Both apolipoproteins B-48 and B-100 are synthesized and secreted by the human intestine

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Abstract Apolipoprotein B (apoB), an apolipoprotein associated with very low density lipoproteins and the atherogenic low density lipoproteins (LDL), directs the metabolism of lipoprotein particles in plasma by interacting with the LDL receptor. Utilizing human intestinal biopsy organ cultures, we have studied the synthesis of intestinal apoB in man. Intestinal organ cultures from normal adults (n = 6) were incubated in the presence of protease inhibitors in media supplemented with [^{35}S]methionine. Media from these cultures were evaluated by sequential $NaDodSO_4$ polyacrylamide gel electrophoresis, radioautography, and Western blot analyses, and intestinal biopsies were studied using immunohistochemistry. The relative abundance of apoB-100 and apoB-48 mRNA was assessed using reverse transcriptase-polymerase chain reaction followed by primer extension. Although apoB-48 was the principal isoprotein that was newly synthesized by intestinal organ cultures, apoB-100 was also synthesized and secreted by human intestinal organ cultures with 16 ± 3% of the intestinal apoB mRNA coding for apoB-100. These results establish that apoB-100 is produced by the human intestine. The synthesis of the atherogenic apoB-100 by the intestine has pathophysiologic implications for the development of diet-induced atherosclerosis. —**Hoeg, J. M., D. D. Sviridov, G. E. Tennyson, S. J. Demosky, Jr., M. S. Meng, D. Bojanovski, I. G. Safonova, V. S. Repin, M. B. Kuberger, V. N. Smirnov, K. Higuchi, R. E. Gregg, and H. B. Brewer, Jr.** Both apolipoproteins B-48 and B-100 are synthesized and secreted by the human intestine. *J. Lipid Res.* 1990. **31:** 1761–1769.

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Dietary fats are absorbed by the intestine, incorporated into chylomicrons within enterocytes and then secreted into the circulation (1–3). ApoB is a principal component of intestinally derived lipoproteins. In contrast with the human liver, which secretes predominately the 512 kDa apoB-100 isoprotein (4, 5), a smaller apoB isoprotein is secreted by the intestine in both rats (6) and humans (7). In humans, the lower molecular weight apoB isoprotein has been designated apoB-48, since it was approximately 48% the size on $NaDodSO_4$ gel electrophoresis of the larger apoB-100 isoprotein (7). Thus, it has been proposed that the human intestine is the source of plasma apoB48 and that apoB-48 can serve as a marker for intestinally derived lipoprotein particles (7–9). ApoB-48 has been reported to be synthesized by organ cultures of human intestine (5). The specific apoB isoprotein present on a lipoprotein particle affects the rate of lipoprotein particle removal from plasma (10–12). The site and mechanism of the synthesis of apoB-100 and apoB-48 may have both physiological and pathophysiological implications. ApoB-100 on LDL, the principal apoB isoprotein present in fasting plasma, has been associated with the development of premature atherosclerosis (13). However, postprandial lipoprotein particles have also been proposed to be atherogenic (14). We have evaluated the synthesis of apoB in human intestinal organ cultures. The data from these studies indicate that, in addition to apoB-48, both the mRNA and protein of apoB-100 are synthesized by the human intestine.

Abbreviations: apoB, apolipoprotein B; LDL, low density lipoproteins; VLDL, very low density lipoproteins; HBSS, Hank's balanced salt solution; PBS, phosphate-buffered saline; MEM, minimum essential medium; RT, reverse transcriptase; PCR, polymerase chain reaction; pHuGln, plasmid of human apoB coding for glutamine.

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METHODS

Isolation and incubation of intestinal mucosa for organ cultures

Specimens for organ cultures were obtained by endoscopy in six adults aged 22–25 years. Informed consent was obtained from all of the subjects. After intestinal biopsy, samples were immediately placed into tissue culture wells (Nunc, Denmark) and oriented with the mucosal surface facing up as described by Trier (15) and Sviridov et al. (16). Three biopsies from each subject were initially incubated together in 0.4–0.5 ml of methionine-free Eagle's minimal essential medium containing 500 μCi of L-[^{35}S]methionine (44.33 TBq mmol) (New England Nuclear, Boston, MA). After a 20-min incubation with radioactive medium, the biopsies were washed twice with complete, serum-free Eagle's minimal essential medium. This was followed by a 3-h, 37°C incubation in 0.4 ml of complete serum-free Eagle's minimal essential medium. After the incubation with the unlabeled medium, the medium was harvested, separated from the cellular debris by a 5-min, 4°C microfuge centrifugation, and transferred to a second microfuge tube. The lysis buffer containing 5 mM EDTA, 0.5% Na deoxycholate, 0.1% $NaDodSO_4$, 1% Triton X-100, 1 mM leupeptin, and 1 mM phenylmethylsulfonylfluoride was added to the medium (0.1 ml). An 80-μl aliquot of polyclonal anti-apoB (Boehringer-Mannheim) was then added to the sample and incubated for 18 h at 4°C. The immunocomplexes were then precipitated as outlined below. After the [^{35}S]methionine pulse-chase, the tissue samples were solubilized with 0.5 ml of buffer which contained: 20 mM MOPS (pH 7.2), 20 mM NaH_2PO_4, 5 mM EDTA, 1% Triton X-100, 0.5% Na deoxycholate, and 0.1% $NaDodSO_4$. The biopsies were then vortexed 3–5 sec, incubated at room temperature for 15 min in a rotating shaker, vortexed again for 3–5 sec, and centrifuged for 5 min at 4°C. Anti-apoB antiserum (80 μl) was added to the supernatant and incubated for 18 h at 4°C.

Following immunocomplex formation, 40 μl of a 10% suspension of prewashed Pansorbin (Calbiochem) was added to the media and cell suspensions. After 1-h, room temperature incubation, the samples were centrifuged (Microfuge^R, 4°C), and the immunoprecipitates were washed repeatedly (cells 5 × and media 3 ×) with the solubilization buffer. ApoB was eluted from the Pansorbin complex by heating the precipitates for 5 min at 90°C in 0.1 ml of the following releasing buffer: 3% $NaDodSO_4$, 5 M urea, 90 mM DTT, 150 mM sucrose, and 150 mM Tris-HCl (pH 6.7). Aliquots of the solubilized apoB-antiapoB reaction mixture were then analyzed by $NaDodSO_4$-PAGE as outlined below. Plasma lipoproteins for use as standards (density <1.25 g/ml) were isolated by preparative ultracentrifugation (17).

Protein analyses

The organ culture apoB immunoprecipitates were subjected to slab gel electrophoresis using 0.1% $NaDodSO_4$ and 7.5% polyacrylamide in 0.1 M Tris-glycine (pH 8.3). The samples were electrophoresed within 1.5-mm-thick gels at 250 volts at 4°C and either directly stained with Coomassie Brilliant Blue G-250, or autoradiographed for 4 h and transferred to nitrocellulose paper for immunoblot analyses (4, 8, 18).

Sequential immunoblot analyses were conducted by first probing for the presence of apoB-100 using the monoclonal antibodies ABB-3 and ABB-5 (Radioimmunoassay Incorporated, Scarborough, Ontario), originally termed 4G3 and 5E11, respectively, which are directed toward epitopes of apoB-100 and do not recognize apoB-48 (19). The apoB isoproteins were visualized by immunoperoxidase staining. After the initial immunoblot, ABB-3 and ABB-5 were stripped from the nitrocellulose filter paper by three sequential 2-h washes with 0.1 M glycine-HCl, 20 mM magnesium acetate, and 50 mM KCl (pH 2.2). The filters were then exposed to a polyclonal apoB antibody that recognized both apoB-100 and apoB-48, and the immunoblot was incubated with either ^{125}I-labeled goat anti-rabbit antibody or ^{125}I-labeled protein A. The [^{35}S]methionine-labeled samples were shielded by lucite during the film exposure of the ^{125}I-labeled Western blots to block the radiation of the endogenous label.

Immunocytochemistry

Freshly obtained intestinal mucosal biopsies were immediately frozen in dry ice and stored in liquid nitrogen until further processing. Sections (10 μm thickness) of intestine, pancreas, liver, and kidney were embedded in OCT compound (Miles Laboratories, Elkhart, IN) and processed as outlined previously (20). Slides were incubated overnight at 4°C with both ABB-3 and ABB-5 antibodies, as well as a monoclonal anti-insulin antibody and a monoclonal antibody derived from a neuroblastoma (HSAN 1.2), both of which served as irrelevant monoclonal antibody controls. Sections were then incubated, washed, reincubated with a sheep anti-mouse second antibody, and stained using the alkaline phosphatase-mouse anti-alkaline phosphatase antibody bridge technique (21) as previously described (19) or by utilizing a biotin/avidin system (Vectastain, Vector Laboratories, Burlingame, CA).

Quantitation of apoB mRNA editing

Total RNA isolation. Intestinal biopsies were obtained and either snap-frozen on dry ice or incubated under identical conditions that were outlined for the organ culture studies, except that radiolabeled methionine was not

used. After the 3.5-h, 37°C incubation, the organ cultures were also snap-frozen on dry ice. Samples were then placed in 500 μl of ice-cold 4 M guanidine isothiocyanate and ground to homogeneity (60 sec) using a Teflon pestle. Each sample was then passed 10 times through a 20-gauge needle followed by three 500-μl acid-phenol (phenol saturated with 100 mM sodium acetate, pH 5.2, 1 mM EDTA)–chloroform (1:1, v/v)) extractions (22), and one 500-μl chloroform extraction. Ten μg of yeast tRNA (ytRNA) was added, the samples were brought to 3.8 ml volume with guanidine isothiocyanate, and layered over 1.1 ml 5.7 M CsCl–5 mM EDTA (23). Samples were centrifuged for 20 h at 36,000 rpm in a Beckman SW 50.1 rotor and the resultant pellets were dissolved in 10 μl sterile H_2O.

Reverse transcription–polymerase chain reaction (RT–PCR). A procedure based on Sanger dideoxynucleotide chain termination (24), originally adapted by Zhuang and Weiner (25) and subsequently modified by Driscoll et al. (26) and Davies et al. (27) and our laboratory, was used for apoB cDNA analysis; the remaining 95 μl of PCR mixture was treated with RNAse A (10 μg) for 30 min at 37°C and applied directly to a Sephadex G-50 spun column (Boehringer-Mannheim). Samples were spun at 3500 *g*, ethanol-precipitated, washed with 70% ethanol, and resuspended in 10 μl sterile 10 mM Tris-HCl, 1 mM EDTA. Three microliters of the resultant purified apoB cDNAs were annealed to 0.3 femtomoles of 5'-^{32}P end-labeled 88PE in the presence of 57 mM Tris-HCl, pH 7.5, 29 mM $MgCl_2$, 71 mM NaCl, and H_2O in a final volume of 7 μl. Samples were heated to 95°C for 5 min, 70°C for 10 min, cooled passively to 42°C; 3 μl containing 1.6 U T7 DNA Polymerase (USB), 33 mM DTT, 0.33 mM dA, dC, and dT, and 0.83 mM ddG was added and extension was allowed to proceed for 10 min at 42°C. Reactions were stopped by adding 10 μl of 95% formamide. Samples were electrophoresed through 7 M urea/17.5% polyacrylamide gels bound to GelBond (FMC), and relative amounts of apoB-100 and apoB-48 extension products were quantitated by direct quantitation of dried gels (Betascope™, Betagen).

One μl of each RNA sample was digested simultaneously with both Sau3A I (5 units, New England Biolabs) and DNAse I (1 unit, RQ1 DNAse, Promega) for 1 h at 37°C, followed by acid-phenol–chloroform, then chloroform extraction and ethanol precipitation. Reverse transcription (RT) of nuclease-treated RNA was performed with either first strand synthesis primed by SO176, t'-ATAATAGGATCCATAGCTATTTTCAAATC ATGTA-3', (an antisense oligonucleotide annealing 34 nucleotides downstream from nucleotide 6666 of the apoB mRNA) or with priming by SO175, 5'-TTTATCTGAAT TCATTCAATTGGGAGAGACAAGTT-3', (a sense oligonucleotide located 132 bases upstream from nucleotide 6666). A reagent cocktail approach was used (28) for both RT and PCR (29), with an aliquot of the cocktail mix being added to each nucleic acid sample directly. For RT of set R, 99 μl of a solution containing 10 mM DTT, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.01% gelatin, 40 U RNAsin (Promega), 200 μM dNTP's, 0.5 μg of SO176, and H_2O were added to each nuclease-treated RNA. A similar RT was performed on set W, substituting SO175 as the oligonucleotide. Samples were heated to 70°C for 10 min, cooled passively to 37°C, 40 U of Moloney murine reverse transcriptase was added (Pharmacia), and extension was allowed to proceed to 2 h at 37°C. Samples were heated to 70°C for 15 min, placed on ice, and 2.5 U of Taq DNA polymerase was added, with 0.5 μg of SO175 added to set R and 0.5 μg of SO176 added to set W. The PCR was performed with cycles as described (30).

Southern blot analysis. Five μl of each PCR was removed, 10 μg of RNAse A (Sigma) was added, the samples were incubated at 37°C for 10 min, and electrophoresed through ethidium bromide–2% NuSieve (FMC)/1% agarose gels. The gels were denatured, neutralized, and blotted on Nytran (Schleicher and Schuell) according to the method of Southern (31). After UV-crosslinking with 0.12 J, the filters were air-dried, prehybridized with 5 × SSPE (SSPE = 150 mM NaCl, 10 mM NaH_2PO_4, pH 7.4, 1 mM EDTA), 5 × Denhardt's (1 × = 0.02% Ficoll, 0.02% polyvinylpyrollidone, 0.02% bovine serum albumin), 0.1% $NaDodSO_4$, and 100 μg/ml salmon sperm DNA, and hybridized for 16 h at 42°C with 1 × 10^6 cpm/ml of 5'-^{32}P end-labeled 88PE, 5'-TATCTT TAATATACTGATC-3' (an antisense oligonucleotide hybridizing 5 nucleotides downstream from nucleotide 6666 of the apoB cDNA). Filters were washed three times at room temperature with 2 × SSPE/0.1% $NaDodSO_4$ followed by a stringent wash in 2 × SSPE/0.1% $NaDodSO_4$ at 46°C for 15 min. Washed filters were exposed to XOMat XAR-5 film at room temperature.

RESULTS

The quantities of lipoproteins that are secreted by intestinal organ cultures are quite small. In order to assess the presence of different isoproteins in the same biopsy sample, it was necessary to develop a sequential technique for apoB immunoblotting with antibodies directed toward the apoB isoproteins (**Fig. 1**). The apoB-100 specific antibodies (ABB-3 + ABB-5) recognized only apoB-100 (Fig. 1, lane B). The polyclonal antibody (Fig. 1, lane C) recognized both the apoB-100 and apoB-48 isoproteins. Thus, this method of sequential immunoblotting permitted detection of both apoB-100 and apoB-48.

Using these techniques, studies were then undertaken to analyze the apoB isoproteins secreted by human intestinal organ cultures in vitro. Intestinal biopsy samples

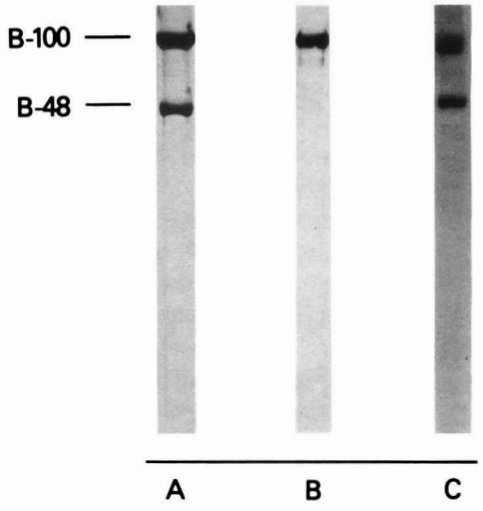

Fig. 1. Identification of apoB-100 and apoB-48 by sequential immunoblot analysis. Lipoprotein standards were separated by $NaDodSO_4$-PAGE and either stained with Coomassie Blue (lane A) or transferred to nitrocellulose paper. The nitrocellulose paper strips were then probed with the ABB-3 and ABB-5 apoB-100-specific monoclonal apoB antibodies (lane B) or a polyclonal antibody that recognizes both apoB-100 and apoB-48 (lane C).

were obtained and the apoB isoproteins in the biopsies and in the medium were assessed (**Fig. 2**). Protease inhibitors and antioxidants were added to the samples in order to prevent extracellular proteolytic cleavage of the secreted apolipoproteins. ApoB-100 was detected by Coomassie Blue protein stain both within the biopsies as well as in the medium (Fig. 2, lanes B and C, respectively). It appeared that apoB-48 was the primary isoprotein within the organ culture and that apoB-100 was the predominant apoB species in the culture medium. Direct radioautography of the radiolabeled ^{35}S-labeled B apolipoproteins established that newly synthesized apoB-100 and apoB-48 were present within the organ cultures (Fig. 2, lane D) as well as in the culture media (Fig. 2, lane E). In contrast to the protein stain, the predominant form of nascent apoB present within the organ culture and the culture media was apoB-48. The apoB monoclonal antibodies that recognize only apoB-100 detected this isoprotein both in the intestinal cell (Fig. 2, lane G) and in the culture medium (Fig. 2, lane H). Immunoblot analysis using the polyclonal anti-apoB antibody detected principally apoB-48 within both the enterocytes (Fig. 2, lane J) and in the medium (Fig. 2, lane K). The difference in

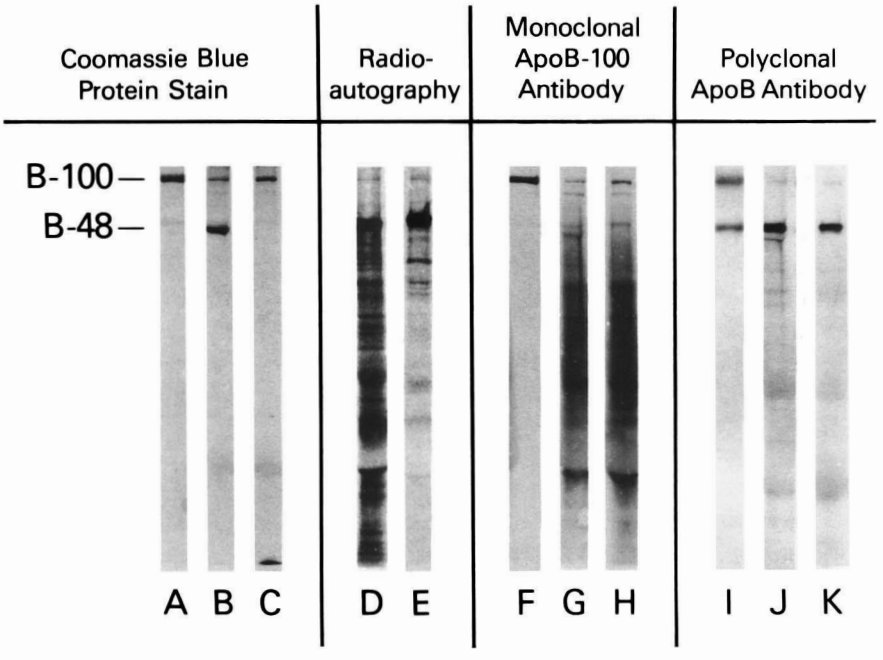

Fig. 2. Analysis of the apoB isoproteins in the cells and media of intestinal organ cultures from adults. Intestinal biopsies from six adults were incubated as organ cultures as outlined in Methods in the presence of 500 μCi [^{35}S]methionine. ApoB within cells or secreted by the intestinal organ cultures was isolated by immunoprecipitation and the same samples were sequentially evaluated by 7.5% $NaDodSO_4$-PAGE and immunoblot analyses. ApoB-100 and apoB-48 standards were analyzed by protein staining (A), apoB-100 specific immunoblot analysis (F), and polyclonal apoB antibody immunoblot analysis (I). Intracellular apoB in the organ cultures was isolated by immunoprecipitation and analyzed by protein staining (B), direct radioautography (D), apoB-100-specific immunoblot analysis (G), and by polyclonal apoB immunoblot analysis (J). Tissue culture media were pooled and the apoB was isolated by immunoprecipitation. Media apoB isoproteins were then evaluated by protein staining (C), direct radioautography (E), apoB-100-specific immunoblot analysis (H), and polyclonal apoB immunoblot analysis (K). Therefore, the blots using monoclonal (G) and polyclonal (J) antibodies were conducted on the gel depicted in D. Similarly, lanes H and K represent the sequential immunoblots performed upon the sample in lane E.

the relative amounts of apoB-100 and apoB-48 that were detected by protein staining (Fig. 2, lanes B and C) and immunoblot analysis (Fig. 2, lanes J and K) was due to more effective transfer of the smaller apoB-48 species during electrotransfer. Therefore, organ cultures synthesize both apoB-48 and apoB-100.

It was of interest that, although newly synthesized ^{35}S-labeled apoB-100 could be detected by radioautography of the Coomassie Blue-stained gel (Fig. 2, lanes D and E), the relative proportion of newly synthesized ^{35}S-labeled apoB-100 to the total medium apoB-100 mass based on protein stain (Fig. 2, lanes B and C) was low. Quantitation of the apoB mass by laser scanning densitometric analysis of direct protein staining from several experiments indicated that extracellular apoB-100 represented from 13% to 40% of the total adult apoB mass. However, quantitation of the autoradiographs demonstrated that only 5% of the total labeled methionine was in the apoB-100 isoprotein. These data indicate that newly synthesized apoB-100 can readily be detected in all intestinal samples, with a large proportion of the aoB-100 in the medium and in the biopsy representing unlabeled apoB-100 released during the time course of the organ culture studies.

Using immunohistochemical techniques, we have demonstrated that apoB-100 was present within the enterocyte (**Fig. 3**). The apoB-100 specific monoclonal antibodies detected apoB-100 in both hepatocytes and enterocytes (Fig. 3, panels B and J). No apoB was observed in the pancreas and kidney (Fig. 3, panel D and H). Insulin staining the pancreatic islet (Fig. 3, panel F) and the lack of staining using the irrelevant HSAN 1.2 antibody (Fig. 3, panels A, C, E, G, and I) represent the positive and negative controls, respectively.

The finding of apoB-100 protein in intestinal organ cultures paralleled the finding of mRNA within human intestinal biopsies that encode apoB-100. Amplification of apoB cDNAs from human intestinal total RNAs was performed with RT-PCR using a cocktail reagent approach. Bands of the predicted size, 234 bp, were seen on ethidium bromide-stained agarose gel electrophoresis of apoB cDNAs amplified from pHuGln, a control plasmid containing apoB-100 cDNA sequence 6378-6594, and from human intestinal RNA amplified by RT-PCR with an antisense primer for first strand synthesis. In contrast, no bands were visible on attempted amplification from either yeast tRNA or intestinal RNA subjected to RT with a sense first strand primer followed by PCR. **Fig. 4** illustrates Southern blot analysis of an agarose gel containing apoB cDNAs amplified from human intestinal RNA using antisense (set R, Materials and Methods) or sense (set W) primers of RT followed by PCR, as well as the PCR product amplified from pHuGln. Positive hybridization signals were present in lanes containing pHuGln and set R amplification products, while no signals were detected in lanes containing yeast tRNA and set W amplification products. Similar results were obtained on Southern blot analysis of all human small intestinal samples reported herein: positive signals were detected on antisense first strand priming followed by PCR, while no signals were observed on attempted sense first strand priming.

Primer extension analysis using three extending deoxynucleotides (dA, dC, and dT) and one terminating dideoxynucleotide (ddG) allowed accurate quantitation of apoB-48:apoB-100 cDNA ratios. **Fig. 5** illustrates primer extension of several apoB cDNAs, with correct extension seen for the pHuGln PCR product, a known apoB-100 cDNA. No extension was visible for the yeast tRNA RT-PCR product, a nonhomologous carrier. Both apoB-100 and apoB-48 sized products were seen on primer extension of human intestinal apoB cDNA prepared from a normal volunteer with apoB-48 predominating (82%). Similar replicate analyses on intestinal biopsies from two other normal volunteers indicated that 84% ± 3% (mean ± SD) of the intestinal apoB mRNA encoded apoB-48 while 16% ± 3% coded for apoB-100. Finally, a time-course study of the effects of media incubation of intestinal biopsies at 37°C showed a similar pattern of apoB-48 (81%) and apoB-100 (19%) mRNA expression after 3.5 h incubation in culture.

DISCUSSION

The metabolism of lipoproteins is directed by the apolipoproteins at the aqueous-lipid interface of the lipoprotein particles. ApoB remains associated with the same lipoprotein particle in the circulation and is the only apolipoprotein that does not undergo exchange between lipoprotein particles (9, 32). Two isoproteins of apoB have been detected in both rats (6, 33-35) and humans (7-9). In humans, the larger of the two isoproteins, apoB-100, is synthesized in the human liver (4, 5). It has been proposed that the smaller apoB-48 isoprotein is a specific marker for intestinally derived lipoprotein particles (7-9).

The human intestine contains mRNA of sufficient size to code for both the apoB-100 as well as apoB-48 isoproteins (36). Using an apoB cDNA probe, Glickman, Rogers, and Glickman (5) demonstrated the presence of an mRNA species compatible with an apoB-48 message in fetal intestine after 16 weeks of age and in adult intestinal biopsies. They also evaluated the synthesis of nascent apoB isoproteins by performing [^{3}H]leucine pulse-chase studies. Analysis of the immunoprecipitates of intracellular apoB by counting the radioactivity present in gel slices revelaed only apoB-48 in samples taken from adults. These studies were conducted by quantifying the radioactivity which co-migrated with apoB-100 and apoB-48 on NaDodSO$_4$/4% PAGE. The conclusions of Glickman et al. (5) are in contrast to the results obtained in our studies. We examined the apoB isoproteins in both the

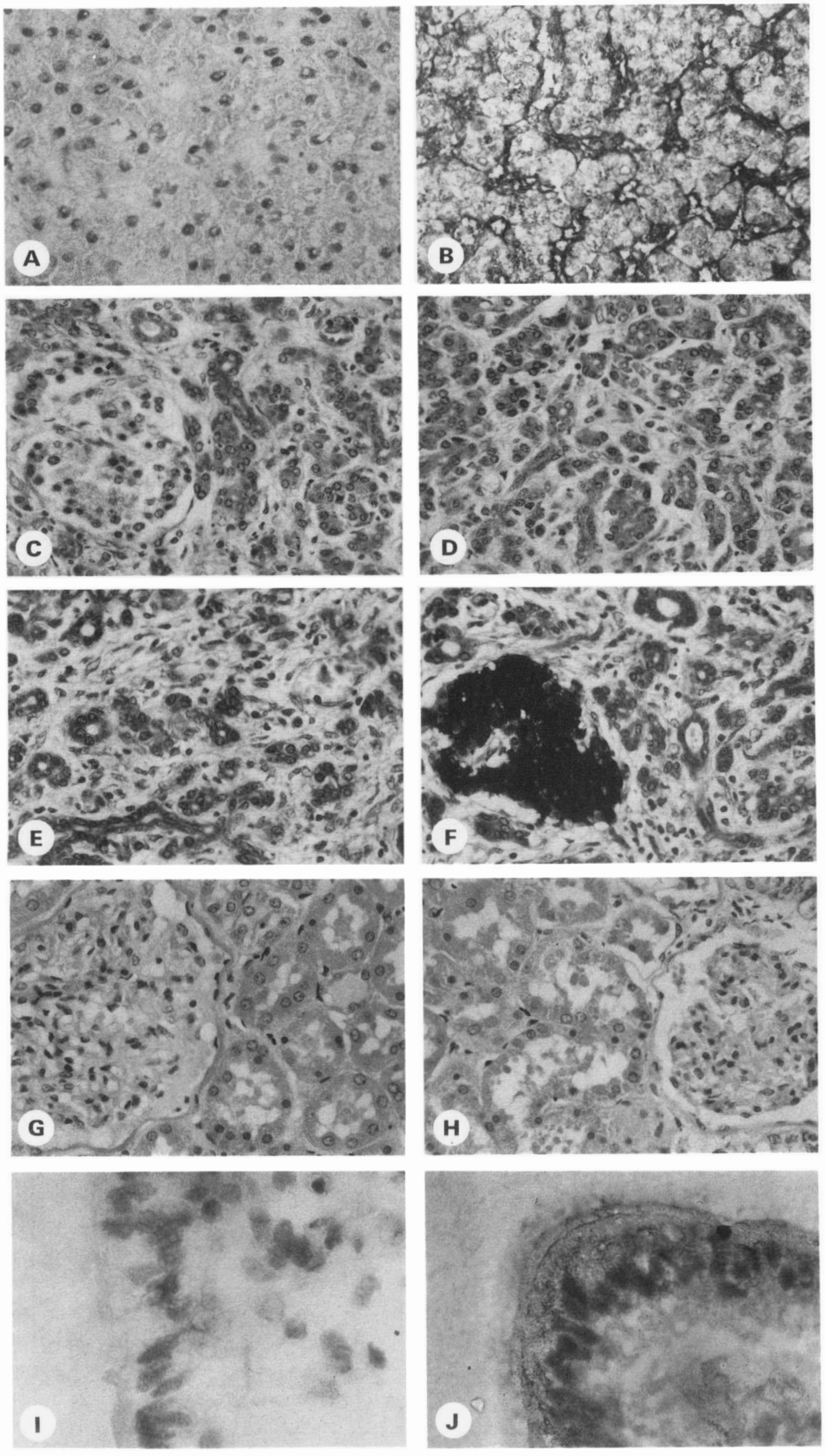

Fig. 3. Evaluation of human intestinal biopsies by immunohistochemical analysis. Liver (A, B), pancreas (C, D, E, F), kidney (G, H), and intestine (I, J) preparations were evaluated using monoclonal antibodies directed against apoB-100 (B, D, H, J), a monoclonal anti-insulin antibody (E, F), or an antineuroblastoma antibody that served as a negative control (A, C, G, I). The staining evident in E and F depicts the presence of insulin in the pancreatic islets, and the staining present in B and J indicates the presence of apoB-100 in adult human liver and intestine, respectively.

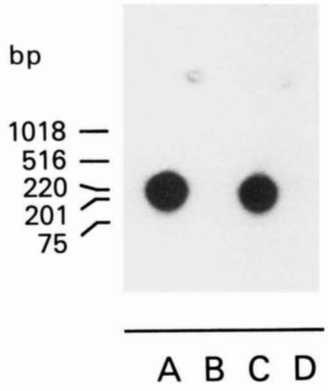

Fig. 4. Southern blot analysis of human apoB cDNAs. Samples were subjected to RT-PCR followed by electrophoresis through a 2% NuSieve (FMC)/1% agarose gel followed by Southern transfer to a nylon membrane and hybridized to 5'- ^{32}P-labeled probe 88PE. Lane A: pHuGln (PCR with primers SO175 and SO176); lane B: yeast tRNA subjected to RT with SO176 followed by PCR; lane C: human intestinal total RNA subjected to RT with SO176 followed by PCR; and lane D: human intestinal total RNA subjected to RT with primer SO175 followed by PCR. The film was deliberately overexposed to assess potential signal in lanes B and D.

biopsy samples and in the medium of adult intestinal organ cultures. Newly synthesized intestinal apoB isoproteins were studied by $NaDodSO_4$ gel electrophoresis of immunoprecipitated apoB isoproteins labeled with [^{35}S]methionine. The apoB-100 and apoB-48 isoproteins present intracellularly and secreted into the culture media were detected by immunoblot analysis using apoB-100 specific monoclonal antibodies and polyclonal apoB antibodies that recognized both apoB-100 and apoB-48. Several protease inhibitors and antioxidants were incorporated into the current study protocol to limit the degree of degradation of the apoB isoproteins that can occur during organ culture incubation. The results from these studies establish that both apoB-100 and apoB-48 are synthesized as well as secreted from the adult human intestine. The major newly synthesized intestinal apoB isoprotein was overwhelmingly apoB-48, only 3–5% being apoB-100 under the conditions utilized in the present studies. However, newly synthesized apoB-100 was also detected.

Based on protein staining of the intracellular and medium apoB, however, the predominant apoB isoprotein present in the cell and the media was apoB-100. The multiple washes of the organ cultures, as well as the identification of apoB-100 in the enterocyte by immunocytochemistry, make it unlikely that the apoB-100 was trapped extracellularly in the organ culture. These studies indicate that there was a pool of preformed apoB-100 within the cell that was released into medium during incubation. This may represent a pathway for retroendocytosis of apoB-100-containing lipoproteins taken up by the enterocyte.

The synthesis of both apoB-48 and apoB-100 by the intestine parallels the studies conducted on the editing of intestinal apoB mRNA. The human small intestine contains mRNA coding for apoB-100 (Fig. 5). ApoB-48 is, however, the predominant apoB mRNA present, in agreement with previous reports from other groups (37, 38) as well as our own (39, 40). ApoB-100 may represent 11–20% of intestinal apoB mRNA. These apoB mRNA ratios are in agreement with the ratios of newly synthesized and secreted protein, with apoB-48 predominating. These data indicate that RNA editing of apoB is a principal site of apoB isoprotein synthesis regulation. The slightly greater ratio of apoB-48:apoB-100 protein synthesis compared to the apoB-48:apoB-100 mRNA content may reflect either a higher translational efficiency of apoB-48 or more rapid degradation of the apoB-100 isoprotein. These findings do not represent artifact due to dedifferentiation of enterocytes during the incubation period, since similar amounts of apoB-100 mRNA were observed both before and after a 3.5 h incubation (Fig. 5). Finally, the signal amplification provided by RT–PCR–primer extension, developed independently by Davies et al. (27) and Driscoll et al. (26) as well as our group, per-

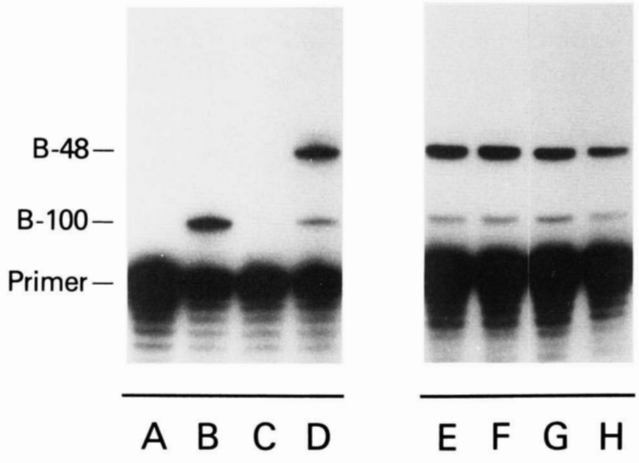

Fig. 5. Primer extension analysis of human intestinal apoB cDNA. Total RNA was isolated from human small intestinal biopsies and subjected to RT-PCR primer extension. This was followed by electrophoresis through 7 M urea/17.5% polyacrylamide gels as described in Materials and Methods. Lane A contains only the primer, while lane B illustrates the primer extension of the PCR product from pHuGln, which contains the apoB-100 sequence. Extension product of yeast tRNA, lacking the region of apoB editing, is shown in lane C. Lanes D, E, and F illustrate RT-PCR primer extension products generated from replicate samples of human intestinal RNA isolated from tissue that had been snap-frozen immediately after biopsy from normal volunteers. Lanes G and H are duplicate samples taken from the same volunteer as illustrated in lanes E and F. In contrast to E and F, which were biopsies that were immediately snap-frozen, and biopsies in G and H were frozen after the biopsies had been incubated for 3.5 h under conditions identical to those used for the protein synthesis studies. The expected migrations of unextended primer (19 nucleotides), apoB-100 (25 nucleotides), and apoB-48 (36 nucleotides) extension products are noted on the left. The expected migrations of the primer (19 nucleotides), apoB-100 (25 nucleotides), and apoB-48 (36 nucleotides) extension products are noted on the right.

mits the detection of minimal mRNA template and should prove useful in quantitation of other nucleic acids that differ by a single base.

The similar primary structure of the mature apoB mRNA and the apoB gene in the region subject to RNA editing (within exon 26) required stringent control for genomic DNA contamination. Previous human intestinal biopsies that we have obtained have contained significant amounts of genomic DNA clearly visible on ethidium-stained formaldehyde gels run for RNA analysis (not shown). The use of acid-phenol–chloroform extractions (22) in concert with nuclease pretreatment of intestinal biopsy RNAs was effective in reducing genomic DNA contamination as evidenced by the absence of signal in RNAs subjected to "wrong" first strand synthesis followed by PCR (Fig. 4). In addition, the cocktail reagent approach was critical in guarding as much as possible against reagent-primer contamination with cDNA or genomic DNA. Control for unwanted DNA contamination, therefore, permitted the definitive identification of apoB-100 mRNA in the human intestine.

In the present studies, the identification of intestinally derived apoB-100 protein may have clinical implications. Lipoprotein analyses are generally performed after a 12–14 h fast in order to assess atherogenic risk. Therefore, the synthesis of an atherogenic apoB-100 by intestine could play a role in the development of diet-induced atherosclerosis which may not be reflected in the fasting lipoprotein profile. In addition, investigation into potential normal and deranged regulation of intestinal apoB-100 synthesis and secretion may provide insight into the pathogenesis of some forms of genetic dyslipoproteinemia.

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