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Contents

Part IV Other Forms of Lipids

Introduction

Although evidence exists on the health benefits associated with the inclusion of certain lipid-rich foods (e.g. nuts, dairy products and fish) in the diet, the mechanisms that explain the physiological effects and the long-term benefits are not well understood. Lipids in themselves are essential nutrients and have many beneficial health effects: they are a source of energy and essential fatty acids, they are structural components of cell membranes, they are required to solubilise fat soluble compounds, and they serve as precursors of hormones. In general, it is only when dietary lipids are consumed in excess that they begin to have a negative impact on our health. The nutritional quality of our diet has a huge influence on our health and well-being, with a plethora of conditions and diseases being associated with a poor diet. It is becoming increasingly apparent that the nutritional quality of our food is a consequence of not only its nutritional composition but also how its structure influences the rate and extent of nutrient availability. The structure of a food influences the way it is transformed during processing and digestion. This, in turn, has an impact on nutrient bioaccessibility (release) and digestibility, and subsequently on the physiological response and health of the individual who consumes that food. Hence, care needs to be taken when assessing nutritional quality based purely on nutrient composition.

Lipids are generally insoluble in water; therefore, to be digested, lipids are required to be dispersed or emulsified to make them more accessible to the lipases which hydrolyse them into components suitable for absorption, for instance, by being released from the food matrix (from the cells of a plant tissue). The rate and extent of lipid digestion is governed by the way lipids are "presented" to the lipases in the different compartments of the gastrointestinal tract. Lipid digestion can be viewed as a colloidal and interfacial phenomenon where digestive agents (e.g. bile salts and lipases) and food components (e.g. dietary fibres, proteins and phytosterols) interact with the lipid at the lipid–water interface and thereby impact their hydrolysis by lipase(s).

Diets containing foods high in fat are considered to be detrimental to health; however, studies have shown that the consumption of certain foods which are high in fat, such as nuts and certain dairy products, led to beneficial effects on risk factors

for cardiovascular disease. A great proportion of lipid in some food matrices, such as almonds, remains undisturbed after mastication and the subsequent digestion processes. Here, the plant cell walls are resistant to digestion in the upper gastrointestinal tract and therefore encapsulate intracellular nutrients, thereby limiting lipid bioaccessibility. In dairy products, the structure of the food matrix can also have an impact on lipid digestibility. Indeed, the composition, microstructure and rheological properties of cheese matrices have been shown to impact the kinetics of the degradation of the cheese and the digestibility of the lipid it contained. Regarding food products where the lipids are readily available, such as milk and plant-based beverages or soups, other parameters can dictate lipid digestibility. These include the size of the droplets and the "quality" of the droplets interface (i.e. surface-active molecules) and the presence of certain compounds that may inhibit lipase activity (e.g. dietary fibres).

Therefore, the overall structure of the food containing the lipids is likely to also play a crucial role, notably by influencing their bioaccessibility and digestibility and ultimately their impact on nutrition and health.

This book aims to cover some of the latest research performed on food structure in the context of lipid digestion. The subjects include lipases, in vitro and in vivo models used to monitor lipid digestion, physiological aspects of lipid digestion and the impact of food structure (plant and animal sources of lipids).

Part I
Digestion of Lipids

Chapter 1
Enzymes Involved in Lipid Digestion

A. Salhi, F. Carriere, Myriam M.-L. Grundy, and A. Aloulou

Lipid digestion is a complex process that takes place at the lipid-water interface and involves various lipolytic enzymes present predominantly in the stomach and the small intestine [34]. These enzymes catalyse the hydrolysis of a variety of dietary lipids from animal and plant sources, such as triacylglycerols (TAGs), phospholipids, galactolipids, cholesterol and vitamin esters. They include gastric lipase, colipase-dependent pancreatic lipase, pancreatic lipase-related proteins 2 (PLRP2), carboxyl ester hydrolase or bile salt-stimulated lipase (CEH, BSSL), and pancreatic phospholipase A2. A debate still exist about the existence of a lingual lipase in human [30, 86, 140], an enzyme that has been demonstrated to be present and active in rat and mice tongue only and which is the product of a gene ortholog [53] to the gene of gastric lipase [24] in humans and many other species. Bakala N'Goma et al. [12] have reviewed the key findings that support the existence of lingual or gastric lipases in several species in term of gene expression, enzyme immunocytolocalization and lipase activity. So far, no supporter of the existence of a lingual lipase in humans has been able to provide similar data.

Contrary to the other major digestive enzymes, i.e. proteases and amylases, lipases act at the lipid-water interface because their substrate is insoluble in water

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[3, 122, 123]. This characteristic makes the development of kinetic models challenging and the usual Michaelis-Menten model no longer applies [115]. As for any enzyme, the rate of lipase activity depends on the initial quantity and type of substrate, the temperature, the pH, but a key parameter is the accessible surface available for lipase adsorption and activity. Indeed, activity increases with the rate of emulsification and the decrease in lipid droplet size, which corresponds to a larger specific surface [17]. The accumulation of lipolysis products and/or surfactants at the interface can however interfere with lipase activity in various ways, including enzyme inhibition or activation. In other words, lipolysis can be affected by environmental conditions and compounds that can alter the structure and/or the integrity of the interface (e.g. surface-active molecules such as proteins, phospholipids and bile salts) [39, 54, 123]. Besides these parameters, the form in which the lipids are delivered to the GI tract, including the food matrix, also impact the rate and extent of lipolysis. This particular topic is reviewed in Chaps. 7, 8, and 9. One important point to consider is that gastrointestinal lipolysis is a dynamic process that starts at the lipid-water interface and progressively proceeds through enzyme reactions on substrates with higher polarity and interaction with water (some lipolysis products like monoacylglycerols (MAGs), phospholipids). The enzymes involved in the steps subsequent to TAG and diacylglycerol (DAG) hydrolysis (carried out by enzymes with high affinity for lipid-water interfaces, the so-called true lipases) have usually a higher affinity for substrates forming mixed micelles and dispersed in the water phase.

This chapter gives a brief overview of the main dietary lipids from both animal and plant origins. Then, the lipolytic enzymes present in the human gastrointestinal (GI) tract and their structure/function relationship are described. Finally, the kinetic models and the sources of enzymes used in vitro models of digestion are discussed.

1.1 Dietary Lipids

Lipids, and in particular TAGs, represent the most condensed form of energy available in biological tissues [71]. Outside of cell membranes, lipids are stored in organelles called lipid bodies, oil bodies or oleosomes in plant seeds, that are present in different cell types. These lipid bodies have a spherical shape and are composed of a core of neutral lipids (TAGs and cholesterol esters) surrounded by a monolayer of phospholipids, cholesterol and proteins [82], as well as other polar lipids in plants and algae. In all organisms, the metabolic pathways responsible for the formation of lipid bodies and their mobilization through hydrolytic reactions have strong similarities. Detailed reviews of lipid bodies in mammals, plants and microorganisms have been published [4, 110, 159].

1.1.1 Membrane Lipids

Eukaryotic cells are compartmentalized into organelles (e.g. endoplasmic reticulum, Golgi apparatus, and mitochondria) that are delimited by membranes with different biological functions.

These membranes are essentially made up of phosphoglycerolipids, with the exception of plant photosynthetic membranes that contain large amounts of galactoglycerolipids. This is the case, for instance, in the plasma membrane which delimits a cell and the endomembrane system which consists of different organelles (e.g. lysosomes and endosomes) dispersed in the cytoplasm of a eukaryotic cell [91]. Phosphoglycerolipids are consisting of two fatty acid residues esterifying a glycerol residue which is itself esterified by a phosphate residue. They are lipids organized around a glycerol-3-phosphate residue, unlike triglycerides which do not contain a phosphate group but are instead formed of three fatty acid residues esterifying a glycerol residue. In contrast to triglycerides, phosphoglycerides are highly amphiphilic molecules, i.e. consist of a hydrophilic polar head (the phosphate group and its substituents, such as choline, ethanolamine, glycerol or inositol) and hydrophobic aliphatic tails (the fatty acid residues).

Other than phosphoglycerides, membranes also contain other lipids such as sphingolipids and sterols, the latter are mainly represented in animals by cholesterol [100]. Biological membranes are organized around a lipid bilayer consisting of two sheets of lipid molecules in which the phosphoglycerides are oriented to present their hydrophilic heads in contact with water and their hydrophobic tails in contact with the other sheet.

Finally, in plants and photosynthetic organisms in general, membranes, such as those of thylakoids present in chloroplast, are mainly composed of galactolipids [5]. The main galactolipids are monoglactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG).

In almost all living organisms, the cell membrane consists of a lipid bilayer, as do the membranes surrounding the cell nucleus and organelles [95]. Enveloped viruses also have a membrane around which they surround themselves during exocytosis. The lipid bilayers are impermeable to ions and hydrophilic molecules, allowing the cells to regulate the pH and salinity of their cytosol with the help of transmembrane proteins that act as membrane transporters capable of generating and maintaining a concentration gradient of various chemical species between the cytoplasm and the extracellular medium. These bilayers also tend to close on themselves (budding) to form vesicles. Depending on the nature and composition of the lipids, their interactions can lead to the formation of a distinct phase that separates from water (e.g. oil droplet and emulsions), micelles, and lamellar phases including liposomes or membrane surfaces [87].

1.1.2 Storage Lipids

In mammals, lipids are found in various forms: constitutional lipids which are the primordial cellular constituents of membranes, represent 10% of the dry weight of tissues, serve to protect vital organs (heart, liver, kidneys, spleen, brain and spinal cord), circulating lipids which appear in the blood in the form of lipoproteins, and then disappear at the end of digestion to settle in the liver and other tissues, and reserve lipids which accumulate in adipose tissue and are very important in the production of energy.

Adipose tissue is made up of fat cells called adipocytes, which are animal cells that specialize in energy storage. They contain more than 95% of the body's TAGs [48]. They store lipids up to a critical cell size between 70 and 120 μm. Beyond this maximum size, the adipocyte, is no longer able to store fat, and divides, leading to the formation of a new adipocyte (mitosis process). Once mobilized by lipolysis, the TAGs represent an energy supply for the other organs and in particular the muscle tissue [47]. Adipose tissue is the most important site for storing metabolic energy. In mammals, there are 2 types of adipocytes: white and brown [144].

White adipose tissue represents one of the body's most important energy reserves, it is the main tissue responsible for the storage of TAGs, while brown adipose tissue is involved in the conversion of TAGs into heat (thermogenesis). When the carbohydrate reserves are depleted (in case of fasting, physical exertion, fighting the cold, etc.), or unusable (in case of severe diabetes), the body calls upon the reserves of white adipose tissue to provide the organs with the necessary energy substrates.

Brown fat is involved in thermogenesis through lipolysis of the TAGs of the adipocytes. Unlike white adipocytes, which contain a single lipid droplet per cell, brown adipocytes contain many smaller droplets and a much higher number of mitochondria that contain iron, giving the tissue its brown colour. Brown adipose tissue also contains more capillaries than white adipose tissue because it has a higher oxygen requirement than most other tissues [110].

Small lipid bodies are also detected in different types of tissues, such as liver, muscles, heart, kidneys, small intestine, mammary glands, cultured fibroblasts and macrophages. Lipid droplets rich in cholesterol esters are also detected in steroidogenic cells of the adrenal cortex and in the testes and ovaries [67, 74].

These lipid bodies, rich in cholesterol esters, provide a source of cholesterol that can be used, for example, for the synthesis of steroid hormones [22, 67, 74] or membrane biogenesis [111]. In specific cells such as liver stellate cells, which are rich in retinol esters, lipid bodies contain significant amounts of vitamin A [11].

Lipid globules in breast milk are another form of transient storage and energy transport. The lipid content of breast milk can vary from 0.2% to 61% (w/w). These lipid globules (0.2 to 2 μm in diameter) are formed mainly by a hydrophobic core (triglycerides, esterified cholesterol and fat-soluble vitamins) surrounded by a triple layer membrane made of proteins and phospholipids, and resulting from the exocytosis of lipid droplets during native milk production by the cells of the mammary

gland [28]. After processing (pasteurization and homogenization), the phospholipids surrounding milk droplets can be re-organized in monolayers [101, 116].

In vertebrates, lipoproteins are essential for lipid transport to the different tissues of the body. These include chylomicrons (CM) synthesized by the enterocyte during digestion and very low density lipoproteins (VLDL) synthesized by the liver [33, 79, 80, 88, 137]. Lipoproteins are large complexes of proteins and lipids, the outer membrane of which is a monolayer of phospholipids and free cholesterol with one or more proteins called apolipoproteins (e.g., Apo-A, Apo-B, etc.); the core of lipoproteins contains TAGs, cholesterol esters and small amounts of other hydrophobic substances, such as fat-soluble vitamins. CM and VLDL are primarily hydrolyzed by lipoprotein lipase (LPL), while the other lipoproteins, low density lipoproteins (LDL) and high density lipoproteins (HDL), are preferentially hydrolyzed by hepatic lipase (HL) and endothelial lipase (EL) [84, 113, 118].

1.2 Lipolytic Enzymes in the Human GI Tract

1.2.1 True Lipases Acting at the Lipid-Water Interface

These water-soluble enzymes show a high affinity for lipid-water interfaces and are essential for initiation the lipolysis of dietary TAG. In the GI tract, gastric and pancreatic lipases belong to this category of lipolytic enzymes.

1.2.1.1 Gastric Lipase

Human gastric lipase (HGL) is produced in the fundic mucosa of the stomach [107] and is co-located with pepsinogen in the chief cells of the fundic glands [106]. HGL is a highly glycosylated, 50 kDa serine enzyme with an α/β hydrolase fold [37, 126]. Several signals trigger the secretion of gastric lipase such as stomach movements, cholinergic stimuli [40], test meals [36] and the gastrin gastrointestinal hormone gastrin, which also stimulates the secretion of pepsinogen and gastric acid [141, 154]. Gastric lipase is active and stable in the acidic environment of the stomach, where the gastrointestinal lipolysis of dietary fat is initiated [2, 36, 154], although HGL can potentially hydrolyze all esters bonds in a TAG molecule in vitro [39]. Nevertheless, in vivo, MAGs are rarely observed in gastric contents because the gastric lipolysis reaction by HGL is rapidly inhibited by reaction products such as FFA [70, 114]. Gastric lipase has high levels of activity on TAG (Fig. 1.1), to a lesser extent on DAG, and its activity on MAG is generally very low compared to its activity on TAG [62, 64]. Gastric lipase also hydrolyzes polyethylene glycol (PEG) mono- and di-esters and is one of the most active lipases on mixtures of acylglycerols and PEG esters such as the pharmaceutical lipid-based excipients Gelucire®

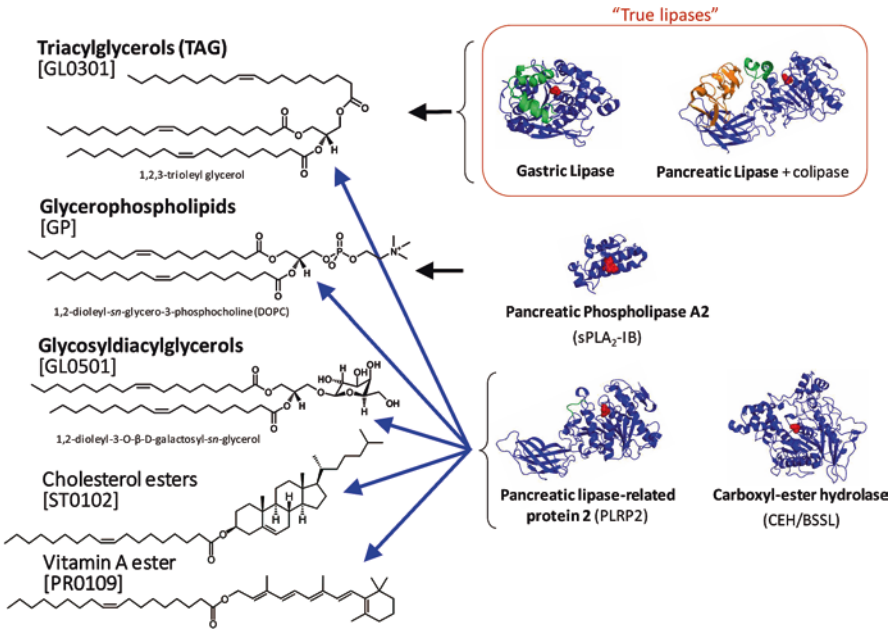

Fig. 1.1 Chemicalstructures of the main lipid classes found in human diet and gastrointestinal lipolytic acting on these lipids. Numbers below the substrate types correspond to the Lipid Maps Classification system (see https://www.lipidmaps.org/) [58, 59]

44/14 and Labrasol® [60–62, 64]. However, HGL does not hydrolyze cholesterol esters or phospholipids (Fig. 1.2) [39].

Gastric lipase shows stereopreference for the hydrolysis of the ester bond at the sn-3 position of TAG, that has been demonstrated both in vitro and in vivo [43]. This enzyme is therefore tailored to release the short and medium chain fatty acids found at this position in milk TAG [125].

In vivo, the level of intragastric lipolysis of TAG ranges from 10% of total TAG acyl chains (normal solid-liquid meal) to 25% (emulsified liquid meal), and DAG and FFAs are primarily generated in the stomach by gastric lipase [36, 38, 42]. During a meal, HGL is always present and stable in the duodenal contents, and can therefore contribute to intestinal lipolysis [36] (Fig. 1.3). Overall, it has been estimated that HGL can release 1 acyl chain out of the four that have to be released for the intestinal absorption of 2 TAG molecules in the form of MAG [36]. This is the reason why it is often mention in literature that HGL can contribute to as much as 25% of fat digestion. Studies in patients with severe exocrine pancreatic insufficiency and no significant levels of pancreatic lipase have confirmed that HGL alone can achieve around 30% of dietary TAG digestion [38].

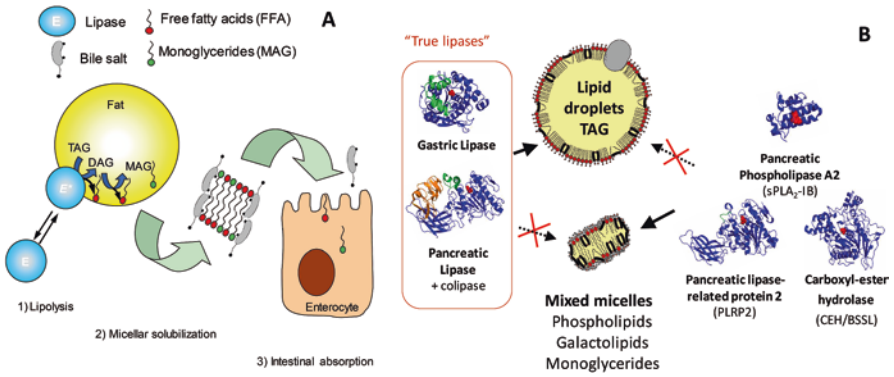

Fig. 1.2 Schematic representation of the essential steps of gastrointestinal lipolysis and intestinal fat absorption (**a**), including (1) the lipolysis reaction after lipase adsorption at the oil-water interface, (2) the micellar solubilisation of lipolysis products, (3) the intestinal absorption of lipolysis products by enterocytes), and substrate specificity of the various lipolytic enzymes found in the GI tract (**b**). Two main categories of lipolytic enzymes can be defined, with the so-called "true lipases" showing a preference for water-insoluble substrates (TAG, DAG) and acting preferentially at the oil-water interface and the enzymes showing a preference for substrates forming mixed micelles with bile salts and dispersed in the aqueous phase like phospholipids, galactolipids and monoglycerides. Adapted from [44, 97]

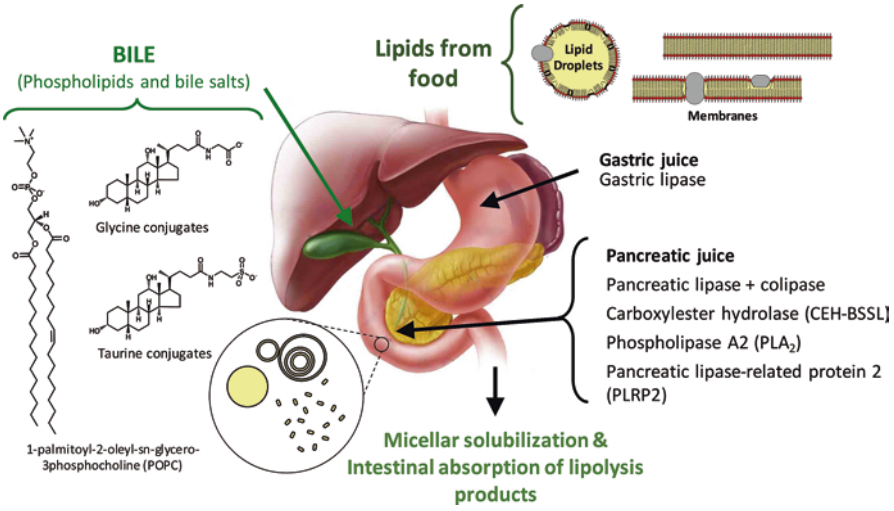

Fig. 1.3 Physiology of gastrointestinal lipolysis

1.2.1.2 Pancreatic Lipase and Colipase

Quantitatively, human pancreatic lipase (HPL) is the major lipase involved in the lipolysis of dietary fat. In the small intestine, HPL acts in the presence of bile with its specific protein cofactor colipase acting as an anchor for the enzyme at the

oil-water interface and counteracting the competitive inhibitory effects of bile salts (Fig. 1.3). The biochemical, structural and physiological properties of pancreatic lipase have been extensively reviewed [45, 150]. HPL is a 50.5 kDa glycosylated serine hydrolase, it is synthesized by the acinar cells of the pancreas in the form of an active enzyme and not as an inactive zymogen like most pancreatic enzymes. It is delivered into the intestinal lumen via the pancreatic duct.

In vitro, HPL alone can act on TAG emulsions (Fig. 1.1), but hydrolysis of TAG is inhibited in the presence of bile salts that compete with lipase for adsorption at the lipid-water interface [20]. In vivo, HPL therefore requires its specific10 kDa colipase cofactor, also produced by the pancreas, to counteract the effects of the bile salts and hydrolyze TAGs. Pancreatic lipase also acts on DAG, which actually could be its major substrate after the action of gastric lipase and ultimately produces 2-MAG together with FFA. When DAG serves as an initial substrate for HPL in vitro, the activity measured is usually lower than the activity on TAG [56, 64]. However, when the relative hydrolysis rates of TAG and DAG are estimated in the course of TAG hydrolysis followed by the subsequent hydrolysis of the generated DAG, the rate of DAG hydrolysis was found to be higher than that of TAG [104]. HPL is 1,3-regioselective lipase tailored for efficiently producing 2-MAG from TAG. It is not active on 2-MAG, is weakly active on medium-chain 1(3)-MAGs and is inactive on long-chain 1(3)-MAGs. HPL is not active on phospholipids (Fig. 1.2b) [45, 142], while phospholipase A1 activity has been reported for porcine pancreatic lipase [50]. HPL has very low levels of activity on PEG mono- and di-esters, and is completely inactive on mixtures of these esters and acylglycerols, such as Labrasol® and Gelucire® 44/14 [62, 64]. HPL has no activity on cholesterol esters [1] or galactolipids [6, 10]. Pancreatic lipase has been extensively studied for many years, however, some basic data such as the amount of HPL produced during a meal and its specific activity on test meal TAG had to wait for clinical studies to be obtained [36, 41].

1.2.2 Other Lipolytic Enzymes Present in Pancreatic Secretion

1.2.2.1 Pancreatic Lipase-Related Protein 2 (PLRP2)

Two pancreatic lipase related proteins (PLRP1 and PLRP2) are produced by the pancreas. Human PLRP1 and PLRP2 have 65% and 68% identity respectively with classical pancreatic lipase HPL [72] and a highly conserved catalytic triad (Ser, Asp, His). The three-dimensional structures of these three proteins are superimposable in their structural domains, but differ at the level of the lid that controls access to the active site for PLRP2 and at the level of steric hindrance in the vicinity of the active site for PLRP1 [57, 127, 157]. As a consequence, both native and recombinant HPLRP1 do not exhibit lipolytic activity and its eventual contribution to lipid digestion is still unknown.

PLRP2 exhibits lipolytic activity on various substrates including TAGs, phospholipids (phospholipase A1 activity), galactolipids and cholesterol esters (Fig. 1.1). The lipase activity of PLRP2 is inhibited by micellar concentrations of bile salts and is not reactivated by colipase, suggesting that PLRP2s does not have lipase activity in vivo but may preferentially act as a phospholipase [142] or a galactolipase [136] (Fig. 1.2b). Indeed, PLRP2 also has a high level of galactolipase activity on both MGDG and DGDG [10]. It has been established that human pancreatic juice contains at least two enzymes that effectively hydrolyze DGDG, HPLRP2 and CEH have been identified as these galactolipases [9]. In addition, HPLRP2 is also active on lipophilic vitamin esters [121], as well as on cholesterol esters [8, 130] and is one of the most active monoacylglycerol lipase identified so far [56, 57]. The broad substrate specificity of HPLRP2 is also illustrated by the fact that this enzyme has significant activities on mixtures of acylglycerols and PEG esters such as Gelucire® 44/14 and Labrasol® [62, 64]. Cholesterol esterase activity is the most recent activity attributed to both recombinant human (rHPLRP2) and guinea pig lipase (rGPLRP2) PLRP2 [130] because HPLRP2 has been described as having cholesterol esterase activity [12] (Fig. 1.1). They confirmed a low cholesterol esterase activity for the latter (18.7 ± 1.1 U/mg at pH 6) and a lower activity for GPLRP2 (3.7 ± 0.6 U/mg at pH 6).

1.2.2.2 Carboxyl Ester Hydrolase – Bile Salt-Stimulated Lipase (CEH/BSSL)

Carboxyl ester hydrolase (CEH, EC 3.1.1.1) is a non-specific esterase with different names: bile salt-stimulated lipase (BSSL) [77, 92], cholesterol esterase [108, 109], carboxyl ester lipase (CEL) [129] and lysophospholipase [146]. In humans CEH/BSSL, which is secreted by acinar cells of the exocrine pancreas, is present in pancreatic juice [66, 73]. It is also produced by the mammary glands, present in breast milk and is often referred to as BSSL in this context [23, 77]. Like PLRP2, CEH-BSSL hydrolyzes various substrates in vitro like cholesterol esters, TAG, MAG, vitamin (A, E) esters, and phospholipids [55, 83, 92–94].

CEH-BBSL also hydrolyses carotenoid esters (lutein and capsanthin diesters, esters of β-cryptoxanthin) [29], galactolipids (monogalactosyldiglycerides) [6] and PEG mono- and di-esters [62–64]. Recently, a new potentiometric test has been developed to measure cholesterol esterase activity using cholesterol acetzate as substrate [130]. To measure cholesterol esterase activity, the preparation of the substrate is not straightforward as cholesterol esters are highly apolar and oil-soluble. Other lipids, such as TAGs and phospholipids, have generally be used to allow the dispersion in water of small lipid aggregates containing cholesterol esters. Since most cholesterol esterase enzymes also have lipase and phospholipase Al (PLA1) activities, they can also act on the TAGs or phospholipids present in the reaction mixture, releasing fatty acids from these substrates and it is therefore impossible to estimate the cholesterol esterase activity by titration of the released fatty acids alone. Long-chain cholesterol esters also have a low affinity for bile salt micelles

and cannot be dispersed in mixed micelles like phospholipids and galactolipids. This problem was solved by a short-chain cholesterol ester which can be dispersed in water using bile salt micelles. Dispersions of cholesterol acetate (CholA) and sodium taurocholate (NaTC) were found to be good substrate for both pancreatic CEH and PLRP2 [130]. The specific activity of human CEH/BSSL measured under these conditions was found to be the highest recorded with cholesterol esters [17, 27, 112].

CEH-BSSL has no regiospecificity on TAG and can hydrolyse all three ester bonds, whatever their position on the glycerol backbone [155].The physiological role of CEH is still subject to debate [81], but it seems likely that CEH plays a major role in the digestion of cholesterol esters and lipophilic vitamins in adults, and probably in that of acylglycerols in newborn children [76].

Its contribution to the lipolysis of 2-MAG seems to be physiologically relevant only during the lactation period in humans [19, 75]. In vitro bile salt-stimulated lipase alone cannot hydrolyze native milk fat globule TAG, but low rates of hydrolysis by gastric lipase trigger the hydrolysis of milk by pancreatic lipase and bile salt-stimulated lipase. HGL and HPL hydrolyze about two thirds of the total ester bonds present in milk, generating MAG and FFA, and adding bile salt-stimulated lipase results in the hydrolysis of MAG. The concerted action of these three lipases results in the complete digestion of milk TAG, generating free glycerol and FFA as the end-products.

1.2.2.3 Pancreatic Phospholipase A2

Pancreatic phospholipase A2 (PLA2; phosphatide 2-acylhydrolase, EC 3.1.1.4) is involved in the digestion of dietary phospholipids. PLA2 is a stereospecific enzyme which catalyzes the hydrolysis of the sn-2 fatty acyl ester bond of 3-sn-glycerophospholipids, generating FFA and 1-sn-lysophospholipids [50]. Pancreatic PLA2 belongs to Group IB of secretory low molecular weight phospholipases A2 (sPLA2-IB) [52]. It is mainly present in pancreatic secretion, but also in the smooth muscle cells of the lungs and vessels. sPLA2 are involved in many biological functions other than digestion, such as cell proliferation, contraction of smooth muscle in the airways and vessels, and in various inflammatory diseases such as rheumatoid arthritis, endotoxic shock, respiratory distress syndrome and certain types of cancer [85, 145]. On the contrary to HGL, HPL, HPLRP2 and CEH-BSSL that are serine hydrolases with a Ser-His-Asp catalytic triad [57, 126, 157], sPLA2 has no catalytic triad, and the nucleophile required for cleavage of the ester bond is an activated water molecule which is hydrogen-bonded to a His residue, which in turn is hydrogen-bonded to an Asp residue [143].

1.3 Main Structural Features of Gastrointestinal Lipases

1.3.1 Gastric Lipase: Lipase with a Single Domain

HGL is a globular protein consisting of a single polypeptide chain of 379 amino acids [24]. The molecular weight of native HGL, purified from gastric juices, is approximately 50 kDa. This mass corresponds to the mass of the mature 379-amino acid polypeptide plus significant N-glycosylation. There are 4 major isoforms of human gastric lipase with isoelectric points between 6.8 and 7.4 [105]. In 1999, the three-dimensional structure of HGL in its closed form was resolved at 3.0 Å, following the production of the recombinant protein in insect cells using the baculovirus expression system. It revealed several structural elements [126]:

– A main globular domain with an α/β hydrolase fold, organized around a central structure composed of eight β-stands surrounded by six α-helices.
– A flap or lid (residues 210 to 267) composed of 58 amino acids, articulated around two α-helices [126] and controlling the accessibility to the active site. The flap reveals near the active site a large hydrophobic patch of 29 amino acids (residues 215–244), which may be involved in the binding of substrate [102].

1.3.2 Pancreatic Lipases: Lipases with Two Domains

Both HPL and HPLRP2 are composed of two domains: a N-terminal and globular catalytic domain, with an α/β hydrolase fold and a lid controlling the access to the active site as in HGL [57, 96, 147, 149], and a C-terminal domain involved in interactions with colipase and lipids. The C-terminal domain of pancreatic lipases has a sandwich structure containing an exposed hydrophobic loop (loop β5'; residues 405–414) inserted between two strands β5 and β6. This structural domain has an important role in the interaction of lipase with its cofactor, colipase [148, 149]. Structure-function studies have shown that the C-terminal domain also has an important role in the interaction with the lipid interface and has some homologies with C2 lipid binding domains [20, 46]. The β5' loop has a large accessible apolar surface area which is of the order of 877 $Å^2$. The hydrophobic (H), charged (C) and semi-polar (S) residues of the β5' loop have accessible surfaces of 488 $Å^2$, 105 $Å^2$ and 284 $Å^2$ respectively. The loop β5' is located on the same side of the enzyme as the hydrophobic loops surrounding the active site (lid and β9 loop). In addition, the hydrophobic surface of the β5' loop is not masked after colipase fixation. In HPL, the β5' loop contributes with the open lid, the β9 loop and the hydrophobic tips of colipase to the formation of a large hydrophobic plateau that may parallel the lipid-water interface [96].

HPLRP2 is organized in two domains similar to those found in HPL, except that the lid is found in an open conformation in the absence of surfactants and inhibitors

[57]. This conformation is different from the one observed in the open HPL, which probably explains differences in kinetic properties between HPLRP2 and HPL. Moreover, most of the amino acid residues of HPL involved in the interaction with colipase, both within the lid and the C-terminal domain, are mutated in HPLRP2 [142], which explains why HPLRP2 has only a weak interaction with colipase [136]. Indeed, the lipase activity oh HPLRP2 on TAG is inhibited by bile salts and is not restored by addition of colipase.

1.4 Important Parameters Governing the Activity of Gastrointestinal Lipases

1.4.1 The Role of Bile Salts

The influence of bile salts on the activity of lipases of different origins has been extensively studied in vitro. It has been established that, at low concentrations, bile salts have an activating effect on the hydrolysis of emulsified long-chain TAG by pancreatic [26] and gastric [69] lipases. In interpreting these results, it is generally accepted that bile salts, by lowering the tension at the water-substrate interface, prevent the interfacial denaturation of the enzyme. In the lipolysis reaction, the enzyme partitions between the aqueous phase and the interface [152] (Fig. 1.2a). It has been assumed that the enzyme adsorbed at the interface would exist in a conformational state with an open lid, in equilibrium with an inactive form with the closed lid remaining in the water phase (Fig. 1.2a). It has been shown however that the lid can open in solution in the presence of bile salts with a maximum aperture in the presence of supramicellar concentrations of bile salts [14]. Under physiological conditions, it is therefore expected that the lid opens in solution prior to HPL adsorption at the interface, which is supported by the exposition of a large hydrophobic plateau upon lid opening. The adsorption and activity of pancreatic lipase is however dependent on the presence of colipase, which anchors the lipase at the interface when the concentration of bile salts exceed its micellar concentration [21, 26]. Contrary to HPL, HGL is not inhibited by micellar concentrations of bile salts and does require a cofactor [89].This is due to a higher tensioactivity of HGL, that has a high penetration capacity at the lipid-water interface, even in the presence of phospholipids [51, 131].

Bile salts have a dual impact on the activity of true lipases. On the one hand, they can impair lipase adsorption and activity. On the other hand, they can ensure the continuity of lipolysis by removing the reaction products from the oil-water interface by a process of micellar solubilisation. Concerning the other lipolytic enzymes acting on more polar lipids (phospholipids, galactolipids, MAG), bile salts have an essential role in the dispersion of these substrates in the aqueous phase in the form of mixed micelles (Fig. 1.2) and the bile salt to substrate molar ratio is critical for the activities of HPLRP2, CEH-BSSL and PLA2 [97–99].

1.4.2 Gastric and Duodenal pH Variations

The variations in the gastric and duodenal pH which occur during digestion have been studied in several test meal experiments on healthy humans and patients with exocrine pancreatic insufficiency [36, 38, 42, 124]. After the ingestion of a meal, the pH of the gastric content ranges between 5.5 and 7, depending on the composition of the meal ingested. Then, the gastric content is diluted by the gastric acid secretion and the pH decreases. Gastric emptying also contributes to this pH decrease by removing the meal components from the gastric contents and thus reducing their buffering effects. The intragastric pH value is found in the 4 to 5 pH range at half gastric emptying time (approx. 60 min) in heathy volunteers, which corresponds to the optimum pH conditions for HGL activity on TAG [69, 89].

The duodenal pH variations are less pronounced and are usually restricted to the 5 to 7 pH range, giving a mean value of 6.25 in healthy volunteers. These pH values also correspond to the optimum pH conditions for HPL activity on TAG in the presence of bile salts [25, 89]. Thus, both HGL and HPL are tailored for acting optimally on TAG at the pH values found in the stomach and small intestine, respectively. In the case of HPLRP2 and CEH-BSSL, the optimum pH of activity depends on the type of substrate hydrolyzed and it is usually found in the neutral to slightly alkaline pH range [56]. Both enzymes show however an optimum activity on cholesterol esters at pH 6 [130].

1.4.3 The Effects of Calcium on Lipolysis

Calcium contributes decisively to the catalytic activity of sPLA2-IB, since a calcium ion interacts with the phosphate group of the phospholipid substrate, with the catalytic water molecule and with the tetrahedral intermediate generated during the deacylation reaction (stabilization of the negative charge in the oxyanion hole) [153]. Calcium may also contribute to the interactions of sPLA2 with lipid aggregates. A second calcium ion has been observed near the N-terminal part of the enzyme and the interfacial binding surface (i-face) in the 3D structure of bovine sPLA2-IB [119], as well as in other sPLA2 [135]. Finally, calcium may also contribute to the interfacial organization of the phospholipid substrate itself by condensing the polar heads of negatively charged phospholipids [138].

Therefore, when studying or using PLA2, it is essential to monitor calcium concentration, whereas lipases do not have absolute calcium requirements. A calcium ion is found in the 3D structures of pancreatic lipases, but it is remote from the active site and interfacial binding sites and has probably only structural functions. However, some non-specific effects of calcium may be observed in lipase assays, as calcium can compensate for the electrostatic repulsion that occurs between the enzyme and a charged lipid-water interface. Calcium and FFAs may also form soaps

during the lipolysis reaction, and lipase kinetics may be affected by the removal of FFAs from the lipid-water interface [16, 18, 150].

1.5 Kinetic Models of Lipolysis and Enzyme Assays

When we speak of the Michaelis-Menten model, we are talking about homogeneous enzymology where the enzymatic reaction takes place in a homogeneous medium, i.e. in a single phase in which the enzyme and the substrate are soluble. For lipolytic enzymes acting essentially at the lipid-water interface (in a heterogeneous medium), we speak of heterogeneous enzymology and the classical Michaelian model of enzyme kinetics can no longer be applied. One then passes from a three-dimensional or volumetric system to a two-dimensional or surface system. In 1973, an interfacial kinetic model was proposed by Verger and de Haas [151]. According to this model, the enzymatic reaction takes place in two elementary stages:

- An adsorption step of the enzyme at the lipid interface (adsorption or penetration) (E: water soluble enzyme, E*: adsorbed enzyme). This step is governed by two rate constants: a constant of penetration (kp) or adsorption of the enzyme at the lipid-water interface and a constant of desorption (kd) of the enzyme. During this step, the enzyme may or may not undergo a conformational change.
- An actual catalytic step analogous to a Michaelis Menten pseudo-reaction that takes place on the surface and no longer in solution. All concentrations of the species involved in the reaction such as the enzyme (E and E*), the lipid substrate (S), the reaction product (P) and the enzyme/substrate complex (E*S) are expressed in surface density (mole.m-2) instead of volume concentration (mole.m-3). In order to differentiate soluble enzyme forms in the aqueous phase (E) from those present at the interface, the latter are denoted with an asterisk (E*, E*S).

1.5.1 Kinetic Phenomenon of Interfacial Activation

Lipases are water-soluble enzymes, which however have very low activity on water-soluble esters. Their activity becomes very high when the concentration of the substrate exceeds solubility and allows the formation of aggregates such as lipid droplets and thus the formation of a water-lipid interface [65, 134]. This phenomenon is called interfacial activation, which distinguishes lipases from esterases acting only on water-soluble carboxylic esters. Its observation requires the use of non-natural short chain TAG with some solubility in water and its relevance for natural long chain TAG has often been questioned. Nevertheless, it has triggered numerous researches to explain this phenomenon at the enzyme molecular level, first with speculations on conformational changes occurring in the presence of a lipid-water