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Apolipoproteins in Human Fetal Colon: Immunolocalization, Biogenesis, and Hormonal Regulation

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Abstract The present investigation aimed at defining the localization of apolipoproteins (apo) A-I, A-IV, B-48, and B-100 along the crypt-villus axis of the human fetal colon, their biogenesis during gestation, and their hormonal regulation. Using immunofluoresence, the distribution of apo A-I and A-IV appeared as a gradient, increasing from the developing crypt to the tip of the villus. On the other hand, apo B-100 staining was found in the crypt and the lower mid-villus region with varying intensities in the upper villus cells, while the 2D8 antibody which recognizes both apo B-100 and B-48, revealed uniform staining along the crypt-villus axis. Apolipoprotein synthesis, determined by [^{35}S] methionine labeling, immunoprecipitation, and SDS-PAGE showed a predominance of apo A-IV (53%), followed by apo A-I (23.9%), apo B-48 (13.4%), and apo B-100 (9.7%). The synthesis of each apolipoprotein was significantly modulated by hydrocortisone, insulin and epidermal growth factor (EGF). Apart from a decrease in apo B-100 exerted by EGF and a reduction in apo A-I resulting from the addition of insulin, the other apolipoproteins were all enhanced. Our data confirm that the fetal colon has the capacity to synthesize apolipoprotein A-I, A-IV, B-48, and B-100 and establish that their synthesis are modulated by hormonal and growth factors known to be involved in the regulatory mechanism of the functional development of human jejunum. J. Cell. Biochem. 70:354–365, 1998.

Key words: human fetal colon; apolipoprotein A-I, A-IV, B-48, B-100; hydrocortisone; insulin; epidermal growth factor

The small intestine plays a primary role in the assimilation and transport of dietary fat [Davidson, 1994; Lévy, 1992a; Tso et al., 1986]. One of its major functions is the synthesis of chylomicrons and their secretion into the plasma, where their triglyceride moiety can be utilized as an energy source or stored. Apolipoproteins serve as integral structural components of lipoproteins, and play an integral role in their normal assembly, secretion, and metabolism [Li et al., 1988]. For example, apo B is essential for the triglyceride-rich lipoprotein assembly/secretion process. Patients lacking the ability to normally produce apo B display the inability to deliver chylomicrons or VLDL to the circulation [Lévy et al., 1994]. Several physiologic roles have also been proposed for apo A-I and apo A-IV, including lecithin:cholestorol acyltransferase activation [Steinmetz et al., 1988], stabilization of lipoprotein (chylomicron and HDL) surfaces [Li et al., 1988], interaction with their own receptors [Li et al., 1988], and stimulation of reverse cholesterol transport [Li et al., 1988].

Functionally polarized absorptive enterocytes are considered the unique cells of the small intestinal epithelium capable of producing extracellular lipid transport proteins, principally apolipoproteins A-I, A-IV, and B-48 [Green et al., 1981]. Although little is known about the distribution of these apolipoproteins in the human small intestine, it is generally assumed that they are not located in cells that populate the crypt of Lieberkühn, the proliferative compartment of the intestinal epithelium. Their expression is suggested to be rather initi-

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ated as enterocytes undertake their upward migration on the villus [Christensen et al., 1981]. The barriers that impede our understanding of the ontogeny and distribution stem partly from the lack of an ideal model to study the human small intestinal epithelium.

Furthermore, there is a virtual lack of knowledge about apolipoprotein location in other intestinal regions, such as the colon. Although the functions of the adult colon are considered to be limited to the conservation of water and electrolytes, bacterial metabolism, and fermentation, and the controlled evacuation of feces [Moran et al., 1992], the fetal colon appears to possess metabolic functions, which resemble those of the mature small bowel [Ménard, 1989]. We have recently demonstrated that the esterification of lipids, and the assembly of lipoproteins occurs in the developing human colon [Lévy et al., 1996]. In the present paper, we have focused our attention on the expression of apolipoproteins. The distribution of apolipoproteins A-I and A-IV along the crypt-to-villus axis of the human fetal colon was compared with fetal jejunum. We have also examined the localization of B-48 and B-100, since both apo B forms are produced during the fetal period. Furthermore, we used the organ culture model [Ménard et al., 1987] to study the ontogeny as well as the regulation of apolipoprotein synthesis in the fetal colon by insulin, epidermal growth factor, and hydrocortisone, hormones known to be involved in fetal gut development [Ménard, 1989].

MATERIALS AND METHODS

Human Colonic Specimens

Small intestine and large bowel specimens from 36 fetuses ranging from 15 to 20 weeks in age were obtained from normal elective pregnancy terminations. No tissue was collected from cases associated with known fetal abnormality or fetal death. The entire gut was immersed in Leibovitz L-15 medium, containing garamycin (40 µg/ml) and mycostatin (40 µg/ml), and brought to the culture room within 30 min. Samples of the jejunum and colon cleansed of mesentery were immediately processed for indirect immunofluorescence and culture. These studies were approved by the Institutional Human Subject Review Board.

Indirect Immunofluorescence

The preparation and embedding of specimens for cryosectioning were performed as previously described [Beaulieu et al., 1990] using optimum cutting temperature embedding compounds (Tissue Tek, Miles Laboratories, Elkhart, IN). Cryosections 2–3 µm thick, cut on a Jung Frigocut 2800N cryostat (Leica Canada Inc., Saint-Laurent, Canada), were fixed on glass slides with acetone-chloroform (1:1) for 5 min at 4°C and washed twice with phosphate-buffered saline (PBS). Following a 30 min incubation with 10% blotto-PBS to quench the remaining aldehyde residues, the glass slides were washed twice in PBS. The staining procedure using antibodies and fluorescein was performed at room temperature in humid chambers.

Monoclonal antibodies 2D8 and 4G3, kindly provided by Drs. R. Milne and Y. Marcel, are directed against different antigenic determinants of both forms of apo B [Milne et al., 1983]: 2D8 recognizes the NH_2-terminal of the amino acid chain common to both apo B-100 and apo B-48, whereas 4G3 is directed against COOH-terminal of the amino acid chain of apolipoprotein B-100. Monoclonal anti-human apo A-I and apo A-IV were obtained from Boehringer Mannheim (Montréal, Canada). Sections were incubated for 1h with the monoclonal antibodies diluted in 10% Blotto (in PBS): 4G3 anti-apo B-100 (1:100), 2D8 anti-apo B-48 and apo B-100 (1:300), anti-apo A-IV (1:100), and anti-apo A-I (1:300). Sections were then washed in PBS and incubated with fluorescein-conjugated goat anti-mouse or donkey anti-sheep immunoglobulin G (Boehringer Mannheim, Laval, Quebec, Canada) used at a dilution of 1:30 and 1:50 in 10% Blotto-PBS. After extensive washing with PBS, the sections were stained with 0.01% Evans blue in PBS. The preparations were then mounted in glycerol-PBS (9:1) containing 10 µg/ml of paraphenylenediamine, and viewed with a Reichert Polyvar 2 microscope (Leica, Montréal, Canada), equipped for epifluorescence. The monoclonal antibodies for apo Bs are reliable tools which have been frequently used to specifically react against apo B in various studies. As previously mentioned [Lévy et al., 1996], immunoblot experiments were performed demonstrating their specificity. Furthermore, several other control experiments were performed including excess of LDL purified by ultracentrigation or purified apo B obtained

after delipidation of LDL by ethanol-ether (1:1) added to the antibody solution. Finally, adsorption of the antibodies with LDL or apo B led to non significant staining. Immunoblot experiments were also carried out with the other antibodies establishing that they recognized only antigenic determinant of apo A-I and A-IV.

An unrelated mouse monoclonal anti-DNA antibody was used followed by fluorescein in order to determine the extent of nonspecific adsorption of mouse immunoglobulins to tissue sections. Finally, the primary antibodies were omitted or replaced by non-immune sheep or mouse serum at 1:800 and 1:700 dilution, respectively (Figs. 1C,D; 2E,F). All these control experiments did confirm the specificity of the results.

Organ Culture

The colon was split longitudinally, washed in culture medium, and cut into explants (3 × 7 mm). Five to seven explants were randomly transferred onto lens paper with the mucosal side facing up in each organ culture dish (Falcon Plastics, Los Angeles, CA). Six dishes were used for each experimental condition. An amount of medium (0.8 ml) sufficient to dampen the lens paper was added. Explants were cultured in serum-free Leibovitz L-15 medium according to the technique described previously [Lévy et al., 1992; Ménard et al., 1990]. After a 3-h stabilization period, the medium was replenished with one containing a final amount of 1.3 µmol/ml of nonlabeled oleic acid attached to albumin and supplemented with hydrocortisone (50 ng/ml, Collaborative Research, Waltham, MA), EGF (100 ng/ml, Collaborative Research, MA), or insulin (3 mU/ml, Novo Nordisk, Mississauga, Ontario, Canada). Colonic explants were cultured for 42-h period which in preliminary experiments was optimal for hormonal studies. These hormonal and growth factor concentrations were chosen based on their known biological effects on the brush border functions and lipoprotein synthesis in the developing human gut [Lévy et al., 1992b; Loirdighi et al., 1992; Ménard, 1988, 1989, 1990].

Pulse Labeling of Intestinal Explants and Immunoprecipitation Procedure

These assays were carried out as described previously [Lévy et al., 1992b, 1994b). Briefly, following the incubation period with nonlabeled oleic acid (42 h) to stimulate the synthesis of apolipoproteins, jejunal, and colonic explants were washed twice with methionine-free Leibovitz medium. They were then incubated in the same medium containing nonlabeled oleic acid and the corresponding hormone, for 45 min in the presence of [^{35}S] methionine (300 µCi/ml). At the end of the labeling period, at 37°C, explants were washed (×3) and homogenized in phosphate-buffered saline (20 mM sodium phosphate, 145 mM NaCl, pH 7.4) containing 1% (wt/vol) Triton x-100, methionine (2 mM), phenylmethylsulfonyl fluoride (PMSF; 1 mM), and benzamide (1 mM). Aliquots of tissue homogenates were precipitated with 20% trichloroacetic acid (TCA). The precipitates were then washed three times with 5% TCA before the radioactivity was determined in a Beckman liquid scintillation spectrometer. The homogenates were also centrifuged (4°C) at 105,000*g* for 60 min in a 50-Ti rotor (Beckman, CA) and supernatants subsequently reacted with excess apolipoprotein monoclonal antibodies (against apo A-I, A-IV, B-48 and B-100) for 18 h at 4°C. Pansorbin (Calbiochem, CA) was then added, and the mixture was re-incubated at 20°C for 60 min. The immunoprecipitates were washed extensively and analyzed by a linear 4–20% acrylamide gradient preceded by a 3% stacking gel, as described previously [Lévy et al., 1994b]. Gels were sectioned into 4-mm slices and counted after an overnight incubation at 20°C with 1 ml BTS-450 (Beckman) and 10 ml of scintillation fluid (Ready Solv. NA, Beckman).

Statistical Analyses

Data are presented as means ± SEM. Significance of differences was evaluated using Student's paired *t*-test.

RESULTS

Immunolocalization of Apolipoproteins Along the Crypt-Villus Axis

To examine the expression of the individual apolipoproteins in the intestine and to determine their distribution along the crypt-villus axis, indirect immunofluorescence was carried out using specific monoclonal antibodies. Figures 1 and 2 illustrate the localization of apo A-IV and A-I along the crypt-villus axis in human fetal jejunum and colon. Immunofluorescence staining was exclusively observed in the columnar epithelial cells of both segments but never in goblet cells as in human adult intes-

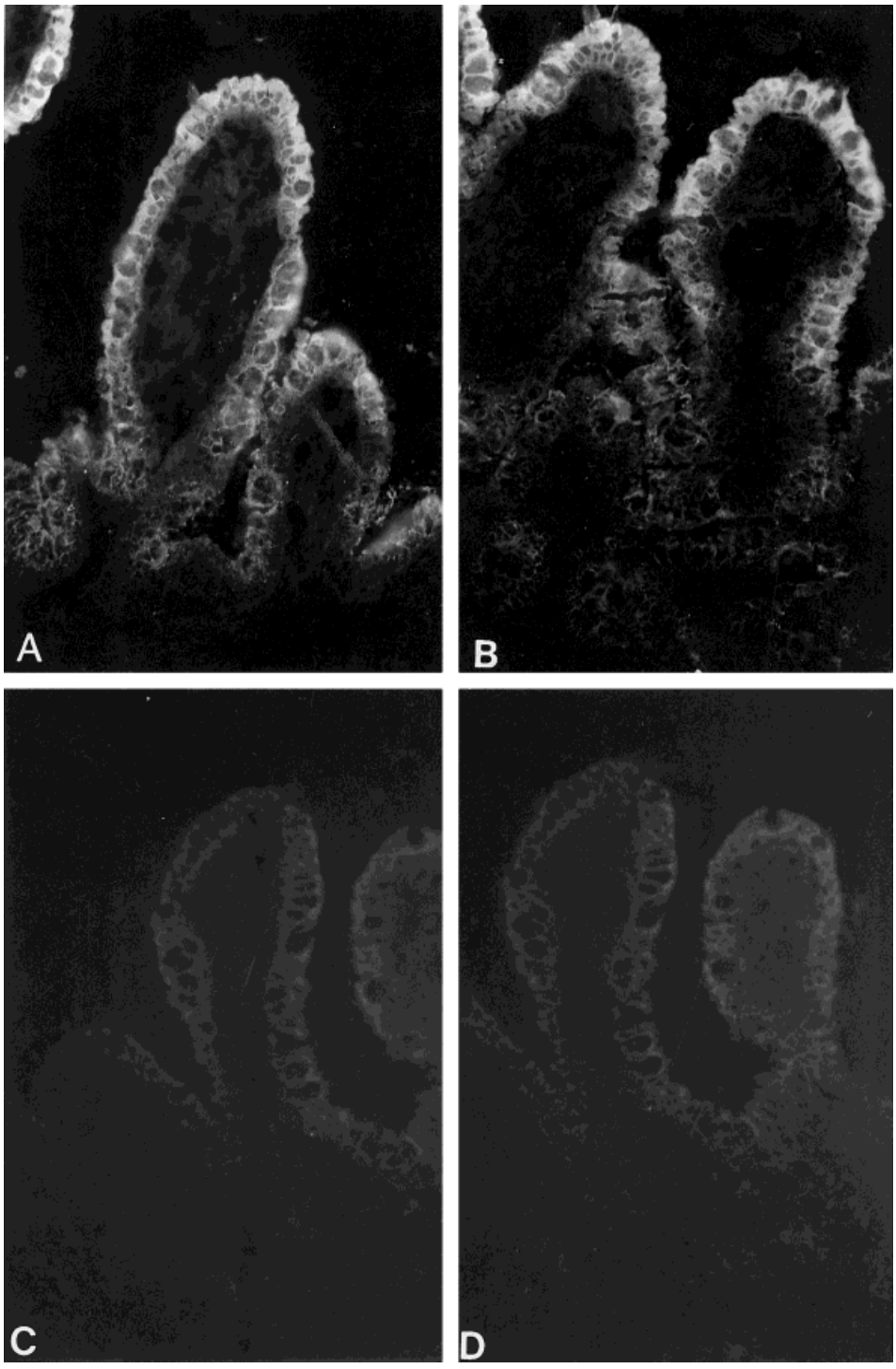

Fig. 1. Expression and distribution of apolipoproteins along the jejunal crypt-villus axis. Representative indirect immunofluorescence micrographs of crysections of human fetal jejunum at 20 weeks of gestation. Crysections were stained with anti-apo A-IV (**A**), anti-apo A-I (**B**) or treated with nonimmune sheep (**C**) or mouse (**D**) serum. Increasing gradients of apo A-IV and A-I were observed from the developing crypt to the villus tip. No fluorescent staining was observed when primary antibodies were replaced by nonimmune serum. Magnification = 115.

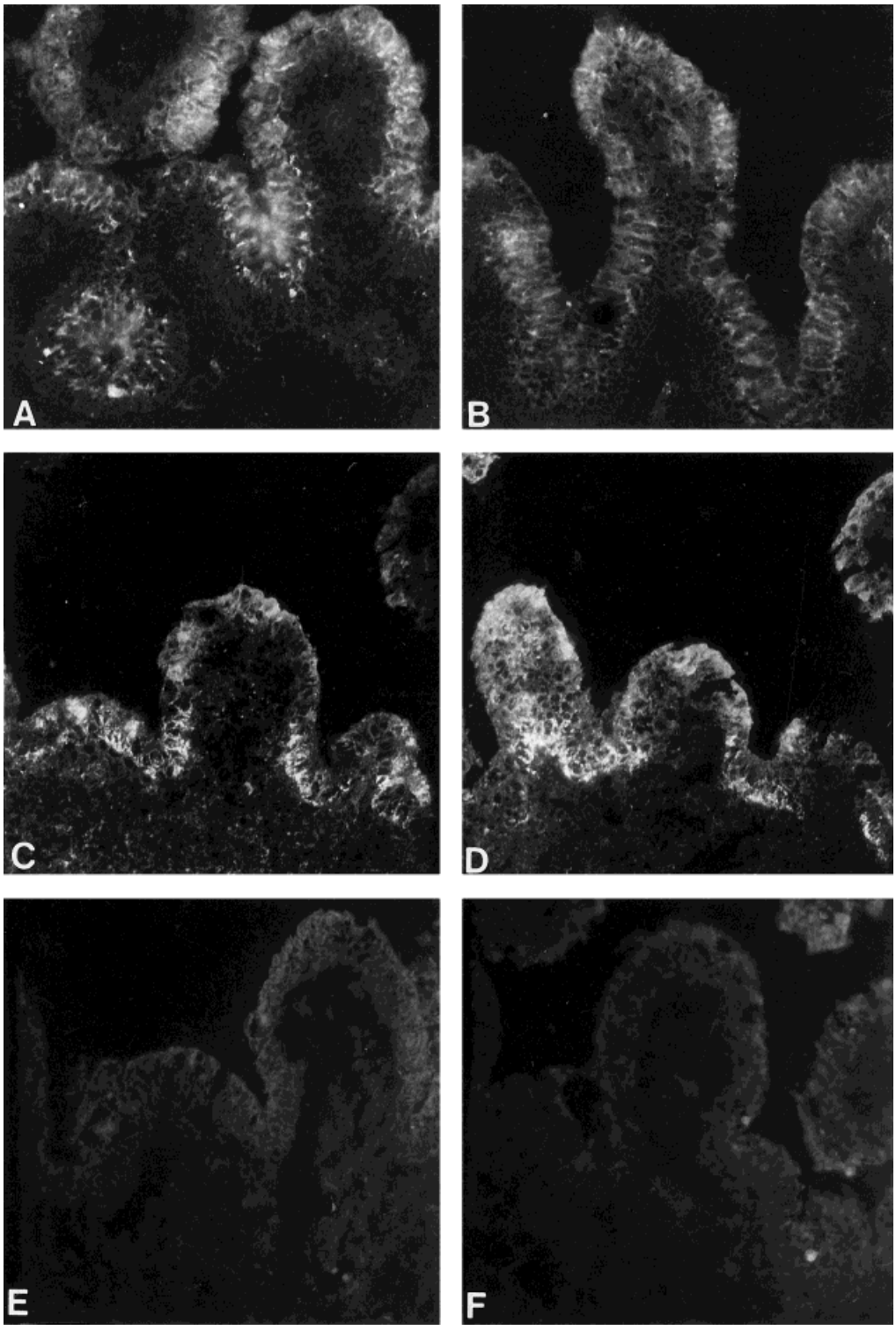

Fig. 2. Expression and distribution of apolipoproteins along the colonic crypt-villus axis. Representative indirect immunofluorescence micrographs of cryosections of human fetal colon at 20 weeks of gestation. Cryosections were stained with anti-apo A-IV (**A**), anti-apo A-I (**B**), 4G3 anti-apo B-100 (**C**), and 2D8 anti-apo B-48 and B-100 (**D**) or treated with nonimmune sheep (**E**) or mouse (**F**) serum. Apo A-IV and apo A-I exhibited an increasing gradient along the crypt-villus axis. Apo B-100 was uniformly distributed in the crypt and lower mid-villus region with varying staining intensities in the upper villus cells clearly visible in the cross section of the villus (upper right corner). On the other hand, the epithelial cell labeling with 2D8 was uniform along the entire length of the crypt-villus axis. Magnification = 115.

tine [Green, 1982]. The distribution of apo A-IV (Figs. 1A, 2A) and apo A-I (Figs. 1B, 2B) along the crypt-villus axis in both jejunum and colon appeared in a gradient fashion increasing from the developing crypt to the villus tip. Apo A-IV and apo A-I staining were detected in the apical, lateral and basal areas of both enterocytes and colonocytes. In all specimens studied, immunodetection of apo B-100 in fetal jejumum (not shown) and colon (Fig. 2C) revealed a staining distribution to the crypt and the lower midvillus region with varying staining intensities in the upper villus cells. On the other hand, using the 2D8 antibody which recognizes the NH_2-terminal of the amino acid chain common to both apo B-100 and apo B-48, the epithelial cell labeling was found to be uniform along the entire length of the crypt-villus axis (Fig. 2D).

De Novo Synthesis and Developmental Profile of Apolipoproteins by Fetal Colon

In addition to documenting the presence of apo B-48, B-100, A-I, and A-IV by immunofluorescence, we assessed their biogenesis by the human fetal colon. For this purpose, colonic explants were cultured in the presence of [^{35}S]-methionine for 45 min, and newly synthesized apolipoproteins were analyzed by NaDodSO_4-PAGE following immunoprecipitation. Radioactivity was found mostly in apo A-IV, followed in decreasing order by apo A-I, apo B-48, and apo B-100 (Fig. 3).

In order to determine whether developmental changes occur in apolipoprotein synthesis, colonic explants were categorized according to fetal age. As illustrated in Figure 4, the synthesis of apolipoproteins B-48 and B-100 peaked at 18 weeks of gestation, while apo A-IV reached its maximal level at 16 weeks. The synthesis of apo A-I appears to be maximum at 16 and 17 weeks and remained relatively elevated thereafter.

Hormonal Regulation of Apolipoprotein Synthesis

The modulation of fetal colonic apolipoprotein synthesis by hydrocortisone, EGF, and insulin was examined using serum-free organ culture methodology. Hydrocortisone (Fig. 5) significantly increased apo A-IV, apo A-I, and apo B-48 synthesis (40, 35, and 71%, respectively). Similarly, the addition of EGF (Fig. 6) produced a significant increase of apo A-IV, apo A-I, and apo B-48 synthesis (47, 50, and 93%, respectively), but decreased apo B-100 synthesis (56% of control).

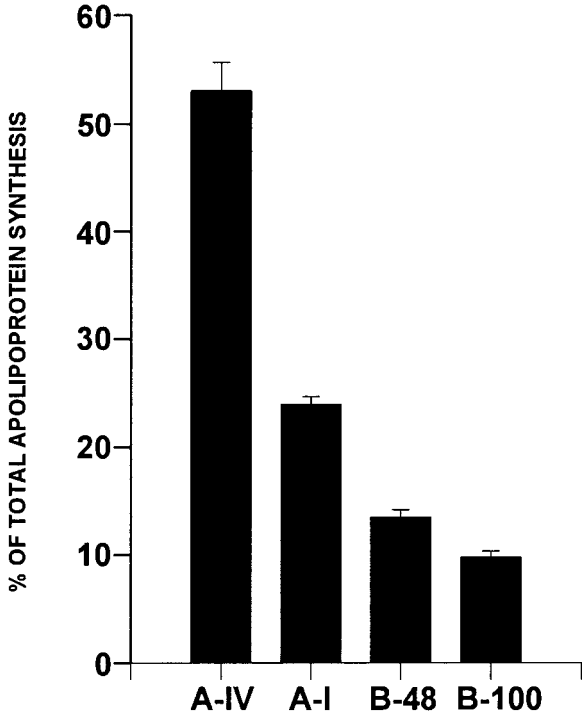

Fig. 3. Quantification of apolipoprotein synthesis in human fetal colon ranging from 15 to 20 weeks of gestation. Colonic explants were cultured for 42 h in the presence of oleic acid, followed by a 45 min incorporation using [^{35}S] methionine. Apolipoproteins were isolated by immunoprecipitation as described in Materials and Methods. Values represent means ± SEM of 19 specimens and are expressed as % TCA-precipitable proteins.

Finally, incubation of fetal colon explants with insulin (Fig. 7) resulted in significant increase of apo A-IV (31%) and apo B-48 (26%), and a significant decrease of apo A-I synthesis (21%).

DISCUSSION

The determination of the sites of apolipoprotein synthesis has been the subject of extensive investigation during the last 2 decades. In mammalian species, the liver and the intestine are thought to produce the majority of circulating apolipoproteins [Havel, 1987]. However, little knowledge is available about the relationship between intestinal apolipoproteins and gut ontogeny. Furthermore, data concerning the localization and the hormonal regulation of apolipoproteins by the colon, an organ capable of the lipid transport during development [Lévy et al., 1996], are virtually absent. Our systematic investigation has demonstrated for the first time that 1) apolipoprotein A-IV, A-I, B-48, and B-100

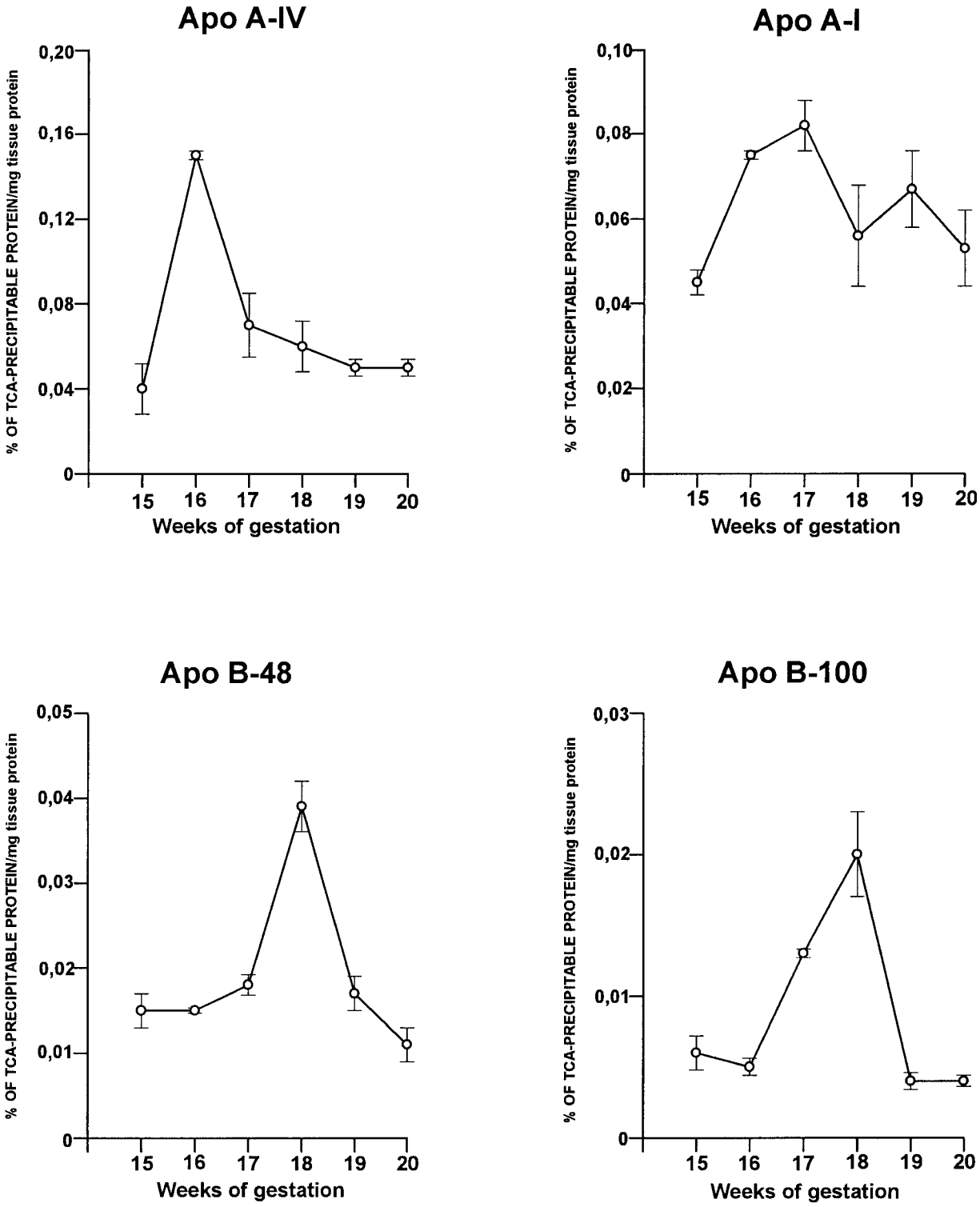

Fig. 4. Patterns of apolipoprotein biogenesis during human fetal colon development. Colonic explants were cultured for 42 h and apolipoproteins were isolated using immunoprecipitation technique as described in Materials and Methods. Results are expressed as means ± SEM and each time-point represents three different tissues.

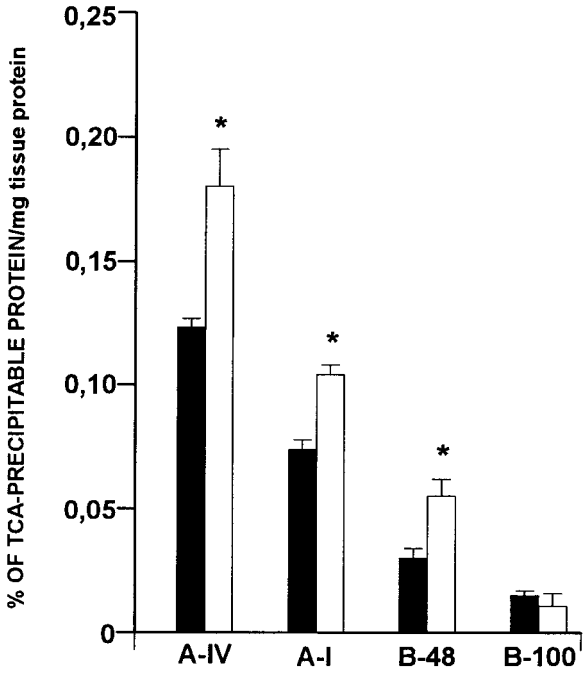

Fig. 5. Effect of hydrocortisone (50 ng/ml) on A-IV, A-I, B-48, and B-100 apolipoprotein synthesis. Explants originating from the same fetuses were split into two groups. One group was cultured 42 h with hydrocortisone (open column) and the second was utilized as a paired control (solid column). Results are expressed as the mean ± SEM of five experiments with fetuses aged 17 to 20 weeks. *Significantly different from controls ($P < 0.05$).

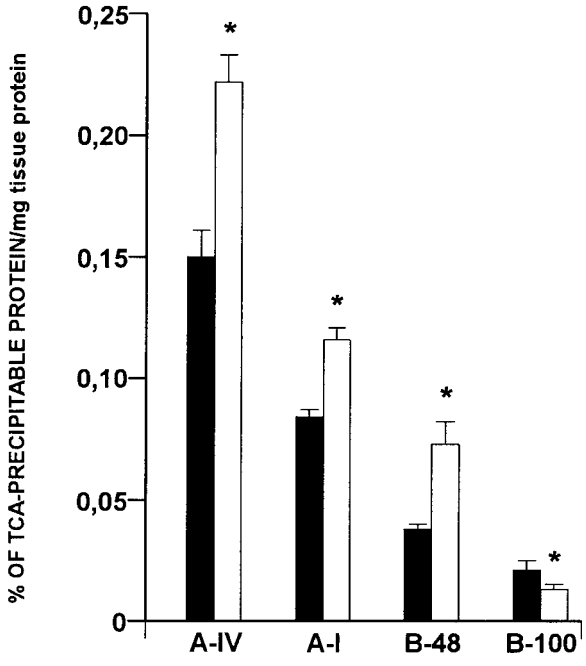

Fig. 6. Effect of epidermal growth factor (100 ng/ml) on A-IV, A-I, B-48, and B-100 apolipoprotein synthesis. Colonic explants were cultured as described in the legend of Figure 5. Results are expressed as the mean ± SEM of five experiments with fetuses aged 17 to 20 weeks. *Significantly different from controls ($P < 0.05$).

are distributed along the entire length of the crypt-villus axis 2) there is a developmental profile in their synthesis, and 3) their production can be regulated by different hormones and growth factors, relevant to the fetal gut environment [Ménard, 1989].

The present investigation reports developmental changes in colonic apolipoprotein synthesis between 15 and 20 weeks of gestation (Fig. 4). Whereas apo A-IV synthesis peaked at 16 weeks, that of apo A-I remained relatively stable throughout the period of study. Apo-B synthesis peaked at 18 weeks of gestation. At the present time, no developmental events occuring at 16–18 weeks that might account for the rise in apo A-IV and apo B synthesis are reported. The appearance or disappearance of colonic villi as possible explanation is excluded since these structures develop between 11 and 14 weeks of gestation along the entire length of the colon and disappear by a still unknown process between 30 weeks of gestation and birth [Ménard et al., 1990]. Furthermore, to our knowledge no changes in systemic or local hormonal or growth factor levels that might correlate these developmental variations have never been reported during this period. Although the physiological meaning of these developmental profiles remains to be elucidated, they may serve as a model for the study of intestinal epithelial cell maturation. The crypt-villus axis represents the functional unit of the absorptive intestinal mucosa. The colonic villi are lined primarily by mature absorptive cells and mucus-secreting goblet cells, and the crypts lined by stem cells, proliferating undifferentiated cells and differentiated secretory cells. The differentiation of each cell type takes place while the cells move up towards the villus tip [Leblond, 1981]. Our data clearly demonstrate a crypt-villus gradient of apolipoprotein expression and they also show that crypt cells may constitute a source of apolipoproteins. The presence of this crypt-villus gradient strongly suggests that the ability of intestinal epithelial cells to synthesize apolipoproteins A-I and A-IV increases with the concomitant migration and differentiation along the villus. This is in accordance with the concept of a crypt-to-villus gradient of functional maturation of enterocytes for some brush

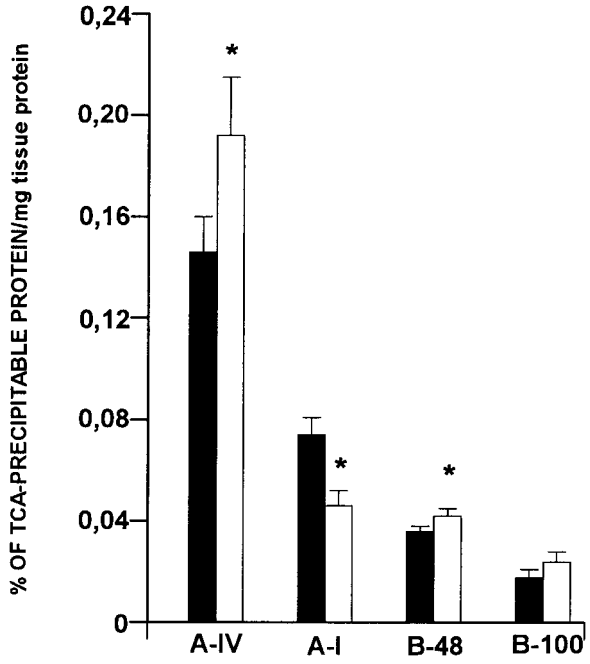

Fig. 7. Effect of insulin (3 mU/ml) on A-IV, A-I, B-48, and B-100 apolipoprotein synthesis. Colonic explants were cultured as described in the legend of Figure 5. Results are expressed as the mean ± SEM of six experiments with fetuses aged 17 to 20 weeks. *Significantly different from controls ($P < 0.05$).

border hydrolytic activities [Ménard et al., 1994]. Other absorptive functions including fatty acid esterification to triacylglycerol mature in parallel, due to a 10-fold greater activity of acyl-CoA synthetase and the 60-fold greater activity of triacylglycerol acyltransferase between crypt and villus cells [Shiau et al., 1980]. It is interesting to note that the gradient of apo A-I and apo A-IV expression reported to occur in the human adult small intestine [Green et al., 1982], is already present in the developing human jejunum and colon.

Using the immunofluorescence technique with the specific 4G3 antibody, apo B-100 expression was found in the crypt and the lower mid-villus region with varying staining intensities in the upper villus cells. However, employing the 2D8 antibody recognizing both apo B-100 and apo B-48, the staining was uniform along the crypt-villus axis. These results establish that both apo B-100 and apo B-48 are also synthesized by the fetal colon. Furthermore, our data also suggest that apo B-100 synthesis is particularly active in crypt and lower mid-villus cells, while apo B-48 by deduction further accumulates in terminally mature cells of the upper mid-villus. These observations parallel the studies conducted on the editing of intestinal apo B mRNA. The editing reaction changes codon 2153 from glutamine (CAA) in the apo B-100 mRNA to an in-frame translational stop codon (UAA) in apo B-48 mRNA [Chen et al., 1987; Powell et al., 1987]. The unedited mRNA encodes apo B-100 (containing 4536 amino acid residues), whereas the edited mRNA encodes apo B-48 (containing 2152 residues). When apo B mRNA editing was examined in Caco-2 cells, its abundance was proportional to the degree of cell differentiation [Boström et al., 1990; Jiao et al., 1990; Teng et al., 1990]. A reasonable speculation is that apo B mRNA editing remains functionally limited in immature crypt cells and in the less differentiated cells of the lower mid-villus region, while its activity is enhanced in the well-differentiated epithelial cells of the upper villus region. Recently, the developmental appearance of APOBEC-1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide #1) was examined in human small intestine [Giannoni et al., 1995]. Their immunocytochemical analysis indicated that all villus-associated enterocytes expressed APOBEC-1 protein.

They also showed a restricted pattern of mRNA distribution predominantly within enterocytes, with little expression in submucosal and adventitial cells [Giannoni et al., 1995]. These observations suggest that mature villus cells are likely to be the major source of APOBEC-1 gene expression in human gut, which support our findings. It is generally accepted that the ability to elaborate apolipoproteins and lipoproteins is restricted to the mature villus enterocytes [Green et al., 1982]. In the same way, it has been long recognized that only mature villus tip enterocytes exhibit full brush border hydrolases and these enzymes have therefore been used as specific markers for studies on enterocyte differentiation. In recent years, this concept has been challenged with the delineation of two classes of brush border markers in the human gut: one specific to differentiated enterocytes, such as maltase-glucoamylase and lactase, and one expressed both in undifferentiated crypt cells and differentiated villus cells, such as sucrase-isomaltase, aminopeptidase A, and dipeptidyl peptidase IV [Ménard et al., 1994]. These markers, expressed in human jejunal crypt cells, are also expressed in colonic crypt cells, suggesting more similarities than anticipated between these two cell populations.

Our observations also clearly establish that crypt cells residing in both the human fetal jejunum and colon, are able to express different apolipoproteins. Whether or not these crypt cells are also able to elaborate lipoprotein species remains to be investigated. Nevertheless, our findings corroborate and extend our previous data [Lévy et al., 1996], unequivocally establishing that the fetal colon is endowed with the capacity for apolipoprotein synthesis. In view of the fact that apolipoproteins provide the structural framework for packaging and transporting lipids, we speculate that the fetal colon represents a functional, albeit temporary extension of the small intestine of absorptive function with respect to lipids. This is in accordance with the efficient uptake and lipid processing by the human fetal colon, we described previously, including the formation and exocytosis of chylomicrons, VLDL, and HDL [Lévy et al., 1996].

The successful maintenance of human fetal gut tissue in defined culture conditions provides a powerful tool to study the modulation of the functional development of the gastrointestinal tract [Ménard et al., 1988; Ménard, 1989]. The use of this organ culture system allowed us to address the nature of the developmental cues that modulate apolipoprotein and lipoprotein synthesis in the developing human gut. We have previously established the implication of hydrocortisone, insulin, and EGF as the modulators of lipid transport and lipoprotein synthesis by the human fetal small intestine [Lévy et al., 1992b, 1994b; Loirdighi et al., 1992, 1997; Thibault et al., 1992]. In the current investigation, we incubated colonic explants with the hormone and growth factor concentrations previously established as influencing lipoprotein synthesis in the small intestine. Hydrocortisone and EGF modulated lipoprotein synthesis in the fetal jejunum [Lévy et al., 1994b; Loirdighi et al., 1997] and colon in a similar fashion (Figs. 5, 6). On the other hand, insulin exhibited opposite effects, increasing apo B-48 and decreasing apo A-I in the colon (Fig. 7). These data at variance with the studies on newborn piglets [Black et al., 1989] illustrate the complexity of the regulatory mechanisms controlling apolipoprotein synthesis in the fore and the hind compartments of the developing gut. Even though both hydrocortisone and EGF increase apolipoprotein synthesis, these actions cannot be interpreted as being the result of nonspecific generalized induction of cell proliferation/differentiation by these additives in organ culture. It is well known that hydrocortisone induces cell proliferation in human fetal jejunum but not in the colon [Ménard et al., 1990]. On the other hand, EGF decreases cell proliferation in both segments [Ménard, 1989; Ménard et al., 1990]. Meanwhile, hydrocortisone exhibits specific induction of lactase and alkaline phosphatase activities in the jejunum without affecting any of the brush border enzymic activities in the colon [Ménard et al., 1994]. However, EGF decreases the synthesis of all brush border enzymes in both segments [Ménard et al., 1988, 1990]. Therefore, hydrocortisone and EGF differentially modulate the cell proliferation, the brush border digestive enzymes as well as apolipoprotein synthesis in the jejunal and colonic epithelial cells. The apo A-IV gene is part of a closely linked, tandemly organized and evolutionarily conserved multigene cluster which also includes apo A-I and C-III [Elshourbagy et al., 1996b; Haddad et al., 1996; Karathanasis, 1985]. In humans, this cluster has been mapped to the long arm of chromosome 11 [Karathanasis, 1988]. The close proximity of these genes has prompted speculation that these proteins may be coordinately regulated [Elshourbagy et al., 1996b; Haddad et al., 1996]. However, the analysis of the methylation patterns of the A-I/C-III/A-IV gene cluster suggests that the three genes despite their close physical association, are independently regulated [Shemer et al., 1991]. In addition several observations in rats indicate no effect of administered lipid on the expression of intestinal apo A-I while apo A-IV is clearly induced by this treatment [Davidson et al., 1985; Hayashi et al., 1990; Steinmetz et al., 1987]. Our data showing differences in the developmental profile and hormonal modulation of apo A-I and A-IV support a separate regulation for the two apolipoproteins. Studies in animals [Black et al., 1992; Elshourbagy et al., 1985a; Shemer et al., 1991] and our own experiments in humans document alteration in apo A-IV expression or synthesis during development.

The molecular mechanism for these changes in apo A-IV are not well defined. Interestingly, a site in the proximal promoter region in the 5′ flanking sequence of the apo A-IV gene has been shown to bind HNF-4, v-erbA-related receptor 3 (EAR-3), and apo A-I regulatory protein (ARP-1) [Ktistaki et al., 1984]. All these

factors appear to influence the transcription of apo A-I, apo A-IV, and apo C-III [Ladias et al., 1991, 1992]. Although our investigation establishes that hormones and growth factors can specially intervene in the functional development of the human gut, further studies are necessary to define whether these trans-factors play a role in the either hormone-dependent or developmental regulation of intestinal apolipoproteins.

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