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Apolipoprotein B synthesis by human liver and intestine *in vitro*

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ABSTRACT **The synthesis of apolipoprotein B (apoB) was examined in human fetal and adult intestine and liver. Intestine and liver were minced and then incubated with [^{3}H]leucine, homogenized, and subjected to immunoprecipitation with antiserum that recognized both apoB-100 and apoB-48 (forms of apoB found in low density lipoproteins and in chylomicrons, respectively). Immunoprecipitates of fetal and adult liver contained radioactivity in a single apoB-100 peak when examined by NaDodSO$_4$/polyacrylamide gel electrophoresis. Intestine from fetuses at 11 weeks of gestation incorporated radioactivity mainly into apoB-100, with little incorporation into apoB-48. Sixteen-week fetal intestine showed both apoB-100 and apoB-48, whereas adult intestine incorporated radioactivity only into apoB-48. Pulse–chase experiments with 11- and 16-week fetal intestine showed no evidence for the conversion of apoB-100 to apoB-48. Incubation of intestinal homogenates with fetal liver apoB-100 did not result in the conversion of apoB-100 to smaller forms of apoB. A cDNA probe to hepatic apoB-100 identified a single, 18-kilobase transcript in poly(A)$^+$ RNA from fetal and adult liver and fetal intestine of all ages. These studies define the developmental pattern of apoB synthesis in human fetal and adult liver and intestine. No evidence could be found for the conversion of apoB-100 to apoB-48. The finding of a single mRNA transcript despite the form of apoB synthesized in each tissue is discussed.**

The intestine and the liver of all species, including human, are the major sites of apolipoprotein synthesis and secretion of triacylglycerol-rich lipoproteins (1, 2). An apparent requisite for the secretion of triacylglycerol-rich lipoproteins from either the liver or the intestine is the synthesis and incorporation into the particles of apolipoprotein B (apoB). Failure to incorporate apoB into triacylglycerol-rich lipoproteins leads to an accumulation of triacylglycerol in both liver and intestine and an absence of all lipoproteins containing apoB in the plasma. This is seen in the genetic disorder abetalipoproteinemia, where there is an apparent failure of both liver and intestine to incorporate apoB into lipoproteins (3). It was therefore proposed that a single gene controlled the synthesis of apoB in liver and intestine. This hypothesis has come into question, since distinctive forms of apoB (apoB-100 and apoB-48) have been described for liver and intestine (4–6) and several patients have been described who presumptively showed either impairment of hepatic (7, 8) or partial impairment of intestinal (9) apoB secretion. The mechanism by which apparently organ-specific forms of apoB are synthesized is less clear.

Recently, both rat (10) and human (11–13) partial cDNA clones for hepatic apoB have been developed, and a partial sequence for the human hepatic apoB gene has been published (14). Both in rat and in man, these clones have identified a single mRNA transcript of 18–20 kilobases (kb) in both liver and intestine. The interpretation of these data is unclear, however, because direct data on the synthesis of organ-specific forms of apoB by human liver and intestine are limited. It is known that Hep G2 cells, derived from a human hepatoma, synthesize only apoB-100 (25); however, no data from normal human liver and intestine are available. Therefore, the finding of a single mRNA transcript in liver and intestine when probed with a cDNA to apoB-100 could be due to (*i*) expression of the apoB-100 gene in the intestine, as has recently been suggested (14), or (*ii*) expression of a single gene for apoB in both tissues, with organ-characteristic forms of apoB (apoB-100 or apoB-48) produced by posttranslational mechanisms yet to be defined. In the present study, we have directly determined the forms of apoB synthesized in both adult and fetal human liver and intestine and have determined that no evidence for specific posttranslational modification of either apoB-100 or apoB-48 could be found.

METHODS

Tissue Samples and Preparation. Fetal tissues were obtained after suction abortion (11–20 weeks of gestation). The liver and intestine were rapidly identified and immediately used for isotope-incorporation studies or RNA extraction as described below. Adult human intestinal biopsy samples were obtained either from the second portion of the duodenum, by using a multiport Rubin suction biopsy tube positioned under fluoroscopic control, or from endoscopic duodenal biopsies of normal-appearing duodenum in the course of diagnostic upper-duodenal endoscopies. Samples of human liver were obtained from normal margins of liver surrounding hepatic resections for either trauma or resective surgery for hepatic tumors.

Isotope-Incorporation Studies. Fetal liver and intestine were coarsely minced into 0.5-mm sections and placed into 1.0 ml of Krebs–Ringer bicarbonate solution (118 mM NaCl/4.7 mM KCl/3.6 mM $CaCl_2$/1.2 mM $MgSO_4$/1.2 mM KH_2PO_4/25 mM $NaHCO_3$, adjusted to pH 7.4 with 95% O_2/5% CO_2) containing glucose (5 mM), penicillin (50 units/ml), streptomycin (50 μg/ml), Trasylol (0.2 unit/ml), and [^{3}H]leucine (1–2 mCi, 120 Ci/mmol; 1 Ci = 37 GBq) at 37°C. The tissues were incubated at 37°C with gentle shaking in an atmosphere of 95% O_2/5% CO_2 for various times. In some experiments, after an initial period of isotopic incorporation, the tissue was rinsed in warm buffer and then incubated in buffer containing 20 mM nonradioactive leucine. Samples were removed for immunoprecipitation at various times.

Immunoprecipitation Studies. At the conclusion of each incubation, tissue samples were homogenized in 1 ml of phosphate-buffered saline (20 mM sodium phosphate/145 mM NaCl, pH 7.4) containing 1% (wt/vol) Triton X-100, 2 mM leucine, 1 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine, using a Polytron tissue homogenizer (Brinkmann Instruments). The homogenate was centrifuged at 125,000 × *g* for 60 min in a 50.3Ti rotor (Beckman Instruments) at 4°C. The supernatant was subjected to immunoprecipitation for apoB, using procedures identical to those

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Abbreviations: apoB, apolipoprotein B; LDL, low density lipoprotein(s); VLDL, very low density lipoprotein(s); kb, kilobase(s).

reported previously (15). The antiserum used for immunoprecipitation was from rabbits that had been injected with purified human low density lipoproteins (LDL). These antisera reacted against apoB-100 and apoB-48, as determined by Ouchterlony analysis and immunoblotting of both LDL and a mixture of human chylomicron apolipoproteins. The antisera recognized both apoB from LDL (apoB-100) and human chylomicron apoB (apoB-48) prepared from chylomicrons isolated from chyluric patients. Antiserum was added to immunoprecipitation mixtures in amounts necessary to ensure precipitation under conditions of antibody excess. This was determined by adding ^{131}I-labeled (26) LDL to aliquots of immunoprecipitation mixtures and adjusting the concentration of antiserum so that all the labeled LDL was immunoprecipitated. These antiserum concentrations were then used for immunoprecipitation of other aliquots of the same samples. Immunoprecipitates were washed extensively and analyzed by $NaDodSO_4$/4% PAGE. Gels were sectioned into 1.5-mm slices, and the radioactivity in each slice was determined as described (15). The specificity of apoB immunoprecipitation was determined by carrying out additional immunoprecipitations on aliquots of the same samples under identical conditions, with the addition of excess unlabeled human LDL prepared by ultracentrifugation according to standard techniques (27). A radioactive species precipitated by anti-apoB antiserum was validated to be apoB only if exogenously added LDL abolished its precipitation. In addition, the migration of apoB-100 (LDL) and apoB-48 (rat chylomicrons) was determined by Coomassie blue staining of gels containing 5–10 μg of each protein and completely coincided with radioactive peaks designated apoB-48 or apoB-100.

RNA Extraction. All tissue for RNA extraction was processed immediately and homogenized, in a Polytron, in 3 M lithium chloride/6 M urea/50 mM sodium acetate, pH 5.3. RNA was extracted according to the method of Auffray and Rougeon (16). Yields from this procedure were superior to guanidinium chloride extraction. Selection of poly(A)$^+$ RNA was carried out according to the method of Bailey and Davidson (17). RNA was fractionated by electrophoresis in 0.6% agarose/6% formaldehyde gel. The fractionated RNA was transferred to a nitrocellulose filter and hybridized to ^{32}P-labeled cDNA to a fragment of apoB-100, kindly provided by J. Breslow, Rockefeller University, New York (12). This probe, pB8, identifies an mRNA transcript of 22 kb when hybridized against human hepatoma (Hep G2) RNA.

RESULTS

Synthesis of apoB by Liver and Intestine. Fig. 1 shows the pattern of apoB immunoprecipitation from fetal and adult liver that had been incubated with [^{3}H]leucine for 15 min. For both fetal and adult liver, a single peak of radioactivity is present at the position of apoB-100 in this $NaDodSO_4$/PAGE system. Radioactive peaks were abolished when an exogenous source of apoB (LDL) was added to the incubation mixture, validating that the radioactivity is contained in immunoreactive apoB.

Fig. 2 shows the pattern of apoB immunoprecipitation in fetal intestine of various ages and in adult intestine. A variable pattern is seen, depending on fetal age. At the earliest times studied (11 weeks), the predominant form appears to be apoB-100, with relatively small amounts of apoB-48 synthesized. With increasing fetal age (16 weeks), both apoB-48 and -B-100 are synthesized in relatively equal amounts in a given intestinal sample. Altering the length of isotope exposure (5–30 min) did not alter the relative proportion of radioactivity in the apoB-100 and -B-48 peaks in a given sample. In a single sample of older fetal intestine (21 weeks), the apoB-100 peak had disappeared and only apoB-48 was radioactive. In adult duodenal mucosa, only apoB-48 is synthesized. To eliminate the possibility of an initial rapid synthesis of apoB-100 followed by a rapid conversion to apoB-48, we incubated adult intestine with [^{3}H]leucine for as little as 2 min without detecting apoB-100. We also wished to eliminate the somewhat unlikely possibility that any *in vitro* conversion of apoB-100 to apoB-48 could have occurred during our routine immunoprecipitation (4°C for 16 hr). We therefore carried out immunoprecipitations for 2 hr, with identical results. In addition, immunoprecipitation of another aliquot of the sample after −70°C storage for 24 hr gave no change in the pattern of apoB. The elimination of protease inhibitors also had no effect on the form of apoB immunoprecipitated.

To further explore the possible posttranslational modification of apoB-100 to apoB-48, we carried out pulse–chase experiments with intestine. After an initial period of [^{3}H]leucine exposure (10–15 min), tissues were rinsed, placed in fresh buffer containing 20 mM nonradioactive leucine, and homogenized after incubation for various times up to 45 min. Figs. 3 and 4 show the results for intestine of 11- and 16-week fetuses. No interconversion of apoB-100 and apoB-48 could be demonstrated. Finally, we reasoned that if apoB-48 were derived posttranslationally from apoB-100, then fetal tissue containing apoB-48 should contain activity(ies) able to effect this conversion. We therefore exposed fetal liver to [^{3}H]-leucine for 10 min and then homogenized this together with a segment of intestine as a putative source of "converting enzyme." Only a single peak of radioactivity corresponding to liver apoB-100 was seen, with no evidence for the

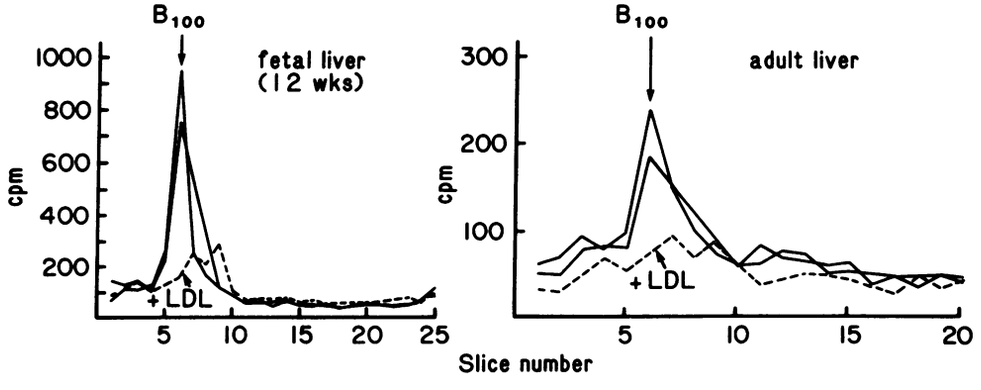

FIG. 1. Synthesis of apoB in fetal (*Left*) and adult (*Right*) liver. Minced fetal and adult liver samples were incubated with [^{3}H]leucine, and apoB was immunoprecipitated. Immunoprecipitates were electrophoresed in $NaDodSO_4$/4% polyacrylamide gels, and the radioactivity in 1.5-mm gel slices was determined. Solid lines represent duplicate immunoprecipitations. Broken lines show the effect of addition of nonradioactive LDL to the immunoprecipitation mixture. The position of LDL apoB-100 (arrow) in a gel run in parallel and stained with Coomassie blue is indicated.

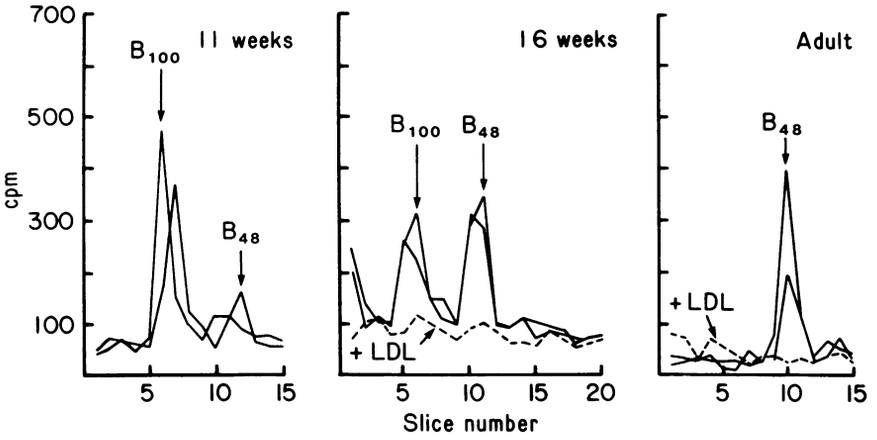

FIG. 2. Synthesis of apoB in fetal (11 and 16 weeks of gestation) and adult intestine. Minced fetal and adult intestine were incubated with [^{3}H]leucine and then analyzed by immunoprecipitation and electrophoresis as described in the legend to Fig. 1. Solid lines show duplicate determinations of a representative experiment. Broken lines show the effect of addition of LDL to the immunoprecipitation mixture. The positions of apoB-100 (LDL) and apoB-48 (chylomicrons) standards are indicated.

formation of apoB-48. When the same intestinal sample was incubated separately with [^{3}H]leucine, radioactive apoB-48 was detected, showing that it should be an appropriate source for "converting enzyme" if it were present.

mRNA Patterns in Fetal and Adult Tissues. Using the pB8 cDNA probe corresponding to a portion of apoB-100 (12), we examined the pattern of mRNA in all the tissues studied. RNA extracts from fetal (16 weeks) liver and intestine and from adult intestine contained a single apoB-related transcript of 18 kb (Fig. 5).

DISCUSSION

The present studies have determined the synthesis of organ-specific forms of apoB in human liver and intestine and compared adult and fetal synthesis of this protein. Previously, evidence for intestinal and hepatic forms of apoB largely rested on the identification of different forms of the protein in major secretory products from each organ. Chylomicrons harvested from rat mesenteric lymph or human thoracic duct lymph (4) contained a smaller form of apoB, apoB-48, than did hepatic very low density lipoproteins (VLDL) or their metabolic products, LDL, which contained apoB-100 (6). A clear organ specificity for apoB-100 and apoB-48 was questioned, however, when it was shown that both forms of apoB are synthesized by rat liver (6, 18). In addition, there has been the suggestion that larger forms of apoB may also be synthesized by rat intestine (5, 19, 20), although recent studies using immunoprecipitation techniques have only shown apoB-48 in rat enterocytes (21).

The situation in humans is less clear, since few direct studies on the synthesis of apoB by human liver and intestine have been reported. The human hepatoma cell line Hep G2 synthesizes apoB-100 (25); however, no data on normal human liver have been available. The lack of a human small-intestinal cell line has impeded efforts to study apoB synthesis directly. Evidence for organ-specific forms of human apoB was strengthened by clinical syndromes where apoB-48 and chylomicron secretion was normal while apoB-100 and hepatic VLDL secretion was impaired (7, 8). This suggested different synthetic mechanisms for hepatic and intestinal apoB synthesis, although the precise mechanisms are not defined. The development of cDNA probes for apoB-100 mRNA has permitted the identification of specific

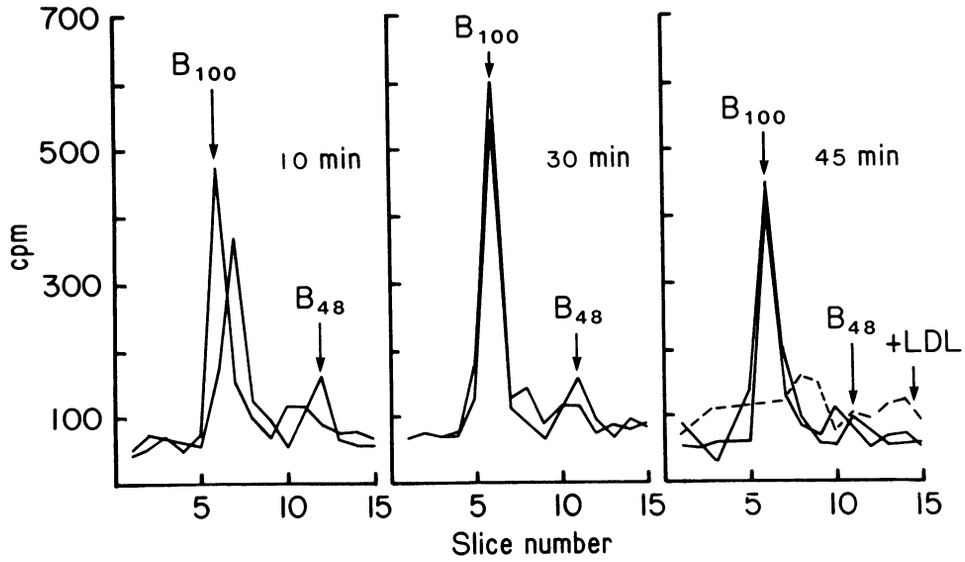

FIG. 3. Pulse–chase analysis of apoB synthesis in fetal intestine (11 weeks). After 10 min of exposure to [^{3}H]leucine, the tissue was rinsed and placed in buffer containing 20 mM nonradioactive leucine. Immunoprecipitations were carried out at the indicated times. Duplicate determinations are shown.

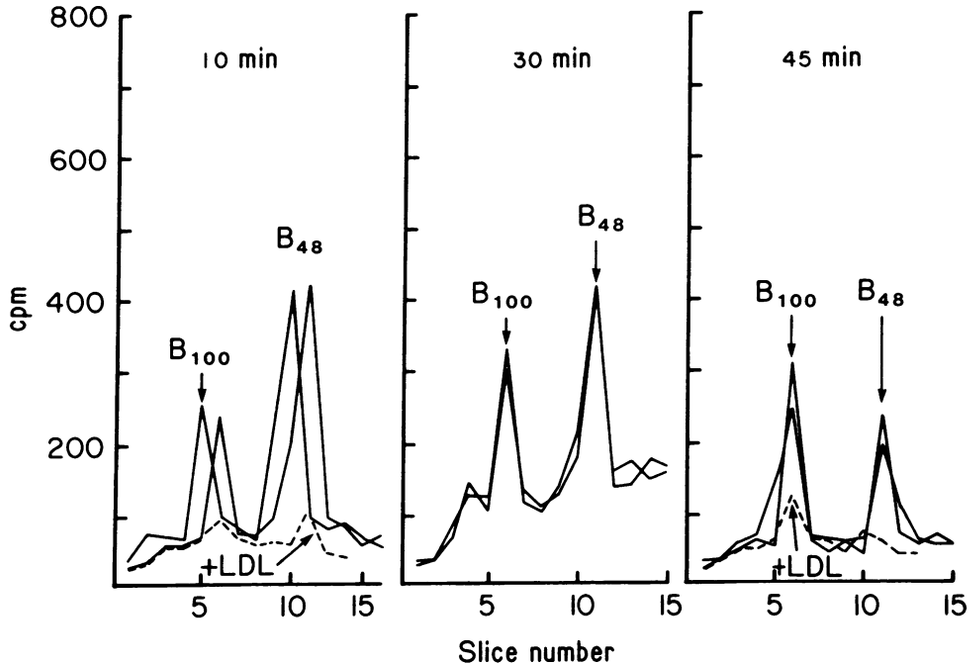

FIG. 4. Pulse–chase analysis of apoB synthesis in fetal intestine (13 weeks). See legend to Fig. 3.

mRNA in each tissue. As expected, a strong hybridization signal was detected in liver RNA samples, implying the synthesis of apoB-100. Somewhat unexpectedly, the apoB100 cDNA also hybridized with RNA from adult intestine, implying that apoB-100 may also be synthesized in intestine (14). Alternatively, it was suggested (14) that apoB-100 mRNA could also eventuate in apoB-48, perhaps by posttranslational modification of apoB-100. The present study addresses some of these uncertainties. Both fetal and adult liver synthesize only apoB-100 (Fig. 1) and are unlike rat liver, where both forms of apoB are synthesized. The events in intestine are more complex: apoB-100 is the predominant form of apoB synthesized in early fetal intestine, with the progressive development of apoB-48 synthesis. In adult intestine, apoB-100 is no longer synthesized and apoB-48 is the sole form present (Fig. 2). We found no evidence for the conversion of apoB-100 to apoB-48 in pulse–chase experiments (Figs. 3 and 4) or in mixing experiments where liver apoB-100 was exposed to intestinal homogenates capable of synthesizing apoB-48. Similar methods have been employed to demonstrate posttranslational modifications of apoA-I and apoA-IV (22, 23).

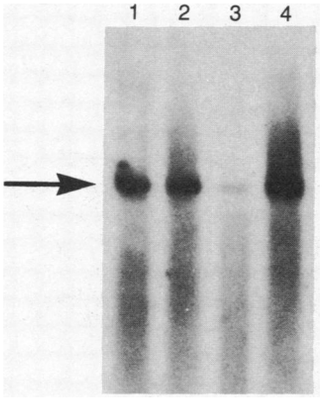

FIG. 5. Detection of apoB in RNA from liver and intestinal tissues. RNA samples were fractionated in 0.6% agarose gels containing formaldehyde transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled nick-translated pB8 under stringent (30 mM NaCl/3 mM sodium citrate, pH 7) conditions. Arrow indicates 18-kb apoB-related band; RNA size markers were 18S and 28S rRNA bands, located by ethidium bromide staining of the same gel. Lanes: 1, total fetal (16 weeks) liver RNA (20 μg); 2, total fetal (16 weeks) intestinal RNA (20 μg); 3, poly(A)$^+$ fetal intestinal RNA (1 μg); 4, total adult intestinal RNA (20 μg).

We confirmed previous observations that a cDNA probe to apoB-100 identifies a single mRNA transcript of 18 kb in both liver and intestine (Fig. 5). Our studies permit a correlation between mRNA transcripts and synthetic forms of apoB in a given tissue. The detection of a single, 18-kb transcript that hybridizes with the apoB-100 cDNA, in tissues containing either form of apoB, or both (fetal intestine), suggests certain possibilities. Although it is possible that the probe recognizes only apoB-100 mRNA, this is unlikely, since no apoB-100 synthesis could be demonstrated in adult intestine and this would require a stable pool of mRNA that is not translated. It is also possible that separate apoB-100 and apoB-48 mRNAs exist that are similar in size and therefore not resolved by electrophoresis.

If, as current evidence suggests, a single apoB mRNA is present in liver and intestine, then the synthesis of both forms of apoB may result. The present studies give no evidence for posttranslational modification of apoB-100 to apoB-48. Alternatively, both apoB-100 and apoB-48 could be products of the same mRNA, with organ-specific factors responsible for translational control of synthesis. Evidence for this must await the development of apoB-48-specific probes and complete elucidation of the entire apoB gene.

An additional finding in the present study was the synthesis of apoB-100 in the early fetus and its disappearance with age. Concomitantly, apoB-48 appears, and apoB-48 is the sole form of apoB present in the adult. Factors responsible for changing gene expression in fetal intestine are of potential interest. A recent report (24) that corticosteroids and insulin can markedly affect apolipoprotein mRNA levels in rat liver suggests that hormonal effects may be important for regulation of apolipoprotein gene expression. The development of apoB in human fetal intestine may be an excellent model for study of the differential expression of apoB-100 and apoB-48 and the subsequent emergence of apoB-48 as the adult form.

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