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Intestinal-fatty acid binding protein and lipid transport in human intestinal epithelial cells

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Abstract

Intestinal-fatty acid binding protein (I-FABP) is a 14–15 kDa cytoplasmic molecule highly expressed in the enterocyte. Although different functions have been proposed for various FABP family members, the specific function of I-FABP in human intestine remains unclear. Here, we studied the role of I-FABP in molecularly modified normal human intestinal epithelial cells (HIEC-6). cDNA transfection resulted in 90-fold I-FABP overexpression compared to cells treated with empty pQCXIP vector. The high-resolution immunogold technique revealed labeling mainly in the cytosol and confirmed the marked phenotype abundance of I-FABP in cDNA transfected cells. I-FABP overexpression was not associated with alterations in cell proliferation and viability. Studies using these transfected cells cultured with [^{14}C]oleic acid did not reveal higher efficiency in de novo synthesis or secretion of triglycerides, phospholipids, and cholesteryl esters compared to cells treated with empty pQCXIP vector only. Similarly, the incubation with [^{35}S]methionine did not disclose a superiority in the biogenesis of apolipoproteins (apo) A-I, A-IV, B-48, and B-100. Finally, cells transfected with I-FABP did not exhibit an increased production of chylomicrons, VLDL, LDL, and HDL. Our observations establish that I-FABP overexpression in normal HIEC-6 is not related to cell proliferation, lipid esterification, apo synthesis, and lipoprotein assembly, and, therefore, exclude its role in intestinal fat transport.

Keywords: Lipid esterification; Apolipoprotein synthesis; Lipoprotein assembly and cell proliferation

Intestinal epithelial cells have the unique ability to assemble and secrete chylomicrons (CM), the major vehicles for the transport of alimentary fat [1–4]. They are also capable of producing different types of lipoproteins, such as very-low-density (VLDL) and high-density (HDL) lipoproteins [3–6]. The formation of CM represents a highly complex task that requires the uptake of lipolytic products, re-esterification and translocation of cellular lipid pools, synthesis and post-translational modifications of various apolipoproteins (apo), and the assembly of lipid and apo moieties [7,8]. Whereas, biochemical, molecular, and cell biological studies of different congenital lipid transport disorders have disclosed the crucial role of apo B-48 [9,10], microsomal triglyceride transport protein (MTP) [11], and Sar-1 GTPase protein [12], little information is available about the function(s) of cytosolic proteins in the enterocyte. Two small fatty acid-binding proteins (FABPs) co-exist in the enterocyte cytosol: intestinal (I) and liver

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(L)-FABP [13–15]. These 14–15-kDa proteins are 29% homologous [16], but differ in their binding specificity and other properties [17–21]. Since I-FABP is highly and exclusively expressed in intestinal epithelial cells, it was proposed to play a fundamental role in the uptake and transport of dietary fatty acids (FAs) in the small intestine [15,22]. This hypothesis was reinforced by the discovery that I-FABP variant deriving from the polymorphism of the FABP2 gene on chromosome 4q28-q31 has a greater affinity for long-chain FAs than the wild-type (wt) [22], is associated with elevated triglyceride (TG) concentrations after a fat meal [23], and predisposes to insulin resistance [22,24]. However, the development of knockout mouse revealed that I-FABP is not essential for dietary fat uptake although a role in lipid absorption and/or secretion may not be ruled out definitely [25]. Indeed, the observations emanating from human intestinal cell line Caco-2 overexpressing I-FABP disclosed a low rate of FA uptake [26]. Nevertheless, the I-FABP overexpression was limited to 15-fold and a thorough characterization of lipid esterification, apo biogenesis, and lipoprotein was not carried out.

In our ongoing effort to further our understanding of the mechanisms governing intracellular lipid transport, we overexpressed I-FABP to determine its function in normal intestinal epithelial cells. In this study, we chose to focus on human crypt intestinal epithelial cells (HIEC) that, in our hands, were capable of key fat transport functions of well-differentiated enterocytes [27].

Methods

Cell culture. Normal human intestinal epithelial cells were generated with the use of the dissociating enzyme thermolysin as previously described [27]. Cell populations were expanded and then kept frozen (in DMEM containing DMSO and FCS) at passage 2 or 3, in liquid nitrogen. For the following studies, cell aliquots were cultured at 37 °C in high glucose DMEM containing 5% FBS, 10 mM Hepes, 4 mM glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 5 μg/ml epidermal growth factor, and 100 μl/ml insulin; transferrin, selenium, all from Gibco-BRL. Confluence was reached by day 13. Media were replaced every 2 days. The experiments were carried out 8 days after confluence.

Virus production and retroviral infections. Wt I-FABP-encoding cDNA (kindly provided by Dr. J.H. Veerkamp) was PCR amplified and HA-tagged using sense primer 5′-GAAGAT CTTCATGTATGATGTTCCTG ATTATGCTATG GCGTT TGACAGCACTTGG-3′ and the antisense primer 5′-CCATCGATGG TCAA TCCTTTTTAAAGATCCTTTT-3′ with the *Taq* Plus Precision PCR system (Stratagene, Vancouver, BC) according to the company's specifications. The amplicon was digested by *Bgl*II and *Cla*I, and inserted downstream of the cytomegalovirus promoter in the retroviral vector pLNCX2 (Clontech) and confirmed by sequencing. The insert was subcloned into the *Eco*RI site of pQCXIP (Clontech) after removal from pLNCX2 using *Bgl*II and *Cla*I and Klenow to fill in the overhang. The Wt-I-FABPHA and empty pQCXIP vectors were used to produce virus in HEK293T cells in cotransfection with helper amphotropic DNA vector (pAmpho) as described previously [28]. Selection using 1 μg/ml puromycin (Calbiochem) was applied 24 h after the end of infection for 10 days to obtain stable populations. All experiments shown were performed within 3 weeks of selection, virus production, and retroviral infection.

Proliferation assay. HIEC-6 cells (6×10^5 cells/ml) were allowed to reach subconfluence over 48 h in 24-well plates (Falcon Plastics, Oxnard, CA). The cells were then washed with PBS and changed to FCS-free supplemented DMEM for a 24-h stabilization period. Cells were cultured for 20-h in supplemented DMEM containing only 1% FCS. Proliferation was studied by monitoring changes in DNA synthesis, as measured by [^{3}H]thymidine uptake (2 mCi/ml) added during the last 2 h of culture. Cells were trypsinized and harvested, and radioactivity was measured in trichloroacetic acid protein precipitates as counts per minute per microgram DNA. Total DNA was determined for each well in sonicated cells by a microfluorometric method using calf thymus DNA as a standard (Boehringer–Mannheim, Germany).

Incorporation of [^{14}C]oleic acid into lipids. Monolayers were washed twice with MEM (serum free) and the [^{14}C]oleic acid (Amersham, Oakville, Ontario, Canada) substrate (equivalent to 1.0 μmol/ml) containing 2–2.5 μCi/well (sp. act. of 53.9 mCi/mmol) was added to the medium. Cells were then incubated for 18 h at 37 °C in order to determine lipid synthesis and secretion. At the end of this incubation period, the cells were washed and scraped off with a rubber policeman into maleate buffer (pH 6.0), and sonicated. Another aliquot was taken for lipid extraction after the addition of unlabeled carriers [phospholipids (PL), monoglycerides (MG), diglycerides (DG), TG, free FA, and cholesteryl ester (CE)], using standard methods [6]. The various lipid classes synthesized from [^{14}C]oleic acid were separated by thin-layer chromatography (TLC) using the nonpolar solvent mixture: hexane–ether–acetic acid 80:20:3 (vol/vol/vol). The area corresponding to each lipid was scratched off the TLC plates. The silica powder was then placed in a scintillation vial with Ready Safe (Beckman Instruments, Fullerton, CA), and radioactivity was measured by scintillation counting (Beckman #LS5000 TD, Ontario, Canada). Cell protein was quantified by the method of Lowry et al. [29], and results were expressed as dpm/mg cell protein. Lipids secreted into the medium were analyzed and quantified as above, after centrifugation (2000 rpm, 30 min, 4 °C), to remove cell debris.

Lipid carrier. Blood was drawn 1.5 h after the oral intake of a fat meal, and postprandial plasma was prepared to serve as a carrier for the lipoproteins synthesized by HIEC, as described previously [6]. The TG-enriched plasma was incubated at 56 °C for 1 h to inactivate enzymatic activity.

Isolation of lipoproteins. For the determination of secreted lipoproteins, HIEC were incubated with lipid substrate (as above), for a culture period prolonged to 18 h, so as to detect an appreciable amount of lipoproteins secreted into the medium. The medium was supplemented with anti-proteases [phenylmethylsulfonyl fluoride (PMFS), pepstatin, EDTA, aminocaproic acid, chloramphenicol, leupeptin, glutathione, benzamidine, dithiothreitol (DDT), sodium azide, and trasylol], all at a final concentration of 1 mM. A plasma lipid carrier was added (2:0.6 vol/vol). Lipoproteins were then isolated by sequential ultracentrifugation using a TL-100 ultracentrifuge (Beckman, Montréal, Quebec, Canada), as per our established method [6]. Briefly, VLDL ($d < 1.006$ g/ml) and low density lipoprotein (LDL) ($d < 1.063$ g/ml) were separated by centrifugation (100,000*g*) for 2.26 h with a TLA 100.4 rotor at 4 °C. The HDL was obtained by adjusting the LDL infranatant to density <1.21 g/ml, and centrifuging for 6.5 h at 100,000*g*. 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. To study newly synthesized apos, HIEC were incubated with [^{35}S]methionine (50 mCi/mmol, Amersham) [30]. In order to induce lipoprotein and apo synthesis, cells were incubated with unlabeled oleic acid (0.8 mM) 18 h prior to [^{35}S]methionine incubation. Cells were then rinsed (three times) with serum-free, methionine-free MEM and incubated with unlabeled oleic acid and [^{35}S]methionine (100 μCi/ml, Amersham). After incubation for 1 h at 37 °C, the cells were scraped off the dish into a cell lysis buffer (Tris 10 mM, NaCl 150 mM, EDTA 5 mM, SDS 0.1%, Triton 1%, and Na deoxycholate 0.5%). The medium and cell lysates were supplemented with the anti-protease mixture as described above. The cell lysates from two dishes were pooled for assays of apo synthesis.

Immunoprecipitation of apolipoproteins. After supplementing the medium and the cell lysate with unlabeled methionine (0.1 mM), immunoprecipitation was performed using polyclonal anti-human apos (Boehringer–Mannheim Biochemicals, Mannheim, Germany) at 4 °C

overnight [30]. Samples were then washed with Nonidet (0.05%) and centrifuged. The immunoprecipitate was resuspended in sample buffer (1.2% SDS, 12% glycerol, 60 mM Tris, pH 7.3, 1.2% β-mercaptoethanol, and 0.003% bromophenol blue) and loaded on a 3% polyacrylamide stacking gel. Radioactive molecular weight standards were purchased from Amersham. Gels were sectioned into 2-mm slices and counted after an overnight incubation with 1 ml BTS-450 (Beckman) and 10 ml of Ready Safe scintillation fluid (Beckman). Results are expressed as % of total [^{35}S]methionine-labeled protein/mg cell protein.

Western blots. To assess the presence of I-FABP, intestinal tissue was homogenized and adequately prepared for Western blotting as described previously [31]. Proteins were denatured in sample buffer containing SDS and β-mercaptoethanol, separated on a 4–20% gradient SDS–PAGE, and electroblotted onto nitrocellulose membranes. Nonspecific binding sites of the membranes were blocked using defatted milk proteins followed by the addition of primary antibodies directed against I-FABP. The relative amount of primary antibody was detected with species-specific horseradish peroxidase-conjugated secondary antibody. Blots were developed and the mass of I-FABP was estimated using an HP Scanjet scanner equipped with a transparency adapter and software.

Statistical analysis. All values were expressed as means ± SE. The differences between the means were assessed using Student's two-tailed *t* test.

Results

Stable transfection of Wt I-FABP in HIEC

The biological consequences of I-FABP overexpression were examined in cultured HIEC after viral infection of cDNA cloned in the retroviral vector pQCXIP. The percentage of retrovirally transduced cells ranged between 60% and 80%, as estimated by parallel infections, using viruses expressing the green fluorescent protein gene product (results not shown). The pQCXIP retroviral vector coexpressed a puromycin resistance gene that allowed selection of pure population of transduced cells within 10 days. As shown in Fig. 1, the selected populations exhibited ~90-fold more I-FABP expression compared to normal HIEC transfected with empty pQCXIP.

Immunocytochemical labeling

Protein A-gold immunocytochemical techniques were employed to detect the presence of I-FABP in HIEC (Fig. 2). The ultrastructural analysis revealed significant labeling in the cytosol. Some particles were seen associated with plasma membrane. Cells overexpressing I-FABP displayed significantly more labeling than control cells.

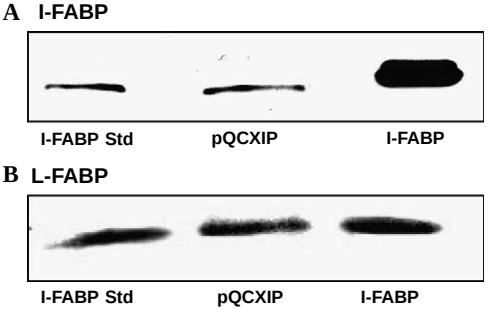

Fig. 1. Expression of I-FABP in HIEC. Populations of HIEC stably expressing pQCXIP (empty vector) or Wt I-FABP in pQCXIP were harvested 2 weeks post selection. Equal amounts of whole cell lysates were separated by SDS–PAGE and proteins were analyzed by Western blotting with specific polyclonal antibody directed against (A) I-FABP and (B) L-FABP.

Cell proliferation and viability

Cell proliferation was assessed by [^{3}H]thymidine incorporation. No significant cell proliferation changes were noted in between transfected I-FABP-HIEC (11.08 ± 1.43 dpm/μg DNA) and cells transfected with empty pQCXIP (10.97 ± 1.27 dpm/μg DNA). Additionally, as assessed by lactic dehydrogenase and trypan blue exclusion, cell transfection did not result in significant variability of cell viability (Results not shown).

Lipid synthesis and secretion

The data mentioned above clearly demonstrated substantial overexpression of I-FABP without affecting HIEC cell integrity. The next step consisted in the understanding of I-FABP involvement in de novo lipid esterification and output. To this end, cells were incubated with [^{14}C]oleic acid for 18 h and their lipid content was analyzed by TLC. As illustrated in Fig. 3, no changes in total radiolabeled lipids were recorded in cells and media from transfected and control cells. Similarly, I-FABP overexpression did not alter the synthesis and secretion of the major lipid classes, i.e., TG, PL, DG, MG, and CE.

Influence of I-FABP overexpression on apo biogenesis

Apo synthesis was evaluated after a 18-h-period incubation with [^{35}S]methionine (Fig. 4). Evidently, I-FABP overexpression did not modify the incorporation of [^{35}S]methionine into apos (A-I, A-IV, B-48, and B-100) both in cells and media.

I-FABP overexpression and lipoprotein assembly

As anticipated from the lack of lipid and apo changes, lipoprotein exocytosis was not altered by I-FABP overexpression (Fig. 5). The secretion of CM, VLDL, LDL, and HDL was not modified by the abundance of I-FABP available in HIEC-6 cells.

Discussion

Intestinal lipoprotein assembly is crucial for dietary fat absorption. More and more progress has been achieved as to the identification of essential components participating in intracellular lipid transport, such as apo B, MTP, and Sar1 ATPase. However, the mechanisms of FA transfer from apical membrane to the endoplasmic reticulum (ER) where their reesterification takes place have not been established. Despite some controversies, many investigators surmised that the I-FABP is responsible for cytosolic

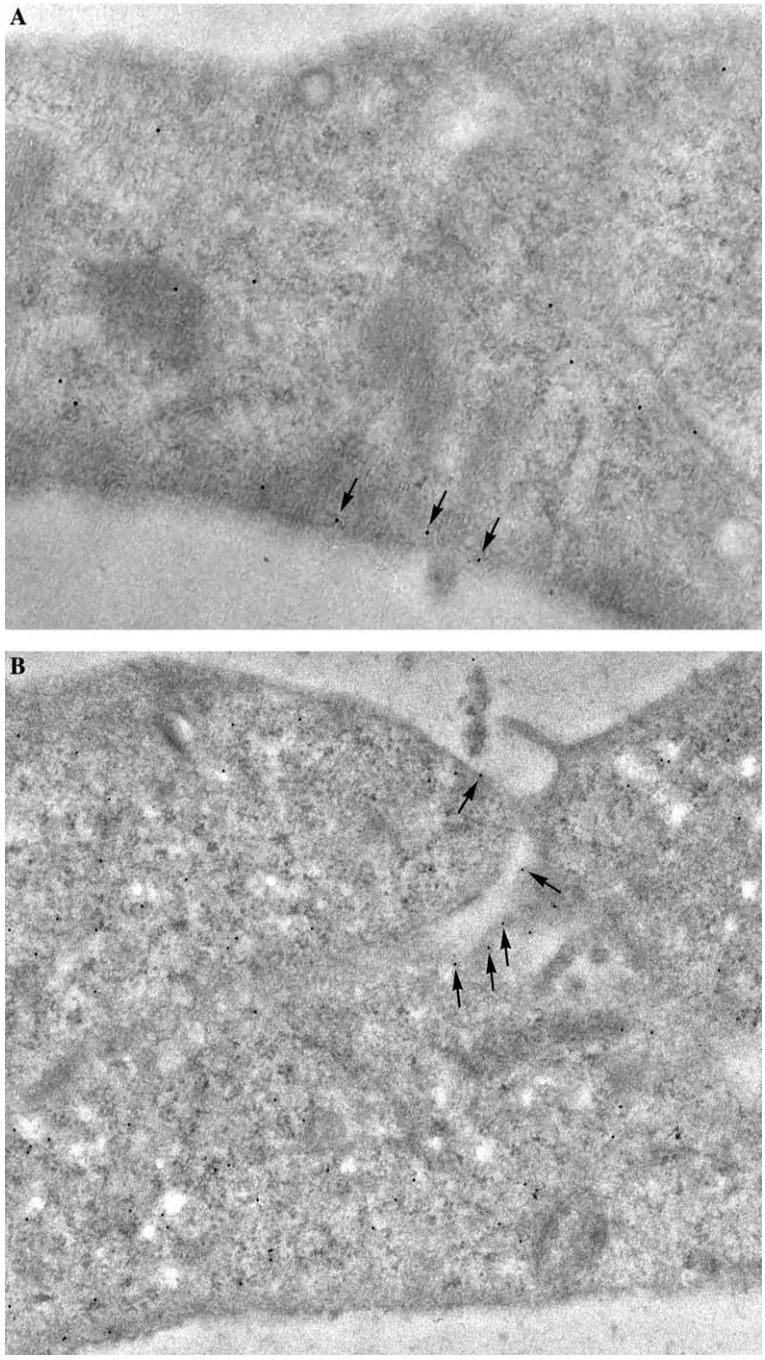

Fig. 2. Immunocytochemical detection of I-FABP in HIEC-6. Protein A-gold immunocytochemical technique was applied with the polyclonal antibody directed against I-FABP. The labeling by gold particles revealed the abundance of I-FABP in cells transfected with cDNA (B) (35,000×) compared to controls treated with empty pQCXIP alone (A) (45,000×).

trafficking. The present investigation does not provide support for this hypothesis since a series of findings have documented the limited capacity of I-FABP to influence the assembly of intestinal lipoproteins. Our observations using HIEC-6 overexpressing sizeable amounts of I-FABP demonstrated negligible influence of this transporter on lipid synthesis, apo biogenesis, and lipoprotein exocytosis. Unlike L-FABP gene, the I-FABP gene is expressed uniquely in the small intestine [18]. Data from biochemistry studies [32], X-ray crystallography [33], and nuclear magnetic resonance spectroscopy [34] have not only defined the physical structure of I-FABP, but have also proposed a collisional model to explain the transfer of lipids within the cell. Accordingly, Baier et al. [22,35] have reported the high affinity for primarily long-chain FAs. Nevertheless, the contribution of I-FABP to intestinal fat assimilation and lipid metabolism has not been well examined. Our data suggest that I-FABP does not represent a critical factor for the delivery of FAs to the ER for the elaboration of lipoproteins. Since no in vivo or in vitro I-FABP

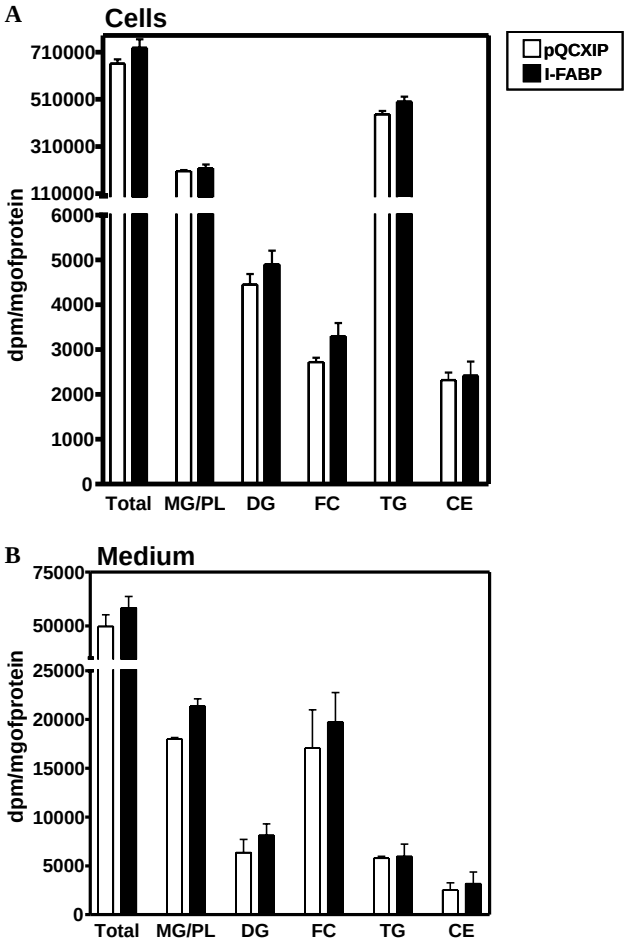

Fig. 3. Effect of I-FABP overexpression on lipid synthesis and secretion. Cells were incubated with [^{14}C]oleic acid as substrate. Following the 18-h incubation period, cells were harvested and media were collected to determine de novo cell (A) and medium (B) lipid synthesis as described in Methods. Values are means ± SE for different experiments. PL, phospholipids; TG, triglycerides; CE, cholesteryl esters, DG, diglyceride; and MG, monoglyceride.

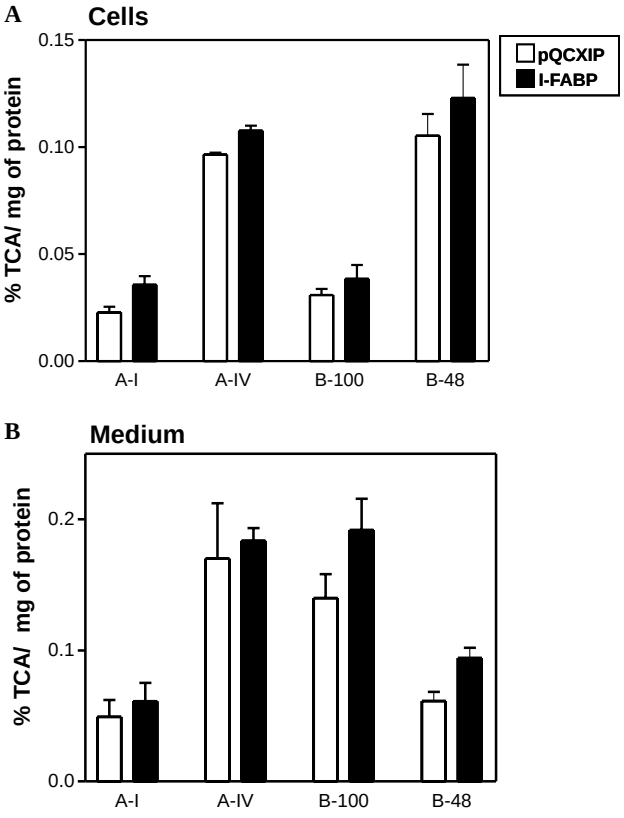

Fig. 4. Apolipoprotein syntheses by HIEC-6 after I-FABP overexpression. HIEC-6 were incubated with [^{35}S]methionine for 18 h. At the end of the labeling period, cells were washed, homogenized, and centrifuged. Samples from cell homogenates (A) and media (B) were analyzed by linear 4–20% SDS–PAGE after immunoprecipitation. Gels were sliced and counted for radioactivity. Values are means ± SE for four separate experiments.

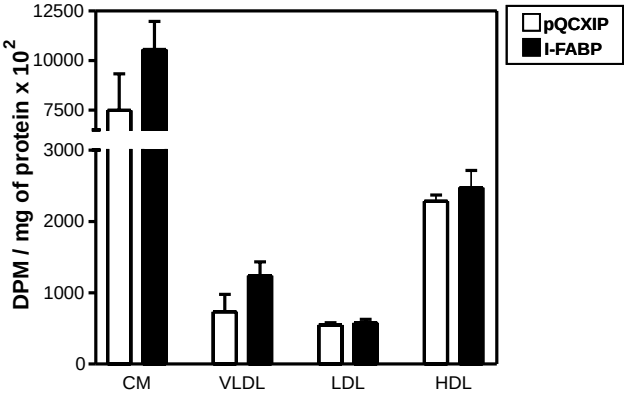

Fig. 5. Lipoprotein secretions by HIEC-6 following I-FABP overexpression. HIEC-6 were cultured in the presence of [^{14}C]oleic acid. After 18-h incubation period, lipoproteins from media were isolated by ultracentrifugation. Values are means ± SE for four separate experiments.

function can be definitively put forward, the puzzling and interesting question pertains to its specific role in the enterocyte. Is it involved in the movement of FAs to specific subcellular compartments other than the ER? Does it in influence mitochondrial β-oxidation in a similar fashion to L-FABP in the liver [36]? It is actually a lipid-sensing component of energy homeostasis as proposed by Vassileva et al. [25]? Undoubtedly, much work is required to provide new insights into the physiological role of I-FABP in the intestine.

Various FABPs can mediate FAs from the site of absorption to intracellular organelles within the enterocyte. The three FABPs identified in the intestine are I-FABP, L-FABP, and ileal lipid-binding protein [37]. The presence of several types of intracellular lipid-binding proteins in the small intestine may represent a carefully devised cooperative plan of action to cope with the considerable daily consumption of alimentary fat. Thus, it is possible that changes in I-FABP concentrations may incite the other local transporters to adjust their levels. In our study, the overexpression of I-FABP did not cause any modification in L-FABP expression and no counterbalance has been expected from ileal lipid-binding protein since its normal localization is limited to the ileum and colon. We conclude

that lipoprotein packaging sluggishness is strictly the result of the weak impact of I-FABP.

As mentioned before, Darimont et al.'s [26] findings indicate that Caco-2 cells overexpressing I-FABP disclosed a low rate of FA uptake. However, the present investigation did not reveal decreased FA uptake in HIEC. Differences in the intestinal model, the level of I-FABP expression, and other experimental techniques may explain the divergence between the two studies.

L-FABP has been found to be involved in cellular mitotic activity and hepatocarcinogenesis [38,39]. Recent studies have also associated increased levels of L-FABP with cell regeneration following partial hepatectomy [40]. Antisense oligodeoxynucleotides directed towards L-FABP reduced cellular proliferation rates and increased apoptosis [41]. The mitoinhibitory action of dexamethasone was due to decreased L-FABP expression [42]. In our studies, overexpression of I-FABP in HIEC-6 did not modify cell proliferation, suggesting that I-and L-FABP have different roles in intestinal epithelial cells.

In conclusion, our systematic investigation has demonstrated for the first time that overexpression of I-FABP expression is not associated with lipid esterification, apo biogenesis, and lipoprotein assembly in human intestinal epithelial cells. Thus, the present studies provide additional in vitro support for the hypothesis that I-FABP does not play a substantial role in intestinal fat transport. Furthermore, I-FABP does not seem to regulate HIEC-6 cell proliferation. Further investigation is obviously needed to unravel the specific function(s) of I-FABP in the small intestine.

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References

[1] M.M. Hussain, S. Fatma, X. Pan, J. Iqbal, Intestinal lipoprotein assembly, Curr. Opin. Lipidol. 16 (2005) 281–285.
[2] C.T. Phan, P. Tso, Intestinal lipid absorption and transport, Front Biosci. 6 (2001) D299–D319.
[3] E. Levy, M. Mehran, E. Seidman, Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion, FASEB J. 9 (1995) 626–635.
[4] F.J. Field, S.N. Mathur, Intestinal lipoprotein synthesis and secretion, Prog. Lipid Res. 34 (1995) 185–198.
[5] G.P. Forester, A.R. Tall, C.L. Bisgaier, R.M. Glickman, Rat intestine secretes spherical high density lipoproteins, J. Biol. Chem. 258 (1983) 5938–5943.
[6] E. Levy, L. Thibault, D. Menard, Intestinal lipids and lipoproteins in the human fetus: modulation by epidermal growth factor, J. Lipid Res. 33 (1992) 1607–1617.
[7] D.D. Black, Intestinal lipoprotein metabolism, J. Pediatr. Gastroenterol. Nutr. 20 (1995) 125–147.
[8] N.O. Davidson, S. Anant, A.J. MacGinnitie, Apolipoprotein B messenger RNA editing: insights into the molecular regulation of post-transcriptional cytidine deamination, Curr. Opin. Lipidol. 6 (1995) 70–74.
[9] E. Levy, C.C. Roy, L. Thibault, A. Bonin, P. Brochu, E.G. Seidman, Variable expression of familial heterozygous hypobetalipoproteinemia: transient malabsorption during infancy, J. Lipid Res. 35 (1994) 2170–2177.
[10] X. Lin, P. Yue, Y. Xie, N.O. Davidson, N. Sakata, R.E. Ostlund Jr., Z. Chen, G. Schonfeld, Reduced intestinal fat absorptive capacity but enhanced susceptibility to diet-induced fatty liver in mice heterozygous for ApoB38.9 truncation, Am. J. Physiol. Gastrointest. Liver Physiol. 289 (2005) G146–G152.
[11] J.R. Wetterau, L.P. Aggerbeck, M.E. Bouma, C. Eisenberg, A. Munck, M. Hermier, J. Schmitz, G. Gay, D.J. Rader, R.E. Gregg, Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia, Science 258 (1992) 999–1001.
[12] B. Jones, E.L. Jones, S.A. Bonney, H.N. Patel, A.R. Mensenkamp, S. Eichenbaum-Voline, M. Rudling, U. Myrdal, G. Annesi, S. Naik, N. Meadows, A. Quattrone, S.A. Islam, R.P. Naoumova, B. Angelin, R. Infante, E. Levy, C.C. Roy, P.S. Freemont, J. Scott, C.C. Shoulders, Mutations in a Sar1 GTPase of COPII vesicles are associated with lipid absorption disorders, Nat. Genet. 34 (2003) 29–31.
[13] J.I. Gordon, J.B. Lowe, Analyzing the structures, functions and evolution of two abundant gastrointestinal fatty acid binding proteins with recombinant DNA and computational techniques, Chem. Phys. Lipids 38 (1985) 137–158.
[14] R.K. Ockner, J.A. Manning, R.B. Poppenhausen, W.K. Ho, A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues, Science 177 (1972) 56–58.
[15] D.A. Sweetser, E.H. Birkenmeier, I.J. Klisak, S. Zollman, R.S. Sparkes, T. Mohandas, A.J. Lusis, J.I. Gordon, The human and rodent intestinal fatty acid binding protein genes. A comparative analysis of their structure, expression, and linkage relationships, J. Biol. Chem. 262 (1987) 16060–16071.
[16] J.H. Veerkamp, R.A. Peeters, R.G. Maatman, Structural and functional features of different types of cytoplasmic fatty acid-binding proteins, Biochim. Biophys. Acta 1081 (1991) 1–24.
[17] J.F. Glatz, A.M. Janssen, C.C. Baerwaldt, J.H. Veerkamp, Purification and characterization of fatty-acid-binding proteins from rat heart and liver, Biochim. Biophys. Acta 837 (1985) 57–66.
[18] N.M. Bass, The cellular fatty acid binding proteins: aspects of structure, regulation, and function, Int. Rev. Cytol. 111 (1988) 143–184.
[19] M.P. Bansal, R.G. Cook, K.G. Danielson, D. Medina, A 14-kilodalton selenium-binding protein in mouse liver is fatty acid-binding protein, J. Biol. Chem. 264 (1989) 13780–13784.
[20] P. Tso, A. Nauli, C.M. Lo, Enterocyte fatty acid uptake and intestinal fatty acid-binding protein, Biochem. Soc. Trans. 32 (2004) 75–78.
[21] L.B. Agellon, M.J. Toth, A.B. Thomson, Intracellular lipid binding proteins of the small intestine, Mol. Cell. Biochem. 239 (2002) 79–82.
[22] L.J. Baier, J.C. Sacchettini, W.C. Knowler, J. Eads, G. Paolisso, P.A. Tataranni, H. Mochizuki, P.H. Bennett, C. Bogardus, M. Prochazka, An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance, J. Clin. Invest. 95 (1995) 1281–1287.
[23] J.J. Agren, R. Valve, H. Vidgren, M. Laakso, M. Uusitupa, Postprandial lipemic response is modified by the polymorphism at codon 54 of the fatty acid-binding protein 2 gene, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 1606–1610.
[24] B.D. Mitchell, C.M. Kammerer, P. O'Connell, C.R. Harrison, M. Manire, P. Shipman, M.P. Moyer, M.P. Stern, M.L. Frazier, Evidence for linkage of postchallenge insulin levels with intestinal fatty acid-binding protein (FABP2) in Mexican-Americans, Diabetes 44 (1995) 1046–1053.
[25] G. Vassileva, L. Huwyler, K. Poirier, L.B. Agellon, M.J. Toth, The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice, FASEB J. 14 (2000) 2040–2046.
[26] C. Darimont, N. Gradoux, E. Persohn, F. Cumin, A. De Pover, Effects of intestinal fatty acid-binding protein overexpression on fatty acid metabolism in Caco-2 cells, J. Lipid Res. 41 (2000) 84–92.

[27] E. Levy, J.F. Beaulieu, E. Delvin, E. Seidman, W. Yotov, J.R. Basque, D. Menard, Human crypt intestinal epithelial cells are capable of lipid production, apolipoprotein synthesis, and lipoprotein assembly, J. Lipid Res. 41 (2000) 12–22.
[28] M.J. Boucher, D. Jean, A. Vezina, N. Rivard, Dual role of MEK/ERK signaling in senescence and transformation of intestinal epithelial cells, Am. J. Physiol. Gastrointest. Liver Physiol. 286 (2004) G736–G746.
[29] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[30] E. Levy, D. Sinnett, L. Thibault, T.D. Nguyen, E. Delvin, D. Menard, Insulin modulation of newly synthesized apolipoproteins B-100 and B-48 in human fetal intestine: gene expression and mRNA editing are not involved, FEBS Lett. 393 (1996) 253–258.
[31] E. Levy, D. Menard, I. Suc, E. Delvin, V. Marcil, L. Brissette, L. Thibault, M. Bendayan, Ontogeny, immunolocalisation, distribution and function of SR-BI in the human intestine, J. Cell Sci. 117 (2004) 327–337.
[32] K.T. Hsu, J. Storch, Fatty acid transfer from liver and intestinal fatty acid-binding proteins to membranes occurs by different mechanisms, J. Biol. Chem. 271 (1996) 13317–13323.
[33] J.C. Sacchettini, J.I. Gordon, L.J. Banaszak, Crystal structure of rat intestinal fatty-acid-binding protein. Refinement and analysis of the *Escherichia coli*-derived protein with bound palmitate, J. Mol. Biol. 208 (1989) 327–339.
[34] F. Zhang, C. Lucke, L.J. Baier, J.C. Sacchettini, J.A. Hamilton, Solution structure of human intestinal fatty acid binding protein: implications for ligand entry and exit, J. Biomol. NMR 9 (1997) 213–228.
[35] J.B. Lowe, J.C. Sacchettini, M. Laposata, J.J. McQuillan, J.I. Gordon, Expression of rat intestinal fatty acid-binding protein in *Escherichia coli*. Purification and comparison of ligand binding characteristics with that of Escherichia coli-derived rat liver fatty acid-binding protein, J. Biol. Chem. 262 (1987) 5931–5937.
[36] D. Linden, K. Lindberg, J. Oscarsson, C. Claesson, L. Asp, L. Li, M. Gustafsson, J. Boren, S.O. Olofsson, Influence of peroxisome proliferator-activated receptor alpha agonists on the intracellular turnover and secretion of apolipoprotein (Apo) B-100 and ApoB-48, J. Biol. Chem. 277 (2002) 23044–23053.
[37] P. Oelkers, P.A. Dawson, Cloning and chromosomal localization of the human ileal lipid-binding protein, Biochim. Biophys. Acta 1257 (1995) 199–202.
[38] R.P. Custer, S. Sorof, Mitosis in hepatocytes is generally associated with elevated levels of the target polypeptide of a liver carcinogen, Differentiation 30 (1985) 176–181.
[39] R.P. Custer, S. Sorof, Target polypeptide of a carcinogen is associated with normal mitosis and carcinogen-induced hyperplasias in adult hepatocytes, Proc. Natl. Acad. Sci. USA 81 (1984) 6738–6742.
[40] R. Hammamieh, N. Chakraborty, R. Das, M. Jett, Molecular impacts of antisense complementary to the liver fatty acid binding protein (FABP) mRNA in DU 145 prostate cancer cells in vitro, J. Exp. Ther. Oncol. 4 (2004) 195–202.
[41] R. Das, R. Hammamieh, R. Neill, M. Melhem, M. Jett, Expression pattern of fatty acid-binding proteins in human normal and cancer prostate cells and tissues, Clin. Cancer Res. 7 (2001) 1706–1715.
[42] G. Rajaraman, F.J. Burczynski, Effect of dexamethasone, 2-bromopalmitate and clofibrate on L-FABP mediated hepatoma proliferation, J. Pharm. Pharmacol. 56 (2004) 1155–1161.