

# Human Pancreatic Digestive Enzymes

David C. Whitcomb · Mark E. Lowe

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**Abstract** A primary function of the pancreas is to produce digestive enzymes that are delivered to the small intestine for the hydrolysis of complex nutrients. Much of our understanding of digestive enzymes comes from studies in animals. New technologies and the availability of the sequence of the human genome allow for a critical review of older reports and assumptions based on animal studies. This report updates our understanding of human pancreatic digestive enzymes with a focus on new insights into the biology of human proteases, lipases and amylases.

**Keywords** Trypsin · Elastase · Lipase · Amylase · Co-lipase

Ivan Pavlov, one of the great surgeons and physiologists of the 19th century, in his book “The Work of the Digestive Gland” remarked,

Our results, I hope, have for ever done away with the crude and barren idea that the alimentary canal is universally responsive to every mechanical, chemical, or thermal agency, regardless of the particular requirements of the each phase of digestion. Instead of this hazy conception, we now see delineated an intricate mechanism which, like everything else in nature, is adapted with

the utmost delicacy and precision to the work which it has to perform. (Quoted in Rothman [1])

This observation continues to be strengthened and expanded, as the details of digestion and absorption are unveiled.

The digestive enzymes of the pancreas play a central role in digestion. Indeed, pancreatic failure from congenital disorders such as cystic fibrosis teaches us that the mixture of digestive enzymes produced by the pancreas is essential for life. This system is so important that redundant systems for digestion of the essential nutrients exist throughout the digestive tract to assist digestion. Furthermore, the content and specificity of the digestive enzymes remain under amazing control so that humans can adapt to a wide range of diets, whether they are carnivorous or vegetarian.

With this redundancy and flexibility in mind, each of the major classes of digestive enzymes are found in both in the upper digestive tract and in the pancreas. The focus of this article will be on the nutrients to be digested (protein, carbohydrates, fats, and other compounds), and the major pancreatic enzymes that digest specific chemical bonds of complex nutrients.

## Protein digestion and human proteases

Proteins typically make up about 10% of caloric intake in Western diets. Proteins, which are eventually digested into amino acids, are critical for proper growth, development, repair, energy, and every type of bodily function. The variety and complexity of dietary proteins and the critical role of amino acids in bodily function is reflected in the fact that nearly 80% the pancreatic enzymes by weight are proteases.

Although the major exocrine products of the pancreatic acinar cells are proteases, a variety of additional proteases

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D. C. Whitcomb (✉)  
Departments of Medicine, Cell Biology and Physiology, and  
Human Genetics, University of Pittsburgh,  
Pittsburgh, Pennsylvania  
e-mail: whitcomb@dom.pitt.edu

M. E. Lowe  
Pediatrics and Molecular Genetics and Biochemistry,  
University of Pittsburgh,  
Pittsburgh, Pennsylvania

are expressed throughout the digestive tract, and especially in the stomach and small intestine. The stomach produces the pepsinogens that hydrolyze peptide bonds involving phenylalanine, tyrosine, and leucine [2]. These enzymes have optimal activity between pH 1.8 and 3.5 and are irreversibly inactivated in neutral or alkaline pH [3]. This class of enzyme is especially useful in digesting muscle, tendons, and other components of meat with a high collagen content.

The enterocytes of the small intestine produce several aminopeptidases, 2 carboxypeptidases, 2 endopeptidases, and  $\gamma$ -glutamyl transpeptidase [2]. These enzymes are most effective in digesting small peptides after initial hydrolysis of complex proteins by gastric and pancreatic enzymes. It is likely that intestinal peptidases are not capable of digesting enough proteins to meet metabolic needs of children (e.g., pancreatic insufficiency owing to cystic fibrosis), and probably not adults (e.g., alcoholic chronic pancreatitis). Indeed, in a minipig model of complete pancreatic exocrine insufficiency it was shown that when animals were fed meals containing approximately 80 g protein per day, approximately 70% protein remains unabsorbed in the small intestine, whereas only 20% is unabsorbed in the small intestine in control animals [4, 5]. Surprisingly, residual protein in feces dropped to only 40% in pancreatic insufficiency and 10% in control pigs, suggesting a possible role for hindgut digestion or fermentation in pigs. In humans with pancreatic insufficiency, there appears to be significant, but insufficient protein digestion, suggesting that there are either unidentified endopeptidases in the brush border [6], or that there are unknown adaptive responses in the intestine in the context of pancreatic failure.

Proteases must be carefully regulated because their activity in the wrong location can lead to digestion of human

tissues, activation of inflammatory pathways, and disruption of a variety of systems that use enzymes as key mediators. An example is the premature activation of trypsin within the pancreas to trigger life-threatening acute pancreatitis [7]. Therefore, issues of protease regulation may be as important as protease activity.

### Pancreatic proteases

The pancreas is the major source of proteases in the digestive system for the digestion of ingested proteins. The primary proteases are synthesized as inactive pro-enzymes (zymogens) and include 3 forms of trypsin; chymotrypsinogen A and B; proelastase, procarboxypeptidase A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub>, B<sub>2</sub>; and other proteases, although the exact nature of the human enzymes compared to animal enzymes has not been fully elucidated (Table 1). The trypsins, chymotrypsins and elastase are endopeptidase of the serine protease family of enzymes. The x-ray crystallographic structure of the serine proteases are highly homologous, but they have different specificity pockets that allow only selected peptide bonds to be hydrolyzed. For example, trypsin-like proteases hydrolyze peptides at the site of basic amino acids (lysine and arginine); chymotrypsin hydrolyses peptides involving aromatic amino acids (phenylalanine, tyrosine, tryptophan), and elastase splits the protein backbone at bonds at uncharged small amino acids (such as alanine, glycine, and serine) [2]. The other major class of proteases are metalloproteinases, which include the carboxypeptidases. Carboxypeptidase-A attacks the last amino acid of a target peptide chain when it is aromatic, neutral, or acidic amino acid, while carboxypeptidase-B attacks basic amino acids. The different

**Table 1** Major proteases in humans pancreas: Pancreatic proteases

Enzyme	Action	Product(s)
Trypsin	Endopeptidase; cleaves internal bonds at lysine or arginine residues; <i>activates other pancreatic proenzymes</i>	Oligopeptides
Cationic trypsinogen (PRSS1)		
Anionic trypsinogen (PRSS2)		
Mesotrypsin (PRSS3)	Endopeptidase – arginine	
Pancreasin (?)		
(Trypsin IV ?)		
Chymotrypsin	Endopeptidase; cleaves bonds at aliphatic amino acid residues	Oligopeptides
Chymotrypsinogen B1		
Chymotrypsin-like protease		
Caldecrin (?) [63]		
Elastase (unclear which form is secreted)	Endopeptidase; cleaves bonds after small amino acid residues – alanine, glycine, serine	Oligopeptides
Elastase 2A, 2B		
Elastase 3A, 3B		
Carboxypeptidase A	Exopeptidase; cleaves aromatic amino acids from carboxyl terminal end of protein and peptides	Aromatic amino acids and peptides
A1, A2, A3		
Carboxypeptidase B	Exopeptidase; cleaves arginine or lysine from carboxyl terminal end of proteins and peptides	Arginine, lysine, and peptides
B1, B2		

isoforms of the enzymes (carb-A and -B) arise from different genes and function optimally under slightly different conditions. However, there is significant overlap in function, such that isolated mutations or deletions of specific pancreatic digestive enzyme genes result in no discernable phenotype. This argues to the importance of the overall system that, like the small intestinal enterocytes, uses multiple parallel and redundant systems to ensure that adequate amounts of all of the key amino acids are available for growth and maintenance of the organisms, despite diversity and inconsistency of diet.

### Serine proteases

The serine proteases (trypsin, chymotrypsin, and elastase) are enzymes that share several important structural features including a serine at the catalytic site [8]. All serine proteases are formed from a single peptide that folds into 2 globular domains connected by a single chain called the *autolysis loop* (trypsin 117–128 in the codon nomenclature, or 112–123 in the chymotrypsinogen numbering system, whereas the autolysis loop for chymotrypsin is 142–153). The catalytic site is formed by 3 peptides, numbered as H57, D102, and Ser 195 (chymotrypsin numbering system) and have a specificity pocket that determines the amino acid side chain recognition determined by 3 positions: 189, 216, and 226 (e.g., DGG for trypsin, SGG for chymotrypsin–chymotrypsin numbering system).

Each of these serine proteases has an activation peptide of differing lengths, with the activation peptide of chymotrypsin remaining attached to the enzyme after cleavage. The activation peptides are all activated by trypsin, and once the activation peptide is cleaved there is a conformational change with opening of the specificity pocket to allow binding of substrate. Each of the peptides has an autolysis site, allowing a second trypsin to attack the trypsin molecule by cleavage R122 (using the codon numbering, or at R117 using the chymotrypsin residue numbering system).

It appears possible that chymotrypsin may, in a manner similar to trypsin autolysis, be able to attack another chymotrypsin by cleavage of Tyr146 or Asn148. Elastase autolysis is more uncertain, although a possible hydrolysis site for trypsin is recognized at R125 [9, 10]. Elastase could possibly inhibit elastase 3a (protease E) and 3B at A117 (unpublished predictions based on GenBank LOCUS AL590556). On the other hand, elastase 1B and 2A appear to have a lysine (K) at the homologous site of trypsin, which remains a possible cleavage target for trypsin (unpublished observation based on GenBank LOCUS AL512883). Thus, these serine proteases are activated by trypsin, and possibly inactivated by either trypsin or their own active enzymes. The factor determining activation versus inactivation for the trypsins is calcium (see below).

### Trypsinogen

Trypsinogen is the most important of all of the digestive enzymes because it plays a central role in regulating all of the other digestive enzymes. All trypsin are endopeptidase and hydrolyze the carboxyl groups of arginine or lysine residues of peptides. Trypsin makes up about 19% of the protein in pancreatic juice, and is the most abundant of all pancreatic digestive enzymes. Much of the critical biochemical features of trypsinogen, and the active form trypsin, appear to be associated with limiting trypsin activity within the pancreas and activating it within the duodenum. The expression of multiple forms of trypsin with nearly identical enzymatic specificities attests to the importance of maintaining functional trypsinogen genes within the genome.

The 3 most common forms of pancreatic trypsin have a similar genetic sequence and protein structure formed by a single peptide containing a 14-amino acid signal peptide, an 8-amino acid activation peptide, and a 174-amino acid mature, active molecule. The most abundant form of trypsinogen, which makes up two thirds of all trypsin activity, is *cationic trypsinogen* [11], which was also termed *trypsinogen I* by Figarella *et al.* [12] and *trypsinogen 3* by Scheele *et al.* [13]. The cDNA for cationic trypsinogen was named *TYR1* by Emi *et al.* [14], the gene *T4* by Rowen *et al.* [15], with the UniGene name and symbol designated as *protease, serine 1*, and *PRSS1*, respectively. Currently, *cationic trypsin* and *PRSS1* are used interchangeably to describe this enzyme and the gene [16].

The second major form of trypsinogen is *anionic trypsinogen*, or *PRSS2*. This makes up about one third of trypsin activity, but may be relatively upregulated compared to cationic in alcoholics [17] or under certain inflammatory conditions [18], even though the ratio between the mRNAs appears to remain constant in rodents [19]. The mRNA expression levels under various controlled conditions cannot easily be determined in humans, but it is clear that anionic trypsinogen is more quickly degraded than cationic trypsinogen [18, 20], accounting for the some of the apparent variations.

The third form of trypsinogen is *mesotrypsinogen* [21] or *PRSS3*. Mesotrypsinogen constitutes <5% of total trypsin activity, but has distinct feature of being resistant to inhibition by the specific cationic and anionic trypsin inhibitor that is expressed in the pancreas known as *pancreatic secretory trypsin inhibitor* (PSTI) or by the UniGene name and symbol, serine protease inhibitor, Kazal-type, 1 (SPINK1). This resistance to inhibition may be caused by substitution of an Arg at 198 of mesotrypsin compared to Gly in the other trypsins [22].

A fourth trypsin-like enzyme has recently been identified called *pancreasin* [75]. Pancreasin was originally identified in placental cDNA and represents a new pancreatic trypsin-like serine peptidase that cleaves peptides after an arginine

residue [75]. It has an *N*-terminal signal peptide, activation peptide, a single active mature peptide, and no membrane anchor. Northern blot analysis revealed expression of pancreas only in pancreas. The biology and physiology of this peptide has not yet been determined.

**Regulation of trypsin function.** Trypsin activity is highly regulated. As noted, trypsin is synthesized in the pancreas as an inactive zymogen, trypsinogen. Trypsinogen normally remains inactive until it reaches the duodenum, where it has been traditionally thought to be activated by enterokinase, although current evidence suggests that the molecule thought to be enterokinase is activated by trypsin rather than being the primary activator of trypsin [23]. Trypsin is both activated and inactivated by trypsin. Activation occurs with cleavage of the 8-amino acid trypsinogen activation peptide (TAP), *APFDDDDK* – IVG... , with cleavage occurring between K23 and I24 (or residue K15–I16 in the chymotrypsin residue numbering system). The 4 aspartic acid (D) residues exert a negative charge that inhibits trypsin activation and facilitates enterokinase mediated activation [24] and forms a binding site for calcium. In the presence of calcium the efficiency of trypsin-mediated cleavage of TAP is markedly enhanced [24, 25]. Upon cleavage of TAP the new *N*-terminus, I16–V17, folds into the trypsin molecule to form an internal salt bridge with D194 [26]. The specific, nonpolar solvation of the ileval cavity is required to stabilize the active conformation of the enzyme and open the specificity pocket so that substrate can bind and hydrolysis proceed. The activation process is pH dependent, with protonation of I16 preventing the interaction of I16–V17 with D194 and the molecule remains in trypsinogen-like conformation. These features contribute to the optimal activity of trypsin being between pH 7.5 and 8.5 [21]. Thus, both calcium concentration and pH define the conditions of trypsinogen activation to trypsin.

As noted, trypsin is also inactivated by trypsin through hydrolysis of the connecting chain between the 2 globular domains at R117 (chymotrypsinogen numbering)/R122 (codon numbering). The enzyme remains active after initial hydrolysis, and the bond may be resynthesized [27]. Nevertheless, this does appear to be the first site of trypsin-mediated inactivation and autodigestion [28–31]. As in the activation site, calcium governs access to R122 by a second trypsin molecule [25]. In this case, binding of calcium to the second calcium binding pocket limits [25, 32], rather than facilitates, a second trypsin's attack on the trypsinogen/trypsin molecule. Hence, calcium binding prevents autolysis and supports survival of the active enzyme [33].

The mechanism appears to involve flexibility and movement of at least 12-amino acid residues of the autolysis loop into a conformation that is available for R122–V123 cleavage [10]. Binding of calcium to the calcium-binding loop adjacent to the autolysis loop limits exposure of the autol-

ysis site. The affinity of the binding site near the autolysis loop appears to be slightly higher than that in the activation peptide (4 mmol versus 50 mmol [30] or  $K_D \sim 10^{-1.8}$  versus  $K_D \sim 10^{-3.2}$  mol [24]). Thus, trypsinogen activation and trypsin inactivation are co-regulated by trypsin and calcium acting at different sites and under reciprocal conditions.

**Intrapancreatic regulation of trypsin activity.** A series of control mechanisms to limit trypsinogen activation and trypsin activity are seen within the pancreas, with our most detailed understanding in the acinar cells and ducts. Within the acinar cell trypsinogen activation may be controlled by low calcium, PSTI/SPINK1 (in the context of ongoing inflammation) and compartmentalization [34]. It has been proposed that trypsinogen may be activated to trypsin through mis-sorting of vesicles allowing trypsinogen to come in contact with cathepsin B or other lysosomal enzymes [35]. However, the fidelity of protein compartmentalization is not perfect, and cathepsin B and other lysosomal enzymes appear in zymogen granules and are secreted in the pancreatic juice [36, 37]. The importance of cathepsin B and other lysosomal enzymes in the premature or physiologic activation or inactivation of trypsinogen continues to be debated [38–40], but they likely play some role in pancreatic disease.

**Trypsin-related pathology.** Trypsin-associated pathology is linked to its activity in the wrong place at the wrong time. Studies in families with hereditary pancreatitis revealed that mutations affecting the calcium-regulated activation site [41, 42] or calcium-regulated inactivation sites [43, 44] of the trypsin molecule lead to recurrent acute pancreatitis and eventually chronic pancreatitis [34]. By extension, these studies suggest that acute pancreatitis associated with acinar cell hypercalcemia are trypsin dependent [45, 46], and pancreatitis associated with failure of duct drainage, including cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations [47, 48], are trypsin dependent [34].

The importance of inappropriate trypsin activity within the pancreas may also explain why the severity of the acute inflammatory reaction in acute pancreatitis is greater than the inflammatory response seen with the injury of other organs [49]. Trypsin may cross-activate components of the immune system (e.g., activating the rennin–angiotensin system by digesting angiotensin 2 and 3, disrupting the blood clotting system by bypassing thrombin and other proteases, directly activate the protease activated receptors (e.g., PAR2) bypassing tryptase [50, 51]; see below). However, the role of trypsin and other digestive enzymes in the pathogenesis of acute pancreatitis is beyond the scope of this review.

Regulation of trypsin and trypsin-like enzymes may be important in the lower GI tract, where pancreatic trypsin

activity is normally minimal. As noted above, inflammation is regulated, in part, by a series of important cell surface receptors called protease-activated receptors (PAR). Four major PARs are known: PAR1, PAR2, PAR3 and PAR4, which differ in the type of activating proteases and disabling proteases, tissue localization and function [50]. The PARs are generally activated in physiologic situations by either thrombin–PAR1, PAR3 and PAR4 (related to blood clotting) or trypsin–PAR2 and PAR4 (a product of mast cell degranulation). PAR2 and PAR4 are also cross-activated by the pancreatic trypsins. PAR1 and PAR2 are expressed on epithelial cells and PAR2 plays an important role in regulating inflammation. The role of PAR2 in the gut has been reviewed elsewhere [50, 52]. Of note, the administration of trypsin to mouse colon causes generalized inflammation in wild-type mice, but NOT in PAR2 knock-out mice. In addition, trypsin and trypsin-like peptides may play a significant role in some types of infectious colitis since symptoms are markedly reduced with administration of soybean trypsin inhibitor in mice [53]. Also, trypsin IV, a form of trypsin that is expressed in tumors and in the lower GI tract but *not* in the pancreas appears to be a potential activator of PAR2 and PAR4 in epithelial tissues, and its resistance to endogenous trypsin inhibitors may permit prolonged signaling [54]. Pancreatic cationic trypsin (PRSS1) and anionic trypsin (PRSS2), on the other hand, are inhibited by PSTI/SPINK1, are highly regulated, and activity is normally lost after the jejunum (possibly because of calcium absorption in the duodenum and jejunum). The best evidence is the study of Layer *et al.* [55] demonstrating loss of enzyme function moving from duodenum to ileum resulting persistence of 74% of amylase activity, 22% of trypsin activity, and 1% of lipase activity. Thus, trypsin and protease activity disappear as the intestinal contents reaches the colon. Based on the location of PAR2 and PAR4 in the colon, there is potential danger of colitis with persistence of pancreatic trypsin activity, or with the therapeutic use of synthetic, bacterial or fungal proteases that remain active in the colon.

### Chymotrypsinogen

Chymotrypsinogen is the second most abundant serine protease in pancreatic secretions and makes up about 9% of total pancreatic juice protein [56]. Chymotrypsin is an endopeptidase that hydrolyzes peptides at amino acids with aromatic side chains, including phenylalanine, tyrosine, and tryptophan. Chymotrypsinogen was 1 of the first serine proteases to be crystallized [30], and the kinetics of activation and inactivation have been studied extensively [57]. Chymotrypsinogen is synthesized in the pancreas with an 18-amino acid signal peptide, a 15-amino acid activation peptide, and a 230-amino acid active molecule.

Activation of chymotrypsinogen to chymotrypsin is accomplished by trypsin acting at the Arg15–Ile16 bond (R33–I16 with codon numbering). However, the activation peptide remains attached to the molecule. Retention of the activation peptide through disulfide bonds stabilizes the active enzyme against sensitivity to heat denaturation and acid unfolding, and increases resistance to inactivation by pepsin [58]. Chymotrypsin is inactivated by chymotrypsin with autolysis at Y146 and 148 (releasing a Thr–Asn dipeptide), but this chymotrypsin-digested chymotrypsinogen is still able to be activated [30], unless further degradation occurs. The chymotrypsin autolysis loop (residues 142–151) is distinct from the trypsin autolysis loop.

Scheele *et al.* [13] and Appelt *et al.* [59] identified a single form of chymotrypsinogen in human pancreatic juice, and Carrere *et al.* [56] identified 2 forms of chymotrypsin immunoreactivity in serum. However, the possibility that the higher molecular weight form represents a chymotrypsin-inhibitor complex was not excluded. Tomita *et al.* [60], who first cloned the human chymotrypsinogen gene, found evidence for 2 forms by Southern blot. A review of GenBank using Blast homology searches revealed only chymotrypsinogen B1, which, according to the OMIM entry, has alternately been termed chymotrypsinogen B, chymotrypsinogen A, and  $\alpha$ -chymotrypsinogen. Of note, a chymotrypsin-like protease (CTRL) has been identified on chromosome 16q22.1. This protease is expressed in the pancreas and secreted in pancreatic juice, has a 54% identity to chymotrypsin B, and has a distinct substrate specificity with similarities to both chymotrypsin and elastase-2 (hydrolyzing at tyrosine, phenylalanine, or leucine) [61].

A peptide that was identified as a hypocalcemic factor in acute pancreatitis and termed *caldecrin* was found to be expressed in the human pancreas, was structurally similar to elastase IV and to had chymotrypsinogen-like activity [62]. This enzyme was later demonstrated to be secreted by the pancreas and activated by trypsin, suggesting that it is a digestive enzyme of the serine protease family [63]. The importance of these last 2 proteins in human pancreatic juice is unknown.

### Elastase

Elastase is the third major class of serine endopeptidase in pancreatic juice and digests peptides at alanine, glycine, and serine residues. The designation of an enzyme as an elastase depends on its ability to digest elastin, a highly insoluble extracellular protein giving many tissues their elastic properties [64]. The classic target molecule, elastin is composed largely of glycine, proline, and other hydrophobic residues and contains multiple lysine-derived cross-links so that the enzymes that digest elastin are generally powerful proteases that can hydrolyze numerous proteins [64]. Of note, elastase

inactivates PAR1 and PAR2, which are normally inactivated by neutrophil elastase as part of an inflammatory feedback loop.

Pancreatic proelastase is structurally similar to trypsinogen and other serine proteases in the pancreas with a signal peptide, activation peptide, and a mature, functional protein formed by a single peptide. Proelastase is activated by trypsin and the active enzyme is also likely inactivated by trypsin [9].

Scheele *et al.* [13] identified 2 forms of elastase in human pancreatic juice, and this was confirmed by Appelt *et al.* [59]. Because the sequence of human elastase has only recently been identified through the human genome project, there is limited information on secretion and regulation of the specific forms in pancreatic juice.

A gene for pancreatic “elastase 1” (EC 3.4.21.36) was identified on chromosome 12q13, but this enzyme is not expressed in the human pancreas owing to a promoter mutation [65]. Elastase 2 (also called *neutrophil elastase*, *leukocyte elastase*, or *medullasin*) is a serine protease located at chromosome 19p13.3, but is primarily expressed in neutrophils and not the pancreas. Macrophage elastase (now known as matrix metalloproteinase 12) is a metalloprotease located chromosome locus 11q22.2–q22.3, is not a serine protease, and is not expressed in the pancreas [64]. Elastase 2A and 2B are serine elastases with 90% amino acid homology and similar structural motifs [66]. The genes are located at chromosomal locus 1p36.2 and these enzymes are expressed in the human pancreas [66]. Genes for pancreatic elastase 3A (protease E) and 3B, and are also on chromosome 1 near elastase IIB (or possibly 2B; see GenBank LOCUS AL590556) and may also contribute to the “2 forms” of elastase and the elastase activity found in human pancreatic juice by Scheele *et al.* [13] and Appelt *et al.* [59].

The identification of the major elastase-like elements in pancreatic juice may be important for understanding “indirect” pancreatic function tests using fecal human elastase-1 measurements. The measurement of inactive fragments of elastase (or chymotrypsin [67]) in the stool using monoclonal antibodies and an ELISA is commercially available and widely used, especially in Europe [68–70]. Interestingly, a new polyclonal elastase 1 test has been developed, but in an independent study the antibodies did not appear to react with purified elastase 1 standards [71]. Pezzilli *et al.* [72] demonstrated that the monoclonal (original commercial kit) and polyclonal (new commercial kit) ELISA assays in stool generally give parallel results and to a chymotrypsin assay, but that the new polyclonal test overestimates stool elastase. On the one hand, it is possible that the polyclonal test is not overestimating fecal elastase, but rather reacting with multiple or alternate forms of pancreatic elastase. It remains unclear as to what either assay is measuring; human elastase 1 (so named because of homology with rat elastase 1) was

reported to be silenced by gene mutations and therefore not expressed in human pancreas [65]. Thus, significant work remains in linking the genes to the enzymes and clarifying the nomenclature.

### *Carboxypeptidase*

Carboxypeptidases are enzymes that are exopeptidases digesting at the C-terminus of peptide chains. Unlike the serine proteases the carboxypeptidases are metalloproteinases that contain a zinc atom at the active site. The pancreatic carboxypeptidase-like class includes carboxypeptidase A1 and A2, carboxypeptidase B1, carboxypeptidase A3, and carboxypeptidase B2. The regulatory B-type carboxypeptidase subfamily includes carboxypeptidase N, carboxypeptidase M, carboxypeptidase E (or H), and AEBP1. The carboxypeptidase As cleave carboxyterminal phenylalanine, tyrosine, and tryptophan, and the carboxypeptidase Bs digest terminal arginine, and lysine. The pancreatic carboxypeptidases are synthesized as proenzymes that are activated by trypsin. These enzymes appear as monomers (e.g., A1 and A2) or complexes (e.g., A3) and differ in activation properties, thermal stability, and activity [73].

In summary, by weight proteases comprise the majority of pancreatic enzymes and are essential to normal protein digestion and metabolism. Trypsin and chymotrypsin are the most extensively studied pancreatic digestive enzymes because they served as model enzymes for x-ray crystallography studies. The discovery that mutations in the cationic trypsinogen gene are associated with hereditary pancreatitis pointed to the trypsin as key initiators of acute pancreatitis, the importance of regulatory mechanisms in controlling activity, and the importance of trypsin in cross-activation of immune, coagulation, and other pathways when regulated activity is lost. Sequencing of the human genome has also facilitated understanding of the number and types of enzymes in humans. These factors should lead to new investigations and a better understanding of pancreatic enzymes in health and disease.

### **Fat digestion and human lipases**

Life and well-being require an adequate intake of dietary fats. On average, the Western diet contains at least 100 g of fat, of which long-chain triglycerides account for 92–96% of the total [75]. Cell membrane lipids including phospholipids, sphingolipids, galactolipids, and cholesterol and cholesterol esters comprise the bulk of the remaining lipids. Other dietary lipids, such as fat-soluble vitamins and provitamins, are quantitatively minor, but contribute importantly to health. Additionally, about 20 g of phospholipids, predominantly phosphatidylcholine, and 1–2 g of cholesterol enter

the duodenum in bile each day [75]. Virtually all of these lipids require digestion before they are efficiently absorbed. Unesterified cholesterol and provitamins like the carotenoids represent exceptions, although the absorptive mechanism for carotenoids remains unclear [76].

Dietary triglycerides and phospholipids comprise a heterogeneous group of molecules with distinct chemical and stereochemical compositions [75, 77]. Triglycerides have 3, usually different, long-chain fatty acids esterified to a glycerol backbone. Triglycerides from plants and marine fish contain more monounsaturated and polyunsaturated fatty acids than animal fats [75, 77]. Some plant species contain triglycerides with medium-chain fatty acids, usually unsaturated. Milk fat triglycerides of all mammals include variable percentages of short- and medium-chain fatty acids [78–80]. Phospholipids invariably contain 2 long-chain fatty acids, 1 saturated and 1 unsaturated [75]. The degree of unsaturation reflects the dietary origin of the fat, just as with triglycerides.

Despite the complexity of dietary triglycerides and phospholipids, digestion proceeds efficiently and >95% of dietary fats are normally absorbed. In humans, digestion begins in the stomach with the action of gastric lipase because humans do not have a lingual lipase [81]. Gastric lipase releases 10–30% of fatty acids before the fat emulsions pass into the intestine where they mix with bile lipids and digestive enzymes from the pancreas [82]. The pancreas secretes a variety of lipases that contribute to dietary fat digestion [83]. Some may act on specific substrates, whereas others may have broader substrate specificity and digest a variety of dietary lipids. When the pancreas fails, steatorrhea appears before protein and carbohydrate malabsorption [84]. The malabsorption of fats often dominates the clinical picture in pancreatic insufficiency and accounts for much of the nutritional morbidity associated with pancreatic exocrine failure.

#### Gastric lipase

In humans, the chief cells of the stomach express gastric lipase. Human gastric lipase has a molecular mass of 50 kDa and contains multiple potential glycosylation sites [85]. Human gastric lipase belongs to the  $\alpha/\beta$  hydrolase fold family of protein structures [86]. In addition to a globular core, human gastric lipase has a cap domain analogous to a domain first identified in serine carboxypeptidases. The Ser–His–Asp catalytic triad is buried beneath a segment of 30 residues that reside in the cap domain. In this position, substrates cannot enter the active site unless the segment moves into a new conformation.

Gastric lipase is most active on sn-3 ester linkages of triglycerides, but will hydrolyze sn-1 positions as well [87]. The enzyme is quite acid stable and has a pH optimum for hydrolysis of triglycerides in the range of 4.5–5.5, depending

on the substrate and assay conditions [88]. Interestingly, the products of digestion, fatty acids, inhibit gastric lipase [89].

#### Human studies

In humans, gastric lipolysis releases 10–30% of fatty acids from dietary triglycerides [82, 90–92]. The action of gastric lipolysis facilitates subsequent hydrolysis by pancreatic lipase [90, 91, 93, 94]. In instances of pancreatic insufficiency, gastric lipase can partly compensate for the deficiency of pancreatic lipase. Preterm and newborn infants, who have a physiologic deficiency of pancreatic lipase, depend on gastric lipase for efficient digestion of dietary fats [95]. Gastric lipase accounts for the residual ability of patients with cystic fibrosis to hydrolyze and absorb fats [96–99]. Furthermore, significant levels of gastric lipase activity were present in the duodenum of these patients [96, 97]. A recent study by Carriere *et al.* [100] showed similar results for adults with chronic pancreatitis. They identified gastric lipase activity in the duodenum and found that gastric lipase hydrolyzes about 30% of dietary fatty acids. These studies show conflicting results regarding increased expression of gastric lipase in patients with pancreatic insufficiency compared with controls. Gastric lipase deficiency has not been described in humans.

#### Animal studies

Available studies on animals confirm that preduodenal lipases, gastric or lingual, contribute to dietary fat digestion [101–104]. No animal model of gastric lipase deficiency exists.

### Pancreatic lipases

The pancreas produces a variety of lipases that are responsible for the majority of fat digestion within the diet. A summary of the major lipases is given in Table 2.

#### Pancreatic triglyceride lipase

##### Structure and function

The best-known and most widely studied triglyceride lipase secreted by the pancreas is pancreatic triglyceride lipase (PTL) [105]. Expression of the mRNA encoding PTL is mainly in the exocrine pancreas of most, if not all, adult vertebrates, although low levels can be detected in other tissues [106]. In contrast, the newborn pancreas expresses little or no mRNA encoding PTL [107]. Like amylase activity, lipase levels in the duodenum rise with age and reach adult levels in the first 1–2 years of life [108, 109]. The mRNA encodes

**Table 2** Classification of human digestive lipases

Lipase	Expression Pattern	Main Site of Action	Substrate Specificity
Gastric	Gastric chief cells	Stomach	<ul style="list-style-type: none"> <li>• sn-3 positions of glycerides</li> <li>• Also sn-1 and sn-2 positions</li> </ul>
Colipase-dependent pancreatic lipase	Pancreatic acinar cells	Small intestine	<ul style="list-style-type: none"> <li>• sn-1 and sn-3 positions of glycerides</li> </ul>
Pancreatic lipase-related protein 1	Pancreatic acinar cells	Small intestine	<ul style="list-style-type: none"> <li>• None known</li> </ul>
Pancreatic lipase-related protein 2	Pancreatic acinar cells	Small intestine	<ul style="list-style-type: none"> <li>• sn-1 and sn-3 positions of glycerides</li> </ul>
Carboxyl ester lipase	Intestinal paneth cells	Small intestine	<ul style="list-style-type: none"> <li>• sn-1 and sn-2 position of galactolipids</li> </ul>
	Lymphocytes		<ul style="list-style-type: none"> <li>• sn-1 position of phospholipids</li> </ul>
	Pancreatic acinar cells Breast milk		<ul style="list-style-type: none"> <li>• All positions of glycerides</li> <li>• sn-1 and sn-2 positions of galactolipids</li> <li>• sn-1 and sn-2 positions of phospholipids</li> <li>• vitamin esters</li> <li>• cholesterol esters</li> <li>• ceramide</li> </ul>
Phospholipase A2	Pancreatic acinar cells	Small intestine	<ul style="list-style-type: none"> <li>• sn-2 position of phospholipids</li> </ul>

a protein of 465 amino acids [106]. The first 16 amino acids comprise the signal peptide and there is no activation peptide. Isolated human PTL has a molecular mass of 48 kDa and a single oligosaccharide chain [110].

The crystal structure of PTL reveals that the protein has 2 domains. The *N*-terminal domain has the  $\alpha/\beta$  hydrolase fold, which is present in other lipases and esterases [111, 112]. This domain contains the Ser–His–Asp catalytic triad, which, like gastric lipase, is buried beneath a surface loop, the lid domain, defined by a disulfide bridge between Cys238 and Cys262 [113]. In this position, the surface loop sterically hinders access of substrate to the active site creating an inactive conformation of PTL. Another crystal structure of the colipase–PTL complex obtained in the presence of octylglucoside and phospholipids in mixed micelles, revealed another conformation of PTL [114]. The surface loop had moved to open and configure the active site, creating an active conformation of PTL. Most likely, the lid of PTL remains closed and inactive until PTL absorbs to an oil-water interface. Because PTL activity is not controlled by trypsin, the closed-loop conformation may be a way to prevent inappropriate PTL activity. The other domain, the *C*-terminal domain, has a  $\beta$ -sandwich structure and provides the major binding surface for colipase (see below).

PTL is a carboxyl esterase that hydrolyzes acylglycerides but not phospholipids, cholesterol esters, or galactolipids [115, 116]. In vitro, PTL has activity against retinyl esters, but this activity has not been demonstrated in vivo [117]. PTL cleaves a broad range of acyl chain lengths from the  $\alpha$ -position of triglycerides showing a preference for acyl chains in the sn-1 and sn-3 positions [116]. The difference in the hydrolysis rates of long chain triglycerides ( $C_{14}$ – $C_{22}$ ) varies only 6-fold [118]. Because the pancreas secretes PTL in large excess, the differences in rates are not a factor and PTL should cleave all dietary long-chain triglycerides efficiently.

### Colipase

Curiously, many constituents of the duodenal lumen, including bile salts, phospholipids, cholesterol esters, dietary proteins, and dietary carbohydrates, inhibit PTL [116]. Another pancreatic exocrine protein, colipase, restores activity to PTL in the presence of inhibitory substances [116]. mRNA encoding colipase appears in the fetal pancreas and, unlike the mRNA encoding PTL, is expressed at birth in rodents [107]. Colipase mRNA is also present in the human fetal pancreas (M. Lowe, unpublished observations). Colipase is a small molecular weight protein of 10 kDa and has no enzymatic activity of its own [119]. The pancreas synthesizes and secretes colipase as a proform, procolipase; a pentapeptide, termed *enterostatin*, is cleaved from the amino terminus, presumably by trypsin in the duodenum [120, 121]. Colipase forms a 1:1 complex with PTL to create an active lipase by anchoring PTL to the substrate through a lipid-binding region and by stabilizing PTL in an active conformation [122].

### Human studies

Little data exist about the in vivo role of PTL in the intraluminal digestion of dietary triglycerides. Like the situation with all pancreatic digestive enzymes, there are few descriptions of patients with isolated PTL deficiency [123]. The few reported patients have steatorrhea, but to date no one has described any mutations in the gene encoding PTL and it remains possible that these patients have other defects as well [124]. Recent in vitro studies suggest that PTL accounts for the majority of the lipase activity in pancreatic juice [125].

Based on much in vitro data, many have proposed that colipase has an essential role in dietary fat digestion. In fact, only a smattering of in vivo data support this role. One report shows that 2 brothers with absent colipase in pancreatic



secretions have fat malabsorption [126]. The steatorrhea corrected after treatment with pancreatic extracts.

#### *Animal studies*

The mouse model of PTL deficiency does not have steatorrhea [127]. On the surface, this finding argues that PTL is not essential for efficient dietary fat digestion. Another explanation, which has not been tested yet, holds that other lipases can compensate for the absence of PTL. Under normal circumstances, PTL may account for the majority of triglyceride digestion. Rats fed the recombinant C-terminal domain of PTL, which specifically inhibits PTL *in vitro*, had fat malabsorption [128, 129]. Presumably the C-terminal domain forms complexes with colipase and competes with endogenous PTL for colipase. This finding supports a role for the PTL–colipase complex in dietary fat digestion *in vivo*.

Studies of procolipase-deficient mice strongly support an essential role for colipase in dietary fat digestion at all ages [130]. Both newborn and adult colipase-deficient mice have steatorrhea, indicating that colipase even functions at an age when PTL is not expressed. Because colipase interacts with PTL in the adult, the steatorrhea at this age argues for an important role of PTL in dietary fat digestion.

#### Pancreatic lipase-related proteins

##### *Structure and function*

PTL belongs to the lipase gene family that contains hepatic lipase and lipoprotein lipase as well as 2 other closely related pancreatic exocrine proteins, pancreatic lipase-related protein 1 and 2 (PLRP1 and PLRP2) [131]. The mRNA encoding both related proteins is expressed in the fetal pancreas as well as in the adult pancreas [107, 132]. The human pancreas secretes both proteins in small amounts [133, 134]. These proteins have 65–70% identical amino acid sequences compared with PTL [135]. Their crystal structures superimpose on that of PTL [136]. Despite these structural similarities, the 2 related proteins differ from PTL in their substrate preference [31]. PLRP1 has no known substrate. The mutation of 2 amino acids near the catalytic site confers triglyceride lipase activity to the mutant PLRP1, but the native enzyme does not hydrolyze triglycerides [137, 138]. In contrast, PLRP2 has broad substrate specificity. It cleaves triglycerides, phospholipids, and galactolipids [139].

##### *Human studies*

The function of human PLRP1 and PLRP2 in dietary fat digestion remains uncertain, although both proteins are present in human pancreatic secretions [134, 139, 140]. There are no reported instances of human deficiency of either protein.

It has been suggested that PLRP2 functions as a galactolipase based on studies of pancreatic juice and recombinant PLRP2 [139, 141]. PLRP2 is expressed at birth in the human, whereas PTL is expressed at very low levels [132]. This finding and the results from the animal studies described in the following section suggest that PLRP2 may be essential for efficient dietary fat digestion in the newborn.

##### *Animal studies*

Mice deficient in PLRP1 have no known phenotype (M. Lowe, unpublished observations). Mice deficient in PLRP2 do have a phenotype [142]. They malabsorb dietary fat in the newborn period and exhibit poor weight gain. The steatorrhea resolves upon PTL expression near weaning and the mice show catch-up weight gain. Adult PLRP2-deficient mice appear normal. They do not have abnormalities in triglyceride digestion. The absorption of galactolipids has not been tested in these mice. In mice, PLRP2 represents the only colipase-dependent lipase activity in the newborn pancreas, which provides an explanation for why newborns require colipase for efficient dietary fat digestion [143].

#### Carboxyl ester lipase

##### *Structure and function*

The presence of a lipolytic activity separate from PTL was recognized many years ago. The enzyme, carboxyl ester lipase (CEL), was purified from human pancreatic juice where it represents about 4% of the total protein [143]. Human CEL has a molecular weight of 100 kDa and is a glycoprotein. The cDNA predicts a protein of 722 amino acids including a 20-amino acid signal peptide [144]. An activation peptide is not present. The protein has high proline content, with the majority of the proline residues located in the last 25% of the protein where a series of proline-rich tandem repeats reside. The mammary gland of some species, including humans, and the exocrine pancreas express mRNA encoding CEL [144–148]. In species that express CEL in the mammary glands, expression of CEL in the newborn pancreas is quite low.

The crystal structure of human CEL has not been solved. A structure of a truncated CEL lacking the proline-rich tandem repeats shows that it belongs to the  $\alpha/\beta$  hydrolase family [149]. CEL has a Ser–His–Asp catalytic triad. Like PTL, CEL has a surface loop that sits over the active site. The CEL loop appears to be highly mobile even in the absence of an oil–water interface.

Many investigators have described CEL independently and, consequently, they have given CEL a variety of names such as *cholesterol esterase* and *bile salt-dependent lipase*. The reason for the multiple names is that the enzyme has

activity against a variety of substrates. In vitro, the enzyme hydrolyzes triglycerides, cholesterol esters, phospholipids, lysophospholipids, ceramides, vitamin esters, and galactolipids [115, 141]. Two potentially important activities of CEL are the ability to hydrolyze monoacylglycerols and glycerolipids containing long chain polyenoic fatty acids [150–152]. Despite years of work on CEL, the in vivo function of this abundant lipase remains enigmatic.

#### *Human studies*

The broad substrate specificity of CEL has led to multiple postulated roles for the enzyme in dietary fat digestion. One role that has been frequently proposed is that of dietary triglyceride digestion by breast milk CEL in the newborn. In low birth weight human infants, fat absorption increased 40% and weight gain improved when the infants were fed raw breast milk or formula supplemented with breast milk containing CEL compared with infants fed boiled milk or formula without supplementation [153, 154]. There are no human studies that suggest a function for CEL in adults and no examples of human deficiency exist.

#### *Animal studies*

A mouse model of CEL deficiency suggested a role in absorption of cholesterol esters, but not in the digestion of retinyl esters or triglycerides or in the absorption of unesterified cholesterol [155, 156]. The digestion of phospholipids and galactolipids was not tested in this model. One group proposed that the function of CEL may be restricted to the hydrolysis of long-chain polyunsaturated fatty acids, but this hypothesis also remains untested [157]. Recent studies in CEL-deficient mice and tissue culture cells, Caco2, suggest a novel function for CEL in chylomicron assembly [158]. The authors propose that the promotion of large chylomicron formation by CEL is mediated by the hydrolysis of ceramide by CEL.

#### Phospholipase A2

##### *Structure and function*

The other major lipase in pancreatic fluid is Phospholipase A2 (PLA2). This enzyme is present in the pancreatic secretions of a wide variety of tissues and species. The human pancreatic enzyme has a molecular weight of about 14 kDa [159, 160]. The predicted amino acid sequence has 125 amino acids, including a signal peptide and a 7-amino acid activation peptide [161]. PLA2 catalyzes the hydrolysis of the sn2-acyl ester bond of phospholipids.

#### *Human studies*

Chen and Innis [162] examined phospholipid malabsorption in patients with cystic fibrosis and found evidence of increased total fecal phospholipids and of fecal phosphatidylcholine in patients with cystic fibrosis compared to controls, even though the patients continued to take pancreatic enzymes. This study suggests that patients with pancreatic insufficiency malabsorb phospholipids, although the amounts appear to be small if the patients are on pancreatic enzyme therapy. No reports of human deficiency exist.

#### *Animal studies*

Although PLA2 has the potential to digest luminal phospholipids, PLA2-deficient mice absorb phospholipids from a test meal at a normal rate, suggesting that other lipases can compensate for the lack of pancreatic PLA2 [163].

In summary, the necessity for each of these individual lipases remains unclear. In vitro digestion of human milk fat globules or of synthetic fat emulsions containing phospholipid and triglyceride proceeds most efficiently with a mixture of lipases [164–166]. The inclusion of both CEL and PTL in a model system doubled the rate obtained with PTL alone, suggesting that CEL may preferentially act on products of PTL digestion [167]. The paucity of reports describing human deficiencies in any of the individual lipases or of colipase can have several explanations. The individual lipases may not function in physiologic fat digestion as predicted by in vitro studies, causing investigators to look for the wrong phenotype. Perhaps the deficiencies are fatal and lead to fetal or neonatal demise. Studies of mice deficient in a variety of individual pancreatic lipases argue against this possibility. Alternatively, other lipases may compensate for the deficiency. This last scenario seems the most likely, although few data exist to help distinguish between these possible explanations. The overlapping substrate specificities of the various lipases suggest that these enzymes can compensate for one another. CEL could compensate for PTL deficiency just as PLRP2 functions during the developmental deficiency of PTL in the newborn and CEL or PLRP2 could digest phospholipids in PLA2-deficient mice. Gastric lipase partially compensates for the lack of pancreatic lipases. In addition, intestinal lipases can theoretically replace some of the pancreatic lipases. For instance, the phospholipase B, which enterocytes synthesize, can potentially digest phospholipids in the intestinal lumen. The presence of steatorrhea in patients with pancreatic insufficiency argues that other organs are not a sufficient source of triglyceride lipases when production of all pancreatic lipases is impaired.

## Carbohydrate digestion and human amylases

Carbohydrates, in the form of starch or simple sugars, account for 40–50% of the calories in the Western diet [168, 169]. Starches, polymers of glucose, are the storage form of carbohydrate in plants accounting for 10–80% of the plant volume. Amylose, a straight-chain  $\alpha$ -1,4-linked glucose polymer, and amylopectin, a branched starch with a backbone of  $\alpha$ -1,4-linked glucose with  $\alpha$ -1,6-linked glucose branches about every 20–25 residues, are the major dietary starches. About 20% of dietary starch is amylose and the remainder is amylopectin. Because the intestinal epithelium only absorbs monosaccharides, dietary starch must be hydrolyzed into glucose by the action of  $\alpha$ -amylase. The predominant sources of  $\alpha$ -amylase are the parotid glands and the pancreas [96].

### Pancreatic $\alpha$ -amylase

#### *Structure and function*

About 5–6% of the total protein in pancreatic secretions is  $\alpha$ -amylase, a glycoprotein of 57 kDa, containing a single oligosaccharide chain [171]. The human cDNA predicts a protein of 512 amino acids with a molecular weight of 57.6 kDa [96]. Unlike other pancreatic zymogens, amylase does not have an inactive proform. X-ray diffraction studies of porcine pancreatic amylase provide insight into the enzymatic mechanism of amylase [98]. The protein contains 3 domains—A, B, and C. The substrate-binding site lies in a cleft between the A and B domains. Residues in the A and B domains bind calcium, which may stabilize the active site cleft. A chloride molecule binds to the A domain near the active site cleft. Domain C forms an all  $\beta$ -structure and seems to be an independent domain with unknown function. Binding of substrate analogs suggest that Asp197 and Asp300 participate in catalysis.

The substrate-binding site contains 5 subsites with the catalytic site positioned at subsite 3 [173]. Substrate can bind with the first glucose residue in subsite 1 or 2, allowing cleavage to occur between the first and second or second and third glucose residues. Consequently, amylase preferentially cleaves interior  $\alpha$ -1,4-glucose linkages [174–180]. Neither terminal glucose residues nor  $\alpha$ -1,6-linkages can be cleaved by  $\alpha$ -amylase. The resulting products of  $\alpha$ -amylase digestion are called *dextrins*, a mixture of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units that contain both  $\alpha$ -1,4 and  $\alpha$ -1,6 linkages. Intestinal brush border enzymes, maltase and isomaltase, finish the digestion of dextrins [181].

### *Human studies*

In vitro studies show that amylase is the only glycosidase in human pancreatic fluid [9, 171]. As such, it is essential for the digestion of dietary starch. Hydrogen breath testing and intestinal intubation show that healthy volunteers absorb 99% of a 50- to 100-g starch load, although it is unclear how much was fermented by bacteria after reaching the colon [182, 183]. When amylase is completely inhibited, about 80% of complex carbohydrates are absorbed in healthy subjects [183]. By comparison, about 10% of a carbohydrate load is malabsorbed by patients with chronic pancreatitis [184]. Patients with isolated pancreatic amylase deficiency, a rarely reported condition, have carbohydrate maldigestion that may produce abdominal distension, flatulence, loose stools, and poor weight gain [185–188]. Symptoms resolve with pancreatic enzyme replacement therapy. There are very few papers on the efficacy of starch absorption in patients with pancreatic insufficiency treated with pancreatic extracts [189]. The efficacy of therapy may depend on the source of the starch [190]. For instance, patients with pancreatic insufficiency efficiently absorbed wheat starch after enzyme therapy, but a group of patients with cystic fibrosis eating their regular diet had increased fecal starch and carbohydrate compared to controls [189].

The rarity of pancreatic amylase deficiency has several potential explanations. Pancreatic amylase deficiency may not have a clinical phenotype and the reported patients may also have an additional, undetected deficiency in another brush border or pancreatic digestive enzyme. Alternatively, the phenotype may be severe and associated with decreased neonatal survival. Given the developmental profile of amylase, this explanation seems unlikely. Newborns and infants have a relative deficiency of amylase compared with older children and adults [108]. Newborns and infants do well because they have diets with little or no starch and mainly disaccharides or short oligosaccharides. Potentially, detrimental mutations in the amylase gene may be rare because they are repaired efficiently or because mutations in the gene may occur infrequently. Finally, products of other genes may compensate for the loss of the pancreatic amylase gene in most patients. With the congenital absence of pancreatic amylase, increased salivary amylase expression or other, yet unidentified gene products, could compensate for the loss of pancreatic amylase [185, 191]. No data exist to distinguish among these possibilities.

### *Animal studies*

Studies in minipigs have shown that small intestinal malabsorption of starch does occur following artificially induced PEI, the extent depending upon the size of the starch meal

[149]. Thus, in pigs fed approximately 125 g of starch per day, there was a malabsorption of 12%, and in pigs fed 270 g of starch per day, there was a malabsorption of 38%. Interestingly, in both cases fecal digestion was complete, again showing the compensatory effect of hindgut fermentation. A mouse model of pancreatic amylase deficiency does not exist.

### Salivary $\alpha$ -amylase

#### *Structure and function*

The predicted amino acid sequences of the salivary and pancreatic isoforms differ by only 6% [96]. Consequently, they also have nearly identical crystal structures, as described [192]. Still, various methods can distinguish between the 2 isoforms [193]. Like pancreatic amylase, salivary amylase is a 1,4- $\alpha$ -D-glucan glucanohydrolase. Human salivary amylase comprises 2 major families, distinguished by the presence or absence of glycosylation [194].

#### *Human studies*

Salivary amylase initiates starch digestion in the mouth [181]. The contribution of salivary amylase to starch digestion remains unclear and probably depends on the length of time the starch is chewed in the mouth and on the source of the starch [195]. Although salivary amylase is degraded in the stomach, small amounts of salivary amylase can be detected in the duodenum [181, 185]. The salivary isoenzyme was detected in almost 18% of duodenal aspirates from 124 consecutive patients [185]. Salivary amylase was detected in the duodenum of 40.5% of patients with pancreatic insufficiency. Another study demonstrated that salivary amylase could produce significant starch digestion in the stomach and small intestine in premature infants, who have a developmental deficiency of pancreatic amylase [196]. Still, the amount of starch digestion falls short of that accomplished when pancreatic amylase is present. The digestion of starch by salivary amylase does not compensate for the lack of pancreatic amylase in pancreatic insufficiency or in isolated deficiency of pancreatic amylase; these patients have starch malabsorption.

#### *Animal studies*

In diabetic rats, about one third of ingested starch was digested in the mouth and stomach, presumably by salivary amylase [197]. Ligation of the parotid duct in the diabetic rats significantly reduced amylase activity and starch digestion in the stomach and small intestine. This same group demonstrated that salivary amylase secretion and gastric starch di-

gestion in rats depended on the dietary consistency and water content of the diet [198]. No animal model of salivary amylase deficiency exists.

In summary, the bulk of available data indicates that efficient dietary starch digestion requires pancreatic amylase, although salivary amylase variably contributes to digestion. No other pancreatic enzymes appear to be necessary. Patients with pancreatic insufficiency need replacement therapy with amylase or diets devoid of starch, a difficult therapy with a high probability of noncompliance. Abdominal symptoms in patients on pancreatic extracts could result from starch malabsorption. Clinically, starch absorption is rarely if ever tested and a common therapy for patients with abdominal complaints is to increase the dose of pancreatic enzymes, usually with the presumption that fat malabsorption contributes to the symptoms. There is a paucity of human studies examining the coefficient of starch absorption in patients with pancreatic insufficiency.

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