to represent the nadir of jejunal apoB synthesis, animals were infused for the indicated times (Fig. 4) with 10 mM Na taurocholate and full reinduction was encountered after 14 (but not 8) hr bile salt replacement. To determine whether the reinduction of jejunal apoB synthesis following bile salt replacement was a reflection of conformational change and enhancement of immunogenicity of cytosolic apoB, the following control study was undertaken. Samples of cytosolic supernatant from a bile-diverted animal were incubated with increasing concentrations of Na taurocholate across a physiologic range from 0.2 to 20 mM. Incubation conditions were varied with respect to time (4–16 hr) and temperature (4°, 26°, 37°C). Immunoprecipitation was conducted in quadruplicate and the results (immunoprecipitable apoB cpm/10 μl) were found to be identical to unincubated samples subjected to immediate immunoprecipitation (for example: no incubation = 845.5 ± 94 cpm/10 μl; 2 mM Na taurocholate for 4 hr at 26°C = 874.5 ± 71.5 cpm/10 μl; 20 mM Na taurocholate for 16 hr at 4°C = 782 ± 53 cpm/10 μl). Thus, while the mechanism of bile salt-mediated reexpression of jejunal apoB synthesis remains unclear, the same observations could not be reproduced by in vitro incubation.

TABLE 1. Total cellular protein and apoB specific activity in jejunal enterocytes: effect of acute triglyceride administration

| | 30-hr Fast | Experimental Group[a] 90 min +0.1 g TG | 90 min Control | 4 hr +0.1 g TG | 4 hr Control | 4 hr +1.0 g TG | 6 hr +1.0 g TG | 6 hr Control |
|---|---|---|---|---|---|---|---|---|
| Total cellular protein[b] (cpm/ng) | 1.28 ± 0.48 | 0.94 ± 0.25 | 1.38 ± 0.71 | 0.86 ± 0.20 | 1.19 ± 0.59 | 1.73 ± 0.65 | 0.94 ± 0.39 | 0.94 ± 0.17 |
| ApoB[c] (cpm/ng) | 7.89 ± 2.23 | 8.81 ± 2.65 | 12.21 ± 6.04 | 5.43 ± 2.43 | 10.23 ± 5.50 | 9.43 ± 3.25 | 10.88 ± 5.16 | 8.61 ± 2.02 |
| Difference from respective control[d] | | NS | | | NS | | | NS |

[a] Animals (4–6 per group) maintained on regular (4.5% by weight triglyceride) or fat-free chow were fasted for 12–16 hr prior to study. Animals received either 0.1 or 1.0 g of triglyceride as described in Methods, while control animals received an isocaloric, fat-free bolus. At the stated times after bolus administration, animals were prepared with in situ jejunal loops and received a 9-min pulse of [$^3$H]leucine. Cytosolic supernatants were subsequently prepared following homogenization of washed enterocytes (Methods).

[b] Total cellular protein specific activity was determined by reacting aliquots of enterocyte homogenate with hot 10% TCA and measuring total protein concentration (11) on a further aliquot. Data are expressed as mean ± SD.

[c] ApoB specific activity was determined by measuring [$^3$H]leucine incorporation into a known volume of cytosolic supernatant apoB by quantitative immunoprecipitation (Methods). ApoB mass was determined on a separate aliquot by RIA. Data are expressed as mean ± SD.

[d] Statistical significance was determined by independent *t*-test; NS, not significant at the $P < 0.05$ level.




**Fig. 3.** ApoB synthesis in jejunal enterocytes: effects of external bile diversion. A: Groups of animals (5–8 per group) were bile-diverted and infused with either 10% dextrose–0.9% NaCl–0.04% KCl (D10-saline, ▲) or D10-saline plus 10 mM Na taurocholate (■) at 2.4 ml/hr for 48 hr. Another bile-diverted group received D10-saline plus 10 mM Na taurocholate for 44 hr and then received 80 mg of micellar lipid (10 mM Na taurocholate–10 mM monoolein–18 mM oleic acid, pH 7.6) over 4 hr (□). Control animals were not bile-diverted but underwent laparotomy and were restrained and infused for 48 hr with D10-saline (●). ApoB synthesis rates are expressed as mean ± SE. *, $P < 0.05$, **, $P < 0.01$, and ***, $P < 0.001$ compared to bile-diverted, D10-saline-infused animals. B: ApoB content was determined by RIA and represents mean ± SE. †, $P < 0.02$ compared to bile-diverted, D10-saline-infused animals.

Bile diversion also produced a profound effect upon apoB synthesis rates in ileal enterocytes (**Fig. 5A**) [sham-operated 0.61% ± 0.15 (SD) vs 0.18% ± 0.10 (SD) for bile-diverted animals, $P < 0.001$]. The fall in synthesis rate was accompanied by a similar fall in enterocyte apoB content (Fig. 5B). However, in contrast to the observations (above) in jejunal enterocytes, continuous replacement with 10 mM Na taurocholate was only partially effective in preventing the fall in ileal apoB synthesis and no further effect was observed with micellar fatty acid–

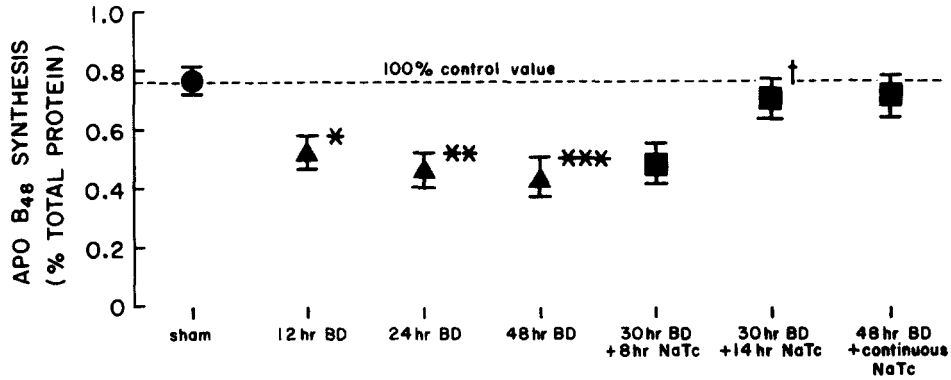


**Fig. 4.** ApoB synthesis in jejunal enterocytes: time course of suppression following bile diversion and reinduction with 10 mM Na taurocholate. Animals (4–8 per group) underwent bile diversion, with or without bile salt replacement as indicated, for various times up to 48 hr. Jejunal apoB synthesis was determined at the conclusion of each experiment. *, $P < 0.02$, **, $P < 0.01$, and ***, $P < 0.001$ compared to sham-operated controls; †, $P < 0.01$ compared to 48-hr bile-diverted animals.

monoolein (Fig. 5A). Taken together, the incomplete response to bile salt repletion and the changes in enterocyte apoB specific activity with altered biliary lipid flux, suggest that aspects of the regulation of apoB metabolism differ fundamentally in jejunal and ileal enterocytes.

Total protein synthesis, as judged by [$^3$H]leucine incorporation into trichloroacetic acid-insoluble radioactivity per mg of protein, was unaltered by the various maneuvers described (**Table 2**).

## DISCUSSION

This report describes the results of studies conducted to examine the regulation of apoB synthesis in vivo in rat small intestine. ApoB is synthesized exclusively in the liver and small intestine of all mammalian species studied to date (1–3). In light of the relative importance of hepatic apoB synthesis (8) and in view of the demonstrated uptake of serum LDL by rat enterocytes (17), a time of 9 min was chosen for pulse-labeling since it was found to maximize enterocyte protein specific activity with no detectable radioactivity in serum LDL (data not shown). The time course of labeling enterocyte apoB was further examined by comparing synthesis rates obtained after a 9- or 30-min exposure to [$^3$H]leucine. Two groups of four animals were compared, there being no detectable differences in the results [0.60 ± 0.04 (SD) vs 0.685 ± 0.26 (SD), $P > 0.05$]. It is recognized that since leucine represents ~11.6 mol % apoB-48 (3), expression of leucine incorporation into apoB as a fraction of its incorporation into trichloroacetic acid-insoluble protein may result in an overestimate of synthesis rate (18). However, given the use of appropriate control animals against which to compare the results of the various perturbations described, the expression of apoB synthesis as described provides a meaningful index of physiological regulation.

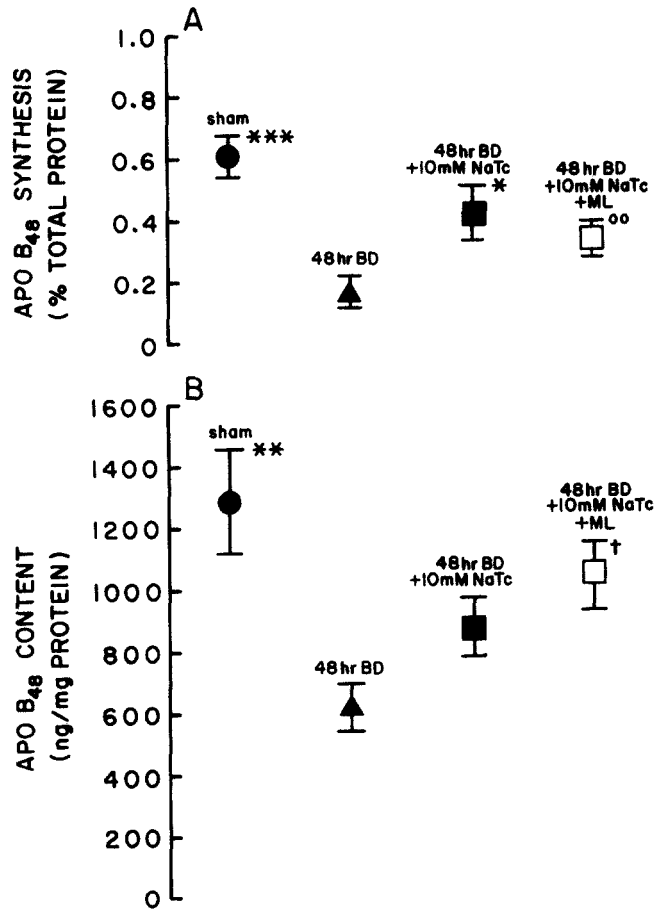


**Fig. 5.** ApoB synthesis in ileal enterocytes: effects of external bile diversion. A: Groups of animals were studied as outlined in the legend to Fig. 3. ***, $P < 0.001$ and *, $P < 0.05$ compared to bile-diverted, D10-saline-infused animals; °°, $P < 0.001$ compared to bile-diverted, D10-saline-infused animals and also to sham-operated controls. B: ApoB content was determined by RIA. **, $P < 0.01$ and †, $P < 0.02$ compared to bile-diverted, D10-saline-infused animals.

TABLE 2. Total cellular protein and apoB specific activity in jejunal and ileal enterocytes following external bile diversion

| | Experimental Group[a] | | | |
|---|---|---|---|---|
| | Sham | 48-hr BD | 48-hr BD + 10 mM NaTC | 44-hr BD + 10 mM NaTC + 4-hr Micellar Lipid |
| Jejunum | | | | |
| Total cellular protein[b] (cpm/ng) | 1.52 ± 0.56 | 1.30 ± 0.55 (NS) | 2.10 ± 0.91 (NS) | 1.18 ± 0.51 (NS) |
| ApoB[c] (cpm/ng) | 8.03 ± 1.57 | 6.14 ± 3.15 (NS) | 11.63 ± 5.5 (NS) | 6.51 ± 2.49 (NS) |
| Ileum | | | | |
| Total cellular protein (cpm/ng) | 1.87 ± 1.30 | 1.24 ± 0.39 (NS) | 1.38 ± 0.55 (NS) | 1.20 ± 0.75 (NS) |
| ApoB (cpm/ng) | 8.51 ± 4.72 | 4.01 ± 1.03* | 7.15 ± 3.9 (NS) | 3.18 ± 1.12** |

[a]Animals (5–8 per group) were subjected to external bile diversion (BD) with constant intraduodenal infusion of 10% dextrose–0.9% NaCl–0.04% KCl for 48 hr, as described in Methods. Where indicated, animals received, in addition, 10 mM Na taurocholate for the duration of the study, either with or without a 4-hr infusion of micellar lipid. Sham-operated controls were similarly restrained and infused with dextrose–saline alone.

[b]Total cellular protein specific activity was determined by reacting aliquots of jejunal or ileal enterocyte homogenates with hot 10% TCA (Methods) and measuring total protein concentration (11) on a further aliquot (Methods). Data are expressed as mean ± SD.

[c]ApoB specific activity was determined by measuring [$^3$H]leucine incorporation into a known volume of supernatant apoB by quantitative immunoprecipitation (Methods). ApoB mass was determined on a further aliquot by RIA. Data are expressed as mean ± SD.

Statistical comparison by independent *t*-test: *, $P < 0.05$ compared to Sham; **, $P < 0.02$ compared to Sham; NS, no significant differences detectable when compared to sham-operated controls.

Aspects of the methodology employed in this report deserve additional comment. In particular, throughout all the maneuvers described, enterocyte apoB synthesis was quantitated by expressing incorporation of radioactive precursor into immunoprecipitable product as a function of precursor incorporation into cellular protein. This fractional estimate of synthesis is valid only to the extent that apoB is uniformly recovered and that total protein synthesis is unchanged. ApoB recovery was directly determined in a setting of variable triglyceride flux since the hydrophobicity of this protein might conceivably have prevented equivalent recovery in the presence (fat-fed) and absence (bile-diverted) of mucosal triglyceride. Under both conditions, homogenization in 1% Triton provided equivalent (>80%) recovery of homogenate apoB mass and immunoprecipitable apoB counts. These figures should be compared to those of Alpers et al. (19) who found only 45% recovery of apoB mass following homogenization in 50 mM barbital buffer, without detergent. Protein synthesis, as judged by the criteria alluded to earlier, was comparable throughout the various groups when compared to appropriate controls (Tables 1 and 2).

The present studies suggest that intestinal apoB synthesis is unaltered by acute triglyceride flux. This is at variance with an earlier demonstration (8) that the intestinal contribution to plasma apoB was significantly depressed following triglyceride elimination. Our studies suggest that such a fall in the apparent intestinal contribution is not the result of a decrease in apoB synthesis; possibly changes in the efflux of apoB from enterocytes—which appears to be linked to active triglyceride flux—might explain this observation (8). Depletion of enterocyte apoB mass with active triglyceride flux is a finding also at variance with previous reports (6, 20–23), including studies from Glickman et al. (20–22) and Green et al. (23), based on immunohistochemical staining. The explanation for this discrepancy most probably resides in the qualitative nature of immunofluorescence quantitation. A recent immunoelectron microscopic study of human jejunal enterocyte apoB following fat infusion (24) concluded that label redistribution (from RER to Golgi) was the predominant finding rather than an overall increase in the amount of staining, alluded to by Green et al. (23). Redistribution of enterocyte apoB and its subsequent export as a chylomicron-associated apolipoprotein would be consonant with the present findings and also with the earlier demonstration by Rachmilewitz, Albers, and Saunders (7) of a decrease in human jejunal enterocyte apoB mass with the onset of triglyceride flux. Thus, in studies where mass has been directly quantitated by RIA, the present study and that of Rachmilewitz et al. (7) both suggest a triglyceride-dependent depletion of enterocyte apoB, although in the present study this was an inconsistent effect at all time points (compare 4 hr and 6 hr, Fig. 2). An earlier study by Schonfeld, Bell, and Alpers (6) concluded that apoB mass increased with active triglyceride flux, but this study employed mucosal scraping and was based on only one animal per time point. Recent studies by Alpers et al. (19) have shown that a substantial extracellular pool of apoB exists; the present studies illustrate that there is also considerable animal-to-animal variation in enterocyte apoB mass, supporting the need to make such measurements in multiple animals and using isolated cells.

The absence of an effect of triglyceride flux on intestinal apoB synthesis is in agreement with studies performed using cultured hepatocytes (25, 26). Thus, Davis and Boogaerts (25) demonstrated no change in hepatic apoB secretion rate or in [$^3$H]leucine incorporation into newly synthesized apoB with enhanced fatty acid uptake and consequent stimulation of VLDL-triglyceride secretion. By contrast to the situation encountered in human abeta-

lipoproteinemia (27), where absent synthesis of apoB results in, among its effects, defective hepatic and intestinal secretion of triglyceride-rich lipoproteins, the current studies together with previous reports (9, 25, 26) based on direct measurements of synthesis rates, suggest that triglyceride flux is not a stimulus to the synthesis of apoA-I or apoB, two of the major chylomicron apoproteins in both rat and humans.

The present studies implicate aspects of biliary lipid flux as playing a major role in the basal expression of intestinal apoB synthesis. The suppression and reinduction of apoB synthesis appear to exhibit a broadly similar time course—approximately 12 hr—but the exact mode of action of Na taurocholate is as yet unclear. Potential mechanisms may relate to facilitated uptake of critical amounts of lumenal cholesterol (28), the source of which presumably would be sloughed enterocytes, since dietary and biliary cholesterol are both eliminated. Alternatively, small amounts of conjugated bile salts may themselves flux across the proximal small intestinal mucosa (29) and thereby act as direct effectors of apoprotein synthesis regulation.

Recent studies (Davidson, N. O., unpublished observations) from our laboratory also suggest that phospholipid flux may exert physiological regulation of apoB synthesis in rat enterocytes. The marked, reversible suppression of jejunal apoB synthesis, related to lumenal bile salt availability, contrasts with the negligible effects of bile diversion on jejunal apoA-I synthesis (9). Taken together, this suggests that the regulation of jejunal apoprotein synthesis involves separate mechanisms for apoA-I and apoB.

The marked suppression of ileal apoB synthesis following bile diversion and its incomplete expression following replacement of Na taurocholate, either alone or together with a brief exposure to micellar fatty acid, suggest that the regulation of ileal apoB synthesis involves factors in addition to the availability of lumenal bile salt. More detailed characterization of the effects of other components of biliary lipid on the regulation of intestinal apoprotein synthesis will be the focus of future reports.

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