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Salivary markers of taste sensitivity to oleic acid: a combined proteomics and metabolomics approach

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Abstract Saliva is a biological fluid playing numerous roles in the oral cavity and increasingly considered as a source of markers. The role of saliva in sensory perception has been known for years but it is only recently that its potential role in oral fatty acids (FA) perception has been suggested. The aim of the present work was to study the relationships between taste sensitivity to oleic acid and the salivary proteome (2D electrophoresis) and metabolome (¹H NMR). This was achieved by comparing saliva from two groups of subjects, highly (sensitive+) and weakly sensitive (sensitive-) to the taste of oleic acid. Partial least squares-discriminant analyses (PLS-DA) were used to model the relationship between sensitivity to C18:1, and the proteome and metabolome data. The two groups could be discriminated by ten spots. In particular, cystatin SN, cystatin D, zinc-alpha-2-glycoprotein and carbonic anhydrase 6 were overexpressed in the sensitive+ group. The latter result was confirmed by ELISA. The overexpression of these proteins, which have been associated to taste perception, supports the argument that C18:1 is perceived by the taste system. The two groups could also be discriminated on the basis of eight

metabolites, with FA, FA/proline, lysine and FA/pyruvate overexpressed in the sensitive+ group and acetate, leucine/ isoleucine and butyrate overexpressed in the sensitive- group. The overexpression of these metabolites suggests a higher bacterial load in this group which could be implicated in perception of FAs.

Keywords Saliva, Proteome, Metabolome, Fat taste, Oleic acid

1 Introduction

Taste is one of the chemical senses that functions as a gatekeeper to the body. In addition to the long-recognized five basic tastes (sweet, bitter, sour, salty and umami), it was recently argued that fat can also be perceived by the taste system in humans. This was suggested first by sensory studies demonstrating that fatty acids (FAs) in their non-esterified forms can be perceived even excluding textural and olfactory cues (Chale-Rush et al. 2007; Mattes 2009).

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The concept was further supported by biological data since free FA receptors such as CD36 (Simons et al. 2011) and GPR120 (Galindo et al. 2012) are found on the tongue of humans. The taste of fat has raised much research interest, especially since it was proposed that gustatory sensitivity to FAs can contribute to food choice and preferences. For example, subjects hypersensitive to oleic acid (C18:1) have a lower energy intake than hyposensitive subjects, and more particularly a lower fat intake (Stewart et al. 2010). It was also shown that both oral sensitivity and gastrointestinal response to C18:1 are compromised in obese subjects (Stewart et al. 2011).

The first biological fluid in contact with food is saliva. Saliva has many roles in the oral cavity, including in food perception (Amerongen and Veerman 2002) by breaking down and/or solubilizing food constituents. Some recent studies have shown a correlation between the enzymatic composition of saliva and fat perception and liking (Neyraud et al. 2012) or sensitivity to FAs (Poette et al., in press). A modification of saliva lipolytic activity and antioxidant capacity after stimulation by C18:1 was observed specifically in highly sensitive subjects compared to less sensitive subjects (Mounayar et al. 2013). These studies highlight the interplay between FA perception and saliva composition. However, they were based on measuring specific characteristics potentially involved in FA perception such as flow rate, protein concentration, antioxidant capacity and enzymatic activities, while ‘omics’ approaches may provide additional information to understand mechanisms at the origin of taste sensitivity. Thus untargeted proteomics analysis revealed differences in salivary profiles between taste-impaired patients and healthy subjects (Igarashi et al. 2008), between 6-*n*-propylthiouracil (PROP) non-tasters/tasters/supertasters (Cabras et al. 2012) and between subjects hyper- and hypo-sensitive to the bitter taste of caffeine (Dsamou et al. 2012). The latter study suggested a mechanism of action whereby in-mouth proteolysis may be linked to sensitivity to bitterness.

In contrast to proteomic studies and to our knowledge, a metabolomics approach has never been applied to the topic of taste, let alone fat taste, sensitivity. Salivary metabolome profiles have nonetheless proved useful to discriminate populations based on pathological conditions (Silwood et al. 1999; Sugimoto et al. 2010; Aimetti et al. 2012), a variety of physiological and environmental parameters (Bertram et al. 2009; Sugimoto et al. 2013) or salivary collection method (Takeda et al. 2009; Neyraud et al. 2013).

In this context, the present study investigates the proteomic and metabolomic profiles of saliva of two groups of subjects highly (sensitive+) or weakly sensitive (sensitive-) to the taste of C18:1, in order to evaluate the contribution of saliva to fat taste perception.

2 Materials and methods

2.1 Subjects selection

Twenty-five male subjects who reported no health problem were selected from an initial panel of 73 subjects according to their taste sensitivity to C18:1. Details of the selection procedure are described in Mounayar et al. (2013). Briefly the screening procedure consisted in presenting four triangle tests to the subjects consisting of two control samples and one sample containing a low concentration of C18:1 (0.099 mM) and ten triangle tests consisting of two control samples and 1 sample containing a high concentration of C18:1 (1.77 mM). Each sample contained 4 mL of an emulsion containing 95 % water fraction and a 5 % oil fraction. The aqueous phase contained a 10 % (w/w) long-life non-fat powdered milk solution (low fat Régilait, France) diluted with mineral water (Evian, France). The fat phase (5 % w/w) was composed of a stable oil (Miglyol 812, Sasol, Germany) and oleic acid (Sigma Aldrich, France) at the required concentration. The subjects were wearing nose clips during the entire session in order to eliminate olfactory cues. The subjects were selected based on either their ability to perceive the low concentration samples in the four triangle tests series or their inability to perceive the high concentration samples in the ten triangle tests series. Two groups were thus created: one sensitive+, consisting of 12 subjects who could perceive C18:1 at low concentration (0.099 mM) and one sensitive-, consisting of 13 subjects who could not perceive C18:1 at high concentration (1.77 mM). Three subjects were excluded from the present study due to analytical difficulties. The data presented thus correspond to 11 subjects in the sensitive+ group, and 11 subjects in the sensitive- group. The entire protocol was approved by the Ethics Committee: Comité de Protection des Personnes Est-1, and by the Direction Générale de la Santé-France 5 (CPP no. 2010/59, AFSSAPS no. 2010-A01225-34).

2.2 Saliva collection and preparation

Resting saliva was collected between 6 and 7 p.m. on three consecutive days, by allowing the subjects to drool into a container at their own rhythm for 10 min. The saliva was then weighed and chilled at -80 °C until further use. Prior to ‘omic’ analyses, saliva was thawed and clarified by centrifugation at 14,000×*g* for 20 min at 4 °C. The three aliquots were pooled.

2.3 Protein content measurement and two-dimensional electrophoresis analysis

Protein concentration was measured in duplicates using the Bradford assay where bovine serum albumin (BSA) was

used as a standard. 2D electrophoresis was performed as in Dsamou et al. (2012) with the following minor modifications. In the first dimension, the protein load per strip was adjusted to 400 μg of proteins. The gels were stained using Sypro Ruby (Bio-Rad), and the images were acquired on a Pharos FX imaging system (Bio-Rad). The image analysis was done using Samespots software (NonLinear Dynamics). For each gel, spot volumes were normalized by the total volume of all spots. Values are expressed in ppm.

2.4 Carbonic anhydrase 6 and cystatin SN quantification

Carbonic anhydrase 6 and cystatin SN were quantified using Enzyme-Linked Immunosorbent Assay kits from USCN Life Science Inc., and Cusabio, respectively. The concentrations were obtained in ng/mL and then converted to ng/mg of proteins.

2.5 ^1H -NMR analysis

Samples were first thawed and 100 μL aliquots were added to 550 μL of deuterium oxide (D_2O) containing 0.25 mM sodium trimethylsilyl-[2,2,3,3- $^2\text{H}_4$]-1-propionate (TSP; as a chemical shift reference at 0 ppm). The solutions were centrifuged at $5,000\times g$ for 10 min before being placed in 5 mm NMR tubes. All ^1H NMR spectra were obtained using a Bruker DRX-600 Avance NMR spectrometer operating at 600.13 MHz for a ^1H resonance frequency and an inverse detection 5 mm ^1H - ^{13}C - ^{15}N cryoprobe attached to a cryoplatfrom.

All ^1H NMR spectra were acquired at 27 $^\circ\text{C}$ using the Carr–Purcell–Meiboom–Gill (CPMG) spin-echo pulse sequence with presaturation, as described previously (Neyraud et al. 2013) using 512 transients.

All NMR spectra were then data reduced using AMIX (version 3.9.11, Bruker, Germany) to integrate 0.01 ppm wide regions corresponding to the δ 9.0–0.7 ppm region. The δ 6.5–4.5 ppm region, which includes water resonance, was excluded. A total of 601 NMR buckets were included in the data matrices. Each integrated region was normalized to the total spectral area.

2.6 Statistical analysis

Partial least squares-discriminant analyses (PLS-DA) were used to model the relationship between the variable of interest (Y), in this case sensitivity to C18:1, and the NMR or 2D electrophoresis profiles (X). Analysis was performed as in Neyraud et al. (2013). Briefly, this includes several steps: (1) OSC filtering to remove variations not linked to the sensitivity (sensitivity was used as a corrective factor); (2) Standardization of filtered data (mean-centered and/or

scaled—unit or Pareto); (3) selection of number of components by cross validation; (4) Evaluation of predictive capacity of the model (Q^2 parameter); (5) assessment of model robustness by permutation test. Discriminant variables selection was done using variable importance in the projection (VIP) with a threshold of 1.0. Kruskal–Wallis test was finally used to determine proteins and metabolites that differed significantly between the two groups considering $p < 0.05$ as the level for significance. Multivariate analysis was performed using SIMCA-P software (V13, Umetrics AB, Umea, Sweden).

The overexpression of CA6 and cystatin SN, measured by ELISA, in sensitive+ subjects was tested using one-tailed Students' t test. This test was done using the Statistica software (StatSoft X). The level of significance was set at 5 %.

2.7 Protein identification by mass spectrometry

Spots of interest were excised using the Ex Quest spot cutter (Bio-Rad). The gel pieces were then washed twice with 0.1 M NH_4HCO_3 and 100 % acetonitrile (ACN) for 10 min. Reduction/alkylation was achieved by incubating the excised spots successively in 10 mM tris(2-carboxyethyl)phosphine (TCEP)/0.1 M NH_4HCO_3 for 30 min at 37 $^\circ\text{C}$, in ACN for a few minutes, in 55 mM iodoacetamide/0.1 M NH_4HCO_3 for 20 min and finally in ACN for 8 min. The digestion step was performed in 20 μL of a solution of 40 mM NH_4HCO_3 /10 % ACN containing 5 ng/ μL of trypsin (V5280, Promega). Eighteen microliters of trypsin were removed after 15 min incubation at 4 $^\circ\text{C}$, and 5 μL of 40 mM NH_4HCO_3 /10 % ACN was added before incubation at 37 $^\circ\text{C}$ for 2 h. Peptides extraction was performed by successive incubation in 1 μL of 0.1 % trifluoroacetic acid (TFA), 13 μL of ACN for 8 min and 3 μL of ACN. The resulting peptide extract was concentrated by evaporation.

For the analysis in MS and MS/MS mode, 1 μL of matrix (3.5 mg/mL α -cyano-4-hydroxycinnamic acid (HCCA)—ref 201344, Bruker Daltonics—in a solution of 50 % ACN/0.2 % TFA) and 0.5 μL of the digest were deposited on a ground steel target plate. Analysis was conducted using a MALDI-TOF/TOF UltrafleXtreme Bruker Daltonics in automatic mode. The database search was performed on SwissProt restricted to human entries. Two missed cleavages were allowed. Carbamidomethyl modification of cysteine was accepted as a stable modification and methionine oxidation as a variable modification. Mass deviation tolerance was set at 30 ppm in MS mode and 0.6 Da in MS/MS mode. Systematic reinterrogation on the list of unmatched peaks was performed until no new protein was identified.

3 Results

3.1 Differences in saliva protein composition between sensitive+ and sensitive- groups

Image analysis resulted in 325 spots detected and matched across all gels. The score-plot of the PLS-DA obtained from proteome data showed a clear separation between the two groups (sensitive+ and sensitive- groups) (Fig. 1a). This analysis generated a two latent components model with $R^2Y = 97.6\%$ and $Q^2 = 0.76$. Fifty-two spots were selected as having a VIP value higher than 1. Ten were significantly ($p < 0.05$, Kruskal Wallis test) different between the two groups (Fig. 2; Table 1) and eight were successfully identified by mass spectrometry (Table 2). Two spots were overexpressed in the sensitive- group, one being identified as containing alpha-amylase 2B and Ig alpha-2 chain C region A higher number of spots (8 out of 10) were over-expressed in the sensitive+ group: three spots correspond to cystatin SN among which spot 94 contained the co-migrating protein Ig alpha-2 chain C region. Spot 289 contained another protein of the

cystatin family (cystatin D). The three remaining spots corresponded respectively to carbonic anhydrase 6 (CA6), zinc-alpha-2-glycoprotein (ZAG), and Ig lambda-1 chain C regions. Quantifications obtained by ELISA confirmed the significant ($p = 0.008$) overexpression of CA6 in the sensitive+ group (mean \pm SEM; 195.8 ± 34.3 ng/mg of proteins) compared to the sensitive- group (110.1 ± 15.2 ng/mg of proteins) (Fig. 3a). Although non-significant ($p = 0.10$), concentration of cystatin SN tended to be higher in the sensitive+ group (mean \pm SEM; 11016 ± 3264 ng/mg of proteins) compared to the sensitive- group (mean \pm SEM; 6669 ± 802 ng/mg of proteins) (Fig. 3b).

3.2 Differences in saliva metabolite composition between sensitive+ and sensitive- groups

The score-plot of the PLS-DA obtained from filtered and centered NMR data showed a clear separation between the two groups (sensitive+ and sensitive- groups) (Fig. 1b). This analysis generated a 2 latent components model with

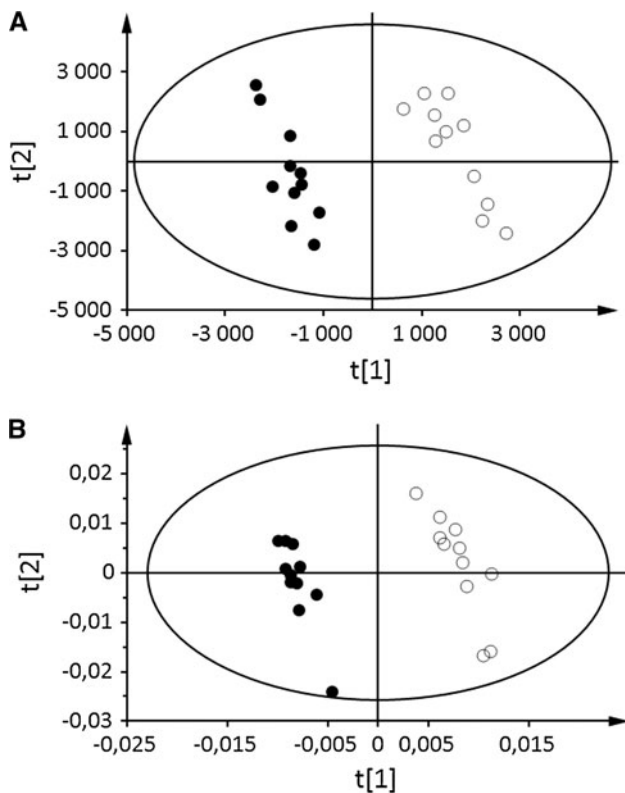


Fig. 1 Two-dimensional PLS-DA scores plot of 2D electrophoresis data (a) and of ^1H NMR spectra (b) from sensitive+ (filled circles, $n = 11$) and sensitive- (empty circles, $n = 11$) subjects. **a** $R^2 = 97.6\%$ and $Q^2 = 0.76$ (first axis $R^2 = 91.7$ and $Q^2 = 0.68$; second axis $R^2 = 5.9$ and $Q^2 = 0.76$). **b** $R^2 = 98.6\%$ and $Q^2 = 0.93$; (first axis $R^2 = 94.8$ and $Q^2 = 0.81$; second axis $R^2 = 3.8$ and $Q^2 = 0.64$)

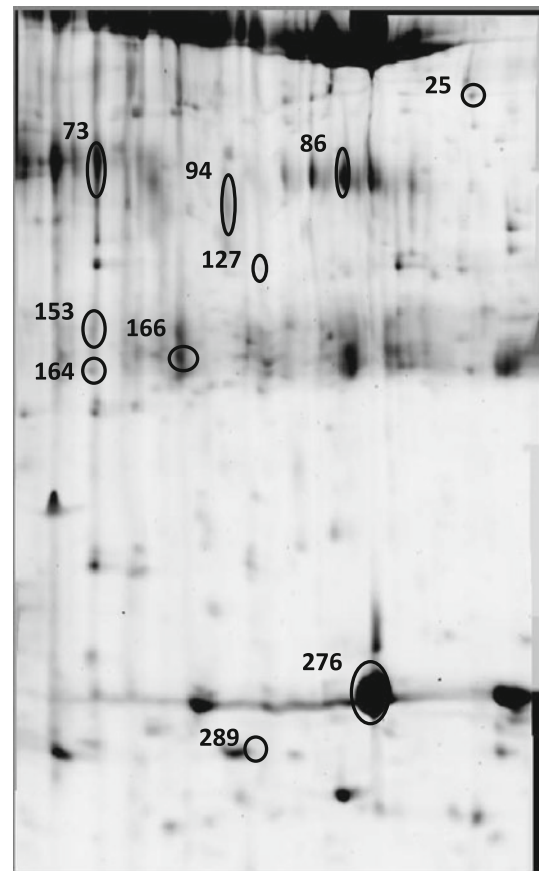


Fig. 2 Two-dimensional electrophoretic profile of human whole saliva. The circled spots are discriminant (VIP > 1, PLS-DA) and differently represented ($p < 0.05$, Kruskal-Wallis test) between the sensitive+ ($n = 11$) and sensitive- ($n = 11$) groups

$R^2Y = 98.6\%$ and $Q^2 = 0.93$, indicating a robust model. Thirty-four buckets were selected as having a VIP value higher than 1. Eighteen were significantly ($p < 0.05$, Kruskal Wallis test) different between the two groups. These corresponded to eight metabolites. The compounds assigned based on their 1D and 2D spectra are listed in Table 3. FA, FA/proline, lysine and FA/pyruvate were overexpressed in the sensitive+ group while acetate, leucine/isoleucine and butyrate were overexpressed in the sensitive- group.

Table 1 Discriminant spots in the PLS-DA model (VIP >1 and $p < 0.05$ for the Kruskal-Wallis test)

Identified proteins	Discriminant spot number	Rank ^a	p value
Overexpressed in the sensitive+ group			
Ig lambda-1 chain C region	153	11	0.004
Cystatin SN	276	1	0.005
ZAG	73	4	0.006
Cystatin SN	166	8	0.009
Cystatin SN/Ig alpha-2 chain C region	94	7	0.013
Carbonic anhydrase 6	86	2	0.016
Non identified	164	33	0.019
Cystatin D	289	9	0.032
Overexpressed in the sensitive- group			
Alpha amylase 2B/Ig alpha-2 chain C region	127	41	0.023
Non identified	25	24	0.027

^a Rank of variable importance in projection (VIP) for the first dimension of the PLS DA

4 Discussion

The aim of the present work was to investigate the link between saliva proteome and metabolome and taste sensitivity to C18:1. Overall, the sensitive+ and sensitive- groups could be discriminated on the basis of ten protein spots and eight metabolites.

Looking first at the proteome analysis, ten spots were particularly important in the discrimination of the two groups but in our view, the biologically most relevant information was provided mainly by five identified spots (73, 86, 166, 276 and 289). Thus, co-migration of proteins in spots 94 and 127 forbade determining whether the differences between the two groups were due to one or the other of the proteins. These two spots also contained immunoglobulin alpha-two heavy chain but since one spot was overexpressed in the sensitive+ group and the other in the sensitive- group, conclusions may not be drawn on the abundance or implication of IgA2 in C18:1 sensitivity. Similarly, alpha-amylase present in spot 127 co-exists in saliva under many isoforms. For example, more than 100 spots correspond to alpha-amylase on 2D electrophoretic gels in conditions comparable to the ones used here (Hirtz et al. 2005). It is therefore very unlikely that this spot alone indicates a more global difference in alpha-amylase abundance. Finally, the result concerning over-expression of immunoglobulin lambda-type light chain (spot 153) is also rather unconvincing since such light chains are not specific to a given type of immunoglobulins. In contrast, the five other spots overexpressed in sensitive+ subjects, corresponding to cystatin SN, cystatin D, ZAG and CA6 are of particular interest. The first technical reason is that one single protein was identified in each spot and these proteins' theoretical molecular weights matched reasonably well the apparent molecular

Table 2 Details of mass spectrometry results (MALDI-TOF or MALDI TOF-TOF) for proteins listed in Table 1

Spot	Protein	Swiss prot entry reference	Theoretical/estimated MW (kDa)	No. of peptides MS mode	Sequence coverage	Mascot score ^a MS mode	No. of peptides identified MS/MS mode	Mascot score ^b MS/MS mode
73	ZAG	P25311	34/38	12	44.4	85	2	76.6
86	Carbonic anhydrase 6	P23280	35/35				2	112.2
94	Cystatin SN	P01037	16/34	6	53.9	81	1	67.7
	Ig alpha-2 chain C region	P01877	38/34				2	135.6
127	Alpha-amylase 2B	P19961	58/31				3	117.0
	Ig alpha-2 chain C region	P01877	36/31				2	155.0
153	Ig lambda-1 chain C regions	P0CG04	11/29				3	187.7
166	Cystatin SN	P01037	16/28	10	70.1	146	2	220.3
276	Cystatin SN	P01037	16/14	10	70.2	150	2	220.1
289	Cystatin D	P28325	16/12				1	67.3

^a In MS mode, the identification is significant if Mascot score >56

^b In MS/MS mode, the identification is significant if Mascot score >40

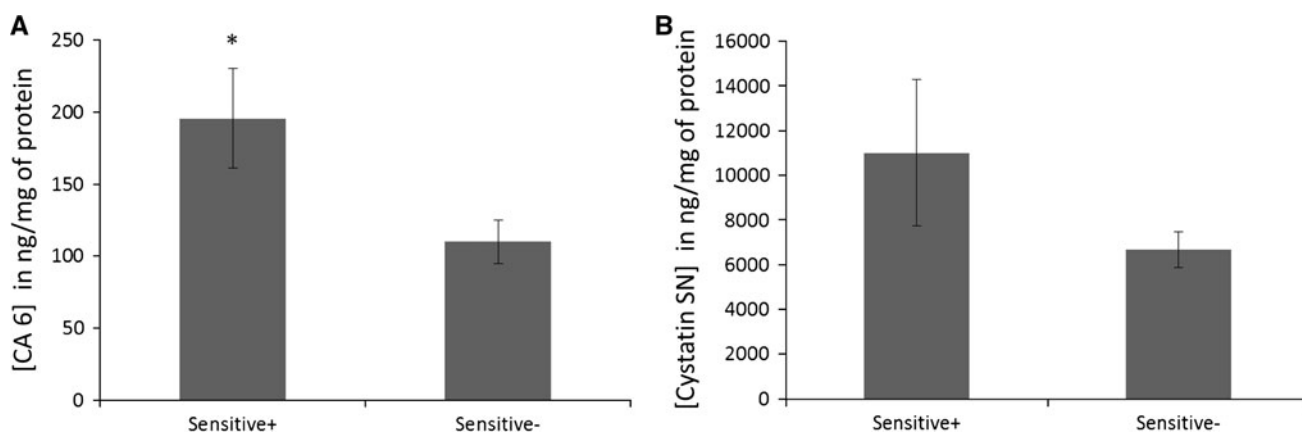


Fig. 3 Salivary concentration of carbonic anhydrase 6 (a) and cystatin SN (b) in the sensitive+ ($n = 11$) and sensitive- ($n = 11$) groups determined by ELISA. Mean values \pm SEM. Means are

significantly different for carbonic anhydrase ($p = 0.008$, Student's t test) and not significantly different for cystatin SN ($p = 0.10$, Student's t test)

Table 3 Discriminant metabolites in the PLS-DA model (VIP >1 and $p < 0.05$ for the Kruskal-Wallis test)

Identified metabolites	Discriminant NMR buckets (chemical shift ppm)	Rank ^a	p value
Overexpressed in the sensitive+ group			
Lysine	1.93; 1.94; 1.95; 1.96; 1.97	10; 13; 18; 27; 31	0.0014
FAs	2.33; 2.34; 2.32; 2.31	15; 17; 20; 32	0.0028
FAs/pyruvate	2.38	30	0.0035
FAs/proline	2.07; 2.04; 2.02; 2.03	6; 7; 8; 9	0.0165
Overexpressed in the sensitive- group			
Acetate	1.91	1	7.1e-05
Leucine/ isoleucine	0.96; 0.95	21; 28	0.0053
Butyrate	0.91	19	0.045

^a Rank of variable importance in projection (VIP) for the first dimension of the PLS DA

weight, suggesting that the spots contained full-length proteins. Spot 166 containing cystatin SN is an exception, but the large spot 276 also identified as cystatin SN confirmed that this protein was probably overall overexpressed in sensitive+ subjects. The second biological reason for paying special attention to these proteins is that cystatin SN, ZAG and CA6 have been previously been linked to the gustatory function performance.

Cystatin SN belongs to the 'Salivary cystatins', which are inhibitors of cysteine proteases contributing to the control of in-mouth proteolysis (Dickinson 2002). Cystatin SN was found to be under-represented in subjects hypersensitive to the bitter taste of caffeine compared to hypsensitive subjects (Dsamou et al. 2012). The hypothesis developed by the authors is that enhanced proteolysis

associated to lower levels of cystatin SN would affect the mucosal pellicle and facilitate access of taste molecules to taste receptors. Abundance of salivary cystatins was also positively correlated to acceptance of a bitter solution in human infants (our unpublished data), acceptance being presumably opposed to sensitivity for this particular taste. Therefore, these two studies suggest that cystatin SN is negatively linked to bitter taste perception. In the present study, in contrast and although not confirmed at a 5 % level of significance by ELISA, cystatin SN is positively linked to sensitivity to C18:1. This is somehow in line with the reduced cystatin SN abundance observed in patients complaining of taste disorders (Igarashi et al. 2008), but the mechanism behind this association remains to be elucidated. Moreover, the overexpression of cystatin D in sensitive+ subjects strengthens the assumption of a global role of cysteine proteases inhibition in sensitivity to C18:1.

ZAG has been reported in two studies correlating salivary protein composition and taste. Thus in patients complaining of taste disorders, this protein was under-expressed compared to healthy controls (Igarashi et al. 2008). In non-pathological conditions, ZAG was more highly represented in infants rejecting a bitter solution, i.e. probably more sensitive to it (our own unpublished data). In line with these two studies, ZAG is also here positively associated to sensitivity to C18:1, which may indicate a generic role of this protein in the taste function. Many diverse biological functions have been attributed to ZAG (reviewed in Hassan et al. 2008), such as regulating melanin production, initiating lipid mobilization or contributing to cell adhesion, none of these obviously linked to sensory perception. However, in the particular case of C18:1 perception, it is noteworthy that ZAG structure exhibits a hydrophobic groove which indicates its capability to bind FAs (Hassan et al. 2008), and in fact ZAG was experimentally shown to bind a variety of FAs such as

arachidonic, linolenic, eicosapentaenoic or docosahexaenoic acids (Kennedy et al. 2001). The FA binding properties of this protein may therefore impact on sensitivity to FA, either by modulating the level of detectable C18:1 in saliva or by acting as a C18:1 solubilizer.

Finally, CA6 is a key salivary protein when it comes to chemosensory perception. Thus, CA6 (which alternative name is gustin) was identified as playing a role in taste perception since it was expressed at lower levels in patients with hypogeusia (Henkin et al. 1975). It was later shown that deficiency in CA6 manifested by both olfactory and gustatory dysfunction, and induced severe taste buds morphological abnormalities (Henkin et al. 1999). More recently, genetic polymorphism in the CA6 gene, affecting the protein functionality through its ability to bind zinc, was described as contributing to the perceptive acuity to the bitter compound PROP (Padiglia et al. 2010; Calo et al. 2011) and this polymorphism may explain the reduced taste bud density in PROP non-tasters. The abundance of CA6 in 3-month-old infant saliva has also been linked to bitter taste acceptance (our own unpublished data). In the present study, quantitative results obtained by ELISA confirmed that the actual level of CA6 was lower in sensitive– compared to sensitive+ subjects. The fact that CA6 is linked to C18:1 detection suggests that it is perceived in the oral cavity through the taste buds. This finding adds to the mounting evidence that FA perception involves indeed a taste component.

Focusing now on salivary metabolome, sensitive+ and sensitive– subjects could be discriminated on the basis of eight metabolites. From a sensory point of view, and based on the “adaptation” mechanism, the over representation of FAs in the sensitive+ group was quite unexpected. Thus, by homology to the adaptation to basal sodium levels, where subjects with higher salivary sodium levels have higher salt detection threshold (Bartoshuk 1978), one could have expected that over representation of FAs would be associated to higher thresholds to C18:1 (sensitive– subjects). However, ¹H NMR spectroscopy is able to discriminate different classes of FA but not individual FAs such as C18:1, and chromatographic techniques are required to identify individual FAs. Based on the high interindividual variability in FA patterns (Neyraud et al. 2013), it is not certain that over representation in FAs is equivalent to over representation in C18:1. Concerning the amino acids, one may hypothesize that the discriminant representation of proline, lysine and leucine/isoleucine reflect differences between the two groups in abundance of proteins rich in these constitutive amino acids. Thus, the higher representation of proline may indicate higher levels of proline rich proteins (PRPs) since they contain up to 40 % of this amino acid (Bennick 1982). PRPs and taste sensitivity have been previously linked in a study where

higher levels of PRPs were found in saliva of subjects highly sensitive to the taste of PROP (Cabras et al. 2012). In a follow-up study, the mechanism of action was attributed in part to the constitutive amino-acids lysine and arginine, since supplementation in either PRPs or free lysine and arginine enhanced PROP perception (Melis 2013). Interestingly in our study, higher levels of lysine were also associated to increased perception. The authors suggested a mechanism involving interaction between the tastant and PRPs. Further investigation is necessary to determine whether this may apply also to C18:1. Alternatively, PRPs and lysine would be associated to global taste acuity though a mechanism yet to be elucidated.

Among the metabolites overexpressed in the sensitive– group, acetate and butyrate are, with propionate, the main secretory products of anaerobic bacteria secreted in the gastro-intestinal tract (Vinolo et al. 2011). In some studies using ¹H NMR, high anaerobic bacterial activity was suggested to be at the origin of the over-representation of acetate and butyrate in saliva of children with caries lesions (Fidalgo et al. 2013), in saliva of patients presenting generalized chronic periodontitis (Aimetti et al. 2012) or in biopsies of active carious dentin of patients with primary root carious lesion (Silwood et al. 1999). In the latter, the authors also found a negative correlation between acetate and pyruvate (mol% content). Such negative correlation is comparable to our observations in the two groups. Thus, it seems reasonable to propose that over-representation of these short chain fatty acids (SCFAs) attest of a higher microbial metabolism. This is also supported by the over-representation of leucine/isoleucine. Indeed, leucine is implicated in the regulation of amino acid metabolism and related processes of a wide range of bacteria through the binding to leucine-responsive regulatory proteins (Lrp) (Brinkman et al. 2003). The higher concentration of leucine/isoleucine would therefore be an indicator of favorable conditions for oral bacteria. Altogether, this suggests that higher bacterial activity is associated to a lower sensitivity to C18:1, and more generally that the oral flora is not independent from perception of FAs.

5 Concluding remarks

Proteomics and metabolomics approaches were applied to whole saliva and proved useful to discriminate groups of subject. Over-representation in the sensitive+ group of salivary proteins like CA6, ZAG, cystatins and possibly PRPs, which have been associated to taste perception, supports the argument that C18:1 is perceived by the taste system. In addition, over-representation in the sensitive– group of metabolites like SCFAs and leucine/isoleucine suggest that the bacterial load is somewhat associated to

taste perception of FAs. Altogether, these results show the benefit of using different “omics” approaches on saliva to open new research perspectives in the field of human chemosensory perception.

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