

Principles of Physiology of Lipid Digestion

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ABSTRACT : The processing of dietary lipids can be distinguished in several sequential steps, including their emulsification, hydrolysis and micellization, before they are absorbed by the enterocytes. Emulsification of lipids starts in the stomach and is mediated by physical forces and favoured by the partial lipolysis of the dietary lipids due to the activity of gastric lipase. The process of lipid digestion continues in the duodenum where pancreatic triacylglycerol lipase (PTL) releases 50 to 70% of dietary fatty acids. Bile salts at low concentrations stimulate PTL activity, but higher concentrations inhibit PTL activity. Pancreatic triacylglycerol lipase activity is regulated by colipase, that interacts with bile salts and PTL and can release bile salt mediated PTL inhibition. Without colipase, PTL is unable to hydrolyse fatty acids from dietary triacylglycerols, resulting in fat malabsorption with severe consequences on bioavailability of dietary lipids and fat-soluble vitamins. Furthermore, carboxyl ester lipase, a pancreatic enzyme that is bile salt-stimulated and displays wide substrate reactivities, is involved in lipid digestion. The products of lipolysis are removed from the water-oil interface by incorporation into mixed micelles that are formed spontaneously by the interaction of bile salts. Monoacylglycerols and phospholipids enhance the ability of bile salts to form mixed micelles. Formation of mixed micelles is necessary to move the non-polar lipids across the unstirred water layer adjacent to the mucosal cells, thereby facilitating absorption. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 2 : 282-295)

Key Words : Gastric Lipase, Pancreatic Triacylglycerol Lipase, Emulsification, Bile Salts, Colipase, Carboxyl Ester Lipase

INTRODUCTION

During the last years, studies on lipid digestion and metabolism in farm animals, particularly pigs, have mainly focussed on the provision of dietary energy, since fat is an excellent energy source, which energy value is approximately 2.25 times higher than that of carbohydrates (Maynard et al., 1979). Although fats are an essential part of the diet, but if consumed in excess, they may exert negative effects on human health (Lowe, 1994). For example, high levels of triacylglycerols are associated with cardiovascular diseases (NIH Consensus Conference, 1993). On the other hand, patients with reduced fat digestion and absorption, e.g. due to pancreas insufficiencies, suffer from fat malabsorption, reduced supply of energy and fat-soluble vitamins (Armand et al., 1999), suggesting the importance of preduodenal lipid digestion induced by gastric lipase. Gastric lipases may also be of great importance for lipid digestion in newborn animals, as it has been already established for infants (Hamosh, 1995). These examples show the importance and complexity of a balanced lipid digestion, underlying basic physiological regulatory and absorption mechanisms.

Recent reviews with regard to lipid digestion have provided deeper insights into the relation between the structure of lipid hydrolysing enzymes and digestive processes (e.g. Miled et al., 2000; Lowe, 2002), or they

have concentrated on mechanisms of lipid absorption and chylomicron assembly (Mu and Høy, 2004). In this review, an overview of the principal mechanisms of lipid digestion is given, thereby considering the complex interactions between pancreatic lipase, colipase and bile salts. Furthermore, attention is also drawn to the role of preduodenal lipid digestion, as well as the physiological role of carboxyl ester lipase (CEL), a lipid hydrolysing enzyme which is able to act on a wide range of lipids, e.g. cholesterol esters.

LIPID DIGESTION: PHYSICOCHEMICAL AND ENZYMATIC STEPS

Dietary lipids consumed by monogastrics are predominantly triacylglycerols. The utilization of fat in animals and humans requires the digestion and absorption of dietary fat sources during the passage through the gastrointestinal tract (GIT). Gastrointestinal lipid digestion consists of several sequential steps that include physicochemical and enzymatic events. Since fat is insoluble in water, emulsification is required for the digestion of fat in an aqueous medium as it exists in the GIT. This emulsification leads to the organization of dietary lipids in the form of droplets in the aqueous digestive system (Carey et al., 1983; Øverland et al., 1993). Digestion of dietary triacylglycerols starts in the stomach with the action of gastric lipase at the lipid-water-interface. Then, fat digestion continues in the duodenum with the synergetic action of gastric and colipase-dependent pancreatic lipase (Hamosh et al., 1975; Carey et al., 1983; Verger, 1984).

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Lipid digestion converts dietary fats into more polar derivatives with a higher degree of interaction with water. The triacylglycerols are mainly transformed into monoacylglycerols, non-polar cholesterol esters are converted to polar non-swelling amphiphiles, and phospholipids are hydrolysed to lysophospholipids. The latter are soluble amphiphiles that form micellar solutions. The fatty acids that are released due to lipid digestion are also amphiphiles when they are ionised. The lipolysis products (mainly monoacylglycerols and ionised fatty acids) leave the surface of the oil droplet and are incorporated into structures consisting of phospholipids or bile salts, thereby forming multi- or unilamellar vesicles and mixed micelles in the aqueous phase, before being absorbed by the enterocytes (Carey et al., 1983; Hernell et al., 1990). The gradual increase in pH in the luminal contents in distal direction of the small intestine facilitates the partitioning of fatty acids into the micellar phase (Borgström, 1974). Some limited lipid digestion may also occur when the micellar lipid comes into contact with the microvillus membranes of the enterocytes, since these structures show phospholipase A2 and carboxyl ester lipase activities, as well as other acylglycerol hydrolases and phospholipases (Brindley, 1974).

LIPASES – ACTING AT THE INTERFACE

Triacylglycerol lipases catalyse a reaction involving water and a water-insoluble substrate that is part of a large aggregate, like a micelle or an emulsion particle. Thus, lipolysis takes place at the lipid-water interface, a quasi two-dimensional phase segregating the bulk lipid phase from the bulk aqueous phase (Entressangles and Desnuelle, 1968; Brockman, 1984). Efficient lipid digestion requires the formation of a relatively stable and finely dispersed emulsion with an interfacial composition favourable to the anchoring of lipases, since the rate of lipolysis seems to be directly proportional to the available surface area. *In vitro* and *in vivo* experiments have shown that the extent of lipid emulsification, which directly affects the lipid/water interface area, modulates the binding onto the droplet surface and thus the activity of digestive lipases. The properties of the interface influence lipid digestion, and therefore the bioavailability of lipid nutrients. These properties are defined by physicochemical properties such as lipid globule organization, lipid droplet size (which influences the lipid interface area), and the molecular structure of the triacylglycerols constituting the lipid droplet (Armand et al., 1992,1999; Borel et al., 1994).

Lipid emulsification must therefore be considered as a fundamental part of lipid digestion by generating a lipid-water interface which is essential for the interaction between water-soluble lipases and insoluble lipids (Carey et

al., 1983; Armand et al., 1994,1996). The lipid droplets consist of a hydrophobic core containing the majority of the triacylglycerol molecules, esterified cholesterol, and fat-soluble vitamins, which is surrounded by an amphipathic surface monolayer of phospholipids, free cholesterol, and a few triacylglycerol molecules. About 2 to 5 mol% of the droplet surface lipid is triacylglycerol, which is available for hydrolysis by lipases at the surface of the lipid droplet (Miller and Small, 1982; Borel et al., 1996).

PREDUODENAL LIPASES – LIPID DIGESTION IN THE STOMACH

The stomach might act as an emulsifying organ given the peristalsis which propels the gastric content against the pylorus, thereby dispersing oil droplets and other solid particles (Malagelada and Azpiroz, 1989). The most detrimental property of the stomach content in terms of lipid emulsification is its acidic pH, which can reduce the ionisation, and might therefore result in emulsion breakage (Prince, 1974).

It was shown a long time ago in humans that dietary triacylglycerols are hydrolysed to a certain degree in the stomach (Borgström et al., 1957). This gastric lipolytic activity was found to be particularly active on milk triacylglycerols and medium chain triacylglycerols in both the suckling rat (Helander and Olivecrona, 1970), and in man (Cohen et al., 1971).

For several species, there have been identified so-called preduodenal lipases originating from the tongue or the pharynx (salivary lipase from salivary glands), or from the fundic region of the stomach. Such preduodenal lipases belong to a family of enzymes that have the ability to withstand acidic conditions, with a general pH optimum ranging between pH 4.0 and 6.0 (Moreau et al., 1988a). For example, acidic lipases have been found in mouse, calf, sheep, rabbit, monkey, horse, and guinea pig (DeNigris et al., 1988; Moreau et al., 1988a; Gargouri et al., 1989). Furthermore, a distinct gastric lipase that has properties which differ from those of pancreatic lipase has been isolated from stomach contents of dogs (Engstrom et al., 1968), and rats (Clark et al., 1969). It has also been found in pigs (Newport and Howarth, 1985), however, no reports exist so far that describe lipases of the upper GIT of birds (Krogdahl, 1985). In humans, gastric lipase (HGL, EC 3.1.1.3) has been identified (Schonheyder and Volquartz, 1946) as a highly glycosylated globular protein with 4 potential N-glycosylation sites, of about 50 kDa (Bodmer et al., 1987). This enzyme is acid-stable and shows activity in a broad pH range (Gargouri et al., 1989). Human gastric lipase was found to be stable in gastric juice at pH values ranging from 2.0 to 7.0, especially between 3.0 and 5.0 (Ville et al., 2002). Studies investigating the tissular and

cellular distribution of HGL have demonstrated that HGL is present in the mucosa only, and is colocalized with the pepsinogen in chief cells of fundic glands of the stomach (Moreau et al., 1988b; Moreau et al., 1989), where it initiates the digestion of triacylglycerols (Hamosh, 1990; Carrière et al., 1993). Given the specific activity reported for purified human gastric lipase (Gargouri et al., 1986a), the concentration of gastric lipase ranges between 0.5 and 1 μM , a value close to that reported for pancreatic lipase (2 μM) in the small intestinal content (Borgström and Hildebrand, 1975).

Contribution of gastric lipolysis to lipid digestion

In humans, the importance of gastric lipase is still debated, however several authors (e.g. Hamosh et al., 1975; Carrière et al., 1993; Armand et al., 1994) found that in healthy humans, gastric lipolysis leads to the hydrolysis of 10 to 30% of the ingested triacylglycerols, generating free fatty acids and a mixture of diacylglycerols and monoacylglycerols. This has also been confirmed for animals, e.g. in rats the capacity of the preduodenal lipases amounts to about 20% at day 60 after birth (Levy et al., 1982). Gastric lipase facilitates subsequent hydrolysis by pancreatic lipase by promoting fat emulsification (Armand et al., 1994, 1996), and by generating fatty acids and diacylglycerols (Bernbäck et al., 1989; Armand et al., 1994, 1996), as well as by promoting enzyme activity. The latter was shown by Gargouri et al. (1986b) *in vitro*, who observed an enhanced PTL activity on a triacylglycerol emulsions after preincubation of the substrate with human gastric juice. This is in accordance with Borel et al. (1994), who showed *in vitro* an increase in activity of PTL after prehydrolysing triacylglycerol emulsions by gastric lipase. According to Armand et al. (1994), gastric lipolysis may modulate the extent of emulsification, and, at the same time, the extent of emulsification may control the rate of lipolysis.

Carrière et al. (1993) have measured separately the gastric and pancreatic lipase outputs and monitored the amounts of lipolytic products produced during the digestion of a liquid test meal in intubated healthy volunteers. They found that most of the HGL secreted in the stomach was still active in the duodenum, acting in association with PTL. The authors estimated the relative contributions of HGL and human pancreatic lipase to overall digestion of dietary triacylglycerols: during the whole digestion process, gastric lipase hydrolyses 17.5% of the acyl chains of the triacylglycerols. Considering that only two acyl chains out of the three from a triacylglycerol molecule need to be hydrolysed to ensure complete intestinal absorption, gastric lipase contribution to the hydrolysis of triacylglycerols is about 25%.

Armand et al. (1994) investigated human gastric aspirates of fasting subjects who were intragastrically

intubated, and received a coarsely emulsified test meal, consisting mainly of emulsified particles with a droplet size ranging between 10 and 100 μm . The authors found that during digestion in the stomach non-emulsified lipids (with a diameter $\geq 100 \mu\text{m}$) represented a minor fraction. A significant amount of the large lipid droplets (70 to 100 μm) disappeared, and fine droplets (1 to 10 μm) were generated. The resulting median diameter of emulsified droplets significantly dropped from the value given by the test meal (median diameter 53 μm) to 22 μm after 1 h digestion. During the following 3 h, until almost complete emptying of the stomach, rather constant intermediate median diameters were observed (35 to 37 μm). In this study, most changes were observed after the first hour of digestion. The results of Armand et al. (1994) indicate that lipolysis mainly occurs within the first hour of digestion in the stomach, in agreement with other authors (Hamosh et al., 1975), who found that the maximal rate of lipolysis was achieved within 4 min and that hydrolysis was nearly completed at this time. In the study of Armand et al. (1994), the fine droplet fraction (1 to 10 μm) that underwent a significant increase in surface area after 1 h of digestion, exhibited a marked enrichment in lipolytic products. This points out that the generation of lipolytic products catalysed by gastric lipase assists in emulsifying dietary lipids in the stomach, as previously suggested by Hamosh et al. (1975).

Gastric lipase attacks primarily the short- and medium-chain fatty acid linkages on the sn-3 position of triacylglycerol rather than long-chain-fatty acids, and the medium-chain length acids that are liberated can be absorbed directly in the stomach (Clark et al., 1969). Therefore, gastric lipase may be particularly important in suckling animals, since the milk of many species (e.g. pigs and ruminants) contains high proportions of medium-chain fatty acids (Helander and Olivecrona, 1970; Drackley, 2000). According to Cohen et al. (1971), in adult humans, preduodenal lipolysis is considered to be a minor component of the overall lipolytic system, and the importance of gastric lipolysis may be limited to the infantile period. Liao et al. (1983) studied the developmental pattern and quantitative contribution of lingual and gastric lipases to intragastric fat digestion, by measuring lipase activity in homogenates of lingual glands and gastric mucosa of rats from birth until 60 days of age. The authors found in the period from birth to weaning a rapid and extensive hydrolysis of triacylglycerol in the stomach, with the triacylglycerol concentration decreasing from 98 mol% in rat milk to 34 to 49 mol % in the stomach contents half an hour after feeding. In this study however, total lipolytic activity in rat gastric mucosa was only 2 to 10% of that in the lingual glands throughout the entire period studied, suggesting the importance of lingual lipase in the newborn rat.

Bernbäck et al. (1989,1990) concluded from *in vitro* studies with purified gastric lipase an exclusive role of this enzyme in the digestion of human milk triacylglycerols. The fat in milk is present in the form of milk fat globules, i.e. the core of triacylglycerols is enveloped by an external surface layer consisting of more polar lipids, such as phospholipids, cholesterol, and proteins. This milk fat globule membrane exerts an inhibitory effect on hydrolytic action of pancreatic lipase (Patton and Keenan, 1975; Bläckberg et al., 1981). However, after a short preincubation with gastric lipase, resulting in a limited lipolysis, the milk fat triacylglycerols were available for an immediate and rapid hydrolysis by pancreatic lipase. Therefore, the importance of gastric lipase consists in its ability to initiate triacylglycerol hydrolysis (Bernbäck et al., 1989).

Roy et al. (1979) carried out *in vivo* studies with young Sprague-Dawley rats to examine the role of gastric lipolysis on fat absorption and bile acid metabolism. The authors concluded that gastric lipolysis seems to be of physiological importance in situations where lipolytic mechanisms are stressed by a large fat intake. Its principal role seems to be to potentiate intestinal lipolysis by facilitating the emulsification of dietary lipids through its formed products and, therefore the contact of pancreatic lipase with its substrates.

Abrams et al. (1987) found, by investigating patients with exocrine pancreatic insufficiency, non-pancreatic lipolytic activity in gastric and duodenal aspirates. Lipolytic activity was measured *in vitro* by hydrolysis of a triacylglycerol emulsion. Non-pancreatic lipolytic activity accounted for about 90% of total lipolytic activity in the duodenum in patients with exocrine pancreatic insufficiency, as opposed to 7% in control subjects. The authors suggested that in physiological (e.g. pre- or full-term infants) and pathologic pancreatic exocrine insufficiency (e.g. cystic fibrosis and pancreatitis), gastric lipolysis might play a key role in digestion of dietary fat, by hydrolysing a substantial part of dietary lipids in the stomach. This is confirmed by the fact, that even in complete absence of pancreatic lipase, e.g. due to exocrine pancreatic insufficiency, such patients still absorb a high percentage of dietary fat (Lapey et al., 1974).

Although human pancreatic lipase (HPL) plays an important role in dietary triacylglycerol digestion in healthy adult humans (Carrière et al., 1994; Brockman, 2000; Miled et al., 2000), it seems that neither HPL nor colipase secretions are mandatory for fat digestion in humans. According to Roussel et al. (1999), the co-administration of acidic lipases, which hydrolyse dietary lipids under acidic conditions, should assist to treat patients with various forms of pancreatic deficiency.

LIPID DIGESTION IN THE DUODENUM

Lipid digestion is completed in the proximal small intestine by enzymes synthesized and secreted by pancreatic acinar cells. The most important of the intestinal lipases to attack lipid droplets passing from the stomach into the small intestine is the pancreatic triacylglycerol lipase (Lindstrom et al., 1988; Lowe, 1994).

Pancreatic triacylglycerol lipase (PTL)

Pancreatic triacylglycerol lipase (PTL, E.C. 3.1.1.3) expression is found in most, if not all, adult vertebrates (Lowe, 2002). The production of this carboxyl esterase by the pancreas varies from species to species and also with age. The pancreatic lipase has a pH optimum of about 8.5, but is still very active at pH 6.5. The enzyme is irreversibly inactivated below pH 4.0 (Holt, 1971). The characteristic feature of this enzyme, secreted by the exocrine pancreas, is its specificity of action on insoluble emulsified substrates. It has much greater activity against water-insoluble substrates such as long-chain triacylglycerols than against water-soluble substrates (Verger, 1997). The enzyme is activated when it encounters an oil/water interface and initiates hydrolysis of triacylglycerols and diacylglycerols. The extent of binding is related to the physicochemical and the compositional structure of the interface, i.e. the 'quality' of the interface. The term 'quality' is as yet undefined but assumed to contain contributions from electrostatic interactions, orientation of substrate, and hydration forces (Wickham et al., 1998).

Pancreatic triacylglycerol lipase adsorbs almost non-specifically and with high affinity to interfaces which comprise only simple acylglycerols or other non-polar compounds. However, it has a strong preference for acylglycerols over phospholipids, cholesterol esters, and galactolipids (Verger, 1984; Andersson et al., 1996). Hydrolysis in the interface is rapid and exhibits modest specificity with respect to substrate (pro-)chirality. It may also contribute to the hydrolysis of retinyl esters *in vivo* (Van Bennekum et al., 2000). Pancreatic lipase specifically attacks triacylglycerol acyl groups which are on the 1(3)-positions of glycerols and shows little specificity for acyl chain length (Brockerhoff and Jensen, 1974). This specificity of pancreatic lipase for the terminal fatty acid groups of triacylglycerols has been well established for pigs (Schonheyder and Volquartz, 1954), and the properties of the chicken PTL appear to be similar (Laws and Moore, 1963). Pancreatic lipase also has been isolated from turkey showing biochemical properties similar to mammalian PTL, e.g. with a maximal activity at pH 8.5, and deactivation at pH less than 5 (Sayari et al., 2000).

Factor influencing lipolysis: emulsification

The physical properties of the lipid emulsion that passes from the stomach to the intestine change after being mixed with the bile, pancreatic juice and the secretions from the small intestine. The pH of the proximal part of the small intestine, where most of the lipid digestion takes place, rises to 5.8 to 6.5 (Borgström, 1974). Important amounts of amphipathic molecules with potential emulsifying properties are found in the duodenal contents (Hernell et al., 1990; Armand et al., 1996). This includes lipolytic products of dietary triacylglycerols and phospholipids (i.e. diacylglycerols, monoacylglycerols, lysophospholipids, and protonated free fatty acids), biliary lipids (i.e. phospholipids and cholesterol in a bile salt-rich medium), and amphipathic peptides derived from protein hydrolysis. The emulsifying properties of phospholipids, monoacylglycerols, and free fatty acids have been well described *in vitro* (Linthorst et al., 1977; Carey et al., 1983). Almost all of the dietary triacylglycerols and diacylglycerols are incorporated into the core of the emulsion particle, which is coated by a monolayer or multilamellar phase of mainly polar lipids, phospholipids, and fatty acids, and a small amount of cholesterol and of triacylglycerols. Bile salts, denatured dietary proteins, and dietary oligosaccharides, also cover the surface of the emulsion particle (Carey and Hernell, 1992). According to Fillery-Travis et al. (1995), bile lipids distribute at the surface of lipid droplets creating a crystalline monolayer preventing aggregation and coalescence of the droplets and thus have a stabilizing rather than an emulsifying effect, allowing a large droplet surface area to be maintained.

According to Holt (1971), emulsification requires an emulsifier, mechanical energy, and a stabilizer to maintain the resulting solution. The principle emulsifiers of triacylglycerol droplets seem to be the bile salts and, according to Holt (1971), sufficient mechanical energy required for emulsification should be provided by the motility of the antrum and duodenum. Stabilization may occur by virtue of lipolytic products of triacylglycerol hydrolysis. However, according to Linthorst et al. (1977), the fact that highly polar bile salts may act *per se* as an emulsifier was not confirmed in any *in vitro* experiment and needs to be verified (Carey et al., 1983). Armand et al. (1996) studied the physico-chemical characteristics of emulsions during fat digestion in the human stomach and duodenum. Gastric and duodenal aspirates of volunteers were collected after receiving intragastrically a coarsely emulsified test meal. Lipid droplet size was determined in the test meal and in gastric and duodenal aspirates. The authors showed that, in the duodenum, most lipids were present as emulsified droplets (1 to 100 μm in size). After 1 h of digestion, large droplets and unemulsified material originating from the test meal ($>100 \mu\text{m}$) disappeared,

whereas smaller droplets (1 to 50 μm) were generated. In the stomach, a comparable emulsion particle size was observed, with unemulsified dietary fat only representing a minor fraction of stomach contents. Most dietary lipids are present in the human duodenum as emulsified droplets ranging in size between 1 to 50 μm , and it seems that, at least in humans, no further marked emulsification of dietary lipids occurs in the duodenum compared with the stomach. Armand et al. (1996) suggest, that although overall excess amounts of amphipaths are present in the small intestine, limiting factors such as mechanical shear forces could largely limit an emulsification process in the small intestine, though some degree of emulsification in the duodenum may occur. According to Kellow et al. (1986), there is not enough mechanical energy in the duodenum to allow further lipid emulsification. Under physiological conditions, emulsification mainly occurs in the stomach with phospholipids and fatty acids as the principal emulsifiers present in stomach, produced by the action of gastric lipase (Carey et al., 1983; Armand et al., 1994,1996).

However, Armand et al. (1996) also determined the extent of triacylglycerol hydrolysis, and they found that the extent of lipolysis was much lower in the stomach (6 to 16%) compared to the duodenum (42 to 45%), where small droplets were enriched with lipolytic products, cholesterol and phospholipids. As previously observed in human stomach content (Armand et al., 1994), the smaller the droplet size, the higher the ratio of lipolytic products in relation to intact triacylglycerol molecules. The main differences observed in this respect between duodenum and stomach droplet composition were an increased monoacylglycerol content as digestion proceeds in the duodenum. This observation is likewise linked to the quantitative and qualitative differences in the generation of lipolytic products under the action of the gastric or pancreatic lipase (Verger, 1984; Hamosh, 1990), and a more negligible small droplet enrichment in cholesterol and phospholipids, which is likely due to the important bile supply of these lipid species. Under very efficient intraduodenal lipolysis (about 3.7-fold higher than in the stomach), neutral lipid depletion gives rise to smaller emulsion droplets, which have a size-dependent lipid composition (Armand et al., 1996).

Role of bile salts

Bile salts are synthesized from cholesterol in hepatocytes of the liver. The main body structure of bile salts is cholic acid (Figure 1) which is conjugated by the liver with the amino acids taurine or glycine, thereby increasing the water solubility and decreasing the cellular toxicity of the bile salts (Borgström, 1974; Gaull and Wright, 1987). In pigs, bile salts are conjugated mainly with glycine, whereas in chicken only taurine conjugates are

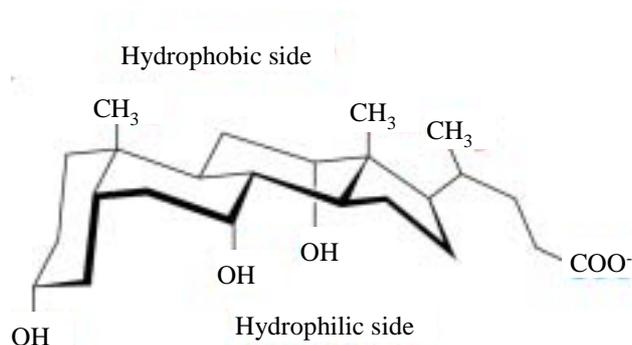


Figure 1. Structure of cholic acid. After McMurry and Castellion (2002).

produced (Alvaro et al., 1986). Bile salts have detergent-like properties, and their structure provides a large, rigid, and planar molecule consisting of a steroid nucleus (hydrophobic side) with a few hydroxyl groups and a ionic head (hydrophilic side) (Figure 1). The hydrophobic side can either interact with the equivalent face of other bile salt molecules, or it can dissolve at an oil/water interface. The hydrophilic groups of the bile salts interact with the aqueous environment. Thus, the bile salts remain at the water-lipid interface and do not penetrate deeply into either surface (Nair and Kritchevsky, 1971; Brindley, 1984).

Although PTL has no specific requirement for bile salts, the increased surface area due to the action of bile salts seems to increase the rate of triacylglycerol hydrolysis induced by pancreatic lipase (Drackley, 2000). However, most likely, the main effect of bile salts is to solubilize the products of lipase action in the bulk-water phase of the intestinal contents, thereby removing them from the site of enzyme action, i.e. the oil-water interface. Otherwise, if the products of this lipolytic action remained at the enzyme site, the progressing activity of the lipolytic system would be inhibited by product inhibition of the reaction. Another role of bile salts is to inhibit the re-formation of triacylglycerol from monoacylglycerol and fatty acids, probably by solubilizing these compounds and removing them from the site of lipase action, thereby pulling the lipase reaction in the direction of continued lipolysis (Holt, 1971). According to Patton and Carey (1981), in the absence of bile, fat digestion and/or absorption might be incomplete and be prolonged into the more distal parts of the intestine. This is in accordance with Demarne et al. (1982), who investigated fat absorption in rats artificially deprived of bile secretion by bile duct ligation. The authors found a decrease of about 50% in the apparent absorption of dietary lipids.

The products of lipolysis are removed from the water-oil interface by incorporation into mixed micelles. These mixed micelles are necessary for effective absorption of dietary lipids, and both monoacylglycerols and phospholipids greatly enhance the ability of bile salts to form the mixed micelles present within the small intestinal

lumen (Holt, 1971). The mixed micelles act as carrier to transport the lipolytic products from the lumen to the absorptive site, thereby overcoming the unstirred water layer present at the surface of the intestinal microvillus membrane which is thought to be the main barrier to lipid absorption (Dietschy, 1978).

The formation of micelles depends on the concentration of bile salts in solution, and there exists a critical minimum concentration which is necessary for a detergent to form a micellar solution (termed "critical micellar concentration", CMC). *In vitro*, pure solutions of bile salts appear to have a CMC of about 2 mM. However, *in vivo*, in the presence of endogenous monoacylglycerol and phospholipid, it may be much lower and in the range of 0.75 to 1 mM (Holt, 1971). Generally, the bile salt concentration in the gut is not constant, but changes over time and in the different parts of the small intestine. According to Northfield and McColl (1973) and Heaton (1985), after a meal, bile salt concentration sharply increases in the duodenum up to ca. 15 mmol/L and then progressively decreases to 6 mmol/L. In the jejunum, the bile salt concentration amounts to 10 mmol/L, and in the ileum, the concentration falls below 4 mmol/L due to active absorption.

RESTORING EFFECT OF COLIPASE ON BILE SALT-MEDIATED INHIBITION OF LIPASE

Of all known lipolytic enzymes in pancreatic juice of vertebrates, only pancreatic lipase requires a polypeptide cofactor (colipase), a 10 kDa non-enzymatic protein (Maylié et al., 1971), for optimal activity (Patton and Carey, 1981). Colipase is an acid and heat stable exocrine protein that is secreted by the pancreas (Borgström and Erlanson-Albertsson, 1984), and is also found in the stomach (Sörhede et al., 1996). Colipase is secreted from the pancreas as procolipase, a protein which is cleaved under the influence of trypsin to colipase and a peptide called enterostatin. This peptide functions as a hormone that regulates satiety, reduces fat intake and inhibits pancreatic secretion (Erlanson-Albertson et al., 1991; Erlanson-Albertson, 1992).

Although bile salts are not essential for dietary lipid absorption, they are required to achieve optimal absorption conditions for dietary lipids (Lowe, 2002). Interestingly, bile salts inhibit PTL activity at higher concentrations, but stabilize lipase at low concentrations against surface denaturation by lowering the free energy of the apolar interface (Verger, 1984; Brockman, 2000). This was shown *in vitro* by Momsen and Brockman (1976), investigating the effects of colipase and taurodeoxycholate on the catalytic and physical properties of PTL at an oil-water interface.

According to Borgström and Erlanson (1971,1973), PTL is completely but reversibly inactivated by conjugated

bile salts in concentrations above their critical micellar concentration. This inhibition is parallel to a displacement of the enzyme from the water/substrate interface to the aqueous phase resulting in a physical separation of lipase from its water-insoluble substrate (Borgström, 1975; Momsen and Brockman, 1976). Several different mechanisms have been suggested to explain the bile salt inhibition: the inhibitory effect of bile salts could be related to the building of a detergent monolayer on the substrate surface, thereby preventing the lipase from reaching its substrate. Furthermore, the bile salts give a negative charge to the substrate surface, which might result in electrostatic repulsion (Borgström and Erlanson, 1973). The inhibition may also reflect a competition between lipase and bile salt for the interface and/or a lowering of the interfacial tension, thereby impeding lipase adsorption to the interface (Nano and Savary, 1976).

Pancreatic lipase, which will not bind to interfaces containing bile salts, phospholipids, proteins or other surface-active constituents of the intestinal milieu, overcomes this inhibition by binding to the interface in a complex with colipase (Brockman, 2000). Though the most pronounced effect of colipase is to reverse the inhibitory effects of bile salts, it causes, in addition, a stimulation of the rate of hydrolysis in the absence or presence of bile salts and it markedly improves the linearity of product formation as a function of time (Brockerhoff and Jensen, 1974). According to Borgström and Erlanson (1973), these effects may be the result of a stoichiometric binding between lipase and colipase which increases the rate of lipase adsorption onto its substrate and stabilizes the enzyme at the interface (Vandermeers et al., 1975). Alternatively, the colipase could cover the substrate surface and thereby block enzyme denaturation, facilitate the binding of enzyme to the interface and/or support the proper orientation of the bound enzyme (Brockerhoff and Jensen, 1974).

Previous models of colipase function suggested that colipase may act as a bridge between PTL and the lipid substrate by binding to both PTL and the lipid emulsion surface. This model is supported by the crystal structures of the porcine colipase-human PTL complex solved under different conditions (Van Tilbeurgh et al., 1992,1993). In the first structure, PTL is present in an inactive conformation, and the catalytic site is sterically blocked by a surface loop, which is termed the lid. The other structure, solved in the presence of mixed micelles, reveals the active conformation of PTL in which the lid moves away from the catalytic site and forms new interactions with colipase. Colipase has similar conformations in both structures (Egloff et al., 1995). Importantly, colipase has two opposing surfaces that can potentially anchor PTL to the substrate. One surface, which contains predominantly hydrophilic residues, forms interactions with the C-terminal domain of

PTL. The opposite surface, which faces away from PTL, has four hydrophobic loops positioned to interact with the substrate surface. In this conformation, colipase directs PTL into the proper proximity and orientation for lipid hydrolysis. It stabilizes the lid in the open, active conformation and may direct PTL to substrate patches on the surface of dietary emulsions (Crandall and Lowe, 2001).

The neutron crystal structure of a colipase-lipase complex, however, has suggested that the stabilization of the enzyme in its active conformation and its adsorption to the emulsified oil droplets are mediated by a preformed lipase-colipase-micelle complex (Hermoso et al., 1997). This is confirmed by Pignol et al. (2000) who observed a similar ternary complex by the use of small angle neutron scattering with D₂O/H₂O contrast variation for characterization of a solution containing colipase-lipase complex and taurodeoxycholate micelles. *In vitro*, in the absence of bile salt, lipase can readily bind the triacylglycerol water interface and evolve its activity. *In vivo*, the physiological triacylglycerol vesicles covered by dietary and biliary phospholipids, cholesterol, and bile salts are at equilibrium with smaller mixed aggregates of micellar size in the aqueous phase (Hofmann and Small, 1967). Pignol et al. (2000) suggest that these physiological mixed micelles may have the appropriate size to form a ternary complex with colipase and lipase. Within this ternary complex, the enzyme is maintained, in the aqueous phase, in its active conformation, and this preformed complex reacts as an entity that defines the degree of lipase adsorption to the emulsified substrate.

Inhibition of PTL, e.g. by phospholipids, and its amelioration by colipase is characterized by a long lag phase in the catalytic activity. This has been shown, for example by Borgström (1980), after PTL addition to a phosphatidylcholine-rich interface. The lag phase, which is accepted as a measure of the ease of penetration of the lipase into the interface (Wickham et al., 1998), is followed by an accelerated hydrolysis of triacylglycerols during which a substantial amount of the substrate is degraded. However, hydrolysis products have to be removed from the interface, as it occurs *in vivo* by the action of bile salts; otherwise the reaction will stop due to the accumulation of fatty acids and monoacylglycerols in the interface. The presence of colipase in the system greatly reduces the duration of the lag phase (Borgström and Erlansson-Albertsson, 1984).

Although there is little information about the importance of colipase under *in vivo* conditions (Brockman, 2000; Lowe, 2002), its role is not simply hypothetical, since a genetic deficiency of colipase leads to severe fat malabsorption, as it has been shown in humans (Hildebrand et al., 1982). By using procolipase-deficient mice, D'Agostino et al. (2002) confirmed the requirement for

Table 1. Potential physiological functions of carboxyl ester lipase in digestion of dietary fat

Physiological function	Reference
Hydrolysis of retinyl esters?	Rigtrup and Ong, 1992; Weng et al., 1999
Hydrolysis of triacylglycerol containing long-chain polyenoic acids	Chen et al., 1989; Chen et al., 1994
Removing of fatty acids in 2-position of monoacylglycerol	reviewed by Rudd and Brockman, 1984
Hydrolysis of cholesteryl ester	Shamir et al., 1995; Fält et al., 2002
Production of lipoproteins	Kirby et al., 2002

colipase in dietary fat digestion. The colipase-deficient mice showed fat malabsorption as newborns and as adults, but only when fed a high-fat diet. However, when fed with a low-fat diet, the colipase-deficient mice had normal fat absorption. The results demonstrate the critical role of colipase in the digestion of dietary lipids by PTL.

According to Bosc-Bierne et al. (1984), the activity of pancreatic lipase in chicken is also inhibited by various bile salts, including sodium taurochenodeoxycholate, which is found in large proportion in chicken bile, but lipase activity is restored by colipase. *In vitro* studies on interspecies activation of lipase by colipase using a mixed lipid substrate system have revealed some difference between the behaviour of chicken lipase with its colipase and that with the porcine cofactor. However, according to Bosc-Bierne et al. (1984), avian and mammalian lipolytic systems seem to be functionally similar, and are probably of comparable importance for lipid digestion (Bosc-Bierne et al., 1984).

CARBOXYL ESTER LIPASE

Another pancreatic enzyme important in duodenal lipid digestion is carboxyl ester lipase (CEL, E.C. 3.1.1.13), also named pancreatic cholesterol esterase, cholesterol ester hydrolase, carboxylic ester hydrolase or bile salt-stimulated lipase. It has a wide pH optimum and is a non-specific lipolytic enzyme able of hydrolysing cholesteryl esters, tri-, di- and monoacylglycerols, phospholipids, lysophospholipids, and ceramides (Rudd and Brockman, 1984; Wang and Hartsuck, 1993; Hui, 1996). Carboxyl ester lipase is synthesized primarily in the pancreatic acinar cells and lactating mammary glands of higher mammals, and as a minor constituent of other tissues, particularly the liver (Harrison, 1988; Winkler et al., 1992), activated macrophages (Li and Hui, 1997), and endothelial cells (Li and Hui, 1998). The hydrolysis of water-insoluble triacylglycerols and cholesteryl esters by CEL requires its activation by bile salts, whereas CEL hydrolysis of water-soluble substrates such as lysophospholipids is not necessarily dependent on bile salt activation (Liang et al., 2000).

According to an *in vitro* study of Tsujita and Okuda (1990), inactivation of CEL occurs at a high energy lipid-water interface, and an important role of bile salts *in vivo* is to stabilize CEL at interfaces against the surface inactivation. The rate constant for surface inactivation

increases above pH 7.0. Bile salts cause dose-dependent protection of the enzyme from surface inactivation, however, they show higher protective effects at acidic pH than at alkaline pH. This confirms previous results of Tsujita et al. (1987), suggesting that bile salts might interact directly with CEL by a 1:1 interaction far below the CMC, and that at higher pH, affinity of bile salts to CEL is substantially reduced.

Role of CEL in dietary fat and cholesterol digestion and absorption

Potential functions of CEL in the digestion and absorption of cholesteryl ester, phospholipid and triacylglycerol have been shown by the use of cell culture assays and other *in vitro* studies (e.g. Shamir et al., 1995). Table 1 summarizes some of the physiological functions of CEL in fat digestion and absorption. However, only the cholesteryl ester hydrolytic activity seems to be a specific function performed only by this enzyme in the digestive tract. Phospholipid hydrolysis in the intestinal lumen can also be accomplished by group 1B phospholipase A2 secreted by the pancreas, and triacylglycerol hydrolysis is, at least in adults, usually attributed to PTL activity (Carey et al., 1983). Thus, it is difficult to conclude, if CEL plays the primary role in the aforementioned digestive processes, or if CEL provides only supplemental or compensatory lipolytic capacity, with group 1B phospholipase A2 and PTL performing the main function. According to studies with CEL-null mice (Hui and Howles, unpubl. data), the latter seems to be confirmed at least with regard to triacylglycerol digestion.

Jensen et al. (1997) investigated the development of digestive enzymes in pigs. The authors showed a high activity of CEL in the pancreatic tissues during the suckling period, and a decrease in CEL activity at weaning, underlying the possible importance of this enzyme in digestion of milk fat. However, CEL is also synthesized and secreted by lactating mammary glands of most mammalian species. In terms of milk fat digestion, the CEL present in milk is probably more important than the CEL secreted by the pancreas. Since CEL is not inactivated during passage through the stomach of neonates (Lee and Lebenthal, 1993), this enzyme also might participate in lipid digestion within the intestinal lumen prior to the maturation of the pancreas. This possible compensatory role of milk lipase activity is confirmed by a human study of Alemi et al. (1981). In low

Table 2. Major steps of lipid digestion, site of action, and involved secretions

Site	Secretion	Physical action	Chemical action	Reference
Stomach		Partial emulsification through peristalsis		Malagelada and Azpiroz, 1989
	Gastric lipase		Hydrolysis of triacylglycerols (10 to 30%)	Borgström et al., 1957 Hamosh et al., 1975 Carrière et al., 1993 Armand et al., 1994,1996
		Products of triacylglycerol hydrolysis promote emulsification		
Proximal duodenum	Pancreatic triacylglycerol lipase, gastric lipase Bile salts Colipase Carboxyl ester lipase		Hydrolysis of triacylglycerols Emulsification, formation of mixed micelles Restoring effect on bile salt-mediated inhibition of triacylglycerol lipase Hydrolysis of cholesteryl esters, tri-, di- and monoacylglycerols, phospholipids, lysophospholipids, and ceramide	Carey et al., 1983 Verger, 1984 Hamosh, 1990 Holt, 1971 Patton and Carey, 1981 Borgström and Erlanson, 1971,1973 Patton and Carey, 1981 Rudd and Brockman, 1984 Wang and Hartsuck, 1993

birth-weight human infants, fat excretion was lower in infants given a mixture of fresh human milk and formula than in infants alimented with pure formula. This is also in accordance with Howles et al. (1998), who demonstrated in a study with CEL-null mice, that pups nursed by CEL-null dams contained large amounts of tri-, di- and monoacylglycerols in their colon contents, in contrast to pups nursed by control dams where none were detectable.

Furthermore, CEL is able to remove fatty acids from the 2-position of monoacylglycerols. While it is generally accepted that monoacylglycerols are absorbed directly by the mucosa via passive diffusion, this assumption is based at least partly on the fact that PTL hydrolyses triacylglycerols only to fatty acids and 2-monoacylglycerol. Thus, it seems that CEL is important for the complete digestion of acylglycerols to fatty acids and glycerol (Friedman and Nylund, 1980; Rudd and Brockman, 1984; Hui and Howles, 2002). While the physiological significance of this activity remains unclear with regard to adults, it has to be considered that approximately 40% of 2-monoacylglycerols are reduced to fatty acids and glycerol before being absorbed (Mattson and Volpenhein, 1972). However, in neonates, digestion of the monoacylglycerols may be relatively important for absorption, since according to a study of Howles et al. (1998), pups nursed by CEL-null dams have significant amounts of monoacylglycerols in their faeces.

Carboxyl ester lipase plays an important role in the conversion of cholesteryl esters into cholesterol (Shamir et al., 1995; Fält et al., 2002) which seems to be an obligatory step for cholesterol absorption. The role of CEL in digestion and absorption of cholesteryl esters was shown by the use

of CEL knock-out mice: in the absence of CEL, intestinal absorption of dietary cholesteryl oleate was diminished by >60% (Howles et al., 1996). However, the absence of CEL seems not to have any influence on intestinal absorption of free cholesterol, as has been shown in CEL knockout mice (Howles et al., 1996). Hui and Howles (2002) reviewed all available data concerning the role of CEL in cholesterol absorption, and they concluded that, at least under normal conditions, CEL plays no direct role for the absorption of free cholesterol. Since both dietary cholesterol and cholesterol originating from bile are predominantly unesterified, the importance of CEL in intestinal cholesterol absorption remains open. However, according to Young and Hui (1999), optimal cholesterol absorption is dependent on sufficient hydrolysis of phospholipids and triacylglycerols. Thus, CEL seems to have a compensatory or supplementary function to ensure sufficient lipolytic activity (Hui and Howles, 2002).

CEL also might be of physiological importance in terms of production of lipoproteins which are necessary for the transport of hydrolysed lipids. In a recent study with fistulated mice, Kirby et al. (2002) investigated the possible influence of CEL in the intestinal lumen on the type of lipoproteins produced. The results of this study revealed that CEL might play an important role in the promotion of large chylomicron production in the intestine. The mechanism of this process seems to be mediated through CEL hydrolysis of ceramide generated during the lipid absorption process. The important role of sphingomyelin and its metabolic product ceramide in controlling lipid trafficking in mammalian cells has already been documented: It has been shown that naturally occurring

long chain ceramides, e.g. originating from sphingomyelin hydrolysis, enhance Golgi apparatus disassembly and are able of affecting protein transport through the secretory pathway (Rosenwald and Pagano, 1993; Fukunaga et al., 2000). The participation of CEL in chylomicron production may be of clinical importance with regard to the relationship between dietary lipid transport and risk of obesity or arteriosclerosis. It has been shown that large chylomicrons are cleared faster from circulation by the liver than the smaller postprandial lipoproteins (Martins et al., 1996). Kirby et al. (2002) suggest that such a delayed hepatic clearance of smaller lipoproteins might promote obesity due to increased transport of dietary fat to adipose tissues. Therefore, CEL in the GIT might have a protective role by enhancing the production of large chylomicrons in response to fat feeding.

SUMMARY

This review summarizes the current knowledge on the physiology of lipid digestion, thereby combining the well-known principles of the mechanisms of lipid digestion with recent studies to give a deeper insight into these processes. Table 2 gives a summary of the major steps of lipid digestion, their site of action, and the secretions which are involved.

By preduodenal digestion, a substantial part of lipids is hydrolysed. This may be of importance at least during infancy and pancreatic insufficiency. Furthermore, emulsification is provided by the products of lipolytic action in the stomach together with the stomach's motility. However most lipolytic activity takes place by pancreatic lipase in the duodenum. Carboxyl ester lipase serves as a compensatory or supplemental enzyme for complete lipid digestion, but also seems to be involved in the complex transport mechanisms of hydrolysed lipids. Additional investigation should aid to identify the distinct functions of this enzyme. A substantial knowledge of the physiology of lipid digestion should provide a base for practical approaches to enhance productivity and health of farm animals, as well as it would be useful in terms of medical fields, e.g. for treatment of diseases inherent to insufficiencies in lipid digestion.

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