Cellular and molecular aspects of fat metabolism in the small intestine

BY PHILIPPE BESNARD, ISABELLE NIOT, ANDRE BERNARD
AND HELENE CARLIER
Laboratoire de Physiologie de la Nutrition, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation (ENSBANA), Université de Bourgogne, 1, Esplanade Erasme, F-21000 Dijon, France

Aspects cellulaire et moléculaire du métabolisme lipidique au niveau de l'intestin grêle

RÉSUMÉ

Le rôle énergétique, structural et fonctionnel des acides gras à longue chaîne (AGLC) dans l'organisme dépend de leur biodisponibilité intestinale. Les données actuelles tendent à montrer que deux mécanismes distincts et complémentaires permettent aux AGLC de traverser la membrane apicale de l'entérocyte: une diffusion simple et un transport faisant intervenir au moins deux protéines membranaires, la plasma-membrane fatty acid-binding protein (FABPpm) et la fatty acid transporter (FAT). On ignore actuellement quelle est l'importance relative de ces deux mécanismes et s'il existe une spécialisation de ces protéines vis à vis des différents AGLC. Ces derniers sont ensuite pris en charge au niveau intracellulaire par les fatty acid-binding proteins cytosoliques (FABPc). L'entérocyte a la particularité d'exprimer deux FABPc differentes, la I-FABPc et la L-FABPc, dont les rôles respectifs restent à élucider. Elles apparaissent cependant déterminantes pour la constitution d'un pool intracellulaire d'acides gras non estérifiés et la protection contre les effets cytotoxiques des acides gras libres. La L-FABPc pourrait être également impliquée dans la modulation de l'activité de certaines enzymes du métabolisme lipidique et dans le contrôle de la differenciation et de la prolifération cellulaire. Après activation par la long-chain acyl-CoA synthétase (ACS), les AGLC sont soit pris en charge par l'acyl-CoA-binding protein (ACBP), soit directement métabolisés (estérification, transformation et oxydation). L'ACBP semble exercer une double fonction à la fois en constituant un pool d'acides gras activés et en assurant leur transport intracellulaire. Les donnés actuelles suggèrent donc que les fonctions des FABPc et ACBP sont coordonnées et complémentaires. Durant la période postprandiale, les AGLC sont majoritairement réestérifiés en triacylglycérols (TAG) à la surface du réticulum endoplasmique lisse par l'intermédiaire de la voie des 2-monoacylglycérols. La microsomal TAG transfer protein (MTP) assure le passage des TAG néo-synthétisés à l'intérieur du réticulum endoplasmique où associés avec les phospholipides, le cholestérol estérifié ou non et les apoprotéines ils constituent les pré-lipoprotéines. La structuration définitive en lipoprotéines (chylomicrons, VLDL et HDL) a lieu au niveau de l'appareil de Golgi avant exportation vers la lymphe. Au niveau de l'entérocyte, la régulation de l'expression des protéines clefs du métabolisme lipidique est complexe et multiple. Par exemple, il est établi que l'induction des apoprotéines intestinales par des régimes hyperlipidiques est d'origine post-transcriptionelle. En revanche, des travaux récents portant sur l'effet d'hypolipémiants

proliférateurs de péroxysomes de la famille des fibrates montrent que certaines lipid-binding proteins (FAT, FABPc, ACBP) sont contrôlées par un mécanisme coordonné d'origine transcriptionnel impliquant une famille de transprotéines nucléaires: les peroxisome proliferator-activated receptors. Ces transrégulateurs sont également activés par certains AGLC. Or il est établi que ces AGLC contrôlent plusieurs gènes impliqués dans le métabolisme lipidique de l'adipocyte via cette famille de transrégulateurs. Les travaux actuels s'orientent donc vers la mise en évidence d'un mécanisme régulateur semblable au niveau l'entérocyte.

It is well established that lipids play essential roles in the cell either as an energy source, membrane components or precursors of lipid mediators which exert a large variety of regulatory effects. More recently, fatty acids (FA) have been shown also to directly modulate both ion-channel activation and synaptic transmission (Ordway et al. 1991) and to exert cellular regulatory functions as signal-transducing molecules (Ailhaud et al. 1994; Sumida, 1995).

Lipids represent about 40% of the daily energy intake in the Western diet. Triacylglycerols (TAG), which are mainly composed of long-chain FA (LCFA, $\geqslant C_{16}$), are the major form of dietary lipids and are referred to as fats. The other lipids found in the diet are cholesteryl esters (CE) and phospholipids (PL). TAG are hydrolysed in the proximal part of the small intestine to form 2-monoacylglycerols (MAG) and FA through the action of the pancreatic juice. FA absorption from intestinal lumen involves several steps: diffusion through the unstirred water layer (UWL), passage through the plasma membrane, cytosolic activation and esterification, lipoprotein formation and exocytosis. Although the present report describes each of these steps, it focuses more particularly on new insights into the area of intestinal LCFA absorption and metabolism in the small intestine: LCFA uptake by enterocytes, lipid-binding proteins (LBP) and LCFA as putative transcriptional regulators of proteins involved in fat metabolism. For this reason, it is additional to recent reviews published in this field of research (Davidson, 1994; Tso, 1994).

FA are hydrophobic molecules which are transported and metabolized in the aqueous environment of the organism. The structure of FA (number of C atoms and degree of unsaturation) determines their relative water solubility which plays a crucial role in their behaviour and metabolic use. During the postprandial period the LCFA, which are more hydrophobic than the short- and medium-chain FA, are after several metabolic steps preferentially directed towards the lymph, while the shorter-chain FA are essentially found in the blood, bound to albumin. Three strategies are used to solubilize LCFA: formation of mixed micelles, binding with cellular proteins and formation of lipoprotein complexes. Under standard diet conditions, these events occur in the proximal part of the gut (upper third of the jejunal villi). Indeed, this complex organ is organized along the cephalo–caudal and crypt-to-villus axes in regions characterized by a variable phenotypic expression producing regional specialization of function.

CELLULAR UPTAKE OF LONG-CHAIN FATTY ACIDS

The first step of absorption is the entry of LCFA from the bulk lumen phase into the aqueous barrier lining the enterocytes. The water overlying the microvilli of enterocytes is not in equilibrium with the bulk aqueous phase observed in the lumen of the small

intestine. It constitutes a diffusion barrier which is referred to as the UWL. The UWL, which has a thickness of several hundred micrometres, requires the presence of mucus and constitutes a low-pH compartment (Shiau *et al.* 1985). Since the solubility of free LCFA in an aqueous medium is very limited, they will gain access to the microvilli of enterocytes as mixed micelles in association with bile salts. The micellar solubilization increases the aqueous concentration of LCFA 100–1000-fold (Tso, 1994). At the surface of the brush border, the presence of an acidic microclimate promotes micellar diffusion and dissociation. Indeed, at low pH the protonation of LCFA reduces their solubility in the micelles and, thus, promotes their release (Shiau, 1981).

How LCFA move across the biological membranes is a subject of controversy. Since they have a lipophilic character, passive diffusion through the phospholipid bilayer was thought to be the exclusive mechanism involved in FA uptake. For instance, all FA from octanoate (C (8:0) to arachidonate (C (20:4 *n*6) exhibit rapid transbilayer movement ($t_{1/2}$ < 1 s) in the un-ionized form across small egg phosphatidylcholine vesicles (Kamp *et al.* 1993). Considering the fast movement of FA across this protein-free phospholipid bilayer, these authors suggest that a need for a transport protein to increase FA movement across a membrane is unlikely. However, this concept is challenged by the isolation and the characterization of several plasma-membrane proteins (plasma-membrane fatty acid-binding protein (FABPpm; Stremmel *et al.* 1985*a*), P22 (Trigatti *et al.* 1991), fatty acid translocase (FAT; Abumrad *et al.* 1993), fatty acid transfer protein (FATP; Schaffer & Lodish, 1994)) which exhibit high binding affinity for LCFA. Among the numerous lipid-binding proteins expressed in the enterocyte (Table 1), two membrane proteins are found: FABPpm and FAT.

Plasma-membrane fatty acid-binding protein

FABPpm is a 40 kDa protein found in various organs including liver (Stremmel *et al.* 1985*a*), small intestine (Stremmel *et al.* 1985*b*), heart and adipose tissue (Potter *et al.* 1987) and placenta (Campbell *et al.* 1994). This membrane carrier protein is related to mitochondrial glutamic-oxaloacetic transaminase (Berk *et al.* 1990) and the mitochondrial isoform of aspartate aminotransferase (Stump *et al.* 1993). FABPpm seems to have large transport competence since it exhibits high binding affinity not only for LCFA but also lysophosphatidylcholine, MAG and cholesterol (Stremmel, 1988). Immunocytochemical studies demonstrate that FABPpm is expressed in the apical tips of the brush-border villus and crypt of the jejunum and ileum; in contrast, none is found in oesophageal and colonic mucosa (Stremmel *et al.* 1985*b*). It is speculated that FA transport across the jejunal microvilli might be driven by an active Na-dependent process similar to the hepatocellular uptake of LCFA (Stremmel, 1987, 1988). However, the fact that pre-incubation with monospecific FABPpm antibody induces only a partial inhibition of the absorption of LCFA by jejunal explants strongly suggests the existence of a dual uptake mechanism consisting of a passive diffusional transport process and active carrier-mediated translocation (Stremmel, 1988). The regulation of FABPpm expression is poorly understood since the cloning of the protein has not yet been performed.

Fatty acid transporter

Recently, an 88 kDa membrane protein, homologous to CD36 glycoprotein (Tandon *et al.* 1989), was identified then characterized in rat adipocytes by labelling with

sulpho-N-succinimidyl derivatives of LCFA under conditions where FA uptake was significantly inhibited (Harmon et al. 1991; Abumrad et al. 1993). This protein, termed FA transporter (FAT), seems to be involved in facilitating membrane permeation of LCFA and is highly expressed in various tissues including small intestine (Abumrad et al. 1993). In the rat, FAT mRNA level is slightly higher in jejunum, the main site of fat absorption, than in duodenum and is low in ileum. No transcript is found in stomach and colon. A gradient of FAT expression also exists along the crypt-to-villus axis since the FAT is constitutively expressed in the jejunal epithelial cells located in the upper half of the villus, while it is undetectable in the crypt and submucosal cells. These results are confirmed by immunofluorescence using a monospecific antibody raised in the rabbit against the rat FAT protein. In the jejunal mucosa, the fluorescence is limited to the brush border of enterocytes. No signal is found in the goblet cells. This localization and the fact that FAT mRNA level is strongly enhanced along the small intestine by chronic high-fat diets are consistent with the postulated role for this protein in membrane transport of FA (P. Besnard, H. Poirier and P. Degrace, unpublished results).

In conclusion, although the LCFA move rapidly across the artificial protein-free lipid bilayer, FA carrier proteins may be required within membranes to promote cellular uptake of LCFA from the complex environment. Indeed, in the particular case of the enterocyte, the lipid bilayer is not freely accessible to FA because of the high glycoprotein level (mucopolysaccharides from mucus and cell coat) found near the microvilli. The membrane FA-binding proteins expressed in the small intestine might sequester the FA, facilitating their membrane translocation by the formation of a FA gradient. The conformation of FAT, with most of the protein in an extracellular hairpin and with a number of stretches of hydrophobic amino acids forming small hydrophobic pockets (Abumrad et al. 1993), suggest that this protein might act as the LCFA cellular uptake promoter in the small intestine.

INITIAL INTRACELLULAR EVENTS

Once translocated across the microvillus membrane of the enterocyte, LCFA are reversibly and non-covalently bound to cytosolic fatty acid-binding proteins ($FABP_c$) before being activated by long-chain acyl-CoA synthetase (EC 6.2.1.3; ACS). The acyl-CoA esters formed will be either metabolized in endoplasmic reticulum, mitochondria and peroxisomes or bound to acyl-CoA-binding protein (ACBP) to constitute an intracellular acyl-CoA pool (Fig. 1).

Cytosolic fatty acid-binding proteins

For several years, the $FABP_c$ have been the focus of extensive studies and several reviews have been performed recently (Glatz et al. 1993; Veerkamp et al. 1993; Veerkamp, 1995; Veerkamp & Maatman, 1995). The $FABP_c$ are 14–15 kDa cytosolic proteins unrelated to $FABP_{pm}$ (Table 1), which exhibit high affinity for saturated and unsaturated LCFA. They belong to a multigenic family of LBP including presently twelve members (Veerkamp & Maatman, 1995). Two different $FABP_c$ are expressed along the small intestine. The first one, termed intestinal $FABP_c$ (I-FABPc; Alpers et al. 1984) is strictly confined to this organ, while the second one, termed liver $FABP_c$ (L-$FABP_c$; Gordon et al. 1983) is also found in liver and kidney. The ileum co-expresses
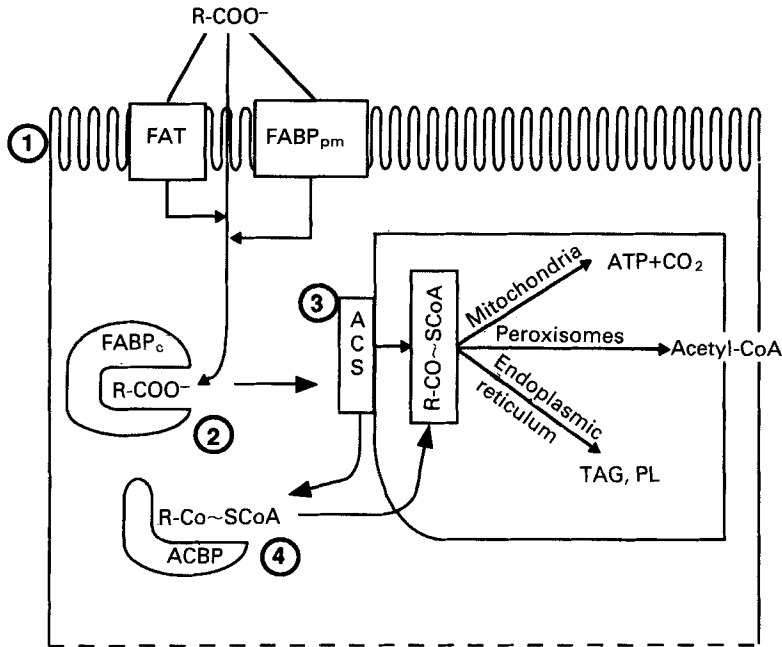
Fig. 1. Putative role of the cytosolic and plasma-membrane fatty acid-binding proteins (FABP$_c$ and FABP$_{pm}$ respectively) and acyl-CoA-binding protein (ACBP) in the small intestine. 1, Membrane transport of long-chain fatty acids (LCFA); 2, cytosolic LCFA pool; 3, activation of LCFA; 4, cytosolic acyl-CoA pool. FAT, fatty acid transporter; ACS, long-chain acyl-CoA synthetase (*EC* 6.2.1.3); TAG, triacylglycerols; PL, phospholipids.

another member of the FABP$_c$ family which binds both bile salts and FA: the ileal LBP (ILBP; Walz *et al.* 1988). I-FABP$_c$ and L-FABP$_c$ are found abundantly in the small intestine (2–3% of total proteins) and their respective mRNA encode 1–2% of the total intestinal mRNA (Bass *et al.* 1985). The intestine maintains a gradient in the expression of fatty acid-binding protein (FABP) genes along the cephalo–caudal and crypt-to-villus axes, the higher expression being found in the tip of the jejunal villi (Shields *et al.* 1986). The organization of the intestinal and liver FABP$_c$ genes is similar since they contain four exons and three introns (Sweetser *et al.* 1987). The introns vary markedly in size. However, they interrupt the coding sequences at the same position. There is an extensive evolutionary conservation of the primary structure of these genes with up to 80% of homology between human and rat L-FABP$_c$. In contrast, I-FABP$_c$ and L-FABP$_c$ are poorly related within a species (only 28% of sequence homology in the rat; Chan *et al.* 1985; Sweetser *et al.* 1987). The three-dimensional structure of the *Escherichia coli*-derived rat I-FABP$_c$ was established by X-ray crystallography. The protein consists of ten anti-parallel β strands organized into two orthogonal β sheets forming a clam shell conformation. The first two β strands are connected by two α helices by which the protonated FA may enter the clam structure. The carboxylate group of the FA is coordinated to the protein through a network of ionic-hydrogen bonding interactions involving several polar amino acid side chains and water molecules within the cavity (Sacchettini *et al.* 1988; Sacchettini & Gordon, 1993). The tertiary structure of the

Table 1. *Major lipid-binding proteins (LBP) expressed in the small intestine*

Protein	Cellular localization	Molecular mass (kDa)	Tissue expression	Ligand(s)	References
Plasma membrane FABP	Plasma membrane	40	Small intestine, liver, adipose tissue, muscle	LCFA, LPC, CS	Stremmel et al. (1985a)
Fatty acid translocase	Plasma membrane	88	Small intestine, adipose tissue, heart	LCFA	Abumrad et al. (1993)
Intestinal cytosolic FABP (FABP$_c$)	Cytosol	14–15	Small intestine	LCFA	Alpers et al. (1984)
Liver FABP$_c$	Cytosol	14–15	Small intestine, liver, kidney	LCFA, haem, CS, eicosanoids, xenobiotics, acyl-CoA, carcinogens	Gordon et al. (1983)
Ileal LBP (I-15-P)	Cytosol	14–15	Ileum	Bile salts, LCFA	Walz et al. (1988)
Cellular retinol-binding protein	Cytosol	15–16	Small intestine	Retinol	Chytil & Ong (1987)
Acyl-CoA-binding protein	Cytosol	10	Ubiquitous	Acyl-CoA	Mogensen et al. (1987)
Sterol carrier protein (SCP2-SCPx)	Cytosol, mitochondria, peroxisome, SER	13–14 58–59	Small intestine, liver, steroidogenic tissues	CS, PL	Noland et al. (1980)
Microsomal triacylglycerol transfer protein	Endoplasmic reticulum	88 + 58	Small intestine, liver	TAG, CE, PC	Lin et al. (1994)

FABP, fatty acid-binding protein; LCFA, long-chain fatty acids; LPC, lysophosphatidylcholine; CS, cholesterol; PL, phospholipids; TAG, triacylglycerols; CE, cholesteryl esters; SER, smooth endoplasmic reticulum.

Table 2. *Binding parameters of cytosolic fatty acid-binding protein (FABP$_c$) expressed in the small intestine*

(Mean values and standard deviations)

	Intestinal FABP$_c$		Liver FABP$_c$				References
No. of binding sites . . .	1		2				Cistola *et al.*
Affinity of binding sites . . .	High		High		Low		(1989, 1990)
	Mean	SD	Mean	SD	Mean	SD	
Dissociation constant (nM)							
Palmitate 16:0	30	2	23	2			
Stearate 18:0	6	1	9	1			
Oleate 18:1*n*-9	39	9	9	2	62	25	Richieri *et al.* (1994)
Linoleate 18:2*n*-6	140	40	23	3	420	250	
Linolenate 18:3*n*-3	480	260	69	12	1000	500	
Arachidonate 20:4*n*-6	350	140	48	9	420	130	

L-FABP$_c$ has not yet been reported, although crystals have been obtained from various mammalian species. The characteristics of ligand binding to I-FABP$_c$ and L-FABP$_c$ have been extensively studied. It is well established that (1) L-FABP has a binding capacity of 2 mol FA/mol protein in contrast to I-FABP$_c$ which has a stoichiometry of 1 mol FA per mol protein (Lowe *et al.* 1987; Cistola *et al.* 1989) and (2) L-FABP$_c$ can bind a large variety of hydrophobic ligands while I-FABP$_c$ only binds FA (Veerkamp *et al.* 1991). Most dissociation constants (K_d) reported in the literature are about 1 mM with little difference in the affinities of FA for I-FABP$_c$ and L-FABP$_c$ and in the binding of various FA to the same FABP$_c$ (for review, see Veerkamp *et al.* 1991). However, recently Richieri *et al.* (1994), using a fluorescent probe technique, have reported quite different findings. They compared the affinities for various FA of six different FABP$_c$, including the intestinal and liver forms. The K_d are low, ranging from about 2 to 1000 nM. I-FABP$_c$ and L-FABP$_c$ exhibit similar K_d values for saturated LCFA. In contrast, one of the binding sites of the L-FABP$_c$ exhibits a higher affinity for unsaturated LCFA than the single binding site of the I-FABP$_c$. The two binding sites of the L-FABP$_c$ are equivalent for saturated FA but differ in affinity by about 10-fold for unsaturated FA, confirming the existence of high- and low-affinity binding sites (Table 2).

The suggested functions generally assigned to FABP$_c$ include: the facilitation of cellular FA uptake and trafficking, FA targeting towards different metabolic pathways, protection against the cytotoxic effects of free FA, modulation of enzyme activity involved in lipid metabolism. Recently, roles in signal transduction pathways (Glatz *et al.* 1995) and in cell proliferation (Sorof, 1994) have also been reported. Although the significance of the two FABP$_c$ types in the intestinal mucosa is always unclear, several explanations have been proposed. In contrast to other cell types using FA, the enterocyte takes up FA from two sources, the intestinal lumen and the blood. In an attractive hypothesis, Alpers *et al.* (1984) suggest that the I-FABP$_c$ might be involved in the transfer of lumen FA for TAG synthesis while L-FABP$_c$ is involved in the transfer of blood-borne FA for oxidation and phospholipid synthesis. Another hypothesis is based on the difference in the binding specificity of the two FABP$_c$. It is suggested that

I-FABP$_c$ would transport only FA of lumen and blood origin, while L-FABP$_c$ would essentially transport other hydrophobic ligands playing the role of intracellular albumin (Cistola *et al.* 1989).

Fatty acid activation

To our knowledge, in contrast to the liver, fatty acid activation is little known in the small intestine. Activation of LCFA is the first reaction in cellular FA metabolism. In the liver, this reaction is catalysed by ACS (Tanaka *et al.* 1979). Mitochondria, endoplasmic reticulum and peroxisomes contain ATP-dependent ACS activity (Krisans *et al.* 1980) which is membrane-bound. In the endoplasmic reticulum and peroxisomes the active site of the ACS is cytosolic. The mitochondrial ACS is an integral enzyme of the outer membrane. In liver ACS has the same amino acid composition in the three organelles, suggesting the existence of a single enzyme (Suzuki *et al.* 1990). It is established that ACS synthesis is down-regulated by acyl-CoA and, thus, enhanced by ACBP and L-FABP$_c$. In liver, ACS mRNA and ACS activity are increased by a high-fat diet and treatment with fat-lowering drugs of the fibrate type (Schoonjans *et al.* 1993).

Acyl-CoA-binding protein

The acyl-CoA-binding protein (ACBP) is an ubiquitous 10 kDa cytosolic protein which binds fatty acyl-CoA with high affinity. Unrelated to the FABP$_c$ family, the ACBP is identical to diazepam-binding inhibitor protein, a mammalian brain neurotransmitter (Knudsen *et al.* 1989). Unlike FABPc, a single form of ACBP is found in organisms. It is particularly highly expressed in the liver, kidney and small intestine (Knudsen, 1990). ACBP has a stoichiometry of 1 mol acyl-CoA per mol protein. *In vitro* binding capacities for different acyl-CoA have been determined using a bovine recombinant ACBP (Rasmussen *et al.* 1994). The binding affinity depends on the length of the acyl chain (K_d, 0·24 (SD 0·02) 10^{-6} M and 0·45 (SD 0·02) 10^{-13} M for octanoyl-CoA and hexadecanoyl-CoA respectively; Rasmussen *et al.* 1994), with the higher affinity for long-chain acyl esters, but it is independent of the number of double bonds (Rosendal *et al.* 1993). ACBP has a high degree of binding specificity since it binds neither free CoA nor FA with measurable affinities. The promoter region of the rat ACBP gene reveals several characteristic features of housekeeping genes (Mandrup *et al.* 1993), which suggests that the protein exerts a basic biochemical function. The ACBP gene consists of four exons and three introns. The eighty-six amino acid residues are organized in four α helices which are folded into a boomerang structure (Knudsen *et al.* 1993; Kragelund *et al.* 1993). ACBP seems to exert a dual function of acyl-CoA pool former and intracellular acyl-CoA transporter. Indeed, the over-expression of ACBP in yeast increases dramatically the pool of long-chain acyl-CoA esters (Mandrup *et al.* 1992) and *in vitro*, the ACBP is able to transport acyl-CoA esters to mitochondria or microsomes and donate them to β oxidation or TAG synthesis (Rasmussen *et al.* 1994).

In conclusion, FABP$_c$ and ACBP might play complementary and coordinate functions in the cell: first, bringing the LCFA to membrane ACS and second playing a role of shuttle between the organelles removing the acyl-CoA from a membrane to offer them to another membrane.
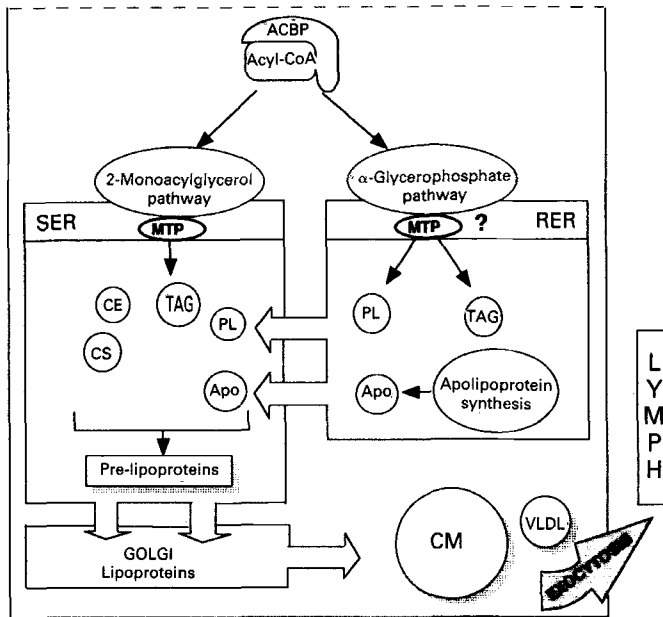
Fig. 2. Lipoprotein synthesis and packaging in the small intestine. SER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum; MTP, microsomal triacylglycerol transfer protein; TAG, triacylglycerols; CS, cholesterol; CE, cholesteryl esters; PL, phospholipids; apo, apoprotein; CM, chylomicrons.

LIPOPROTEIN FORMATION

Although the acyl-CoA esters may also be oxidized and/or transformed (saturated, desaturated, elongated or shortened), they are mainly used in the small intestine for further esterification. The esterified products are associated with apolipoproteins to form lipoproteins.

The esterification step

Two distinct biochemical pathways are involved in TAG synthesis in the enterocyte (Fig. 2). The former, the 2-MAG pathway, uses 2-MAG as an acyl acceptor and the latter, the phosphatidic acid pathway, utilizes α-glycerophosphate as acceptor (for review, see Tso, 1994). During the postprandial period, the 2-MAG pathway is by far the more important, while the α-glycerophosphate pathway becomes dominant during the interprandial period and fasting. The enzymes involved in the 2-MAG pathway are located in the cytoplasmic face of the smooth endoplasmic reticulum (Friedman & Cardell, 1977; Bell *et al.* 1981), whereas those involved in the phosphatidic acid pathway are associated with the rough endoplasmic reticulum (Higgins & Barnett, 1971). After esterification, the newly synthesized TAG enter the cisternae of the endoplasmic reticulum via the microsomal TAG transfer protein (MTP; Lin *et al.* 1994; Table 1 and Fig. 2). This protein, which can also promote cholesteryl ester and phosphatidylcholine transfer, is an heterodimer with two subunits of 58 and 88 kDa. The lack of MTP expression in

Table 3. *Main characteristics of intestinal lipoproteins*

	Chylomicrons	VLDL	HDL
Size (μm)	0·07–0·6	0·028–0·07	0·005–0·015
Major apolipoproteins	B48, AI, AIV	B48, AI, AIV	AI
Major lipids (% of total lipids)	TAG 95	TAG 90	CS 32
Protein content (% of total lipoprotein)	2	10	50

TAG, triacylglycerols; CS, cholesterol.

abetalipoproteinaemic patients whose apo B synthesis was normal, suggests that MTP plays an essential role in lipoprotein assembly and secretion (Wetterau *et al.* 1992).

Lipoprotein assembly and secretion

Since several recent reviews have been devoted to lipoprotein metabolism (Glickman & Sabesin, 1994; Shepherd, 1994; Black, 1995), only the main characteristics of intestinal lipoprotein assembly and exocytosis are reported here.

Morphological aspects of the assembly, intracellular transport and exocytosis of lipoproteins in the small intestine have been investigated by electron microscopy and electron microscope radioautography (Cardell *et al.* 1967; Carlier, 1976; Sabesin & Frase, 1977; Bernard *et al.* 1979). The ultrastructural features of murine enterocytes after the ingestion of a high-fat diet are illustrated in Plate 1. Within minutes of the delivery of the fat bolus, large lipid droplets appear in the endoplasmic reticulum saccules located in the apical cytoplasm of enterocytes (Plate 1 (A)). Then the extension and the swelling of Golgi vesicles containing many structured lipoproteins in the supranuclear area is observed (Plate 1 (B)); these secretory vesicles fuse with the lateral plasma membrane of the enterocyte and deliver the newly synthesized lipoproteins by exocytosis along the lateral plasma membrane (Plate 1 (C)). Finally, lipoproteins may be observed successively in the intercellular spaces, lymphatic capillaries of the villus and in mesenteric lymph (Plate 1 (D–E); Bernard *et al.* 1979; Caselli *et al.* 1979). As shown in Plate 1 (A), no obvious synchronous relationship exists between adjacent enterocytes since one cell may be actively engaged in lipid absorption while others show no indications of such activity (Cardell *et al.* 1967). Several immunological and biochemical observations also demonstrate that lipoproteins are progressively constructed through the endo-membrane system of the cell (Kessler *et al.* 1975; Christensen *et al.* 1987; Fig. 2). However, the molecular mechanism by which the apolipoproteins and the lipid particles are associated is still unknown.

The small intestine produces three different types of lipoproteins which transport and deliver lipids to peripheral tissue and liver (Table 3). In contrast to apo AI and AIV, apo B exhibits two isoforms: apo B-100 and apo B-48 which are produced by the liver and small intestine respectively. A single gene produces these two forms by a post-transcriptional modification of the mRNA, called mRNA editing (Powell *et al.* 1987). Apo B-48 consists of the amino-terminal 48% of apo B-100.

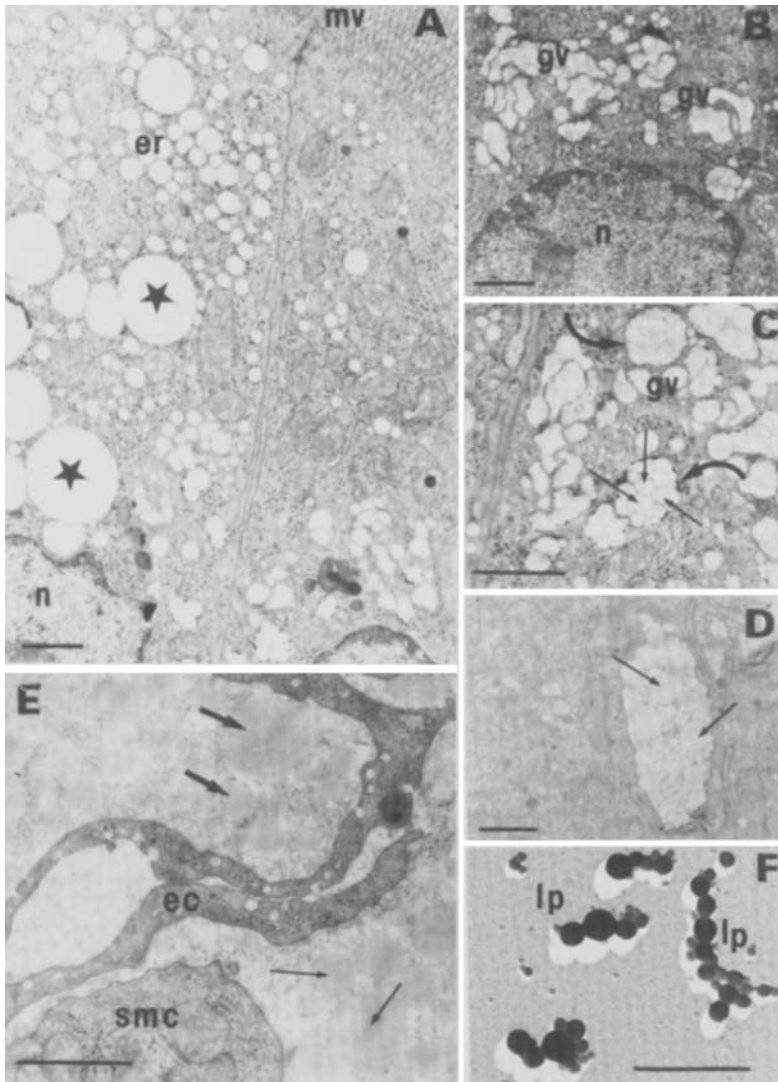Chylomicrons (CM) are large spherical particles produced exclusively by the small

Plate 1. Lipoprotein formation and secretion. (A) Micrograph of jejunum of rat 2 h after ingestion of a high-fat diet containing 200 g canbra oil/kg (×10 000) showing the cytoplasm between microvilli (**mv**) and nucleus (**n**) and many endoplasmic reticulum vesicles (**er**) containing large lipid droplets (★). (B) Micrograph of supranuclear area of rat jejunum 0·5 h after ingestion of a high-fat diet containing 200 g peanut oil/kg. The extension of Golgi vesicles (**gv**) can be observed (×10 000). (C) Micrograph of secretory rat jejunum 0·5 h after ingestion of a high-fat diet containing 200 g canbra oil/kg showing Golgi vesicles (➤) containing structured lipoproteins (→) before their exocytosis (×20 000). (D) Micrograph of an intercellular space between rat jejunum enterocytes 2 h after ingestion of a high-fat diet containing 200 g canbra oil/kg. Many lipoproteins of different size (→) were formed (×10 000). (E) Micrograph of rat mesenteric lymph capillary, 2 h after ingestion of canbra oil. The capillary is largely dilated by the presence of lipoproteins (➤, →). **ec**, endothelial cell; **smc**, smooth muscle cell (×20 000). (F) Micrograph of lymph lipoprotein particles (**lp**) collected by fistulation of the main mesenteric lymph vessel in rat during the third hour after ingestion of a high-fat diet containing 200 g peanut oil/kg (×20 000). All micrographs were obtained using a transmission electron microscope (Hitachi HU 11E).

intestine (Plate 1 (F)). They are TAG-rich lipoproteins mainly comprising dietary lipids and are the major form produced by the small intestine. A smaller TAG-rich molecule, the intestinal VLDL, is synthesized during both the inter-prandial periods and fasting. It transports absorbed biliary lipids and lipids from sloughed enterocytes. The molecular composition of intestinal VLDL is distinct from that of liver VLDL (for details, see Black, 1995). Finally, the small intestine also synthesizes nascent HDL which are involved in reverse cholesterol transport.

REGULATION OF GENES INVOLVED IN FAT METABOLISM

Among all the proteins involved in intestinal LCFA metabolism, the regulation of gene expression of LBP and apolipoproteins has been studied particularly. Knowledge of the molecular events controlling the expression of these proteins is crucial to understanding the different steps of intestinal fat absorption and in the future to correcting some disorders.

Regulation of lipid-binding proteins (LBP)

The fibrates are hypolipidaemic drugs triggering peroxisome proliferation in most rodents. They are strong inducers of several genes involved in lipid metabolism. Recently, we have demonstrated that the L-FABP$_c$ gene is transcriptionally induced by bezafibrate both in liver and small intestine (Besnard et al. 1993; Mallordy et al. 1995; Fig. 3). Interestingly, ACBP and L-FABP$_c$ are enhanced by peroxisome proliferators in the liver (Van Den Heuvel et al. 1993; Sterchele et al. 1994) and FAT mRNA are also highly increased in the duodeno-jejunum from rats or mice chronically treated with bezafibrate (P. Besnard, H. Poirier and P. Degrace, unpublished results). It is well established that peroxisome proliferators exert their genomic effects through a class of transnuclear regulators termed peroxisome proliferator-activated receptors (PPAR; Issemann & Green, 1990). The PPAR are members of the steroid and thyroid superfamily of nuclear receptors (see Keller & Wahli, 1993; Motojima, 1993). Several distinct PPAR have been described which show a distinct spatial and temporal pattern of expression. The binding of a PPAR to a peroxisome proliferator-responsive element (PPRE) found in the promoter of a target gene triggers the transcription process. PPRE consensus sequences are found in the promoter of the L-FABP$_c$ (Issemann et al. 1992; Simon et al. 1993), ACBP (Mandrup et al. 1993). The repeated fourteen-nucleotide sequences found in the promoter of I-FABP$_c$ (Sweetser et al. 1988) exhibit high homology with the PPRE consensus sequence and a direct repeat-like element is found in the promoter of human CD 36, a homologue of FAT (Armesilla & Vega, 1994). Interestingly, PPAR are activated also by physiological concentrations of LCFA (Göttlincher et al. 1992; Schmidt et al. 1992; Dreyer et al. 1993). In adipocytes, LCFA lead to transcriptional induction of various LBP including ALBP (Amri et al. 1991) and Mal 1 (Ibrahimi et al. 1994), the two isoforms of FABP$_c$ expressed in the adipose tissue and FAT (Amri et al. 1995) through the activation of a new member of the PPAR family, termed fatty acid-activated receptor (FAAR; Amri et al. 1995). Since PPARα and FAAR are found in the small intestine (Beck et al. 1992; Amri et al. 1995) and L-FABP$_c$ is transcriptionally up-regulated by dietary fats (Mallordy et al. 1995; Fig. 3), it is tempting to speculate that LBP involved in intestinal FA absorption are regulated via a transprotein activated by LCFA. The fact that jejunal FAT mRNA level (P. Besnard,
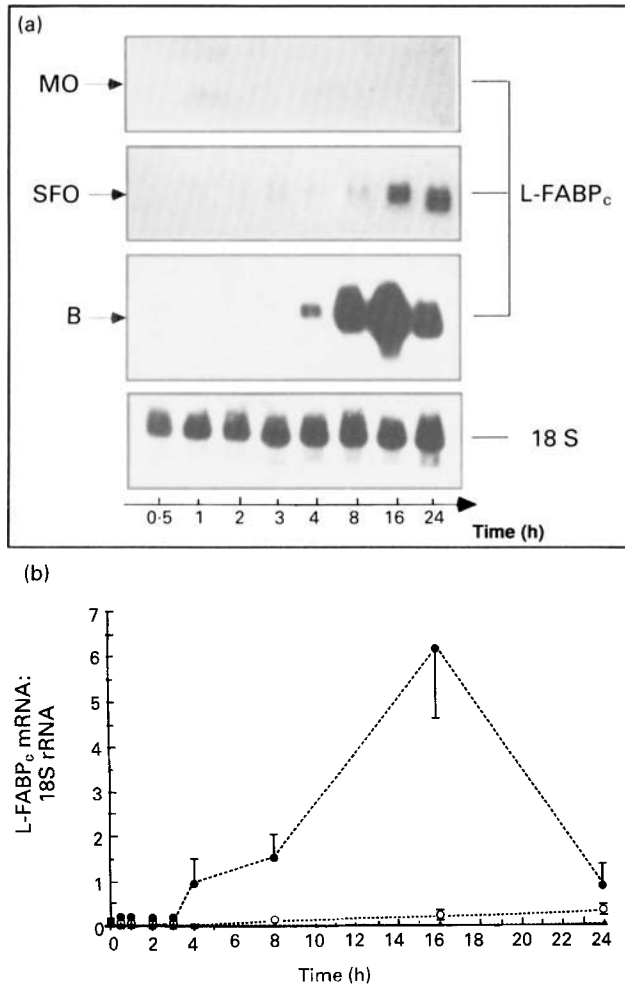
Fig. 3. Time-course of liver fatty acid-binding protein (L-FABP) mRNA production in mouse terminal ileum after direct infusion of mineral oil (MO), sunflower oil (SFO) or bezafibrate (B). (a), Northern-blot analysis of 20 μg total RNA; (b), means of three independent experiments. Mice were infused with 0·2 ml MO (▲), 0·2 ml SO (○), 400 mg B/kg body mass plus SO (●), or were untreated (■). The amounts of total mRNA sampled were checked using an 18S mRNA probe. Values are means with their standard errors represented by vertical bars. (From Mallordy *et al.* 1995. Reproduced with the permission of the *European Journal of Biochemistry*.)

H. Poirier and P. Degrace, unpublished results) and MTP expression (Lin *et al.* 1994) are highly induced in rats fed on various high-fat diets, provides further support for this hypothesis.

Regulation of apolipoproteins

The molecular mechanisms involved in the regulation of the main apolipoproteins expressed in the small intestine are still unknown. However, it has been established that apo B, apo AI and apo AIV genes are up- or down-regulated by members of the orphan

nuclear hormone transcription factors family such as ARP1, HNF4, Ear3/COUP-TF and RXRα (Laudet *et al.* 1992) via unknown factors. Some of these transproteins, such as HNF4 and Ear3/COUP-TF or RXRα and ARP1, exert functional antagonist effects through direct competition for binding to the same DNA response element of the apo AIV (Ochoa *et al.* 1993) and apo A1 (Ge *et al.* 1994) genes respectively. These results suggest that these receptors are closely related to lipid metabolism.

The effect of drugs such as fibrates and high-fat diets on intestinal lipoproteins and LBP expression seem to involve different regulatory mechanisms, since fibrates repress the apo AI gene through a PPAR-independent mechanism (Vu-Dac *et al.* 1994) and dietary lipids induce the apo genes via a post-transcriptional mechanism. Indeed, the results of studies with human subjects indicate that the significant increase in apo B-48 within TAG-rich lipoproteins in postprandial plasma is not due to alterations in apo B gene transcription rate or editing, the major regulatory event being the modulation of the protein intracellular degradation (Lopez-Miranda *et al.* 1994). Moreover, immuno-fluorescence studies have demonstrated the presence of apo-B and apo-A pools in the enterocytes before feeding fat (Glickman *et al.* 1976). In rat pups, the administration of Intralipid promptly increases the apo AIV mRNA level. Since the transcription rate of the apo AIV gene tested by nuclear run-on assay is unchanged, the dietary-fat-dependent level of intestinal apo AIV mRNA appears to be regulated mainly by post-transcriptional events inducing the stabilization of the apo AIV mRNA (Sato *et al.* 1992). Similar results are reported in the piglet (Black *et al.* 1990). In mice, unaltered apo AI mRNA levels found in liver after high-fat diet administration suggest that the increases in plasma apo AI and HDL levels were not the result of a transcriptional mechanism (Srivastava, 1994). In piglets, dietary lipids induce a 2-fold increase in apo AI synthesis in jejunum, but the regulation takes place at a translational level (Trieu *et al.* 1992).

In conclusion, several key proteins involved in intestinal lipid metabolism seem to be regulated by members of the steroid-receptor superfamily which are activated by unknown ligands. These receptors might bind intracellular molecules generated during lipid metabolism, reflecting the instantaneous metabolic state of the cell and bringing the appropriate changes in the gene expression. Identification of such ligands may provide important insights into the molecular mechanisms linking physiological signals to lipid metabolism. Recent findings have shown that LCFA or their derivatives may play this role since they may coordinately regulate several genes involved in lipid metabolism in adipose tissue. The data reported for the LBP strongly suggest that similar regulatory mechanisms also exist in the small intestine.

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