Effect of intestinal chylomicron secretory blockade on apolipoprotein synthesis in the newborn piglet

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Pluronic L-81 is a hydrophobic surfactant which blocks intestinal chylomicron secretion at the pre-Golgi level without affecting triacylglycerol uptake and re-esterification. To study the effects of such blockade on apolipoprotein synthesis, newborn female piglets received 24 h intraduodenal infusions of low-triacylglycerol, or high-triacylglycerol with or without Pluronic L-81, diets, followed by determination of apolipoprotein (apo) B-48, A-I and A-IV synthesis and content and apo B and A-IV mRNA levels in the small intestine. Jejunal apo B-48 content, synthesis and mRNA levels were down-regulated below basal levels by the addition of Pluronic to the high-triacylglycerol infusion. The normal increase in apo A-I synthesis induced by triacylglycerol absorption was ablated in both jejunum and ileum, even though the expected increase in apo A-I content in jejunum still occurred. Although attenuated, the expected increase in jejunal apo A-IV synthesis and mRNA levels with triacylglycerol absorption was still present with Pluronic treatment. These results suggest very different mechanisms of cellular regulation and trafficking for the various apolipoproteins incorporated into nascent intestinal chylomicrons. Apo B may be specifically down-regulated by the chylomicron secretory blockade induced by Pluronic L-81.

INTRODUCTION

Intestinal chylomicron biogenesis is an incompletely understood process involving the intracellular packaging of exogenous lipid, principally triacylglycerol, with apolipoproteins for secretion from the enterocyte [1–4]. The regulatory mechanisms and subcellular localization of many steps of this process remain unknown, particularly with regard to incorporation of the apolipoproteins into the nascent lipoprotein particle.

Pluronic L-81 is a hydrophobic surfactant which quickly and reversibly blocks chylomicron secretion at the pre-Golgi level [5–10]. When Pluronic L-81 is infused into the small intestine with a triacylglycerol emulsion, digestion by pancreatic lipase, uptake of lipolytic products and re-esterification of the lipid occur normally [5–10]. However, the re-esterified triacylglycerol accumulates in large smooth-endoplasmic-reticulum vesicles within the enterocyte, and chylomicrons are not secreted [6]. When the Pluronic is removed from the infused lipid, the accumulated triacylglycerol is immediately secreted in the form of chylomicrons into the lymphatics without residual effects [5,7,10]. Pluronic L-81 may be a useful model for inherited defects in lipoprotein secretion, such as abetalipoproteinaemia.

The present studies were designed to determine the effects of this secretory blockade on the intestinal synthesis and content of the apolipoproteins of nascent chylomicrons: apo B-48, apo A-I and apo A-IV. The newborn piglet was used for these studies since we have previously characterized the normal apolipoprotein-synthetic regulatory patterns induced by lipid absorption in this model system [11,12].

MATERIALS AND METHODS

Animals

Female domestic pigs (2 days old) were obtained from Research Industries Corp., Monee, IL, U.S.A. The use of animals in this

study was approved by the University of Chicago Institutional Animal Care and Use Committee.

Preparation of the animal model

From the time of arrival to the time of surgery the next day, the 2-day-old animals were kept in heated incubators. Animals were fed on artificial sow's milk (SPF-LAC, Pet-Ag Inc., Hampshire, IL, U.S.A.) by gavage during this period. Anaesthesia was induced by intramuscular ketamine (40 mg/kg) and maintained by a face mask delivering 1 litre of O₂/min and 0.5–0.8% halothane. A longitudinal midline abdominal incision was made, and the peritoneal cavity opened. The second portion of the duodenum was cannulated with silicone rubber tubing [(internal diam. = 0.076 cm (0.030 in), external diam. = 0.165 cm (0.065 in)] through an incision in the gastric antrum. The tubing was then secured in place with a purse-string suture, exteriorized through the right flank, tunneled subcutaneously to the mid-dorsum, and secured through a swivel tether. Post-operatively the animals were allowed to recover in the heated isolettes. An intraduodenal infusion of 5% (w/v) glucose in 45 mM-NaCl/20 mM-KCl at 100 ml/kg per 24 h was started during recovery. After a 24 h recovery period animals were awake, alert and mobile. Also at this time animals were noted to be tolerating the infusion well without vomiting or abdominal distension and to be passing stool. Experimental infusions were started at the end of this recovery period.

After 24 h recovery period three groups of animals were studied.

Group 1: piglets receiving a 24 h intraduodenal infusion of dilute Vivonex (Norwich Eaton Pharmaceuticals, Norwich, NY, U.S.A.), a high-carbohydrate low-fat (carbohydrate 226 g/l, protein 21 g/l and fat 1.45 g/l in undiluted form) elemental formula, at 209 kJ (50 kcal)/kg per 24 h (–TG group, *n* = 7).

Group 2: animals receiving a 24 h intraduodenal infusion of Intralipid (Cutter, Berkeley, CA, U.S.A.), a triacylglycerol emulsion, at 209 kJ (50 kcal)/kg per 24 h (TG–Plu group, *n* = 7).

Group 3: animals receiving a 24 h intraduodenal infusion of

Intralipid at 209 kJ (50 kcal)/kg per 24 h containing Pluronic L-81 (BASF Wyandotte Corp., Wyandotte, MI, U.S.A.) at 1 mg/ml (TG + Plu group, $n = 5$).

All groups received a total fluid volume of 100 ml/kg per 24 h during the experimental infusion by supplementing with 0.9% NaCl solution. An energy intake of 209 kJ (50 kcal)/kg per 24 h during the 24 h experimental infusions was used in order to keep the infusions isoenergetic and to take into account the fact that Vivonex has a high osmolality when used at full strength (550 mosmol/kg), which was generally not tolerated by the animals. During the infusions the animals were kept in the heated isolettes and allowed to move freely within the limits of their tethers. At no time during the recovery phase or during the experimental infusions did the animals receive peroral feedings.

In order to document that Pluronic L-81 produces the same effect in the neonatal piglet as described previously in the adult rat, a TG - Plu and a TG + Plu animal were studied in the same way as the above groups, except that [^3H]triolein ([9,10- ^3H](n)triolein, 26.8 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.) was added to the infusate with sonication during the last 6 h of the experimental infusion (total 500 μCi infused). At the end of this period, proximal jejunal mucosa was harvested for quantitative lipid extraction and t.l.c. to document the accumulation of infused radioactive triacylglycerol in the mucosa of the animal treated with Pluronic, as compared with the untreated animal.

Determination of intestinal apolipoprotein synthesis

At the end of the experimental infusions the animals were anaesthetized, and a 10 cm segment of proximal jejunum was isolated 10 cm distal to the ligament of Treitz by two ligatures. Likewise, a 10 cm segment of distal ileum was isolated 10 cm from the ileocaecal valve. Radiolabelling was performed by instilling 1.5 mCi of L-[4,5- ^3H]leucine (> 120 Ci/mmol) (Amersham, Arlington Heights, IL, U.S.A.) into each segment; 9 min later the segments were removed and prepared for immunoprecipitation as described below. This labelling time has previously been shown to be optimal in similar experiments in the adult rat, and we have used it previously in the piglet [11–15]. Segments of jejunum immediately distally adjacent to the above segments were placed in liquid N_2 for RNA extraction.

Preparation of mucosal supernatants for immunoprecipitation

Radiolabelled intestinal segments were flushed with 50 ml of iced phosphate-buffered saline (PBS; 50 mM-phosphate/100 mM-NaCl, pH 7.4) 20 mM-leucine, and the mucosa was scraped and homogenized on ice in 1 ml of PBS/1% Triton X-100/2 mM-leucine/1 mM-phenylmethanesulphonyl fluoride/1 mM-benzamidine, pH 7.4, as previously described [11–13]. Samples of the homogenate were taken for measurement of total protein concentration and trichloroacetic acid-precipitable radioactivity. The remainder was pelleted at 105000 g for 60 min in a Beckman 50.3 Ti rotor, followed by collection of the supernatant. Although most intracellular apolipoprotein is membrane-associated, this technique has been shown previously to result in extraction and solubilization of 84–94% of total recoverable apolipoprotein mass with no discernible effect of the state of lipid flux [15]. All procedures were performed at 0–5 $^{\circ}\text{C}$, and the mucosal supernatant samples were stored at -80°C until analysis.

Apolipoprotein immunoprecipitation

Intestinal supernatant fractions were subjected to specific immunoprecipitation of apo B, apo A-I and apo A-IV under

conditions of antibody excess as described [11–13]. Samples of supernatants were mixed with washed IgG-Sorb (The Enzyme Center, Malden, MA, U.S.A.) and subsequently made to react with excess rabbit anti-(pig apolipoprotein) antiserum for 18 h at 4 $^{\circ}\text{C}$. After a second addition of IgG-Sorb and extensive washing, the liberated immunocomplex was applied to either 5.6% (apo A-I and apo A-IV) or 4% (apo B) SDS/polyacrylamide tube gels. After electrophoresis, gels were sliced into 1 mm slices and incubated in 3% Protosol/97% Econofluor (New England Nuclear) at 37 $^{\circ}\text{C}$ overnight before liquid-scintillation counting in a Packard model 2000 liquid-scintillation counter. Apolipoprotein species were identified by comparison with stained co-electrophoresed apolipoproteins. Apolipoprotein synthesis was expressed as the percentage of specific immunoprecipitated apoprotein radioactivity (c.p.m.) as compared with total protein trichloroacetic acid-precipitable radioactivity. Apolipoprotein synthesis was thereby expressed as a percentage of total protein synthesis. All samples were subjected to re-immunoprecipitation to ensure the completeness of the first antigen-antibody reaction.

Apolipoprotein quantification by e.l.i.s.a.

Competitive e.l.i.s.a. assays were performed for mucosal apo B and apo A-I contents as previously described [12], by using serial dilutions of supernatants prepared from the tissue homogenates as the competing antigen. Standard antigens consisted of pig plasma low-density lipoprotein (apo B) and high-density lipoprotein (apo A-I) applied to the microtitre-plate wells in serial dilutions. Assay plates were read at 492 nm by a Bio-Rad ELISA reader (Bio-Rad, Richmond, CA, U.S.A.) interfaced with an Apple Macintosh Plus microcomputer and associated Bio-Rad software. All semi-logarithmic inhibition plots for the samples were parallel to the standard plots. All samples were run in duplicate in the same assay, and variability between duplicates was less than 5%.

Mucosal lipid extraction and t.l.c.

In the animals infused with [^3H]triolein, segments of proximal jejunum were isolated and rinsed as described above. Mucosa was scraped and homogenized, followed by Folch extraction of total lipids [16]. Samples of the extracted lipid were separated by t.l.c. [13]. The various lipid classes were identified by exposure to iodine vapour and comparison with co-chromatographed lipid standards. The bands were then scraped, and radioactivity content was determined by scintillation counting.

Mucosal RNA extraction and slot-blot hybridization

Segments of jejunum distal to and adjacent to the radiolabelled segments from two animals in each experimental group were removed and immediately frozen in liquid N_2 . These segments were later homogenized in 4 M-guanidine thiocyanate/25 mM-sodium citrate, pH 7.0, as the initial step in RNA extraction as described [17]. Integrity of each RNA preparation was verified by methylmercury-gel electrophoresis and observation of ribosomal subunits. Serial dilutions of RNA were applied to nitrocellulose filters by using a slot-blot apparatus (Schleicher and Schuell, Keene, NH, U.S.A.). Total RNA in each slot was made constant by the addition of yeast tRNA. Filters were pre-hybridized and hybridized at 37 $^{\circ}\text{C}$ by using final concentrations of 50% (v/v) formamide, 6 $\times$ SSC (SSC = NaCl/sodium citrate), 5 mM-sodium phosphate, 1 mM-EDTA (disodium salt), 1.5 $\times$ Denhardt's solution, 100 μg of sonicated salmon sperm DNA/ml and 10% (w/v) dextran sulphate. The filters were probed with human apo B (provided by J. Scott, MRC Clinical Research Centre, Harrow, U.K.) [18] and apo A-IV (provided by J. Gordon, Washington University, St Louis, MO, U.S.A.) [19]

cDNAs. We have previously established the validity of these human cDNA probes for slot-blot hybridization with pig RNA by Northern-blot analysis. The human apo B cDNA hybridizes with a single approx. 14 kb transcript, and the human apo A-IV cDNA hybridizes with a single 1.8 kb transcript [11]. The probes were labelled by random priming [20]. Washes were performed at room temperature under low stringency. Autoradiograms were developed after exposure to XAR-5 film (Eastman Kodak Co., Rochester, NY, U.S.A.) at -70°C for 3 days, and scanned with a laser densitometer (LKB Instruments, Rockville, MD, U.S.A.).

Protein measurement

Protein was measured by a modified Lowry technique [21].

Statistical analysis

Data in experimental groups were analysed by one-way analysis of variance, followed by the Student–Newman–Keuls test to compare specific groups. The null hypothesis was rejected at $P < 0.05$.

RESULTS

Intestinal mucosal triacylglycerol radioactivity content

In order to confirm the accumulation of absorbed triacylglycerol in intestinal mucosa of animals infused with triacylglycerol plus Pluronic L-81, both a TG–Plu and a TG+Plu animal received

[^3H]triolein in the duodenal infusate during the final 6 h of the 24 h experimental infusion. Quantitative extraction of total jejunal-mucosal homogenate lipid revealed that the Pluronic-treated jejunum contained 4503 d.p.m./mg of total protein, compared with 2181 d.p.m./mg in the TG–Plu jejunal mucosa. In both animals, approx. 80% of the mucosal radioactivity was in the triacylglycerol fraction as assessed by t.l.c. analysis. Thus Pluronic treatment induces the mucosal accumulation of absorbed triacylglycerol in the piglet, as has been noted previously in the adult rat [5–8,10].

Intestinal apolipoprotein synthesis and content

Incorporation of [^3H]leucine into mucosal total protein in both jejunal and ileal segments in all three experimental groups was comparable, as shown in Table 1. As we have reported previously in the newborn and older suckling piglet, the protein precipitated with antibodies to pig apo B had an apparent molecular mass of 220 kDa (apo B-48) in all of the intestinal samples; no apo B-100 (apparent molecular mass approx. 330 kDa) peaks were seen [12,13]. The protein precipitated with specific antibodies to pig apo A-I had the expected apparent molecular mass of 26 kDa, and the protein precipitated with specific antibodies to pig apo A-IV had the expected apparent molecular mass of 42 kDa, as reported previously in experiments in both newborn and suckling pigs [11–13]. In addition, competition with the appropriate excess of pig apolipoprotein completely blocked the apolipoprotein immunoprecipitations in the present study.

Jejunal apo B-48, apo A-I and apo A-IV synthesis as determined by [^3H]leucine incorporation for the three experimental groups is shown in Table 2. Absorption of dietary triacylglycerol (TG–Plu group) had no effect on apo B-48 synthesis as compared with the –TG group. However, addition of Pluronic L-81 to the duodenal infusion (TG+Plu group) resulted in a significant decrease in apo B synthesis. Consistent with a post-translation mechanism of regulation, as we have reported previously [12], jejunal apo B content doubles with triacylglycerol absorption (Table 2), despite the lack of increase in synthesis. However, the addition of Pluronic to the triacylglycerol infusion

Table 1. Jejunal and ileal total protein specific radioactivities

Values are expressed as c.p.m./ μg of total protein in mucosal cytosolic supernatants (means $\pm$ S.E.M.). No statistically significant difference was found among any of the values.

Experimental group	Jejunum	Ileum
–TG	1138 $\pm$ 212	1097 $\pm$ 275
TG–Plu	872 $\pm$ 168	867 $\pm$ 144
TG+Plu	1120 $\pm$ 260	1364 $\pm$ 281

Table 2. Jejunal apolipoprotein synthesis and content

Result significantly different from the –TG group at: ^a $P = 0.014$; ^d $P = 0.005$; ^f $P = 0.010$; ^g $P = 0.0001$; ^h $P = 0.0001$. Result significantly different from the TG–Plu group at: ^a $P = 0.005$; ^e $P = 0.005$; ^c $P = 0.004$.

Group...	Synthesis (% of total protein synthesis)			Content (ng/ μg of total protein)		
	–TG	TG–Plu	TG+Plu	–TG	TG–Plu	TG+Plu
Apo B-48	1.98 $\pm$ 0.46	1.93 $\pm$ 0.21	0.96 $\pm$ 0.09 ^a	1.12 $\pm$ 0.21	2.73 $\pm$ 0.52 ^b	0.48 $\pm$ 0.13 ^c
Apo A-I	5.37 $\pm$ 0.83	10.52 $\pm$ 1.22 ^d	4.28 $\pm$ 0.91 ^e	2.58 $\pm$ 0.49	5.69 $\pm$ 0.90 ^f	5.18 $\pm$ 0.53
Apo A-IV	1.40 $\pm$ 0.27	6.94 $\pm$ 1.12 ^g	4.81 $\pm$ 0.58 ^h			

Table 3. Ileal apolipoprotein synthesis and content

Result significantly different from the –TG group at: ^a $P = 0.014$; ^b $P = 0.025$; ^c $P = 0.044$.

Group...	Synthesis (% of total protein synthesis)			Content (ng/ μg of total protein)		
	–TG	TG–Plu	TG+Plu	–TG	TG–Plu	TG+Plu
Apo B-48	0.66 $\pm$ 0.26	0.70 $\pm$ 0.15	0.25 $\pm$ 0.09 ^a	1.10 $\pm$ 0.24	1.82 $\pm$ 0.22 ^b	0.87 $\pm$ 0.11
Apo A-I	1.26 $\pm$ 0.83	2.23 $\pm$ 0.40 ^c	0.91 $\pm$ 0.11	2.32 $\pm$ 0.40	2.74 $\pm$ 0.36	2.82 $\pm$ 0.34
Apo A-IV	0.72 $\pm$ 0.09	0.73 $\pm$ 0.20	0.46 $\pm$ 0.18			

results in a significant decrease in jejunal apo B content (Table 2), paralleling the observed decrease in synthesis.

Jejunal apo A-I synthesis approximately doubled with triacylglycerol absorption (Table 2), and mucosal content significantly increased (Table 2). Therefore, in contrast with apo B-48, jejunal apo A-I synthesis is regulated by dietary triacylglycerol absorption and parallels mucosal content. Pluronic L-81 treatment (TG + Plu group) prevented the normal increase in jejunal apo A-I synthesis with lipid absorption (Table 2), although the expected increase in mucosal content of apo A-I still occurred with Pluronic treatment (Table 2).

Jejunal apo A-IV synthesis increased 5-fold with lipid absorption (Table 2), in the same range as we have previously reported in the newborn pig [11]. Pluronic L-81 treatment blunted this increase slightly, but the value for the TG + Plu animals was not significantly different from that of the TG – Plu animals.

In ileal mucosa (Table 3), the same pattern for apo B regulation as observed in jejunum was found, although all values were lower in ileum as compared with jejunum. Also similar to the jejunal pattern, ileal apo A-I synthesis doubled with triacylglycerol absorption, and this increase was completely ablated with Pluronic treatment (Table 3). However, as we have reported previously, fat feeding had no effect on ileal apo A-I content (Table 3), despite the observed increase in synthesis, and in the present study Pluronic treatment did not change ileal content. With regard to ileal apo A-IV, synthesis was unaffected by lipid absorption with or without Pluronic. As with apo B, apo A-I and apo A-IV synthesis and content generally exhibited a proximal-to-distal (high to low) gradient from jejunum to ileum.

Intestinal apolipoprotein mRNA levels

Jejunal apo B and apo A-IV mRNA abundances from two animals in each of the three experimental groups were determined by slot-blot hybridization using human apo B and apo A-IV cDNA probes. An arbitrary absorbance unit (A.U.) of 1.0 was assigned to the experimental group with the highest mean absorbance by densitometry for each apolipoprotein. Apo B mRNA abundance parallels synthesis, with a decrease in the TG + Plu animals to 40 % (0.34–0.46 A.U.) of the –TG (0.89–1.11 A.U.) and TG – Plu (0.86–1.08 A.U.) values. Therefore, the decrease in apo B synthesis with Pluronic treatment may be mediated at the pre-translational level, although our small sample number precludes statistical analysis, and slot-blot hybridization is generally semi-quantitative. Jejunal apo A-IV mRNA abundance generally parallels synthesis, with the –TG group having 21 % (0.15–0.27 A.U.) of the TG – Plu value (0.84–1.16 A.U.) and the TG + Plu group having 71 % (0.59–0.83 A.U.).

DISCUSSION

The intracellular synthesis, assembly, processing and secretion of lipoprotein particles is a complex process. Apolipoproteins are synthesized in the rough endoplasmic reticulum and packaged with lipids in the smooth endoplasmic reticulum [1–4]. The nascent lipoprotein particles then move from the endoplasmic reticulum to the Golgi apparatus for final processing, including apolipoprotein terminal glycosylation and phospholipid modification [1–4]. The particles are then transported in secretory vesicles to the plasma membrane for secretion by reverse pinocytosis [1]. Particularly, the assembly of the apolipoprotein peptides with the lipid moiety and the regulation of this process have not been well characterized. The present study was designed to observe specifically the effect of a pre-Golgi chylomicron secretory block on the intestinal synthesis and content of apo B-48, apo A-I and apo A-IV. This blockade was achieved by the

intestinal infusion of Pluronic L-81 with a lipid emulsion in the newborn piglet. Pluronic L-81 is a hydrophobic non-ionic surfactant which allows normal triacylglycerol digestion, uptake of lipolytic products, and re-esterification within the enterocyte [5–10]. The lipid then accumulates as large droplets within vesiculated smooth endoplasmic reticulum, and the movement of pre-chylomicrons into the Golgi complex is blocked [6]. The mechanism of this effect is unknown at present. The presence of the Pluronic effect in our piglet model was documented by the observation of radiolabelled triacylglycerol accumulation within jejunal mucosa when lipid plus Pluronic was infused intraduodenally.

Piglet intestinal apo B content is normally regulated post-translationally by lipid flux without change in synthesis [12,13]. Chylomicron secretory blockade by Pluronic decreases both apo B synthesis and total mucosal content without affecting total protein synthesis. Furthermore, this down-regulation appears to occur at the pre-translational level. Inability to incorporate apo B normally into nascent intracellular chylomicrons may cause this down-regulation. Although the apo B content of the triacylglycerol inclusions accumulating in Pluronic-treated animals has not been determined, it is possible that the metabolic block occurs before or simultaneous with the aggregation of apo B with the lipid particles, preventing the assembly process. In livers from rats treated with orotic acid, intracellular accumulation of triacylglycerol also occurs [22]. Using the orotic acid model and subcellular-fractionation techniques, Hay *et al.* [23] have demonstrated that the contents of these lipid inclusions are relatively depleted of apo B as compared with rat plasma very-low-density lipoprotein (VLDL). Studies in cultured rat hepatocytes suggest that significant amounts of newly synthesized apo B are degraded intracellularly [24]. Furthermore, oleate added to the medium of cultured HepG2 cells has been shown to stimulate apo B secretion without an accompanying increase in apo B mRNA abundance [25]. It is an attractive hypothesis that in the absence of luminal lipid absorption newly synthesized apo B, not needed for lipoprotein packaging and secretion, is rapidly degraded intracellularly. This would explain the normally observed increase in mass in the face of no change in synthesis or RNA levels with intestinal lipid flux. If such degradation occurs very early in the apo B synthesis pathway (even coincident with translation), it is conceivable that our method of measuring synthesis is really quantifying the net effect of true synthesis (incorporation of the radiolabel into nascent apolipoprotein) and concurrent degradation (proteolysis of labelled protein). Therefore, the effect of Pluronic on apo B synthesis in our study could be due to increased early degradation. This process may be accentuated with Pluronic treatment resulting in down-regulation of apo B content, synthesis and mRNA levels, to below basal levels. It is not likely that Pluronic treatment selectively directly inhibits apo B synthesis, since total mucosal protein synthesis was not affected by Pluronic treatment and, as discussed below, synthesis of the other apolipoproteins was not inhibited to the same degree as that of apo B.

The expected increase in apo A-I synthesis with triacylglycerol absorption in both jejunum and ileum in the piglet is inhibited by Pluronic L-81 treatment, even though the expected increase in jejunal apo A-I content still occurs. Although slightly attenuated, the expected increase in jejunal apo A-IV synthesis with triacylglycerol absorption is still present with Pluronic treatment. We have previously shown that the increase in jejunal apo A-IV synthesis induced by triacylglycerol absorption is mediated at the pre-translational level [11], and this increase appears to be unaffected by Pluronic. The less dramatic effects of chylomicron secretory blockade on apo A-I and A-IV may be due to the presence of alternative pathways of secretion for these

apoproteins, such as intestinal very-low density lipoprotein [9] and high-density lipoprotein [26,27], which may be unaffected by Pluronic. Although not addressed in the present studies, significant amounts of apo A-I and apo A-IV might be secreted not bound to lipoprotein particles. Our results with regard to apo B and apo A-IV differ from observations by Hayashi *et al.* [28,29] in the adult rat. In their studies Pluronic treatment completely blocked the expected increase in apo A-IV intestinal synthesis and lymphatic output with lipid absorption. Although in their studies apo B synthesis was not statistically significantly affected by Pluronic treatment, this group found intestinal apo B synthesis to be 0.98% of total protein synthesis in rats receiving only lipid and 0.74% in rats receiving lipid plus Pluronic. Possible explanations for these conflicting observations include species differences, differences in maturational stage, and the fact that apo A-IV synthesis and mRNA is only up-regulated 2–3-fold normally with fat feeding in the rat [30,31] compared with a much greater increase observed normally in the newborn piglet [11].

In contrast with apo A-I and apo A-IV, apo B appears to be an integral component of the lipoprotein particle and does not dissociate at any time during the peripheral metabolism of the lipoprotein [32]. Apo B also is associated only with triacylglycerol-rich lipoproteins secreted by the intestine (chylomicrons and VLDL) and not secreted in the unbound state and is therefore totally dependent on the secretion of these lipoproteins for exit from the enterocyte [2–4,32]. It should be noted that Pluronic treatment selectively blocks chylomicron secretion with no apparent effect on intestinal VLDL secretion [9], supporting the concept that the synthetic and secretory pathways for these two particles may be segregated. Therefore, some apo B associated with VLDL does exit from the cell [9].

The results of the present study suggest very different mechanisms of cellular regulation and trafficking for the various apolipoproteins incorporated into nascent intestinal chylomicrons. We speculate that in disorders of intestinal lipoprotein secretion, such as abetalipoproteinaemia, the underlying defect might be one of impaired cellular lipid transport with a secondary down-regulation of apo B mass and synthesis, rather than defective synthesis of apo B. This concept is especially relevant in light of recent reports of patients with abetalipoproteinaemia with an intact apo B gene [33,34], detectable intracellular apo B [33,34], and lack of linkage of the apo B gene with that of abetalipoproteinaemia in family studies [35]. Further studies to delineate the mechanism of the action of Pluronic L-81 at the subcellular level are needed.

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