

Salivary lipids: A review

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Abstract

Saliva is produced by both large and small salivary glands and may be considered one of the most important factors influencing the behavior of oral cavity homeostasis. Secretion of saliva plays an important role in numerous significant biological processes. Saliva facilitates chewing and bolus formation as well as performs protective functions and determines the buffering and antibacterial prosperities of the oral environment. Salivary lipids appear to be a very important component of saliva, as their qualitative and quantitative composition can be changed in various pathological states and human diseases. It has been shown that disturbances in salivary lipid homeostasis are involved in periodontal diseases as well as various systemic disorders (e.g. cystic fibrosis, diabetes and Sjögren's syndrome). However, little is known about the role and composition of salivary lipids and their interaction with other important ingredients of human saliva, including proteins, glycoproteins and salivary mucins. The purpose of this review paper is to present the latest knowledge on salivary lipids in healthy conditions and in oral and systemic diseases.

Key words: lipids, saliva, salivary glands, salivary lipids

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Salivary glands and saliva

Saliva is produced by 3 pairs of major salivary glands (parotid, submandibular and sublingual) and numerous minor unpaired salivary glands scattered within the oral submucosa.^{1–4} Depending on the nature of saliva and its constituent cell types, the following glands can be distinguished: purely serous salivary glands, known as the alveolar salivary glands (e.g. the parotid gland and von Ebner's glands, located at the base of the tongue); purely mucous glands called tubular salivary glands (a plurality of small submucosal glands); and mixed tubulo-alveolar salivary glands.^{3,4} The latter glands may occur with a greater number of mucous cells (in the sublingual glands mucosal cells represent approx. 70%) or a predominance of serous cells (in the submandibular glands serous cells represent 80%).

Within a day, the salivary glands produce an average of 1000–1500 mL of saliva when at rest; stimulation may increase the amount of saliva secreted by several times.^{5,6} In the absence of stimulation, the parotid, sublingual and minor salivary glands provide about 25%, 7–8% and 7–8%, respectively, of the whole saliva flow. The submandibular gland produces 60% of the unstimulated whole saliva (UWS) flow. When the salivary flow is stimulated, the parotid gland contribution increases by 10–15%, while the remaining salivary glands do not significantly increase saliva secretion.^{3,4,6,7} The fluids secreted by the serous parotid glands contribute most of the peroxidase, proline rich glycoprotein (PRG) and amylase, while the mixed, submandibular, sublingual and minor salivary gland secretions are rich in mucin, which is responsible for the viscoelastic properties of saliva and for the blood-group activity of saliva.^{8–11,15}

Saliva fulfills a variety of functions in the oral cavity. It is the liquid medium of the oral cavity ecosystem, providing hydration of the teeth and mucous membrane surfaces, thereby allowing articulation and swallowing. Saliva determines the protection of the oral tissues against biological, mechanical and chemical stimuli; allows the perception of taste and temperature; and is responsible for initial food digestion.¹⁶ These functions and properties of saliva are attributed to electrolytes, buffering systems, proteins, glycoproteins, and lipids.^{17–22} Salivary proteins, including glycoproteins, and lipids create a “network” which signals the presence of fats in the oral cavity and provides diagnostic and prognostic information.^{23–25} Salivary proteins are well characterized, but data for characterizing salivary lipids and their functions is scarce and controversial.^{16,21}

A brief review of lipids

There are many definitions of lipids. Previously, lipids were defined as non-polar compounds insoluble in water but easily soluble in organic solvents.²⁶ Christie describes

lipids as “fatty acids and their derivatives, and compounds biosynthetically or functionally related to them”.²⁷

To facilitate a comprehensive worldwide classification of lipids, the International Lipid Classification and Nomenclature Committee, with the participation of the LIPID MAPS Consortium, divided lipids into 8 main groups: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides.^{28,29} The first 5 of these groups of lipids are found in saliva, so the authors decided to present briefly the biochemical characteristics of these lipids. It is worth mentioning that many previous works about salivary lipids use the old division of lipids into neutral lipids, glycolipids and phospholipids, which can be quite confusing.^{28,29}

Free fatty acids

Free fatty acids are the primary component of salivary lipids.³⁰ Free fatty acids are composed of a hydrocarbon chain terminated by a COOH group. The presence of a repeating series of CH₂ determines the hydrophobic nature of the fatty acids, while the COOH group is hydrophilic.^{26,31} The proportion of hydrophilic and hydrophobic groups in the molecule accounts for the amphipathic nature of free fatty acids.

Due to the number of carbon (C) atoms, free fatty acids can be divided into short- (< 6 C), medium- (8–14 C), and long-chain (> 16 C) free fatty acids; however, the most common molecules usually contain from 4 to 30 carbon atoms.³² Furthermore, saturated and unsaturated free fatty acids can be distinguished. In the body only unsaturated free fatty acids can be produced, in which the double bond is at position ω -9. Linoleic acid (ω -6) and α -linolenic acid (ω -3) cannot be synthesized by humans and must be provided by food. They are referred to as essential unsaturated fatty acids.³²

Glycerolipids

Glycerolipids include all glycerol-containing lipids, among which the largest group are acylglycerols (esters formed from fatty acids and glycerol). Acylglycerols exist in various physical forms: monoacylglycerols (MGs), diacylglycerols (DAGs), and triacylglycerols (TGs); triacylglycerols are more plentiful than the other 2 groups.^{28,29} In contrast to MGs and DAGs, TGs do not form dispersed micelles.³²

Glyceroglycans are another subclass of glycerolipids, characterized by one or more sugar moieties attached to glycerol via a glycosidic linkage.^{29,33}

One of the most numerous subgroups of glycerolipids are glycerophospholipids (phospholipids, PH). The common structural element of this class of compounds is

phosphatidic acid (PA). The phosphate group of PA can be combined through an ester bond with a compound containing a hydroxyl group, for example choline, ethanolamine, serine, inositol or another glycerol, forming a phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol. Among linked fatty acids, acids attached at the C1 carbon atom are typically saturated, while those attached at the C2 atom are unsaturated with one or more double bonds.^{29,31,32}

Glycerophospholipids consist of a subset of ether phospholipids, which may contain both ester and ether linkages. This group includes plasmalogens and a platelet activating factor (PAF).^{29,31,32} In the plasmalogens, glycerol is attached at carbon atom C1 by an ether linkage to the long chain in the aldehyde enol form. There are 3 classes of plasmalogens: phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.^{29,31,32}

Sphingolipids are lipids that comprise aminoalcohol instead of glycerol: sphingosine or dihydrosphingosine.^{29,31,32} The amino group of sphingosine may communicate via an amide bond with fatty acids, forming a ceramide.^{34–36} A ceramide is a precursor of sphingomyelin and glycosphingolipids. Glycosphingolipids form a polar sugar chain (consisting of one or more monosaccharides), which is bonded O-glycosidically to the ceramide by a hydroxyl group at the C1 atom of sphingosine. Depending on the type of sugar chain, the following are distinguished: neutral glycosphingolipids, containing neutral saccharides (e.g. galactosylceramide and glucosylceramide); acidic glycosphingolipids (gangliosides containing one or more sialic acid residues); and sulphatides, containing a sulfonic acid group. Another group of glycosphingolipids, which has not yet been demonstrated in saliva, consists of basic glycosphingolipids and amphoteric glycosphingolipids.^{28,29}

Cholesterol is a monohydroxy cyclic unsaturated alcohol, which is of exogenous origin or which may be synthesized *de novo* in the cytoplasm and endoplasmic reticulum of hepatocytes.³⁷ In body fluids, cholesterol is associated with lipoproteins. It is a major component of cell membranes (with the exception of the mitochondrial membrane) and is present in the cytoplasm in the esterified form with fatty acids.^{31,32,38}

Phospholipids, cholesterol and sphingolipids are a structural and functional building material for the cell and intracytoplasmic membranes. Their presence determines the flexibility, fluidity and permeability of these membranes. It should be emphasized that phospholipids may play an important role as second messengers and in the processes of salivation.³⁹ This group includes inositol phospholipids. Neurotransmitters, such as acetylcholine, norepinephrine and substance P, connecting with their membrane receptors, activate G proteins, in particular protein GP(q). This protein activates phospholipase C, which hydrolyzes membrane phosphoinositol to form

phosphatidylinositol-4,5-diphosphate (IP2). Phospholipase C phosphorylates cellular proteins that stimulate the processes of gene expression and cell proliferation, as well as activate membrane channels in the cell. It also increases the synthesis and secretion of proteins by the follicle cells and ducts of the salivary glands. IP3 is released from the membrane into the cytoplasm, where it binds to specific receptors in the membrane of the endoplasmic reticulum, which opens the channels for calcium ions and initiates the production of initial saliva.^{6,10}

Salivary lipids in health

Lipids in saliva obtained from the major salivary glands were first detected by Doubleday in 1909.⁴⁰ During subsequent years, a quantitative analysis of the lipids in saliva began, focusing on particular classes of lipids.^{41–45} Later precise quantitative analyses of lipids focused not only on the major groups of lipids, but also on fatty acids constituting individual groups of lipid. The data on the total lipid concentration in stimulated submandibular and parotid saliva is controversial, since they vary from 0.91 mg/100 mL to 9.52 mg/100 mL and from 0.21 mg/100 mL to 9.24 mg/100 mL, respectively.^{46,47} However, early qualitative analyses of the lipids in saliva are similar in all available works in this field. Larsson et al. and Slomiany et al. demonstrated that in parotid and submandibular saliva more than 99% and 98% of lipids, respectively, were non-polar.^{46,47} The neutral lipids were represented by cholesterol and its esters, free fatty acids and tri-, di- as well as monoglycerides, which was confirmed by subsequent studies by Tomita et al. and Brassler et al.^{48,49} Brassler et al. also identified other neutral lipids, namely squalene and wax esters, and they found that salivary lipids were similar to the profile of skin surface lipids. The only polar lipids identified by Larssen et al. were phosphatidylcholine, phosphatidylethanolamine and sulphatide, present in both parotid and submandibular fluid; these findings were in agreement with Slomiany et al.^{46,50} In another work, Slomiany et al. demonstrated that parotid and submandibular saliva also contain glycolipids: glyceroglycolipids and glycosphingolipids.⁵¹ The latter were represented by galactosyl ceramide, glucosylceramide and lactosylceramide, and their content varied from 0.5% to 2% of the total salivary lipids. Neutral glyceroglycolipids accounted for approx. 60% of the total glyceroglycolipids, where hexa- and octaglycosylglyceroglycolipids predominated. Sulfated compounds were represented by tri- and tetraglycosylglycolipids.⁵¹ The non-esterified (so-called free) salivary fatty acids – primarily palmitic acid – were the most abundant, followed by stearic, oleic and linoleic acids. Other non-esterified fatty acids were present at concentrations of less than 1 μ M.⁵² Tri-, di-, and monoglycerides were composed mainly of palmitic and

stearic acid, while cholesteryl esters were characterized by the presence of large amounts of residues of palmitic, stearic and oleic acids. Salivary phospholipids included both saturated and unsaturated fatty acids ranging in size from 12 to 26 carbon atoms, wherein palmitic, stearic, oleic and erucic acids were present in the largest quantities.⁴⁷ The lipid core of the glyceroglycolipids consisted mainly of glyceryl monodocosyl, monoheneicosyl and monoheicosyl alkyl ethers as well as palmitic, stearic and erucic fatty acids.⁵³

While the content and the composition of parotid saliva lipids do not differ significantly from those of submandibular saliva, and stimulation does not significantly change the fatty acid content or the lipid profile of saliva, considerable differences have been found in minor salivary gland lipids.⁴⁵ Slomiany et al. showed that 32.4% of the total lipids were neutral lipids, 44.6% were glycolipids and 23% phospholipids. The total amount of lipids in labial saliva was estimated to be 423.8 µg/mL of saliva.⁵⁴ Of the total neutral lipids, 43.8% was comprised of free fatty acids, 26.9% was cholesteryl esters, 15.4% triglycerides, 11.6% cholesterol and approx. 3% was made up of mono- and diglycerides. The free fatty acids, cholesteryl esters and triglycerides mainly included palmitic, oleic and erucic acids. Glycolipids were represented by glyceroglycolipids and glycosphingolipids. Glycosphingolipids were represented by glucosylceramide and lactosylceramide; their content in saliva ranged from 0.5% to 1.3% of the total lipids. Sulphated glyceroglycolipids, amounting to 25.4% of the total glyceroglycolipids, were primarily tri- and tetraglucosyl compounds. Neutral glyceroglycolipids were demonstrated to be mono-, di-, tetra-, hexa- and octaglucosyl glyceroglucolipids; the last two were predominant, and accounted for 61.6% of the total glyceroglucolipids. The glycolipids were rich in oleic (30.1%) and erucic (19%) acids. Oleic acids (38.9%) were the main lipid component of the phospholipids, which represented 23% of the total labial lipids, (mainly phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine).

The total labial salivary lipids are 4–5 times more numerous than parotid and submandibular salivary lipids, and show a higher percentage of phospholipids and glycolipids. One possibility is that these differences may reflect the different processes in which serous and mucous cells secrete their products, which was explained by Tandler and Poulsen.⁵⁵ They suggested that mucous cells of the labial salivary glands secrete their products in a partly apocrine manner in which the release of the contents of the secretory vesicles is accompanied by the loss of part of the cell membrane. Therefore, the discharge of such cells contains lipids as part of the cell membrane (i.e. phospholipids and sphingolipids). It is also possible that the glycoproteins synthesized by cells of the mucous labial gland may combine with lipids of the cell membrane, and these cells are then secreted into the saliva.

On the other hand, the observed differences could be due to the interaction between lipids and proteins suspended in then aqueous solution of saliva. The relationship between salivary lipids and the protein components of saliva and the extent of interaction between them were partly demonstrated by Slomiany et al., who measured the distribution of lipids in the fractions of parotid and submandibular saliva following Bio-Gel A-50 column chromatography.⁵⁶ Over 50% of the submandibular salivary lipids, in particular glycolipids, free fatty acids, phospholipids and cholesterol, were detected in the fraction of saliva rich in mucins. In the parotid saliva, free fatty acids were detected in the fraction deprived of carbohydrates, whereas cholesteryl esters and phospholipids were associated with the carbohydrate-abundant fraction.

The physical state of the lipids present in saliva is unknown. Since lipids are practically insoluble in water, they must be complexed with the carrier in order to exist in the saliva. To date, the existence of 2 types of interactions between proteins and lipids has been demonstrated, i.e. hydrophobic and covalent interactions. Hydrophobically bound lipids are represented by neutral lipids, glycolipids and phospholipids; the only covalently attached lipids are fatty acids.^{57,58} In a hydrophobic amino acid, side chains participate in the hydrophobic binding. These side chains are located on the protein surface, coming in contact with the hydrophobic parts of the lipids. The covalent bond is created, however, by the commonality of one or more pairs of electron-binding atoms.³¹ It has been shown that in the submandibular glands most hydrophobic interactions with lipids are combinations with MUC5B (50%) and MUC7 (30%). These bonds are very strong and resistant to urea and cesium salts.^{59,60} Slomiany et al. thought that lipids produced in the parotid glands exist as lipoproteins, and the main glycoprotein taking part in the interactions between proteins and lipids may be a proline-rich glycoprotein (PRG).⁴⁷

The presence of such lipid carriers was recently demonstrated in the saliva of healthy subjects that showed the presence of apolipoprotein B.⁶¹ However, little is known about the fragments of glycoproteins, where hydrophobic interactions with the lipids present in saliva occur. Salivary mucins contain highly glycosylated regions and regions devoid of carbohydrate moiety.¹¹ The removal of non-glycosylated regions of mucins was associated with a decrease in the phospholipid content as well as an increase in the number of glycolipids (the content of neutral lipids did not change significantly). These observations suggest that phospholipids may interact hydrophobically with non-glycosylated glycoproteins, while glycolipids bind to the glycosylated parts of mucins.^{59,60}

Fatty acids are combined through an ester linkage with non-glycosylated regions of salivary glycoproteins, mainly large- and low-molecular mucins. In this bind-

ing, hydroxyl groups of serine, threonine and tyrosine may be involved.^{62,63} Most frequently, covalent combinations occur: palmitic, stearic, erucic and oleic acids.^{64,65} It has been shown that the attachment of a fatty acid takes place during the biosynthesis of salivary mucin in mucosal cells and occurs at the end of the processing of oligosaccharide chains, just prior to the formation of polymeric high-molecular-weight mucin. In addition, the connection of a fatty acid is catalyzed by a specific fatty acyl transferase and takes place in the region of a non-glycosylated protein chain; however, fatty acids are attached as hydroxy esters rather than thiol esters. Fatty acyl transferase activity was demonstrated not only in the mucus-secreting submandibular, sublingual and minor salivary glands, but also in the serous parotid glands. Because the major proteins synthesized by parotid glands are rich in glycoproteins, it is highly likely that PRG is acylated with fatty acids.^{62,63}

Three sources of lipids in human saliva are considered: serum component transudation, exfoliative cells and salivary gland secretion.^{66,67} It has been demonstrated that saliva, serum and cell membrane do not differ significantly with respect to neutral lipids and phospholipids, but they differ markedly with respect to glycolipids. The glycolipids occurring in serum and cell membranes are comprised mainly of glycosphingolipids, while mainly glyceroglycolipids are encountered in saliva.^{51,68} Moreover, given the fact that serum contains 50–60 times more lipids than saliva, neutral lipids and phospholipids could originate mainly from serum, as has been demonstrated for cholesterol. Salivary cholesterol accounts for 15% of the neutral lipids in saliva, and men show more salivary cholesterol than women, both of which are true of serum; moreover, a positive correlation between cholesterol levels in saliva and in serum suggests that a high percentage of the salivary cholesterol originates from serum, which is in agreement with Slomiany et al.^{37,47} A thorough analysis of the contribution of serum and membrane lipids other than cholesterol demonstrates that only a small amount (less than 2%) is plasma-born or is a result of cell exfoliation.⁴⁵ Several pieces of evidence point to the fact that salivary lipids mostly originate from the salivary glands. It has been shown that the biosynthesis of triglycerides, phospholipids, sulfated lipids and glyceroglycolipids takes place in the salivary glands.^{69,70} Salivary glands contain 2 different sulphonyl transferases, carrying sulfate groups on the sugar chain of glycolipids. The 1st enzyme, present in the microsomal fraction, is transferred by the sulfate groups on the sugar chain of glyceroglycolipids, which are the main glycolipids of human saliva. The 2nd enzyme is present in the cytoplasmic fraction and is responsible for the sulfation of sugar chains of sphingolipids, which represent the main ingredients of the plasma membrane. It is therefore apparent that salivary glands synthesize both secretory and structural lipids.

Salivary lipids and systemic diseases

There is little data on the nature and content of salivary lipids in systemic diseases. Cystic fibrosis is an innate genetically determined human disease involving impaired secretion by the exocrine glands, including the salivary glands. So far, numerous studies have been published on disturbances of the composition and secretion of salivary proteins and glycoproteins in the course of this disease; however, only Slomiany et al. have published a qualitative and quantitative study on neutral lipids, phospholipids and glycolipids in the submandibular saliva of young patients with cystic fibrosis.^{50,71–74} They showed that saliva from cystic fibrosis patients differs from the saliva from healthy controls with respect to the lipid content and composition. Submandibular saliva from cystic fibrosis patients contained 66% more lipids than the saliva of the healthy controls. The same neutral lipid composition was found in the saliva of healthy individuals and in the patients with cystic fibrosis, but their percentages were significantly different. The saliva of the cystic fibrosis patients contained significantly more fatty acids (54%), triglycerides (35%) and cholesterol (42%) than the saliva of the healthy subjects. The phospholipid content in the saliva of the cystic fibrosis patients was twice as high as in the saliva of healthy individuals, with no differences in the proportion of individual phospholipid classes in the 2 types of saliva samples. In the cystic fibrosis patients' saliva the presence of 0.2–0.5% glycosphingolipids was demonstrated, in addition to the glycerolipids occurring in the healthy controls' saliva. These patients' saliva contained significantly higher concentrations of di- and octaglycosyl glyceroglycolipids, while the saliva of the healthy individuals presented significantly higher levels of mono- and hexaglycosyl glyceroglycolipids. The acidic tetraglycosyl glyceroglycolipid constituted 80% of the sulfated glyceroglycolipids in the healthy individuals' saliva and 59% of the sulfated glyceroglycolipids in the cystic fibrosis patients' submandibular saliva. No significant differences were observed in the fatty acid composition of the lipid fraction of normal and cystic fibrosis saliva. Other studies by A. Slomiany et al. and B.L. Slomiany et al. demonstrated, however, that submandibular saliva of cystic fibrosis patients was characterized by significantly elevated levels of covalently-bound fatty acids.^{57,75} Those authors hypothesized that the elevated levels of neutral lipids in the cystic fibrosis saliva were the result of increased transition of lipids from the blood, since lipids are known to be increased in the serum of cystic fibrosis patients.⁷⁶ Another theory was that the increase in neutral lipid levels may be a result of increased synthesis and secretion of salivary proteolipids, since it has been shown that other salivary proteins are also elevated.⁷² Elevated concentrations of

Table 1. An overview of major studies about salivary lipids in health and disease

Aim/study design	Results/endpoints	References
The lipid composition of parotid saliva in young men and changes caused by stimulation	There were no significant differences in the composition of neutral and polar lipids in the parotid saliva as a result of stimulation.	45
The lipid content of parotid and submandibular saliva from caries-susceptible and caries-resistant patients	The levels of phospholipids and neutral lipids (i.e. free fatty acids, triglycerides and cholesterol esters) were significantly lower in the parotid and submandibular saliva of caries-resistant patients.	91
The relationship between high-molecular-weight mucus glycoprotein and covalently bound lipids from stimulated submandibular saliva of caries-susceptible and caries-resistant patients with blood type A	The submandibular saliva from caries-susceptible and caries-resistant patients are characterized by a different quantitative composition and different physiochemical characteristics of salivary mucus glycoproteins and covalently bound lipids.	51
The relationship between salivary lipids and proteins and glycoproteins in human saliva	Parotid and submandibular lipids show specific associations with the proteins and glycoproteins in human saliva.	56
The lipid composition of stimulated human labial gland saliva	Of all the salivary lipids, 44.6% were glycolipids, 32.4% neutral lipids, 23% phospholipids.	50
The lipid composition of dental plaque from caries-susceptible and caries-resistant patients	Plaque maturation is associated with elevated levels of neutral lipids and phospholipids, as well as reduced glycolipid content.	92
The quality and quantity of neutral lipids in parotid, submandibular and whole stimulated human saliva	Cholesteryl esters, cholesterol, triglycerides, diglycerides, monoglycerides and free fatty acids accounted for 96-99% of the total salivary lipids.	46
The lipid and protein content in stimulated parotid and whole saliva from caries-susceptible and caries-resistant women	The lipid and protein content was significantly elevated in the parotid saliva from caries-susceptible patients.	48

phospholipids and the presence of glycosphingolipids in cystic fibrosis patients' submandibular saliva may be due to the presence of membrane lipids in an aqueous solution of saliva, and may also be the evidence of the existence of salivary abnormalities associated with the secretion of important proteins by cystic fibrosis patients.

Intracellular lipid accumulation in the salivary glands of diabetic rats has been observed, but it was greater in the parotid than in the submandibular or sublingual glands.^{77,78} Morris et al. were the first to investigate the fatty acid profile, the time course of fatty acid accumulation and the effect of insulin treatment on salivary gland lipids in induced diabetes in a rat model.⁷⁷ They observed that stearic acid (C18:0) and linoleic acid (C18:2w6) showed a significant increase in all the salivary glands over 4 weeks after the streptozotocin injection to induce diabetes. In contrast, oleic acid (C18:1w9) was significantly down-regulated in the parotid gland after 3 and 4 weeks, which was in agreement with Mahay et al.⁷⁹ Decreases in arachidonic acid were significant in the submandibular gland in 3 weeks and in the parotid gland in 2 weeks of experimental diabetes. Insulin treatment decreased the amount of stearic and linoleic acids in the submandibular and parotid glands as compared to the controls. The authors concluded that the decreases in C18:1w9 and C20:4w6 may be a result of inhibition of desaturase enzymes, which are known to be stimulated by insulin.⁸⁰ A lack of insulin results in the accumulation of saturated (C18:0) and less unsaturated fatty acids (C18:2w6) as well as an increase in C18:2w6/C18:1w9 ratios. The latter is probably a result of the fact that the amount of linoleic acid is constant,

because it comes from the diet, but cannot be desaturated and elongated, while oleic acid cannot be produced from stearic acid. The authors also posited that the accumulation of saturated and less unsaturated fatty acids may be due to a decrease in the consumption of lipids in the synthesis of cell membranes of secretory vesicles. The accumulation of saturated and less unsaturated fatty acids alter membrane structure and fluidity, as well as membrane enzyme function and the secretory function mechanism.^{79,81} Treatment with insulin showed that the effects of diabetes on salivary lipids are rapid and totally reversible.

Salivary glands are a major target organ of inflammation in the course of Sjögren's syndrome (SS).^{82,83} An immunohistological analysis of the salivary glands of SS patients has disclosed lymphocytic infiltration with a predominance of T lymphocytes, especially CD4 + cells (the ratio between CD4 + and CD8 = 2:1), as well as a reduced number of B lymphocytes and macrophages, which may be the immediate cause of destruction and dysfunction of the salivary glands.⁸⁴ Changes in the total amount of saliva secreted are accompanied by changes in the quality of the saliva; however, most researchers have analyzed the protein component of saliva in the course of SS, and data on lipids in the saliva of SS patients is scarce.⁸⁵⁻⁸⁸ Parotid salivary lipid analyses have demonstrated that patients with SS had twice as many total lipids as healthy controls, 4 times as many glycolipids and 20 times more phospholipids. Neutral lipids in SS patients contain a higher percentage of mono-, di- and triacylglycerols and cholesterol than healthy controls, and a lower percentage of cholester-

ol esters. A phospholipids profile showed a significant increase in phosphatidylcholine in SS, while a profile of glycolipids revealed an increase in sphingomyelin.⁸⁹ More recently, Tishler et al. identified eicosanoids in the saliva of SS patients.⁹⁰ They demonstrated a significant increase of prostaglandin E₂ and thromboxane E₂ in mixed saliva from SS individuals as compared to healthy controls and xerostomic patients. They stated that elevated concentrations of eicosanoids in the saliva of SS patients could be a good marker of an inflammatory process in the salivary glands in the course of this disease and could help to identify patients suffering from xerostomia, which may result from other diseases.

The results of the main studies about human salivary lipids in health and disease are summarized in Table 1.

Summary

Salivary lipids are among the most essential cellular components of human saliva. In the oral environment they determine the flexibility, fluidity and permeability of cellular membranes and participate in intercellular transport and the signal transduction pathways between the salivary glands and other tissues. Although the qualitative and quantitative content of salivary lipids can change in various pathological states and diseases (e.g. cystic fibrosis, diabetes and Sjögren's syndrome), little is known about the role and composition of salivary lipids, and about their interaction with other important ingredients of human saliva, including proteins, glycoproteins and salivary mucins. Only accurate knowledge of the qualitative and quantitative composition of lipids and their role in the oral cavity will make it possible to understand better the pathological processes occurring in the oral cavity and in the parotid and submandibular salivary glands. There is, therefore, a need for further research on the role of salivary lipids in both health and disease.

References

- Amano O, Mizobe K, Bando Y, Sakiyama K. Anatomy and histology of rodent and human major salivary glands: An overview of the Japan Salivary Gland Society – sponsored workshop. *Acta Histochem Cytochem*. 2012;45:241–250.
- Kontis TC, Johns ME. Anatomy and physiology for the salivary glands. In: Bailey BJ. *Head & neck surgery – otolaryngology*. Philadelphia, PA; 2001.
- Sonesson M, Eliasson L, Matsson L. Minor salivary gland secretion in children and adults. *Arch Oral Biol*. 2003;48:535–539.
- Eliasson L, Carlén A. An update on minor salivary gland secretions. *Eur J Oral Sci*. 2010;118:435–442.
- Malamud D. Salivary diagnostics: The future is now. *Am J Dent Assoc*. 2006;137:284–286.
- Turner RJ, Sugiya H. Understanding salivary fluid and protein secretion. *Oral Dis*. 2002;8:3–11.
- Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. *J Am Dent Assoc*. 2008;139:185–245.
- Zalewska A, Knaś M, Waszkiewicz N, et al. Salivary antioxidants in patients with systemic sclerosis. *J Oral Pathol Med*. 2014;43:61–68.
- Zalewska A, Knaś M, Żendzian-Piotrowska M, et al. Antioxidant profile of salivary glands in high fat diet-induced insulin resistance rats. *Oral Dis*. 2014;20:560–566.
- Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*. 2009;34:486–496.
- Zalewska A, Zwierz K, Żółkowski K, Gindziński A. Structure and biosynthesis of human salivary mucins. *Acta Biochim Polon*. 2000;47:1067–1079.
- Sonesson M, Wickström C, Kinnby B, Ericson D, Matsson L. Mucins MUC5B and MUC7 in minor salivary gland secretion of children and adults. *Arch Oral Biol*. 2008;53:523–527.
- Strechfus CF. *Advances in salivary diagnostics*. Springer-Verlag Berlin Heidelberg; 2015.
- Sonesson M, Ericson D, Kinnby B, Wickström C. Glycoprotein 340 and sialic acid in minor-gland and whole saliva of children, adolescents, and adults. *Eur J Oral Sci*. 2011;119:435–440.
- Sonesson M, Hamberg K, Wallengren ML, Matsson L, Ericson D. Salivary IgA in minor-gland saliva of children, adolescents and young adults. *Eur J Oral Sci*. 2011;119:15–20.
- Dawes C, Pedersen AM, Villa A, et al. The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. *Arch Oral Biol*. 2015;60:863–874.
- Hopcraft MS, Tan C. Xerostomia: An update for clinicians. *Aust Dent J*. 2010;55:238–244.
- Jankowska A, Waszkiel D, Kobus A, Zwierz K. Ślina jako główny składnik ekosystemu jamy ustnej. Część II. Mechanizmy odpornościowe. *Wiad Lek*. 2007;60:253–257.
- Jankowska A, Waszkiel D, Kowalczyk A. Ślina jako główny składnik ekosystemu jamy ustnej. Część I. Mechanizm wydzielania i funkcje. *Wiad Lek*. 2007;60:148–154.
- Fabian TK, Fejérdy P, Csermely P. Salivary genomics, transcriptomics and proteomics: The emerging concept of the oral ecosystem and their use in the early diagnosis of cancer and other diseases. *Curr Genomics*. 2008;9:11–21.
- Fabian TK, Hermann P, Beck A, Fejérdy P, Fabian G. Salivary defense proteins: Their network and role in innate and acquired oral immunity. *Int J Mol Sci*. 2012;13:4295–4320.
- Fejerskov O, Kidd E. Wydzielanie i skład śliny. In: *Próchnica zębów. Choroba próchnicowa i postępowanie kliniczne*. Wrocław; 2006.
- Mattes RD. Oral detection of short-, medium-, and long-chain free fatty acids in humans. *Chem Senses*. 2009;34:145–150.
- Higashi T, Shibayama Y, Fuji M, Shimada K. Liquid chromatography-tandem mass spectrometric method for the determination of salivary 25-hydroxyvitamin D₃, a noninvasive tool for the assessment of vitamin D status. *Anal Bioanal Chem*. 2008;391:229–238.
- Izawa S, Saito K, Shirotaki K, Sugaya N, Nomura S. Effects of prolonged stress on salivary cortisol and dehydroepiandrosterone: a study of two-week teaching practice. *Psychoneuroendocrinology*. 2012;37:852–858.
- Ridgway N, McLeod R. *Biochemistry of lipids, lipoproteins and membranes*. Amsterdam; 2016.
- Christie WW. *Gas chromatography and lipids: A practical guide*. Dundee: The Oily Press; 1989.
- Fahy E, Subramaniam S, Brown HA, et al. A comprehensive classification system for lipids. *J Lipid Res*. 2005;46:839–861.
- Fahy E, Subramaniam S, Murphy JRC, et al. Update of the LIPID MAPS comprehensive classification system for lipids. *J Lipid Res*. 2009;50:59–514.
- Calder PC. Functional roles of fatty acids and their effects on human health. *J Parenter Enteral Nutr*. 2015;39:185–325.
- Bańkowski E. *Biochemia: Podręcznik dla studentów uczelni medycznych*. Wrocław; 2009.
- Żak I. Lipidy i pochodne. In: *Chemia Medyczna*. Katowice; 2001.
- Påhlsson P, Spitalnik SE, Spitalnik PF, et al. Characterization of galactosyl glycerolipids in the HT29 human colon carcinoma cell line. *Arch Biochem Biophys*. 1998;396:187–198.
- Di Paola M, Cocco T, Lorusso M. Ceramide interaction with the respiratory chain of heart mitochondria. *Biochemistry*. 2000;39:6660–6668.
- Symons JD, Abel ED. Lipotoxicity contributes to endothelial dysfunction: A focus on the contribution from ceramide. *Rev Endocr Metab Disord*. 2013;14:59–68.

36. Van Brocklyn JR, Williams JB. The control of the balance between ceramide and sphingosine-1-phosphate by sphingosine kinase: Oxidative stress and the seesaw of cell survival and death. *Comp Biochem Physiol.* 2012;163:26–36.
37. Karjalainen S, Sewón E, Soderling E, et al. Salivary cholesterol of healthy adults in relation to serum cholesterol concentration and oral health. *J Dent Res.* 1997;76:1637–1643.
38. Kanazawa A. Sterols in marine invertebrates. *Fish Sci.* 2001;67:997–1007.
39. Mandel ID. The functions of saliva. *J Dent Res.* 1987;66(Spec. Is.):623–627.
40. Doubleday AW. Plodding toward diagnosis by salivary analysis. *Dent Cosmos.* 1909;51:412–421.
41. Dirksen TR. Lipid constituents of whole and parotid saliva. *J Dent Res.* 1963;42:920–924.
42. Krasnow F. Cholesterol and lecithin in teeth and saliva. *J Dent Res.* 1934;14:226–227.
43. Krasnow F. Physiological significance of phospholipid in human saliva. *J Dent Res.* 1945;24:319–326.
44. Mandel ID, Einstein A. Lipids in human salivary secretions and salivary calculus. *Arch Oral Biol.* 1969;14:231–233.
45. Rabinowitz JL, Shannon IL. Lipid changes in human male parotid saliva by stimulation. *Arch Oral Biol.* 1975;20:403–406.
46. Larsson B, Olivecrona G, Ericson T. Lipids in human saliva. *Arch Oral Biol.* 1996;41:105–110.
47. Slomiany BL, Murty VL, Slomiany A. Salivary lipids in health and disease. *Prog Lipid Res.* 1985;24:311–324.
48. Tomita Y, Miyake N, Yamanaka S. Lipids in human parotid saliva with regard to caries experience. *J Oleo Sci.* 2008;57:115–121.
49. Brassler AJ, Barwacz CA, Dawson DV, Brogden KA, Drake DR, Wertz PW. Presence of wax esters and squalene in human saliva. *Arch Oral Biol.* 2011;56:588–591.
50. Slomiany BL, Slomiany A, Mandel ID. Lipid composition of human submandibular gland secretion from light and heavy calculus formers. *Arch Oral Biol.* 1980;25:749–751.
51. Slomiany BL, Murty VL, Mandel ID, Zalesna G, Slomiany A. Physicochemical characteristics of mucus glycoproteins and lipids of the human oral mucosal mucus coat in relation to caries susceptibility. *Arch Oral Biol.* 1989;34:229–237.
52. Kulkarni BV, Wood KV, Mattes RD. Quantitative and qualitative analyses of human salivary NEFA with gas-chromatography and mass spectrometry. *Front Physiol.* 2012;3:1–6.
53. Slomiany BL, Slomiany A, Glass GB. Glyceroglucolipids of the Human Saliva. *Eur J Biochem.* 1978;84:53–59.
54. Slomiany BL, Zdebska E, Murty VL, Slomiany A, Petropoulou K, Mandel ID. Lipid composition of human labial salivary gland secretions. *Arch Oral Biol.* 1983;28:711–714.
55. Tandler B, Poulsen JH. Fusion of the envelope of mucous droplets with the luminal plasma membrane in acinar cells of the cat submandibular gland. *J Cell Biol.* 1976;68:775–781.
56. Slomiany BL, Witas H, Murty VL, Slomiany A, Mandel ID. Association of lipids with proteins and glycoproteins in human saliva. *J Dent Res.* 1983;62:24–27.
57. Slomiany BL, Murty VL, Slomiany A, Zielenski J, Mandel ID. Mucus glycoprotein of human saliva: Differences in the associated and covalently bound lipids with caries. *Biochim Biophys Acta.* 1986;882:18–28.
58. Slomiany BL, Murty VL, Takagi A, Tsukada H, Kosmala M, Slomiany A. Fatty acid acylation of salivary mucin in rat submandibular glands. *Arch Biochem Biophys.* 1985;242:402–410.
59. Witas H, Slomiany BL, Zdebska E, Kojima K, Liau YH, Slomiany A. Lipids associated with dog gastric mucus glycoprotein. *J App Biochem.* 1983;5:16–24.
60. Witas H, Sarosiek J, Aono M, Murty VL, Slomiany A, Slomiany BL. Lipids associated with rat small-intestinal mucus glycoprotein. *Carbohydr Res.* 1983;120:67–76.
61. Defagó MD, Garcés NR. New contribution about lipid carriers in human saliva. *Biozell.* 2008;32:105.
62. Liau YH, Murty VL, Gwozdziński K, Slomiany A, Slomiany BL. In vitro fatty acid acylation of mucus glycoprotein from sublingual salivary glands. *Biochim Biophys Acta.* 1986;880:108–116.
63. Slomiany BL, Liau YH, Carter SR, Zielenski J, Slomiany A. Enzymic acylation of mucus glycoprotein with palmitic acid in rat submandibular salivary gland. *Arch Oral Biol.* 1986;31:463–468.
64. Slomiany A, Jozwiak Z, Takagi A, Slomiany BL. The role of covalently bound fatty acids in the degradation of human gastric mucus glycoprotein. *Arch Biochem Biophys.* 1984;229:560–567.
65. Slomiany A, Witas H, Aono M, Slomiany BL. Covalently linked fatty acids in gastric mucus glycoprotein of cystic fibrosis patients. *J Biol Chem.* 1983;258:8535–8538.
66. Defagó MD, Valentich MA, Actis AB. Lipid characterization of human saliva. *J Calif Dent Assoc.* 2011;39:874–880.
67. Kensch A, Reich M, Kümmerer K, Hanning M, Hanning C. Lipids in preventive dentistry. *Clin Oral Investiq.* 2013;17:669–685.
68. Cullis PR, Hope MJ. Physical properties and functional roles of lipids in membranes. In: Vance DE, Vance JE, eds. *Biochemistry of lipids and membranes*; 1985.
69. Galanti N, Baserga R. Glycolipid synthesis in the early prereplicative phase of isoproterenol-stimulated salivary glands of mice. *J Biol Chem.* 1971;246:6814–6821.
70. Pritchard ET. Sulpholipid formation in rat submandibular gland. *Biochem J.* 1977;166:41–144.
71. Carnoy C, Ramphal R, Scharfman A, et al. Altered carbohydrate composition of salivary mucins from patients with cystic fibrosis and the adhesion of *Pseudomonas aeruginosa*. *Am J Respir Cell Mol Biol.* 1993;3:323–340.
72. Livnat G, Bentur L, Kuzmisky E, Nagler RM. Salivary profile and oxidative stress in children and adolescents with cystic fibrosis. *J Oral Pathol Med.* 2010;39:16–21.
73. Nie S, Benito-Peña E, Zhang H, Wu Y, Walt DR. Multiplexed salivary protein profiling for patients with respiratory diseases using fiber-optic bundles and fluorescent antibody-based microarrays. *Anal Chem.* 2013;85:9272–9280.
74. Shori DK, Genter T, Hansen J, et al. Altered sialyl- and fucosyl-linkage on mucins in cystic fibrosis patients promotes formation of the sialyl-Lewis X determinant on salivary MUC-5B and MUC-7. *Pflugers Arch.* 2001;443:S55–61.
75. Slomiany BL, Jozwiak Z, Slomiany A, Takagi A, Mandel ID. Occurrence of fatty acids covalently bound to protein in human submandibular saliva IRCS. *Med Sci.* 1983;11:765.
76. Davis PB, di Sant'Agnese PA. A review. Cystic fibrosis at Forty – Quo Vadis? *Pediatr Res.* 1980;83–87.
77. Morris PA, Prout RES, Proctor GB, Garrett JR, Anderson LC. Lipid analysis of the major salivary glands in streptozotocin-diabetic rats and the effects of insulin treatment. *Arch Oral Biol.* 1992;37:489–494.
78. Anderson LC, Garrett JR. Lipid accumulation in the major salivary glands of streptozotocin-diabetic rats. *Arch Oral Biol.* 1986;31:469–475.
79. Mahay S, Adeghate E, Lindley MZ, Rolph CE, Singh J. Streptozotocin-induced type 1 diabetes mellitus alters the morphology, secretory function and acyl lipid content in the isolated rat parotid salivary gland. *Moll Cell Biochem.* 2004;261:175–181.
80. Sprecher H. Biochemistry of essential fatty acids. *Prog Lipid Res.* 1981;20:13–22.
81. Christon R, Fernandez Y, Cambon-Gros C, et al. The effect of dietary essential fatty acid deficiency on the composition and properties of the liver microsomal membrane of rats. *J Nutr.* 1988;118:1311–1318.
82. Bierć J, Borzym K, Waszkiel D, Daniszewska I, Zalewska A. Patogeneza zespołu Sjögrena. *Wiad Lek.* 2014;65:519–526.
83. Carr AJ, Ng WF, Figueiredo F, Macleod RI, Greenwood M, Staines K. Sjögren's syndrome - An update for dental practitioners. *Br Dent J.* 2012;213:353–357.
84. Nikolov NP, Illei GG. Pathogenesis of Sjögren's syndrome. *Curr Opin Rheumatol.* 2009;21:465–470.
85. Delaleu N, Jonsson R, Koller MM. Sjögren's syndrome. *Eur J Oral Sci.* 2005;113:101–113.
86. Enger TB, Palm Ø, Garen T, Sandvik L, Jensen JL. Oral distress in primary Sjögren's syndrome: Implications for health-related quality of life. *Eur J Oral Sci.* 2011;119:474–480.
87. Fox RI. Sjögren's syndrome. *Lancet.* 2005;366:321–331.
88. Fox RI. The salivary gland epithelial cell in Sjögren's syndrome: What are the steps involved in wounding or killing their secretory function? *J Rheumatol.* 2012;39:1117–1119.

89. Slomiany BL, Kosmala M, Nadziejko C, et al. Lipid composition and viscosity of parotid saliva in Sjögren syndrome in man. *Arch Oral Biol.* 1986;31:699–702.
90. Tishler M, Yaron I, Raz A, Meyer FA, Yaron M. Salivary eicosanoid concentration in patients with Sjögren's syndrome. *Ann Rheum Dis.* 1996;55:202–204.
91. Slomiany BL, Murty VL, Aono M, Slomiany A, Mandel ID. Lipid composition of human parotid and submandibular saliva from caries-resistant and caries-susceptible adults. *Arch Oral Biol.* 1982;27:803–808.
92. Murty VL, Slomiany BL, Laszewicz W, Slomiany A, Petropoulou K, Mandel ID. Lipids of developing dental plaque in caries-resistant and caries-susceptible adult people. *Arch Oral Biol.* 1985;30:171–175.