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Selective Effects of Hydrocortisone on Intestinal Lipoprotein and Apolipoprotein Synthesis in the Human Fetus

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Abstract Studies employing human fetal intestine have yielded much interesting information on the role of polarized enterocytes in fat absorption and transport. Using the organ culture model, we examined the influence of hydrocortisone on the synthesis and secretion of lipids and lipoproteins. Human jejunal explants were cultured for 5 days at 37°C in serum-free medium containing either [^{14}C]-oleic acid or [^{14}C]-acetate, alone or supplemented with hydrocortisone (25 or 50 ng/ml). The uptake of [^{14}C]-oleic acid was associated with the production of triglycerides, phospholipids, and cholesteryl esters, which were all affected by hydrocortisone. This hormonal agent (50 µg) led to the marked reduction of secreted triglycerides (43%, $P < 0.01$), phospholipids (39%, $P < 0.01$), and cholesteryl esters (36%, $P < 0.05$) without altering the characteristic distribution of tissue and medium lipid classes. Similarly, hydrocortisone significantly ($P < 0.01$) decreased (~60%) the incorporation of [^{14}C]-acetate into secreted free and esterified cholesterol in the medium. With [^{14}C]-oleic acid as a precursor, hydrocortisone significantly diminished the delivery of chylomicrons and very low density lipoproteins to the medium while consistently enhancing the secretion of high density lipoproteins. In parallel, [^{35}S]-methionine pulse-labeling of jejunal explants revealed the concomitant inhibitory effect of hydrocortisone on apo B-100 synthesis and hydrocortisone's stimulatory effect on apo B-48 and apo A-I. These studies suggest that glucocorticoids play a critical role in lipoprotein processing during intestinal development. J. Cell. Biochem. 66:65–76 1997. © 1997 Wiley-Liss, Inc.

Key words: chylomicron; very low density lipoprotein; high density lipoprotein; apoprotein B-100; apoprotein B-48; apoprotein A-I; fat transport; ontogeny

Plasma lipoproteins transport water-insoluble neutral lipids and phospholipids from their formation sites to different organs for storage or utilization [Davidson and Magun, 1993; Tso and Balint, 1986; Levy, 1992]. The intestine is a major source for the biosynthesis of plasma-destined lipoproteins. Therefore, inherited disorders in their intracellular elaboration, owing for example to a simple structural modification or to a defect in apolipoprotein B, result in fat malabsorption and profound alterations in their plasma composition and concentration [Levy et al., 1987a,b; Glickman et al., 1979; Levy, 1996].

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There is now ample evidence, especially from animal species, that the intestine is able to produce different types of lipoproteins. Chylomicrons (CM) are essentially formed postprandially, while very low density lipoproteins (VLDL) are found mostly in the fasting state to carry endogenous lipids [Levy and Roy, 1989; Risser et al., 1985]. The third class of lipoproteins synthesized by the enterocyte is the high density lipoprotein (HDL), which is the smallest and the densest lipoprotein particle [Mehran et al., 1992; Green et al., 1978; Magun et al., 1985].

Whereas a substantial body of literature covers gastrointestinal development, including morphology and various digestive functions [Ménard, 1989; Koldovsky, 1981; Henning, 1981], limited information is available on the

ontogeny of intestinal lipid transport. However, fetuses as well as premature and newborn children are faced with considerable nutritional requirements for growth and development [Levy, 1992]. In particular, dietary lipids serve as a vital source of energy and as an essential component of cell membranes. Our meager knowledge in this field is probably due to the absence of an appropriate human model. The Caco-2 cell line, which derives from a human colonic adenocarcinoma, has been very useful in investigating intestinal lipid transport and lipoprotein metabolism [Levy et al., 1996]. However, this model cannot obviously offer a system for exploring lipoprotein processing as a function of development. Recently, employing the organ culture technique, we have been able to demonstrate the high capacity of the human fetal intestine to elaborate lipoprotein fractions for the transport of newly synthesized lipids [Levy et al., 1992]. The lipoprotein fractions present in the culture medium correspond to their plasma counterparts CM, VLDL, and HDL. Our observations also stressed the progressive increase in certain classes of these lipoprotein fractions and the ontogenic changes in their lipid moieties [Thibault et al., 1992]. Furthermore, our data highlighted, for the first time, the implication of epidermal growth factor [Levy et al., 1992, 1994] and insulin [Loirdighi, 1992] in the modulation of the intracellular events governing the assembly and delivery of lipoproteins in normal human intestine.

Other important hormones that might be involved in the control of the packaging and transport of dietary lipids, at the stage of gestation, are glucocorticoids. Experiments conducted in vivo in the rat have suggested that glucocorticoid administration stimulated the hepatic production of plasma VLDL and impaired the removal of plasma triglyceride-rich lipoproteins [Reaven et al., 1974; Bagdade et al., 1976; Cole et al., 1982]. Since these results might be related rather to the insulin resistance and hyperinsulinemia produced by glucocorticoids, additional studies were thus carried out in perfused liver [Cole et al., 1982] and isolated hepatocytes [Duerden et al., 1989] and showed a stimulation of VLDL secretion by glucocorticoids. Moreover, it is well documented that glucocorticoids regulate important aspects of enterocyte differentiation and maturation [Ménard, 1989; Koldovsky, 1981; Henning, 1981; Ménard and Carvert, 1990]. However, the role of glucocorticoids in the regulation of the intestinal de novo synthesis of long-chain fatty acids, esterification into different lipid forms, and transport by lipoprotein carriers remains elusive. The present studies were therefore undertaken primarily to shed light on this subject. The work was performed in intestinal organ culture, since this system has been well characterized in terms of the metabolic viability of the tissue and its hormonal responsiveness regarding lipid and lipoprotein formation.

MATERIALS AND METHODS

Specimens

Tissues from 25 fetuses ranging from 17–20 weeks in age were obtained from normal elective pregnancy terminations. No tissue was collected from cases associated with known fetal abnormality or fetal death. Studies were approved by the institutional Human Subject Review Board. The entire small intestine was immersed in Leibovitz L-15 medium (room temperature) containing garamycin (40 μg/ml) and brought immediately to the culture room. The proximal half of the intestine excluding the first 3 cm was used and defined as jejunum.

Organ Culture

The jejunum was cleansed of mesentery, split longitudinally, washed in culture medium, and cut into explants (3×7 mm). An average of 10–15 explants was 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. Leibovitz L-15 medium (0.8 ml) was added to the central well of each culture dish, a volume sufficient to dampen the tissue without immersing it [Ménard and Arsenault, 1985]. Explants were cultured in serum-free Leibovitz L-15 medium according to the technique described previously [Levy et al., 1992; Ménard and Arsenault, 1985; Ménard et al., 1988]. After an initial 3 h stabilization period, the medium was changed with a fresh one, containing a final amount of either 1.3 μmol/ml of nonlabeled oleic acid with 0.3 μCi of [^{14}C]-oleic acid (sp. act. 53.9 mCi/mmol) (Amersham, Montreal, Canada) attached to albumin or [^{14}C]-acetate (sp. act. 50–62 mCi/mmol) (Amersham). Oleate/albumin complex was prepared according to the method of Fielding et al. [1979]. Hydrocortisone (Collaborative Research, Waltham, MA), dissolved in redistilled water, was added at concentrations of 25 and 50 ng/ml. The medium was replaced every day with a

fresh one containing the same concentrations of hydrocortisone. Explants originating from the same fetuses were split into two groups: half incubated with hydrocortisone and half used as a paired control. Lipid analyses were performed on the tissue and media. The general morphology of fetal jejunal explants was examined at the onset and after 5 days of culture, as previously described [Ménard and Arsenault, 1985]. The mucosal epithelium was always found intact, and no damaged cells were present.

Lipid Carrier

Blood was drawn 3 h after the oral intake of 50 g of fat per 1.72 m^2 (flavored commercial cream), and postprandial plasma was prepared in order to serve as a carrier for the lipoproteins synthesized by the organ culture, as described previously [Levy et al., 1992, 1994; Thibault et al., 1992; Loirdighi et al., 1992]. The CM-enriched plasma was obtained after an oral fat loading [Levy et al., 1992, 1994; Thibault et al., 1992; Loirdighi et al., 1992] and was incubated at 56°C for 1 h to inactivate enzymatic activity.

Isolation of Lipoproteins

After incubation, the explants were homogenized in isotonic saline containing antibacterial and antiprotease agents (sodium azide 0.01%, EDTA 0.1%, and Trasylol 10.000 lk/ml). The medium was mixed with the plasma lipid carrier (2:1, v/v), and the lipoproteins were isolated by sequential ultracentrifugation using a TL-100 ultracentrifuge (Beckman Instruments Inc., Montreal, Quebec, Canada), as described previously [Levy et al., 1992]. Briefly, after the removal of 0.97 g/ml density fraction by short centrifugation in a TLS 55 rotor (20,000 rpm for 20 min), lipoproteins of $d < 1.006$ g/ml and $d < 1.063$ g/ml were separated by spinning at 100,000*g* for 2.26 h with TL 100.3 at 5°C. The high density fraction was obtained by adjusting the 1.063 g/ml infranatant to density 1.21 g/ml and by centrifuging for 6.5 h at 100,000 rpm. Each lipoprotein fraction was exhaustively dialyzed against 0.15 M NaCl, 0.001 M EDTA, pH 7.0, at 4°C for 24 h.

De Novo Apolipoprotein Synthesis: Pulse-Labeling of Intestinal Explants and Immunoprecipitation Procedure

Following the 5 day incubation period in the presence or absence of hydrocortisone and with nonlabeled oleic acid to stimulate the synthesis of lipids and apoproteins, jejunal explants were washed twice with methionine-free Leibovitz medium. They were then incubated in the same medium, containing unlabeled oleic acid, for 45 min in the presence of [^{35}S]-methionine (300 μCi/ml sp. act. 1,062 Ci/mmol) with or without hydrocortisone [Levy et al., 1987a, 1994]. 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 (1 mM), and benzemidine (1 mM). Aliquots of tissue homogenates were treated with 20% trichloroacetic acid (TCA), and the protein precipitates were washed three times with 5% TCA before the radioactivity was determined in a Beckman liquid scintillation spectrometer. An aliquot of the homogenate was also centrifuged (4°C) at 105,000*g* for 60 min in a 50-Ti rotor (Beckman, CA), and the supernatant was subsequently reacted with excess specific monoclonal antibodies for 18 h at 4°C. The immunoprecipitation of apo A-I and apo B was carried out with polyclonal antibodies obtained commercially (Boehringer, Quebec, Canada). Pansorbin (Calbiochem, San Diego, CA) was then added, and the mixture was reincubated at 20°C for 60 min. The immunoprecipitate was washed extensively and analyzed by a linear 4–20% acrylamide gradient preceded by a 3% stacking gel, as described previously [Levy et al., 1987a, 1990, 1994]. Gels were sectioned into 4 mm slices and counted after an overnight incubation at 20°C with 1 ml of BTS-450 (Beckman) and 10 ml of liquid scintillation fluid (Ready solv.; Beckman).

Analyses

Aliquots of explant homogenates and their respective incubation media were lipid-extracted with chloroform-methanol 2:1 (v/v) [Folch et al., 1957]. Small amounts of lipid standards were added to the samples before separation of individual lipid classes by one-dimensional silica gel thin layer chromatography (TLC) (Eastman Kodak, Rochester, NY), as described previously [Levy et al., 1992]. The nonpolar solvent system was hexane-diethylether–glacial acetic acid 80:20:3 (v/v/v), and the polar solvent was chloroform-methanol-water–acetic acid 65:25:4:1 (v/v/v/v). The radio-

activity of the separated fractions was measured in a Beckman liquid scintillation spectrometer. Quenching was corrected using computerized curves generated with external standards. An aliquot of the tissue homogenate was used for protein determinations [Lowry et al., 1951].

Statistics

Statistical evaluation of the results was performed by Student's *t*-test for paired data. Values are expressed as means ± SEM.

RESULTS

Morphological Studies

The general morphology of human fetal jejunal explants, both at light and electron microscopic levels, was examined at the onset and after 5 days of culture (results not shown). In the two conditions, jejunal mucosa exhibited well-developed villi and forming crypts. At the ultrastructural level, the absorptive cells showed a well-developed brush border. The mucosal epithelium was always intact, and no damaged cells were present. The addition of different concentrations of hydrocortisone did not modify the overall morphology, in accordance with previous studies [Arsenault and Ménard, 1985].

Measurement of Lipid Synthesis With [^{14}C]-oleic Acid as a Precursor: Modulation by Hydrocortisone

The incorporation of labeled oleic acid was used to estimate the synthetic rate of lipid classes in intestinal organ culture (Fig. 1). Jejunal explants, incubated for 5 days, incorporated [^{14}C]-oleic acid mainly into phospholid (PL) and triglyceride (TG), with lesser amounts incorporated into diglyceride (DG) and cholesteryl ester (CE). However, PL was the predominant glycerolipid in the tissue, while TG represented the major glyceride form in the medium. The amount of [^{14}C]-oleic acid incorporated into medium triglycerides was greater than its counterpart in the tissue, suggesting efficient TG transport.

In order to elucidate the role of hydrocortisone in lipid esterification and delivery, jejunal explants were cultured for 5 days with [^{14}C]-oleic acid in the presence of two concentrations of hydrocortisone. As shown in Figure 2, this hormonal factor reduced the incorporation of

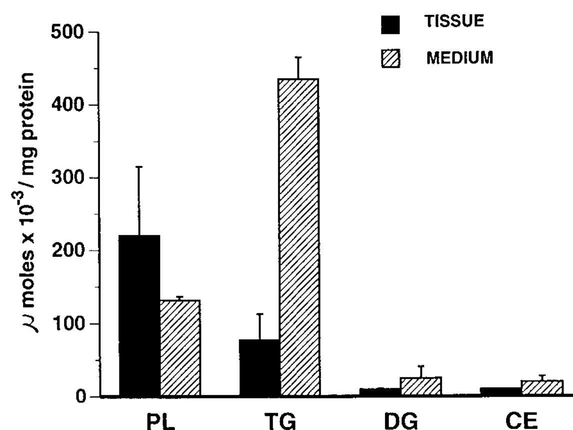

Fig. 1. Tissue accumulation and release of newly synthesized lipids in intestinal organ culture. Jejunal explants were incubated with [^{14}C]-oleic acid substrate for 5 days. Lipids of tissue homogenate and media were then extracted with chloroform-methanol (2:1), isolated by TLC, and quantitated as described in Materials and Methods. Results are expressed as micromoles per milligram of tissue protein. Values represent means ± SEM of five experiments. CE, cholesteryl ester; DG, diglyceride; PL, phospholipid; TG, triglyceride.

the label into total lipids in general below control values, and into TG, CE, and PL in particular. Although the tissue (Fig. 2A) and medium (Fig. 2B) clearly displayed the inhibitory effect of hydrocortisone, the secretion process seems more affected.

Little influence of hydrocortisone on the lipid (Table I) and phospholipid (Table II) composition was noted with oleic acid as a substrate. Moreover, the effect of hydrocortisone at shorter periods of incubation was less marked than that at longer periods (results not shown).

Measurement of Cholesterol Synthesis With [^{14}C]-Acetate as a Precursor: Effect of Hydrocortisone

The ability of fetal intestinal tissue to synthesize and secrete cholesterol forms from [^{14}C]-acetate is documented in Figure 3. An active incorporation of [^{14}C]-acetate was detected mainly in free cholesterol (FC). In contrast, only limited radioactivity was recovered in cholesteryl esters (CE). Although this pattern of distribution was valid for the tissue and medium, the medium/tissue radioactivity ratio, an index of secretion, shows values greater than 1.0 for FC (1.21), which indicated that FC was preferentially exported to the medium. On the other hand, the low ratio for CE (0.70) suggested a decreased export to the medium.

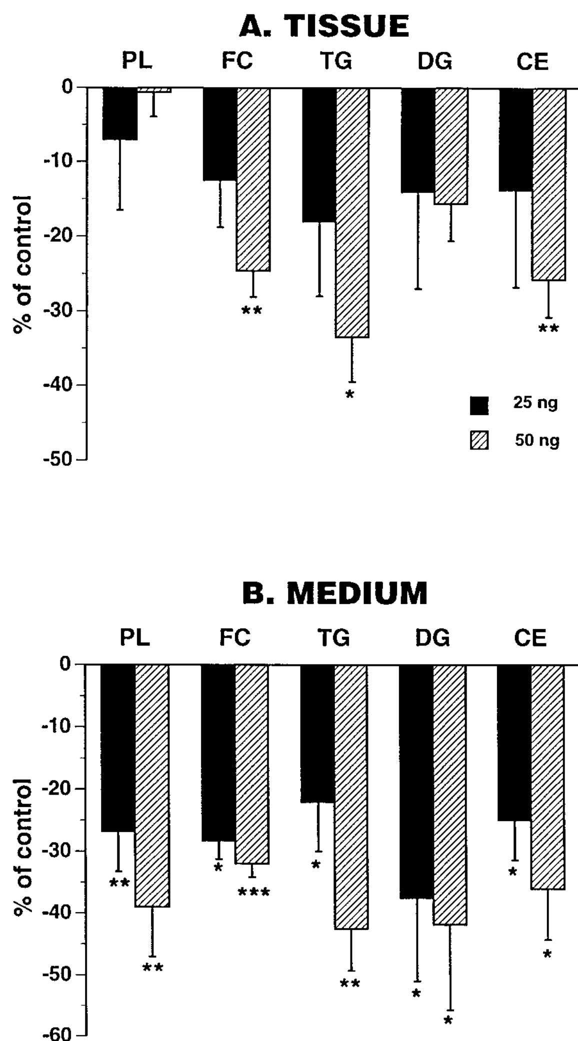

Fig. 2. Effects of hydrocortisone on lipid synthesis (**A**) and secretion (**B**) with [^{14}C]-oleic acid as precursor. Jejunal explants were cultured as described in the legend of Fig. 1. Hydrocortisone (HC) was added to the medium at 25 (hatched bar) and 50 (solid bar) ng/ml. Data are expressed as a percentage of decrease relative to their own control values in five experiments. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

In order to gain insight into these aspects, jejunal explants were incubated with [^{14}C]-acetate in the presence of two concentrations of hydrocortisone, 25 and 50 ng/ml. Addition of this hormone provoked a significant reduction in the production of labeled cholesterol either in the tissue (Fig. 4A) or medium (Fig. 4B). The inhibition was somewhat more marked at the higher hydrocortisone concentration regarding FC and CE. Furthermore, the incorporation of labeled substrate into cell and medium lipids in the presence of hydrocortisone was examined at shorter times (results not shown). Although a similar trend of decrease was noticed, the effect of hydrocortisone was less pronounced than that observed at longer periods.

Lipoprotein Formation and Secretion

Experiments were also conducted to examine the role of hydrocortisone in the transport of newly synthesized lipids by lipoproteins (Fig. 5). As expected from the lipid changes mentioned above, alterations in lipoprotein exocytosis were fully expressed in the presence of hydrocortisone. The secretion of CM and VLDL decreased by 20 and 35%, respectively, in response to hydrocortisone, whereas the release of HDL fractions was enhanced by 23% compared with control explants. One can conclude that hydrocortisone exerted a selective effect on lipoprotein classes. No significant modifications of the lipoprotein-lipid composition were noted with the addition of hydrocortisone to culture medium (results not shown), suggesting that the number of lipoprotein particles, rather than size, was altered by hydrocortisone.

Apoprotein Biogenesis

To address the effect of hydrocortisone on the synthesis of apoproteins, we incubated jejunal explants for 5 days with nonlabeled oleic acid and hydrocortisone. Thereafter, they were pulsed with [^{35}S]-methionine for 45 min. Apoproteins were immunoprecitated and separated by SDS–polyacrylamide gel electrophoresis, and their radioactivities were determined. Figure 6A shows a representative gel scan, which reveals the ability of jejunal explants to produce apo A-I as the predominant apolipoprotein, followed in decreasing order by apo B-48 and apo B-100. In six individual experiments, hydrocortisone diminished apo B-100 synthesis by approximately 30% and enhanced apo B-48 biosynthesis by 50% (Fig. 6B). In agreement with our data on HDL, the incorporation of [^{35}S]-methionine into intestinal apo A-I was raised by hydrocortisone treatment. These data point to the specificity of the response of individual apoproteins to hydrocortisone.

Distinct Effect of Hydrocortisone on a Small Number of Fetal Intestines

As shown before, jejunal explants from most fetuses (n = 23, 88%) efficiently responded to hydrocortisone by reducing the synthesis of lip-

TABLE I. Effect of Hydrocortisone on the Lipid Composition of Jejunal Explants Incubated With [^{14}C]-Oleic Acid*

Samples	Hydrocortisone (ng/ml)	% distribution PL	FC	DG	TG	CE
Tissue (n = 9)	0	59.8 ± 3.8	1.7 ± 0.2	2.5 ± 0.4	26.9 ± 3.7	2.6 ± 0.2
	25	66.0 ± 2.8	1.8 ± 0.1	2.3 ± 0.2	23.5 ± 2.2	2.4 ± 0.2
	50	67.7 ± 2.6	1.7 ± 0.1	2.3 ± 0.2	21.9 ± 2.1	2.4 ± 0.2
Medium (n = 9)	0	27.7 ± 1.8	2.1 ± 0.5	2.2 ± 0.4	58.8 ± 1.1	2.4 ± 0.1
	25	26.5 ± 2.1	1.4 ± 0.3	2.1 ± 0.4	60.1 ± 1.6	2.3 ± 0.1
	50	28.7 ± 1.6	1.6 ± 0.4	2.3 ± 0.4	58.4 ± 1.4	2.5 ± 0.2

*Jejunal explants were incubated with [^{14}C]-oleic acid and treated in similar conditions as those described in the legend of Figure 1. Values are mean ± SEM for nine experiments.

TABLE II. Effect of Hydrocortisone on Phospholipid Composition of Jejunal Explants Incubated With [^{14}C]-Oleic Acid*

Samples	Hydrocortisone (ng/ml)	% distribution Sphingomyelin	Phosphatidyl-choline	Phosphatidyl-serine	Phosphatidyl-inositol	Phosphatidyl-ethanolamine
Tissue (n = 9)	0	4.6 ± 0.2	63.8 ± 1.2	7.3 ± 0.8	10.1 ± 3.7	14.2 ± 2.7
	25	4.0 ± 0.3	63.2 ± 0.9	6.9 ± 0.2	7.3 ± 0.6	18.6 ± 0.3
	50	4.7 ± 0.4	62.0 ± 0.9	5.5 ± 1.4	7.0 ± 0.3	20.8 ± 1.5
Medium (n = 9)	0	8.5 ± 2.0	65.8 ± 6.0	5.9 ± 1.1	9.5 ± 3.1	14.2 ± 2.2
	25	9.0 ± 1.4	67.3 ± 3.4	5.3 ± 0.7	7.0 ± 1.2	14.5 ± 0.8
	50	7.6 ± 1.2	71.4 ± 4.2	6.0 ± 0.6	9.3 ± 0.6	14.4 ± 2.6

*Jejunal explants were incubated with [^{14}C]-oleic acid and treated in similar conditions as those described in the legend of Figure 1. Values are mean ± SEM for nine experiments.

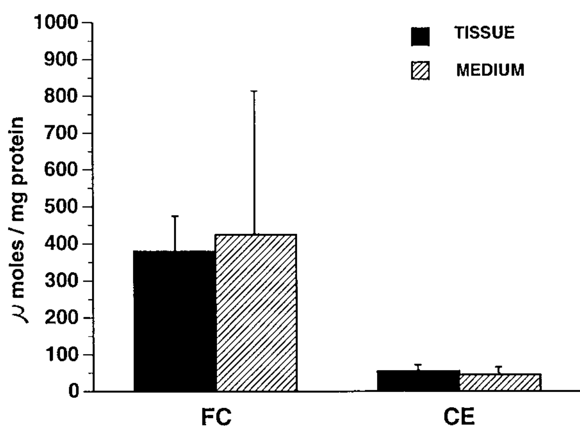

Fig. 3. Newly synthesized and secreted lipids with [^{14}C]-acetate as a precursor. Samples of tissue and medium were analyzed essentially as described in Figure 1. Values are means ± SEM of nine experiments.

ids, CM, VLDL, and apo B-100. A concomitant rise of HDL and apo A-I resulted from the addition of hydrocortisone. In contrast, explants from few fetuses (n = 3, 12%) exhibited opposite metabolic behavior to hydrocortisone, which was characterized by enhanced secretion of lipids, CM, and HDL and unchanged VLDL output (Table III). These modifications were accompanied by an equal elevation of de novo apo B synthesis and a fall of apo A-I biogenesis. The present data clearly demonstrate hypo- and hyperresponsiveness of fetal intestines to hydrocortisone under strictly controlled conditions.

DISCUSSION

The intestine plays a central role in the metabolism of lipoproteins [Davidson and Magun, 1993; Tso and Balint, 1986; Levy, 1992]. Recently, we reported on the optimum conditions for the study of dietary fat transport during development [Thibault et al., 1992]. Thus, our experiments provided strong evidence that human fetal intestine is a reliable and useful model to study the synthesis and secretion of lipoproteins [Levy et al., 1992, 1994]. Physiologic regulation was examined, and our findings established the implication of EGF and insulin in the modulation of the intracellular events guiding the assembly and release of lipoproteins during intestinal ontogeny in humans [Levy et al., 1992, 1994, 1996]. The pres-

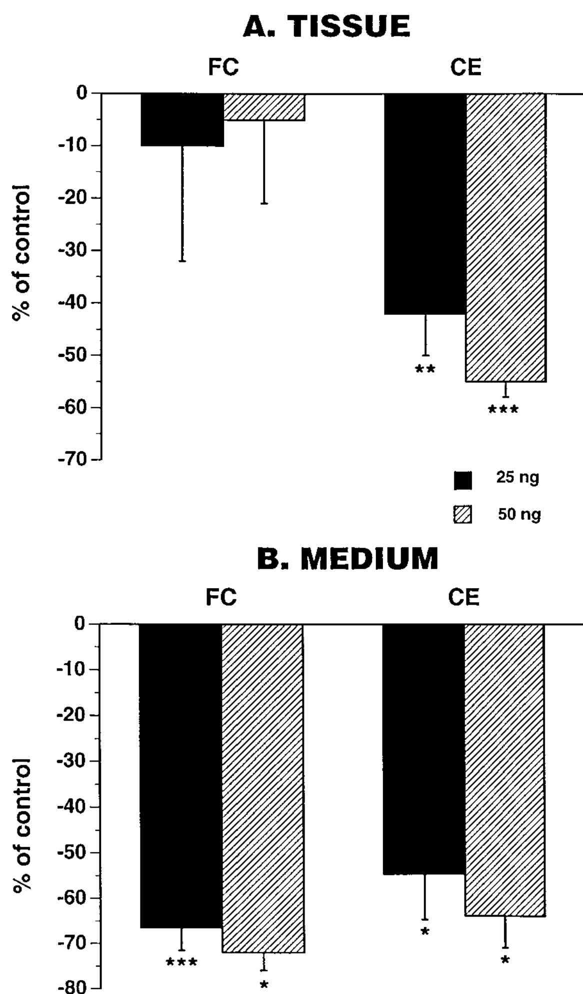

Fig. 4. [^{14}C]-acetate incorporation into tissue (**A**) and medium (**B**) during 5 days of culture supplemented with hydrocortisone. Data are expressed as a percentage of decrease relative to their own control value in five experiments. *$P < 0.02$. **$P < 0.01$. ***$P < 0.001$.

ent investigation demonstrates, for the first time, several aspects of the action of glucocorticoid on intestinal fat transport, including an inhibitory effect on the synthesis of various lipid classes, TG-rich lipoproteins, and apo B-100 as well as a stimulatory influence on HDL, apo A-I, and apo B-48.

To study the potential regulatory role of hydrocortisone, we chose an incubation period of 5 days. Based on previously reported studies, this period was most suitable for optimal response to glucocorticoids [Arsenault and Ménard, 1985; Ménard et al., 1990]. Furthermore, our own experiments, which tested shorter times (data not shown), resulted in modest effects of hydrocortisone and clearly supported the selection of 5 day incubation as a more favorable condition.

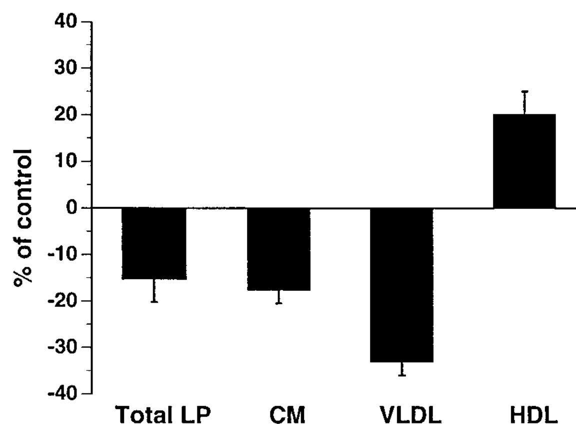

Fig. 5. Effect of hydrocortisone on lipoprotein synthesis and secretion. Intestinal explants were cultured in the presence of [^{14}C]-oleic acid. After 5 days of incubation, the media of two to three experiments were pooled and lipoproteins (LP) isolated by ultracentrifugation, as described in Materials and Methods. Values are means of three experiments and are expressed as a percentage change relative to their own control values ($P < 0.05$).

It is also noteworthy that after 5 days in culture the epithelial cells retained their morphological characteristics, and the addition of hydrocortisone did not affect the morphology of the intestinal explants. Studies were carried out in order to determine whether a remodeling of cell and even medium lipids occurs over this time period (results not shown). These experiments could not document marked hydrolysis or substantial reuptake of secreted lipoproteins. Hydrocortisone was unable to affect these processes over this time period. Furthermore, it should be noted that the intestines from the same fetuses were used for hydrocortisone experiments and their paired controls.

In our experiments, the effects of hydrocortisone on lipids, apolipoproteins, and lipoproteins were specific, since hydrocortisone 1) decreased chylomicron and VLDL delivery while enhancing the secretion of HDL, 2) diminished the biogenesis of apo B-100, whereas it raised that of apo B-48 and apo A-I, and 3) it reduced the overall synthesis of proteins (results not shown). The concentration of hydrocortisone was chosen because the range 25–50 ng/ml was optimal for other physiologically relevant responses [Ménard et al., 1990; Villa et al., 1992]. Furthermore, the hydrocortisone levels used were representative of gestational periods [Ménard, 1989]. We therefore consider that our reported effects are physiological rather than pharmacological.

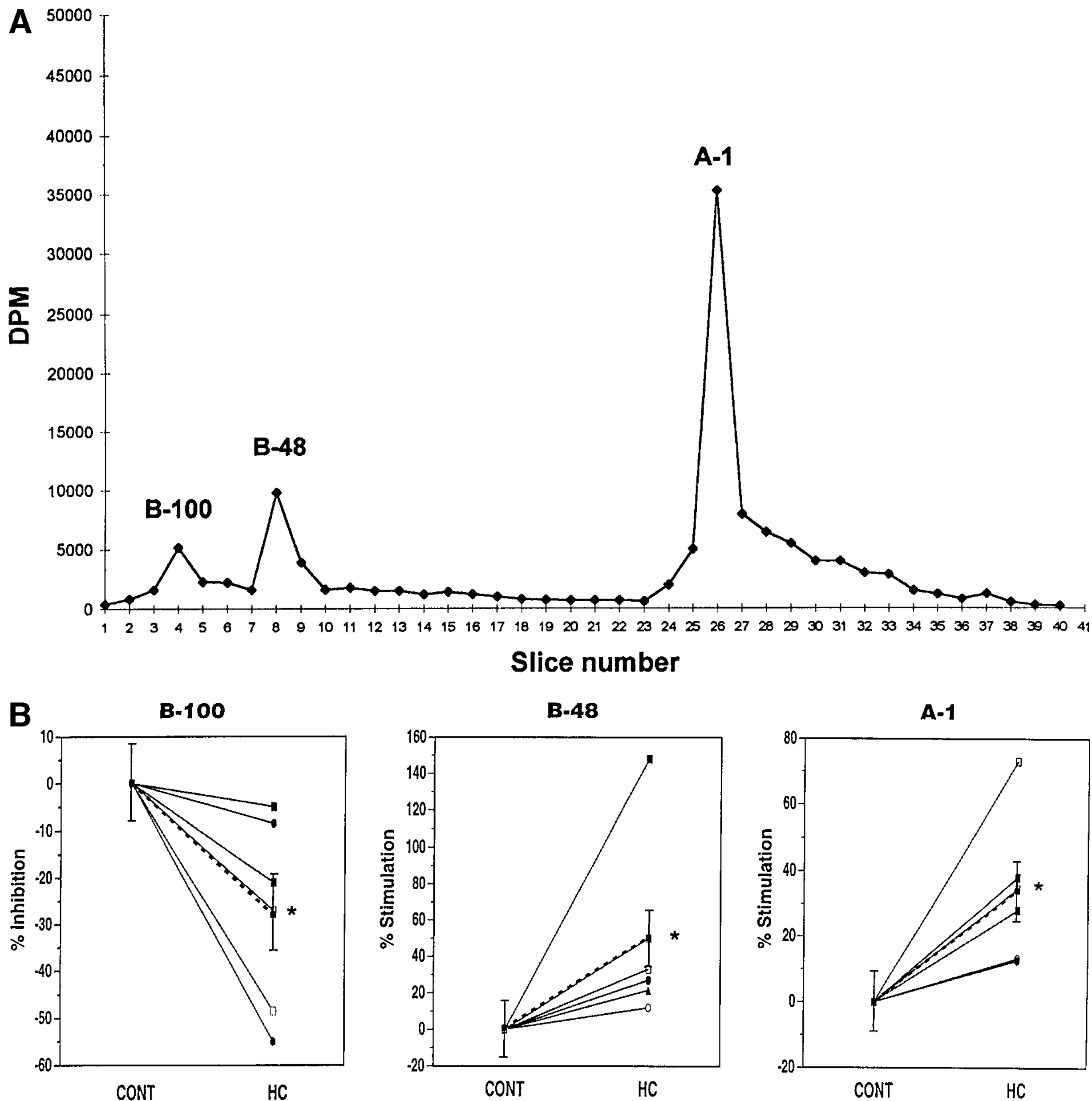

Fig. 6. [^{35}S]-methionine incorporation into immunoprecipitable apolipoprotein B-100, B-48, and A-I. The incubation of individual explants with hydrocortisone (50 ng/ml) and their own controls was performed as described in Materials and Methods and is presented in **B**. Apolipoprotein synthesis is expressed as a percentage of total protein synthesis. The individual experiments are represented (solid lines). The dotted line represents the mean ± SEM. Data from a representative experiment are illustrated in **A**. *$P < 0.05$.

To investigate the modulation of the formation and release of newly synthesized lipids by hydrocortisone, we incubated jejunal explants with two different lipid precursors. Our results indicate that hydrocortisone was potent in decreasing the production of newly synthesized lipids and phospholipids with [^{14}C]-oleic acid. Furthermore, similar results were obtained with [^{14}C]-acetate, which represents a more appropriate substrate for cholesterol synthesis. It is noteworthy that only limited amounts of cholesteryl ester were elaborated by jejunal explants, suggesting a minor activity of acyl-CoA: cholesterol acyltransferase, responsible for cholesterol esterification.

One of the most striking novel findings from this work is the relatively large decrease in the secretion of lipids, particularly transported by

TABLE III. Fetal Intestines With Opposite Response to Hydrocortisone*

% change relative to control values								
Medium lipids			Medium lipoproteins			Tissue apolipoproteins		
PL	TG	CE	CM	VLDL	HDL	B-100	B-48	A-I
↑ 36.5 ± 8.7	↑ 35.3 ± 9.2	↑ 45.2 ± 10.5	↑ 80	—	↑ 36	↑ 42.9 ± 14.2	↑ 70.5 ± 37.3	↓ 22.1 ± 19.7

*The effect of hydrocortisone on the listed parameters was obtained in 12% of the fetal intestines studied. Jejunal explants were cultured as described in Figures 2, 5, and 6.

CM and VLDL, when jejunal explants were exposed to hydrocortisone. The situation described herein for human intestinal explants is not similar to the findings reported for rat hepatic VLDL stimulation in vivo [Reaven et al., 1974; Taskinen et al., 1983]. Among the hypotheses that could explain these conflicting results, one can argue that, in contrast to our intestinal system, the in vivo effects do not prove that glucocorticoids act as key players, since glucocorticoids may produce changes in other hormones or substances, which in turn may be the true effectors. Synergistic stimulation between insulin and glucocorticoids occurs for the synthesis of glycogen [Whitton and Hems, 1976], fatty acids [Minshull and Strong, 1985], and the activity of lipoprotein lipase [Ashby and Robinson, 1980]. Moreover, an alternative hypothesis that may reconcile these discrepancies is the proposition that the in vivo situation reflects a responsiveness of the liver to glucocorticoids, given that dexamethasone, a synthetic glucocorticoid, increased the output of TG and VLDL in perfused liver [Cole et al., 1982] and isolated hepatocytes [Duerden et al., 1989]. Furthermore, those discrepancies that do exist between our work and others' probably relate to the experimental conditions as well as to the species or models used.

Our observations have indicated opposite regulatory patterns for intestinal expression of apo B. Pulse experiments with [^{35}S]-methionine of the explants, cultured in the presence of the hormone, documented an increase of apo B-48 synthesis concomitant with decreased levels of radiolabeled apo B-100. The mechanism involved in this modulatory segregation by hydrocortisone is not clear. One must recall that apo B-48 and apo B-100 are products of the same gene and that apo B-48 is produced from the apo B gene by a novel mechanism involving mRNA editing [Chen et al., 1987; Powel et al., 1987]. A single C → U base change is introduced at nucleotide position 6666 of apo B-100 mRNA, resulting in a change of the codon CAA, encoding glutamine to the stop codon UAA [Chen et al., 1987; Powel et al., 1987]. The translation of this mRNA produces the shorter apo B-48. It is possible that hydrocortisone results in the induction of the stop codon and the stimulation of apo B mRNA editing, thereby generating apo B-48. Previously, apo B mRNA editing was shown to be developmentally regulated in the adult rat liver by nutritional and hormonal factors [Wu et al., 1990; Higuchi et al., 1992; Thorngate et al., 1994; Davidson et al., 1988; Baum et al., 1990; Seishima et al., 1991]. Thyroid hormone treatment in hypothyroid rats [Davidson et al., 1988a,b] and carbohydrate refeeding diet following fasting [Baum et al., 1990] modify the amount of rat hepatic apo B-48 mRNA. It is possible that glucocorticoids play a role in the switch in dominance from apo B-100 to apo B-48 mRNA, which occurs during fetal development. However, the data of Inui et al. [1992] demonstrated that hepatic apo B mRNA editing responded to developmental area in the neonatal rat but appeared to be uninfluenced by dexamethasone administration. One should note that these findings were obtained in the liver of a whole animal administered dexamethasone at the neonatal period.

Several studies have reported the regulation of liver apo B–containing lipoprotein secretion at various levels, such as apo B gene transcription [Baum et al., 1990], apo B mRNA translation [Dashti, 1992], apo B protein translocation across the membrane of the endoplasmic reticulum [Davis et al., 1990], and apo B lipid assembly [Russinol et al., 1993]. Ample evidence has also stressed the role of intracellular degradation in controlling the exocytosis of hepatic apo B [Sparks et al., 1992; Dixon et al., 1991]. Recently, Wang et al. [1995] have shown that pretreatment of hepatocytes with dexamethasone significantly decreased the degradation of apo B, which was compatible with the observed increase in apo B secretion. These workers suggested that dexamethasone may inhibit the protease that degrades apo B. More experiments

are required to clarify 1) whether these mechanisms are operative in the small intestine, 2) how they modify each form of apo B, and 3) if they are compatible with the mechanisms of action for glucocorticoids, which regulate the expression of a subset of steroid-responsive genes by interacting with specific intracellular receptors [Yamamoto, 1985].

Apo A-I, the major apolipoprotein of HDL, is synthesized in the intestine and liver and is crucial to lipoprotein metabolism. In the current investigation, cultured explants were used to study the effects of hydrocortisone on the production of apo A-I. The results suggest that, consistent with the raised HDL secretion, the synthesis of apo A-I is stimulated by hydrocortisone. In a similar fashion, dexamethasone stimulated apo A-I biosynthesis in primary rat hepatocytes incubated with [^{35}S]-methionine [Wang et al., 1995]. In the HepG2 cell line, the regulation of apo A-I synthesis is dependent on the level of the mRNA, which is controlled by an approximately 2.5 kb DNA region located immediately upstream of the TATA box of the gene [Theriault et al., 1992; Chao et al., 1988; Fennewald et al., 1988]. Nuclear proteins that activate transcription have also been isolated from these cells [Fennewald et al., 1988]. However, the relationship between the control of apo A-I synthesis by hydrocortisone and these agents requires much greater definition in the cultured human intestine.

Different responsiveness of fetal intestines to hydrocortisone was identified in our investigation. Two groups of intestines were described: those (88%) with low lipid and TG-rich lipoprotein secretion and those with opposite trends (12%). Currently, the underlying cause for this variability is not understood. Interestingly, large interindividual responses of plasma cholesterol and lipoprotein concentrations within various species consuming a diet of similar composition and cholesterol content were reported [Morrisett et al., 1982; Katan and Beynen, 1987; Safonova et al., 1993]. It was suggested that the difference in plasma between high and low responding humans and animals on a cholesterol-rich diet can be almost completely accounted for by the variability in the rate of absorption of dietary cholesterol [Bhattacharyya and Eggen, 1981]. Evidence exists that much of this variability is under genetic control [McGill et al., 1988].

There is convincing evidence that glucocorticoids have an important role in regulating the development of mammalian small intestinal functions, mainly those characterizing the brush border membrane [Ménard, 1989; Koldovsky, 1981; Henning, 1981; Ménard and Calvert, 1990]. Experimental data have also documented the involvement of glucocorticoid hormones in the regulation of perinatal human intestinal lactase expression at a posttranscriptional level [Ménard et al., 1988; Villa et al., 1992]. Although the mechanism of action of glucocorticoids in modulating lipid and apolipoprotein synthesis at the cellular level remains to be elucidated, the results of the current study stress that hydrocortisone may be pivotal in the lipoprotein processing in the human small intestine during development.

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