

Physiological parameters governing the action of pancreatic lipase

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The most widely used pharmacological therapies for obesity and weight management are based on inhibition of gastrointestinal lipases, resulting in a reduced energy yield of ingested foods by reducing dietary lipid absorption. Colipase-dependent pancreatic lipase is believed to be the major gastrointestinal enzyme involved in catalysis of lipid ester bonds. There is scant literature on the action of pancreatic lipase under the range of physiological conditions that occur within the human small intestine, and the literature that does exist is often contradictory. Due to the importance of pancreatic lipase activity to nutrition and weight management, the present review aims to assess the current body of knowledge with regards to the physiology behind the action of this unique gastrointestinal enzyme system. Existing data would suggest that pancreatic lipase activity is affected by intestinal pH, the presence of colipase and bile salts, but not by the physiological range of Ca ion concentration (as is commonly assumed). The control of secretion of pancreatic lipase and its associated factors appears to be driven by gastrointestinal luminal content, particularly the presence of acid or digested proteins and fats in the duodenal lumen. Secretion of colipase, bile acids and pancreatic lipase is driven by cholecystokinin and secretin release.

Pancreatic lipase: Gastrointestinal enzymes: Small intestine: Lipolysis: Colipase

Introduction

A myriad of factors of dietary, endogenous and bacterial origin occur in the lumen of the small intestine, and therefore have the potential to affect the digestive processes there within. Pancreatic lipase (also referred to as TAG acyl hydrolase⁽¹⁾ and originally named steapsin⁽²⁾; EC 3.1.1.3) is one of a range of enzymes secreted by the pancreatic acinar cells that is involved in digesting dietary lipids for subsequent absorption in the small intestine.

cDNA for human pancreatic lipase was first isolated in the 1980s⁽³⁾. The primary and tertiary structures of human pancreatic lipase were described soon after⁽⁴⁾. Three subgroups of human pancreatic lipases have been identified which share about 70% sequence identity⁽⁵⁾. Pancreatic lipase-related proteins 1 and 2 have similar structure and action to pancreatic lipase, although it is possible they may have different substrate specificity. These enzymes are also produced by the pancreas and secreted into the duodenum, and are characterised in detail elsewhere^(6–13). The term ‘pancreatic lipase’ in the present review refers to ‘classical’ or ‘colipase-dependent’ pancreatic lipase from this point on.

Human pancreatic lipase is a 50 kDa peptide chain consisting of two structural domains: the catalytically active,

336-amino acid long N-terminal domain, and the shorter (113 residues) C-terminal domain⁽¹⁴⁾, which binds to the cofactor colipase (see below). The lipase family of enzymes acts at the interface of lipid and aqueous mixtures. This interfacial binding is unique in digestive enzymes. The majority of lipases are water soluble and catalyse cleavage of water-insoluble TAG⁽¹⁵⁾. Pancreatic lipase differs from other mammalian digestive hydrolase systems in that it is not a single domain enzyme. Its catalytic activity is increased in the presence of the cofactor colipase. As pancreatic lipase acts at the interface of lipid and water, its action is also dependent on the presence of bile acids or other moieties capable of emulsifying lipids⁽¹⁾. As a result of these factors, the classic lock-and-key model does not lend itself well to understanding lipase catalytic behaviour and activity.

Plasma levels of pancreatic lipase are routinely measured in the diagnosis of pancreatic dysfunction⁽¹⁶⁾. In addition, pancreatic enzyme mixtures are often given therapeutically to patients with pancreatic exocrine deficiency, including those with chronic pancreatitis and cystic fibrosis⁽¹⁷⁾. In measuring the levels of pancreatic lipase activity, a number of tests that use synthetic substrates have been developed. To reduce interference of other lipolytic factors

Abbreviation: CCK, cholecystokinin.

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in plasma, these lipase activity assays are carried out at high (often unphysiological) pH. While such models are clearly useful diagnostic tools, it must be noted that these types of assays do not adequately predict the action of pancreatic lipases under gastrointestinal conditions.

Tetrahydrolipstatin (commonly referred to as orlistat) is a hydrogenated derivative of a naturally occurring compound originally isolated from *Streptomyces toxytricini*⁽¹⁸⁾. It was developed as a pharmacological agent to inhibit dietary lipid absorption during the 1980s and was approved for drug use in 1998⁽¹⁹⁾. Orlistat inhibits a range of human lipases (including gastric and pancreatic) through covalent binding to the active site serine residue that is essential for enzymic action⁽¹⁸⁾.

Previous clinical data suggest that orlistat reduces fat absorption by about 30% in human subjects. Double-blind, placebo-controlled evidence also demonstrates the efficacy of orlistat in reducing body weight in obese individuals^(20–22), as well as further benefitting other anthropometric and plasma biomarkers of CVD risk in this population group^(23,24). The clinical efficacy of orlistat is tempered by common gastrointestinal side effects, including steatorrhoea and faecal incontinence that result from excess dietary lipids entering the colon. While poor compliance for orlistat usage has been reported as a result of these unwanted side effects⁽²⁵⁾, inclusion of extra viscous, non-fermentable fibre in the diet appears to greatly reduce their occurrence⁽²⁶⁾.

The above evidence for orlistat would suggest that inhibition of pancreatic lipase is an effective strategy for benefitting weight management (and therefore obesity therapy). Through the study of the physiological and biochemical processes involved in intestinal lipid hydrolysis, it may be possible to develop novel pharmaceutical and/or food products that effectively inhibit pancreatic lipase but do not cause unwanted gastrointestinal side effects.

The rest of the present review will summarise evidence on how pancreatic lipase functions, including details on how physiological factors affect its activity and secretion.

Pancreatic lipase

Secretion

Pancreatic acinar cells are highly polarised, with a small apical membrane domain, and a much larger basolateral domain⁽²⁷⁾. They synthesise, store and secrete a variety of enzymes into the duodenal lumen on demand. These enzymes, including pancreatic lipases, are stored in zymogen granules within the apical pole of the cells. A wide range of secretagogues, including acetylcholine and cholecystokinin (CCK), stimulate basolateral membrane receptors in the acinar cells. This generates an intracellular response driven by Ca^{++} , diacylglycerol and cAMP⁽²⁸⁾. These secondary messengers stimulate fusion of the zymogen granules with the apical membrane, leading to digestive enzyme secretion from the cells. These processes are discussed in greater detail elsewhere^(27,29). While the intracellular pathways involved in pancreatic enzyme release (including protein sorting and zymogen granule binding) are not fully understood^(27,28), a number of pathways

have been suggested for control of the luminal factors relevant to lipolytic activity.

While it is currently unsure whether the release of separate pancreatic enzymes is independent of each other or not⁽³⁰⁾, CCK appears to be the main hormonal drive for increased zymogen secretion and, therefore, lipase secretion. CCK is released from I cells and enteric nerves in the duodenal and jejunal mucosa in response to the presence of fats and amino acids in the intestinal lumen⁽²⁸⁾. Released CCK is believed to affect intestinal stimulation through contact with CCK_A receptors found within the pancreas and the intestinal crypt epithelia^(31,32).

Further stimulation for release of pancreatic enzymes comes from parasympathetic acetylcholine release from pre- and postganglionic neurones. This acetylcholine release stimulates muscarinic M₃ receptors, which in turn triggers the intracellular cascades driving zymogen granule exocytosis. Cholinergic stimuli will occur within cephalic, gastric and intestinal phases of digestion⁽³³⁾.

Pancreatic lipase appears to lose catalytic activity below pH 5. This observation would suggest a potential exacerbation of low lipase activity in disorders causing pancreatic exocrine insufficiency, as not only will lower amounts of lipase be secreted into the duodenal lumen, but it may also be catalytically inactive due to low intestinal pH, caused by a lack of pancreatic bicarbonate secretion.

Previous observations in fasting (healthy) human subjects have noted that peak levels of pancreatic enzyme secretion (including pancreatic lipase) appear to occur at the commencement of phase III of the duodenal migrating motor complex (characterised by regular, high-amplitude phasic contractions). Additional peak levels of secretion also appear to occur at non-specific time points within the interdigestive (fasting) stages of motility^(34,35).

During the fed state, intestinal motor responses to the presence of nutrients in the duodenum appear to occur over the same period of time as pancreatic secretory responses⁽³⁶⁾. This is to be expected, as both responses are elicited by similar hormonal drives.

Secretin which is released from duodenal S cells in response to the presence of luminal contents and parasympathetic stimulation may also work in synergy with CCK in up-regulating the release of pancreatic enzymes⁽³⁷⁾. Release of pancreatic polypeptide and somatostatin from the pancreas results in local inhibition of acinar cell exocytosis⁽³⁸⁾. Cholinergic stimulation of pancreatic enzyme exocytosis is also inhibited by elevated circulating concentrations of pancreatic polypeptide⁽³⁹⁾, glucagon-like peptide-1^(40,41) and peptide YY (released from the ileum) and ghrelin and leptin^(42,43).

Catalytic action

The pancreatic lipase complex catalyses hydrolysis of the ester bonds that attach fatty acids to the glycerol backbone in di- and triacylglycerols. Glycerol contains an alcohol –OH group at each of its three carbons to which fatty acids can be linked by ester bonds⁽⁴⁴⁾. Pancreatic lipase has no activity towards the central *sn*-2 ester bonds, but is specific to the cleavage of the outer *sn*-1 and *sn*-3 esters. However, *sn*-2 ester bonds slowly undergo a non-enzymic

isomerisation to 1-monoacylglycerols under the alkaline conditions of the small intestine, subsequently making them available for hydrolysis as well, potentially allowing full TAG hydrolysis⁽¹⁾.

Pancreatic lipase retains the same serine 152–histidine 263–aspartate 176 triad that is conserved within other members of the lipase family^(4,45). Serine–histidine–aspartate triads also drive catalytic activity in serine proteases⁽¹⁴⁾. Four main steps, involving changes in conformation or charge, occur to the pancreatic lipase molecule⁽¹⁴⁾:

- (1) Initial closed conformation: a loop structure within the N-terminal (residues 237–261), often referred to as the ‘lid’ domain, initially covers the active site. Two other domains maintain the lid in this closed confirmation by van der Waal’s forces; $\beta 5$ (75–84) and $\beta 9$ (203–223).
- (2) Transition to open confirmation: the $\beta 5$ domain moves away from the lid domain, causing the lid domain to uncover the active site (the open conformation).
- (3) Formation of an oxyanion hole: the movement of the $\beta 5$ domain also creates an electrophilic region around the triad serine residue. This oxyanion hole helps stabilise the intermediate catalytic product formed during the reaction. Aromatic side chains from residues tyrosine 114, phenylalanine 215 and phenylalanine 77 endow the oxyanion hole with hydrophobicity, as does the presence of proline 180, isoleucine 209 and leucine 202.
- (4) Binding to substrate and catalysis: two acyl-binding sites in the $\beta 9$ domain allow the carbonyl carbon of the primary ester bond access to the triad serine 152 residue. The *sn*-1 (or because of molecular symmetry, the *sn*-3) acyl chain is held within the oxyanion hole. The *sn*-2 chain lies within a second hydrophobic groove formed by side chains of lid domain residues (251 to 259) and by isoleucine 78 in the $\beta 5$ domain⁽⁴⁶⁾. This results in hydrolysis of the acylglycerol substrate. Two residues (leucine 213 and phenylalanine 215) on the $\beta 9$ domain contact with alkyl chains are implicated in increasing hydrolysis product stability⁽¹⁴⁾.

Interestingly, pancreatic lipase still shows appreciable *in vitro* activity to hydrolyse dietary TAG in the absence of colipase, but cannot function without the presence of bile salts, as shown in Fig. 1.

Effect of pH on pancreatic lipase activity

As they must maintain activity under a wide range of physiological conditions, gastrointestinal enzymes show a much greater resistance to irreversible denaturation. In particular, pancreatic lipase has to be stable under a wide range of pH within the small intestine. The enzyme is secreted as part of the pancreatic juice at about pH 8. The approximate pH within the small intestine is suggested to be from about 6.5 in the duodenum to over 7 in the distal ileum. However, previous assessments agree that there is a generally higher pH more distally in the small intestine, and a much wider pH range occurs in both the healthy and diseased state (see Table 1).

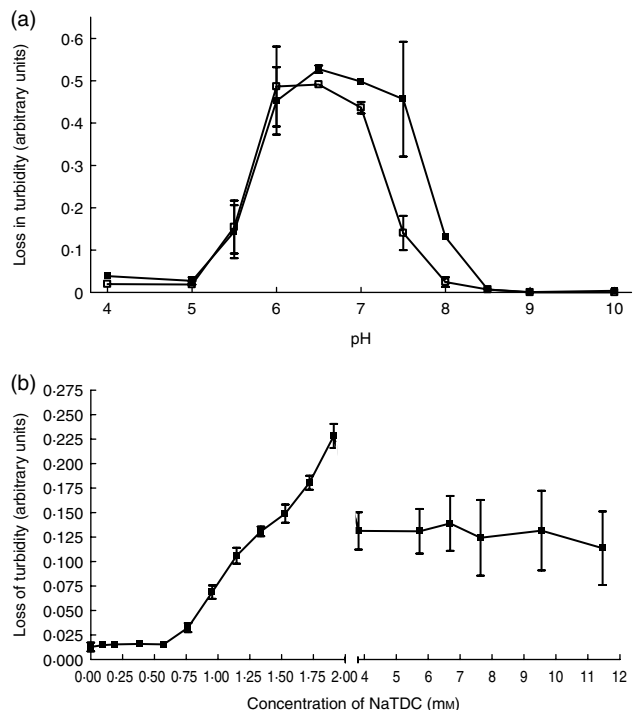


Fig. 1. Effect of colipase presence and bile salt concentration on porcine pancreatic lipase activity. (a) Lipase activity over a pH range in the presence (■; 23.8 μg/ml) and absence (□) of colipase. (b) Lipase activity over a range of bile salt (sodium taurodeoxycholate; NaTDC) concentrations at pH 7. Values are means, with standard errors represented by vertical bars. Olive oil micelles were used as a substrate using procedures modified from Vogel & Zieve⁽⁹⁷⁾.

Control of small-intestinal pH

Control of acid output of the stomach (i.e. pH of the digesta entering the small intestine) is reviewed in detail elsewhere^(47,48). However, as pancreatic lipase activity, and that of other hydrolytic enzymes in the small intestine, favours a more neutral pH, processes must be in place to quickly and effectively raise intestinal pH. The pH of the small-intestinal lumen can be raised by secretion of bicarbonate ions at three separate sites: pancreatic and hepatic ducts and small-intestinal epithelial cells (particularly those located in intestinal crypts). The model of bicarbonate secretion is analogous in these three sites at a cellular level (see Fig. 2), although it must be noted that the small-intestinal epithelia also allows paracellular migration of bicarbonate, due to the intercellular junctions being considerably leakier⁽⁴⁹⁾ than hepatic and pancreatic duct junctions. Stimulated output of bicarbonate from the luminal epithelium has been suggested to vary along the length of the small intestine, with the highest in outputs in the proximal duodenum equating to approximately 200 μmol/cm per h⁽⁵⁰⁾. This suggests (assuming small-intestinal length of 5 m) an intestinal output of bicarbonate of up to 100 mmol/h. Secretin stimulation of healthy human participants did not appear to affect hepatobiliary bicarbonate concentration (30 mmol/l pre-stimulation *v.* 35 mmol/l post-stimulation), but secretion volume rose from 20 ml/h to nearly 80 ml/h⁽⁵¹⁾, suggesting a

Table 1. Reported pH range in the human small intestine

Reference	Measured by	Population group	pH range
Hernell <i>et al.</i> (1990) ⁽¹⁰⁰⁾	Intestinal aspiration	Four healthy adult males	6.1–8.1
Kay <i>et al.</i> (1983) ⁽¹⁰¹⁾	Intestinal aspiration	Mixture of twenty-five chronic pancreatitis and twenty-five non-pancreatic patients	5.45–6.30*
Watson <i>et al.</i> (1972) ⁽¹⁰²⁾	pH telemetry	Two healthy participants and seven gastroenterology patients	4–8.2
Evans <i>et al.</i> (1988) ⁽¹⁰³⁾	pH telemetry	Sixty-six healthy subjects	6.1–7.1†
Zentler-Munro <i>et al.</i> (1984) ⁽¹⁰⁴⁾	Intestinal aspiration	Twelve adult cystic fibrosis patients	Of the samples, 18% were < pH 5 and 56% were > pH 6
Fallingborg <i>et al.</i> (1989) ⁽¹⁰⁵⁾	pH radiotelemetry	Thirty-nine healthy participants	6.4–7.3‡
Sasaki <i>et al.</i> (1997) ⁽¹⁰⁶⁾	pH telemetry	Four healthy participants	6.3–7.9
		Four Crohn's patients	6.9–8.6
Fallingborg <i>et al.</i> (1998) ⁽¹⁰⁷⁾	pH radiotelemetry	Nine patients with inactive Crohn's disease	6.2–7.5
		Thirteen healthy participants	5.9–8.1
Press <i>et al.</i> (1998) ⁽¹⁰⁸⁾	pH radiotelemetry	Twenty-three inflammatory bowel disease patients	6.1–9.2
		Twelve healthy participants	6.2–7.9
Brouwers <i>et al.</i> (2005) ⁽¹⁰⁹⁾	Intestinal aspiration	Five healthy participants	6.5–8.2§
Perez De La Cruz Moreno <i>et al.</i> (2006) ⁽¹¹⁰⁾	Intestinal aspiration	Six healthy participants	6.3–7.5
Ibekwe <i>et al.</i> (2008) ⁽¹¹¹⁾	pH radiotelemetry	Eight healthy participants	5.9–7.8

* Range includes highest and lowest mean duodenal pH values for participants with varying duodenal chymotrypsin activity.

† Range includes highest and lowest mean segmental values given.

‡ Only mean proximal and distal values cited.

§ Range includes highest and lowest mean pH values over a 240 min time-course in duodenum and jejunum.

total stimulated bicarbonate output of < 3 mmol/h. Similar studies on pancreatic juice suggest a stimulated volume output of 200 ml/h⁽⁵²⁾ with bicarbonate output reaching 90 mmol/l⁽⁵³⁾ (total stimulated output of < 20 mmol/h).

Secretin is the principal hormonal stimulant driving bicarbonate and fluid secretion into the small intestine^(42,54,55). Secretin acts through attachment to G-protein-coupled receptors that also show affinity for vasoactive intestinal peptide⁽⁵⁶⁾. Unlike pancreatic enzyme secretions, pancreatic release of bicarbonate appears to be unaffected by pancreatic polypeptide⁽³⁹⁾.

Bile acids

Bile acids are secreted by the liver, and stored in the gallbladder in man. From here, they are ejected into the duodenum via the biliary duct by contraction of the surrounding smooth muscle. Bile acids are synthesised from cholesterol within hepatocytes. About 95% of bile acids within secreted bile are actually from the recirculating pool of bile that occurs from liver to duodenum to distal ileum/large bowel to liver⁽⁵⁷⁾. Recycled bile acids are reabsorbed at the hepatocyte serosal (or sinusoidal) membrane by a range of specific Na-dependent and Na-independent transporters.

The majority of bile acids secreted (both recycled and new) in man are conjugated within the hepatocytes to glycine and taurine amino acid residues. This process is catalysed by bile acyl-CoA:amino acid N-acyltransferase, which is localised in peroxisomes and the cytosol⁽⁵⁸⁾. Conjugated bile acids are considerably more soluble in the intestinal milieu than unconjugated bile acids⁽⁵⁹⁾, thus endowing bile salts with greater solubility to benefit their role in making dietary lipids more available to pancreatic

lipase. Bile acids and salts act as biological detergents to emulsify dietary lipids, thereby greatly increasing the surface area of the lipid–aqueous interface for pancreatic lipase action. Critical micellar concentrations of different types of bile acids tend to occur within a low millimolar range, but bile acids occur well in excess of this concentration in the small intestine^(60,61).

Following conjugation, bile salts are then secreted into hepatic canaliculi – secretory lumens formed between two or more adjacent hepatocytes and their tight junction complexes. Transport of conjugated bile across the canalicular membrane occurs through ATP-driven bile salt export pumps⁽⁵⁹⁾. The canaliculi empty into larger bile ductules that eventually drain into the common bile duct⁽⁶²⁾. In man, and many other mammals, the bile drains into the gallbladder for subsequent release. The rat does not have a gallbladder, and so is considered a poor model of human bile circulation, and hence a poor model of fatty acid and cholesterol metabolism.

CCK appears to be the major effector of bile acid secretion, acting to mediate bile release through sphincter of Oddi relaxation and contraction of the smooth muscle surrounding the gallbladder⁽⁶³⁾. Other stimuli for gallbladder contraction/sphincter of Oddi relaxation appear to be motilin and vagal efferent impulses, whereas pancreatic polypeptide and somatostatin release acts to inhibit gallbladder output⁽⁶⁴⁾.

Previous reports suggests that bile acid action results in an emulsion of stable lipid particles of less than 0.5 µm in diameter⁽¹⁾. *In vitro*, higher ratios of unconjugated bile acids appear to inhibit lipase activity⁽⁶⁵⁾, while conjugated bile acids stimulate lipolysis below critical micellar concentrations, but appear to be strongly inhibitory at higher concentrations⁽⁶⁰⁾.

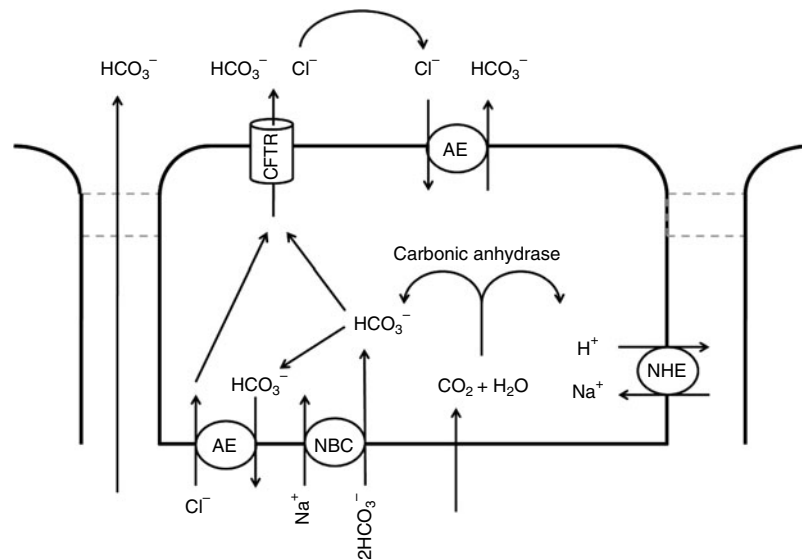


Fig. 2. Putative cellular mechanisms involved with bicarbonate secretion from pancreatic duct, hepatic duct and small-intestinal epithelial cells. Intracellular accumulation of bicarbonate occurs through conversion of CO_2 (which passively diffuses into the cell from the blood) by carbonic anhydrase and the action of the basolateral $\text{Na}^+\text{--HCO}_3^-$ co-transporter (NBC). In the unstimulated cell, bicarbonate is removed from the cell by anion exchangers (AE) at the apical and basolateral membranes. Within the stimulated cell, the basolateral AE action is halted. The conductance of the apical chloride leak channel (CFTR) is raised, which results in higher localised chloride concentrations apically, thus driving increased bicarbonate release through the apical AE. During the latter stages of stimulation of bicarbonate release (i.e. when the apical and luminal concentration of bicarbonate is high), it is believed that the apical AE becomes inhibited. Bicarbonate efflux then occurs through the CFTR. Passive diffusion of bicarbonate from the blood to the lumen (left of figure) can only occur in the leaky epithelia of the intestine. Adapted from details in Allen & Flemström⁽⁵⁴⁾, Kanno *et al.*⁽⁹⁸⁾ and Steward *et al.*⁽⁹⁹⁾. Other membrane transporters indirectly involved in driving these processes, such as the basolateral $\text{Na}^+:\text{K}^+:2\text{Cl}^-$ ATPase and K leak channels, are not included for clarity. NHE, $\text{Na}^+\text{--H}^+$ exchanger.

A number of reports have suggested that bile acids actually inhibit lipase activity as a result of the observed effect of reducing interfacial adhesion (for example, Gargouri *et al.*⁽⁶⁶⁾; Patton & Carey⁽⁶⁷⁾). In the physiological situation, however, the detergent action of bile will greatly increase the surface area within the small-intestinal lumen, thereby increasing total accessibility⁽⁵⁾.

Colipase

Human procolipase is a ninety-five-amino acid protein secreted by the pancreatic acinar cells. It is rapidly converted to colipase by tryptic and proteolytic action⁽⁶⁸⁾, causing cleavage at the Arg 5–Gly 6 bond⁽⁶⁹⁾. Within this conversion, a five-amino acid fragment is lost. This pentapeptide, also referred to as enterostatin⁽⁷⁰⁾, has been suggested to be important in appetite control in animal studies⁽⁷¹⁾.

Due to shared pathways of secretion, a similar range of neurohumoral mediators is likely to affect up- and down-regulation of procolipase release as that of pancreatic lipase (see above).

Colipase is an 11 000 Da protein that is catalytically inactive on its own, and binds to pancreatic lipases in a 1:1 ratio⁽⁷²⁾. Structurally, colipase is a small amphipathic protein, stabilised against acid denaturation by five disulfide bridges⁽⁷³⁾. As it can interact with both aqueous and non-aqueous compounds, it is believed to be important in helping ‘anchor’ pancreatic lipases to lipid droplets and micelles. The presence of NEFA in lipid micelles has been suggested to favour colipase interfacial binding^(72,74).

Murine knock-out studies have shown that procolipase-deficient mice had a 60 % lower postnatal survival rate (presumably due to a decrease in the available energy during weaning) and also had reduced body weight (by 30 %) compared with wild types during and after weaning. In addition, when knock-out mice were fed a high-fat diet, they exhibited fat malabsorption through steatorrhea⁽⁷¹⁾.

Crystallographic analysis suggests that colipase binds to the non-catalytic, C-terminal domain of pancreatic lipase⁽⁵⁾. This interaction between colipase and the C-terminal is through the amino acids in two colipase hairpin loops. The weak association between the two proteins is increased in the presence of a lipid interface⁽⁵⁾. The binding of colipase to pancreatic lipases is also believed to play a role in stabilising the open-lid conformation⁽⁷²⁾. Mutations at the Glu 15 residue of the colipase protein, which occurs within the area that binds to the lipase lid, greatly reduced the potential of colipase to maintain the open-lid confirmation⁽⁵⁾.

The main area of the colipase that interacts with micelles is believed to be the 70–85 loop⁽⁵⁾. Further residues have been suggested to be important in this interaction through a range of studies. Computational modelling of colipase binding with lipid droplets and bile salt micelles hypothesises that hydrophobic finger areas of the colipase molecule are the interfacial binding site⁽⁶⁸⁾. Interfacial interactions of colipase have been shown by *in vitro* study to be dependent on the lipid substrate involved, as well as the ionic strength and pH of the aqueous phase⁽⁶⁸⁾. Previous studies have demonstrated that colipase–lipase binding occurs in such a way that the hydrophobic regions of the

colipase molecule are orientated towards the lipid–aqueous interface^(5,75). Site-directed mutagenesis towards this area revealed that Tyr 55 and 59 residues appeared to be important in the ability of colipase to act as a cofactor⁽⁷⁵⁾ in pancreatic lipase produced from human cDNA. Further suggestion that this hydrophobic region of colipase was integral in interface binding came from NMR analysis of conserved areas of porcine and equine colipases. Within these studies, two aromatic residues (Tyr 55 and either Trp or Phe residues at the 52 position) were also associated with interaction with bile salt micelles⁽⁷⁶⁾.

Calcium ions

Unlike other factors involved in intestinal lipolysis, Ca⁺⁺ concentration within the small-intestinal lumen is mainly as a result of dietary intake. Ca is believed to be involved in the catalytic activity of the pancreatic lipase–coenzyme complex. Previous *in vitro* lipolysis studies have demonstrated an increase in purified human pancreatic lipase activity of about 8% in the presence of 0.5 mM–Ca compared with a Ca-free solution. No further increase in activity was seen with higher concentrations of Ca up to 4 mM⁽⁷⁷⁾. Early work in this area using radioisotope studies suggested that Ca has no binding affinity for either colipase or lipase. However, low concentrations (plateau of maximal activity at 40 μM–Ca⁺⁺) still appeared to increase lipolysis V_{max} (maximum velocity of reaction) and decrease the apparent K_m for the olive oil emulsion substrate⁽⁷⁸⁾. It has been postulated that the presence of Ca⁺⁺ reduces the lag phase of pancreatic lipase-mediated lipolysis⁽⁷⁹⁾. The presence of Ca ions was shown to reduce the surface charge of micelle droplets, but this did not correlate with lag phase duration⁽⁸⁰⁾. From the above evidence, it appears that Ca⁺⁺ is not essential for intestinal lipolysis. It is unlikely that luminal concentrations (largely governed by dietary intake) will fall below micromolar levels, and even if they do, this only has minor consequence to pancreatic lipase activity.

Luminal control of intestinal lipolytic activity

Enteroendocrine system

While control of intestinal lipolysis is mediated by endocrine, neural and paracrine factors basolaterally, the initial stimulus that affects the release of these factors comes from the composition of the digesta within the gut lumen. Conditions that drive the release of these agonists and antagonists of pancreatic exocrine secretion and other processes involved in intestinal lipolysis are mediated through sampling (chemo-sensing) of the luminal contents by enteroendocrine cells^(81,82). Mechanical and luminal stimuli cause release of enteroendocrine intracellular Ca⁺⁺ stores, resulting in the release of humoral mediators of digestive control. Within the small intestine, specific luminal factors are known to drive lipolytic activity.

Factors such as the presence of an acidic bolus and digested proteins and fats have been demonstrated to cause the release of CCK or secretin from the small-intestinal mucosa in human studies⁽⁸³⁾. In particular, strong CCK

releases have been noted in response to the presence of luminal fatty acid with a chain length of > eleven carbons⁽⁸⁴⁾ or the amino acids tryptophan and phenylalanine⁽⁸³⁾. Secretin release appears to be mainly driven by intestinal luminal acidity^(85,86), particularly below pH 4.5.

Luminal down-regulation of lipolytic secretions comes from intestinal tryptic and proteolytic activity^(87,88). This feedback regulation was shown to only affect CCK-mediated pathways, not cholinergic pathways, in human studies⁽⁸⁹⁾. Tryptic activity has also been shown to reduce pancreatic lipase activity *in vitro*, due to proteolysis of the active enzyme⁽⁹⁰⁾.

Dietary inhibitors of lipolytic activity

More recently, a number of other naturally derived compounds have been noted to have an impact on pancreatic and gastric lipase activity *in vitro* or in animal studies, including those from seaweed^(91,92), green tea⁽⁹³⁾, berries⁽⁹⁴⁾ and wheat flour⁽⁹⁵⁾. A recent review has also highlighted a number of other plant products that inhibit pancreatic lipase action, including saponins and polyphenols⁽⁹⁶⁾. It must be noted that such compounds are likely to have similar unwanted gastrointestinal side effects to orlistat unless consumed at lower amounts or in the presence of bulking agents⁽²⁶⁾.

Summary

Intestinal lipolysis is perhaps the most complex endogenous enzyme-driven process that occurs within the human gut. Three major factors (pancreatic lipase, colipase and bile acids) are released in response to various luminal conditions that drive the rate of dietary lipid digestion. The formation of lipid micelles and their potential to bind to lipase–colipase complexes are important rate-determining steps in this catalytic process.

The present review highlights the cascade of luminal, local and neurohumoral factors associated with the rate of lipid hydrolysis in the small intestine. Novel treatments that aim to target lipolytic pathways to treat obesity should aim to do so in a way that does not produce unwanted gastrointestinal side effects in order to increase patient compliance.

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