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Abstract

Objectives: This systematic literature review aims to summarize the existing scientific evidence on the association of oral lipolysis with diet and with the perception and digestion of lipids in humans and rodents. *Methods*: A validated search strategy of two databases (PubMed and ISI Web of Knowledge) was carried out and the contents were screened by two independent reviewers. The quality of the included studies was critically evaluated on the basis of the Quality Assessment Criteria for Evaluating Primary Research Papers. *Results*: From the originally identified studies (n=2295), 17 articles met the eligibility criteria for inclusion in the analysis. Among them, only 6 articles received the maximum assessment score. The main reason for this finding was the absence of a control for the confounding bias between lipases and esterases. In rodents, oral lipolysis was principally due to the activity of lingual lipase, which was associated with the 3 selected parameters. In humans, the association parameters were principally established through indirect evidence without a clear demonstration of cause. Moreover, no specific lipase, such as lingual lipase in the case of rodents, was identified at the oral level. *Conclusions*: Future research efforts should focus on (i) establishing a standard procedure for oral lipolytic activity evaluation and, in

Abbreviations: SLR: systematic literature review; BMI : body mass index ; THL : tetrahydrolipstatin; PMSF: phenylmethanesulfonyl fluoride

particular, a methodological control to address the lipase *vs* esterase confounding bias and (ii) identifying the main lipases that contribute to the lipolytic activity in humans at the oral level and their respective contribution to the association parameters defined in this review.

Keywords: lipolysis; saliva; lingual lipase; fat taste; diet; fat digestion

Introduction

Whole saliva is a complex mixture of fluids from major (parotid, submandibular, sublingual) and minor salivary glands (e.g., Von Ebner), gingival crevicular fluid, oral bacteria and food debris (Humphrey & Williamson, 2001). Saliva is 99% water and contains numerous proteins, peptide metabolites and ions (Carpenter, 2013). Saliva contributes not only to the protection of mucosa against pathogens but also to the ability to taste, detect and digest macronutrients and micronutrients in food (Dawes et al., 2015). For instance, in the salt taste in humans, sensitivity is directly related to salivary sodium levels (Feron, 2018).

With respect to digestion, salivary alpha-amylases contribute to the hydrolysis and post-prandial metabolism of complex carbohydrates (Joubert et al., 2017; Kurahashi & Inomata, 1999; Lebenthal, 1987; Mandel & Breslin, 2012).

Moreover, salivary alpha-amylases can adapt to the carbohydrate content of the diet (Squires, 1953). Recently, Perry et al. (2007) showed that populations with high starch diets have more copies of the salivary alpha-amylase gene (AMY1) than those with traditionally low-starch diets.

In regard to fats and other lipids, the role of salivary lipolysis in the oral detection and digestion of dietary fat in rodents was discovered (Kawai & Fushiki, 2003; Sclafani & Ackroff, 2018) with the identification and characterization of a lingual lipase secreted by Von Ebner

glands (Hamosh & Scow, 1973). In humans, lipolysis occurs in the oral cavity and may be substrate-specific (Lai, Chua, Gill, & Brownlee, 2019). However, the role of oral cavity lipolysis in the detection and digestion of lipids is debated, with only indirect evidence showing that oral lipolysis has an impact on lipid detection (Feron & Poette, 2013). Moreover, the involvement of specific lipases, i.e., gastric lipase, in oral lipolysis remains highly questionable.

In this context, the aim of this work was to review systematically original articles on studies of oral lipolysis and its association with diet and with the perception and with the digestion of lipids in humans and/or rodents.

Materials and Methods

- **Search strategy**

A review of the literature was conducted in May 2017 for all published articles containing information on the association between oral lipolysis and diet and the perception and digestion of lipids in humans and/or rodents.

The electronic databases PubMed and ISI Web of Knowledge were used to search for relevant articles without date restrictions. A regular search was updated until June 2019, and in this way, articles were added to the previous results.

Additionally, studies were identified from other sources (authors' literature base and the reference lists in the screened articles).

The search strategy consisted of a set of Medical Subject Headings (MeSH), and the terms and free text words were subsequently combined. The search terms were defined based on studies on the respective topics, keywords and the expertise of the working group members. The following groups of key words were used (* indicates that the term was used as a

wildcard to search for certain terms that might represent any group of words associated with these terms):

1) lipase*, lipolysis, lipolytic, (lipid* and hydrolysis), esterase;
2) oral, saliva, salivary, (saliva and gland), tongue, (buccal and mucosa), lingual, (oral and mucosa), (oral and (microbiota, microbiome, bacteria, and flora)), (buccal and (microbiota, microbiome, bacteria, and flora));
3) fat*, fatty*, (fatty and acid*), lipid*,oil*, (dietary fat*), triglyceride*, omega 3, omega 6, oleic, linoleic, linolenic, palmitoleic, stearic, palmitic, butyric, caprylic, lauric, myristic, arachidic, LCFA (long chain fatty acids), OR myristoleic OR vaccenic OR stearidonic OR gondoic OR arachid* OR behenic OR *pentaenoic OR erucic OR *hexanoic;
4) detection, digestion, perception, suckling, eating, feeding, consumption, intake, intensity, sensitivity, orosensitivity, liking, taste, flavour, preference, (taste and threshold); and
5) human, mice, rat, (guinea and pig), mammals.

Articles identified in the limited initial search were screened by title, abstract and full text following, whenever relevant, the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for systematic reviews (Moher, Liberati, Tetzlaff, Altman, & Grp, 2009). Protocol and PRISMA checklist form (table S1) are presented in the supplementary material file.

- **Selection criteria**

Articles were included only if they explored an association between lipolysis in the oral cavity and one of the following characteristics: fat taste perception and/or digestion and/or

diet in human subjects or rodents.

Only articles with abstracts (no proceedings papers or reviews) and written in English were included. There was no limitation on date. The study design and settings were not defined with exclusion criteria because of the exploratory character of this review.

Two reviewers (GF and HB) independently screened titles and abstracts based on the selection criteria. If the abstract did not provide enough information to decide upon inclusion or exclusion, the full paper was retrieved for further screening.

- **Article extraction and synthesis**

Search database results were combined into a master reference database which is End Note X7 (Thomson Reuters) and duplicate references were deleted. Two reviewers (GF and HB) independently reviewed the initial list and compared selections, evaluating all against the pre-defined criteria. Reviews, proceedings papers and non-English articles were set aside manually. Variables for which data were sought were: control of specificity (lipase *vs* esterase), biological material, substrate of the lipase, pH and T° of lipolysis measurement, methodology for lipolysis measurement (titrimetry, spectrophotometry, etc ..), model (human or rodent) and associated effect (lipid taste and preference, lipid digestion, lipid diet)

- **Quality assessment**

The quality assessment of the articles was adapted for this review based on, “The quality assessment criteria for evaluating primary research papers from a variety of fields” (Kmet, Cook, & Lee, 2004). The checklist of criteria included the following questions:

1. Is the objective of the study sufficiently described?
2. Is the study design evident and appropriate?
3. Are the outcomes related to lipolysis evaluation well defined and robust with regard

to the measurement(s) used? Was misclassification bias evident? Were the means of assessment reported?

4. Are the analytical methods described, justified and appropriate?
5. Is some estimate of variance reported for the main results?
6. Are the results reported in sufficient detail?
7. Are the conclusions supported by the results?

Each question represents a parameter of quality and can be answered with 'yes', 'partial', 'no' or 'not applicable'. A score of 1 was allocated to each parameter for rigorous studies with high standards. A score of 0 was allocated to a parameter that was not described, was insufficiently described or was inadequately justified. Finally, a score of 0.5 was allocated to a parameter that was insufficiently described or inadequately justified.

The summary score for each review was calculated by the total sum ((number of 'yes' scores x 1) + (number of 'partial' scores x 0.5) + (number of 'no' or 'not applicable' scores x 0)) divided by total possible score (7).

Results

- **Selected articles**

The overview of the search strategy is shown in figure 1.

In May 2017, a total of 2295 articles were identified: PubMed (n=838) and ISI Web of Knowledge (n=1457). Duplicate articles (n=257) were excluded. Another 1940 articles were excluded because the inclusion criteria were not met (based on the title). Thus, the abstracts of 97 articles were screened.

Eighty articles were excluded based on the following unmet criteria in the abstract: not written in English (n=1), proceedings paper (n=4), a review (n=15), not on humans or rodents (n=7), not on oral cavity or with no reference to lipolysis (n=28), only sensory experiments

144 are described (n=1) or no associations with oral lipolysis (as described previously) (n=24).
145 An alert in each database was created for backward citation chasing because of the delay in
146 writing. As a consequence of this approach, two new articles were found to be of interest for
147 this review and have been included. Moreover, two additional articles from sources other
148 than the databases were included. From these 21 articles, 4 articles were excluded after full
149 text screening for the following reasons: not in the oral cavity (n=1) and not linked with
150 associated parameters (n=3). The final group consisted of 17 articles (Table 1). All of them
151 were subjected to a methodological quality assessment.

152 • **Article quality evaluation**

153 The methodological quality of the included studies was judged to be good, in general.
154 Thirteen of the 17 selected articles had quality scores between 0.9 and 1 (Table 2).
155 The maximum score according to the abovementioned scoring procedure was attributed to
156 6 articles (Besnard et al., 2018; Méjean et al., 2015; Mounayar, Septier, Chabanet, Feron, &
157 Neyraud, 2013; Poette et al., 2014; Sclafani & Ackroff, 2018; Voigt et al., 2014). The lowest
158 score of the list was attributed to one article (Stewart et al., 2010).
159 In all articles, the objectives were clearly explained, and each earned the maximum score on
160 the rating scale. Of the 17 articles, a score of 1 was given for the study design and the
161 analytical methods for 13 and 14 articles, respectively.
162 In 11 articles, oral lipolysis evaluations (main outcome) are well defined and robust
163 according to the measurements without misclassification bias and with means of assessment
164 reported. In 6 articles, oral lipolysis evaluation did not meet all the requirements necessary
165 to earn the maximum score. The main reason for submaximal scoring was the absence of an
166 analytical procedure, i.e., an appropriate inhibitor of lipase or esterase was not chosen for
167 discriminating between lipase and esterase activity.

For 4 articles, the score for "results reported in sufficient detail" did not reach the maximum possible. This lower score was attributed to papers that lacked a table showing the results with standard deviation values of the oral lipolysis measured, or detailed lipolytic activity data were not provided.

Finally, 5 articles showed conclusions that were not fully supported by the results, essentially due to the absence of robust statistical analysis.

- **Study characteristics**

Seven of the selected articles were published after 2010 (table 1), and only 4 of them were published before 1990. Therefore, most of the selected studies were carried out recently.

Humans were used as the system model in the studies described in 11 articles (table 3), and people with obesity had been recruited in 4 of the 11 articles.

Six other articles were based on rodent studies. Among these, five studies had focused on rats, and one had focused on mice.

- **Analytical methods for lipolysis evaluation**

Oral lipolysis was either determined or evaluated across all the selected studies (Table 3). The studies differed by approach (*in vitro* or *in situ*), the control(s) (specificity), the condition(s) of measurement and/or the substrate.

- *Control for lipase specificity*

Lipases and esterases belong to the same enzyme classification group EC 3.1.1: carboxylic ester hydrolases. However, unlike esterases, lipases have substrate specificity towards fatty acid esters (i.e., mono-, di-, tri-glycerides) and preferences for medium (C8 to C14) and long (C>16) chains because of their insolubility in polar media (Robb, 1966). Thus, a control for specificity, which eliminates the case of esterase activity, leads to the selection of only lipase activity measurements. A inhibitor of lipase activity is THL (Tiss, Lengsfeld, Carriere, &

192 Verger, 2009), which was used in the studies described in 11 articles. In one case (Mennella,
193 Fogliano, & Vitaglione, 2014), the control consisted of adding 0.4 mM PMSF to inhibit
194 esterase activity to quantify the lipase activity – the 2 enzymes acted on the same substrate
195 (Kurooka, Okamoto, & Hashimoto, 1977). Contrary to applications for THL, PMSF is used to
196 measure lipase activity after esterase activity is suppressed. Indeed, it was demonstrated
197 that PMSF inhibits acetylcholine esterases (EC 3.1.1.7) of brain and hepatic serum origin
198 (Fahrney & Gold, 1963; Turini, Kurooka, Steer, Corbascio, & Singer, 1969); the type B
199 carboxylesterases are in the brain, and the aryl esterases (EC 3.1.1.2), which belong in the
200 type A carboxylesterase classification, are in hepatic serum (Kurooka et al., 1977). The
201 mechanism of inhibition involves the binding of PMSF to the active site of the esterase
202 (Fahrney & Gold, 1963). Nevertheless, it has not been proven that PMSF inhibits the esterase
203 activity in the oral cavity; for example, saliva contains other esterases, such as carbonic
204 anhydrases (EC 4.1.1.2), in abundance, and bacterial esterase, which could not be inhibited
205 by PMSF and could not have the same active site. Thus, in the attempt to control for the
206 confounding effects of esterase and lipase activity, this indirect method presents more
207 limitations than are presented by methods that use THL to measure lipolytic activity.

208 • *Approaches*

209 <u>In vitro measures</u>

210 Measurements described in 13 articles were taken from experiments performed *in vitro*.
211 Among them, the biological material was free saliva except in three: Armand et al. (1990)
212 and Hamosh (1978), in which the posterior part of the lingual tissue was collected, and
213 Kawai & Fushiki (2003) in which measurements was conducted on exposed circumvallate
214 papillae from collected tongues.

215 In Hamosh and Scow (1973), two types of biological materials were tested, i.e., free saliva

and tissue from the white tissue of the tongue in the region of circumvallate papillae which consists of serous lingual (Von Ebner) glands and secretory ducts and various other parts of the oral cavity.

Whole saliva was used in the oldest studies (Hamosh & Scow, 1973; Plucinski, Hamosh, & Hamosh, 1979), and clarified saliva was used in the papers published in the past 10 years. Whole saliva was frozen directly after collection, whereas clarified saliva was obtained after centrifugation at 14 000 g for 10 to 20 minutes at 4°C (Neyraud, Palicki, Schwartz, Nicklaus, & Feron, 2012). Indeed, pre-treatment standardization has become common practice because the current techniques, such as fluorometry, require homogeneous micro volume samples without large particles (Schipper, Silletti, & Vingerhoeds, 2007).

Saliva was collected according to the objective of the studies in stimulated or unstimulated conditions. The stimulation was mechanical (Besnard et al., 2018) or was conferred by cream consumption and rinsing (Stewart et al., 2010). Four articles used only unstimulated saliva as biological material, 3 articles used unstimulated and stimulated saliva and three articles used only stimulated saliva.

Evaluation in situ

Four studies were conducted *in situ*. Two studies measured lipase activity. In one case, the substrate was placed on filter disk paper, and the amount of non-esterified fatty acids released was measured *in situ* at the proximity of the secretory glands (Von Ebner gland cells) (Voigt et al., 2014).

An alternative method that did not clearly measure lipase activity measured the non-esterified fatty acids released from a natural and high-fat food matrix in an expectorated food bolus (Kulkarni & Mattes, 2014).

Instead of the direct biochemical method described above, indirect evaluation of the role of

lipolytic activity was measured by taste sensitivity to triolein in humans (Pepino, Love-Gregory, Klein, & Abumrad, 2012) or a preference for corn, soybean or triolein oil by rodents (Sclafani & Ackroff, 2018); both studies conducted experiments in the presence or absence of THL.

- *Conditions of measurement*

The measurement of lipolytic activity was made using the following techniques alone or in combination:

- Measurements of the pH variations due to the release of fatty acids from triglycerides by the lipolytic reaction: the pH-stat method was used with a method derived from Taylor (1985), and the results were established through titrimetry.

- Radiometry assay of radioactively labelled fatty acids

- Mass and optical spectroscopy (Beisson et al., 2001; Imamurra, 1989; Kurooka et al., 1977).

- *Substrates used for evaluation of lipolytic activity*

The substrates used for measuring lipolytic activity were diglycerides (in 1 article only), natural triglycerides from food, synthetic triglycerides, radiolabelled triglycerides or synthetic free fatty acid esters. The chain length of the fatty acid substrates ranged from 4 to 18 carbons, and oleic acid was often used. Notably, the use of long-chain triglycerides enabled better activity quantification of the lipolytic reaction mechanism.

In the selected publications, we did not find any information about the degree of substrate emulsification that affects interface development for micelle formation, which is a determinant of lipase activity (Verger, 1997).

Most of the reactions were carried out at pH 7, including those carried out (i) with saliva, which is considered to have a pH of approximately 7 (Schipper et al., 2007), (ii) in a food bolus (1 article), and (iii) fixed onto a matrix (filter disks) (2 articles).

The determination of the optimal pH for lipase activity enables the differentiation of the lipases that act directly at oil-water interface (e.g., acid-like gastric lipases) from the lipases that act on micellar structures with the required biliary salts (e.g., pancreatic lipases) (Eydoux et al., 2007; Lombardo, Fauvel, & Guy, 1980). The pH at which the measurements were taken shows that salivary lipases act as acidic lipases in acidic and neutral environments at oil-water interfaces.

- **Oral lipolysis and association parameters (diet, digestion and taste) (Table 4)**
    - *Relationships with diet*

Four publications were identified as describing a link between diet and oral lipolysis. Two were conducted on rats with the objective of evaluating the effect of a fat-related diet on the level of lingual lipase (Armand et al., 1990; Hamosh, 1978). Both papers reached the same conclusion, i.e., increasing the fat content in the diet led to an increase in lingual lipase activity in a dose-dependent response, suggesting an adaptive response to the level of fat in the diet.

In addition, 2 other papers focused on the relationships between salivary lipolysis and fat preference and intake in humans. These papers reached contradictory conclusions. Mennella et al. (2014) observed higher lipolytic activity, fat preference and fat intake in overweight subjects compared with lean subjects. The authors suggest that high salivary lipolytic activity in overweight subjects could be an adaptive response to low-fat taste perception due to a low level of salivary zinc. In contrast, Méjean et al. (2015) did not identify relationships between salivary lipolytic activity on one hand and fat preferences and

intake on the other hand, regardless of BMI. These discrepancies in the results can be explained by (i) the populations differing significantly between the two studies in terms of age, sex and BMI, and (ii) the methodology used to evaluate salivary lipolytic activity in saliva and, in particular, the controls for the specificity of the reaction, which differed between the two studies, i.e., THL was used in the study by Mejean et al. and PMSF was used in the study by Mennella et al., which could have led to different estimations of lipolysis activity, as explained previously.

- *Relationships with digestion and metabolism*

Three papers described the role of oral lipolysis in lipid digestion and/or metabolism. Two studies were conducted on rats, and only one showed direct evidence of lipid digestion in the stomach, duodenum and ileum due to lingual lipase activity (Hamosh & Scow, 1973). Another study found that lingual lipase is pH-dependent and remains active in the stomach (Plucinski et al., 1979), which suggests that it can contribute to lipid digestion in this compartment.

Only one study was conducted on humans (Vors et al., 2015). It described significant associations between the level of stimulated salivary lipolysis and explained the post-prandial lipaemia profile after fat intake depended on BMI. In particular, obese subjects showed lower salivary lipolysis activity and a delay in post-prandial lipaemia compared with lean subjects. The authors suggested that this lower level of salivary lipolysis in obesity can impair lipid detection in the mouth and thus lead to lipaemia.

- *Relationships with perception and preference*

Ten studies were aimed at evaluating the role of oral lipolysis on taste of and preference for fat. Two studies were conducted on rodents (rats or mice) *in situ* (Kawai & Fushiki, 2003; Sclafani & Ackroff, 2018). In both studies, the inhibition of oral lipolysis by THL led to a

decrease in fat preference, indirectly indicating the role of lingual lipase in the release of free fatty acids from dietary fat such that they could be detected at the oral level.

Eight studies were conducted with humans. Three studies were conducted *in situ* by using THL for the control of oral lipolysis (Kulkarni & Mattes, 2014; Pepino et al., 2012; Voigt et al., 2014). The other 5 studies evaluated oral lipolysis in saliva samples *in vitro*. A control for specificity was included in 6 experiments using THL. With respect to sensory evaluation, 7 studies evaluated taste sensitivity to triolein (*in situ* only) or to free fatty acids (mostly C18:2). One study evaluated the preference intensity ratings for fat emulsion (Neyraud et al., 2012). Two of the three studies conducted *in situ* showed an inhibition of triolein sensitivity in the presence of THL, which provides indirect evidence that oral lipolysis is involved in the detection of dietary fat in humans. Studies conducted *in vitro* have led to contradictory conclusions. Two of them showed a positive correlation between the level of lipolysis in saliva and fat sensitivity (Neyraud et al., 2012; Poette et al., 2014), while two others did not find a statistical correlation (Besnard et al., 2018; Mounayar et al., 2013).

Discussion

In this work, two aspects were considered. In the first part, we considered the methods used for lipase measurements and evidence gathered by functional testing. In the second part, we focused on different associations with oral lipolysis, i.e., taste sensitivity, digestion and diet.

- **Methodological considerations**

One main conclusion of this SLR is that the methods used were diverse and heterogeneous for evaluating oral lipolysis, which made comparing the different papers difficult, particularly the comparisons of study results that were published by different research groups.

This challenge to the comparisons led to the determination of a standardized protocol for the evaluation of lipolytic activity with different constraints.

First, the measurements for lipolytic activity aimed to take into account enzymatic specificity. Among the various controls used to overcome the confounding results from esterase and lipase activity, the most relevant method involved measures with and without THL. The robustness of inhibition by THL (Luthipeng, Marki, & Hadvary, 1992) is due to covalent binding to the active site, which consists of a catalytic triad formed by the serine, aspartate, and histidine that are characteristic of the lipase family, that inactivates the lipases (Beer, Wohlfahrt, McCarthy, Schomburg, & Schmid, 1996); these binding sites are not present in esterases (Chahinian & Sarda, 2009). This difference explains why THL is highly discriminant, as has been demonstrated in the case of digestive lipases (Ransac et al., 1997).

Second, the choice of substrate enabled specific lipases to be targeted for study. The fatty acid chain length is primordial, and medium (from C8 to C14) or long chain (>C16) fatty acid glycerides are used as substrates, although the long-chain fatty acid glycerides are difficult to solubilize. Foremost, researchers compromised between using natural or radiolabelled triglycerides and synthetic esters, such as 4-methylumbelliferyl-oleate, which are less specific but safer and faster for detecting and quantifying. Furthermore, substrates in the form of micelles in aqueous medium ensure an optimal interface (Paiva, Balcão, & Malcata, 2000) for lipase reactions. Thus, the conditions were adapted to study weak lipase activity, as is the case for salivary lipases.

Third, evaluation of lipolysis activity was performed at fixed pH, and some studies conducted experiments at non-physiological pH levels. Additionally, no measurement method for screening several pH values was used, which means that the lipases could not be differentiated.

Finally, sample standardization is preferable in the case of salivary analysis. Clarified saliva samples with a fixed protocol were described in 6 studies (Méjean et al., 2015; Mounayar et

al., 2013; Neyraud et al., 2012; Poette et al., 2014; Vors et al., 2015) and seemed to be adapted for use in these types of analyses.

In summary, a literature review (Beisson et al., 2001) described the screening assays for quantifying lipase activity on the principles and practical aspects of this methodological consideration. It was concluded that the pH-stat method, which enabled screening over an extended range of pH values due to the inclusion of several pH indicators, is suitable for pure lipase assays, and a method based on natural fluorescent substrates, as described in Beisson et al. 1999, is more suitable for crude biological sample assays (Beisson et al., 1999). Recently, a more sensitive method than the original pH-stat has been developed (Camacho-Ruiz, Mateos-Diaz, Carriere, & Rodriguez, 2015). Interestingly, both methodologies are appropriate for screening a large number of biological samples.

- **Oral lipolysis and the association parameters**
    - *Origin of salivary lipolysis*

Lingual lipase was notably involved in salivary lipolysis in rats and mice. It is secreted from the serous glands found on the dorsal tongue (Hamosh, 1978; Hamosh & Scow, 1973; Triantafyllou, Fletcher, & Scott, 2003) and has a high level of activity compared with gastric lipase (DeNigris, Hamosh, Kasbekar, Lee, & Hamosh, 1988). Rat lingual lipase has a molecular weight of 270 000-300 000, which suggests extensive self-aggregation, and it is active from pH 2 to 8. Its activity is not influenced by the concentration levels of phospholipids or bile salts, and therefore it differs significantly from the pancreatic lipases (Roberts, Montgomery, & Carey, 1984). In contrast to that in rats, lingual lipase has never been clearly demonstrated in humans, and attempts to identify its secretion at the serous gland level have always failed (Spielman, D'Abundo, Field, & Schmale, 1993; Voigt et al., 2014). Hence, it is likely that salivary lipolysis is due to the activity of other lipases. Indeed, the expression of putative

secreted lipolytic enzymes has been shown in human Von Ebner gland cells (Voigt et al., 2014).

- *Adaptation of salivary lipolysis to diet lipid content and quality*

The adaptation of salivary properties to the human diet has been clearly established on salivary alpha-amylase activity and carbohydrate consumption, i.e., the level of salivary alpha-amylase is higher, and the consumption of complex carbohydrates is higher in the diet (Squires, 1953). More recently, the number of gene copies of salivary alpha-amylase (AMY1) has been associated with a starch diet in a way that aligns with the findings from the diet studies (Perry et al., 2007) but it is also associated with BMI such that a low AMY1 copy number is associated with a high BMI (Falchi et al., 2014). With respect to lipids in the diet and lingual lipase activity, few papers have suggested salivary adaptation in rodents, but the conclusions are consistent between studies. In contrast, no clear conclusions could be made on salivary adaptation in humans, which is a topic that warrants further attention.

- *Role of salivary lipolysis in fat perception and digestion*

Both in rodents and humans, only indirect evidence suggests a contribution of salivary lipolysis to the orosensory detection of fat. Indeed, salivary lipases hydrolyse triglyceride, and the resulting free fatty acids can be detected by putative fat sensors, such as the CD36 fatty acid transporter, which is involved in the detection of long chain fatty acids at the taste bud level in rodents (Laugerette et al., 2005). In humans, it has been shown that oronasal exposure to dietary fat can influence post-prandial metabolism, particularly the stimulation of lipid digestion and absorption (Mattes, 1996, 2011). With respect to taste, the oral fatty acid threshold measurement of different long chain fatty acids showed that they are perceived through the taste modality only (Chale-Rush, Burgess, & Mattes, 2007), but whether sensors, such as CD36, are involved in long chain fatty acid detection is still

406 debated. However, both in rodents and humans, the contribution of salivary lipolysis to the
407 modulation of oral fat perception is merely speculative. Moreover, factors not involved in
408 lipolysis, namely, lipocalin or carbonic anhydrase VI, have been proposed as other putative
409 salivary components that are involved in the modulation of free fatty acid perception
410 (Besnard et al., 2018; Feron & Poette, 2013). Unravelling the mechanism by which salivary
411 lipolysis impacts fat detection at the oral level should be studied further.
412 With respect to digestion, lingual lipase participates in digesting as much as 6% of the lipids
413 during the oral phase in rats (Kawai & Fushiki, 2003). Compared with triglycerides,
414 diglycerides might be more easily digested by lingual lipase, suggesting a different substrate
415 specificity compared with other lipases of the digestive tract (Osaki et al., 2005). In contrast
416 to that in rats, the level of salivary lipolysis activity in humans is such that it cannot
417 contribute to dietary fat digestion. However, as suggested by Vors et al., 2015, a low level of
418 salivary lipolysis could indirectly contribute to lipid metabolism in humans through the
419 release of a small amount of free fatty acids that are detected in the mouth and thus
420 stimulate anticipatory digestive and metabolic responses for lipids prior to nutrient
421 absorption. Such a mechanism has been proposed for salivary alpha-amylase, as subjects
422 with a higher level of salivary alpha-amylase showed a lower level of post-prandial glucose
423 concentrations after oral intake of a starch load (Mandel & Breslin, 2012). The authors
424 suggested that the production and detection of glucose and/or maltose and/or short-chain
425 oligosaccharides through amylolytic activity in the oral cavity signals the body to prepare for
426 incoming starch and the ensuing glucose.
427 **Limitations of this SLR**
428 A bias cannot be excluded in the selection phase. First, it is possible that not all relevant
429 studies are indexed in the searched databases (ISI Web of Knowledge and PubMed). Second,

the search was based on a list of terms describing the potential association of oral lipolysis with different parameters. The possibility that additional articles would have been identified by adding other terms cannot be excluded; although the search was intended to be as extensive as possible. Third, article eligibility was restrictive. We chose to include papers on oral lipolysis and specific parameters associated with it. This criterion created a restraint that limited our analysis and such an investigation conducted on oral lipolysis outcome was only expected to substantiate and enlarge our conclusions. Fourth, in this review, only studies in English were included, which could have led to biased conclusions. However, it is noteworthy that only one study was excluded for language; therefore, the bias was presumably low. Finally, we chose to include studies submitted to a peer review procedure, which could have limited the results through publication bias. Indeed, studies with positive results tend to be selected for publication.

Conclusion

With respect to methodological considerations, this SLR highlights the fact that a consensus for a normative method to evaluate lipase activity is highly necessary. This methodology must be based on considerations for both specificity (lipase versus esterase substrates) and pH measurements. It will also need to be suitable for high output screening of small volume biological samples. Regarding the associations studied (diet, digestion or taste), this SLR highlights the small number of papers that address salivary lipolysis either in animal models or in humans. In fact, most of the studies conducted in humans or animals focused on the mechanisms leading to lipid and free fatty acid detection rather than on those involved in upstream or peri-receptor biological events. This finding was unexpected in light of the considerable controversy and debate around oral lipolysis and its role in humans.

In contrast to animal lingual lipase, human oral lipases could not be identified with specificity associated with lipid detection, digestion or diet. Moreover, no proteomic studies have been published with conclusions that confirm or dispute the presence of a human lingual lipase. It is likely that in humans, different lipases such as from bacteria are involved in the overall salivary lipolysis activity, rendering any relationships with physiological effects and diet difficult to discern, which explains the extensive discrepancies observed in the literature on this scientific subject.

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Conflicts of interest

The authors state no conflicts of interest

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Figure caption:

Figure 1: overview of the research strategy

656 **Table 1** : References of the 17 selected articles
657

Reference	N°
Articles describing salivary lipolysis in relation to diet	
Armand, M., Borel, P., Cara, L., Senft, M., Chautan, M., Lafont, H., & Lairon, D. (1990). *J Nutr, 120*(10), 1148-1156.	**1**
Hamosh, M. (1978). *Am J Physiol, 235*(4), E416-421.	**2**
Méjean, C., Morzel, M., Neyraud, E., Issanchou, S., Martin, C., Bozonnet, S., . . . Feron, G. (2015). *PloS one, 10*(9), e0137473.	**3**
Mennella, I., Fogliano, V., & Vitaglione, P. (2014). *Food Research International, 66*, 463-468.	**4**
Articles describing salivary lipolysis in relation to lipid digestion and metabolism	
Hamosh, M., & Scow, R. O. (1973). *J Clin Invest, 52*(1), 88-95.	**5**
Plucinski, T. M., Hamosh, M., & Hamosh, P. (1979). *Am J Physiol, 237*(6), E541-547.	**6**
Vors, C., Drai, J., Gabert, L., Pineau, G., Laville, M., Vidal, H., . . . Feron, G. (2015). *Int J Obes (Lond), 39*(9), 1425-1428.	**7**
Articles describing salivary lipolysis in relation to lipid taste and preferences	
Besnard, P., Christensen, J. E., Brignot, H., Bernard, A., Passilly-Degrace, P., Nicklaus, S., . . . Burcelin, R. (2018). *Sci Rep, 8*(1), 6742.	**8**
Kawai, T., & Fushiki, T. (2003). *Am J Physiol Regul Integr Comp Physiol, 285*(2), R447-454.	**9**
Kulkarni, B. V., & Mattes, R. D. (2014). *Am J Physiol Regul Integr Comp Physiol, 306*(12), R879-885.	**10**
Mounayar, R., Septier, C., Chabanet, C., Feron, G., & Neyraud, E. (2013). *Chemosensory Perception, 6*(3), 118-126.	**11**
Neyraud, E., Palicki, O., Schwartz, C., Nicklaus, S., & Feron, G. (2012). *Arch Oral Biol, 57*(5), 556-566.	**12**
Pepino, M. Y., Love-Gregory, L., Klein, S., & Abumrad, N. A. (2012). *J Lipid Res, 53*(3), 561-566.	**13**
Poette, J., Mekoue, J., Neyraud, E., Berdeaux, O., Renault, A., Guichard, E., . . . Feron, G. (2014). *Flavour and Fragrance Journal, 29*(1), 39-49.	**14**
Sclafani, A., & Ackroff, K. (2018). *Am J Physiol Regul Integr Comp Physiol, 315*(3), R434-r441.	**15**
Stewart, J. E., Feinle-Bisset, C., Golding, M., Delahunty, C., Clifton, P. M., & Keast, R. S. (2010). *Br J Nutr, 104*(1), 145-152.	**16**
Voigt, N., Stein, J., Galindo, M. M., Dunkel, A., Raguse, J. D., Meyerhof, W., . . . Behrens, M. (2014). *J Lipid Res, 55*(5), 870-882.	**17**

Table 2 : Quality assessment of the 17 selected studies

N°	Question/ objective sufficiently described?	Study design evident and appropriate?	Outcome measures(s) well defined and robust to measurement/ misclassification bias? Means of assessment reported?	Analytic methods described/justified and appropriate?	Some estimate of variance is reported for main results?	Results reported in sufficient detail?	Conclusions supported by results?	Summary Score[a]	Reason
	Articles describing salivary lipolysis in relation to diet								
1	1	1	0.5	1	1	1	1	0.9	No control for lipase *vs* esterase
2	1	1	0.5	1	1	1	1	0.9	No control for lipase *vs* esterase
3	1	1	1	1	1	1	1	1	
4	1	0.5	0.5	1	1	0.5	0.5	0.7	Control for lipase esterase not specific enough. pH for enzymatic evaluation far from salivary pH No quantitative data from evaluation of products from lipolysis by GC methodology
	Articles describing salivary lipolysis in relation to lipid digestion and metabolism								
5	1	1	0.5	1	0.5	1	0.5	0.8	No control for lipase *vs* esterase
6	1	0.5	0.5	0.5	1	1	1	0.8	No control for lipase *vs* esterase
7	1	1	1	0.5	1	1	1	0.9	Methodology for lipolysis measurement not detailed in the paper, only a reference was

									mentioned
	Articles describing salivary lipolysis in relation to lipid taste and preferences								
8	1	1	1	1	1	1	1	1	
9	1	1	1	1	0.5	1	1	0.9	Results for lipase activity are in abundance ratio of fatty acids without standard deviation
10	1	1	1	1	1	0.5	0.5	0.9	No detailed statistics on sensory values and their relations with lipolysis
11	1	1	1	1	1	1	1	1	
12	1	1	1	1	1	1	0.5	0.9	Indirect relationships (through PCA analysis) between sensory data and lipolysis, no direct correlation.
13	1	0.5	1	1	1	0.5	1	0.9	No measure of lipolytic activity, no description of the effect of THL between the 3 groups of obese subjects (the 3 groups were polled)
14	1	1	1	1	1	1	1	1	
15	1	1	1	1	1	1	1	1	
16	1	0.5	0.5	0.5	0.5	0.5	0.5	0.6	No control for lipase *vs* esterase , no detail regarding lipolytic activity, no statistics between lipolytic activity and sensitivity to fat
17	1	1	1	1	1	1	1	1	

[a]Summary score for each review was calculated by the total sum ((number of 'yes' scores x 1) + (number of 'partial' scores x 0.5) + (number of 'no' or 'not applicable' scores x 0)) divided by total possible score (7)

663

664 **Table 3:** Descriptive analysis of the objectives and the methodology used for the lipolytic activity measurements in the 17 selected articles (NR:
665 not relevant)
666

N°	Objectives	Lipase activity measured	Control of specificity	Biological material	Substrate	pH and T° of measurement	Methodology for lipolysis measurement	Model
	Articles describing salivary lipolysis in relation to diet							
1	To study the adaptative response of lingual lipase and pancreatic lipase to dietary fat.	Yes	No	Lingual tissue of posterior part of the tongue	Tributyrin	pH 5.4, 37°C	Titration	Rats
2	To study the subcellular localization of the lipase from the lingual serous (Von Ebner) glands of rat tongue and the factors affecting its activity (diet) and secretion.	Yes	No	Whole homogenate of lingual serous (Von Ebner) glands or subcellular fractions	Triglycerides doubly labelled [2-^{3}H] glyceryl and [l-^{14}C] tripalmitin	pH 5.4, 37°C	Radiometry	Rats
3	To evaluate the association of salivary flow and composition with both preferences for fat, saltiness and sweetness and the usual nutrient intake in an adult French population.	Yes	THL	Clarified unstimulated saliva	4-methyl-umbelliferyl-oleate	pH 7.5, 37°C	Fluorescence	Human
4	To verify the relationships among salivary lipase, α-amylase activities and zinc concentration with food preference and choice of people with different body mass indices.	Yes	PMSF	Whole unstimulated saliva	2,3-Dimercapto-1-propanol tributyrate, tripalmitin	(1) pH 8.5, 37°C (2) pH of saliva 37°C	Spectrophotometry and GC-FID	Human
	Articles describing salivary lipolysis in relation to lipid digestion and metabolism							

5	To study the secretory tissues of the mouth of suckling and adult rats and examine their lipolytic activity.	Yes	No	Tissues from serous glands of the tongue, from the soft palate, oral pharynx wall including glands, clarified unstimulated saliva and stomach content	[1-^{14}C] palmitic acid and trioleoyl-[2-^{3}H] glycerol, corn oil triglycerides, and natural milk fats	pH 3-8, 37°C and pH 5.4, 37°C	Radiometry and titrimetry	Rats
6	To test the effect of oesophagus ligation (i.e., absence of lingual lipase) on intra-gastric triglyceride hydrolysis and in the small intestine of adults rats.	Yes	No	Whole stimulated food bolus	Bovine milk-cream mixture with 12 to 14% triglycerides	pH 5.4, 37°C	Titration and GC-FID	Rats
7	To test the hypothesis that salivary lipolysis differs according to BMI and post-prandial lipid metabolism.	Yes	THL	Clarified stimulated saliva	4-Methyl-umbelliferyl-oleate	pH 7.5, 37°C	Fluorescence	Human
	Articles describing salivary lipolysis in relation to lipid taste and preferences							
8	To test if subjects with obesity could be characterized by an impaired fatty taste sensitivity linked to a change in the gustatory papillae in the microbial and salivary environment.	Yes	THL	Clarified unstimulated and stimulated saliva	4-Methyl-umbelliferyl-oleate	pH 7.5, 37°C	Fluorescence	Human
9	To focus on the gustatory sense and investigate the significance of the lingual lipase released in the clefts of foliate and vallate papillae, where there are many taste bud cells, to establish a link with the perception of a fat taste.	Yes	THL	Tongues	[carboxyl-^{14}C] triolein on filter paper	NR	Radiometry	Rats

10	To determine the role of lingual lipase in oral fat detection as dependent on the food matrix.	Yes	THL	Saliva in food bolus	Natural high-fat food matrix: almond, almond butter, olive oil, walnut, and coconut	NR	GC-MS	Human
11	To study whether saliva composition is different in groups of subjects having low or high oral sensitivity to oleic acid. To determine whether oral stimulation with oleic acid could modify the composition of saliva.	Yes	THL	Clarified unstimulated and oleic acid stimulated saliva	4-methyl-umbelliferyl-oleate	pH 7.5, 37°C	Fluorescence	Human
12	To study intra- and inter-individual variabilities over time in the composition of molecules likely to interact with food in the mouth, with particular focus on molecules that might interact with fat.	Yes	THL	Clarified unstimulated and stimulated saliva	4-methyl-umbelliferyl-oleate	pH 7.5, 37°C	Fluorescence	Human
13	To evaluate whether a common single nucleotide polymorphism (rs1761667) in the CD36 gene that reduces CD36 expression and the addition of THL to reduce fatty acid release from triacylglycerols would attenuate fat orosensory sensitivity in obese subjects.	No	THL	In situ study	Triolein	NR	NR	Human
14	To better understand how human oral physiology may govern the sensory sensitivity to non-esterified fatty acids.	Yes	THL	Clarified unstimulated saliva	4-methyl-umbelliferyl-oleate	pH 7.5, 37°C	Fluorescence	Human

15	To determine if fat preference is influenced by the inhibition with THL of triglyceride lipolysis at both oral and post-oral levels.	No	THL	In situ study	Triolein, corn oil, soybean oil	NR	NR	Mice
16	To investigate oral fatty acid sensitivity, food selection and BMI in human subjects.	Yes	No	Clarified stimulated saliva	1,2-Diglycerides	pH 8, 35°C	Spectrophotometry	Human
17	To investigate whether triglycerides represent an adequate “fatty” stimulus *in vivo* and *in vitro* and to determine if lipolytic enzymes are present in the human oral cavity.	Yes	THL	In situ study on circumvallate papillae	Triolein	NR	HPLC-MS/MS	Human

677 **Table 4**: Descriptive analysis of the main results about lipolytic activity and the associated parameters studied in the 17 selected articles (NR:
678 not relevant)
679

N°	Associated effects	Main results about lipolytic activity	Main results of the association between the parameters studied and lipolytic activity
	Articles describing salivary lipolysis in relation to diet		
1	Level of fat in the diet	NR	There is a positive adaptive response of the lingual lipase to the level of fat in the diet. The type of fat has no effect on lipolytic activity.
2	Level of fat in the diet	Lingual lipase is located in the secretory granules of the lingual serous (Von Ebner) glands. Lingual lipase secretion is stimulated by isoprenaline.	There is positive adaptive response of lipase lingual to the level of fat in the diet.
3	Lipid intake	NR	Lipolysis is not associated with the intake of lipids, mono-unsaturated or polyunsaturated and saturated fatty acids, whereas sensory preference suggests the influence of saliva characteristics in food acceptance.
4	Dietary fat consumption	NR	Salivary lipolytic activity is higher in the overweight subjects than in the normal weight subjects. High salivary lipase activity in the overweight subjects may be an adaptive response to the low-fat taste perception related to reduced zinc concentration.
	Articles describing salivary lipolysis in relation to lipid digestion and metabolism		
5	Fat digestion	The lipase is secreted from serous gland from the posterior part of the tongue and fewly detected in the stomach. It is pH dependant.	Salivary lipase contributes significantly to the digestion of triglyceride in the stomach.

6	Fat digestion	NR	Lipolytic activity contributes significantly to the hydrolysis of fat droplets not only in the stomach but also in the duodenum and the ileum.
7	Lipaemia	NR	Lipolysis in the obese subjects is significantly lower than in the normal weight subjects and is associated with a delay in post-prandial metabolism, as observed in the obese subjects compared to the normal weight subjects.
	Articles describing salivary lipolysis in relation to lipid taste and preference		
8	oleic acid detection threshold	NR	In the obese subjects, BMI is related to taste sensitivity, but no association is observed between the sensitivity to oleic acid and lipolytic activity in the obese subjects. Whatever the BMI, no link between lipolysis and BMI is observed.
9	Fat taste preference	Lingual lipase is released continuously from the papillae	The addition of THL diminished the preference for triglycerides but not for free fatty acids. Lingual lipase is involved in finding nutritive lipids in food.
10	Fat taste evaluation	NR	THL inhibits the release of non-esterified fatty acids from the food matrix in the food bolus and does not affect the sensory evaluation of almond butter.
11	oleic acid taste sensitivity	Significant decrease in lipolytic activity is observed after stimulation with oleic acid for hypersensitive subjects	No differences are found in the saliva characteristics according to sensitivity to oleic acid. The oleic acid stimulation compared to a control stimulation shows modified salivary composition in the sensitive group only. No difference in lipolytic activity is observed between the hypersensitive and hyposensitive subjects.
12	Fat taste preference	Subject salivary lipolysis is stable over time, and no sampling effect was found.	A positive relationship is found between the level of lipolysis and fattiness, and a negative relationship for the level of lipolysis and preference is observed.
13	oleic acid and triolein orosensory detection thresholds	NR	The presence of THL decreases the sensitivity to triolein in obese subjects.

14	oleic acid orosensory detection threshold	Subject salivary lipolysis is stable over time, and no sampling effect is observed during 4-month collection.	Taste sensitivity to oleic acid is explained by the oral volume and the level of lipolysis in saliva. The higher the lipolytic activity is, the higher the threshold is.
15	Fat taste preference	NR	Inhibition of oral triglyceride hydrolysis leads to a decrease in preferences for fat, but triglyceride hydrolysis is not essential for fat preferences.
16	Orosensory detection thresholds for oleic acid, linoleic and lauric acids	NR	Lipolytic level is sufficient to produce micromolar levels of fatty acids, which can stimulate oral sensors.
17	Sensory sensitivity to triolein or oleic acid	Oleic acid is liberated from triglycerides upon exposure to saliva secreted from foliate papillae. THL reduces the generation of oleic acid. The expression of different lipases at the level of the circumvallate papillae is observed. Gene coding for lingual or gastric lipase is not expressed in human lingual tissue but genes coding for other lipases are expressed.	Triglyceride perception is attenuated by concomitant THL administration. Lipolytic activities in minor salivary gland secretions directly supplying gustatory papillae are correlated to individual sensitivities for triglycerides.

Identification

ISI Web of Knowledge (All databases)
1457

PubMed
838

Excluded because of duplicates: 257

Screening

Records after duplicates removed, screened on titles
2038

Excluded after screening titles: 1940

Records screened on abstracts
97

Excluded after screening abstracts: 80

- Not English: 1
- Proceedings papers: 4
- Reviews: 15
- Not human, neither rodents: 7
- Not in oral cavity or no lipolytic enzyme: 28
- Only sensory experiment: 1
- Not linked with perception, only lipase or lipolysis or only FFA: 24

Eligibility

Full-text screened
17

Additional full-text identified between 31/05/2017-01/06/2019
2

- Neyraud *et al.*, 2017
- Sclafani *et al.*, 2018

Additional records identified through other sources
2

- Stewart *et al.*, 2010
- Besnard *et al.*, 2018

Excluded after screening full-text: 4

- Not in oral cavity or no lipolytic enzyme: 1
  - Sclafani *et al.*, 2018
- Not linked with perception, only lipase or lipolysis or only FFA: 3
  - Staggers *et al.*, 1981
  - Kulkarni *et al.*, 2013
  - Neyraud *et al.*, 2017

Included

Studies included in qualitative synthesis **17**