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Lipid-based delivery systems and intestinal lymphatic drug transport: A mechanistic update ☆

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Abstract

After oral administration, the majority of drug molecules are absorbed across the small intestine and enter the systemic circulation via the portal vein and the liver. For some highly lipophilic drugs (typically log $P > 5$, lipid solubility > 50 mg/g), however, association with lymph lipoproteins in the enterocyte leads to transport to the systemic circulation via the intestinal lymph. The attendant delivery benefits associated with lymphatic drug transport include a reduction in first-pass metabolism and lymphatic exposure to drug concentrations orders of magnitude higher than that attained in systemic blood. In the current review we briefly describe the mechanisms by which drug molecules access the lymph and the formulation strategies that may be utilised to enhance lymphatic drug transport. Specific focus is directed toward recent advances in understanding regarding the impact of lipid source (both endogenous and exogenous) and intracellular lipid trafficking pathways on lymphatic drug transport and enterocyte-based first-pass metabolism.

Keywords: Drug; Lymph; Absorption; Intestine; Formulation; Lipid; Delivery; Oral

Contents

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1. Introduction

It is becoming evident that pressure to identify increasingly potent lead compounds is leading to the identification of increasingly lipophilic drug candidates [1]. In response, and as described throughout this theme issue, a growing number of formulation technologies have been developed to support the absorption of compounds with very low water solubility and high lipophilicity. In combination, these factors have led to more common, and more confident, pre-clinical and clinical progression of compounds with physicochemical characteristics that are consistent with the potential for lymphatic transport (typically log $P > 5$, long chain triglyceride (TG) solubility > 50 mg/g) [2]. As such an understanding of the mechanisms of drug access to the intestinal lymph, the potential ramifications of drug transport to the systemic circulation via the intestinal lymph rather than the portal blood and the impact of formulation changes on intestinal lymphatic transport is of significance to a much broader audience.

The area of intestinal lymphatic transport has been reviewed by ourselves and others in recent years [3–6] and the aim of the current article is therefore not to revisit in detail many of the general aspects that have been well covered previously. Rather we focus here on recent advances in our understanding of the mechanisms of drug access to the intestinal lymph and the importance of enterocyte-based lipid processing in determining patterns of lymphatic drug transport.

2. Overview of intestinal lymphatic drug transport

2.1. General aspects of drug access to the intestinal lymph

The basic principles that dictate the mechanism by which drugs enter the intestinal lymph following oral delivery are summarised in Fig. 1. The gastrointestinal tract is richly supplied with both lymphatic and blood vessels and therefore materials that are absorbed across the small intestinal epithelial cells (enterocytes) can potentially enter either lymphatic or blood capillaries. The majority of absorbed materials are transported into the portal blood because the rate of fluid flow in the portal blood is approximately 500-fold higher than that of the intestinal lymph. However, where facile diffusion across the blood capillary endothelium is limited, for example for high molecular weight or colloidal materials, selective transport into the intestinal lymph may occur since the endothelial architecture of the lymphatics dictates that lymphatic capillaries are significantly more permeable than the neighbouring blood capillaries [7]. Absorption of macromolecular drug constructs into and across the enterocyte however, is limited, and drug access to the intestinal lymph more commonly occurs as a result of post-absorptive association with colloidal lipoproteins during transport across the enterocyte (see Fig. 1). The physical size of the lipoproteins subsequently dictates that diffusion across the vascular endothelium is limited and that preferential access of lipoproteins (and associated drug) to the lymphatics occurs. This mechanism is supported by studies demonstrating that the majority of lymphatically transported DDT [8], aryl and alkyl hydrocarbons [9] and halofantrine [10] are solubilised within the apolar lipid core of lymph lipoproteins.

Lymphatic transport has been shown to be a contributor to the oral bioavailability of a number of highly lipophilic drugs and other xenobiotics following oral delivery, including: two lipophilic cannabinoids [184], halofantrine [11,12], moxidectin [13], mepitiostane [14,15], testosterone derivatives [16], MK-386 (a 5α-reductase inhibitor) [17], penclomedine [18], naftifine [19], probucol [20], cyclosporine [21], ontazolast [22], CI-976 [23], fat soluble vitamins and their derivatives, retinoids [24], lycopene, DDT and analogs [8,25], benzopyrene, PCBs (polychlorinated biphenyls) [26] and a number of lipophilic prodrugs [27–29]. In contrast, only very small quantities of more hydrophilic drugs such as salicylic acid, isoniazid and caffeine are recovered in lymph following oral delivery [8].

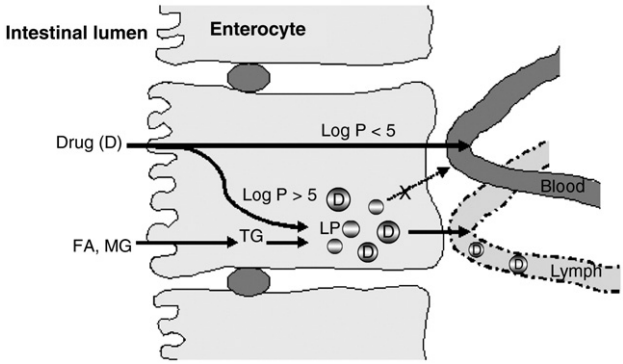

Fig. 1. Drug absorption via the intestinal lymphatic system and portal vein. Both lymph and blood vessels are present in the lamina propria underlying the intestinal absorptive cells (enterocytes) of the intestine. The rate of flow of portal blood, however, is some 500-fold higher than that of the mesenteric lymph and as such, most drugs enter the portal blood more avidly than the mesenteric lymph. In contrast, following uptake into the enterocytes, fatty acid (FA) and monoglyceride (MG) digestion products are resynthesised to triglyceride (TG) and assembled into colloidal lipoproteins (LP) within the endoplasmic reticulum. These LP are exocytosed across the basolateral membrane of the enterocytes and preferentially access the mesenteric lymph vessels as their size precludes easy diffusion across the vascular endothelium. Highly lipophilic drugs (log $P > 5$ and long chain TG solubility > 50 mg/g) may therefore access the intestinal lymph via association with developing lipoproteins in the enterocyte, with the properties of the lipoprotein, rather than the drug, dictating lymphatic access.

2.2. Strategies to enhance lymphatic drug transport

Realisation that drug access to the intestinal lymph is dependent on drug association with developing lipoproteins in the enterocyte suggests that the provision of an appropriate lipid source to drive lipoprotein assembly is a key strategy to enhance lymphatic drug transport. Indeed the efficiency of lipid digestion and solubilisation in the intestinal lumen (reviewed elsewhere in this issue and previously [5,6,30]) and subsequent uptake and transport across intestinal absorptive cells is likely to significantly influence the access of lipophilic drugs to the lymph. Both the type and mass of co-administered lipid can alter the extent of lymphatic drug transport and this subject has been well reviewed previously [3–5,31]. Briefly, fatty acids (FA) with chain lengths of 14 or greater are more highly lymphatically transported (~40–60% of the lipid dose is transported to the systemic circulation via the intestinal lymph with the remainder absorbed via the portal vein blood [32–34]), whereas shorter chain FA, which are more water soluble, are primarily absorbed via the portal blood [35–37]. As such, long chain FA and triglycerides (TG) composed of long chain FA more effectively support lymphatic drug transport than their medium and short chain counterparts [20,24,38–46]. The degree of unsaturation of administered FA also influences the extent of lymphatic lipid and drug transport. In general, mono- and poly-unsaturated FA (MUFA and PUFA, respectively) promote lymphatic lipid transport more readily, produce larger sized lipoproteins and therefore enhance lymphatic drug transport more effectively when compared with the equivalent saturated FA [41–47]. Phospholipids (PL) and in particular phosphatidylcholine (PC) and its digestion product, lyso-phosphatidylcholine (LPC) also enhance lymphatic lipid transport and LPC has been shown to enhance the lymphatic transport of α-tocopherol [48] and more recently, halofantrine [49].

A prodrug approach may also be taken to enhance lymphatic drug transport via the covalent coupling of drugs to lipid moieties including fatty acid, diglyceride or phosphoglyceride [28]. Simple fatty acid esters aim to enhance lipophilicity and therefore drug association with lipoproteins in the enterocyte, however the instability of the ester linkage in the intestine and enterocyte typically results in relatively inefficient lymphatic targeting. Glyceride or phospholipid-based prodrugs, however, are designed to mimic, and intercalate into, the glyceride or phospholipid resynthetic pathways within the enterocyte and are typically more stable and therefore lead to enhanced lymphatic recovery. Lymph-directing prodrug strategies have been reviewed in detail elsewhere [27–29].

2.3. Advantages of intestinal lymphatic drug transport

Due to the unique anatomy and physiology of the lymphatics, intestinal lymphatic drug transport can provide a number of advantages over drug absorption via the portal blood. For example, drugs which are absorbed via the intestinal lymphatic system are essentially protected from hepatic first-pass metabolism since the mesenteric lymph, in contrast to the portal blood, enters the systemic circulation directly without first passing through the liver. For drugs which are highly metabolised on first pass through the liver, transport via the lymphatic system can therefore significantly enhance oral bioavailability. The lymphatic system is also the principle systemic transport pathway for B and T lymphocytes as well as the primary route of metastatic spread of a number of solid tumours [50,51]. As such it has been suggested that immunomodulatory and anticancer compounds may be more effective when absorbed via the lymphatic route [52,53]. Recent evidence further suggests that lymph and lymphoid tissue, and in particular gut associated lymphoid tissue, play a major role in the development of human immunodeficiency virus (HIV) [54,55] and antivirals which target AIDS may therefore be more effective when absorbed via the intestinal lymphatics. Indeed, a recent communication describes the synthesis of lipidic prodrugs of didanosine designed to improve the treatment of HIV [56]. Other viruses may also spread via the lymphatic network including hepatitis B [57], morbillivirus [58] (which also replicates in gut associated lymphoid tissue) and the closely related canine distemper virus [59], severe acute respiratory syndrome (SARS) associated coronavirus [60] and the chronic persistence of hepatitis C is believed to result from uptake into systemic lymphocytes and sequestration into the lymph [61]. Conversely, drug transport via the intestinal lymph results in an altered pattern of local drug exposure to the lymphatics and a changed mode of delivery to the systemic circulation, and therefore potential changes to toxicological profiles are also possible.

3. Recent advances in the understanding of lymphatic drug transport

3.1. Research models

3.1.1. In vivo models

Evaluation of intestinal lymphatic drug transport requires invasive and largely irreversible surgery to access and cannulate the intestinal lymphatic duct. As such, lymphatic drug transport cannot be studied directly in humans. Various animal models have therefore been described in an attempt to quantitate the contribution of the lymphatic system to overall drug absorption [62]. In the majority of cases these pre-clinical models collect the entire volume of lymph flowing through mesenteric or thoracic lymph duct cannulas and therefore provide an absolute indication of the extent of lymphatic transport. Other models have also examined the use of a lympho-venous shunt which has the advantage of allowing sampling of lymph over much longer periods, although in this case the relatively small database for lymph flow rates makes estimation of the absolute extent of lymphatic transport difficult [63]. The majority of lymphatic transport studies described in the literature have utilised rats [62], reflecting the relative ease of sourcing and housing small laboratory animals, however, larger animal models such as dogs [11–13], pigs [64], sheep [65,66] and rabbits [67] have also been described. The advantages of larger animal models include the capacity to dose more clinically relevant full-sized human dosage forms and the ability to administer compounds under more representative fed and fasted

states (rodents do not eat on command). The gastrointestinal tract, transit profile and biliary secretion patterns of dogs and pigs are also more similar to that of humans when compared with rats (in which bile is continuously secreted into the intestine). However, the complexity and cost of larger animal models typically limits widespread application.

Recently, an alternate in vivo approach to the estimation of intestinal lymphatic drug transport has been described in which the systemic exposure of drug is assessed after drug administration in the presence and absence of an inhibitor of intestinal chylomicron flow (e.g. Pluronic-L81 or colchicine) [68]. Comparison of systemic drug exposure profiles in the presence and absence of a functional intestinal lymphatic system provides an indication of the importance (or otherwise) of lymphatic drug transport to overall bioavailability. This approach has the advantage of not requiring the surgical interventions inherent in lymph duct cannulation, however, the broader implications of blocking chylomicron flow and intestinal lipid processing on drug exposure (and indirectly, lymphatic transport) are yet to be studied in detail.

3.1.2. In vitro models

The use of in vitro models as an alternate to in vivo models of assessment of lymphatic drug transport has also been described. For example, Caco-2 cells are well recognised in the pharmaceutical arena as an in vitro model of intestinal epithelium and are widely utilised to screen for intestinal permeability properties. However, Caco-2 cells have also been employed in the lipid biochemistry literature to examine aspects of intracellular lipoprotein assembly [69] and have recently been evaluated as a prospective in vitro model to examine the influence of lipids and lipidic excipients on drug incorporation into lipoproteins and lymphatic transport [70–72]. These data are reviewed in more detail elsewhere in this theme issue [73].

Gershkovich and Hoffman [74] have also suggested that the degree of ex vivo association of drugs with chylomicrons harvested from plasma may be used as a simple predictive tool as to the likely extent of lymphatic drug transport. In these studies, a reasonable linear correlation ($r^2 = 0.94$) was obtained between the extent of lymphatic transport of several lipophilic drugs and their degree of association with plasma chylomicrons ex vivo. Importantly, this correlation was substantially better than that between the extent of lymphatic drug transport and TG solubility or log *P*. An in silico method aimed at developing a quantitative relationship between molecular structure and the extent of intestinal lymphatic drug transfer has also recently been described [75]. The authors found that a relatively complex set of molecular descriptors was required to predict the likelihood of lymphatic transport, although once again the approach appeared to give more accurate predictions than that obtained using traditional descriptors (such as log *P* and TG solubility).

3.2. Lipid transport processes and drug absorption

3.2.1. Lipid transporters and binding proteins

A number of lipid transport proteins have been identified on both the apical and basolateral membranes of enterocytes which, together with several families of intracellular lipid binding proteins, facilitate the absorption and intracellular transport of endogenous and dietary lipids. These transporters and binding proteins have been reviewed in detail elsewhere [76,77]. Fig. 2 depicts a schematic which summarises the lipid transporters and binding proteins that have been implicated in the uptake and transport of various lipids across intestinal epithelial cells. Briefly, lipid uptake across the apical membrane of enterocytes may proceed by either active transport [78,79] or passive diffusion [80] and a number of transporters have been implicated in apical membrane uptake of FA (e.g. CD36/FAT [81,82], scavenger receptor BI (SR-BI) [83,84] and $FABP_{pm}$ [85]) and cholesterol [86] (e.g. SR-BI [87,88], caveolin [89], CD36/FAT [90], aminopeptidase N [86,91], caveolin-1/annexin

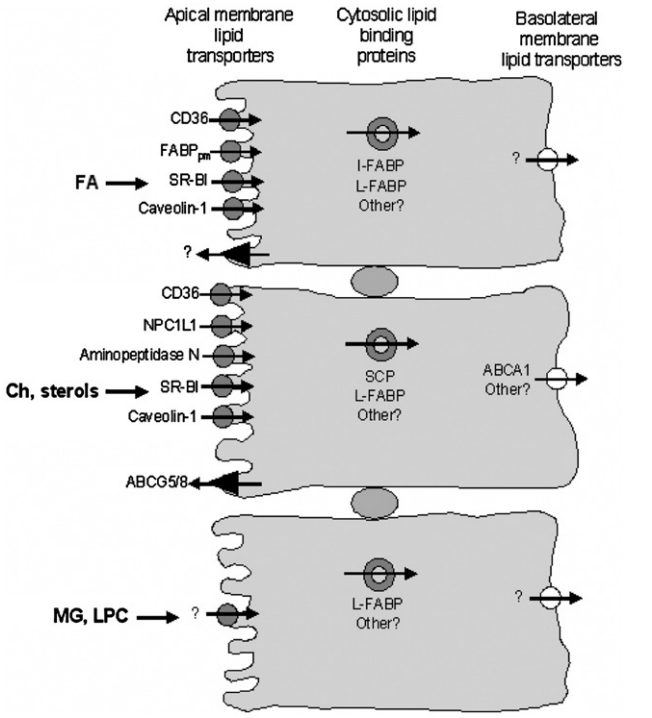

Fig. 2. Lipid transporters and binding proteins involved in intestinal lipid absorption. A number of transporters and binding proteins have been implicated in lipid uptake into and transport across intestinal epithelial cells (enterocytes) although the relative contribution of each of these proteins and indeed the role of passive vs active uptake in the overall intestinal absorption of lipids is yet to be fully resolved. Apical membrane lipid transporters (including CD36/FAT (cluster determinant 36/fatty acid translocase), $FABP_{pm}$ (plasma membrane fatty acid binding protein), SR-BI (scavenger receptor BI), caveolin-1 (which may act in concert with annexin-2), aminopeptidase N, NPC1L1 (Niemann Pick C1-Like 1), (dark circles)) are believed to play a role in the uptake of lipid digestion products including fatty acid (FA), cholesterol (Ch) and other sterols, monoglycerides (MG) and lyso-phosphatidylcholine (LPC) from the intestinal lumen into the enterocytes. Lipid digestion products may also be effluxed from enterocytes back into the intestinal lumen by ABC (ATP-binding cassette) efflux transporters (black triangles). Transport across the enterocyte cytoplasm is thought to be facilitated by intracellular lipid binding proteins (including I-FABP (intestinal fatty acid binding protein), L-FABP (liver fatty acid binding protein), SCP (sterol carrier protein)) (dark rings). Ch exit from enterocytes across the basolateral membrane is facilitated by ABCA1 and a number of as yet unidentified transporters may facilitate exit of other lipids across the basolateral membrane (white circles).

2 complex (the role of which is less clear) [86] and NPC1L1 [92,93]). The relative importance of each of these transporters to overall transport, however, is yet to be distinguished. Protein mediated transfer across the apical membrane of enterocytes has also been demonstrated for lyso-phospholipids and phospholipids [94], cholesterol ester [95] and monoglyceride [96–98] although the transporters involved are less well defined that those for FA and cholesterol.

Several ATP-binding cassette (ABC) transporters have been implicated in lipid uptake across plasma membranes and intracellular lipid trafficking at sites other than the small intestine [99–102]. ABC transporters may therefore be involved in intestinal lipid absorption, although currently, the role of only a few of theses transporters has been demonstrated. For example P-glycoprotein is believed to influence intestinal lipoprotein formation [70,101,103] and it has been suggested that P-glycoprotein facilitates the absorption and intracellular trafficking of cholesterol although the evidence for this is still circumstantial [86]. Additionally, ABCA1 appears to facilitate exsorption of cholesterol across the basolateral membrane of enterocytes to plasma ApoA-1 which enhances the formation of nascent HDL [86,102,104] and ABCG5 and ABCG8 are thought to reduce excess intestinal cholesterol and sterol absorption by facilitating efflux from enterocytes [86,102,105].

More recently, evidence has also been documented of the intact transfer of macromolecular lipid complexes (such as lipoproteins or fatty acids bound to albumin) across the plasma membrane of hepatocytes, adipocytes and endothelial cell lines by incorporation into vesicles derived from the plasma membrane via clathrin- or caveolae-mediated endocytosis [106–108]. As such, endocytosis may play a role in the uptake of dietary lipid from the small intestine in the form of mixed micellar or vesicular species.

Following absorption, lipid digestion products such as FA, Ch, MG and LPC appear to cross the enterocyte cytoplasm by passive diffusion. During cytoplasmic diffusion, intracellular solubilisation of lipids is facilitated by association with intracellular lipid binding proteins (ILBPs) including intestine and liver fatty acid binding proteins (I-FABP and L-FABP respectively) [77,109,110], sterol carrier protein (SCP) [111,112], retinol and retinoic acid binding proteins [113] and ileal bile acid binding protein (I-BABP) [110].

3.2.2. Lipid transporters, binding proteins and lymphatic drug transport

There is increasing, albeit limited, evidence that both membrane-resident lipid transporters and intracellular lipid binding proteins (ILBPs) may impact on the uptake and intracellular disposition and trafficking of drug molecules. This may occur either indirectly via changes to the patterns of intracellular trafficking and disposition of lipids, which in turn alter patters of drug association with intracellular lipid pools; or via direct drug interaction with lipid transporters or ILBPs.

For example, structural studies have demonstrated that I-FABP and L-FABP bind with relatively high affinity to certain drugs, and in particular those with structural similarities to the endogenous ligand FA [114,115]. Drug binding to I-FABP has in addition been shown to enhance the transport of lipophilic drug molecules across a model artificial membrane, where the degree of transport enhancement was related to both drug lipophilicity and I-FABP binding affinity (100). A recent study has also demonstrated a correlation between the level of expression of I-FABP and L-FABP mRNA in small intestinal epithelial cells and the rate of transport of lipid and a model drug (halofantrine) into the intestinal lymph [116]. These results suggest that I-FABP and L-FABP may influence lymphatic drug transport although further data is required to confirm a causal relationship between FABP levels and lymphatic drug transport. It is also unclear whether FABP influences lymphatic drug transport directly by binding to and facilitating drug transport or indirectly by facilitating lymphatic lipid transport. Interestingly, L-FABP and to a lesser extent, I-FABP were recently shown to initiate the ER budding of a pre-chylomicron vesicle which transports lipids from the ER to the Golgi and is the primary rate limiting step of lipid transport into the lymph [117]. L-FABP and I-FABP may therefore have a broader and more influential role in lymphatic lipid transport than previously suspected.

The levels of I-FABP and L-FABP mRNA may be up-regulated acutely by administration of relatively small quantities of lipid over a time course (2–5 h) of potential relevance to the absorption of lipid-based drug formulations, particularly under multiple or chronic dosing situations [116]. These acute data are consistent with previous studies which have demonstrated transcriptional up-regulation of I-FABP and L-FABP following chronic ingestion of high fat diets by both rats and mice [118–120]. Interestingly, the regulatory proteins (e.g. peroxisome proliferator activated receptors (PPAR) [118,121]) which influence FABP transcription and expression in response to lipid ingestion further influence the transcriptional expression of a number of proteins implicated in intestinal lipid absorption [122]. FABP may therefore be only one of a number of co-ordinately regulated proteins which are involved in an acute intestinal response to lipid ingestion and which in turn influence the rate and extent of intestinal lymphatic lipid and drug transport. Clearly, significantly more data is required to confirm (or refute) these suggestions.

3.3. Triglyceride resynthesis and lipoprotein assembly

The pathways which dictate the intestinal uptake and resynthesis of lipid digestion products and subsequent access of lipids to the systemic circulation via either the intestinal lymphatic system or portal vein blood are described in Fig. 3. Essentially, following uptake into the enterocyte, lipid digestion products either diffuse across the cell and enter the portal vein capillaries directly, or are trafficked to the endoplasmic reticulum (ER) where they are resynthesised to TG, PL or CE. TG resynthesis occurs via 2 pathways; the 2-monoglyceride (2-MG) pathway (located in the smooth endoplasmic reticulum (SER)) or the glycerol-3 phosphate (G3P) pathway (located in the rough endoplasmic reticulum (RER)) [30]. In comparison, LPC is resynthesised to PC [123–125] by lyso-phospholipid: Acyl-CoA acyltransferase in the SER and a proportion of the LPC is also hydrolysed to form glycerol-3-phosphorylcholine

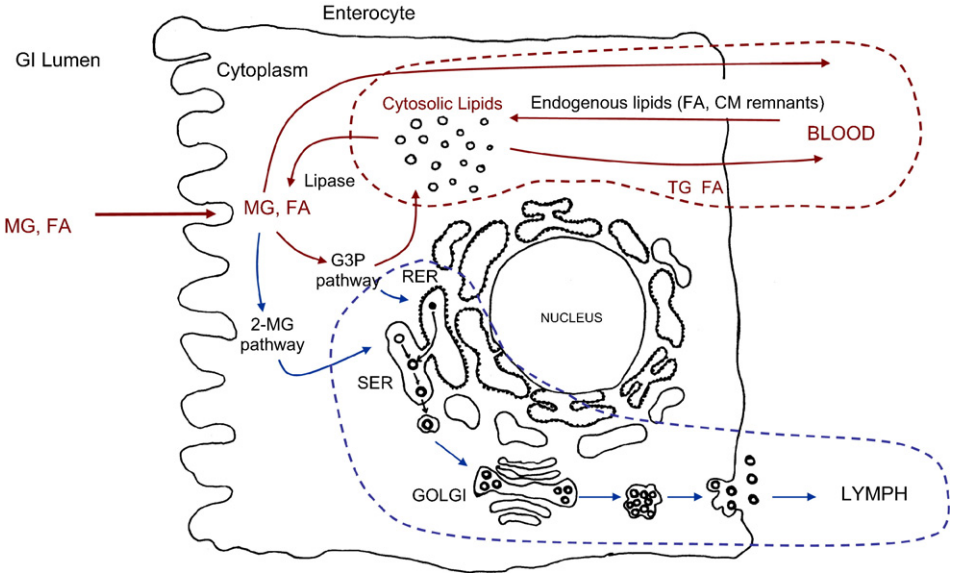

Fig. 3. Intracellular processing of lipids in the enterocyte. Following uptake across the apical membrane of the enterocyte, the products of gastrointestinal (GI) lumen lipid digestion (e.g. monoglyceride (MG) and fatty acid (FA)) may either diffuse across the enterocyte and enter the portal vein blood [34] or be resynthesised to triglyceride via either the 2-monoglyceride (2-MG) pathway associated with the smooth endoplasmic reticulum (SER) or the glycerol-3 phosphate (G3P) pathway associated with the rough endoplasmic reticulum (RER) [134,183]. Triglyceride formed via these pathways may enter the endoplasmic reticular lumen where the triglyceride is assembled into lipoproteins (LP, represented by circles). LP are then transported to the Golgi, exocytosed from the enterocyte and taken up into the intestinal lymphatic system [6]. Since lipid contained within the lipoprotein assembly pathways and the Golgi is destined for transport to the systemic circulation via the intestinal lymphatic system, this pool of lipids is referred to as the lymph lipid precursor pool (dashed blue line) [49,140]. A cytosolic pool of lipids is also located within the enterocytes [49,140]. This lipid pool comprises excess triglyceride formed via the G3P pathway [135] and endogenous lipids taken up from the intestinal blood supply in the form of either FA or chylomicron remnants [137,141]. These cytosolic lipids are subject to hydrolysis by cytosolic lipase [140] and the digestion products so formed may be re-circulated into TG assembly pathways [135]. However, the majority of lipids from this pool exit the enterocyte in the form of TG or free FA and are taken up into portal vein blood [49,140]. The pool of lipids which is transported from the enterocyte via the portal vein is therefore referred to as the portal lipid precursor pool (dashed red line) [49,140]. This figure is modified from reference [5].

which is transported via the portal vein to the liver [126,127]. Ch absorbed from the luminal side of the enterocyte, particularly in the fed state, is esterified to CE by acyl-coA: cholesterol-*O*-acyltransferase 2 (ACAT2) [128–130] and preferentially incorporated into lipoproteins for absorption into the intestinal lymph [42,131,132]. In the fasted state very little Ch is transported into the lymph [131] and most enters a free Ch pool within the enterocyte [133].

The contribution of the 2-MG and G3P pathways to intestinal TG resynthesis is in large part dictated by the sources of lipid present. Thus, the 2-MG pathway is the primary pathway for TG synthesis when exogenous 2-MG is available e.g. following ingestion of TG, diglyceride (DG) or 2-MG [134–136]. In contrast, in the absence of exogenous glycerides (e.g. in the fasted state or following administration of FA, rather than glycerides), the G3P pathway is the major pathway of TG synthesis [134–136]. The major portion of TG formed via the 2-MG pathway crosses the SER membrane and enters lipoprotein assembly pathways [135,136]. Lipids which enter lipoprotein assembly pathways within the enterocyte (such as those resident within the ER and Golgi) and are destined for transport from the enterocyte via the intestinal lymph have been referred to as residing in the lymph lipid precursor pool [49] or chylomicron precursor pool [137]. Since the majority of lipids which enter the lymph lipid precursor pool are formed via the 2-MG pathway, the composition of the lymph lipid precursor pool consists primarily of endogenous lipids in the fasted state but increasingly reflects that of exogenous lipids following ingestion of lipids [49,138].

In addition to the TG formed via the 2-MG pathway, a (relatively minor) fraction of the TG produced via the G3P pathway is also incorporated into lipoproteins [139]. However, the majority of TG synthesised via the G3P pathway enters a pool of lipid droplets which is diffusely distributed throughout the enterocyte cytoplasm. This cytosolic pool of lipids has been referred to as the 'portal lipid precursor pool' since the majority of lipids in this pool are transported to the systemic circulation via the portal vein [140]. Endogenous fatty acids and CM remnants from the intestinal blood supply which are taken up into the enterocytes across the basolateral membrane also enter the portal lipid precursor pool [137,141]. As such, the portal lipid precursor pool consists primarily of basolaterally-sourced endogenous lipids from the intestinal blood supply [137,141] and also endogenous lipids synthesised via the G3P pathway. Since the portal lipid precursor pool is located in the cytoplasm and not surrounded by a membrane, the lipids in the portal lipid precursor pool may be hydrolysed [134,136] by cytosolic lipase [142]. Once hydrolysed, the lipids may be transported to the portal vein or resynthesised via the 2-MG pathway and also incorporated into lipoprotein assembly pathways and the lymph lipid precursor pool [140].

Aspects of lipoprotein assembly occur within both the SER and RER of enterocytes. The first step in the sequential assembly

of lipoproteins involves formation of a primordial lipoprotein within the RER. This is initiated by the co-translational integration of ApoB48 (an apo-protein found on the surface of intestinal lipoproteins and which is believed to be essential for the formation of intestinal lipoproteins) into the RER membrane [143,144] followed by facilitated association of phospholipid with ApoB48 which is mediated by microsomal triglyceride transport protein (MTP) [145,146]. The 'lipidated' ApoB48 (or primordial lipoprotein) is then released into the RER lumen [139]. The second step in the sequential assembly of lipoproteins involves formation of TG droplets within the SER. TG synthesised on the surface of the SER via the 2-MG pathway is believed to enter the SER membrane via a process facilitated by MTP [147,148]. Saturation of the SER membrane by TG occurs relatively rapidly and the TG forms a small lens within the SER membrane which eventually pinches off from the membrane and forms a TG droplet on the luminal side of the SER [149]. The size of the TG droplets formed within the SER and thus the subsequent size of the assembled lipoproteins is believed to depend on the mass and type of administered lipid. The final step of lipoprotein assembly involves fusion of the TG droplets (SER derived) with primordial lipoproteins (RER derived) [139] at the junction of the SER and RER leading to the formation of a nascent lipoprotein. The nascent lipoprotein is subsequently transported in association with a 'pre-chylomicron transport vesicle' (PCTV) to the Golgi apparatus [150] and from the Golgi to the basolateral membrane in Golgi derived vesicles which contain multiple lipoproteins [151]. These vesicles fuse with the basolateral membrane of the enterocytes and the lipoproteins are discharged into the intercellular space underlying the enterocyte from where they are free to diffuse through the connective tissue of the lamina propria to the lymphatic capillaries.

The size, density and therefore types of lipoproteins (e.g. very low density lipoproteins (VLDL) or chylomicrons (CM)) formed in the intestine are thus largely dependent on the mass and type of lipid ingested. In the fasted state or following administration of PL [152], the TG droplets formed within the lymph lipid precursor pool are relatively small leading to the formation of VLDL (diameter 60–80 nm, sedimentation coefficient (S_f) 20–60, $0.93 < \rho$ (density) < 1.006 g/mL) [139,152] which comprise primarily of endogenous lipid resynthesised to triglyceride via the G3P pathway. In contrast, in the postprandial state or after administration of exogenous lipid, the TG droplets formed within the lymph lipid precursor pool are larger leading to the progressive formation and secretion of relatively large and exogenous lipid enriched CM [138,153] (diameter 75–400 nm, $S_f \geq 60$, $\rho < 0.93$ g/mL), in addition to VLDL, into the lymph [43,154,155].

3.4. Endogenous lipids and lymphatic drug transport

3.4.1. Biological sources of endogenous lipids

Whilst lymphatic lipid transport and lipoprotein formation increases substantially following a fatty meal, even in the fasted state, endogenous lipid flux from the intestine to the mesenteric lymph is maintained [156–158]. For example, intestinal VLDL assembled from endogenous lipid sources contribute approximately 11–40% of total fasted plasma TG [137]. These endogenous lipids may enter the enterocyte across the apical (luminal) or basolateral membranes [141]. Apically-sourced endogenous lipids include those in bile and from desquamated enterocytes. Basolaterally-sourced endogenous lipids include fatty acid and CM remnants taken up from the intestinal blood supply [159–161]. CM remnant uptake is mediated by ApoB100 and ApoE receptors on the basolateral membrane [156]. Endogenous lipids may also be synthesised de novo in the enterocyte.

Of these potential sources of endogenous lipid, apically-sourced, biliary derived lipids are the major contributor to lymphatic lipid transport in the fasted state (~50% in rats) and bile diversion substantially reduces fasted lymphatic lipid transport [137]. Cell desquamation provides a minor source of apically-derived materials [157] and the contribution of de novo lipid synthesis in the enterocyte is thought to be even lower [157]. Basolaterally-sourced endogenous lipids from the intestinal blood are thought to predominantly supply the cytosolic portal lipid precursor pool [157], although portal lipid precursor pool lipids may be redirected to the lymph lipid precursor pool via hydrolysis and resynthesis.

Bile-derived PL is also thought to be required for the formation of lipid-rich (CM) lipoproteins [159–164] and appears to enhance both endogenous and exogenous lipid transport into lymph [165]. The ability of PL to enhance lymphatic transport has been elegantly demonstrated in studies using Mdr2 (−/−) mice where biliary phosphatidylcholine secretion is depleted (but normal biliary BS secretion remains). In these animals, postprandial formation of CM does not occur [8,14,166].

3.4.2. Endogenous lipids support lymphatic drug transport

Historically a large mass of exogenous lipid was assumed to be required to stimulate sufficient lymphatic lipid flux and lipoprotein formation to support appreciable lymphatic drug transport. Khoo et al., however, have shown in greyhound dogs that significant fasted state lymphatic drug transport is possible following drug administration with a single unit capsule containing a long chain (LC) lipid-based formulation [12]. The extent of lymphatic drug transport (28.3% of the dose of a model highly lipophilic antimalarial, halofantrine (Hf)) supported by a single unit capsule of formulated LC lipid was substantially greater than the 1.3% recovered in lymph after administration of a lipid free formulation of Hf in the fasted state but was less than the 54% recovered in lymph when dosed following a lipid meal [11]. Interestingly, following administration of the LC lipid formulation, the mass of TG recovered in the lymph (3.4 g over 10 h) was greater than the combination of endogenous TG transport in the lymph in the fasted state (0.5 g over 10 h) and the mass of exogenous lipid dosed (0.58 g), suggesting that administration of the LC lipid formulation led to recruitment of endogenous lipid transport into the lymph. The relatively high extent of lymphatic drug transport therefore appeared to be supported by recruitment of endogenous lymph lipids. The stimulation of endogenous lymphatic transport was

consistent with previous studies in rats where continuous infusion of increasing quantities (from 2.59 mg/h to 26.1 mg/h) of oleic acid over a period of 24 h was shown to increase both endogenous and exogenous lipid transport into intestinal lymph in a dose dependent manner [156,157].

More recently, experiments in lymph-cannulated and bile-duct cannulated rats have further examined the role of endogenous fatty acid recruitment in lymphatic drug transport [167]. In these studies, rats were administered a series of lipid formulations containing halofantrine (Hf), as a model drug, and either 4 mg or 40 mg of oleic acid (OA) over 2 h. The effect of addition of 5 mM bile salt (BS, sodium taurocholate) and lyso-phosphatidylcholine (LPC) was also examined. Administration of 40 mg OA increased both endogenous and exogenous lipid transport into the lymph when compared with administration of either normal saline or 4 mg OA and subsequently enhanced lymphatic drug transport. In contrast, administration of the smaller lipid dose (4 mg of OA) did not stimulate endogenous lymphatic lipid transport above baseline suggesting that endogenous lipid recruitment was (lipid) dose dependent.

Interestingly, addition of 5 mM BS to the 4 mg OA formulation in rats did enhance endogenous lymphatic lipid output and lymphatic transport of Hf (from 7 to 15% of the dose), and in parallel stimulated an increase in biliary lipid secretion. The data therefore suggest that BS infusion stimulates biliary lipid secretion which in turn supports enhanced lymphatic drug transport. In contrast, whilst addition of LPC to 4 mg OA dispersed in BS solution substantially enhanced endogenous lipid transport into lymph, biliary lipid output and lymphatic drug transport did not increase above that obtained after administration of the 4 mg OA/BS formulation alone. Luminal LPC therefore appears to recruit endogenous lipid into the lymph from non-biliary derived sources (possibly via uptake of lipids from the intestinal blood supply across the enterocyte basolateral membrane) and these basolaterally derived lipids are less able to support lymphatic drug transport when compared with biliary derived lipids (see Fig. 4).

3.5. Mucosal lipid pools and lymphatic drug transport

As described in Section 3.3, there are two mucosal pools of lipid within intestinal absorptive cells. The portal lipid precursor pool consists of a number of discrete lipid droplets distributed throughout the enterocyte cytoplasm and the lipids in this pool are predominantly transported to the systemic circulation via the portal vein. In contrast, the lymph lipid precursor pool consists of lipid droplets destined for transport to the systemic circulation via the intestinal lymphatic system and encompasses lipids within lipoprotein assembly pathways in the ER and Golgi. Due to the close relationship between the sources of lipid and synthetic pathways involved in the formation of the portal and lymph lipid precursor pools (see Section 3.3), the size of the two pools is inter-related. Thus, in the fasted state a relatively small amount of lipid is found in both lipid pools [165], whereas, on administration of exogenous lipids the size and turnover rate of both lipid pools may change significantly. For example, on infusion of 135 μmol/h of triolein (TO) to anaesthetised rats the total mass of lipid contained in both the lymph and portal lipid pools increased 6-fold and the amount of lipid in the portal lipid precursor pool increased 8-fold when compared to the fasted state [140,142]. Interestingly, addition of phosphatidylcholine to the infusion of 135 μmol/h TO reduced the mass of lipid in the portal lipid precursor pool and redirected lipids to the lymph lipid precursor pool. This in turn led to a significant increase in lymphatic lipid output in parallel with the increase in lipid in the lymph lipid precursor pool, suggesting that the size of the portal lipid precursor pool was inversely related to the efficiency of lymphatic lipid output [140,142].

Due to the likely high affinity of lymphatically transported drugs for intracellular lipidic domains we recently initiated a series of studies to examine the potential impact of changes to the size and turnover kinetics of the mucosal lipid pools on the intracellular disposition and lymphatic transport of lipophilic drugs [49]. A steady state lymph-cannulated rat model was employed based on models used previously in the lipid

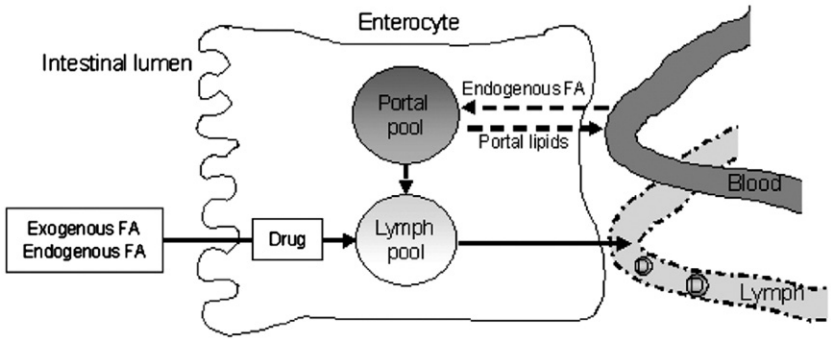

Fig. 4. Schematic representation of the proposed mechanism by which different sources of endogenous lipids support lymphatic drug transport. Administration of exogenous fatty acid (FA) appears to lead to recruitment of endogenous FA into the intestinal lymph. These endogenous FA may enter the enterocyte across the apical (or luminal) membrane (e.g. FA derived from lipids secreted in bile) or the basolateral membrane (e.g. FA from the intestinal blood supply). However, apically- and basolaterally-sourced lipids are trafficked through the pools of lipid within the enterocyte and subsequently enter the lymph in a different manner. Thus, basolaterally-sourced endogenous lipids appear to largely enter the portal lipid precursor pool, from where the majority are transported from the enterocytes to the systemic circulation via the portal vein. A proportion of lipids in the portal lipid precursor pool are also redirected to the lymph lipid precursor pool from where they subsequently enter the lymph. These lipids do not, however, appear to support drug transport into the lymph. In contrast, apically-sourced lipids (either endogenous or exogenous) are trafficked directly into the lymph lipid precursor pool and support drug uptake into the lymph lipid precursor pool and transport into the lymph.

biochemistry literature [168]. To the best of our knowledge, however, this was the first time that the technique had been applied to the study of lymphatic drug transport. A series of lipid-based drug formulations (containing radiolabelled fatty acid (FA) and Hf as a model drug) were administered continuously by intraduodenal infusion to lymph-cannulated rats until steady state rates of FA and drug transport into lymph were achieved. Once steady state was achieved the radiolabelled FA and drug (but not other formulation components) were removed from the infusate allowing assessment of the 'washout profiles' of FA and drug transport into lymph. The first order rate constants describing FA and drug transport from the lymph lipid precursor pool into the lymph were determined from the washout profiles and the mass of FA and drug in the lymph lipid precursor pool was subsequently calculated from the rate of transport of FA and drug into lymph and the rate constants from the washout profiles. The source of endogenous FA in the lipid pool was also probed by comparing the endogenous lipid output in bile in bile-duct cannulated rats with the rate of endogenous lymphatic lipid flux in animals administered the same formulations.

Following continuous administration of low lipid dose formulations containing a long chain length FA (2 or 5 mg of oleic acid (FA)/h) to steady state, the lymph lipid precursor pool and lymph contained primarily endogenous FA rather than exogenous FA. Consistent with previous results [167], the mass of drug solubilised in the lymph lipid precursor pool following administration of small lipid doses was not related to the total mass of lipid in the lymph lipid precursor pool but rather, appeared to depend on the mass of biliary derived endogenous FA (but not other sources of endogenous FA) in the lymph lipid pool. In contrast, exogenous FA was the major lipid source in the lymph lipid pool and lymph following administration of higher lipid dose formulations (20 mg FA/h) and exogenous FA was the primary driver of lymphatic drug transport. The mass of drug in the lymph lipid precursor pool and available for lymphatic transport was proportional to the mass of total (endogenous plus exogenous) FA in the lipid pool following administration of the higher lipid dose formulations.

Increases in the mass of endogenous and exogenous FA in the lymph lipid precursor pool (e.g. following an increase in lipid dose or on addition of BS and LPC to the formulations), were accompanied by increases in the rate of FA transport into the lymph at steady state. However, the rate of lymphatic FA transport did not increase in direct proportion with the increase in size of the lymph lipid pool. Since the rate of FA transport into the lymph is a product of the mass of FA in the lymph lipid pool and the rate constant describing turnover of FA from the pool into the lymph, the greater fractional increase in the mass of lipid in the pool relative to the rate of transport in the lymph indicated a decrease in the magnitude of the turnover rate constant as the pool expanded. This suggested that FA turnover from the lymph lipid precursor pool into the lymph may have a finite capacity which is saturated as the lymph lipid pool expands. This is consistent with previous studies that have suggested that lipid transport through the enterocyte into the lymph may be limited by the rate at which a transport vesicle which carries premature lipoproteins from the ER to the Golgi buds off from the ER membrane [169].

Increases in the mass of drug in the lymph lipid precursor pool were similarly accompanied by increases in the steady state rate of drug transport into the lymph. However, unlike the data with FA, the first order turnover rate constants describing drug transport from the lymph lipid pool into the lymph were relatively constant, regardless of the mass of lipid administered or the size of the lymph lipid pool. Furthermore, in all cases the rate constants describing drug turnover were lower than the corresponding rate constants for lipid. Given that drug is thought to be transported from the lymph lipid precursor pool into the lymph in conjunction with lipid (i.e. in association with lymph lipoproteins) these data were unexpected and suggested the possibility that drug removal from the lymph lipid pool occurred not only via transfer into the lymph, but also via an additional rate process (hence the lower than expected lymph transport rate constant). Subsequent studies suggested that this additional process was that of enterocyte-based metabolism [170]. The impact of lipoprotein association on enterocyte-based metabolism is described in more detail in Section 3.6.2.

Results from these studies therefore demonstrate that the rate and extent of lymphatic drug transport is dependent on the size and turnover kinetics of the lymph lipid precursor pool and that formulation excipients which expand the lymph lipid precursor pool (such as phospholipids [49,140,142]) may enhance lymphatic drug transport. Whilst this early research suggests that relatively small lipid doses (of a size relevant to the development of lipid-based formulations) may alter the intracellular pooling of lipids and thereby impact on intracellular drug disposition, further research is required to define more carefully how these changes impact on lymph-portal drug partitioning as well as overall absorption and bioavailability of lipophilic drugs.

3.6. Lymphatic drug transport and first-pass metabolism

3.6.1. Hepatic first-pass metabolism

The impact of lymphatic drug transport on hepatic first-pass metabolism has been well described in the literature [11,16]. Briefly, however, lymphatically transported drugs are protected from first-pass hepatic metabolism because the mesenteric lymph, unlike the portal blood, empties directly into the systemic circulation without first passing through the liver. For example, testosterone (T) has extremely limited oral bioavailability due to extensive pre-systemic clearance in the intestine and liver [171,172]. By contrast, testosterone undecanoate (TU) [173–175], a highly lipophilic prodrug of T, is orally bioavailable and exhibits androgenic activity after oral administration. The androgenic activity of orally administered TU is generally attributed to systemic T and the active metabolite 5α-dihydrotestosterone (DHT) which are formed from TU after entry into the systemic circulation via the intestinal lymph [16]. The importance of lymphatic transport of TU to systemic T exposure has been examined in greyhound dogs where following postprandial administration of TU, 83–84% of the systemically available T was found to result from

systemic hydrolysis of lymphatically transported TU [16]. Systemic exposure of T in humans also increases substantially following oral TU administration in the fed state, when compared with administration in the fasted state [176,177]. The increase in postprandial exposure of T, therefore likely reflects both enhanced lymphatic transport of TU and an increase in luminal solubilisation of the poorly water soluble steroid.

3.6.2. Enterocyte-based first-pass metabolism

In addition to hepatic first-pass metabolism, enterocyte-based drug metabolism may also be influenced by drug association with lymph lipoproteins. For example, Vetter et al. examined the impact of enterocyte-based metabolism on the lymphatic transport of benzo(*a*)pyrene (BP) following oral delivery to killifish [178,179]. After oral administration, BP and lipid were dispersed in the SI lumen, co-transported across the microvillus membrane and accumulated together within the enterocyte. Eventually, however, the lipid was transported from the intestine into the lymph in the form of lipoproteins, whereas BP was dispersed throughout the cell and did not become associated with lipid. The authors suggested that BP was converted to a more hydrophilic metabolite on contact with the metabolic enzymes situated on the SER and therefore that BP was absorbed into the systemic circulation as a metabolite via the portal vein [178,179]. A further study indicated that the formation of larger fat droplets within the enterocyte following a fatty meal reduced transfer of BP from lipid droplets associated with the SER membrane to microsomal enzymes thereby reducing BP metabolism [178].

As described in Section 3.5, data obtained in our laboratories using the steady state lymph-cannulated rat model have also suggested that drug association with lymph lipoproteins in the lymph lipid pool may alter patterns of drug metabolism in the enterocytes. In these studies, the first order rate constants describing Hf transport from the lymph lipid precursor pool into the lymph were significantly lower than the equivalent rate constants describing FA turnover, following administration of a series of lipid formulations to anaesthetised rats (Table 1) [49,170]. The difference been the rate constants initially suggested a divergence of the two transport processes (lipid vs drug), a contention seemingly at odds with the likely co-transport of drug–lipoprotein complexes into the lymph. An alternative explanation, however, lies in the possibility that removal of Hf from the lymph lipid pool occurred not only by transport into the lymph but also by an additional first order metabolic process. Consistent with this suggestion, Hf is susceptible to first-pass metabolism to desbutylhalofantrine (Hfm) by CYP3A (an enzyme which is also present in the enterocytes), however the contribution of enterocyte-based and hepatic first-pass metabolism to the overall metabolic profile of Hf has not been examined previously. Subsequent studies therefore probed the basis for the difference between the first order rate constants describing Hf and FA transport into the lymph by administering Hf with or without pre-doses of the CYP3A inhibitor, ketoconazole (KC), and also via substitution of Hf with an essentially non-metabolisable probe (DDT). The data confirmed that the first order rate constants for drug and FA turnover into the lymph were not significantly different after administration of either Hf in the presence of KC or DDT, supporting the suggestion that Hf was removed from the lymph lipid precursor pool by enterocyte-based metabolism.

Table 1
The total mass of fatty acid (FA) in the lymph lipid precursor pool (μmol), the first order rate constants describing FA (K_X) and drug (K_D) transport from the lymph lipid precursor pool into the lymph (h^{-1}) and the fractional difference between the first order rate constants describing FA and drug transport from the lymph lipid precursor pool into the lymph $\frac{K_X-K_D}{K_X}$ following continuous intraduodenal infusion of lipid formulations containing a dose of either 100 μg/h halofantrine (Hf) or DDT in 5 mg/h oleic acid (OA) or 20 mg/h OA/5.2 mg/h lyso-phosphatidylcholine (LPC) dispersed in 5 mM bile salt solution, to mesenteric lymph duct cannulated rats (n=4, Mean±SEM)

	Hf in 5 mg /h OA	DDT in 5 mg/h OA	Hf in 20 mg/h OA/LPC	DDT in 20 mg/h OA/LPC
Total FA in the lymph lipid precursor pool (μmol)	23.1±1.5	22.8±2.6	143.3±13.4 [a]	139.0±16.2 [a]
K_X (h^{-1})	0.84±0.07	0.79±0.03	0.46±0.04 [a]	0.49±0.07 [a]
K_D (h^{-1})	0.43±0.03 [b, c]	0.76±0.04	0.30±0.04 [a, b, c]	0.47±0.07 [a]
$\frac{K_X-K_D}{K_X}$	0.48±0.06	0.04±0.07 [d]	0.33±0.08 [a]	0.04±0.03 [d]

This table is reproduced with permission [170].
[a] Statistically different compared to administration of the equivalent formulations containing 5 mg OA ($P<0.05$).
[b] Statistically different to K_X following administration of the same formulation ($P<0.05$).
[c] Statistically different to K_D following administration of the same formulation containing DDT ($P<0.05$).
[d] Statistically different compared to $\frac{K_X-K_D}{K_X}$ following administration of the same formulation with Hf ($P<0.05$).

These data allowed further examination of the relationship between enterocyte-based metabolism and lymphatic drug transport by using the difference between the first order rate constants obtained for Hf and FA transport into the lymph as an indirect indicator of enterocyte-based metabolism. Re-examination of the data in Table 1 therefore suggests that co-administration of Hf with a larger lipid load (20 mg OA/5.2 mg LPC per h compared to 5 mg OA per h) increases the size of the lymph lipid precursor pool (and increases the extent of Hf lymphatic transport), but also reduces the difference between the first order rate constants for Hf and FA turnover into the lymph suggesting a reduction in the rate of enterocyte-based metabolism of Hf in the presence of a larger lymph lipid precursor pool. The mechanism by which co-administration of increasing quantities of lipid reduced enterocyte-based metabolism was not studied but may reflect the sequestration of drug into larger lipid droplets that are formed in the SER at higher lipid doses in turn reducing drug accessibility to metabolic enzymes located on the SER surface [180–182].

Previous studies have shown that the plasma ratio of Hf metabolite (Hfm) to Hf is lower following administration of Hf with a fatty meal [11] and have further suggested that this is, at least in part, due to avoidance of first-pass hepatic metabolism by stimulating lymphatic transport of Hf. This most recent data [49,170] further suggests that stimulation of lymphatic transport of Hf via co-administration with lipid may enhance bioavailability by avoiding both enterocyte-based and hepatic first-pass metabolism.

4. Summary and future perspectives

The identification of increasingly lipophilic drug candidates has dictated a recent increase in interest in the mechanisms by which drugs access the lymph, the formulation approaches that may be taken to maximise or minimise lymphatic transport, and the potential impact of lymphatic transport on drug processing both within the enterocyte and the liver. Stimulation of intestinal lymphatic transport has potential advantages including a reduction in first-pass metabolism and the delivery of high concentrations of drug to the lymphatic system. Whilst recent studies have increased our understanding of the role of lipid precursor pools in lymphatic drug transport and have started to probe the importance of the source of endogenous lipids that might support lymphatic drug transport, the level of mechanistic understanding of drug access to the lymph at a cellular level remains relatively poor. Further increases in useful application of the intestinal lymph as an alternate mode of transport to the systemic circulation are therefore dependent on studies addressing the fundamental mechanism of drug association with lipoproteins in the enterocyte, and the impact of lipids and formulation excipients on this process.

References

[1] C.A. Lipinski, Drug-like properties and the causes of poor solubility and poor permeability, J. Pharmacol. Toxicol. Methods 44 (2000) 235–249.
[2] W.N. Charman, V.J. Stella, Estimating the maximum potential for intestinal lymphatic transport of lipophilic drug molecules, Int. J. Pharm. 34 (1986) 175–178.
[3] C.M. O'Driscoll, Lipid-based formulations for intestinal lymphatic delivery, Eur. J. Pharm. Sci. 15 (2002) 405–415.
[4] C.J.H. Porter, W.N. Charman, Intestinal lymphatic drug transport: an update, Adv. Drug Deliv. Rev. 50 (2001) 61–80.
[5] C.J.H. Porter, N.L. Trevaskis, W.N. Charman, Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs, Nat. Rev. Drug Discov. 6 (2007) 231–248.
[6] C.T. Phan, P. Tso, Intestinal lipid absorption and transport, Front. Biosci. 6 (2001) D299–D319.
[7] L.V. Leak, The structure of lymphatic capillaries in lymph formation, Fed. Proc. 35 (1976) 1863–1871.
[8] S.M. Sieber, V.H. Cohn, W.T. Wynn, The entry of foreign compounds into the thoracic duct lymph of the rat, Xenobiotica 4 (1974) 265–284.
[9] A. Vost, N. Maclean, Hydrocarbon transport in chylomicrons and high-density lipoproteins in rat, Lipids 19 (1984) 423–435.
[10] M.P. McIntosh, C.J.H. Porter, K.M. Wasan, M. Ramaswamy, W.N. Charman, Differences in the lipoprotein binding profile of halofantrine in fed and fasted human or beagle plasma are dictated by the respective masses of core apolar lipoprotein lipid, J. Pharm. Sci. 88 (1999) 378–384.
[11] S.M. Khoo, G.A. Edwards, C.J.H. Porter, W.N. Charman, A conscious dog model for assessing the absorption, enterocyte-based metabolism, and intestinal lymphatic transport of halofantrine, J. Pharm. Sci. 90 (2001) 1599–1607.
[12] S.M. Khoo, D.M. Shackleford, C.J.H. Porter, G.A. Edwards, W.N. Charman, Intestinal lymphatic transport of halofantrine occurs after oral administration of a unit-dose lipid-based formulation to fasted dogs, Pharm. Res. 20 (2003) 1460–1465.
[13] A. Lespine, G. Chanoit, A. Bousquet-Melou, E. Lallemand, F.M. Bassissi, M. Alvinerie, P.L. Toutain, Contribution of lymphatic transport to the systemic exposure of orally administered moxidectin in conscious lymph duct-cannulated dogs, Eur. J. Pharm. Sci. 27 (2006) 37–43.
[14] T. Ichihashi, H. Kinoshita, Y. Takagishi, H. Yamada, Effect of bile on absorption of mepitiostane by the lymphatic system in rats, J. Pharm. Pharmacol. 44 (1992) 565–569.
[15] T. Ichihashi, H. Kinoshita, Y. Takagishi, H. Yamada, Effect of oily vehicles on absorption of mepitiostane by the lymphatic system in rats, J. Pharm. Pharmacol. 44 (1992) 560–564.
[16] D.M. Shackleford, W.A. Faassen, N. Houwing, H. Lass, G.A. Edwards, C.J.H. Porter, W.N. Charman, Contribution of lymphatically transported testosterone undecanoate to the systemic exposure of testosterone after oral administration of two andriol formulations in conscious lymph duct-cannulated dogs, J. Pharmacol. Exp. Ther. 306 (2003) 925–933.
[17] G.Y. Kwei, L.B. Novak, L.H. Hettrick, E.R. Reiss, E.K. Fong, T.V. Olah, A.E. Loper, Lymphatic uptake of MK-386, a sterol 5-alpha reductase inhibitor, from aqueous and lipid formulations, Int. J. Pharm. 164 (1998).
[18] R.A. Myers, V.J. Stella, Factors affecting the lymphatic transport of penclomedine (NSC-338720), a lipophilic cytotoxic drug; comparison to DDT and hexachlorobenzene, Int. J. Pharm. 80 (1992) 51–62.
[19] R.C. Grimus, I. Schuster, The role of the lymphatic transport in the enteral absorption of naftifine by the rat, Xenobiotica 14 (1984) 287–294.
[20] K.J. Palin, C.G. Wilson, The effect of different oils on the absorption of probucol in the rat, J. Pharm. Pharmacol. 36 (1984) 641–643.
[21] C.T. Ueda, M. Lemaire, G. Gsell, K. Nussbaumer, Intestinal lymphatic absorption of cyclosporin A following oral administration in an olive oil solution in rats, Biopharm. Drug. Dispos. 4 (1983) 113–124.
[22] D.J. Hauss, S.E. Fogal, J.V. Ficorilli, C.A. Price, T. Roy, A.A. Jayaraj, J.J. Keirns, Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor, J. Pharm. Sci. 87 (1998) 164–169.
[23] D.J. Hauss, S. Mehta, G.W. Radebaugh, Targeted lymphatic transport and modified systemic distribution of CI-976, a lipophilic lipid-regulator drug, via a formulation approach, Int. J. Pharm. 108 (1994) 85–93.
[24] R. Nankervis, S.S. Davis, N.H. Day, P.N. Shaw, Intestinal lymphatic transport of three retinoids in the rat after oral administration: effect of lipophilicity and lipid vehicle, Int. J. Pharm. 130 (1996) 57–64.
[25] W.N. Charman, V.J. Stella, Effect of lipid class and lipid vehicle volume on the intestinal lymphatic transport of DDT, Int. J. Pharm. 33 (1986) 165–172.
[26] J.M. Laher, M.W. Rigler, R.D. Vetter, J.A. Barrowman, J.S. Patton, Similar bioavailability and lymphatic transport of benzo(*a*)pyrene when administered to rats in different amounts of dietary fat, J. Lipid. Res. 25 (1984) 1337–1342.
[27] V.J. Stella, N.L. Pochopin, Lipophilic prodrugs and the promotion of intestinal lymphatic drug transport, in: W.N. Charman, V.J. Stella (Eds.), Lymphatic Transport of Drugs, CRC press, Boca Raton, 1992, pp. 181–210.
[28] W.N. Charman, C.J.H. Porter, Lipophilic prodrugs designed for intestinal lymphatic transport, Adv. Drug Deliv. Rev. 19 (1996) 149–169.
[29] D.M. Shackleford, C.J.H Porter, W.N. Charman, Lymphatic absorption of orally-administered prodrugs, in: V. J. Stella, R.T. Borchardt, R. Hageman, J.W. Oliyai, J.W. Tilley, H. Magg (Eds), Prodrugs: Challenges and Rewards, AAPS Press, Washington DC, in press.
[30] B.K. Nordskog, C.T. Phan, D.F. Nutting, P. Tso, An examination of the factors affecting intestinal lymphatic transport of dietary lipids, Adv. Drug Deliv. Rev. 50 (2001) 21–44.
[31] W.N. Charman, C.J.H. Porter, S. Mithani, J.B. Dressman, Physiochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH, J. Pharm. Sci. 86 (1997) 269–282.
[32] G.B. McDonald, D.R. Saunders, M. Weidman, L. Fisher, Portal venous transport of long-chain fatty acids absorbed from rat intestine, Am. J. Physiol. 239 (1980) G141–G150.
[33] G.B. McDonald, M. Weidman, Partitioning of polar fatty acids into lymph and portal vein after intestinal absorption in the rat, Q. J. Exp. Physiol. 72 (1987) 153–159.
[34] C.M. Mansbach II, R.F. Dowell, D. Pritchett, Portal transport of absorbed lipids in rats, Am. J. Physiol. 261 (1991) G530–G538.
[35] I.L. Chaikoff, B. Bloom, B.P. Stevens, W.O. Reinhardt, W.G. Dauben, Pentadecanoic acid-5-C14; its absorption and lymphatic transport, J. Biol. Chem. 190 (1951) 431–435.

[36] B. Bloom, I.L. Chaikoff, Reinhardt, Intestinal lymph as pathway for transport of absorbed fatty acids of different chain lengths, Am. J. Physiol. 166 (1951) 451–455.
[37] J.Y. Kiyasu, B. Bloom, I.L. Chaikoff, The portal transport of absorbed fatty acids, J. Biol. Chem. 199 (1952) 415–419.
[38] I. Holmberg, L. Aksnes, T. Berlin, B. Lindback, J. Zemgals, B. Lindeke, Absorption of a pharmacological dose of vitamin D3 from two different lipid vehicles in man: comparison of peanut oil and a medium chain triglyceride, Biopharm. Drug Dispos. 11 (1990) 807–815.
[39] K.J. Palin, C.G. Wilson, S.S. Davis, A.J. Phillips, The effect of oils on the lymphatic absorption of DDT, J. Pharm. Pharmacol. 34 (1982) 707–710.
[40] S.M. Caliph, W.N. Charman, C.J.H. Porter, Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats, J. Pharm. Sci. 89 (2000) 1073–1084.
[41] D.M. Sheehe, J.B. Green, M.H. Green, Influence of dietary fat saturation on lipid absorption in the rat, Atherosclerosis 37 (1980) 301–310.
[42] E.B. Feldman, B.S. Russell, R. Chen, J. Johnson, T. Forte, S.B. Clark, Dietary saturated fatty acid content affects lymph lipoproteins: studies in the rat, J. Lipid Res. 24 (1983) 967–976.
[43] P.H. Green, R.M. Glickman, Intestinal lipoprotein metabolism, J. Lipid Res. 22 (1981) 1153–1173.
[44] M. Cheema, K.J. Palin, S.S. Davis, Lipid vehicles for intestinal lymphatic drug absorption, J. Pharm. Pharmacol. 39 (1987) 55–56.
[45] R.K. Ockner, J.P. Pittman, J.L. Yager, Differences in the intestinal absorption of saturated and unsaturated long chain fatty acids, Gastroenterology 62 (1972) 981–992.
[46] S.E. Bergstedt, H. Hayashi, D. Kritchevsky, P. Tso, A comparison of absorption of glycerol tristearate and glycerol trioleate by rat small intestine, Am. J. Physiol. 259 (1990) G386–G393.
[47] R. Holm, A. Mullertz, E. Christensen, C.E. Hoy, H.G. Kristensen, Comparison of total oral bioavailability and the lymphatic transport of halofantrine from three different unsaturated triglycerides in lymph-cannulated conscious rats, Eur. J. Pharm. Sci. 14 (2001) 331–337.
[48] S.I. Koo, S.K. Noh, Phosphatidylcholine inhibits and lysophosphatidylcholine enhances the lymphatic absorption of alpha-tocopherol in adult rats, J. Nutr. 131 (2001) 717–722.
[49] N.L. Trevaskis, C.J.H. Porter, W.N. Charman, The lymph lipid precursor pool is a key determinant of intestinal lymphatic drug transport, J. Pharmacol. Exp. Ther. 316 (2006) 881–891.
[50] H.A. Cense, C.H. van Eijck, H.W. Tilanus, New insights in the lymphatic spread of oesophageal cancer and its implications for the extent of surgical resection, Best Pract. Res. Clin. Gastroenterol. 20 (2006) 893–906.
[51] M. Arya, S.R. Bott, I.S. Shergill, H.U. Ahmed, M. Williamson, H.R. Patel, The metastatic cascade in prostate cancer, Surg. Oncol. 15 (2006) 117–128.
[52] S. Muranishi, Lymphatic delivery of drugs and its application to cancer chemotherapy, Yakugaku Zasshi 100 (1980) 687–698.
[53] A. Garzon-Aburbeh, J.H. Poupaert, M. Claesen, P. Dumont, G. Atassi, 1,3-dipalmitoylglycerol ester of chlorambucil as a lymphotropic, orally administrable antineoplastic agent, J. Med. Chem. 26 (1983) 1200–1203.
[54] G. Pantaleo, C. Graziosi, J.F. Demarest, O.J. Cohen, M. Vaccarezza, K. Gantt, C. Muro-Cacho, A.S. Fauci, Role of lymphoid organs in the pathogenesis of human immunodeficiency virus (HIV) infection, Immunol. Rev. 140 (1994) 105–130.
[55] G. Pantaleo, C. Graziosi, A.S. Fauci, The role of lymphoid organs in the immunopathogenesis of HIV infection, Aids 7 Suppl 1 (1993) S19–S23.
[56] M. Lalanne, A. Paci, K. Andrieux, N. Dereuddre-Bosquet, P. Clayette, A. Deroussent, M. Re, G. Vassal, P. Couvreur, D. Desmaele, Synthesis and biological evaluation of two glycerolipidic prodrugs of didanosine for direct lymphatic delivery against HIV, Bioorg. Med. Chem. Lett. 17 (2007) 2237–2240.
[57] M. Umeda, H. Marusawa, H. Seno, A. Katsurada, M. Nabeshima, H. Egawa, S. Uemoto, Y. Inomata, K. Tanaka, T. Chiba, Hepatitis B virus infection in lymphatic tissues in inactive hepatitis B carriers, J. Hepatol. 42 (2005) 806–812.
[58] V. von Messling, N. Svitek, R. Cattaneo, Receptor (SLAM [CD150]) recognition and the V protein sustain swift lymphocyte-based invasion of mucosal tissue and lymphatic organs by a morbillivirus, J. Virol. 80 (2006) 6084–6092.
[59] N.T. Lan, R. Yamaguchi, A. Inomata, Y. Furuya, K. Uchida, S. Sugano, S. Tateyama, Comparative analyses of canine distemper viral isolates from clinical cases of canine distemper in vaccinated dogs, Vet. Microbiol. 115 (2006) 32–42.
[60] M. Spiegel, K. Schneider, F. Weber, M. Weidmann, F.T. Hufert, Interaction of severe acute respiratory syndrome-associated coronavirus with dendritic cells, J. Gen. Virol. 87 (2006) 1953–1960.
[61] A. Kessel, E. Toubi, Chronic HCV-related autoimmunity: a consequence of viral persistence and lymphotropism, Curr. Med. Chem. 14 (2007) 547–554.
[62] G.A. Edwards, C.J.H. Porter, S.M. Caliph, S.M. Khoo, W.N. Charman, Animal models for the study of intestinal lymphatic drug transport, Adv. Drug Deliv. Rev. 50 (2001) 45–60.
[63] L. Kagan, P. Gerschkovich, A. Mendelman, S. Amsili, N. Ezov, A. Hoffman, The role of the lymphatic system in subcutaneous absorption of macromolecules in the rat model, Eur. J. Pharm. Biopharm. (in press).
[64] D.G. White, M.J. Story, S.G. Barnwell, An experimental model for studying the effects of a novel lymphatic drug delivery system for propranolol, Int. J. Pharmaceut. 69 (1991) 169.
[65] M. Onizuka, T. Flatebo, G. Nicolaysen, Lymph flow pattern in the intact thoracic duct in sheep, J. Physiol. 503 (Pt 1) (1997) 223–234.
[66] A.M. Segrave, D.E. Mager, S.A. Charman, G.A. Edwards, C.J.H. Porter, Pharmacokinetics of recombinant human leukemia inhibitory factor in sheep, J. Pharmacol. Exp. Ther. 309 (2004) 1085–1092.
[67] V. Bocci, M. Muscettola, G. Grasso, Z. Magyar, A. Naldini, G. Szabo, The lymphatic route. 1) Albumin and hyaluronidase modify the normal distribution of interferon in lymph and plasma, Experientia 42 (1986) 432–433.
[68] A. Dahan, A. Hoffman, Evaluation of a chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs, Eur. J. Pharm. Sci. 24 (2005) 381–388.
[69] E. Levy, M. Mehran, E. Seidman, Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion, Faseb. J. 9 (1995) 626–635.
[70] F. Seeballuck, M.B. Ashford, C.M. O'Driscoll, The effects of pluronics block copolymers and Cremophor EL on intestinal lipoprotein processing and the potential link with P-glycoprotein in Caco-2 cells, Pharm. Res. 20 (2003) 1085–1092.
[71] F. Seeballuck, E. Lawless, M.B. Ashford, C.M. O'Driscoll, Stimulation of triglyceride-rich lipoprotein secretion by polysorbate 80: in vitro and in vivo correlation using Caco-2 cells and a cannulated rat intestinal lymphatic model, Pharm. Res. 21 (2004) 2320–2326.
[72] D.M. Karpf, R. Holm, C. Garafalo, E. Levy, J. Jacobsen, A. Mullertz, Effect of different surfactants in biorelevant medium on the secretion of a lipophilic compound in lipoproteins using Caco-2 cell culture, J. Pharm. Sci. 95 (2006) 45–55.
[73] C.M. O'Driscoll, B.T. Griffin, Biopharmaceutical challenges associated with drugs with low aqueous solubility — the potential impact of lipid based formulations, Adv. Drug Deliv. Rev. 60 (2006) 617–624.
[74] P. Gershkovich, A. Hoffman, Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability, Eur. J. Pharm. Sci. 26 (2005) 394–404.
[75] R. Holm, J. Hoest, Successful in silico predicting of intestinal lymphatic transfer, Int. J. Pharm. 272 (2004) 189–193.
[76] W. Stremmel, L. Pohl, A. Ring, T. Herrmann, A new concept of cellular uptake and intracellular trafficking of long-chain fatty acids, Lipids 36 (2001) 981–989.
[77] L.B. Agellon, M.J. Toth, A.B. Thomson, Intracellular lipid binding proteins of the small intestine, Mol. Cell. Biochem. 239 (2002) 79–82.
[78] W. Stremmel, Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein, J. Clin. Invest. 82 (1988) 2001–2010.
[79] S.L. Chow, D. Hollander, A dual, concentration-dependent absorption mechanism of linoleic acid by rat jejunum in vitro, J. Lipid Res. 20 (1979) 349–356.
[80] E.W. Strauss, Electron microscopic study of intestinal fat absorption in vitro from mixed micelles containing linolenic acid, monoolein, and bile salt, J. Lipid Res. 7 (1966) 307–323.

[81] G. Endemann, L.W. Stanton, K.S. Madden, C.M. Bryant, R.T. White, A.A. Protter, CD36 is a receptor for oxidized low density lipoprotein, J. Biol. Chem. 268 (1993) 11811–11816.
[82] M. Febbraio, D.P. Hajjar, R.L. Silverstein, CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism, J. Clin. Invest. 108 (2001) 785–791.
[83] M. Krieger, Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems, J. Clin. Invest. 108 (2001) 793–797.
[84] S.T. Thuahnai, S. Lund-Katz, D.L. Williams, M.C. Phillips, Scavenger receptor class B, type I-mediated uptake of various lipids into cells. Influence of the nature of the donor particle interaction with the receptor, J. Biol. Chem. 276 (2001) 43801–43808.
[85] G. Rajaraman, M.S. Roberts, D. Hung, G.Q. Wang, F.J. Burczynski, Membrane binding proteins are the major determinants for the hepatocellular transmembrane flux of long-chain fatty acids bound to albumin, Pharm. Res. 22 (2005) 1793–1804.
[86] E. Levy, S. Spahis, D. Sinnett, N. Peretti, F. Maupas-Schwalm, E. Delvin, M. Lambert, M.A. Lavoie, Intestinal cholesterol transport proteins: an update and beyond, Curr. Opin. Lipidol. 18 (2007) 310–318.
[87] H. Hauser, J.H. Dyer, A. Nandy, M.A. Vega, M. Werder, E. Bieliauskaite, F.E. Weber, S. Compassi, A. Gemperli, D. Boffelli, E. Wehrli, G. Schulthess, M.C. Phillips, Identification of a receptor mediating absorption of dietary cholesterol in the intestine, Biochemistry 37 (1998) 17843–17850.
[88] F. Bietrix, D. Yan, M. Nauze, C. Rolland, J. Bertrand-Michel, C. Comera, S. Schaak, R. Barbaras, A.K. Groen, B. Perret, F. Terce, X. Collet, Accelerated lipid absorption in mice overexpressing intestinal SR-BI, J. Biol. Chem. 281 (2006) 7214–7219.
[89] M. Murata, J. Peranen, R. Schreiner, F. Wieland, T.V. Kurzchalia, K. Simons, VIP21/caveolin is a cholesterol-binding protein, Proc. Natl. Acad. Sci. U S A 92 (1995) 10339–10343.
[90] H. Poirier, P. Degrace, I. Niot, A. Bernard, P. Besnard, Localization and regulation of the putative membrane fatty-acid transporter (FAT) in the small intestine. Comparison with fatty acid-binding proteins (FABP), Eur. J. Biochem. 238 (1996) 368–373.
[91] W. Kramer, F. Girbig, D. Corsiero, A. Pfenninger, W. Frick, G. Jahne, M. Rhein, W. Wendler, F. Lottspeich, E.O. Hochleitner, E. Orso, G. Schmitz, Aminopeptidase N (CD13) is a molecular target of the cholesterol absorption inhibitor ezetimibe in the enterocyte brush border membrane, J. Biol. Chem. 280 (2005) 1306–1320.
[92] H.R. Davis Jr., L.J. Zhu, L.M. Hoos, G. Tetzloff, M. Maguire, J. Liu, X. Yao, S.P. Iyer, M.H. Lam, E.G. Lund, P.A. Detmers, M.P. Graziano, S.W. Altmann, Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis, J. Biol. Chem. 279 (2004) 33586–33592.
[93] S.W. Altmann, H.R. Davis Jr., L.J. Zhu, X. Yao, L.M. Hoos, G. Tetzloff, S.P. Iyer, M. Maguire, A. Golovko, M. Zeng, L. Wang, N. Murgolo, M.P. Graziano, Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption, Science 303 (2004) 1201–1204.
[94] Z. Zhang, J.W. Nichols, Protein-mediated transfer of fluorescent-labeled phospholipids across brush border of rabbit intestine, Am. J. Physiol. 267 (1994) G80–G86.
[95] S. Compassi, M. Werder, D. Boffelli, F.E. Weber, H. Hauser, G. Schulthess, Cholesteryl ester absorption by small intestinal brush border membrane is protein-mediated, Biochemistry 34 (1995) 16473–16482.
[96] S.Y. Ho, L. Delgado, J. Storch, Monoacylglycerol metabolism in human intestinal Caco-2 cells: evidence for metabolic compartmentation and hydrolysis, J. Biol. Chem. 277 (2002) 1816–1823.
[97] S.Y. Ho, J. Storch, Common mechanisms of monoacylglycerol and fatty acid uptake by human intestinal Caco-2 cells, Am. J. Physiol. Cell Physiol. 281 (2001) C1106–C1117.
[98] K. Murota, J. Storch, Uptake of micellar long-chain fatty acid and *sn*-2-monoacylglycerol into human intestinal Caco-2 cells exhibits characteristics of protein-mediated transport, J. Nutr. 135 (2005) 1626–1630.
[99] B. Sarkadi, L. Homolya, G. Szakacs, A. Varadi, Human multidrug resistance ABCB and ABCG transporters: participation in a chemoimmunity defense system, Physiol. Rev. 86 (2006) 1179–1236.
[100] K. Wakabayashi, A. Tamura, H. Saito, Y. Onishi, T. Ishikawa, Human ABC transporter ABCG2 in xenobiotic protection and redox biology, Drug Metab. Rev. 38 (2006) 371–391.
[101] P. Borst, N. Zelcer, A. van Helvoort, ABC transporters in lipid transport, Biochim. Biophys. Acta 1486 (2000) 128–144.
[102] G. Schmitz, T. Langmann, S. Heimerl, Role of ABCG1 and other ABCG family members in lipid metabolism, J. Lipid Res. 42 (2001) 1513–1520.
[103] F.J. Field, S.N. Mathur, Intestinal lipoprotein synthesis and secretion, Prog. Lipid Res. 34 (1995) 185–198.
[104] J.J. Repa, S.D. Turley, J.A. Lobaccaro, J. Medina, L. Li, K. Lustig, B. Shan, R.A. Heyman, J.M. Dietschy, D.J. Mangelsdorf, Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers, Science 289 (2000) 1524–1529.
[105] K.E. Berge, H. Tian, G.A. Graf, L. Yu, N.V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, H.H. Hobbs, Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters, Science 290 (2000) 1771–1775.
[106] A. Ring, J. Pohl, A. Volkl, W. Stremmel, Evidence for vesicles that mediate long-chain fatty acid uptake by human microvascular endothelial cells, J. Lipid Res. 43 (2002) 2095–2104.
[107] J. Pohl, A. Ring, W. Stremmel, Uptake of long-chain fatty acids in HepG2 cells involves caveolae: analysis of a novel pathway, J. Lipid. Res. 43 (2002) 1390–1399.
[108] J. Pohl, A. Ring, R. Ehehalt, H. Schulze-Bergkamen, A. Schad, P. Verkade, W. Stremmel, Long-chain fatty acid uptake into adipocytes depends on lipid raft function, Biochemistry 43 (2004) 4179–4187.
[109] N.M. Bass, The cellular fatty acid binding proteins: aspects of structure, regulation, and function, Int. Rev. Cytol. 111 (1988) 143–184.
[110] P. Besnard, I. Niot, H. Poirier, L. Clement, A. Bernard, New insights into the fatty acid-binding protein (FABP) family in the small intestine, Mol. Cell. Biochem. 239 (2002) 139–147.
[111] E.J. Murphy, Sterol carrier protein-2: not just for cholesterol any more, Mol. Cell. Biochem. 239 (2002) 87–93.
[112] U. Seedorf, P. Ellinghaus, J. Roch Nofer, Sterol carrier protein-2, Biochim. Biophys. Acta. 1486 (2000) 45–54.
[113] E.H. Harrison, Mechanisms of digestion and absorption of dietary vitamin A, Annu. Rev. Nutr. 25 (2005) 87–103.
[114] T. Velkov, J. Horne, A. Laguerre, E. Jones, M.J. Scanlon, C.J.H. Porter, Examination of the role of intestinal fatty acid-binding protein in drug absorption using a parallel artificial membrane permeability assay, Chem. Biol. 14 (2007) 453–465.
[115] T. Velkov, S. Chuang, J. Wielens, H. Sakellaris, W.N. Charman, C.J.H. Porter, M.J. Scanlon, The interaction of lipophilic drugs with intestinal fatty acid-binding protein, J. Biol. Chem. 280 (2005) 17769–17776.
[116] N.L. Trevaskis, C.M. Lo, L.Y. Ma, P. Tso, H.R. Irving, C.J. Porter, W.N. Charman, An acute and coincident increase in FABP expression and lymphatic lipid and drug transport occurs during intestinal infusion of lipid-based drug formulations to rats, Pharm. Res. 23 (2006) 1786–1796.
[117] I. Neeli, S.A. Siddiqi, S. Siddiqi, J. Mahan, W.S. Lagakos, B. Binas, T. Gheyi, J. Storch, C.M. Mansbach II, Liver fatty acid-binding protein initiates budding of pre-chylomicron transport vesicles from intestinal endoplasmic reticulum, J. Biol. Chem. 282 (2007) 17974–17984.
[118] H. Poirier, I. Niot, M.C. Monnot, O. Braissant, C. Meunier-Durmort, P. Costet, T. Pineau, W. Wahli, T.M. Willson, P. Besnard, Differential involvement of peroxisome-proliferator-activated receptors alpha and delta in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine, Biochem. J. 355 (2001) 481–488.
[119] H. Poirier, I. Niot, P. Degrace, M.C. Monnot, A. Bernard, P. Besnard, Fatty acid regulation of fatty acid-binding protein expression in the small intestine, Am. J. Physiol. 273 (1997) G289–G295.
[120] P. Besnard, I. Niot, H. Poirier, L. Clement, A. Bernard, New insights into the fatty acid-binding protein (FABP) family in the small intestine, Mol. Cell. Biochem. 239 (2002) 139–147.
[121] T.M. Willson, P.J. Brown, D.D. Sternbach, B.R. Henke, The PPARs: from orphan receptors to drug discovery, J. Med. Chem. 43 (2000) 527–550.
[122] A. Chawla, J.J. Repa, R.M. Evans, D.J. Mangelsdorf, Nuclear receptors and lipid physiology: opening the X-files, Science 294 (2001) 1866–1870.

[123] A. Nilsson, Intestinal absorption of lecithin and lysolecithin by lymph fistula rats, Biochim. Biophys. Acta 152 (1968) 379–390.
[124] Y. Sato, The metabolic fate of lysolecithin administered into rat duodenal lumen, Tohoku J. Exp. Med. 100 (1970) 277–287.
[125] R.O. Scow, Y. Stein, O. Stein, Incorporation of dietary lecithin and lysolecithin into lymph chylomicrons in the rat, J. Biol. Chem. 242 (1967) 4919–4924.
[126] D. Le Kim, H. Betzing, Intestinal absorption of polyunsaturated phosphatidylcholine in the rat, Hoppe-Seyler Z. Physiol. Chem. 357 (1976) 1321–1331.
[127] A. Ottolenghi, Estimation and subcellular distribution of lecithinase activity in rat intestinal mucosa, J. Lipid Res. 53 (1964) 532–537.
[128] S.B. Clark, A.M. Tercyak, Reduced cholesterol transmucosal transport in rats with inhibited mucosal acyl CoA:cholesterol acyltransferase and normal pancreatic function, J. Lipid Res. 25 (1984) 148–159.
[129] L.L. Gallo, T. Newbill, J. Hyun, G.V. Vahouny, C.R. Treadwell, Role of pancreatic cholesterol esterase in the uptake and esterification of cholesterol by isolated intestinal cells, Proc. Soc. Exp. Biol. Med. 156 (1977) 277–281.
[130] C.R. Borja, G.V. Vahouny, C.R. Treadwell, Role of bile and pancreatic juice in cholesterol absorption and esterification, Am. J. Physiol. 206 (1964) 223–228.
[131] E.F. Stange, J.M. Dietschy, The origin of cholesterol in the mesenteric lymph of the rat, J. Lipid Res. 26 (1985) 175–184.
[132] C. Pool, D.F. Nutting, W.J. Simmonds, P. Tso, Effect of Pluronic L81, a hydrophobic surfactant, on intestinal mucosal cholesterol homeostasis, Am. J. Physiol. 261 (1991) G256–G262.
[133] B. Borgstrom, Luminal digestion of fats, in: V.L. Go (Ed.), The exocrine pancreas, Raven press, 1986, pp. 361–373.
[134] R. Lehner, A. Kuksis, Biosynthesis of triacylglycerols, Prog. Lipid Res. 35 (1996) 169–201.
[135] L.Y. Yang, A. Kuksis, Apparent convergence (at 2-monoacylglycerol level) of phosphatidic acid and 2-monoacylglycerol pathways of synthesis of chylomicron triacylglycerols, J. Lipid Res. 32 (1991) 1173–1186.
[136] L.Y. Yang, A. Kuksis, J.J. Myher, Biosynthesis of chylomicron triacylglycerols by rats fed glyceryl or alkyl esters of menhaden oil fatty acids, J. Lipid Res. 36 (1995) 1046–1057.
[137] C.M. Mansbach, R.F. Dowell, Uptake and metabolism of circulating fatty acids by rat intestine, Am. J. Physiol. 263 (1992) G927–G933.
[138] C.M. Mansbach, P. Nevin, Intracellular movement of triacylglycerols in the intestine, J. Lipid Res. 39 (1998) 963–968.
[139] M.M. Hussain, A proposed model for the assembly of chylomicrons, Atherosclerosis 148 (2000) 1–15.
[140] A.D. Tipton, S. Frase, C.M. Mansbach, Isolation and characterization of a mucosal triacylglycerol pool undergoing hydrolysis, Am. J. Physiol. 257 (1989) G871–G878.
[141] C.M. Mansbach, R.F. Dowell, Role of the intestine in chylomicron remnant clearance, Am. J. Physiol. 269 (1995) G144–G152.
[142] P. Nevin, D. Koelsch, C.M. Mansbach II, Intestinal triacylglycerol storage pool size changes under differing physiological conditions, J. Lipid. Res. 36 (1995) 2405–2412.
[143] M.M. Hussain, R.K. Kancha, Z. Zhou, J. Luchoomun, H. Zu, A. Bakillah, Chylomicron assembly and catabolism: role of apolipoproteins and receptors, Biochim. Biophys. Acta 1300 (1996) 151–170.
[144] T.A. Rapoport, Transport of proteins across the endoplasmic reticulum membrane, Science 258 (1992) 931–936.
[145] D.A. Gordon, H. Jamil, D. Sharp, D. Mullaney, Z. Yao, R.E. Gregg, J. Wetterau, Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability, Proc. Natl. Acad. Sci. U S A 91 (1994) 7628–7632.
[146] S.B. Patel, S.M. Grundy, Interactions between microsomal triglyceride transfer protein and apolipoprotein B within the endoplasmic reticulum in a heterologous expression system, J. Biol. Chem. 271 (1996) 18686–18694.
[147] M.M. Hussain, J. Iqbal, K. Anwar, P. Rava, K. Dai, Microsomal triglyceride transfer protein: a multifunctional protein, Front. Biosci. 8 (2003) s500–s506.
[148] M.M. Hussain, J. Shi, P. Dreizen, Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly, J. Lipid Res. 44 (2003) 22–32.
[149] D. Atkinson, D.M. Small, Recombinant lipoproteins: implications for structure and assembly of native lipoproteins, Annu. Rev. Biophys. Biophys. Chem. 15 (1986) 403–456.
[150] N.S. Kumar, C.M. Mansbach II, Prechylomicron transport vesicle: isolation and partial characterization, Am. J. Physiol. 276 (1999) G378–G386.
[151] R.W. Mahley, B.D. Bennett, D.J. Morre, M.E. Gray, W. Thistlethwaite, V.S. LeQuire, Lipoproteins associated with the Golgi apparatus isolated from epithelial cells of rat small intestine, Lab. Invest. 25 (1971) 435–444.
[152] P. Tso, D.S. Drake, D.D. Black, S.M. Sabesin, Evidence for separate pathways of chylomicron and very low-density lipoprotein assembly and transport by rat small intestine, Am. J. Physiol. 247 (1984) G599–G610.
[153] C.M. Mansbach II, S. Parthasarathy, A re-examination of the fate of glyceride-glycerol in neutral lipid absorption and transport, J. Lipid Res. 23 (1982) 1009–1019.
[154] N.O. Davidson, M.E. Kollmer, R.M. Glickman, Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid, J. Lipid Res. 27 (1986) 30–39.
[155] P. Tso, M.B. Lindstrom, B. Borgstrom, Factors regulating the formation of chylomicrons and very-low-density lipoproteins by the rat small intestine, Biochim. Biophys. Acta 922 (1987) 304–313.
[156] J. Baxter, Origin and characteristics of endogenous lipid in thoracic duct lymph in rat, J. Lipid Res. 7 (1966) 158–166.
[157] Y.F. Shiau, D.A. Popper, M. Reed, C. Umstetter, D. Capuzzi, G.M. Levine, Intestinal triglycerides are derived from both endogenous and exogenous sources, Am. J. Physiol 248 (1985) G164–G169.
[158] R.K. Ockner, F.B. Hughes, K.J. Isselbacher, Very low density lipoproteins in intestinal lymph: origin, composition and role in lipid transport in the fasting state, J. Clin. Invest. 48 (1969) 2079–2088.
[159] P. Tso, J.A. Balint, Formation and transport of chylomicrons by enterocytes to the lymphatics, Am. J. Physiol. 250 (1986) G715–G726.
[160] P. Tso, J.A. Balint, W.J. Simmonds, Role of biliary lecithin in lymphatic transport of fat, Gastroenterology 73 (1977) 1362–1367.
[161] C.M. Mansbach II, A. Arnold, M.A. Cox, Factors influencing triacylglycerol delivery into mesenteric lymph, Am. J. Physiol. 249 (1985) G642–G648.
[162] P.J. Voshol, D.M. Minich, R. Havinga, R.P. Elferink, H.J. Verkade, A.K. Groen, F. Kuipers, Postprandial chylomicron formation and fat absorption in multidrug resistance gene 2 P-glycoprotein-deficient mice, Gastroenterology 118 (2000) 173–182.
[163] P. Tso, J. Lam, W.J. Simmonds, The importance of the lysophosphatidylcholine and choline moiety of bile phosphatidylcholine in lymphatic transport of fat, Biochim. Biophys. Acta 528 (1978) 364–372.
[164] C.M. Mansbach II, R.F. Dowell, Portal transport of long acyl chain lipids: effect of phosphatidylcholine and low infusion rates, Am. J. Physiol. 264 (1993) G1082–G1089.
[165] S.M. Sabesin, S. Frase, Electron microscopic studies of the assembly, intracellular transport, and secretion of chylomicrons by rat intestine, J. Lipid Res. 18 (1977) 496–511.
[166] T. Noguchi, Y. Jinguji, T. Kimura, S. Muranishi, H. Sezaki, Mechanism of the intestinal absorption of drugs from oil-in-water emulsions. VII. Role of bile in the lymphatic transport of lipid-soluble compounds from triolein emulsions, Chem. Pharm. Bull. (Tokyo) 23 (1975) 782–786.
[167] N.L. Trevaskis, C.J.H. Porter, W.N. Charman, Bile increases intestinal lymphatic drug transport in the fasted rat, Pharm. Res. 22 (2005) 1863–1870.
[168] C.M. Mansbach II, A. Arnold, Steady-state kinetic analysis of triacylglycerol delivery into mesenteric lymph, Am. J. Physiol. 251 (1986) G263–G269.
[169] C.M. Mansbach, R. Dowell, Effect of increasing lipid loads on the ability of the endoplasmic reticulum to transport lipid to the Golgi, J. Lipid Res. 41 (2000) 605–612.
[170] N.L. Trevaskis, C.J.H. Porter, W.N. Charman, An examination of the interplay between enterocyte-based metabolism and lymphatic drug transport in the rat, Drug Metab. Dispos. 34 (2006) 729–733.
[171] F. Murad, R.C. Haynes, Androgens and anabolic steroids, in: A.G. Gilman, L.S. Goodman, A. Gilman (Eds.), The Pharmacological Basis of Therapeutics, MacMillan, New York, 1980, p. 1448.
[172] P.R. Daggett, M.J. Wheeler, J.D. Nabarro, Oral testosterone, a reappraisal, Horm. Res. 9 (1978) 121–129.

[173] H. Frey, A. Aakvaag, D. Saanum, J. Falch, Bioavailability of oral testosterone in males, Eur. J. Clin. Pharmacol. 16 (1979) 345–349.
[174] T. Noguchi, W.N. Charman, V.J. Stella, The effect of drug lipophilicity and lipid vehicles on the lymphatic absorption of various testosterone esters, Int. J. Pharm. 24 (1985) 173–184.
[175] A. Coert, J. Geelen, J. de Visser, J. van der Vies, The pharmacology and metabolism of testosterone undecanoate (TU), a new orally active androgen, Acta Endocrinol. (Copenh) 79 (1975) 789–800.
[176] H. Frey, A. Aakvaag, D. Saanum, J. Falch, Bioavailability of oral testosterone in males, Eur. J. Clin. Pharmacol. 16 (1979) 345–349.
[177] W.M. Bagchus, R. Hust, F. Maris, P.G. Schnabel, N.S. Houwing, Important effect of food on the bioavailability of oral testosterone undecanoate, Pharmacotherapy 23 (2003) 319–325.
[178] P.A. Van Veld, R.D. Vetter, R.F. Lee, J.S. Patton, Dietary fat inhibits the intestinal metabolism of the carcinogen benzo[*a*]pyrene in fish, J. Lipid Res. 28 (1987) 810–817.
[179] R.D. Vetter, M.C. Carey, J.S. Patton, Coassimilation of dietary fat and benzo(*a*)pyrene in the small intestine: an absorption model using the killifish, J. Lipid Res. 26 (1985) 428–434.
[180] F.A. Reubsaet, J.H. Veerkamp, S.G. Bukkens, J.M. Trijbels, L.A. Monnens, Acyl-CoA oxidase activity and peroxisomal fatty acid oxidation in rat tissues, Biochim. Biophys. Acta 958 (1988) 434–442.
[181] N.H. Haunerland, Fatty acid binding protein in locust and mammalian muscle. Comparison of structure, function and regulation, Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 109 (1994) 199–208.
[182] J.H. Veerkamp, H.T. van Moerkerk, Fatty acid-binding protein and its relation to fatty acid oxidation, Mol. Cell. Biochem. 123 (1993) 101–106.
[183] J.M. Johnston, G.A. Rao, Triglyceride biosynthesis in the intestinal mucosa, Biochim. Biophys. Acta. 106 (1965) 1–9.
[184] P. Gershkovich, B. Qadri, A. Yacovan, S. Amselem, A. Hoffman, Different impacts of intestinal lymphatic transport on the oral bioavailability of structurally similar synthetic lipophilic cannabinoids: dexanabinol and PRS-211,220, Eur. J. Pharm. Sci. 31 (2007) 298–305.