Apolipoprotein A-I synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid

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Abstract Techniques were developed to provide direct quantitation of apolipoprotein A-I (apoA-I) synthesis rates in rat small intestine. Following intralumenal administration of a pulse of [^{3}H]leucine, newly synthesized enterocyte apoA-I was quantitated by specific immunoprecipitation and compared to [^{3}H]leucine incorporation into total trichloroacetic acid-precipitable protein. ApoA-I synthesis rates (% total protein) were found to be significantly higher in jejunal enterocytes (1.84 ± 0.20) compared to ileal enterocytes (0.91 ± 0.25) from the same, fasting, animals, $P < 0.01$. It was found that rats consuming regular (4.5% w/w fat) rodent chow had apoA-I synthesis rates, 30 to 240 min after receiving an intraduodenal bolus of 100 mg of triglyceride, that were indistinguishable from control animals receiving either saline or an isocaloric, but fat-free, enteral preparation. By contrast, animals consuming a fat-free chow for 8 days prior to study had a small but significant response to acute reintroduction of dietary triglyceride. Four hours after 100 mg of triglyceride was administered, jejunal apoA-I synthesis (% total protein) was 1.84 ± 0.1 compared to 1.37 ± 0.04 for animals exposed to an isocaloric, fat-free enteral preparation, $P < 0.01$. External bile diversion for 48 hr, which effectively removed all lumenal sources of lipid, reduced apoA-I synthesis in jejunal enterocytes but produced no more depression than that found in sham-operated controls infused for 48 hr with dextrose-saline or control animals fasted for 30 hr. By contrast, apoA-I synthesis in ileal enterocytes was reduced significantly by external bile diversion (0.59 ± 0.20) in comparison to sham-operated controls (1.19 ± 0.32) $P < 0.01$. Continuous infusion of 10 mM Na taurocholate for 48 hr or 10 mM Na taurocholate for 44 hr and 80 mg of micellar lipid for 4 hr produced results similar to those obtrained by bile diversion alone (0.56 ± 0.2 and 0.61 ± 0.25, respectively) suggesting that bile salt deficiency alone was not responsible for the observed depression in ileal apoA-I synthesis. These results suggest that, under conditions of physiological dietary triglyceride intake, apoA-I synthesis in jejunal enterocytes is not actuely regulated by changes in triglyceride flux. After prolonged dietary triglyceride withdrawal, the reintroduction of fat produces a small, but significant, increase in jejunal apoA-I synthesis. The data further suggest that apoA-I synthesis in jejunal enterocytes is regulated in part by the availability of lumenal lipid, but that the presence of bile does not exert an additional level of control. By contrast, the data obtained with ileal enterocytes suggest that a major aspect of the regulation of apoA-I synthesis in this location is related to biliary lipid flux, although not to the presence of lumenal bile salts. The study suggests fundamental differences exist in the regulation of apoA-I synthesis in jejunal and ileal enterocytes of the rat. —**Davidson, N. O., and R. M. Glickman.** Apolipoprotein A-I synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* 1985. **26:** 368–379.

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Apolipoprotein A-I (apoA-I) is the major protein component of circulating high density lipoprotein (HDL) in a variety of animals including man. Evidence from a number of studies in experimental animals (1–3) and man (4–6) indicates that apoA-I is synthesized in both the liver and small intestine, and is secreted by these organs into plasma as a component of either triglyceride-rich lipoprotein (chylomicrons and VLDL) or HDL. To date, however, details of the metabolic events relating synthesis and secretion of apoA-I from the intestine and the effects of triglyceride flux have been gathered from largely indirect evidence (for a review, see refs. 5 and 6). While early studies employing immunohistochemical techniques (1, 7) suggested that apoA-I content appeared to increase after a fat load, separate reports have suggested acute fat-feeding to be associated with either a steady increase (8) or a fall (9) in apoA-I content, as measured by RIA. Studies examining mesenteric lymph secretion rates of apoA-I (1, 10) have generally shown an increased secretion in the face of active triglyceride absorption, but the time course of this response has left open the question of whether increased apoA-I synthesis actually occurs in response to mucosal triglyceride flux.

By contrast, studies from this laboratory showed that apoA-I secretion into rat mesenteric lymph was unchanged following external bile diversion (11), a procedure known to deplete intestinal lymph of triglyceride-rich lipoproteins.

Abbreviations: HDL, high density lipoprotein; apo, apolipoprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PMSF, phenylmethyl sulfonyl fluoride; DTT, dithiothreitol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; RIA, radioimmunoassay.

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While this report again left unanswered the question of apoA-I synthesis by the intestine, it suggested that apoA-I secretion may be independent of mucosal triglyceride flux. This suggestion was strengthened by a report that showed that the proportion of apoA-I derived from the small intestine was unaltered by the absence of dietary or biliary lipid (3).

More recently, Gordon et al. (12) have characterized the effect of acute triglyceride feeding upon translatable mRNA levels for apoA-I in rat small intestine and have shown no change up to 8 hr after exposure to intragastric corn oil.

We now report the development and validation of techniques which allow direct quantitation of apoA-I synthesis, in vivo, in rat small intestine. We further report the results of studies conducted to investigate the role of acute triglyceride flux as a stimulus to apoA-I synthesis by jejunal enterocytes. In addition, we have characterized the response of apoA-I synthesis within both jejunal and ileal enterocytes to external bile diversion. The results suggest that only following prolonged dietary triglyceride withdrawal does apoA-I synthesis in jejunal enterocytes increase in response to acute triglyceride administration. The results of studies examining the role of biliary lipid flux suggest that apoA-I synthesis in jejunal and ileal enterocytes may be independently regulated.

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 standard pelleted rat chow (Ralston-Purina, St. Louis, MO) containing a mimimum of 4.5% fat by weight as determined by the supplier. Where indicated, animals were fed a fat-free pelleted diet (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 the study.

Experimental protocol to test the effects of acute triglyceride flux

Animals were fasted for 12–14 hr prior to study and were anesthetized with diethyl ether. A small laparotomy incision was made and, under direct vision, 2 ml of isotonic saline or 2 ml of 10% Intralipid (Cutter, Berkeley, CA) diluted 1:1 with isotonic saline was instilled into the duodenum, using a 1½-in 25-G needle, via a transpyloric approach. The incision was closed using skin clips, the entire procedure taking less than 2 min. Where indicated, some control animals received an isotonic, isocaloric bolus of a high nitrogen, fat-free elemental formula (Criticare HN, Mead Johnson, Evansville, IN) to avoid artefacts related to caloric load.

Isotope administration and preparation of enterocytes

At appropriate times, animals were reanesthetized and a loop of proximal small intestine, taken from the ligament of Treitz to 10 cm distal, was created with ligatures around both ends. Approximately 500 μCi of L[4,5-^{3}H]leucine (>120 Ci/mmol, Amersham) was instilled into the loop and the intestine was carefully replaced intraabdominally. Animals were kept under a heating lamp for 9 min, whereupon they were killed by cervical dislocation; the intestinal loop was removed and the contents were flushed with 20 ml of iced PBS (50 mM phosphate, 100 mM NaCl, pH 7.4) containing 20 mM leucine and 1.0 mM DTT. Isolated enterocytes were subsequently prepared by a modification of the method described by Weiser (13). A cotton ligature was tied around one end of the intestinal loop and buffer A (KCl, 1.5 mM; NaCl, 96 mM; Na citrate, 27 mM; KH_2PO_4, 8 mM; Na_2HPO_4, 5.6 mM; pH 7.3) containing 10 mM leucine was instilled so as to fill the loop and the intestine was clamped. The loop was placed in PBS at 37°C in a shaking water bath set at 80 oscillations per min, for 15 min. The mucosal contents were then decanted and discarded and the loop was filled with buffer B (Na_2EDTA, 1.5 mM; DTT, 1.0 mM; KCl, 2.69 mM; NaCl, 137 mM; KH_2PO_4, 1.47 mM; Na_2HPO_4, 8.10 mM; pH 7.2) containing 5 mM leucine at 37°C and reincubated for two periods, each of 15 min, with the mucosal contents being harvested each time. This procedure yielded a population of enriched villus cells, as determined by enzyme markers, which were then pelleted briefly by low speed centrifugation and washed three times in 5 ml of iced PBS–20 mM leucine. The final pellet was homogenized in 1 ml of PBS–1% Triton–2 mM leucine–1 mM PMSF–1 mM benzamidine, pH 7.4, using a Polytron (Brinkmann Instruments, NY) tissue homogenizer. Aliquots of the homogenate were removed and the remainder was pelleted at 105,000 *g* for 60 min in a 50 Ti rotor (Beckman, CA). All procedures were performed at 0–4°C and the resulting supernatant samples were stored at −75°C until analysis. In some studies, where indicated in the text, intestinal mucosa was scraped to yield a rapid preparation of enterocytes. In these studies, the loop was flushed as described and gently everted over a glass rod. The mucosa was gently scraped using a glass microscope slide, and the pellet was washed and homogenized as described above.

External bile diversion: surgical procedures and infusion protocol

Animals were anesthetized with sodium pentobarbital and the common bile duct was cannulated with poly-

ethylene 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 restaining 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; 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 reanesthetized. In each animal a proximal loop of jejunum (as above, Methods) and a 10-cm loop of distal ileum (distal end 5 cm proximal to the ileocecal valve) were prepared as described above and enterocytes were harvested after [^{3}H]leucine administration. Samples were subsequently handled as described above (Methods).

Radioimmunoassay (RIA) of apoA-I

The methods for preparation of rat apoA-I and rabbit antisera to apoA-I have been published previously (1, 11). ApoA-I was iodinated by the chloramine T procedure (14) yielding material that was maximally 70–80% immunoprecipitable. RIA was performed in a final Triton concentration of 0.83% in an assay mixture containing 100 μl of unknown diluted appropriately with PBS–1% BSA (50 mM phosphate, 100 mM NaCl, 0.02% NaN_3, 1% bovine serum albumin, pH 7.4), 20,000 cpm ^{125}I-labeled apoA-I, 0.04% anti-apoA-I, and 0.04% nonimmune rabbit serum per tube in a final volume of 500 μl. After 48 hr at 4°C, goat anti-rabbit serum was added at 1% volume per tube and the assay was harvested after a further 18-hr incubation at 4°C. Standard curves were prepared from a calibrated pool of rat plasma stored in multiple aliquots at −75°C. Results were calculated directly from the standard curve using the logit function as part of the data reduction software supplied by LKB (Rackgamma II, LKB Instruments, Gaithersburg, MD).

Quantitative immunoprecipitation of intestinal apoA-I

Intestinal supernatant fractions were reacted with excess anti-apoA-I antiserum under conditions largely as outlined (15, 16) but with some modification. Inactivated *Staphylococcus aureus* cells (Cowan I strain) (Pansorbin, Calbiochem, La Jolla, CA) were washed extensively in NETTAL buffer (0.15 M NaCl, 5 mM Na_2 EDTA, 1% Triton, 65 mM Tris HCl, 0.1% BSA, 2 mM leucine, 0.02% NaN_3, pH 7.4) within 24 hr of use (15, 16). Aliquots of intestinal supernatant were added to 50 μl of NETTAL buffer at room temperature in microfuge tubes (Sarstedt, Princeton, NJ) and 30 μl of washed Pansorbin was added. The mixture was incubated at room temperature for 15 min and then at 4°C for 30 min. After centrifugation at 12,000 *g* for 10 min at 4°C, the supernatant was added to 200 μl of NETTAL buffer containing 20,000 cpm of ^{125}I-labeled apoA-I. Anti-apoA-I antiserum was added (5–10 μl per tube) and the mixture was incubated at 4°C for 18 hr. Pansorbin was subsequently added and the mixture was reincubated for 40 min at 4°C. The cell pellet was collected by centrifugation, resuspended in 100 μl of NETTAL buffer, and layered over a cushion of 500 μl of 1 M sucrose–NETTAL. This was followed by centrifugation and resuspension, a total of three times. Completeness of immunoprecipitation was judged by comparing ^{125}I-labeled apoA-I cpm in the final washed pellet to cpm in the pellet of parallel, control incubations run with ^{125}I-labeled apoA-I and an identical volume of antiserum alone. Recovery of immunoprecipitable ^{125}I-labeled apoA-I, when subjected to this procedure, consistently exceeded 95%. The procedure was then repeated in duplicate, without ^{125}I-labeled apoA-I. Additional samples were run with nonimmune rabbit serum as a control for nonspecific immunoprecipitation. Following the final wash, the cells were suspended in 100 μl of 2.5% SDS, 10% glycerol, 5% 2-mercaptoethanol, 65 mM Tris-HCl, pH 8.0, heated at 95°C for 3 min, and pelleted at 12,000 *g* for 15 min.

The entire supernatant fraction was then applied to 5.6% SDS-polyacrylamide disc gels (17) and subjected to electrophoresis. Gels were sliced into 2-mm fractions with a razor blade and counted after an overnight incubation at 35°C with 6 ml of Econofluor–3% Protosol (New England Nuclear). Liquid scintillation counting was performed in a Beckman LS 7500 counter (Beckman, Berkeley, CA) with automatic quench correction and an external standard. Incorporation of [^{3}H]leucine into total intestinal homogenate protein was measured by precipitating an aliquot, along with carrier BSA (200–500 μg), with hot 10% trichloroacetic acid. The final pellet was dissolved in 1 ml of Protosol and 10 ml of Econofluor was added; the mixture was incubated at 35°C overnight and counted as above. Samples treated this way gave identical counting efficiency for ^{3}H either as a TCA-precipitate or liberated from the interstices of a polyacrylamide gel slice.

Data expression

ApoA-I content is expressed as ng of apoA-I per mg of homogenate protein. ApoA-I synthesis is expressed as a

relative rate; thus, ^{3}H-cpm incorporated into immunoprecipitable supernatant apoA-I, as judged by SDS-PAGE, divided by TCA-precipitable ^{3}H-cpm incorporated into the same volume of homogenate protein. Immunoprecipitable ^{3}H-cpm in apoA-I represents the average of at least two separate assays and reflects the subtraction of nonspecific counts (from incubations with pre-immune rabbit serum) in addition to background.

Miscellaneous

Protein concentration was measured by the method of Lowry et al. (18). Alkaline phosphatase activity in the homogenates from isolated intestinal cells was determined using *p*-nitrophenylphosphate as substrate (13).

Chemicals and reagents

Oleic acid and monoolein were purchased from Nu-Chek-Prep, Inc., Elysian, MN, and used as supplied. Sodium taurocholate was purchased from Calbiochem-Behring, CA and was >96% pure as determined by the manufacturer. All other materials were of analytical grade.

Statistical analysis

Data are expressed as mean ± 1 SEM. Comparisons were made by Student's *t*-test for paired and unpaired means.

RESULTS

Characterization of immunoprecipitation procedure for determining apoA-I synthesis rate

The basic parameters for establishing the technique as a reliable analytical tool are presented in **Fig. 1, A–C**. Analysis of the immunoprecipitate by SDS-PAGE (Fig. 1A) revealed a single monomorphic peak with the mobility of authentic plasma apoA-I. Reprecipitation of the supernatant yielded no further apoA-I counts, indicating quantitative immunoprecipitation (Fig. 1A). The immunoprecipitation protocol was shown to exhibit saturation with respect to antiserum addition (Fig. 1B) and to demonstrate competitive inhibition of ^{3}H-labeled apoA-I binding with addition of authentic unlabeled plasma apoA-I (Fig. 1C). Additional control studies (data not shown) indicated that no detectable (<2% total) apoA-I mass (by RIA) was liberated upon re-extraction of the 105,000 *g* pellet, suggesting that recovery into the first supernatant was essentially quantitative.

ApoA-I synthesis in jejunal enterocytes: evidence for regulation by triglyceride flux

The distribution of apoA-I synthesis within the small intestine was investigated in animals fasted for 12 hr. ApoA-I synthesis was found to be significantly higher in jejunal enterocytes (1.84 ± 0.20, n = 5) as compared to ileal enterocytes (0.91 ± 0.25, n = 5) from the same animals, $P < 0.01$. This pattern of distribution could conceivably reflect the committed role of the proximal small bowel in lipid absorption. Studies were designed, therefore, to test this hypothesis more directly.

Groups of animals were studied to examine the effects of an acute triglyceride bolus upon apoA-I synthesis rates in jejunal enterocytes. Animals maintained on regular (4.5% w/w fat) chow, up to 14 hr prior to study, were found to exhibit similar rates of apoA-I synthesis following 30 to 240 min exposure to intraduodenal triglyceride or saline (**Fig. 2A**). In order to avoid the 45-min time lag inherent in the enterocyte harvest protocol using calcium chelation (Methods), a group of animals was studied by preparing scraped intestinal mucosa from the isolated loops after pulse labeling with [^{3}H]leucine. Again, there were no differences between animals exposed to triglyceride or saline (Fig. 2A). The absolute values for apoA-I synthesis, however, were substantially lower than those usually encountered with isolated enterocytes (Fig. 2A). In addition to the absence of detectable changes in apoA-I synthesis, attributable to acute tryglyceride flux, there was no change in enterocyte apoA-I content up to 4 hr after acute triglyceride exposure (Fig. 2B).

A group of animals was then fed a fat-free diet for 8 days and pairs of animals were challenged with an intraduodenal bolus of either Intralipid or an isocaloric, but essentially fat-free, formula (Criticare HN). Animals receiving the triglyceride bolus were found to have significantly increased levels of apoA-I synthesis, 1.84 ± 0.1 (n = 4) compared to animals receiving the fat-free elemental formula, 1.37 ± 0.04 (n = 5), $P < 0.01$ (Fig. 2A). These results indicate that, under conditions where the intestine is deprived of triglyceride for several days, the abrupt reintroduction of triglyceride is associated with a small but significant increase in apoA-I synthesis within jejunal enterocytes. In concluding that a true increase in apoA-I synthesis is responsible for this observation, it should be noted that total protein synthesis, as judged by [^{3}H]leucine incorporation into TCA-precipitable protein (cpm/mg), was not significantly different between the groups (1.03 ± 0.24 × 10^6, versus 1.55 ± 0.58 × 10^6 for fat-fed and control animals, respectively), $P > 0.05$. At other times, more consistently with time points of 30 min or less, incorporation of [^{3}H]leucine into TCA-precipitable protein was lower in fat-fed compared to saline-exposed enterocytes. For example, at 30 min, values were 0.93 ± 0.07 × 10^6 versus 1.83 ± 0.26 × 10^6 cpm/mg for fat-fed and control animals, respectively, $P < 0.001$. This was attributed to the effects of the residual Intralipid forming a relatively less permeable barrier to isotope uptake. Despite differences in overall isotope uptake, the relative incorporation into immunoprecipitable apoA-I, when

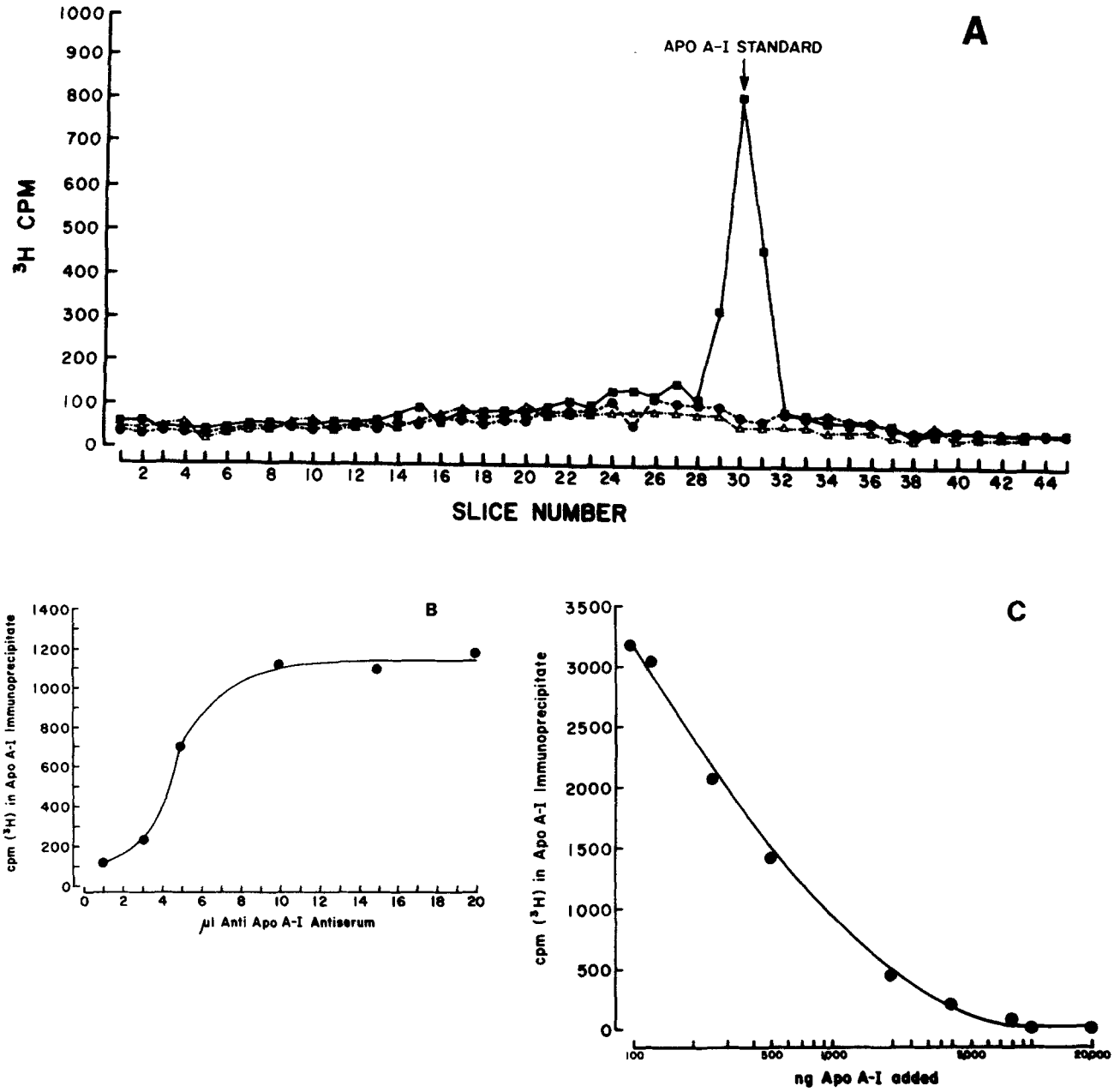

Fig. 1. A, SDS polyacrylamide gel electrophoretic profile of immunoprecipitate. Aliquots of ^{3}H-labeled intestinal supernatant were submitted to quantitative immunoprecipitation with anti-apoA-I antiserum (■-■-■) and the resulting immune complex (Methods) was subjected to SDS-PAGE on 5.6% polyacrylamide gels. An identical aliquot was subjected to precipitation using nonimmune rabbit serum (●---●---●). Following removal of the immune complexes in the sample treated with anti-apoA-I antiserum, the remaining supernatant was resubmitted to immunoprecipitation with a further aliquot of anti-apoA-I antiserum and the immune complex was again characterized by SDS-PAGE (△--△--△). The migration of authentic, unlabeled apoA-I from plasma HDL is indicated by the arrow. B, Immunoprecipitation of ^{3}H-labeled apoA-I with increasing volumes of anti-apoA-I antiserum. Multiple aliquots of a ^{3}H-labeled intestinal supernatant were submitted to immunoprecipitation using progressively larger volumes of anti-apoA-I antiserum. In control experiments, using ^{125}I-labeled apoA-I as an internal standard for quantitative immunoprecipitation (Methods), 10 µl of antiserum had been found to provide quantitative precipitation of both tracer and unknown. C, Competitive displacement of immunoprecipitable ^{3}H-labeled apoA-I counts by authentic unlabeled plasma apoA-I. Multiple aliquots of a ^{3}H-labeled intestinal supernatant were subjected to immunoprecipitation using a fixed volume of anti-apoA-I antiserum. Authentic unlabeled apoA-I was added to the incubation in amounts from 62.5 ng to 20 µg and the resultant immune complex was subjected to SDS-PAGE as described (Methods). Immunoprecipitable ^{3}H-labeled apoA-I was determined following gel slicing and the selective displacement of ^{3}H counts by authentic apoA-I is illustrated.

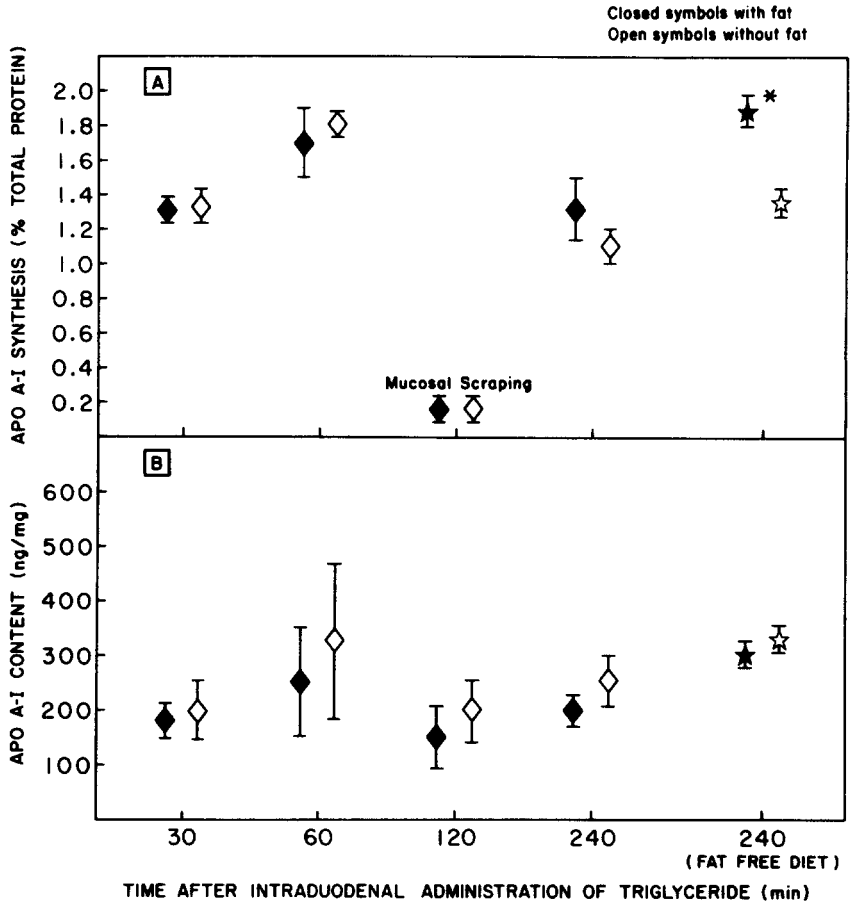

Fig. 2. A, ApoA-I synthesis in jejunal enterocytes: effects of acute triglyceride flux. Animals were allowed ad libitum access to either standard rat chow, or a fat-free diet for 8 days prior to study. After a 12-hr fast, animals received either fat-containing or fat-free isotonic mixtures intraduodenally. At the indicated times, animals were re-anesthetized and [^{3}H]leucine was administered into a closed loop of jejunum as described in Methods. Enterocytes were harvested and immunoprecipitation was performed on the cytosolic supernatant. Immune-precipitated counts incorporated into apoA-I per volume of supernatant (as determined by SDS-PAGE) were compared to TCA-precipitable counts per volume homogenate to yield values for apoA-I synthesis. Each set of values for fat-fed or control animals represents the mean ± 1 SE for groups of four to six animals. *Indicates a significant difference from animals fed a fat-free formula and challenged with a fat-free bolus (Criticare HN), $P < 0.01$. B, ApoA-I content within jejunal enterocytes: effects of acute triglyceride flux. ApoA-I content, was determined on intestinal supernatant samples by RIA. The data are expressed as ng of apoA-I per mg of homogenate protein and each point represents the mean ± 1 SE for groups of four to fifteen animals.

normalized for TCA-precipitable counts, was no different between the groups (Fig. 2A).

ApoA-I specific activity was compared among the different groups of animals studied. As shown in **Table 1**, there were no differences in the degree to which the jejunal enterocyte pool of apoA-I was labeled after a 9-min pulse and 45-min chase (the prerequisite time for enterocyte harvest) in any of the groups.

ApoA-I synthesis in jejunal enterocytes: effect of external bile diversion

The demonstration of an increased synthesis of apoA-I upon reintroduction of dietary triglyceride into animals fed a fat-free diet for 8 days, but not by acute triglyceride flux in animals eating fat-containing chow, suggested that apoA-I synthesis may be maximally expressed in the fasting state in these latter animals. To determine the extent to which the presence of biliary lipid contributes to the expression of apoA-I synthesis in jejunal enterocytes, groups of animals were surgically bile-diverted and perfused via a duodenal cannula for 48 hr with physiological buffers as detailed in the Methods section and the legend to Fig. 3.

As illustrated in **Fig. 3A**, apoA-I synthesis in jejunal enterocytes from animals bile-diverted for 48 hr was no different than that in sham-operated control animals similarly infused and restrained. The addition of 10 mM Na taurocholate to the infusate for the duration of the

TABLE 1. ApoA-I specific activity in jejunal enterocytes following [^{3}H]leucine administration: effect of acute triglyceride feeding[a]

	Specific Activity (cpm/ng apoA-I)[b]				
	0 min	30 min	60 min	240 min	Fat-Free Diet (8 days) 240 min
	82.5 ± 10.0				
Acute triglyceride bolus		77.5 ± 8.3	61.7 ± 3.7	61.9 ± 9.4	62.8 ± 9.5
Control		98.6 ± 10.7	47.6 ± 8.1	70.5 ± 9.0	61.9 ± 9.6
P[c]		NS	NS	NS	NS

[a]Animals were fasted for 12–14 hr prior to receiving an intraduodenal bolus of either Intralipid or saline. Animals studied at 240 min received an isocaloric but fat-free bolus (Criticare HN) as an appropriate control. At the stated time intervals following bolus administration, animals were prepared with in situ jejunal loops and received a 9-min pulse of [^{3}H]leucine. Enterocyte supernatants were subsequently prepared (Methods) using leucine-containing buffers.

[b]ApoA-I specific activity was determined by measuring [^{3}H]leucine incorporation into a known volume of cytosolic supernatant apoA-I using the quantitative immunoprecipitation technique described (Methods). ApoA-I mass was determined on a subsequent aliquot by RIA. Data are expressed as mean ± 1 SE for groups of four to six rats.

[c]*P* determined by pair analysis was found to be nonsignificant (NS), $P > 0.05$.

study or 10 mM Na taurocholate (44 hr) plus 80 mg of micellar lipid (4 hr) produced similar values for apoA-I synthesis in jejunal enterocytes (Fig. 3). These data suggest that external bile diversion has no effect above and beyond that observed by witholding dietary triglyceride (30-hr fast) or perfusing the intestine with glucose–saline for 48 hr (Fig. 3A). There was a trend toward somewhat higher enterocyte content of apoA-I in the bile-diverted

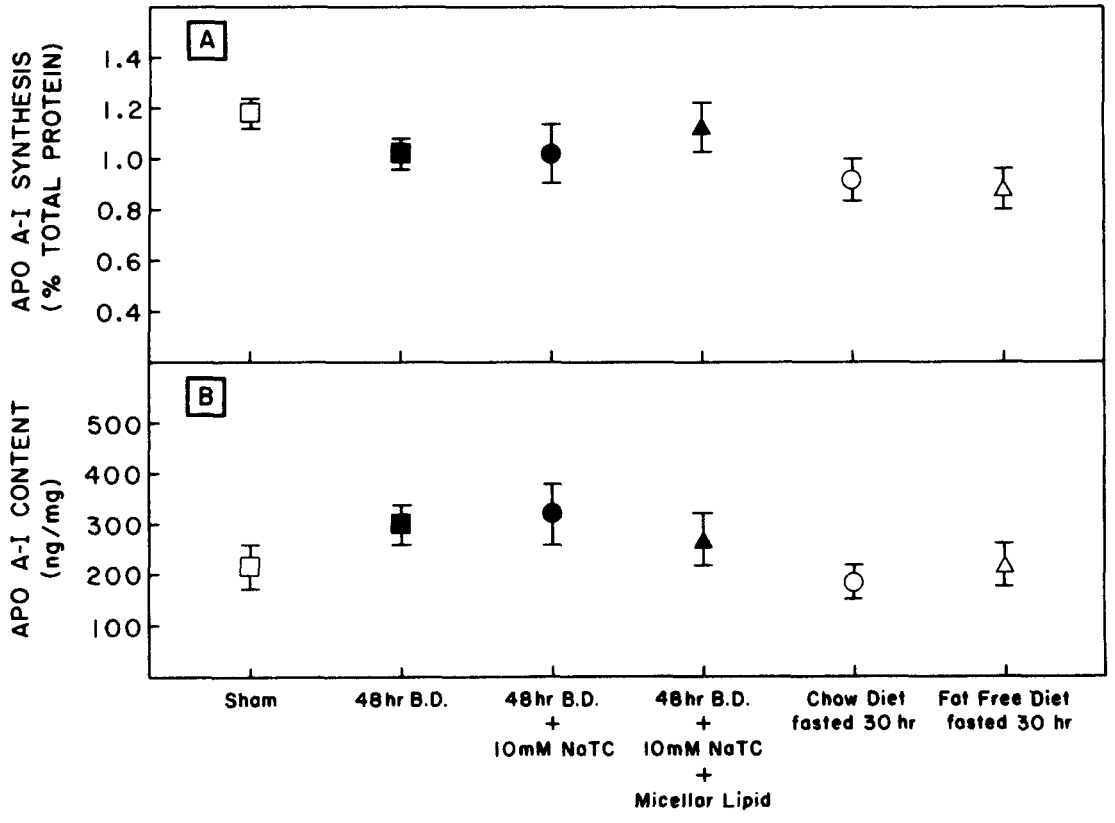

Fig. 3. A, ApoA-I synthesis in jejunal enterocytes: effects of external bile diversion. Groups of animals were surgically bile-diverted and fitted with a duodenal cannula through which they were perfused with either 10% glucose–0.9% NaCl–0.04% KCl (D10 saline) at 2.4 ml/hr (■) or D10 saline plus 10 mM Na taurocholate (●) for 48 hr. A further group was perfused with D10 saline plus 10 mM taurocholate for 44 hr and then received 80 mg of micellar lipid over 4 hr (▲). Control animals were not bile-diverted but were similarly restrained and infused for 48 hr with D10 saline (□). Two additional groups of animals were fasted for 30 hr—one group previously fed regular stock chow (○) and another fed a fat-free diet for 8 days prior to fasting (△). ApoA-I synthesis was determined on cytosolic supernatants from jejunal enterocytes from each of the animals (Methods). The data are expressed as mean ± 1 SE for groups of four to eight animals. There were no significant differences between the groups. B, ApoA-I content in jejunal enterocytes: effects of external bile diversion. ApoA-I content was determined on intestinal supernatant samples by RIA. The data are expressed as ng of apoA-I per mg of homogenate protein and each point represents the mean ± 1 SE for groups of four to eight animals.

groups (Fig. 3B) but this did not achieve statistical significance. Nevertheless, when expressed as specific activity (immunoprecipitable apoA-I cpm/ng), sham-operated animals exhibited higher values than both the bile-diverted and bile-diverted, micellar lipid-infused group (**Table 2**). This finding may reflect differences in turnover of enterocyte apoA-I with the various maneuvers.

Incorporation of [^{3}H]leucine into TCA-precipitable protein (cpm/mg) in the various experimental groups was no different than that in sham-operated animals (Table 2). However, as alluded to above, incorporation was somewhat lower in animals infused with micellar lipid, and indeed a significant difference was evident in [^{3}H]leucine incorporation when this latter group was compared to animals receiving 10 mM Na taurocholate alone (Table 2).

ApoA-I synthesis in ileal enterocytes: effect of external bile diversion

In contrast to the results obtained with jejunal enterocytes, apoA-I synthesis in ileal enterocytes was significantly depressed by external bile diversion for 48 hr (**Fig. 4A**). The reduction in apoA-I synthesis—to levels approximately 50% of that found in either sham-operated or fasted controls (Fig. 4A)—was also discovered in animals receiving 10 mM Na taurocholate or 10 mM Na taurocholate (44 hr) plus micellar lipid (4 hr). These data (Fig. 4A) suggest that apoA-I synthesis in ileal enterocytes was significantly depressed by the withdrawal of biliary secretions. Furthermore, the component(s) of bile mediating this effect have yet to be determined but the diminished synthesis of apoA-I appears not to be affected by the presence of either luminal bile salts alone or in conjunction with brief exposure (4 hr) to bile salt–monoolein–fatty acid micelles. ApoA-I content was also significantly reduced following 48 hr bile diversion (Fig. 4B). This effect was reversed following infusion of 10 mM Na taurocholate (Fig. 4B), but no additional effect was seen with the inclusion of micellar lipid. The changes observed in apoA-I synthesis were proportionally greater than those in enterocyte content, as evidenced by the generally lower apoA-I specific activity in ileal enterocytes from bile-diverted animals (Table 2). Incorporation of [^{3}H]leucine into TCA-precipitable protein was no different among the various treatment groups (Table 2).

The gradient of apoA-I synthesis within the small intestine namely, jejunum > ileum (Results, above), was maintained in all animals undergoing external bile diversion (**Fig. 5**). In contrast to animals fasted for 12 hr (Results, above and Fig. 5), animals fasted for 30 hr or restrained sham-operated controls were found to have values for apoA-I synthesis in ileal enterocytes that were indistinguishable from jejunal enterocytes (Fig. 5).

These findings, taken together, suggest that key aspects of apoA-I metabolism within the ileal enterocyte may be regulated in a manner fundamentally different from that within the jejunal enterocyte.

TABLE 2. Total cellular (TCA-precipitable cpm/mg) protein and apoA-I (immunoprecipitable cpm/ng) 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/mg × 10^{-6})	1.52 ± 0.20	1.30 ± 0.21 (NS)	2.10 ± 0.40 (NS)	1.18 ± 0.18* (NS)
Apo-I[c] (cpm/ng × 10^{-3})	82.3 ± 14	44.7 ± 6.0**	67.0 ± 14 (NS)	44.3 ± 5.3***
Ileum				
Total cellular protein (cpm/mg × 10^{-6})	1.87 ± 0.46	1.24 ± 0.15 (NS)	1.38 ± 0.25 (NS)	1.20 ± 0.26 (NS)
ApoA-I (cpm/ng × 10^{-3})	85.5 ± 23	53.0 ± 6.8 (NS)	34.0 ± 5.4 (NS)	31.0 ± 4.6****

[a]Animals (five to eight per group) were subjected to external bile diversion with constant intraduodenal infusion of 10% dextrose–0.9% NaCl–0.04% KCl for 48 hr, as described (Methods). Where indicated, animals received, in addition, 10 mM Na taurocholate (NaTc) 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 protein concentration on a further aliquot (Methods). Data are expressed as mean ± SEM.

[c]ApoA-I specific activity was determined by measuring [^{3}H]leucine incorporation into a known volume of cytosolic supernatant apoA-I (Methods). ApoA-I mass was determined on a further aliquot by RIA. Data are expressed as mean ± SEM.

*Statistical comparison by independent *t*-test, $P < 0.05$ compared to 48 hr BD + 10 mM NaTc; **, $P < 0.05$ compared to sham-operated; ***, $P < 0.02$ compared to sham-operated; ****, $P < 0.05$ compared to sham-operated; NS, no significant differences detectable when compared to sham-operated controls.

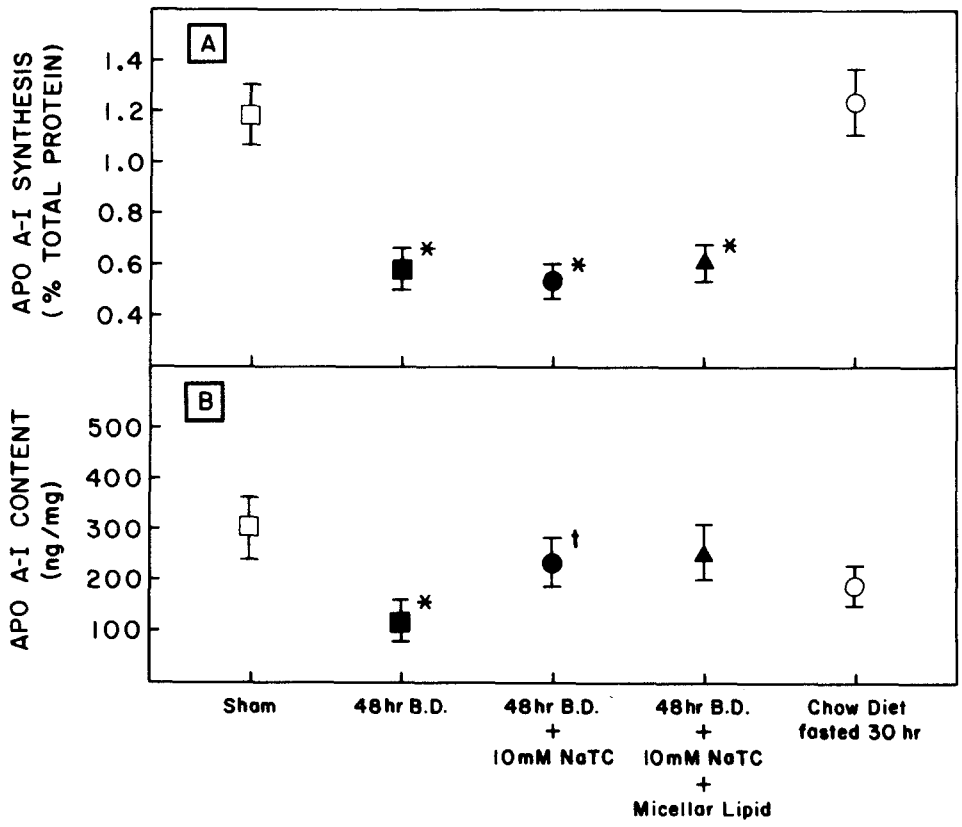

Fig. 4. A, ApoA-I synthesis in ileal enterocytes: effects of external bile diversion. Groups of animals were studied as outlined in the legend to Fig. 3. After 48 hr, animals were anesthetized and [^{3}H]leucine was instilled into a loop of distal ileum. Ileal enterocytes were harvested and apoA-I synthesis was determined by immune precipitation of duplicate aliquots of cytosolic supernatant (Methods). The data are expressed as mean ± 1 SE for groups of four to eight animals. (■), Bile-diverted, perfused with D10 saline (48 hr); (●), bile-diverted, perfused with D10 saline plus 10 mM Na taurocholate (48 hr); (▲), bile-diverted, perfused with D10 saline plus 10 mM Na taurocholate (44 hr) and 80 mg of micellar lipid (4 hr); (□) sham-operated, perfused with D10 saline; (○) chow-fed, fasted for 30 hr. B, ApoA-I content in ileal enterocytes: effects of external bile diversion. ApoA-I content, shown in the lower panel, was determined on intestinal supernatant samples by RIA. The data are expressed as ng of apoA-I per mg of homogenate protein and each point represents the mean ± 1 SE for four to eight animals. *, Indicates statistically different from sham-operated controls, as determined by independent *t*-test, $P < 0.01$. †, Indicates significantly different from 48-hr bile-diverted animals as determined by independent *t*-test, $P < 0.01$.

DISCUSSION

This report describes the results of studies conducted to examine the regulation of apoA-I synthesis in vivo in rat small intestine. Aspects of the methodology bear detailed comment. ApoA-I is synthesized exclusively in the liver and small intestine of all mammalian species studied to date (1–4, 19–21). Given the dual site of origin of apoA-I and the recent demonstration (22) that a small but significant amount of plasma apoA-I is taken up by enterocytes, the timing of the pulse of [^{3}H]leucine appeared critical. A time of 9 min was chosen since it maximized enterocyte labeling while minimizing hepatocyte isotope uptake and secretion of newly synthesized apoA-I from the liver. Earlier work (23) showed that chylomicron particles were visible within Golgi cisternae approximately 8 min after labeled fatty acid administration, implying that chylomicron synthesis is a rapid process. The method described in the present report also includes a 45-min chase period (the obligatory time for enterocyte isolation) with an overwhelming source of unlabeled leucine (20 mM decreasing to 2 mM) to prevent isotope reutilization. Studies were conducted to determine the effect of increasing the pulse labeling time to 30 min. A group of four animals was studied 4 hr after receiving an intraduodenal bolus of triglyceride. ApoA-I synthesis (% total protein) was 0.48 ± 0.20 compared to 1.87 ± 0.20 in animals studied after the same stimulus had been applied with a 9-min pulse ($P < 0.001$). Thus, increasing the length of pulse actually resulted in a lower apparent rate of apoA-I synthesis, suggesting that the turnover of newly synthesized enterocyte apoA-I is extremely rapid.

The expression of apoA-I synthesis data as % incorporation into TCA-precipitable protein is founded on the assumption that [^{3}H]leucine incorporation into apoA-I occurs at a physiologically representative rate compared

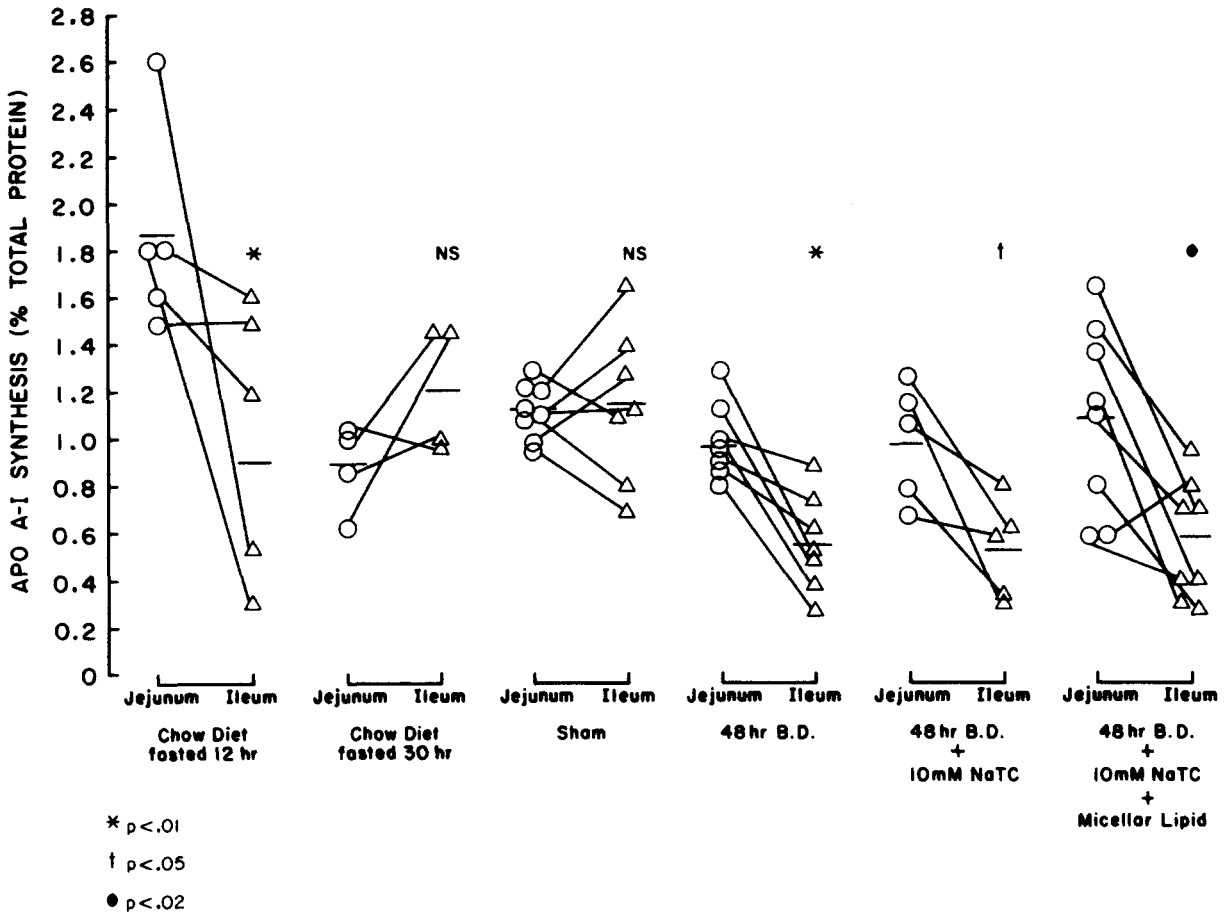

Fig. 5. ApoA-I synthesis in jejunal versus ileal enterocytes: paired observations in animals undergoing prolonged external bile diversion or fasting. Animals were studied as outlined in the legends to Figs. 3 and 4. Jejunal and ileal enterocytes were harvested following a brief pulse of [^{3}H]leucine and apoA-I synthesis was determined on aliquots of cytosolic supernatant (Methods). Individual paired results for each animal studied are illustrated. Results were compared statistically by Student's *t*-test for paired observations; *, $P < 0.01$; †, $P < 0.05$; ◆, $P < 0.02$.

to its incorporation into total cell protein. There are important caveats to this assumption, prominent among which is that leucine represents ~12 mol % of apoA-I (24)—a value which may result in overestimation of apoA-I synthesis (25, 26). It is an additional assumption that the administration of a pulse of [^{3}H]leucine produces homogenous labeling of the enterocyte leucine pool and that compartmentalization does not occur. Nevertheless, given the use of appropriate control animals against which to compare the results of the various perturbations described, the expression of apoA-I synthesis as described provides a meaningful index of physiological regulation.

Recent work has suggested that apoA-I is synthesized in a variety of extrahepatic, extraintestinal sites in chickens and that synthesis undergoes a control process temporally related to key developmental events (26, 27). The question of whether apoA-I synthesis in mammalian small intestine undergoes physiological regulation has been the focus of considerable work.

ApoA-I is an abundantly synthesized intestinal apoprotein (28, 29) whose secretion into mesenteric lymph appears to increase in association with active triglyceride absorption (1, 10). The viewpoint that this increased secretion was the result of increased synthesis was substantiated by early studies which suggested that enterocyte apoA-I content, as measured immunochemically or by RIA, increased in response to acute triglyceride flux (1, 8). These results, however, were challenged by others reporting a decrease in apoA-I content following fat feeding in the rat (9). The specific question of whether apoA-I synthesis in rat small intestine undergoes regulation related to dietary or biliary lipid flux has been addressed by several investigators. Windmueller and Wu (3) reported that the production of apoA-I was not regulated by triglyceride transport or the absence of biliary lipid. These workers used a combination of differential labeling with [^{3}H]- and [^{14}C]leucine to conclude that the contribution of the small intestine was uninfluenced by maneuvers

designed to test the importance of various sources of intestinal lipid (3). More recently, Gordon et al. (12) have reported that translatable mRNA levels for apoA-I in rat small intestine do not change up to 8 hr following an acute triglyceride bolus (12).

The present work, which suggests that under conditions of zero dietary triglyceride intake an acute triglyceride bolus can be demonstrated to produce a small but significant increase in apoA-I synthesis, supports aspects of both reports cited above (3, 12). Gordon et al. (12) and Blaufuss et al. (29) chose to examine mRNA levels in animals consuming fat-containing chow up to the night before study. Furthermore, their data for translatable mRNA levels was gathered by pooling scraped intestinal mucosa from the entire small bowel of the animals studied. Our data suggest that animals fed a regular, fat-containing chow do not respond to an acute triglyceride bolus by increasing jejunal apoA-I synthesis. The present studies also indicate that apoA-I synthesis occurs at a significantly lower rate (of % total protein synthesized) in the ileum, so that Gordon et al. (12, 29) may have been unable to detect small changes in apoA-I mRNA abundance in one region of the small intestine. This same caveat also pertains to the studies of Windmueller and Wu (3, 20) who were unable to show a change in the production of apoA-I by the entire small intestine in response to triglyceride, even after their animals had been placed on a fat-free chow for 12 days prior to study.

Our results with the effects of bile diversion on apoA-I synthesis by jejunal enterocytes are in broad agreement with those reported previously from our laboratory (11) and support some of the conclusions proposed by others (3). Thus, the demonstration that complete elimination of biliary and dietary lipid produced no additional suppression of jejunal apoA-I synthesis above fasting alone (Fig. 3) suggest that biliary lipid flux does not exert an independent effect upon jejunal apoA-I synthesis.

By contrast, the results obtained with ileal enterocytes suggest that apoA-I synthesis in different regions of the small intestine may be subject to independent control. Bile diversion was associated with a significant reduction in ileal apoA-I synthesis (Fig. 5), an effect that appeared to be uninfluenced by the continuous availability of 10 mM Na taurocholate. The basis for this effect is not apparent but is clearly unrelated to the presence of lumenal bile salts per se. Other aspects of external bile diversion, such as alterations in cholesterol homeostasis within the enterocyte (30, 31) or changes in phospholipid metabolism, could potentially contribute to this observation.

The small intestine is an organ with sophisticated evolutionary development. The proximal portion appears to be committed to the absorption of various nutrients including fatty acids and other lipids. The distal small bowel is unique in respect to its active, receptor-mediated uptake of cobalamin and bile salts. The demonstration of apoprotein synthesis in a region of small intestine normally uninvolved with active triglyceride transport is unexplained. The demonstration that the intestine elaborates HDL particles, however, (32, 33) suggests that enterocyte synthesis of apoA-I may occur unrelated to bulk triglyceride transport. It seems likely also that the proposal that apoA-I synthesis in mammalian small intestine occurs as a more on less unregulated process (3) may be an oversimplification. The detailed aspects of the events involved in expression of intestinal apoprotein synthesis will be the focus of future reports.

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