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OBESITY (J MCCAFFERY, SECTION EDITOR)

Salivary Amylase: Digestion and Metabolic Syndrome

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Abstract Salivary amylase is a glucose-polymer cleavage enzyme that is produced by the salivary glands. It comprises a small portion of the total amylase excreted, which is mostly made by the pancreas. Amylases digest starch into smaller molecules, ultimately yielding maltose, which in turn is cleaved into two glucose molecules by maltase. Starch comprises a significant portion of the typical human diet for most nationalities. Given that salivary amylase is such a small portion of total amylase, it is unclear why it exists and whether it conveys an evolutionary advantage when ingesting starch. This review will consider the impact of salivary amylase on oral perception, nutrient signaling, anticipatory metabolic reflexes, blood sugar, and its clinical implications for preventing metabolic syndrome and obesity.

Keywords Salivary amylase · Starch digestion · AMY1 copy number variation · Glucose homeostasis · Insulin · Metabolic syndrome

Introduction

Saliva has many crucial roles in promoting health, including protecting the oral cavity and facilitating eating. Within the mouth, saliva hydrates mucosal tissues, removes cell and food debris, buffers oral pH, lubricates the oral cavity aiding mastication and preventing dental wear, forms food boli to assist swallowing, protects against teeth demineralization, has antimicrobial activity and prevents infections, and closes wounds while stimulating healing [1, 2]. Saliva also plays essential roles in food perception and digestion. The exact mechanisms of digestion remain unclear. For taste, the physical and compositional characteristics of saliva facilitate perception. For example, the fact that saliva is an aqueous liquid makes it an ideal vehicle for carrying taste stimuli and nutrients to the taste receptors [3], which are widely distributed on the tongue, soft palate, and pharynx. Unstimulated saliva also presents low levels of taste stimuli, such as salts and glucose, in comparison to plasma, which enables low detection threshold [1, 4]. Taste perception guides dietary choices as well as influences physiological processes pre- and post-absorptively [5, 6]. The anticipatory phase of digestion is labeled the "cephalic phase responses" and serves to prime the body to metabolize ingested nutrients efficiently, making it an important step in food digestion and the prevention of dysglycemia and dyslipidemia.

Additionally, saliva contains a large number of proteins involved in lipase, peptidase, and hydrolase activities. When comparing the saliva and plasma proteomes, it is clear that the distributions of the salivary proteins are geared toward metabolic and catabolic processes. This indicates that saliva has a major physiologic role in food digestion [7]. The most abundant protein in human saliva is the digestive enzyme α-amylase [8]. This enzyme cleaves large starch molecules into dextrin and subsequently into smaller maltooligosaccharides (MOS) containing α-D-(1,4) linkages, isomaltooligosaccharides (IMOS) containing α-D-(1,6) linkages, the trisaccharide maltotriose, and the disaccharide maltose [9]. Glucose will then be generated from maltose via the action

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of disaccharide enzymes, such as maltase. In the human body, amylase is predominantly produced by the salivary glands and the pancreas. Although salivary and pancreatic amylases are similar, they are encoded by different genes (*AMY1* and *AMY2*, respectively) and show different levels of activity against starches of various origins [10].

The physiological significance of salivary amylase is still being uncovered and aspects of it are controversial, for example, its normative secretory function in plasma remains a mystery. Salivary amylase has a relatively short active contact time with starch. Once a food bolus is swallowed and infiltrated with gastric juice, its catabolic activity is mostly stopped by low acidic pH. Some activity remains within particles due to the barrier protection provided by partially digested starch on the outside of the particle [11], but the majority of the starch is digested by the abundant pancreatic amylase, which is released into the duodenal portion of the small intestine. Nevertheless, studies have demonstrated that considerable starch hydrolysis occurs within seconds in the oral cavity, transforming the gelatinous texture of starch into a semi-liquid [12, 13]. This change of texture might itself influence starch digestion, sensory preferences, and starch intake. Additionally, recent studies have also demonstrated that the small MOS amylolytic products can be detected in the oral cavity via the taste system [14]. These findings strongly support a physiological pre-absorptive role of salivary amylase in starch digestion.

In this review, we will discuss the evolutionary forces that drive the existence of salivary amylase, the benefits of generating higher salivary amylase levels, the possible physiological consequences of early oral starch breakdown, and its roles in protecting blood glucose profile and blood insulin, as well as the disease states of metabolic syndrome, diabetes, and obesity.

Evolutionary Perspective on Salivary Amylase's Role in Starch Digestion

Salivary amylase has been detected in saliva of many omnivorous animals and a few herbivores. In contrast, obligate carnivores, such as cats, never have salivary amylase [15]. Animals feeding on unripe fruits, seed, roots, and bulbs, all rich in starch, exhibit higher salivary amylase activity. This supports the notion that a key role of salivary amylase is starch digestion. Human salivary amylase activity is by far the highest among primates. It has been attributed to the fact that *Homo sapiens sapiens* possess multiple copies of the *AMY1* gene, whereas the other primates, including our nearest living relative the chimpanzees, display the normative two copies per cell (one from each parent) [16]. *AMY1* copy number variation (CNV) is not the sole driver of amylase production in animals, since environmental factors such as stress level [17], circadian rhythms [18], and diet [15, 19] significantly contribute to quantitative variation among species and individuals. Nevertheless, a good correlation exists between *AMY1* copy number and the amount and activity of amylase in saliva [13, 20–22]. Each duplicated segment of the *AMY1* gene contains the regulatory sequences necessary for salivary-specific expression [23] and thus can indeed directly influence messenger RNA (mRNA) and protein expression levels. This is not the case of all gene-containing CNVs [24, 25]. Humans can carry anywhere from 2 to 17 *AMY1* gene copies [21, 26, 27]. High *AMY1* copy numbers have been observed in populations in which ancestors consumed diets rich in starch [21]; this suggests a directional selection toward higher salivary amylase for starch digestion. The development of cooking and, more recently, greater access to starchy foods with the advent of agriculture would have favored *AMY1* CNV, as the increased availability of digestible starch would have resulted in salivary and possibly pancreatic amylase levels becoming a limiting factor in starch digestion [28]. It is important to note that even if the pancreatic amylase genes in humans had not undergone such extensive duplication, individual *AMY2* CNV exists and varies from 0 to 4 for *AMY2A* and from 2 to 6 for *AMY2B* [26, 27].

Like many animals, domesticated dogs, a non-obligate carnivore, only express amylase in the pancreas and not in the saliva [29]. Interestingly, wolves, which are obligate carnivores, carry only 2 copies of *AMY2B*, whereas diploid copy numbers in dogs range from 4 to 30; higher CNV is associated with increased pancreatic amylase activity [30••, 31]. This increase in amylase activity via duplication of *AMY2B* is believed to explain why domesticated dogs can thrive on a diet rich in starch, whereas its closest feral relatives cannot. Thus, the same molecular mechanism (duplication) has acted on similar genes in different species exposed to the same dietary pressure (domestication for dogs) [30••].

A parallel evolutionary event can be drawn between rodents and humans. Rodents, like humans, possess salivary amylase. Studies have shown that both species have acquired amylase activity in the saliva independently via the insertion of a foreign retrovirus into the primitive amylase cluster, diverting a pancreatic gene to become a salivary gene [32]. Because this retroviral insertion occurred after the separation of the primate and rodent orders, it implies that some of the elements required for salivary amylase gene expression have evolved independently in the mouse and human genomes. This supports the idea of a very strong evolutionary selection for amylase to be excreted in saliva [33]. Those are striking examples of parallel evolution and strongly confirm the importance of salivary amylase in starch digestion for humans. But the questions remain, "What is this evolutionary pressure to express amylase in saliva?" Or, "What does salivary amylase precisely do for us that conveys such an advantage?" As the enzymatic activities of pancreatic and salivary amylases

are quite similar, and all mammals produce pancreatic amylase, there is no obvious advantage to duplicate this starch digestion mechanism. We attempt to show in the next sections that perhaps an argument for advantage can be made.

Pre-absorptive Role of Salivary Amylase in Starch Digestion

Salivary amylase greatly impacts the textural characteristics of starch. Enzymatic cleavage of starch produces a rapid decrease in glucose-polymer chain length and viscosity after relatively few glycosidic bonds have been cleaved [34]. These changes in viscosity can play a significant role in determining liking and preference for food. The degree to which the viscosity of starch is thinned in the oral cavity could, therefore, be of nutritional importance. Our group showed that individuals with high *AMY1* copy number had a higher salivary amylase activity and reported faster and larger decrease in perceived starch viscosity than individuals with low *AMY1* copy number [13]. Improved palatability of starchy food might have been one way that salivary amylase CNV helped to increase starch consumption during hominid evolution. To this point, people with ancestors who ate a more starch-rich diet carry higher number of AMY1 CNVs [21].

Whereas starch does not have a clear taste to humans, oral detection of starch or its degradation products via specialized taste receptors would be highly beneficial because of its importance in the human diet. Considerable evidence exists that rodents can orally perceive starch degradation products (oligo and polysaccharides) [35–37] and that their detection is independent of the sweet taste receptor T1R2/T1R3 [38, 39]. There is some evidence that people may also perceive glucose polymers as having a distinct taste from that of sweet-tasting sugars. Humans can discriminate the taste of high concentrations of maltose from the taste of glucose or fructose when matched for intensity [40]. More recently, humans have been shown to respond to short MOS in the mouth as not sweet tasting but perceptible [14]. Thus, humans may perceive a weak glucose polymer taste that is a unique quality of taste, distinguishable from sweet.

In addition, a T1R-independent metabolic pathway for monosaccharides has been recently identified in taste receptor cells of mice. It consists of glucose transporters (GLUTs) and a metabolic sensor pathway (sodium glucose cotransporter 1 (SGLT1) and the ATP-gated K^+ channel (K_{ATP})) that serve as metabolic sugar sensors in other tissues, notably the gut and the pancreas, and may be a key step in the physiological differentiation between caloric and non-caloric sweeteners [41]. Thus, caloric sweeteners in rodents would act on two signaling pathways, T1Rs and a metabolic sensor, whereas non-caloric sensors would only act on T1Rs. The metabolic pathway could not explain by itself the gustatory responses to starchy foods, since starch degradation products are not substrates for GLUT or SGLT1 transporters, only glucose is transported, and salivary amylase does not generate glucose. But this necessary step for the metabolic detection of starch via the T1R-independent pathway in the oral cavity has been identified as membrane bound disaccharidase enzymes [42••]. The authors hypothesized that since gustatory and intestinal epithelia share many chemoreceptors and signal transduction pathways (i.e., taste receptors in the gut [43], metabolic sensors in the taste receptors cells [41]) and since the enterocytes of the intestinal epithelium express GLUTs and SGLT1 as well as disaccharide-hydrolyzing enzymes and contain pancreatic amylase [44], the taste cells may also likely express those enzymes. They showed that taste cells express the enzymes maltase-glucoamylase (MGAM), sucrase-isomaltase (SIS), lactase (LCT), and trehalase (TREH), which hydrolyze the disaccharides maltose, sucrose, lactose, and trehalose, respectively, to generate monosaccharides that can be readily detected by GLUTs and SGLT1. They also showed that *AMY1* is expressed at low levels in taste tissue and at high levels in the salivary parotid glands and lingual Von Ebners glands (VEG), confirming previous findings [45]. Thus, all the necessary machinery to elicit taste signals from starch is present in the gustatory tissues. As VEG secrete their contents directly into the trenches of the circumvallate and foliate papillae, VEG produced *AMY1* may be important in generating locally elevated amount of oligosaccharides and disaccharides in close proximity of the taste pore where MGAM, SIS, and GLUTs are localized, eliciting the metabolic signal even with very low amounts of monosaccharides [42••]. It is interesting to note here that Axelsson et al., when comparing wolf and domestic dog genomes, reported evidence for gain-of-function alterations in *AMY2B* gene and also in the MGAM and SGLT1 genes in dogs [30••]. So, carbohydrate cleavage enzymes capable of generating transportable monosaccharides can increase together during evolution.

In addition to conscious taste perception, gustatory activation stimulates physiological responses (cephalic phase responses), such as increased secretion of saliva [46], gastric acid [46, 47], and pancreatic secretions [46, 48]. Such responses prepare the digestive system to metabolize and absorb nutrients [5] and enable better maintenance of plasma nutrient homeostasis [5, 6]. Cephalic phase insulin release (CPIR) is one such pre-absorptive response to eating [49]. Though it is a relatively minor component of total insulin secretion, CPIR has been shown to be an extremely important determinant of overall glucose tolerance [50]. In one study, our group showed that individuals with low salivary amylase activity due to low *AMY1* copy number did not exhibit CPIR in response to starch and consequently had a higher glycemic response. After ingesting a glucose solution, those individuals, however, exhibited CPIR, which indicates that they could do so [51]. Those results suggest that salivary amylase may be important

for enhanced glucose tolerance and individuals with higher *AMY1* copy number better adapted to ingest starch. Recently, Glendinning et al. [52] not only confirmed that rodents possess two taste transduction pathways for sugars but also demonstrated that if the T1R2/T1R3 pathway is required for attraction to sugar, a pathway independent from T1R2/T1R3 mediates sugar-induced CPIR, presumably the T1R independent metabolic pathway involving GLUTs, SGLT1, and KATP and the carbohydrate-digesting enzymes in the case of di- or oligosaccharide detection.

Therefore, starch break-down products released by salivary amylase can be detected in the oral cavity and elicit an early release of insulin, possibly after disaccharidases generate transportable monosaccharides. Via either or both the metabolic taste pathway and the MOS taste pathway, the salivary amylase activity in the oral cavity could improve starch metabolism pre-absorptively. Those pathways need to be confirmed in humans and the association between high salivary amylase activity and starch-induced CPIR confirmed in larger populations.

Post-absorptive Role of Salivary Amylase in Starch Digestion

Large amounts of pancreatic amylase are released into the duodenum via the pancreatic duct to continue the digestion of the incoming starch. The digestive enzymes are produced and transported by acinar cells which are exocrine cells of the pancreas. The second functional component of the pancreas is the endocrine pancreas. The endocrine pancreas is composed of small islands of cells, called the islets of Langerhans. The endocrine cells do not release their secretions into the pancreatic ducts. Rather they release hormones, such as insulin, into the blood to help control blood glucose levels. In a coordinated manner, insulin directly regulates the acinar pancreas via a portal system that conveys islet blood to acinar cells [53]. The acini have insulin receptors, and it has been demonstrated that insulin is necessary for normal acinar cell function. Thus, insulin regulates pancreatic amylase secretion into the duodenum and by extension starch digestion in the guts.

Curiously, amylase may not only serve exocrine functions but also have endocrine functions as well. The presence of amylase in blood was historically attributed to pathological leakage of pancreatic and salivary glands due to inflammation and disease. The serum content of amylase would, therefore, simply reflect the amylase content in the digestive glands: low in the case of insulin deficiency and higher after feeding [54] or after artificial activation of exocrine secretion of the gastrointestinal glands [55, 56]. However, it has become clear that the level of amylase in blood is in fact tightly regulated [56]. So one must wonder, what is its physiological secretory function? The presence of amylase in blood results from a very active circulation process, a balance between rate of entry and rate of clearance [57]. When the parotid glands, a major source of plasma amylase in rats, are removed, the resting level of salivary-type amylase does not change, and an increase is still found to occur on feeding [58]. Other sources of the enzyme compensate their loss. One of the sources might be the liver, which also produces low amounts of "salivary" amylase [59, 60] and can secrete amylase into the plasma [61]. Moreover, Cloutier et al. [44] have demonstrated in rats that circulating levels of amylase (and lipase) are related to their presence in the intestinal lumen. Internalization by enterocytes and progression of the absorbed enzymes along a transcytotic pathway allows them to reach the blood circulation. In this study, amylase was present in higher concentrations in the intestinal mucosa and in blood after feeding, as has been observed by others. This type of internalization and transfer has not been studied for oral epithelium; it would be interesting to know if it occurs in oral mucosa. Amylase could serve digestive or nondigestive purposes in blood. The serum amylase concentration is very low compared (ng per mL) with its concentration in secretory glands (millimolar). Nevertheless, it is sufficient to detect enzymatic activity and because of the high volume of blood (5 l) this concentration still represents a substantial amount in the range of 1–10 % of tissue levels [56]. It is also possible that the circulation of amylase through the bloodstream provides a means of transporting these proteins from one organ to another one. When labeled exocrine pancreatic proteins were injected into the bloodstream of conscious rats, the majority (approximately 97 %) were taken up by a variety of body tissues, particularly kidney, liver, spleen, and lung [62].

Association between Salivary Amylase and Obesity/Metabolic Syndrome

Recent studies have associated *AMY1* CNV to obesity in European and Asian populations [63••, 64–66]. They found that increased salivary *AMY1* copy number is positively associated with lower body mass index (BMI) and obesity risk, thus providing a genetic link between efficiency of starch digestion and low BMI, due to the *AMY1* gene. But other research teams have questioned those findings, claiming that the authors used molecular methods, such as qPCR, that are not able to provide a precise absolute count of CNV, leading to inaccurate copy numbers at the AMY locus [26]. When using methods with higher genotyping resolution, the pattern of CNV at the amylase locus is very precise and differs from the one obtained in the studies using qPCR [67]. More importantly, they did not observe the reported negative association between *AMY1* and obesity or BMI in large cohorts when using high resolution counting methods [26, 27, 68]. Thus, it appears that the effect of *AMY1* CNV on obesity risk is not as strong as initially

surmised. Yet, the association is likely present nonetheless. Counts of gene copy number and measurements of body mass are at opposite ends of the spectrum of explanatory levels with a multitude of regulatory and environmental factors intervening and diluting any causal link. Hence, it will likely be difficult to find strong associations between salivary amylase CNV and obesity. Also, the copy numbers of *AMY1* and *AMY2A* are correlated, so that phenotypic associations caused by variation in pancreatic amylase copy number could be detected indirectly as an association with *AMY1* copy number [26].

At the protein level, low serum amylase has been observed for many years within clinical settings in patients with obesity, type 1 and 2 diabetes, and metabolic syndrome [69••]. Serum amylase consists of an almost equal proportion of pancreatic and salivary amylase isoforms [63••, 70]. Traditionally, serum amylase has been measured by physicians to identify the presence of acute pancreatitis or the degree of advanced chronic pancreatitis. Conversely, the exhaustion of acinar cells and restricted flow of enzymes from pancreatic parenchyma into the circulation [71, 72] or the destruction of β-cells, due to repeated pancreatitis, can lead to low serum amylase as a result of low pancreatic amylase [69••]. Low serum amylase has also been associated with an increased risk of cardiometabolic disorders in large populations of asymptomatic adults [73–77]. Obesity and diabetes have a common pathology of insufficient insulin function, due to insulin resistance and/or diminished insulin production, and insulin is known to be critical to the production of pancreatic amylase [53, 78]. Therefore, low serum amylase may reflect a manifestation of insufficient pancreatic insulin secretion in asymptomatic people [69••]. Schneeman [79] proposed that insulin resistance may prevent the amplifying effect of insulin on amylase synthesis, leading to lower amylase levels. Unfortunately, in almost all those studies and clinical observations, only the total serum amylase was measured; so we do not know what isoforms were present in serum. It would be very useful to differentiate systematically pancreatic amylase from salivary amylase in blood. It is not known if insulin has also a monitoring role on amylase produced by the salivary glands or other salivary-type amylase producing tissues, such as the liver, which makes small amounts of salivary amylase, but some studies point to a causal insulin action on salivary amylase production in the salivary glands [80, 81]. Thus, there may be a direct functional link between insulin function and amylase production, thereby creating a causal link between starch digestion, glucose homeostasis, and metabolic syndrome.

Conclusions

Salivary amylase affects oral perception of starches, preabsorptive metabolic signaling, and plasma glucose responses to ingested starch. These early controls of digestion result in differences in the efficiency with which starch is handled metabolically. These metabolic controls appear to be of sufficient importance that the pancreatic amylase gene has been copied and expressed in the salivary glands in primates and in rodents independently. In humans, who since the advent of agriculture greatly increased starch intake, the salivary amylase gene has greatly expanded as a copy number variant. Yet, in modern society people tend to eat the same amounts of starch on average whether they make high or low levels of salivary amylase. This appears to put those who produce low levels of salivary amylase and eat high amounts of starch at risk for developing metabolic syndrome.

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Compliance with Ethical Standards

Conflict of Interest Catherine Peyrot des Gachons and Paul A.S. Breslin declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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