Lipases are water-soluble enzymes that hydrolyze water-insoluble lipid molecules, such as triglycerides, phospholipids, and galactolipids. They are ubiquitous in nature and are present in humans, animals, insects, plants, fungi, and microorganisms. While we commonly consider pancreatic lipase, this review provides an overview of several lipases that are important for the digestion and metabolism of lipids in veterinary species. All of these enzymes have specific functions but share a common α/β-hydrolase fold and a catalytic triad where substrate hydrolysis occurs. The pancreatic lipase gene family is one of the best characterized lipase gene families and consists of 7 mammalian subfamilies: pancreatic lipase, pancreatic lipase related proteins 1 and 2, hepatic lipase, lipoprotein lipase, endothelial lipase, and phosphatidylserine phospholipase A1. Other mammalian lipases that play integral roles in lipid digestion include carboxyl ester lipase and gastric lipase. Although most enzymes have preferred substrate specificity, much overlap occurs across the plethora of lipases because of the similarities in their structures. This has major implications for the development and clinical utilization of diagnostic assays. These implications are further explored in our companion Currents in One Health article by Lim et al in the August 2022 issue of the Journal of American Veterinary Medical Association, which focuses on pancreatic lipase assays for the diagnosis of pancreatitis.

Brief Overview of Lipid Digestion, Absorption, and Metabolism

While lipases are commonly used as disease biomarkers, they play critical roles in the digestion of dietary fats among other physiologic roles. The most abundant dietary fats are neutral fats, also known as triglycerides. Each molecule of triglyceride is composed of a glycerol backbone with 3 fatty acids.
acid side-chains (Figure 1). Other than triglycerides, small amounts of phospholipids and cholesterol esters are also present in the diet. Depending on the species, lipid digestion begins in the mouth or stomach through its mechanical and enzymatic function.\(^8\) In dogs and cats, lipid digestion begins in the stomach through the action of gastric lipase.\(^8,9\) Gastric peristalsis breaks dietary lipid into small droplets and mixes it with other macronutrients and gastric lipase. Approximately 25% of ingested triglycerides are hydrolyzed by gastric lipase.\(^8,10\) This digestion is further completed in the small intestines by pancreatic enzymes.\(^10\) Emulsification, which is crucial for lipid digestion, is achieved by mixing dietary fat with bile salts, which are secreted into the lumen of the duodenum. Emulsification enhances lipid digestion by producing micelles, which are small droplets of lipid dispersed in an aqueous matrix, thus increasing surface area for the hydrolytic action of pancreatic enzymes. Dietary fat is hydrolyzed by various pancreatic enzymes, which will be detailed in later sections of this review. Pancreatic enzymes hydrolyze dietary fat (eg, triglycerides, cholesterol esters, and phospholipids) to lipolysis products, such as monoglycerides, cholesterol, and free fatty acids, that are then being solubilized and transported in micelles that are readily absorbed by enterocytes.\(^11\) Within the enterocytes, lipid digestive products are re-esterified with fatty acids to form triglycerides, cholesterol esters, and phospholipids.\(^11\) Inside the enterocytes, these lipids are packaged into chylomicrons.\(^11\) A chylomicron is a large packet of lipid containing triglycerides and cholesterol ester in its core, with phospholipids and apolipoproteins on
the outside. Each chylomicron is assembled within secretory vesicles of the Golgi apparatus and then migrates to the basolateral membrane of the enterocyte. This allows for exocytosis of chylomicrons that then will enter the lymphatic capillaries by moving in between endothelial cells that line the lacteals. Chylomicrons travel through the lymphatic circulations and empty into the circulatory system via the thoracic duct. Lipid metabolism within the circulatory system is then carried out predominantly by hepatic lipase, lipoprotein lipase, and endothelial lipase. A complete overview on lipid metabolism is beyond the scope of this article, and readers are encouraged to refer to readily available resources for a more comprehensive discussion on this topic.

The role that various lipases play in both physiologic and pathologic states is an important consideration when determining the impact of these lipases on various lipase assays. These lipases are further discussed below.

**Lipases Important for Digestion and Metabolism of Lipids**

**Pancreatic lipase gene family**

The pancreatic lipase gene family is the largest and, arguably, the most important family of lipases in veterinary medicine. Pancreatic lipase was discovered by the French physiologist Claude Bernard in 1848 and was the first mammalian lipase of its gene family to be identified. The partial primary sequence of pancreatic lipase (porcine) was first reported in 1973 and completed in 1981. Hepatic (rat) and lipoprotein lipases (bovine) were discovered later and added to this gene family. Subsequently, pancreatic lipase-related proteins 1 (PLRP1; human) and 2 (PLRP2; human), endothelial lipase (mice), and phosphatidylserine phospholipase A1 (rat and human) were identified and the gene family was expanded further.

These mammalian and invertebrate lipases are grouped in the pancreatic lipase gene family because of the homology in their amino acid sequence and gene organization (Figure 2). Within this gene family, PLRP1 and PLRP2 are most closely related to classical pancreatic lipase. They share 68% and 65% amino acid homology with pancreatic lipase, respectively. Comparison of the intron-exon organization of genes encoding hepatic, lipoprotein, and pancreatic lipases shows that hepatic and lipoprotein lipases are more closely related to each other than to pancreatic lipase. However, they still share 30% amino acid homology with pancreatic lipase. Endothelial lipase is more closely related to lipoprotein lipase than to hepatic lipase. Phosphatidylserine phospholipase A1 diverged earlier from pancreatic lipase and lipoprotein lipase and thus shows less homology. In addition to their similarity of the primary amino acid sequence, most lipases of this gene family share a common 3-dimensional structure comprising of 2 domains (the N-terminal and C-terminal domains), a catalytic triad, and a lid structure covering this catalytic triad. Lipases have 2 crucial functions: substrate binding and substrate hydrolysis. Lipase structure is closely related to its function. Therefore, knowing the 3-dimensional structure of a lipase helps us understand its function and substrate selectivity. These structures will be discussed using pancreatic lipase as a general model with notable similarities and differences between members of the gene family highlighted along the way.

**Structure of pancreatic lipase**

Pancreatic lipase has 2 distinct domains, an N-terminal domain consisting of amino acid residues 1 to 336 and a C-terminal domain consisting of amino acid residues 337 to 449 (Figure 3). The N-terminal domain functions as the catalytic domain, and the C-terminal domain is important for noncatalytic functions, such as binding of cofactors, lipids, and heparin. The N-terminal domain contains the α/β-hydrolase fold, which is common to esterases and thioesterases. This domain also contains the catalytic triad, consisting of 3 amino acid residues: serine 152, aspartic acid 176, and histidine 263 (in human pancreatic lipase). This catalytic triad is the active...
site where substrate hydrolysis occurs. It is common to all mammalian lipases of the pancreatic lipase gene family, carboxyl ester lipase, and gastric lipase.17,20 Pancreatic lipase may be present in the inactive or active form, based on the position of a mobile lid structure originating from the N-terminal domain.18,21 In the inactive form, the lid covers the catalytic triad, blocking access of substrates to the active site (Figure 3). When in the active form, the lid opens to expose the catalytic triad.

**Interfacial activation of pancreatic lipase**

When dietary fat is present in the intestinal lumen, bile salt lines up along the surface of this insoluble substrate, forming micelles (Figure 4). Colipase is secreted together with pancreatic lipase and is a cofactor for optimal pancreatic lipase activity. In the presence of micelles, pancreatic lipase changes from the inactive to the active form, with the 2 forms being present in equilibrium in the intraluminal fluid matrix.2 Colipase, which also undergoes conformational changes, acts as a cofactor by binding to the C-terminal domain of pancreatic lipase and anchoring pancreatic lipase to the surface of the micelle. Colipase also favors formation of active pancreatic lipase by stabilizing the mobile lid structure.2 The movement of the lid structure and binding of colipase to the C-terminal domain create a hydrophobic plateau. This plateau is important for the interaction of water-soluble pancreatic lipase with water-insoluble lipid at the lipid-water interface. This interaction results in the interfacial activation of pancreatic lipase.21 Pancreatic lipase can then hydrolyze dietary triglycerides to diglycerides, and subsequently to monoglycerides, and fatty acids that can then be absorbed by enterocytes (Figure 1). Because lipases of this gene family have a large degree of homology, there is much overlap in their function. Generally speaking, apart from PLRP1 that has no apparent enzymatic activity, all other family members display variable substrate selectivity for triglycerides, phospholipids, and galactolipids.14,22

**Pancreatic lipase and its related proteins**

The pancreatic lipase gene family consists of 3 lipase subfamilies of pancreatic origin, namely, pancreatic lipase, PLRP1, and PLRP2.14 Although pancreatic lipase is expressed in all mammals, the expression of PLRP1 and PLRP2 differs between vertebrate species.23 Immunohistochemistry has demonstrated that pancreatic lipase is found exclusively in zymogen granules of pancreatic acinar cells and is thus pancreatic specific.24 Pancreatic lipase and its cofactor, colipase, together with other pancreatic enzymes and zymogens, are secreted into the pancreatic duct system by exocytosis of zymogen granules when acinar cells are stimulated mainly by cholecystokinin and secretin in response to free fatty acids produced by lipolysis in the stomach.11 Pancreatic juice flows along the pancreatic duct system and is ultimately emptied into the intestinal lumen, where the zymogens are activated and together with the enzymes in pancreatic juice begin to catalyze the digestion
of these macronutrients.\textsuperscript{11} Pancreatic lipase is the enzyme responsible for hydrolysis of approximately 75% of dietary triglycerides, while gastric lipase, which will be discussed later, is responsible for the remaining 25%.\textsuperscript{12} Under physiological conditions, most (about 99%) of the pancreatic lipase and colipase are released from the apical pole into pancreatic juice for digestion of dietary fat. Less than 1% diffuses from the basolateral aspect of the acinar cells into circulation. However, when the pancreas is inflamed, apical secretion is blocked, and large amounts of pancreatic lipase are released into the circulation via the basolateral aspect.\textsuperscript{25} Therefore, the measurement of pancreatic lipase in circulation is commonly used as a noninvasive diagnostic marker for acinar cell damage during pancreatitis. Readers are referred to the companion Currents in One Health article by Lim et al in the August 2022 issue of the Journal of American Veterinary Medical Association, focusing on lipase assays for the diagnosis of pancreatitis.

Pancreatic lipase-related proteins 1 and 2 are found in the pancreas at lower concentrations than pancreatic lipase.\textsuperscript{26} The presence of pancreatic lipase-related protein 1 has been reported in many species, including humans, dogs, cats, rats, and guinea pigs.\textsuperscript{14,26–28} It has been isolated from both the pancreas and pancreatic juice, indicating that it is a secreted protein.\textsuperscript{26,27} Nevertheless, neither native nor recombinant PLRP1 displays significant lipolytic activity due to the presence of 2 mutations in the vicinity of the active site.\textsuperscript{29,30} Because PLRP1 has been found to compete for colipase, which pancreatic lipase needs for activity, it has been suggested that PLRP1 acts as a pancreatic lipase inhibitor, thus protecting the pancreas from the consequences of excessive digestion and absorption of fatty acids.\textsuperscript{26,30} The role of PLRP1 in dogs and cats remains unknown, and it will be interesting to explore whether individual animals with an increased expression of PLRP1 are less prone to developing pancreatitis.

Compared with pancreatic lipase, PLRP2 has a wider substrate specificity. In addition to triglycerides, PLRP2 also hydrolyzes phospholipids, galactolipids, retinyl esters (vitamin A), and cholesterol esters.\textsuperscript{27} Its substrate-specific activity varies among different species of animals.\textsuperscript{31} The presence of pancreatic lipase-related protein 2 has been reported in many species, including humans, dogs, cats, rats, mice, guinea pigs, coyus, horses, and rabbits.\textsuperscript{14,23,28,30,32} but has not yet been described in the dog.\textsuperscript{23} Similar to pancreatic lipase, PLRP2 is also expressed in the pancreatic acinar cells.\textsuperscript{31} Immunohistochemistry of mouse pancreas shows patchy staining for PLRP2, suggesting that its levels of expression are uneven throughout the pancreatic acinar cells, in contrast to its uniform mRNA expression as shown by in situ hybridization.\textsuperscript{33} Intracellularly, rat PLRP2, also named GP3, has been found tightly associated with the zymogen granule membrane. Despite this tight association, it appears in media from cultured rat acinar cells and in pancreatic juice. In contrast to pancreatic lipase, which has low expression in neonates, PLRP2 and carboxyl ester lipase are highly expressed in neonates and are the 2 dominant lipases in milk-fat digestion.\textsuperscript{34}

Apart from the pancreas, PLRP2 is present in cultured mouse cytotoxic T-cells, enteroctyes, Paneth cells of the human small intestines, seminal fluid from goats, THP-1 human monocyte cell line, and mouse antigen presenting cells.\textsuperscript{35–37} These studies suggest that PLRP2 functions not only as a gastrointestinal digestive enzyme but may also play roles in immune response, such as T-cell mediated cytotoxicity as well as processing of lipid antigens. Further studies on the functions and potential diagnostic utility of PLRP2 are urgently required.

**Hepatic lipase and lipoprotein lipase**

Hepatic lipase and lipoprotein lipase share a closer sequence homology with each other than with pancreatic lipase.\textsuperscript{3} Both of these lipases play important roles in the metabolism of triglycerides and phospholipids, present in circulating lipoproteins.\textsuperscript{38,39} Hepatic lipase is synthesized almost exclusively by hepatocytes and is found on the cell surface of hepatocytes and sinusoidal endothelial cells.\textsuperscript{40} Hepatic lipase is also found at low levels in the adrenal glands and ovaries.\textsuperscript{40} It has been isolated in several species, including humans, rats, dogs, and cats. Lipoprotein lipase is synthesized by adipose tissue and muscle and is anchored to capillary endothelial cells.\textsuperscript{38,41} This lipase has also been isolated in many species.\textsuperscript{5}

Similar to pancreatic lipase, hepatic and lipoprotein lipases are 2-domain enzymes (i.e., they both have N and C terminals) with a catalytic triad located in their N-terminal domain.\textsuperscript{4,41} Both enzymes have additional heparin-binding sites on their C-terminal domains.\textsuperscript{5} Hepatic lipase binds via this heparin-binding site to heparan sulfate proteoglycans (HSPG) located on hepatocytes and sinusoidal endothelial cells of the liver.\textsuperscript{42} Hepatic lipase is inactive when bound to HSPG. It is mobilized into circulation and subsequently activated by high concentration of circulating high-density lipoproteins.\textsuperscript{39} Circulating hepatic lipase is an active enzyme that does not require cofactors. When activated, it preferentially hydrolyzes triglycerides in high-density lipoproteins. Similar to hepatic lipase, lipoprotein lipase binds via its heparin-binding site to HSPG located on endothelial cells. It hydrolyzes triglycerides in triglyceride-rich lipoproteins, such as very low-density lipoproteins and chylomicrons. Lipoprotein lipase, however, requires activation by cofactor apolipoprotein C-II present on these triglyceride-rich lipoproteins.\textsuperscript{3}

Because of their preferential binding to exogenous heparin, both hepatic and lipoprotein lipases are released into circulation by injection of heparin.\textsuperscript{50} Postheparin plasma lipase activity is commonly used to measure hepatic and lipoprotein lipases in people with dyslipidemias. The activity level of circulating hepatic and lipoprotein lipases is dependent on various factors, including species and breed,\textsuperscript{52} body condition score,\textsuperscript{53} presence of postprandial hypertriglyceridemia, and sex hormone concentrations.\textsuperscript{50,51} Of
dietary lipids. Its function is unclear but is believed to activate mast cells by hydrolyzing cell-surface phosphatidylserine of apoptotic cells. Instead, it shares 30% amino acid homology with phospholipase A2.

Phosphatidylserine phospholipase A1

The remaining mammalian member of the pancreatic lipase gene family, phosphatidylserine phospholipase A1 was identified in rat platelets and is identical to a protein called NMD expressed in a human melanoma cell line. It has not been reported in dogs or cats. This enzyme shares closer genetic resemblance with hepatic and lipoprotein lipases when compared to pancreatic lipase. It has been isolated in several species, including humans, rats, and mice, but its presence has not yet been reported in dogs or cats. This enzyme shares closer genetic resemblance with hepatic and lipoprotein lipases when compared to pancreatic lipase. It has been shown to have phospholipase activity when compared to that for neutral lipids (triglycerides and cholesterol esters) and is important for metabolism of high-density lipoproteins in circulation.

Given that endothelial lipase was the most recent lipase to be discovered, our understanding of its physiologic roles and potential diagnostic utility is more limited than that for hepatic lipase and lipoprotein lipase. Currently, it appears that other knowledge deficits, with more overt links to clinical disease, are taking precedence over research into endothelial lipase. However, additional research in this area may elucidate both physiologic and diagnostic uses in the future.

Carboxyl ester lipase

Carboxyl ester lipase (previously cholesterol esterase or bile salt stimulated lipase) is another enzyme belonging to the α/β-hydrolase family. Like pancreatic lipase, carboxyl ester lipase has a catalytic triad (i.e., serine 194, aspartic acid 320, and histidine 353). It is synthesized in abundance in acinar cells of the exocrine pancreas and in lactating mammary glands. Similar to pancreatic lipase, it is stored in zymogen granules and secreted with pancreatic juice when stimulated by gastrointestinal hormones, such as cholecystokinin, secretin, and bombesin. Carboxyl ester lipase is also synthesized in low levels in the liver, eosinophils, macrophages, and endothelial cells.

Carboxyl ester lipase is a nonspecific lipolytic enzyme. Its main function in the intestinal lumen is to hydrolyze cholesterol esters to free cholesterol and fatty acids. Additionally, it can also hydrolyze triglycerides, diglycerides, monoglycerides, phospholipids, lysophospholipids, and ceramide. While pancreatic lipase is the main enzyme hydrolyzing triglycerides, carboxyl ester lipase hydrolyzes up to 40% of dietary monoglycerides derived from triglycerides to glycerol and free fatty acids for absorption (Figure 1). Carboxyl ester lipase, which is present in abundance in the pancreas and lactating mammary gland, plays an important role in milk-fat digestion and retinyl palmitate (vitamin A) absorption in neonates prior to the expression of pancreatic lipase. Because carboxyl ester lipase not only is present in the intestinal lumen but also in circulation, it also plays a role in lipoprotein metabolism and development of atherosclerosis.

Gastric lipase

Gastric lipase is an acid-stable lipolytic enzyme belonging to the acid lipase gene family. This family has 2 other members, lingual lipase and lysozyme lipase. Although the acid lipase gene family shows no amino acid sequence homology with any other known lipase family, it shares the common α/β-hydrolase fold. Gastric lipase is a serine enzyme with a catalytic triad (i.e., serine 153, aspartic acid 324, and histidine 353) similar to that of pancreatic lipase. Unlike pancreatic lipase and lipoprotein lipase, gastric lipase does not need a cofactor for lipolysis.

Gastric lipase is synthesized and secreted in the stomach, where it hydrolyzes dietary fat. Depending on the species, gastric lipase is synthesized and secreted in different locations and by different gastric cell types. In people, gastric lipase is secreted by the fundic chief cells, with pepsinogen also being cosecreted. In dogs, gastric lipase is secreted throughout the gastric mucosa with decreasing concentrations from the cardia to the pylorus. In cats, it is also secreted throughout the gastric mucosa, but its concentration is uniform from the cardia to the pylorus. Gastric lipase secretion is stimulated by gastric motility, cholinergic stimuli, a meal, and gastrin, which also stimulates pepsinogen and gastric acid secretion. Gastric lipase contributes up to 25% of dietary lipid hydrolysis, which is completed by pancreatic lipase and carboxyl ester lipase in the small intestines. It hydrolyzes triglycerides at all 3 ester bonds, but with marked preference for the ester bond at carbon 3 of glycerol (Figure 1). In contrast,
pancreatic lipase is less stereoselective and hydrolyzes ester bonds at carbon 1 and 3 of glycerol. The specific activity of gastric lipase for hydrolysis of tri-glyceride is 5 to 6 times less than that of pancreatic lipase. In the absence of pancreatic lipase in EPI patients, gastric lipase plays an important role as its secretion can increase 3- to 4-fold and can contribute up to 30% or more of overall dietary fat digestion. Further research into the expression of extra-pancreatic lipases in patients with nonpancreatic disease conditions will provide invaluable knowledge that will assist in the interpretation of nonsubstrate specific lipase assays.

Summary and Future Directions

Lipases are important for lipid digestion and metabolism in dogs and cats. Triglyceride is the main type of dietary lipids, with minor contributions by phospholipids and cholesterol esters. The most notable lipases are subfamilies of the pancreatic lipase gene family (i.e., pancreatic lipase and its related proteins, hepatic lipase, lipoprotein lipase, endothelial lipase, and phosphatidylserine phospholipase), carboxyl ester lipase, and gastric lipase. While pancreatic lipase, carboxyl ester lipase, and gastric lipase are present in the circulatory system, they exert their role in lipid digestion within the intestinal lumen. In contrast, the major lipases for lipid metabolism in the circulatory system are hepatic lipase, lipoprotein lipase, and endothelial lipase. Although most enzymes have their preferred substrate(s), much overlap occurs across the plethora of lipases because of the similarities in their structures, and this may have clinical implications, as further discussed in our companion Currents in One Health in this issue.

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