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Associations between food consumption patterns and saliva composition: Specificities of eating difficulties children

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HIGHLIGHTS

- Saliva composition and diet were characterized in eating difficulties vs healthy children.
- Patients showed lower food consumption frequency scores and higher food selectivity.
- Consumption of sweets and drinks was linked to carbonic anhydrase 6 in both groups.
- Different associations between saliva and diet were identified in the two groups.

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ABSTRACT

Identifying objective markers of diet would be beneficial to research fields such as nutritional epidemiology. As a preliminary study on the validity of using saliva for this purpose, and in order to explore the relationship between saliva and diet, we focused on clearly contrasted groups of children: children with eating difficulties (ED) receiving at least 50% of their energy intake through artificial nutrition vs healthy controls (C). Saliva of ED and C children was analyzed by various methods (targeted biochemical analyses, 2-D electrophoresis coupled to MS, ^{1}H NMR) and their diet was characterized using food frequency questionnaires, considering 148 food items grouped into 13 categories. Complete datasets were obtained for 16 ED and 16 C subjects (median age 4.7 y and 5.0 y, respectively) and the statistical link between salivary and dietary characteristics was studied by Multiple Factor Analysis (MFA).

Overall, ED children showed as expected lower consumption frequency scores and higher food selectivity. The two groups of children differed in "diet/saliva" associations. Some distinctive salivary variables were common to both groups of children. For example, carbonic anhydrase 6 and the consumption frequency of biscuits & sweets and drinks were positively associated with the MFA axis 1 in C children, but oppositely associated in ED children. Specifically for ED children, abundant salivary proteins (cystatins, amylase, amylase fragments) and some metabolites (amino acids, galactose, lactate) correlated with axis 1, together with the consumption frequency of sauces & seasonings, bread & cereal products, ready-to-eat meals, fish, biscuits & sweets, drinks and potatoes. Specifically for C children, several proteins (serum albumin, haptoglobin, Igκ, apolipoprotein A-1, α-1 antitrypsin) correlated with axis 1, together with the consumption frequency of biscuits & sweets, milk & dairy products, drinks, fruit, meat and vegetables.

This study demonstrates that the qualitative aspect of diet is linked to saliva composition, and that the associations between dietary consumption and salivary composition differ between groups of subjects with contrasted diets.

1. Introduction

Saliva is gaining interest in the broad field of sensory physiology and dietary behavior, first because it has an impact on perception of food

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products during consumption. For example, saliva dilutes taste molecules and thus facilitates their access to taste receptors. Salivary proteins can bind flavor compounds such as tannins [1,2] or aroma compounds [3,4], with consequences on sensory attributes such as astringency or aroma persistence. Saliva is also necessary to form a bolus that is easy to swallow, and contributes to texture perception of solid or semi-liquid foods [5,6]. For all these reasons, saliva is a factor of food acceptance and enjoyment. For example, a drop in saliva production after chemoradiation has been related to long-term avoidance of crunchy foods [7], despite the fact that the decreased salivary flow did not result in inefficient swallowing. Some saliva compositional indicators have also been statistically associated to liking for fat, saltiness and sweetness measured through hedonic tests on a variety of food products [8]. On the other hand, saliva secretion and composition are partly controlled by diet. It has for example long been known that some specific dietary constituents can influence saliva composition. In rodents in particular, enrichment of diet in capsaicin results in increased levels of cystatin S-like proteins [9] and diet enrichment in tannins modifies the relative abundance of several proteins [10]. In human subjects, the intake of macronutrients has also been linked to saliva characteristics, for example fat intake and fatty acid profiles [11], carbohydrate intake and antioxidant capacity and amylase activity [8] or more generally energy and flow rates as observed in patients suffering from early childhood protein-energy malnutrition [12] or anorexia [13]. Concerning the type of food consumed, data are rather scarce. The transition from a milk diet to a diversified diet in infants induced modifications of protein and peptide profiles [14,15] which were interpreted as a protective mechanism against excessive proteolysis. In adults, adopting a lactovegetarian diet also modified saliva flow rate and buffering capacity [16], while the same microbiota but discriminant metabolome are reported in the saliva of omnivorous, vegetarian and vegan subjects [17]. Another recent study compared saliva of children with eating difficulties receiving at least 50% of their energy intake through artificial nutrition vs healthy children [18]. Thanks to the use of artificial nutrition in eating difficulties patients, energy and nutrient needs were covered in all children. Nevertheless, proteomics and metabolomics methods allowed discriminating the two groups confirming that oral stimulation by food intake plays a role in shaping the composition of saliva.

Finding objective markers of diet in saliva would represent an advance in research fields such as nutritional epidemiology where most studies rely on questionnaires and self-assessment of dietary intake, which are prone to recall and social desirability biases. A study on serum metabolome [19] successfully identified markers of consumption of specific food groups (e.g. citrus fruit, coffee…). However, saliva is increasingly recognized as a valuable fluid for biomarker analysis, mainly because it offers the advantages of simple and non-invasive sampling. As a preliminary study on the validity of using saliva to define objective markers of diet, and with the objective to explore how saliva may reflect the qualitative aspects of diet and oral intake of food, we focused on clearly contrasted groups of children, with eating difficulties vs healthy. Specifically, our objective was to document whether the associations between saliva composition and food consumption patterns differed between eating difficulties and healthy children.

2. Material and methods

2.1. Participants

The study included 21 children (12 females, 9 males) presenting eating difficulties which developed after the use of artificial nutrition in the neonatal period. The specific inclusion criterion was that patients had received artificial nutrition for at least 3 months in their first 6 months of extra-uterine life. At the time of the study, the patients were aged 4.8 y ± 1.9 (median ± STD) and were still fed via the enteral or parenteral route for at least 50% of their total energy intake. The controls were 23 healthy children (11 females, 12 males) aged 5.3 y ± 2.6 (median ± STD). Thanks to nutritional support in eating difficulties patients, the two groups of children were overall comparable in their energy and macronutrients intake. The study was approved by the local ethical committee (Comité de Protection des Personnes Sud-Est II, no. 2011-039-2) and by the Direction Générale de la Santé (AFFSAPS, no. B111079-90). Written informed consent was obtained from the children's parents. In the remaining of the text, patients with eating difficulties are referred to as “ED” and healthy controls as “C”.

2.2. Saliva sampling and analysis

Saliva was aspirated from the floor of the mouth through soft plastic tubing into a container placed on ice. The targeted duration of aspiration was 5 min but was shortened in case of distress or discomfort. Shortly after sampling, saliva was clarified by centrifugation (10,000*g*, 15 min, 4 °C). The supernatants were aliquoted and stored at −80 °C until analyzed. Analyses were performed as described in details elsewhere [18]. Briefly, total protein concentration measured using a Bradford protein assay (Bio-Rad) was expressed in $mg \cdot ml^{-1}$. Amylolytic, lipolytic and proteolytic activities were expressed as in milli International Enzyme Activity Units per mg of protein ($mIU \cdot mg^{-1}$). Total antioxidant status (TAS) measured using an ORAC assay kit (Zen-Bio) was expressed as micromoles Trolox equivalents per mg of protein (μmol Trolox eq. mg^{-1}).

The abundance of 163 protein spots was measured by image analysis following 2-dimensional electrophoretic separation and expressed as arbitrary units (AU). Proteins of interest were identified by mass spectrometry, either by MALDI-TOF MS and MS/MS analyses as previously described [20] or by nanoLC-ESI MS/MS for spots that remained unidentified by MALDI TOF-TOF. In that case, peptides of the tryptic digest of the spot were separated with a nanoRSLC (ThermoScientific) fitted with a C18 trapping column (5 μm particle diameter, 300 μm inner diameter, 5 mm length; ThermoScientific) and a C18 analytical column (2 μm particle diameter, 75 μm inner diameter, 150 mm length; ThermoScientific). A 60-min gradient was applied, using the two following buffer solutions: 2% acetonitrile/0.1% formic acid and 80% acetonitrile/0.1% formic acid. As peptides eluted from the column, they were electrosprayed directly into an LTQ-Orbitrap Elite mass spectrometer (ThermoScientific) equipped with the TriVersa NanoMate nanospray source (Advion). Full-scan spectra (400–1700 *m/z*, resolution of 120,000) were acquired, followed for each spectrum of fragmentation MS/MS spectra for the 20 ions with the highest intensity. Identifications were achieved with Proteome Discoverer (1.4) software, searching the UniProt databank restricted to human entries. For each spot, the protein providing the highest score was retained.

The abundance of 536 metabolite buckets was acquired after analysis by ^{1}H NMR.

In addition to these analyses, lysozyme activity and the concentration of carbonic anhydrase 6 were also measured. Lysozyme activity was determined using an EnzCheck Lysozyme Assay Kit (Molecular Probes, The Netherlands), which consists in measuring lysozyme activity on a *Micrococcus lysodeikticus* substrate labelled with fluorescein. The intensity of fluorescence proportional to lysozyme activity is read against a lysozyme standard and expressed in AU/mg^{-1}. Carbonic anhydrase 6 (CA6) was quantified using an ELISA kit (USCN Life Science Inc.) and results were expressed as $ng \cdot mg^{-1}$.

2.3. Food consumption habits

Food consumption was evaluated using food frequency questionnaires. A total of 148 food items was included in the questionnaire (Supplementary data S1). These were grouped into 13 categories: vegetables (15 items), potatoes (5 items), meat (6 items), eggs & processed meats (10 items), fish (9 items), bread & cereal products (10 items), ready-to-eat meals (5 items), cheese (12 items), milk & dairy products