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New perspective on taste: exploring association among oral perception, tongue physiology and oral microbiota composition

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Smile often.

Think positively.

Give thanks.

Laugh loudly.

Dream big.

Abstract

Background: Food choices and eating habits are a complex behavior mediated by a number of biological and environmental factors. Taste is considered one of the main predictor of individual food selection and varies greatly among individuals. Thus, there is a considerable interest in understanding how, and to what extent, individual variability can determinately contribute to explain food preferences and behaviors. Moreover, with increasing prevalence of diseases related to over nutrition, there is considerable interest in identifying the factors that could predispose individuals to such disease by influencing dietary decisions. Given that it has been recently proposed that the microbiota could affect individuals' eating behaviors and food preferences, and the composition of such microbiota appears to have an important but still unclear role in obesity development, a novel approach to inquiry into the relationship between obesity, taste sensitivity and oral microbiota composition seems required.

Aim: The general aim of this thesis is to explore taste perception in relation to different variables, using a multidisciplinary approach. Specifically, the activities were devoted to: i) explore inter individual differences in taste perception and their relationship with the composition of oral bacteria and food intake; iii) investigate cross-cultural preferences in oral processing behaviors in Asian and Caucasian consumers and how these may be related to lingual tactile acuity and the density of fungiform papillae on the anterior part of the tongue; iii) investigate among host related factors (such as taste perception and oral microbiota composition) that are proposed as potential causes affecting childhood weight gain.

Results: The findings of the present thesis confirmed that 6-n-propylithiuracil (PROP) responsiveness could be used as an reliable index for general taste sensitivity. Moreover, interindividual differences in taste perception were found to influence habitual food consumption and intake.

No direct correlations between tactile acuity and PROP responsiveness or Fungiform Papillae Density (FPD) were found. Moreover, cross-cultural differences in preferred oral processing behaviors and taste sensitivity have been found in the two population cohorts considered, with Asian subjects predominantly preferred to manipulate foods between the tongue and roof of the mouth and showed a greater FPD and PROP responsiveness.
Regarding the factors that could predispose individuals to obesity disease, the present results showed that taste sensitivity occurred differently accordingly to subjects' nutritional status. In particular, obese children and adolescents presented a lower ability in correctly identifying taste qualities compared to the group of normal-weight. Moreover, the oral microbiota composition seems to have a role in influencing and modulating taste perception, as well as some taxa are found to be positively associated with vegetable-rich (*Prevotella*) or protein/fat-rich diets (*Clostridia*).

Conclusions: The present thesis could help shed light on the complexities of human eating behavior, understanding how and which host-related factors could affect people food choices and habits. Moreover, these outcomes could be used as a starting point to: i) help food industries in developing food products that match different consumers' needs and ii) aid to develop further strategies for obesity prevention and therapy. Lastly, the potentiality of this multidisciplinary approach opens new avenues of research by highlighting associations between sensory and consumer science, food technology and nutrition.

Riassunto

Introduzione: Le scelte e le abitudini alimentari sono un comportamento complesso mediato da una serie di fattori biologici e ambientali. Tra questi, il gusto è considerato uno dei principali fattori coinvolti nelle scelte alimentari e varia notevolmente da individuo a individuo. Pertanto, vi è un notevole interesse nel comprendere come, e in che misura, la variabilità individuale possa contribuire in modo determinante a spiegare le preferenze e i comportamenti alimentari. Con la crescente prevalenza di malattie legate all'eccessiva alimentazione si è sviluppato, inoltre, un notevole interesse nell'individuare i fattori che potrebbero predisporre gli individui a tali patologie, influenzando le loro scelte. Recentemente, è stato proposto che anche il microbiota potrebbe influenzare i comportamenti e le preferenze alimentari degli individui e che la sua composizione possa avere un ruolo importante, tuttavia ancora poco chiaro, nello sviluppo dell'obesità. Pertanto, ci è apparso necessario e innovativo approfondire l'ancora inesplorata relazione tra la sensibilità gustativa, l'obesità e la composizione batterica orale.

Obiettivo: l'obiettivo generale di questa tesi di dottorato è quello di esplorare la percezione sensoriale in relazione a differenti variabili, applicando un approccio multidisciplinare. In particolare, le attività svolte sono state indirizzate a: i) esplorare le differenze inter-individuali nella percezione gustativa e studiare la loro relazione con la composizione batterica orale e l'assunzione di cibo; ii) studiare le differenze interculturali tra consumatori asiatici e caucasici nelle preferenze di consistenza e sensibilità gustativa e tattile; iii) indagare in che misura la percezione gustativa e la composizione del microbiota orale possano essere considerate potenziali cause nell'aumento del peso corporeo in età scolare.

Risultati: I risultati hanno confermato come la sensibilità al 6-n-propiltiuracile (PROP) risulti un indice affidabile per valutare la sensibilità gustativa generale. Inoltre, è stato evidenziato come le differenze interindividuali nella percezione gustativa influenzino il consumo e l'assunzione di cibo.

Non sono state trovate correlazioni dirette tra sensibilità tattile, sensibilità al PROP o la densità di Papille Fungiformi (FPD). Sono state riscontrate, inoltre, differenze legate alle preferenze di consistenza e sensibilità gustativa tra le due popolazioni considerate, evidenziando come i soggetti asiatici preferiscano manipolare prevalentemente gli alimenti tra la lingua e il palato e presentino una maggiore sensibilità al PROP e una maggiore densità di PF. Per quanto riguarda i fattori che potrebbero predisporre gli individui all'obesità, i risultati attuali hanno mostrato come i soggetti obesi presentino una distorta sensibilità gustativa rispetto ai soggetti normopeso. In particolare, i bambini e gli adolescenti obesi sembrano essere caratterizzati da una minore capacità di identificare correttamente i gusti rispetto al gruppo di normopeso. Inoltre, la composizione del microbiota orale sembra avere un ruolo nell'influenzare e modulare la percezione gustativa e, inoltre, alcuni *taxa* sono risultati positivamente associati a diete ricche di verdure (*Prevotella*) o ricche di proteine/grassi (*Clostridia*).

Conclusioni: La presente tesi potrebbe aiutare a comprendendo come e quali fattori abbiano un'influenza sulle scelte e sulle abitudini alimentari delle persone. Questi risultati, inoltre, potrebbero i) essere un punto partenza per le industrie alimentari nello sviluppo di prodotti che soddisfino le diverse esigenze dei consumatori e ii) aiutare a sviluppare ulteriori strategie per la prevenzione e la terapia dell'obesità. Infine, le potenzialità di questo approccio multidisciplinare potrebbero aprire nuove possibilità di ricerca mettendo in luce associazioni ancora non indagate tra le scienze sensoriali, le tecnologie alimentari e la nutrizione.

Table of contents

FOREWORD

Foreword

The present thesis is organized in six chapters. After an introductory part (**Chapter 1**), in which a literature review about the topics of the present thesis has been provided, the rationale and aims are described in **Chapter 2**. **Chapters 3-5** are devoted to present the specific research activities conducted during the three years of project.

The first study was carried out to explore inter individual differences in taste perception, applying different widely used sensory methodologies, and their relationship with the oral microbiota composition and food intake. This study was performed in collaboration with Simone Guglielmetti, Patrizia Riso, Giorgio Gargari and Ranjan Koirala all enrolled at our Department (DeFENS). The collaboration resulted in two publications in *Scientific Reports* and *Nutrients*, respectively.

The second study was carried out to investigate among host related factors that are proposed as potential causes affecting childhood weight gain. The study was performed in collaboration with Gian Vincenzo Zuccotti and Chiara Mameli enrolled at Dept. of Pediatrics (Buzzi Children's Hospital, Milan), and with Claudio Bandi, Simona Panelli and Francesco Comandatore enrolled at Clinical Pediatric Research Center Romeo and Enrica Invernizzi (Milan). The collaboration resulted in one publication in *PLOS One*.

Over a period abroad spent at University of Copenhagen, a study was carried out to investigate cross-cultural differences in oral processing behaviors and taste and texture perception between Asian and Caucasian consumers. The study was financially supported by Arla Foods amba and performed in collaboration with Wender Bredie and Jing Liu enrolled at Dept. of Food Science and with Jon Sporring and Chenhao Wang enrolled at Dept. of Computer Science. The collaboration resulted in one publication in *Food Quality and Preference*.

The general conclusions drawn from this thesis and future perspectives have been reported in **Chapter 6**.

CHAPTER 1

LITERATURE REVIEW

Literature review

Taste perception

The gustatory system is responsible for the regulation of taste perception and aids individuals in evaluating the food nutrient content and in discriminating between safe and harmful foods (Bachmanov and Beauchamp, 2007; Chandrashekar et al., 2006). Moreover, in humans, taste contributes to the overall enjoyment of a meal. It is generally assumed that humans perceive five taste modalities: sweet, umami, salty, bitter and sour and, recently, it has been proposed that additional qualities, such as fatty and metallic, might also be considered basic tastes (Chaudhari and Roper, 2010). Each taste modality is linked with different nutritional or physiological requirements, or indicates a potential dietary risk (Chaudhari and Roper, 2010). Indeed, sweet taste is supposed to detect calorie-rich foods, umami allows to identify foods rich in amino acids, salty taste is associated with electrolytes, while sour taste warns against spoiled or unripen foods and bitter taste helps in identifying potential poisonous or toxic substances.

The ability to detect and differentiate between food-derived chemical stimuli is mediated by receptor cells within taste buds, whose activities may be heavily impacted by variation in receptor genes (Chandrashekar et al., 2006). Taste buds have a ‘garlic bulb’ structure and primarily reside within the gustatory papillae (fungiform, foliate and circumvallate) of the tongue. Among the gustatory papillae, are the fungiform papillae (FP) the anatomical structures major involved in the detection and transduction of oral stimuli. Indeed, both gustatory (chorda tympani nerve - cn. VII) and trigeminal (cn. V) nerves co-innervate lingual FP (Mistretta and Liu, 2006), transducing gustatory, somatosensory and irritant sensations (Prescott and Tepper, 2004). Given the double innervation of FP, these anatomical structures has been selected as one of the phenotypic markers of taste sensitivity, due to their relative

abundance and accessibility on the tongue anterior part, and their association with the density of taste buds (Miller and Reedy 1990a, 1990b).

Each taste bud contains around 50-100 Taste Receptor Cells (TRCs) which are classified into four subtypes: i) *type I cells* which are considered to be the major mediator of perception of salt (for example, NaCl or KCl) (Chandrashekar et al., 2010); ii) *type II cells* which express receptors for sweet, umami and bitter tastants (De Fazio et al., 2006; Yoshida et al., 2006; Tomchik et al., 2007); iii) *type III cells* (presynaptic cells) which are the only type of TRCs that form conventional neuronal synapses with sensory afferent intragemmal nerve fibers and are involved in perception of sour taste (LopezJimenez et al., 2006); iv) *taste cell precursors* which are quiescent precursor cells and immature taste cells present at the base of taste buds (Chaudhari and Roper, 2010). The three different type cells are reported in Figure 1.

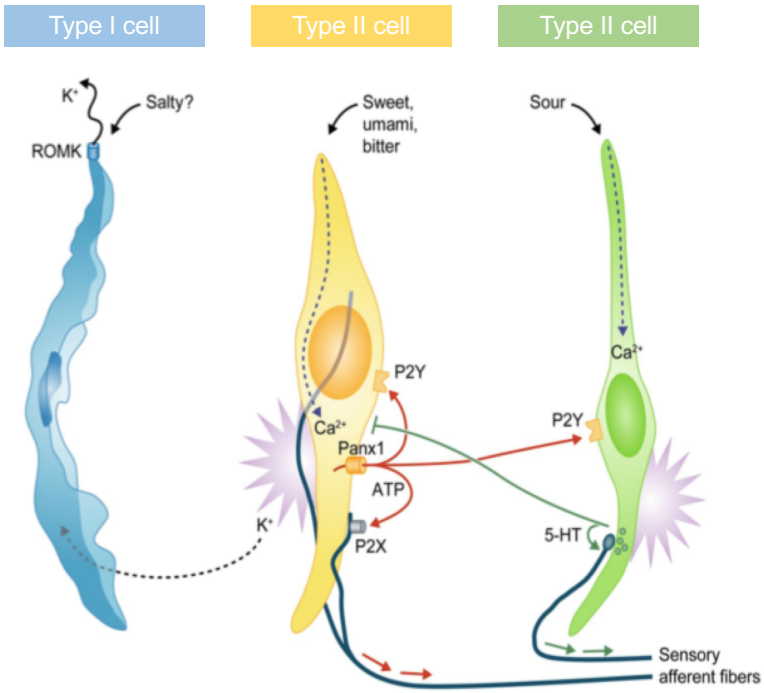

Figure 1. The three major classes of taste cells (modified by Chaudhari and Roper, 2010).

Data in literature provide evidences that the taste buds in the FP contain both mechanoreceptors and fatty acid receptors (i.e. the CD36 and GPR120 receptors) (Galindo et al., 2012; Mattes, 2009; Simons et al., 2011). Hence, it is possible to argue that a higher number of FP may enhance tactile and chemosensory perception, generally increasing the perception of texture. However, much of our understanding related to the physiological mechanism behind the perception of texture in the mouth is derived from findings in the skin (for a review: Abraira and Ginty, 2013). In non-hairy skin, four specialized mechanoreceptor nerve endings have been identified to convey specific sensations of touch. The Merkel cell disks are in the basal layer of the epidermis and consist of clusters of Merkel cells which are slowly adapting and respond to edges and points. Meissner corpuscles are localized in the dermal papillae and consist of horizontal lamellar cells, rapidly adapting, which respond to motion as well as light touch (Roudaut et al., 2012; Foegeding et al., 2015). Ruffini endings and Pacinian corpuscles are both located in deeper layers of the dermis. Ruffini endings are slowly adapting and have been associated with sensations of stretch, while those terminating in Pacinian corpuscles are rapidly adapting and detect high-frequency vibration (Roudaut et al., 2012; Foegeding et al., 2015).

The same nerve fibers present in the non-hairy skin have been recognized in the oral surfaces, with the possible exception of Pacinian corpuscle mechanoreceptors (Linne and Simons, 2017). Moreover, contrary to taste, each type of mechanoreceptor is not responsible for directly coding specific texture modalities such as smoothness, roughness, or viscosity, which are instead coded by a combination of signals (Foegeding et al., 2015; Linne and Sions, 2017).

Genetic differences in taste perception

Taste perception varies greatly between individuals in function of genetic variation in the genes encoding taste receptors. These allelic variations are proposed to be important determinants of individual differences in perceived

taste intensity and food preference, with important consequences for food selection, nutrition, and health (Duffy, 2007; Tepper, 2008; Tepper et al., 2009). Thus, understanding the genetic causes of these variations may contribute to predict individual taste function and potentially dietary patterns followed by such individuals.

As reported previously, bitter taste evolved as a sensing mechanism to identify and avoid a wide range of potential poisonous or toxic substances. In humans, thousands of bitter compounds are detected by a family of 25 bitter receptors (TAS2Rs) from the G protein-coupled receptor (GPCR) superfamily (Chandrashekar et al., 2006; Chaudhari and Roper, 2010) as reported in Figure 2.

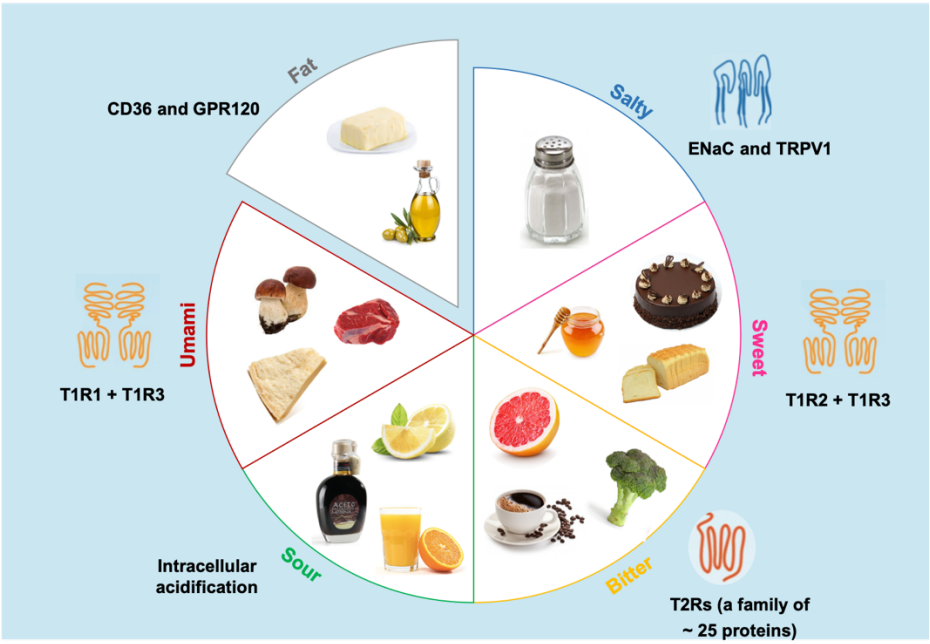

Figure 2. Taste qualities and related taste receptors (modified by Chaudhari and Roper, 2010).

Genetic variations in TAS2R bitter receptor may contribute to the observed high variability in bitter taste (Bachmanov and Beauchamp, 2007; Kim et al., 2003). The most widely studied is the single nucleotide polymorphisms of the

TAS2R38 gene. This genetic variation is associated with different perception abilities for compounds containing the thiocyanate group (NC = S) responsible for bitter taste, such as phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) (Blakeslee and Fox, 1932). There are two major forms of TAS2R38, PAV (Proline, Alanine, Valine) and AVI (Alanine, Valine, Isoleucine) haplotypes.

This combination of alleles underpins the broad segregation of the population into three different phenotypes, generally referred as PROP taster status (Bartoshuk, 1993): PROP Non-tasters (AVI/AVI alleles, less responsive), PROP medium-tasters (PAV/AVI alleles, medium responsive), and PROP Super-tasters (PAV/PAV alleles, most responsive). Subsequent studies went on to estimate the frequency of Non-tasters in hundreds of people worldwide (see Guo and Reed, 2001 for a review). These studies showed that the prevalence of a lack of sensitivity to bitter taste ranges from ~3% in Africa to 10-20% in China and Japan and 40% in India, whereas the estimated proportion of non-tasters in Caucasian populations is about 25-30%.

The PROP responsiveness has been linked to the sensitivity to many other natural compounds, such as caffeine, quinine, and urea, sucrose, sodium chloride and fat (Hayes and Duffy, 2007; Prescott et al., 2004; Tepper et al., 2001), suggesting that PROP responsiveness could be considered another general index of wider sensitivity to taste sensations. Moreover, many studies reported that the density of fungiform papillae and associated taste buds positively correlated with the perceived bitterness intensity of PROP, tough considerable variation in the magnitude of this association has been found (Duffy et al., 2010; Essick et al., 2003; Hayes et al., 2010; Nachtsheim and Schlich, 2013, 2014). However, this relationship is still unclear, and some recent investigations conducted on a wide number of observations found that PROP responsiveness, TAS2R38 haplotype and perceived taste intensity were not related to FP density (Dinnella et al., 2018; Fischer et al., 2013; Garneau et al., 2014).

A number of compounds such as sugars, artificial sweeteners, d-amino acids and sweet proteins can be perceived as sweet (Boughter and Bachmanov, 2007; Nelson et al., 2001). Unlike bitter compounds, sweet substances are inherently perceived as pleasant and are clustered separately from taste receptor cells related to bitter taste. Sweet taste sensation occurred to detect foods high in carbohydrates, particularly sugars, which are usually rich in energy (Hladik et al., 2002). As in the case of sweet taste, umami taste is perceived as pleasant and has an important role in diet since aids individuals in identify foods rich in amino acids. The main substance eliciting umami taste is the amino acid L-glutamate, which generally occurs in food as monosodium glutamate (MSG). Both tastes processing seem to be closely related to each other at molecular level, since are perceived by receptors belonging to the T1R family (Figure 2). In particular, the sweet taste receptor consist of a dimer formed by T1R2 and T1R3, while T1R1 combined with T1R3 forms the dimer responsible for the perception of umami taste (Precone et al., 2019; Nelson et al., 2002). Evidences exist suggesting that both sweet and umami tastes are modified by genetic difference between individuals. In particular, variants in the TAS1R3 gene have been associated with a reduced ability in perceiving sweet and umami tastes (Fushan et al., 2009), while variations in the TAS1R1 gene lead to an increased sensitivity to umami (Shigemura et al., 2009).

Salty taste mostly refers to sensation elicited by sodium chloride (NaCl). Many other cations (e.g. NH_4^+, K^+, and Li^+) could elicit a salty response (DeSimone and Lyall, 2006), albeit their taste has been associated with other taste sensations such as bitterness, sourness, or astringency, rather than salty (Roper, 2007). While the basis of salty taste perception has been studied for years, its molecular mechanism remains unclear. The Epithelial Sodium Channel (ENaC) and the TRPV1 nonspecific cation channel has been identified as putative salty taste receptors (Bachmanov and Beauchamp, 2007) as reported in Figure 2. Genetic variations in these genes encoding for ENaC channel have been linked to changes in salty taste perception (Dias et al., 2013). To date, variability in responses to salty stimuli has been examined for

decades, but a direct genetic link to human salt taste perception has yet to be found (Garcia-Bailo et al., 2009).

It is commonly accepted that sour taste perception is triggered when acidic tastants stimulate the taste buds, initiating the depolarization of acid-sensitive Taste Receptor Cells (Richter et al., 2003). However, taste receptors for sour tasting are not well-characterized and very little is known about interindividual variation in the perception of this basic taste, and how such variation may be genetically explained.

In contrast to taste perception, there are no single and specific texture receptors due to its multiparameter nature and tissues involved (e.g. periodontal, skin, in the temperomandibular joint). Given its complexity, the mechanisms involved in the perception of texture in the mouth remain poorly understood. Although some findings suggested that pressure sensitivity on the tongue varies across people (Breen et al., 2019; Yackinous and Guinard, 2001), works on interindividual differences in oral tactile perception are relatively limited.

Taste and texture perception assessment

Sensory testing can be used to measure the ability of a subject to taste given stimuli. Taste thresholds and supra-threshold taste sensitivity are the most used measurements to test individual's gustatory ability. Threshold measures permit individual comparisons of sensitivity to certain stimuli, while supra-threshold measures examine an individual's perceived taste intensity (ASTM, 2011). Two main kind of threshold measurements have been recognized: detection and recognition thresholds. Detection threshold is defined as the lowest point at which a concentration can be detected, even if the nature of the substance may not be recognized by an individual (Bartoshuk, 1987). Instead, recognition threshold allow to test the lowest concentration an individual can detect a substance and its nature (Wardwell et al., 2009). The basis for both threshold testing is to have participants presented with a range of concentrations of a tasting aqueous solution in an increasing order (ASTM,

2011). In the supra-threshold measurements, subjects assesses the intensity of the stimuli, presented in solutions or are infused on filter papers, using a scale (e.g. the 9-point hedonic scale, the Visual Analogue Scale, VAS, or the General Labeled Magnitude scale, gLMS).

In the last decades, various methods and different tasks have been used to determine oral tactile acuity to gain further insight into its contribution to food texture perception. These have included two-point discrimination task (Engelen et al., 2004), oral letter recognition (Essick, et al., 1999; 2003; Steele, et al., 2014), and other physiological measures (Bangcuyo and Simons, 2017; Linne and Simons, 2017). Among them, the oral letter recognition task is considered the popular one for measuring oral touch sensitivity across subjects, using alphabet letters of varying sizes embossed onto Teflon strips which subjects are asked to identify with their tongue. An alternative method concerns a punctate touch test with von Frey hairs (Semmes-Weinstein monofilaments), which are reported to be repeatable, accurate, and most reliable for measuring light touch–deep pressure sensibility of the tongue and the hard palate (Henkin and Banks, 1967; Cordeiro et al., 1997; Bell-Krotoski and Tomancik, 1987; Bodin et al., 2004).

The role of taste perception in food preferences and intake

It is well know that all the sensory attributes, such as smell, taste, appearance and texture, have a strong influence on eating behaviors and dietary intake. Among them, taste is considered one of the most determinants of food acceptance and consumption (Cox et al., 2016). Taste is modulated by both environmental and genetic factors, and it has been shown these genetic variations in taste receptor genes are linked not merely to variability in taste perception but as well to variability in food preferences and dietary habits (Feeney et al., 2011).

Even tough bitter compounds generally cause a natural rejection response and many bitter-tasting foods should be avoided, some others contain healthy compounds. Indeed, phenols in tea, citrus fruits and wine, organosulfur compounds in cruciferous vegetables and phytonutrients in fruits and vegetables are a case in point. It has been hypothesized that individuals with increased bitter taste sensitivity would rather prefer to consume food rich in saturated fatty acids and added sugars than antioxidant-rich vegetables due to their perceived bitterness (Stevenson et al., 2016). On the other hand, researches have also linked increased sensitivity to bitterness to heightened taste acuity, which may generally prevent food overconsumption (Duffy, 2004). A number of negative associations have been found between PROP responsiveness and preference for different types of foods, such as vegetables, coffee, beer and alcohol consumption (e.g. Dinehart et al., 2006; Duffy et al., 2004). However, the potential interaction between bitterness sensitivity and food intake has yet to be fully understood (Feeney et al., 2011; Garcia-Balio, 2009).

Opposite to bitter taste perception, sweet substances elicit pleasant response, possibly echoing evolutionary pressures to detect high energy foods (Hladik et al., 2002). In line with this assumption, studies have shown that humans present an universal preference for sweet taste, but the preferred intensity is modulated by many factors such as gender, age, race and genetics

(Collaku et al., 2004; Drewnowski et al., 2012). It has been reported that variation in sweet taste perception between individuals could influence food selection and overall dietary intake (see Tan and Tucker, 2019 for a review). However, even if this relationship have been extensively studied, conflicting results have been reported (e.g. Jayasinghe et al., 2017; Low et al., 2016). Indeed, in a recent study published by Jayasinghe and colleagues (2017), subjects characterized as more sensitive to sweet taste had a lower consumption frequency of sweet foods compared to those who perceived the solution proposed as less sweet. On the contrary, Low and colleagues (2016), failed in finding any significant differences between the most and least sensitive participants in terms of dietary intake for a range of various sweeteners.

The inconsistency in the various studies previously cited could be probably due to differences in study participants' characteristics (e.g., gender, ethnicity, age), in sweet taste perception assessment (e.g., psychophysical measurement, type of sweet stimuli) or in dietary intake assessment (e.g., food record, food frequency questionnaire).

Salty taste aids individuals in detecting essential micronutrients, especially Na^+, which is required for maintaining physiological electrolyte balance as well as for regulating blood pressure and water homeostasis. However, humans are different from other animals, since consume salt for pleasure rather than to meet physiological needs (Leshem, 2009) and, also for this reason, sodium intakes are above recommended levels. The perceived intensity of sodium chloride varies greatly among individuals as a specific concentration could elicit weak saltiness perception in one individual and a strongly saltiness to another (Stone and Pangborn, 1990). These different responses to saltiness have led to query what causes these inter-individual differences and they appear to be determined especially by cultural or environmental factors, including exposure to NaCl and consumption of specific nutrients (Durack et al., 2008; Kim and Lee, 2009). Furthermore, it has been hypothesized that part of this variability in sodium intake might be caused by genetic differences associated with salty taste (Chandrashekar et al.,

2006) and a connection between individuals' salt taste sensitivity and sodium-rich foods acceptance and consumption has been suggested (Garcia-Balio, 2009; Hayes et al., 2010; Kim and Lee, 2009). However, this relationship remains unclear and more studies are needed on this topic as well as remain to be explored the poor studied relationship between genes variability, sour taste perception, and subsequent food choices.

Taste perception and the obesity phenomenon

The global prevalence of obesity has increased substantially over the past 40 years and, according to the World Health Organization (WHO), in 2016 over 1.9 billion adults were overweight and over 650 million being obese (WHO, 2016). Once considered a high-income country problem, overweight and obesity are now on the rise in low- and middle-income countries. Indeed, it is not uncommon to find undernutrition and obesity co-existing within the same country, the same community and the same household. Moreover, it is no longer a relevant adult disorder since obesity prevalence in children has accelerated rapidly and, in Italy, its rates are among the highest (36% for boys and 34% for girls) (Mameli et al., 2017; Mameli et al., 2018). At this stage, it seems now no exaggeration to state that obesity is an worldwide epidemic.

Essentially, obesity is the results of an imbalance between the quantity of the energy introduced and the amount expended. The changings in society and the industrialized food system, which produces and promotes convenient and highly-processed foods have led to today's obesogenic food environment that fosters food preferences inconsistent with dietary guidelines.

Obesity is considered a multifactorial aetiology disease, which seems to be genetically based, but requires environmental, psychological and social influences to exhibit (Pozza and Isidori, 2018). It seems clear that important portion of such environmental influences is represented by diet and related eating behaviors. Appetite and consumption are directly influenced by the taste system which determines food acceptance or rejection. Several studies have reported differences in taste sensitivity between obese and non-obese adults (Bartoshuk et al., 2006; Duffy et al., 2007; Goldstein et al., 2005; Pagliarini et al., 2008; Proserpio et al., 2016; 2018; Tepper and Ullrich, 2002) as well as children (Keller and Tepper, 2004; Overberg et al., 2012), showing that perception of taste stimuli (e.g. basic tastes and PROP compound) is reduced in obese individuals. Consequently, this decreased sensitivity to taste stimuli lead obese subjects to consume more to offset the impaired stimulation of their

taste and oral somatosensory system, leading to increases in intake and body weight (Donaldson et al., 2009). However, data concerning correlations between taste sensitivity and obesity are still inconsistent (Cox et al., 2016) and centered mainly on the PROP responsiveness, whereas little is currently known about other taste qualities, especially in children.

Taste perception and oral microbiota: living with a permanent guest

The term *microbiota* was introduced for the first time by Joshua Lederberg in 2001 to mean 'the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and can be determinants of health and disease'. Specifically, the term *microbiota* refers to the microbial taxa associated with human body and this set of microbes and their genes is identify with the term *microbiome*. The Human Microbiome Project and Metagenomics of the Human Intestinal Tract (MetaHIT; www.metahit.eu) and the Human Oral Microbiome Database (HOMD; www.homd.org) clearly demonstrated that to better understand human health and disease is necessary to fully understand the collective human microbiome, since microorganisms are not just passive residents but are responsible of a range of biological functions linked with nutrition and individual well-being (Dewhirst et al., 2010; Gevers et al., 2012). Indeed, the microbial ecosystem within the digestive tract contributes substantial benefit to the host (e.g. to digest otherwise indigestible plant complex carbohydrates), but not all interactions may be advantageous (Tilg and Kaser, 2011). Alcock and colleagues (2014) suggested that 'evolutionary conflict between host and microbes in the gut could lead microbes to divergent interests over host eating behavior'. The authors hypothesize that gut microbes could manipulate host eating behavior promoting their fitness to the detriment of host fitness, shaping individuals' eating behavior and food preferences.

One way to manipulate host eating behavior is to alter hosts' preferences through changing the expression or transduction mechanism of some receptors (Alcock et al., 2014). Duca and colleagues (2012) shown that the tongue and intestine fat taste receptors of germ-free mice are altered compared to the receptors of mice with a normal microbiome. In another study, it has been reported that germ free mice had greater numbers of sweet taste receptors in the gastrointestinal tract and seemed to prefer more sweets compared to normal mice (Swartz et al., 2012). Moreover, it has been reported

that taste receptor expression and activity have been changed after gastric bypass surgery, a procedure that also varies gut microbiota, reduces hunger, increases satiation and affects food preferences (Miras and le Roux, 2013). However, the majority of studies regarding the human microbiota focused especially on the composition of distal gut, while little attention has been paid to microbial communities present along other sites of the digestive tract.

Recently, it has been shown that differences in oral microbiota composition could be linked to interindividual differences in taste perception.

Thus, the potentiality of nongenetic factors to interact with genetic predisposition and influence food habits should be adequately considered in order to provide further insights into the complexities of human eating behaviors.

CHAPTER 2

RATIONALE AND AIM OF THE THESIS

Rationale and aim of the thesis

Rationale

As mentioned in the previous chapters food choices and eating habits are a complex behavior mediated by a number of biological and environmental factors. Taste is considered the number one modifier of individual food selection and varies greatly among individuals. Since it has been shown that impairments in taste perception (e.g. reduced/increased sensitivity) could modulates our response to food preferences and consequently diet, there is a considerable interest in understanding how, and to what extent, individual variability can determinately contribute to explain food preferences and behaviors.

Moreover, with increasing prevalence of diseases related to over nutrition, such as obesity, there is considerable interest in identifying the factors that could predispose individuals to such disease by influencing dietary decisions. Given that the microbes in the gastrointestinal tract could affect individuals' eating behaviors and food preferences, and the composition of microbiota appears to have an important but still unclear role in obesity development, an approach to inquiry into the relationship between obesity, taste sensitivity and oral microbiota composition seems required.

Aim

In the context of the above, the general aim of this thesis is to explore sensory perception in relation to different variables, using a multidisciplinary approach. The potentiality of this multidisciplinary approach opens new avenues of research by highlighting associations between sensory and consumer science, food technology and nutrition, presenting a challenge for future works in this area.

Outline

To start with, *Chapters 3* covers two studies published in international journals. The first one (*Chapter 3a*) explored whether taste perception varies among subjects characterized by different taste responsiveness to the bitter compound PROP (Supertasters vs Non-tasters). Moreover, the composition of oral microbiota were determined and compared in these two groups of subjects. In the second study (*Chapter 3b*), the relationships between the sensitivities for the basic taste qualities (salty, sweet, sour and bitter) were determined for the same groups of subjects and the impact of variation in taste sensitivity and its putative influence on food habits and intake have been investigated. Moreover, gustatory functions and dietary patterns were studied in relation to oral microbiota composition.

Chapter 4 is focused on host related factors with a proposed link to weight gain. To this purpose, taste sensitivity, salivary microbiota composition and food neophobia were compared between children and adolescents with and without obesity in a cross-sectional study.

In *Chapter 5* the hypothesis that ethnicity and population cultural factors may play a role in taste and texture perception and preferences was tested. The study was performed in collaboration with University of Copenhagen and investigated whether differences exist in PROP responsiveness, FP density and touch detection ability on the anterior dorsal part of the tongue among Asian and Caucasian adults. Furthermore, the importance of such sensory differences for preferred oral food processing behaviors in these populations has been explored.

CHAPTER 3

TASTE PERCEPTION, ORAL MICROBIOTA AND EATING BEHAVIORS

3a. New insights into the relationship between taste perception and oral microbiota composition

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New insights into the relationship between taste perception and oral microbiota composition

Abstract

Fairly poor data are available on the relationship between taste perception, food preferences and oral microbiota. In the present study, we investigated the hypothesis that subjects with higher responsiveness to 6-n-propylthiuracil (PROP) might be characterized by a different taste sensitivity and tongue microbiota composition. Indeed, the bacterial metabolism may modulate/enhance the concentration of tastants near the taste receptors, modifying taste perception through a sensorial adaptation mechanism or by a broad range of microbial metabolic pathways. The detection thresholds of sweet, sour, salty and bitter, the Fungiform Papillae Density (FPD) and the composition of bacteria lining the tongue were determined in Supertasters (high PROP responsiveness, ST) and Non-tasters (low PROP responsiveness, NT). An important inter-individual variability was found for all taste stimuli and FPD between the two groups, with NT subjects showing significant higher threshold values and a lower FPD than with STs. We found five bacterial genera whose relative abundances were significantly higher in STs than NTs. This study opens new avenues of research by highlighting associations between parameters usually studied independently.

Introduction

The contribution of taste perception in individual capacity to recognize the energy and nutrient content of foods and discriminate between safe and poisonous food-substances is well known [1, 2]. From birth, people are hard-wired to crave sweet and salty flavours and reject bitter foods [3]. However, later in life, preferences change as a result of repeated food experiences, which can partially explain the great difference in food preferences among

human subjects [4]. There is large inter-individual variation in taste perception [5] and it has been shown that impairments in taste perception and hedonic experience of taste can even cause unhealthy eating habits, which can lead to poor-nutrition or over-nutrition, both representing major public health issues [5]. The most studied and best-understood genetic source of individual variation in oral sensation is 6-n-propylthiouracil (PROP) responsiveness [6-8], which is influenced by TAS2R38 haplotypes [9]. The TAS2R38 gene is a member of the TAS2R bitter taste receptor gene family. Three single nucleotide polymorphisms (rs714598, rs1726866, rs10246939) at positions encoding amino acids 49, 262 and 296 represent the most common variant alleles of TAS2R38, and encodes two major forms of the PROP receptor, PAV (Proline, Alanine, Valine) and AVI (Alanine, Valine, Isoleucine) haplotypes. Individuals that carry the AVI haplotype (AVI/AVI alleles) are minimally or non-responsive to PROP, while individuals with the PAV haplotype (PAV/PAV alleles or PAV/AVI alleles) demonstrate stronger or intermediate responsiveness [10]. Bartoshuk [11] expressed the PROP responsiveness as PROP taster status and identified three groups of subjects: PROP Non-tasters (NTs; AVI/AVI alleles), who perceived this compound as weak or tasteless, PROP medium-tasters (MTs; PAV/AVI alleles), who perceived it as moderately bitter, and PROP Super-tasters (STs; PAV/PAV alleles), who perceived it as extremely bitter. PROP responsiveness has long been used as general marker for sensitivity to a variety of sensory stimuli [7]. It has been reported that STs rate the intensity of other bitter compounds, as caffeine and quinine, and other tastants (e.g. salt, sugar, and acid), as more intense than NTs do [e.g. 8, 12-14]. Moreover, PROP taster status has been associated with greater perception of a variety of orosensory stimuli, including sensations from bitter/astringent fruits and vegetables, fruit juices, and alcoholic beverages compared to NTs [15-17]. It has been suggested that this increased sensitivity could be associated to a greater density of fungiform papillae (FP) located on the tongue, despite data are controversial. Subjects characterized by a greater density of FP (FPD) seem to perceive greater

responsiveness when exposed to PROP, sugar, salt and fat creaminess [18-20].

Since genetic variation in taste receptors may explain some of the observed variability in taste perception, it has been hypothesized that this variability could affect food choice(s) and dietary habits, influencing nutritional and health status, as well as the risk of chronic diseases [for a review: 21]. In this context, literature data provided mixed results and two major hypotheses were suggested. On one hand, a greater PROP responsiveness seems to be associated with diets rich in saturated fatty acids and added sugars, in contrast to plant-based diets rich in antioxidant and protective phytochemicals generally affecting bitterness of plant foods [i.e. 22]. On the other hand, research has also linked higher PROP responsiveness with decreased preferences for high fat and high energy foods and reduced body weight [i.e. 23-24].

Interestingly, it has been suggested that also microbes in the gastrointestinal tract could have a potential direct role in shaping individuals' eating behaviour and food preferences [25]. The majority of studies of the human microbiota have been focused on the distal gut composition whereas little attention has been paid to microbial communities at other sites along the digestive tract. Notably, a relationship between taste sensitivity and specific oral bacteria has been proposed [26-28]. Particularly, at oral level, papillary structure of the dorsal tongue constitutes one of the major microbial reservoirs of the mouth [29]. However, fairly poor literature about this topic is available and, to our knowledge, the relationship between taste perception and tongue microbiota has not been systematically investigated so far.

In this context, the general aim of the present study was to investigate the relationship among host related factors that are proposed as potential modulators of eating behaviour. In particular, we hypothesized that ST and NT subjects might be characterized by a different taste sensitivity and tongue microbiota composition. Therefore, the orosensory detection thresholds of

sweet, sour, salty and bitter, the FPD and the composition of bacteria lining the tongue were determined in these two groups of subjects.

Results

One hundred and five subjects (52F and 53M; age: 23.4 ±2.5; BMI: 21.9 ±2.4) were tested in a screening procedure according to their PROP responsiveness. NTs were 28.6% of total sample (n=30; 15F and 15M; age: 24.2 ±2.8; BMI: 21.6 ±2.7), whereas STs were 27.6% (n=29; 17F and 12M; age: 22.5 ±2.1; BMI: 21.5 ±2.3). The rest of subjects were MTs and were not included in this investigation so that more extreme tasters (super-tasters and non-tasters) could be compared. Thus, only NTs and STs (n=59, 32F and 27M, age: 23.3 ± 2.6 years) were admitted to the main experiment consisting in the assessment of taste sensitivity and oral microbiota composition.

*Taste sensitivity assessment***.** The mean taste threshold values in ST and NT subjects are shown in Fig. 1.

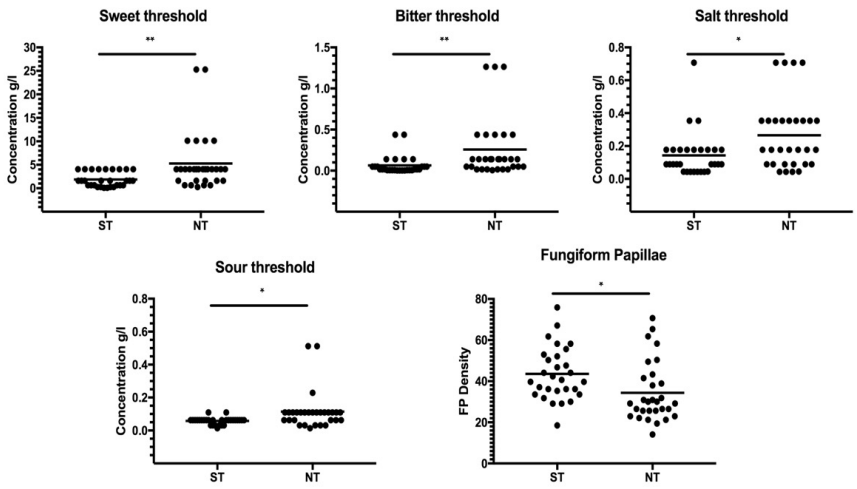

Fig. 1 Scatter plots representing the BET of the four-basic taste (sweet, bitter, salt and sour) and the Fungiform Papillae Density (FPD) in super-taster (ST) and non-taster (NT) subjects. Statistics according to unpaired, two tailed Student's t-test; **, $p < 0.01$; *, $p < 0.05$.

An important inter-individual variability was found for all taste stimuli between the two groups, with NTs subjects showing significant higher threshold values (lower sensitivity) compared with STs (sweet taste: t_{57}= 2.90, p= 0.005; salty taste: t_{57}= 2.63, p= 0.011; bitter taste: t_{57}= 2.69, p= 0.009; sour taste: t_{57}= 2.60, p= 0.012). A significant difference was also found in FPD between the two groups of subjects (t_{57}= 2.58, p= 0.013), with NTs subjects showing a significantly reduced FPD compared with STs.

Characterization of the tongue dorsum microbiota of NT and ST subjects. To infer possible differences in the tongue microbiota composition between STs and NTs, the DNA extracted from swabs of dorsal tongue surface were analysed by 16S rRNA gene profiling. Intra-sample (α) diversity did not differ significantly between ST and NT samples in term of both taxonomic richness and evenness as calculated through five different indexes (see Supplementary Fig. S1A online). In addition, inter-sample (β) diversity measured through UniFrac algorithms did not permit the separation of ST and NT samples (see Supplementary Fig. S1B online). At taxonomic level, we identified a total of 141 taxonomic units, with a minimum number of 26 and a maximum of 60 per sample. Overall, 10 taxonomic units (17%; i.e., the genera Streptococcus, Veillonella, Neisseria, Haemophilus, Prevotella, Rothia, Actinomyces, Granulicatella, Alloprevotella, and Gemella) were detected in all 59 samples, and 25 (42%) were found in at least 90% of samples. The bacterial community structure of all analysed samples (determined through DADA2 pipeline, the SILVA ribosomal RNA gene database, and speciateIT taxonomic assignment; “DADA2/SILVA/speciateIT”) was similar and independent from PROP taster status (Fig. 2).

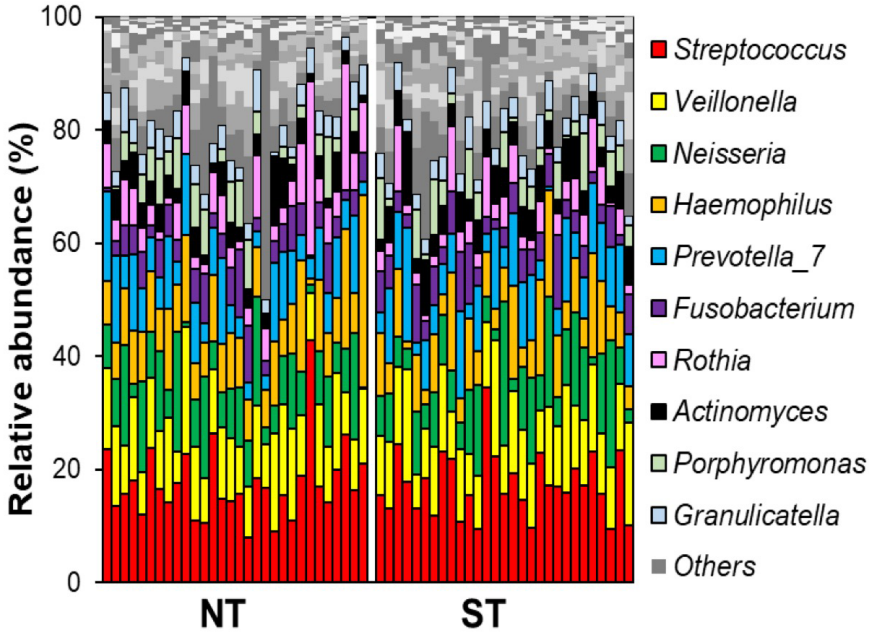

Fig. 2. Stacked histograms of bacterial composition in each tongue dorsum sample to the genus level of taxonomic resolution. Each column refers to the bacterial composition in a single sample. NT, non-taster; ST, super-taster. Only the 10 most abundant bacterial genera are shown; other genera are shown in greyscale color.

Nonetheless, at the level of single taxonomic units, we found that five bacterial genera were significantly higher in ST compared to NT samples, namely the Gram-positive *Actinomyces* (belonging to the phylum *Actinobacteria*; P=0.012 according to Mann-Whitney test), *Oribacterium* (*Firmicutes*; P=0.034), *Solobacterium* (*Firmicutes*; P=0.040) and *Catonella* (*Firmicutes*; P=0.009), and the Gram-negative *Campylobacter* (*Proteobacteria*; P=0.009) (Fig. 3).

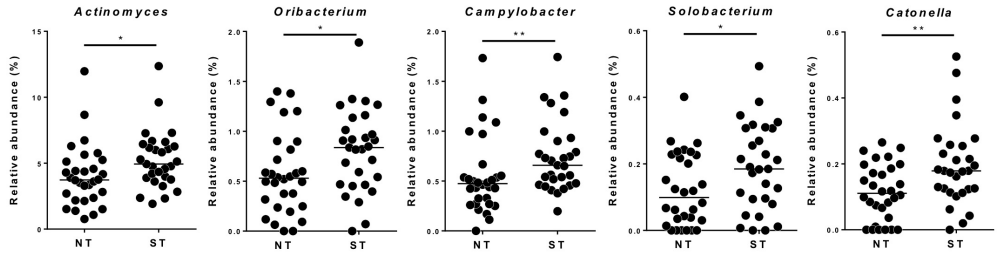

Fig. 3. Scatter plots representing the relative abundance of bacterial genera that resulted significantly different between non-taster (NT) super-taster (ST) samples. Statistics according to Mann-Whitney test; **, $p < 0.01$; *, $p < 0.05$.

Due to the potential impact of the bioinformatic pipeline selected on results in microbiomic analyses, we also compared the microbiota of tongue dorsum of NTs and STs through LEfSe analysis with data generated through QIIME pipeline with Greengenes 16S rRNA gene database; as represented in the resulting cladogram, three genera, i.e. *Actinomyces*, *Oribacterium* and *Campylobacter* were confirmed to be significantly different between the two groups (Fig. 4). In addition, LEfSe analysis found that in ST samples also the *Erysipelotrichaceae* genus *Bulleidia* (*Firmicutes*) was significantly overrepresented, whereas the family *Lachnospiraceae* and an undefined *Erysipelotrichaceae* genus were significantly reduced (Fig. 4). Finally, we also performed Linear Mixed Model analysis to identify potential dependency between the microbiota composition and FPD, also taking into consideration the NT or ST clustering. Using this model, we only observed a trend (p-value 0.059) for the genus *Corynebacterium*.

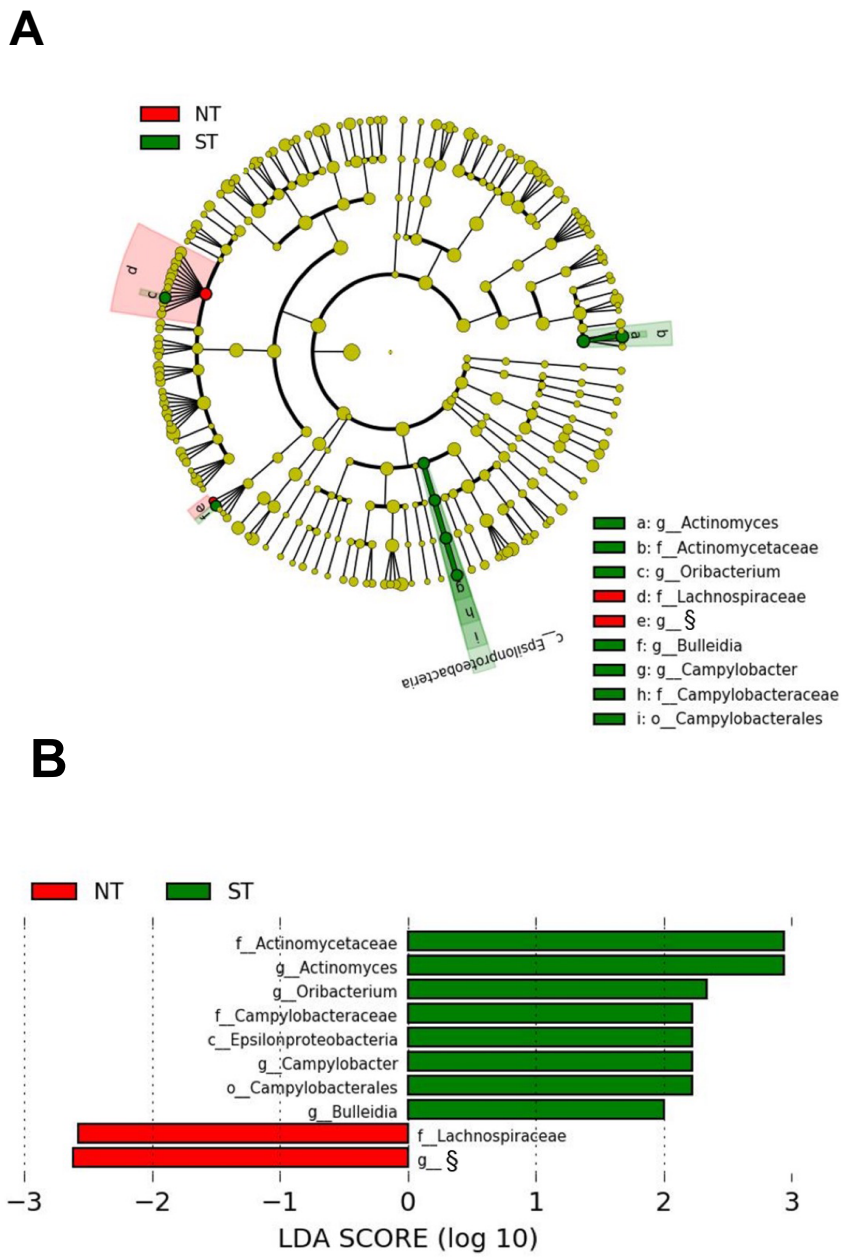

Fig. 4. LEfSe analysis of tongue dorsum microbiota in non-taster (NT) and super-taster (ST) subjects. (A) Cladogram indicating significantly different taxa (LDA score >2; $p < 0.05$) at phylum (p_), class (c_), order (o_), family (f_) and genus (g_) levels between NT and ST groups. (B) Bar graph displaying LDA scores. Green regions indicate taxa enriched in STs while regions in red indicate taxa enriched in NTs. Differing taxa are listed on the right side of the cladogram. §, undefined genus belonging to the family *Erysipelotrichacea.*

Taken together, these results indicate that, although the overall community structure of the tongue dorsum microbiota is not dissimilar, specific bacterial taxa with recognized ecological importance at oral level (i.e., the genera *Actinomyces*, *Campylobacter* and *Oribacterium*) are significantly different between NT and ST subjects.

Discussion

The general aim of the present study was to investigate the relationship among aspects that are proposed as potential modulators of eating behaviour. Over the last decade, the PROP phenotype has received considerable attention for understanding individual differences in taste perception and has also been considered as a marker for food preferences, which could influence dietary behaviour and nutritional status.

Present findings confirmed that STs and NTs differ in their taste ability, with NT subjects showing a significantly lower sensitivity than STs for all tastes. In line with these results, a great number of studies showed that STs rate the bitterness of caffeine as more intense, sucrose as sweeter, sodium chloride as saltier, and citric acid as sourer than NTs do [20, 30, 31].

Because the fungiform papillae contain the taste buds of the anterior tongue, it has been suggested that the greater sensitivity of STs subjects could be due to higher FPD [12, 23, 32-34]. However, this association has not been confirmed in several recent studies [35-40]. The present results support the existence of a relation between PROP sensitivity and FPD and are in line with previous findings which reported that subjects who differ in their response to PROP presented anatomical differences in the tongue.

Considering the above-mentioned literature, in the last decades particular attention has been focused on tongue's physiology, genetics and related phenotypes in order to provide greater insights into the complexities of human eating behaviour. However, much less attention has been paid to the composition of oral microbiota, which might have an unknown role in taste

perception as mouth's permanent host. Indeed, the papillary structure of the tongue dorsum forms a unique ecological oral site that provides a large surface area for the accumulation of saliva, oral microorganisms and debris [41]. Thus, it appears plausible that oral bacteria lining the tongue may influence and modulate taste perception.

In this context, studies which focused on the identification of specific oral microbial community and its relationship with taste perception are scarce and, generally, did not apply exhaustive methods for the whole taste perception evaluation [27] or oral microbiota analysis [26]. Solemdal and colleagues [26] studied variables related to oral health and taste ability in acutely hospitalized elderly. Whole mouth gustatory function was assessed with the "taste strips" method [42], whereas oral bacteria were assessed with the CRT® Bacteria Kit [43], which consists cultivation-dependent method for the exclusive determination of *mutans streptococci* and *lactobacilli* in saliva. They found that taste perception (especially for sour) was particularly reduced in acutely hospitalized elderly with high growth of lactobacilli, suggesting that the organic acids produced by bacteria (e.g. lactic, acetic, and propionic acids) may cause adaptation in sour taste perception, and thus increasing the taste threshold for sour. However, this assumption was not supported by microbiomic or predicted metagenome analyses [26]. On the contrary, Besnard and colleagues [27] applied a microbiomic analysis to study the composition of microbiota and saliva surrounding the circumvallate papillae in combination with the lipid detection threshold in a group of normal weight and obese adults. The multivariate approach highlighted that specific bacteria and salivary signature discriminated between lipid NTs and lipid STs. However, the authors only determined the orosensory detection threshold of linoleic acid (LA) by using the 3-AFC procedure. To our knowledge, our study is the first one that investigated both taste responsiveness and taste detection thresholds for all the basic tastes, applying reliable and sensitive methods, and studied oral microbiota using microbiomic analysis of tongue microbial ecosystem.

In the present study, the analysis of tongue microbiomic profiling data revealed that there was no significant difference in the intra (α)- and inter (β)-subject ecological diversity between ST and NT groups of subjects, confirming previous observations that the tongue dorsum microbiota is characterized by a limited microbial community variation among healthy adults compared to other body sites [44]. The most abundant bacterial groups found in our study are similar to those found in most other studies on healthy subjects. Indeed, 20% of our sequences belonged to the genus *Streptococcus*, confirming the preponderance of this genus within a healthy mouth [45]. Nevertheless, we found that the relative abundance of some taxa was significantly different among STs and NTs. In particular, we identified that major differences exist in five bacterial genera, including the Gram-positive genera *Actinomyces*, *Oribacterium*, *Solobacterium* and *Catonella*, and the Gram-negative *Campylobacter*, which are overrepresented in the STs group.

Moreover, LEfSe analysis confirmed those same taxa to be prevalent as well, but, in addition, showed that the *Erysipelotrichaceae* genus *Bulleidia* was also abundant in STs, whereas the family *Lachnospiraceae* and an undefined *Erysipelotrichaceae* genus were underrepresented.

To infer potential links between bacteria on tongue dorsum and taste responsiveness, we supposed to look into the possibility that bacterial metabolism may modulate/enhance the concentration of tastants near the taste receptors, modifying taste perception through a sensorial adaptation mechanism or by a broad range of microbial metabolic pathways [28, 46]. Indeed, it is well known that polysaccharides can be hydrolysed into oligosaccharides, disaccharides, and monosaccharides by host and bacterial glycosidases. In this context, Feng et al. [28] showed a positive correlation between taste sensitivity and some bacterial phyla. In fact, it has been suggested that the presence of *Actinobacteria* and *Bacteroidetes* in the tongue film are linked to an increase sensitivity, especially to bitterness. These bacteria could degrade carbohydrates into disaccharides, monosaccharides and organic acids [47], which could lead to an enhancement in taste

perception near the taste buds. Moreover, many bacteria (e.i. *Actinobacteria*), are known to produce secondary metabolites which are precursors of some bitter acids or bioactive non-nutrient substances, such as phenols, which can enhance the sensation of astringency and the bitter taste in food products [27,48, 49], and cause an adaptation in bitterness and astringency perception. However, the oro-sensory consequences of such changes remain to be determined. Future research is needed using robust analysis on predicted metagenomics data to infer the possibility that some microbial metabolic pathways could discriminate between ST and NT individuals.

In brief, the data reported herein suggest that the microbial composition of the tongue microbiota of people with higher taste responsiveness are different from those of people of a reduced taste responsiveness, and provide new references that these differences of oral bacteria lining the tongue may influence and modulate taste perception. Corroboration of these results using a larger sample size of ST and NT subjects and a deepen study of subjects' eating habits might achieve a better understanding into several aspects, considered as potential modulators of eating behaviour. Nevertheless, our results offer new insights into the reciprocal impact between the oral microbiota and taste perception. Based on an analysis of tongue microbiota, taste responsiveness and detection threshold of the four-basic taste, this study opens new avenues of research by highlighting associations between parameters usually studied independently.

Material and methods

Participants

One hundred and five normal-weight young adults were recruited from the University community (i.e. through public advertisement). Individuals were excluded if they were pregnant or lactating women, had medical conditions, treatments that could modify taste perception or were habitual smokers. Moreover, subjects who consumed any medication, probiotics or antibiotics

two months before the study were also excluded. Habitual use of mouthwash was also considered a criterion for volunteer exclusion.
Informed, written consent was obtained from all subjects on the first test day. The present study was performed according to the principles established by the Declaration of Helsinki and the protocol was approved by the Institutional Ethics Committee of the University of Milan.

Procedure
Subjects were instructed to refrain from ingestion of all foods, beverages, and oral care products for a minimum of 3 h before arrival to the laboratory. All testing was completed in 2 test sessions. Subjects were familiarized with all procedures and rating scales at the start of the first session, when a screening procedure with PROP solution was performed. Participants (n=105) were selected according to their thiourea taste sensitivity (PROP status) and they required to be NTs or STs. Then, if a subject was identified as NT or ST was admitted to the second session, in which microbiota sampling and taste threshold were evaluated. FP were also counted at this time.

Screening procedure
A method proposed by Prescott and colleagues [31] was used as an initial screen for PROP status. The intensity of bitterness of a supra-threshold 3.2 mmol/L solution of PROP (European Pharmacopoeia Reference Standard, Sigma-Aldrich, Milano, Italy) was rated using the Generalized Labeled Magnitude Scale (0–100), gLMS [50]. Subjects were presented with 2 identical samples (10 ml) coded with a three-digit number and were instructed to hold each sample (10 ml) in their mouth for 10 s, then to expectorate the solution and wait 20 s before evaluating the intensity of bitterness. In order to control for carry-over effect after the first sample evaluation, subjects had a 90s break to rinse their mouths with water. The average bitterness score was used for each subject.
Respondents were grouped according to their PROP status based on arbitrary cut-offs as proposed by Laureati and colleagues [51]. Participants were

categorized as NTs if they rated the PROP solution lower than 17 mm on the gLMS, whereas they were categorized as STs if they rated the PROP solution higher than 53 mm on the gLMS. According to previous studies the Medium-tasters (MTs) were not included in this investigation so that more extreme tasters (super-tasters and non-tasters) could be compared [52-54].

Taste sensitivity evaluation

Stimuli. Tastants were sucrose, sodium chloride, citric acid, and caffeine (Sigma-Aldrich) dissolved in mineral water (Levissima, San Pellegrino spa), representing the four basic tastes - sweetness saltiness, sourness, and bitterness, respectively. For each taste 7 concentrations were prepared in successive dilutions. The total range of concentrations was chosen on the basis of threshold values reported in the literature [55-57] and were adjusted according to preliminary tests. Concentration ranges were established such that the lowest concentration was clearly below and the highest concentration was clearly above the level at which subjects could detect or recognize the stimulus. This resulted in the following ranges of tastants in water in g/l: sodium chloride 6.25×10^{-2} - 4 (0.4 log steps); sucrose 1.6×10^{-1} - 40 (0.4 log steps); citric acid 2×10^{-2} - 1.5 (0.3 log steps); caffeine 3×10^{-3} – 2 (0.4 log steps). The solutions were tested at room temperature and kept at 5° C in the dark for no longer than 2 days.

Taste threshold assessment. Taste thresholds were evaluated using the 3-AFC (Alternative Forced Choice) method reported in ISO/DIS 13301:2018 [60]. This international standard describes a reliable procedure to estimate the value of a threshold for any stimulus presented in an aqueous medium. For each stimulus, subjects were presented with 7 triads of samples coded with a three-digit number. Each triad consisted of 1 sample containing the stimulus and 2 identical samples (10 ml) of a blank solution (mineral water). The 7 triads proceeded from a weaker to an increasingly stronger concentration, and the position of the sample containing the stimulus was randomized over trials and assessors. For each triad, participants were

instructed to select the sample which was different from the other 2 [58]. If the assessors were uncertain, they were instructed to guess (forced choice procedure). At the beginning of each session, and before each triad, the assessors were instructed to rinse their mouth with mineral water.

Fungiform papillae density (FPD) assessment. The individual FPD was calculated following the procedure previously described by Monteleone and colleagues [59]. In brief, the tongue was swabbed with household blue food colouring, using a cotton-tipped applicator, to make fungiform papillae (FP) easily visible on the anterior portion of the dorsal surface of the tongue. Digital pictures were recorded using a digital microscope (MicroCapture, version 2.0 for 20x-400x) and the clearest image was selected. Then, the number of FP was counted in two 0.6 cm diameter circles, one on right side and one on left side of tongue, 0.5 cm from the tip and 0.5 cm from the tongue midline, following the Denver Papillae Protocol [60]. The average of these values was used for each subject. The individual FPD was then calculated by reporting the number of FP to a common unit area of 1 cm^2.

Microbiomic evaluation

Oral sample collection and DNA Extraction. Volunteers were restricted for at least 3 h of food intake prior to sample collection as mentioned previously. The following instructions for self-tongue swab collection were given: volunteers were asked to sit in front of a mirror, bulge out the tongue and gently press the swab on the surface rolling and touching edges, tip and all defined area of the tongue (about 2/3 of the length) for two minutes using a sterile flocked swab (FLOQSwabs™, COPAN S.p.A., Brescia, Italy). The swab samples were immediately placed in 750 µl of Power Bead solution provided in the DNeasy PowerLyzer PowerSoil DNA extraction kit (Qiagen, Hilden, Germany) and stored at -80 °C. For DNA extraction, samples were thawed on ice and homogenized for five minutes to release all the bacterial cells in the solution. Then, swabs were dried pressing several times on the interior wall of the tube. Finally, samples were processed by means of the DNA extraction kit

mentioned above following manufacturer's instructions with a minor modification consisting of incubating samples at 65 °C for 10 min after addition of C1 solution. Mechanical bacterial cell disruption has been performed using a Precellys bead beater kept in a cold room (3 cycles of 6800 rpm × 30 s; Advanced Biotech Italia s.r.l., Seveso, Italy). Quantification and verification of the 260/280 ratio of the extracted DNA was carried out with a Take3 Micro-Volume plate in a Gen5 microplate reader (BioTek Instrument Inc., Winooski, VT, USA). Finally, DNA samples were stored at -80 °C.

Tongue microbiota analysis. The DNA extracted from tongue swabs was analysed at the Institute for Genome Sciences (University of Maryland, School of Medicine, Baltimore, MD, USA) through 16S rRNA gene profiling with Illumina HiSeq 2500 rapid run sequencing of the V3-V4 variable region. Sequencing reads were analysed following a pipeline comprehensive of two main steps: (i) pairing and filtering of raw amplicon sequencing data by DADA2 (R package); (ii) taxonomic assignment of each amplicon sequence with speciateIT on SILVA database according to the custom pipeline freely available on GitHub (https://github.com/Ravel-Laboratory/speciateIT). A total of 1,938,469 filtered high-quality sequence reads were generated with a mean ± standard deviation (SD) of 32,855 ± 21494 reads per sample; maximum of 69359 for sample C55 and minimum of 808 for sample C26. The negative control introduced internally to the entire process gave 187 reads (183 ascribed to *Lactobacillus gasseri* and 4 to *Lactobacillus reuteri*). Analysis and taxonomic assignment of sequencing reads were also performed by means of the bioinformatic pipeline Quantitative Insights Into Microbial Ecology (QIIME) version 1.9.0 [61] with the GreenGenes database (version 13_5). Metadata have been deposited in the European Nucleotide Archive (ENA) of the European Bioinformatics Institute under accession code PRJEB28769.

Statistical analysis

The matrix of the correct and incorrect answers produced separately by each judge was used to calculate the individual taste threshold. The individual's

Best Estimate Threshold (BET) for each sensory stimulus was calculated as the geometric mean of the highest concentration missed and the next higher concentration that was correctly recognized (ISO/DIS 13301:2018). After verifying that taste sensitivity data (taste thresholds and FPD) were normally distributed, the differences between two groups (STs vs NTs) were assessed using an unpaired, two tailed Student's t-test using IBM SPSS statistical software version 25 (SPSS Inc, Chicago, IL, USA). For microbiomic data, significant differences between NT and ST were determined according to Mann-Whitney test with Benjamini-Hochberg correction. In addition, microbial composition differences between groups have been defined with QIIME/Greengenes-generated data through LDA Effect Size (LEfSe) [62]. Linear mixed model was carried out as regression analysis between the microbiota and FPD factors taking into account the binary clustering NT/ST. $p < 0.05$ was considered to be significant and the range $0.05 \leq p < 0.10$ was accepted as a trend. For microbiomic data, statistical calculations were performed using the software program GraphPad Prism 5.

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Author Contributions: CC, ML, PR, SG, EP designed the study. CC carried out the experiment and collected samples. CC and RK assayed samples. CC, GG, SG performed statistical analysis. CC, SG, ML, PR wrote the manuscript. CC, ML, SG, EP regularly discussed the experiments, analysed the results, and provided useful suggestion during the project. All authors read and approved the final manuscript.

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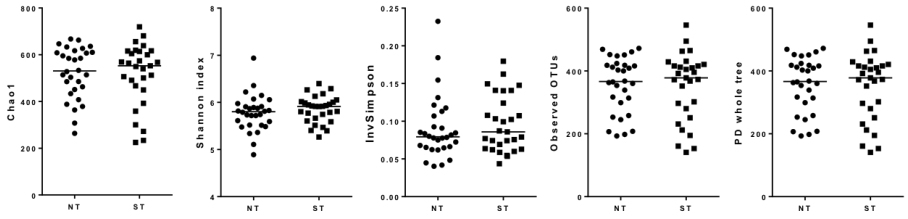
Supplemental Figures and Tables

A



B

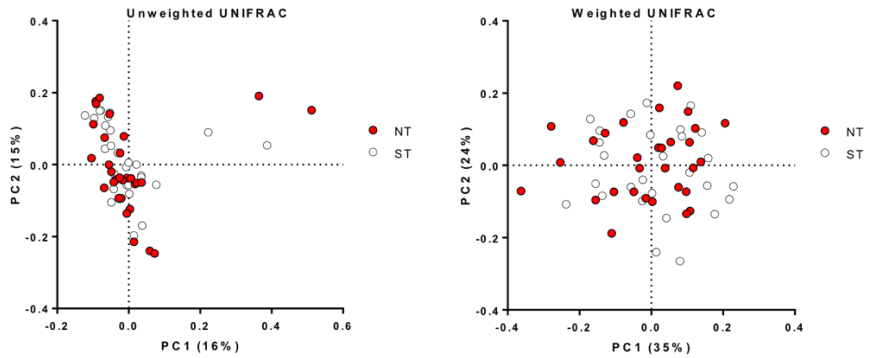


Fig. S1. Diversity analyses of the microbiota composition of tongue dorsum in PROP super-taster (ST) and non-taster (NT) subjects. **A**, intra-sample diversity as determined through five different α-diversity; **B**, β-diversity analyzed through weighted and unweighted UniFrac.

Tab. S1. Number of reads per sample after filtering.

Sample	nr. of reads
C01	30293
C02	29367
C03	69359
C04	46565
C05	54926
C06	4897
C07	56247
C08	35973
C09	2394
C10	11381
C11	59968
C12	4054
C13	50651
C15	24255
C16	53572
C17	3195
C18	50395
C19	39925
C20	34501
C21	46723
C22	10267
C23	34424
C24	52696
C25	3032
C26	808
C27	814
C28	68470
C29	43714
C30	45225
C31	47584
C32	12408
C33	66066
C34	27485

C35	36198
C36	987
C37	5665
C38	8094
C39	51913
C40	53132
C41	54532
C42	51846
C43	54572
C44	67383
C45	22440
C46	41566
C47	39163
C48	27742
C49	35895
C50	51456
C51	34603
C52	23132
C53	9041
C54	8662
C55	64944
C56	27864
C57	33872
C58	2253
C59	2117
C60	7763

CHAPTER 3

TASTE PERCEPTION, ORAL MICROBIOTA AND EATING BEHAVIORS

3b. Exploring Associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake

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Exploring Associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake. ***Nutrients***. 2019:11(5), 1167.

Exploring Associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake

Abstract

The role of taste perception, its relationship with oral microbiota composition, and their putative link with eating habits and food intake were the focus of the present study. A sample of 59 reportedly healthy adults (27 male, 32 female; age: 23.3 ± 2.6 years) were recruited for the study and taste thresholds for basic tastes, food intake, and oral microbiota composition were evaluated. Differences in taste perception were associated with different habitual food consumption (i.e., frequency) and actual intake. Subjects who were orally hyposensitive to salty taste reported consuming more bakery and salty baked products, saturated-fat-rich products, and soft drinks than hypersensitive subjects. Subjects hyposensitive to sweet taste reported consuming more frequently sweets and desserts than the hypersensitive group. Moreover, subjects hypersensitive to bitter taste showed higher total energy and carbohydrate intakes compared to those who perceived the solution as less bitter. Some bacterial taxa on tongue dorsum were associated with gustatory functions and with vegetable-rich (e.g., *Prevotella*) or protein/fat-rich diets (e.g., *Clostridia*). Future studies will be pivotal to confirm the hypothesis and the potential exploitation of oral microbiome as biomarker of long-term consumption of healthy or unhealthy diets.

Introduction

There are many known drivers of food choice and habits, however, taste is considered one of the main predictors [1]. It is generally assumed that humans perceive five different taste modalities: bitter, sweet, umami, sour, and salty. Each taste quality is associated with different nutritional or physiological requirements, or indicates a potential dietary risk [2]. Sweet, salty, and umami

are supposed to signal the nutrient composition of foods, with sweet taste representing carbohydrates, salty taste associated with electrolytes, and umami with proteins. On the contrary, stimuli categorized as bitter and sour are associated with compounds that could be potentially harmful, and are generally regarded as innate aversions [3,4]. Taste perception varies greatly among individuals, strongly influencing food preferences and selection, and therefore nutritional status and health [5]. In particular, during the last decades, research has been focusing on bitter taste perception, and the genetic predisposition to perceive the bitter taste of 6-n-propylthiouracil (PROP) has gained considerable attention as a prototypical taste stimulus and an oral marker of food preferences and eating behavior [6]. Some additional markers include the density of fungiform papillae on the tongue tip [7] and thermal tasting [8].

Previous studies suggested a connection between individuals' taste sensitivity and food acceptance and consumption [9–11]. It has been conclusively demonstrated that PROP-sensitive individuals detect more bitterness from glucosinolate-containing vegetables than non-sensitive individuals and an association between variation in bitter taste perception and food preferences has been documented [12–15]. However, the potential interaction between bitterness sensitivity and food intake has yet to be fully understood [10,16]. Furthermore, it has been reported that variation in sweet taste perception between individuals (see [17] for a review) could influence food selection and overall dietary intake. However, even if a number of previous studies [18–23] have investigated this relationship, conflicting results have been reported, probably due to differences in study participants' characteristics (e.g., gender, ethnicity, age), in sweet taste perception assessment (e.g., psychophysical measurement, type of sweet stimuli) or in dietary intake assessment (e.g., food record, food frequency questionnaire).

The variability in response to salty stimuli has been examined for decades, but a direct genetic link to human salt taste perception has yet to be discovered [16]. The relationship between salty sensitivity and food intake has

been studied much less but a connection between individuals' salt taste sensitivity and sodium-rich foods acceptance and consumption has been suggested [18,19]. Moreover, salty sensitivity appears to be determined more by environmental factors, including exposure to NaCl and consumption of specific nutrients, than by heritability components [24–28].

Recently, in addition to the study of the perception of basic tastes, increasing attention has been focused on the sensitivity to fat stimulus since various evidence indicated that humans can perceive non-esterified, long-chain fatty acids in the oral cavity [29,30]. Moreover, it has been suggested that fat perception may influence the choice and consumption of some high-fat foods and, thus, possibly affects body weight [31,32]. However, additional studies are needed to confirm this assumption.

In this context, the potentiality of nongenetic factors to interact with genetic predisposition and influence food habits should be adequately considered. Recently, it has been suggested that differences in oral microbiota could be involved in the interindividual differences in taste perception. Indeed, in agreement with other reports [33,34], we previously reported a relationship between reduced taste perception and specific oral bacteria's growth [35].

The impact of taste sensitivity and its putative influence on food intake were the focus of the present study. We explored whether variation in gustatory functions among individuals could be related to different dietary patterns and intake. Moreover, gustatory functions and dietary patterns were studied in relation to oral microbiota composition.

Material and Methods

Participants

Healthy, normal-weight volunteers, 18–30 years of age, were recruited from the University of Milan community through public advertisement. They received oral and written explanations of the protocol and answered questionnaires aimed at applying exclusion criteria. The following exclusion

criteria were considered: (1) smokers; (2) pregnant or lactating women; (3) subjects on medication that may interfere with their ability to taste; (4) history of food allergies that may interfere with the study evaluations; (5) subjects on antibiotics two months before the study. Participants were asked to refrain from eating, drinking (except room temperature water), brushing teeth, and chewing gum for 3 h prior to testing.

Power analysis was conducted to determine an appropriate sample size to achieve adequate power. Using data from previous studies [17,20,21] and an α of 5% and a β of 10% (90% power), it was calculated that a sample size of 51 would be required to classify at least 20% of the subjects as hypersensitive to all basic taste.

Informed, written consent was obtained from all subjects. The present study was performed according to the principles established by the Declaration of Helsinki and the protocol was approved by the Institutional Ethics Committee of the University of Milan (protocol number 16/17).

Gustatory Function Assessments

Seven concentrations for each taste stimulus were prepared to determine the recognition thresholds. These concentration ranges covered the published threshold values [36–38], and were adjusted according to preliminary tests. Concentration ranges were established such that the lowest concentration was clearly below and the highest concentration was clearly above the level at which subjects could detect or recognize the stimulus, and allowed for interindividual threshold differences. The dilution factor and the final concentration range were reported in Table 1.

For each taste, participants received the samples at each concentration as a three-alternative forced-choice (3-AFC) ascending series, according to ISO/DIS 13301:2018 [39]. Starting from the lowest concentration, three samples (10 mL each), one containing the sample with stimulus and distilled water and two background samples (only with distilled water), were presented at each concentration. Participants were asked to take the whole 10 mL of

sample into their mouth, swirl the solution around for 3 s, and expectorate. Using the forced-choice method, participants were instructed to select, or guess, the sample which was different from the other two. All samples were given a three-digit number, and the position of the samples with stimuli was randomly allocated. Participants were asked to rinse their mouth with distilled water between each concentration step.

Table 1. Concentrations (g/L) of sucrose, sodium chloride, caffeine, and citric acid used to determine recognition thresholds.

Taste Quality	Reference Stimuli	Sample Concentration (g/L)[a]						
		1	2	3	4	5	6	7
Sweet	Sucrose	$1.6 \cdot 10^{-1}$	$4.0 \cdot 10^{-1}$	1.02	2.56	6.4	16.0	40.0
Salty	Sodium chloride	$6.25 \cdot 10^{-2}$	$1.25 \cdot 10^{-2}$	$2.5 \cdot 10^{-1}$	$5.0 \cdot 10^{-1}$	1.0	2.0	4.0
Bitter	Caffeine	$3.0 \cdot 10^{-3}$	$9.0 \cdot 10^{-3}$	$3.0 \cdot 10^{-2}$	$8.0 \cdot 10^{-2}$	$2.4 \cdot 10^{-1}$	$8.0 \cdot 10^{-1}$	2.0
Sour	Citric acid	$2.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-2}$	$8.0 \cdot 10^{-2}$	$1.5 \cdot 10^{-1}$	$3.5 \cdot 10^{-1}$	$7.5 \cdot 10^{-1}$	1.5

[a] The concentration series for sucrose, sodium chloride and caffeine were prepared with successive 0.4 log dilution steps. The concentration series for citric acid were prepared with successive 0.3 log dilution steps. Reference chemical details: sucrose (Sigma Aldrich srl, Milano, Italy), sodium chloride (Sigma Aldrich srl, Milano, Italy), caffeine (Sigma Aldrich srl, Milano, Italy), and citric acid (Sigma Aldrich srl, Milano, Italy).

Food Intake Evaluation

A Food and Beverage Frequency Questionnaire (FB-FFQ) was used to assess the consumption frequency of specific categories of foods and beverages over the previous month. The questionnaire was developed by considering a previous validated questionnaire for the Italian population [40] but specifically focusing on the main important food and beverage classes contributing to identify “consumers’ behavior according to taste sensitivity” more than actual energy and nutrient intake. In fact, the main purpose was to assess in a qualitative way the habitual intake of foods and beverages. Participants indicated their frequency of intake of 22 food categories (i.e., sweets, salty snacks, dairy products, meats, fish, fruit, vegetables) using the

following frequency categories: less than once a month, 1–3 times per month, 1–4 times per week, 5–7 times per week, 2-4 times per day, and 5 or more times per day [20,41]. In addition, each participant completed a seven-day food diary to assess their food and nutrient intake. Participants were given verbal instructions and written examples on how to fill in the diary recording the type and amount of foods consumed and possibly the recipes and method of preparation. All participants were given a food record booklet.

Oral Sample Collection, DNA Extraction, and Microbiota Composition Evaluation

Oral sample collection was performed as previously reported in Cattaneo et al. [35]. In brief, volunteers, sitting in front of a mirror, were asked to protrude the tongue and gently press the swab on the surface, rolling and touching edges, tip, and all defined areas of the tongue (~2/3 of tongue length) for 2 min using a sterile flocked swab (FLOQSwabsTM, COPAN S.p.A., Brescia, Italy). The swab samples were immediately placed in 750 µL of Power Bead solution provided in the DNeasy PowerLyzer PowerSoil DNA extraction kit (Qiagen, Hilden, Germany) and stored at −80 ◦C. For DNA extraction, samples were thawed on ice, homogenized for five minutes, and dried. Then, samples were processed using the DNA extraction kit and following manufacturer's instructions with a minor modification (e.g., samples were incubated at 65 ◦C for 10 min after adding C1 solution). Bacterial cell disruption was performed mechanically using a Precellys bead beater kept in a cold room (3 cycles of 6800 rpm × 30 s; Advanced Biotech Italia s.r.l., Seveso, Italy). Quantification and verification of the 260/280 ratio of the extracted DNA was carried out with a Take3 Micro-Volume plate in a Gen5 microplate reader (BioTek Instrument Inc., Winooski, VT, USA). Finally, DNA samples were stored at −80 ◦C. The bacterial taxonomic composition of oral swabs was assessed in a previous study by 16S rRNA gene profiling using Illumina HiSeq technology [35]. Analysis and taxonomic assignment of sequencing reads were also performed by means of the bioinformatic pipeline Quantitative Insights Into Microbial

Ecology (QIIME) version 1.9.0 with the GreenGenes database (version 13_5). Metadata were deposited in the European Nucleotide Archive (ENA) of the European Bioinformatics Institute under accession code PRJEB28769.

Data Analysis

The matrix of the correct and incorrect answers produced separately by each judge was used to calculate the individual thresholds. The individual's Best Estimate Threshold (BET) for each sensory stimulus was calculated as the geometric mean of the highest concentration missed and the next highest concentration that was correctly recognized (ISO/DIS 13301:2018) [39].

Participants were divided according to their taste sensitivity into three groups, using basic taste thresholds as a grouping variable. Participants were defined as hypersensitive if they presented threshold values in the lower percentile (25th percentile): Salty ≤ 0.088 g/L; Sweet ≤ 0.639 g/L; Bitter ≤ 0.0164 g/L; Sour ≤ 0.0316 g/L, and as hyposensitive if they presented threshold values in the higher percentile (75th percentile): Salty ≥ 0.0353 g/L; Sweet ≥ 4.040 g/L; Bitter ≥ 0.1385 g/L; Sour ≥ 0.1095 g/L. The remaining subjects were considered as medium sensitive.

Food record data were used to estimate total energy and macronutrient intake by using the software MètaDieta developed using Italian food composition tables (METEDA srl, Italy).

The FB-FFQ data registered for the 22 food item categories were converted to daily frequency equivalents (DFE) calculated by allocating proportional values to the original frequency categories with reference to a base value of 1.0, equivalent to once a day [20,41]. The scores were calculated as reported in Table 2.

Table 2. The six frequency response options and their conversion into Daily Equivalent Frequency.

Original Frequency used in FFQ	Daily Equivalent Frequency
Less than once per month	0.02
1-3 times per month	0.07
1-4 times per week	0.43
5-7 times per week	0.86
2-4 times per day	3.00
5 or more times per day	5.00

Pearson's coefficients correlations were conducted to analyze the relationship between the gustatory functions.

Mixed ANOVAs were carried out considering "Taste sensitivity" (hyper, medium, and hypo) to basic tastes, "gender" (female, F and male; M) and their interaction as fixed factors and dietary intake, total energy and macronutrient intake as dependent variables, followed by pairwise comparisons using the Bonferroni test adjusted for multiple comparisons ($p < 0.05$). Participants were added as random factor in all the analyses. These statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA).

Correlation analyses between the tongue microbial ecology data, gustatory functions, and dietary intake, total energy and macronutrient intake were performed using the Kendall and Spearman formulas as predictors and dependent variables. Significance was set at $p \leq 0.05$ ($\alpha = 5\%$); significance in the range $0.05 < p < 0.10$ was accepted as a trend.

Results

Participant Characteristics

Fifty-nine volunteers (27 males, 32 females) were recruited for this study. The characteristics of all participants are detailed in Table 3.

Table 3. Baseline characteristics and gustatory functions (taste thresholds, PROP responsiveness e FPD) presented as mean, standard error of study participants.

	Mean	SEM
Age (years)	23.30	0.30
BMI (kg/m^2)	21.55	0.33
Gustatory Functions		
Sweet threshold (g/L)	3.61	0.62
Salty threshold (g/L)	0.20	0.02
Bitter threshold (g/L)	0.16	0.04
Sour threshold (g/L)	0.09	0.01
Food intake		
Total Energy (kcal)	1828.88	60.18
Protein (% En)[a]	15.62	0.35
Fat (% En)	35.23	0.85
Carbohydrates (% En)	45.23	0.73
Protein (g/die)[b]	68.00	2.25
Fat (g/die)	70.06	2.70
Carbohydrates (g/die)	215.96	8.64
Total Fiber (g/die)	15.14	6.74

[a] Calculated as % of total energy intake (kcal); [b] Calculated as gram per day.

Association among Gustatory Functions and Their Relationship with Food Intake

Significant correlations were found between tastes that share many common features in the transduction mechanisms. In particular, the bitter threshold showed a significant correlation with the sweet threshold ($r = 0.34$, $p < 0.01$), while the sour threshold had significant correlations with the recognition thresholds of salty ($r = 0.34$, $p < 0.01$). Moreover, a significant correlation was found between sour and bitter thresholds ($r = 0.31$, $p < 0.05$).

As previously described in the "Material and Methods" section, basic taste thresholds were used as grouping variables and respondents were divided into three groups according to their sensitivity (hypersensitive, medium sensitive, hyposensitive). Among the whole samples, no more than 14 subjects switched from hypo- to hypersensitive within the different stimuli. For salty sensitivity, the group with low sensitivity corresponded to 27.1% of the total sample (10 M, 6 F), the medium sensitive group accounted for 27.1% (7 M, 9 F) and the group with high sensitivity corresponded to 45.8% (10 M, 17 F) of the total sample. For sweet sensitivity, the group with high sensitivity corresponded to 28.8% (9 M, 8 F) of the total sample, while the medium and hyposensitive groups accounted for 22.1% (6 M, 7 F) and 49.1% (12 M, 17 F) of the total sample, respectively. For bitter sensitivity, the hypersensitive group corresponded to 40.7% (15 M, 9 F) of the total sample, while the medium and hyposensitive groups accounted for 18.6% (4 M, 7 F) and 40.7% (8 M, 16 F) of the total sample, respectively. For sour sensitivity, the hypersensitive group corresponded to 22.0% (5 M, 8 F) of the total sample, while the medium and hyposensitive groups accounted for 44.1% (10 M, 17 F) and 33.9% (12 M, 8 F) of the total sample, respectively.

Salty Sensitivity. The elaboration of the results on potential impact of "Salty sensitivity" on food and beverage consumption frequency is reported in Table 4.

Consumption frequency of bakery and salty baked products, legumes, fats, and soft drinks seemed to be associated with "Salty sensitivity". *Post hoc* comparisons showed that, in general, hyposensitive subjects consumed these products significantly more than did medium and hypersensitive subjects. The main factor "gender" was significant for various food categories. In all cases, females have been found to consume significantly less salty baked products ($F_{(1,53)} = 8.46$, $p < 0.01$), cured meats ($F_{(1,53)} = 11.25$, $p < 0.001$), and soft drinks ($F_{(1,53)} = 10.19$, $p < 0.01$), but more fish ($F_{(1,53)} = 9.88$, $p < 0.01$), fruit ($F_{(1,53)} = 8.15$, $p < 0.01$), and nuts ($F_{(1,53)} = 7.02$, $p < 0.05$) than males. The "Salty sensitivity" × "gender" interaction was significant only in a few cases (cereal and cereal-derived products: $F_{(2,53)} = 5.52$, $p < 0.01$; cured meats: $F_{(2,53)} = 3.62$, $p < 0.05$; nuts: $F_{(2,53)} = 3.27$, $p < 0.05$; soft drinks: $F_{(2,53)} = 5.06$, $p < 0.01$).

When food record data were considered, a significant association with "Salty sensitivity" was found ($F_{(2,53)} = 3.52$, $p < 0.05$) on fat (as% energy intake), with hyposensitive subjects showing a higher intake compared to medium and hypersensitive subjects. A significant "gender" association was found ($F_{(2,53)} = 5.76$, $p < 0.05$), underlying a higher fat intake in female subjects with respect to males.

Sweet Sensitivity. The elaboration of the results on potential impact of "Sweet sensitivity" on food and beverage consumption frequency is reported in Table 4.

Consumption frequency of legumes and sweets and desserts seemed to be associated with "Sweet sensitivity". *Post hoc* comparisons showed that, in general, hypersensitive subjects consumed these products significantly less than did medium and hyposensitive subjects. The main factor "gender" was significant for various food categories. In all cases, females reported consuming significantly less salty baked products ($F_{(1,53)} = 14.29$, $p < 0.001$),

cured meats ($F_{(1,53)}$ = 5.89, p < 0.05), sweets and desserts ($F_{(1,53)}$ = 4.06, p < 0.05), alcoholic beverages ($F_{(1,53)}$ = 5.19, p < 0.05), and soft drinks ($F_{(1,53)}$ = 5.16, p < 0.05), but more fish ($F_{(1,53)}$ = 5.70, p < 0.05) than males. The "Sweet sensitivity" × "gender" interaction was significant only in a few cases (dairy products: $F_{(2,53)}$ = 3.48, p < 0.05; candies and gums: $F_{(2,53)}$ = 3.17, p < 0.05).
When food record data were considered, significant differences were found between female and male subjects on total energy and carbohydrates and fat consumptions. Energy (kcal) ($F_{(2,53)}$ = 4.71, p < 0.05) and carbohydrate intakes (g) ($F_{(2,53)}$ = 5.70, p < 0.05) were significantly lower in female subjects compared to male subjects. By contrast, fat intake (as% energy intake) ($F_{(2,53)}$ = 8.60, p < 0.01) was significantly higher in females than males.

Bitter Sensitivity. The elaboration of the results on potential impact of "Bitter sensitivity" on food and beverage consumption frequency is reported in Table 5.
"Bitter sensitivity" had a significant association with consumption frequency of oils ($F_{(2,53)}$ = 5.41, p < 0.01). *Post hoc* comparisons showed that hyposensitive subjects consumed these products significantly more than did medium and hypersensitive subjects. The main factor "gender" was significant for some food categories. In all cases, females reported consuming significantly less salty baked products ($F_{(1,53)}$ = 6.63, p < 0.05) and cured meats ($F_{(1,53)}$ = 8.47, p < 0.01), but more fish ($F_{(1,53)}$ = 8.79, p < 0.01), fruit ($F_{(1,53)}$ = 4.87, p < 0.05), and nuts ($F_{(1,53)}$ = 4.02, p < 0.05) than males.
The "Bitter sensitivity" × "gender" interaction was significant only in a few cases (oils: $F_{(2,53)}$ = 5.32, p < 0.01; salty snacks: $F_{(2,53)}$ = 3.54, p < 0.05).
When food record data were considered, a significant association with "Bitter sensitivity" was found on energy (Kcal) ($F_{(2,53)}$ = 3.30, p < 0.05) and carbohydrates (g) ($F_{(2,53)}$ = 3.59, p < 0.05) intakes, with hypersensitive subjects showing higher intakes compared to medium and hyposensitive subjects. A significant "gender" association was found with carbohydrates ($F_{(2,53)}$ = 6.97, p < 0.01) and fat intakes (as% energy intake) ($F_{(2,53)}$ = 11.77, p < 0.001),

underlying a lower carbohydrates but a higher fat intake in females than males.

Sour Sensitivity. The elaboration of the results on potential impact of "Sour sensitivity" on Food and Beverage consumption frequency is reported in Table 5.

"Sour sensitivity" had a significant association only with consumption frequency of fish ($F_{(2,53)} = 6.14$, $p < 0.01$). Post hoc comparisons showed that subjects characterized by medium sensitivity to sour consumed fish significantly less than did hypo- and hypersensitive subjects.

The main factor "gender" was significant for some food categories. In all cases, females reported consuming significantly less salty baked products ($F_{(1,53)} = 8.21$, $p < 0.01$), cured meats ($F_{(1,53)} = 6.47$, $p < 0.05$), and soft drinks ($F_{(1,53)} = 5.29$, $p < 0.05$), but more fish ($F_{(1,53)} = 10.42$, $p < 0.01$) and nuts ($F_{(1,53)} = 4.04$, $p < 0.05$) than males. The "Sour sensitivity" × "gender" interaction was significant only in one category (salty snacks: $F_{(2,53)} = 3.54$, $p < 0.05$).

When food record data were considered, significant differences were found between female and male subjects for carbohydrates (g) ($F_{(2,53)} = 3.86$, $p < 0.05$) and fat consumptions (as% energy intake) ($F_{(2,53)} = 9.12$, $p < 0.01$). Carbohydrates intake (g) was significantly lower in female subjects compared to male subjects. By contrast, fat intake (as% energy intake) ($F_{(2,53)} = 8.60$, $p < 0.01$) was significantly higher in females than males.

Table 4. Mean values of daily equivalent frequency consumption for the 22 food categories by salty and sweet taste sensitivity level.

Items	Daily Equivalent Frequency				Daily Equivalent Frequency			
	p value	Salty Taste Sensitivity Level			*p value*	Sweet Taste Sensitivity Level		
		Hyper	Normal	Hypo		Hyper	Normal	Hypo
Cereal and cereal-derived products (e.g., pasta, rice, barley, spelt)	0.07	1.67	1.33	2.23	0.88	1.92	1.58	1.49
Salty baked products (e.g., bread, pizza, focaccia)	**0.007**	0.99 ^{b}	1.45 ^{ab}	1.99 ^{a}	0.69	1.37	1.12	1.40
Bakery products (e.g., bakery and breakfast cereals, biscuits, croissants)	**0.04**	0.76 ^{b}	1.39 ^{a}	1.42 ^{a}	0.48	1.12	1.34	0.94
Meats	0.98	0.50	0.51	0.49	0.85	0.48	0.54	0.50
Cured meats	0.64	0.36	0.40	0.31	0.09	0.27	0.48	0.37
Fish	0.16	0.41	0.28	0.43	0.35	0.39	0.28	0.39
Milk and yoghurts	0.47	0.84	0.75	1.11	0.27	0.75	0.61	1.04
Dairy products	0.32	0.51	0.79	0.79	0.19	0.52	0.62	0.86
Eggs	0.22	0.34	0.23	0.29	0.72	0.33	0.27	0.30
Vegetables	0.41	1.80	1.85	2.40	*0.08*	2.62	1.72	1.71
Legumes	**0.05**	0.36 ^{b}	0.67^{a}	0.41 ^{ab}	**0.03**	0.69 ^{a}	0.41 ^{ab}	0.35 ^{b}
Potatoes	0.20	0.35	0.58	0.47	0.65	0.49	0.35	0.41
Fruit	0.22	1.65	1.84	2.42	0.79	2.07	1.78	1.78
Fruit juices	0.31	0.22	0.43	0.48	0.80	0.40	0.26	0.36
Nuts	0.27	0.23	0.45	0.36	0.76	0.33	0.22	0.33
Sweets and desserts (e.g., cakes, ice creams, chocolate)	0.53	0.60	0.86	0.78	**0.02**	0.32 ^{b}	0.98 ^{a}	0.87 ^{a}
Fats	**0.05**	0.26 ^{b}	0.22 ^{b}	0.45 ^{a}	0.18	0.19	0.35	0.34
Oils	0.47	1.99	1.54	1.72	0.87	1.83	1.94	1.74
Salty snacks (e.g., chips, salty peanuts)	0.23	0.22	0.22	0.35	0.23	0.17	0.27	0.30
Alcoholic beverages	0.21	0.49	0.29	0.31	0.18	0.30	0.56	0.35
Soft drinks	**0.007**	0.26 ^{b}	1.13 ^{a}	0.16 ^{b}	0.82	0.59	0.39	0.38
Candies and gums	0.82	0.63	0.54	0.78	0.32	0.82	0.81	0.40

Significant *p*-values are shown in bold. Different letters indicate significant differences according to Bonferroni's post hoc test.

Table 5. Mean values of daily equivalent frequency consumption for the 22 food categories by bitter and sour taste sensitivity level.

Items	Daily Equivalent Frequency				Daily Equivalent Frequency			
	p value	Bitter Taste Sensitivity Level			*p value*	Sour Taste Sensitivity Level		
		Hyper	Normal	Hypo		Hyper	Normal	Hypo
Cereal and cereal-derived products (e.g., pasta, rice, barley, spelt)	0.58	1.75	1.29	1.66	0.08	2.19	1.31	1.78
Salty baked products (e.g., bread, pizza, focaccia)	0.70	1.45	1.25	1.20	0.11	0.90	1.43	1.66
Bakery products (e.g., bakery and breakfast cereals, biscuits, croissants)	0.81	0.92	1.10	1.10	0.21	0.69	1.20	1.29
Meats	0.65	0.47	0.48	0.54	0.75	0.53	0.51	0.46
Cured meats	0.41	0.34	0.46	0.33	0.37	0.35	0.40	0.29
Fish	0.22	0.42	0.26	0.36	**0.004**	0.49 [a]	0.27 [b]	0.46 [a]
Milk and yoghurt	0.66	0.72	0.97	0.92	0.96	0.91	0.84	0.82
Dairy products	0.07	0.52	0.52	0.95	0.55	0.49	0.75	0.69
Eggs	0.52	0.33	0.24	0.30	0.54	0.36	0.31	0.27
Vegetables	0.08	1.92	1.04	2.23	0.33	1.84	1.77	2.39
Legumes	0.51	0.53	0.42	0.38	0.09	0.32	0.61	0.40
Potatoes	0.53	0.49	0.39	0.35	0.23	0.61	0.42	0.36
Fruit	0.76	2.06	1.70	1.81	0.94	1.77	1.94	1.94
Fruit juices	0.24	0.48	0.39	0.19	0.70	0.22	0.31	0.39
Nuts	0.42	0.43	0.26	0.26	0.41	0.43	0.23	0.36
Sweets and desserts (e.g., cakes, ice creams, chocolate)	0.85	0.73	0.61	0.78	0.44	0.46	0.75	0.79
Fats	0.84	0.29	0.29	0.25	0.26	0.26	0.36	0.22
Oils	**0.007**	1.46 [b]	1.11 [b]	2.22 [a]	0.35	1.64	1.72	2.16
Salty snacks (e.g., chips, salty peanuts)	0.40	0.24	0.20	0.32	0.44	0.18	0.28	0.22
Alcoholic beverages	0.34	0.46	0.33	0.29	0.70	0.31	0.43	0.37
Soft drinks	0.22	0.68	0.20	0.16	0.81	0.40	0.56	0.36
Candies and gums	0.91	0.68	0.73	0.57	0.76	0.48	0.63	0.78

Significant *p*-values are shown in bold. Different letters indicate significant differences according to Bonferroni's post hoc test.

Correlation between Tongue Dorsum Microbiota, Gustatory Functions, and Dietary Intake

To infer potential links between bacteria on tongue dorsum, gustatory functions, and dietary intake, we performed correlation analyses between taste thresholds, total energy, and macronutrient intake and the DADA2/SILVA/speciateIT-determined bacterial relative abundances.

Several bacterial taxa abundances were correlated with taste thresholds. In particular, one taxon negatively correlated with sweet, three with sour, and six with salty thresholds. In summary, bacterial taxa abundances increase in subjects characterized by lower taste thresholds. On the contrary, the genus *Rothia* was the only taxon positively associated with taste thresholds, specifically salty. No taxon was correlated with the bitter threshold (Figure 1 – left side).

Finally, we performed correlation analyses between the oral microbiota and dietary intake. We found that energy and macronutrient intake were significantly correlated with several bacteria taxa (Figure 1 – right side).

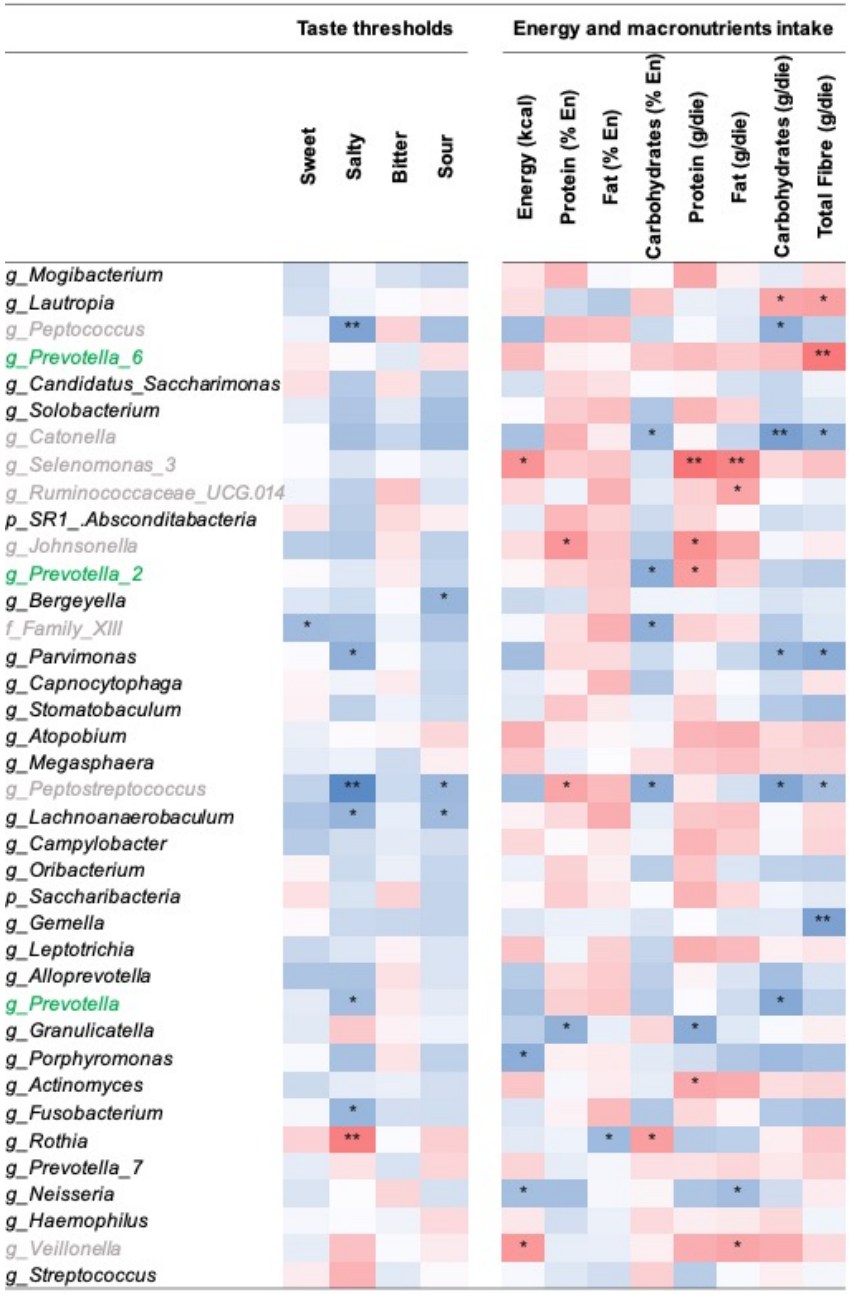

Figure 1. Correlations between the relative abundance of bacterial taxa on tongue dorsum and taste thresholds for the four basic tastes (left side) and nutritional variables (right side). The heatmap represents the Spearman's correlation R-values. Asterisks relate to the Kendall rank correlation P values: * p <0.05; ** p <0.01; *** p <0.001. *Prevotella* genera and *Clostridia* class, which shown significant correlation with energy and macronutrients intake, were highlighted in green and in grey colors, respectively.

Notably, we found that several taxa in *Clostridia* class (e.g., genera *Selenomonas, Ruminococcaceae, Johnsonella*, and *Veilonella*) were positively correlated with total energy, protein, and fat intake and negatively correlated with carbohydrates and total fiber intake (e.g., genera *Catonella* and *Peptostreptococcus*). Contrarily, *Prevotella* genus was positively correlated with total fiber intake (Figure 1 – right side). Overall, these results seem to indicate that some microbial taxa are positively associated with vegetable-rich (*Prevotella* genus) or protein/fat-rich diets (*Clostridia* class).

Discussion

The present study evaluated interindividual differences in recognition thresholds for basic tastes and examined to what extent these variations in gustatory functions among individuals could be related to food intake in a sample of reportedly healthy adult women and men. Additionally, these variables were further evaluated in relation to individual oral microbiota composition.

The present study shows that recognition thresholds for the basic tastes were associated with each other, albeit in different ways. Indeed, significant correlations were found between tastes that share many common features in the transduction mechanisms. The perception of both sweet and bitter tastes is mediated via G-coupled protein receptors, encoded by TAS1R and TAS2R taste receptor gene families, while salty and sour tastes are transduced via ion channels [42]. Thus, these findings seem to confirm the presence of the well-known dichotomy in taste coding for perception of pleasant (e.g., sweet and savory compounds) vs noxious stimuli (e.g., sour and bitter tastants) [43].

The results of the present study showed that interindividual differences in taste perception may influence habitual food consumption and intake. This assumption supports various observations that taste sensitivity may play an important role in dietary habits and body energy balance [37,44–46]. Indeed, it has been suggested that subjects characterized by a reduced or distorted

taste sensitivity could increase the willingness to ingest foods that involve greater stimulation of the taste and oral somatosensory system (e.g., high-energy dense foods rich in sugars and fats), leading to unhealthy food choices, and thus pathogenesis of weight excess.

As far as salt intake is regarded, a few studies [47–49] examined the association between salt hedonics and sodium intake, but the relationship between salty sensitivity and food intake has been poorly investigated. Kim and Lee [25] reported an association between individuals' salty taste sensitivity and sodium-rich fast foods acceptance and consumption in a sample of Korean adolescents. Moreover, Hayes and colleagues [47] reported that variation in salt perception was associated with differences in preferences to high-sodium foods and, indirectly, to sodium intake. Accordingly, in the present study, subjects who were orally hypersensitive to sodium chloride solution reported consuming less bakery and salty baked products than those who were defined as hyposensitive. Moreover, hyposensitivity to salty taste seems to increase consumption of less healthy foods, like saturated-fat-rich products and soft drinks. This assumption is supported by food record data, in which fat intake (expressed as a percentage of total energy intake) was found to be higher in the hyposensitive group.

Previous studies have failed to find associations between sweet taste and diet parameters [21,23]. Contrarily, the present data from the FB-FQ and sweet sensitivity suggests that participants who have a higher threshold for sweet taste (hyposensitive) reported consuming more frequently sweets and desserts than the hypersensitive group. This is supported by findings of recent studies, where positive relationships were found between reduced perceived intensity and increased desire for higher energy providing taste stimuli [50]. Accordingly, Jayasinghe and colleagues [20] showed that participants who perceived as sweeter the highest glucose concentrations are reported to be more sensitive to sweet taste and had a lower consumption frequency of sweet foods compared to those who perceived the solution proposed as less sweet.

Interestingly, this study did not find any relationship between sweet taste sensitivity and energy or macronutrient intakes expressed as a percentage of total energy or grams. This result was in contrast with previous findings suggesting an inverse correlation between glucose taste perception and total energy and carbohydrate intakes [20]. However, as recently discussed by Webb and colleagues [38] it is necessary to use a combination of sweet taste measurements (e.g., glucose, sucrose, and fructose) to better characterize the overall perception and the relationships between sweet taste perception and food intake.

Nevertheless, we observed a relationship between bitter taste sensitivity and total energy and carbohydrates intakes. Participants who were orally hypersensitive to caffeine solutions showed higher total energy and carbohydrate intakes compared to those who perceived the solution as less bitter, suggesting a potential shift towards less healthy dietary patterns in the hypersensitive group of subjects. These results seem to support the hypothesis that higher taste sensitivity to bitter compounds can elicit rejection responses in subjects leading to a reduced selection and intake of some vegetable foods in favor of high-energy-dense foods [51].

Regarding the relationship between sour taste sensitivity and food consumption frequency, our results failed to underline any significant association. It is important to note that, in literature, the attention has been mainly focused on individual variation in sour taste perception and preferences for sour foods [52], suggesting that low preference for sour foods could eventually lead to limited choices or inadequate intake of fruit and berries. However, even if these results demonstrated a genetic contribution to preference for sour foods, the authors underlined that sour taste perception and related preferences for sour foods are mediated by both genetic and environmental factors (e.g., food habits of the family). Thus, the potential relationship among sour taste perception and subsequent food choices and intake remains to be explored [16].

As expected, gender-related differences in food consumption frequency and intake were found, confirming previous studies in which differences in the nutritional quality of the diet of men and women were highlighted [53–56]. Indeed, men reported significantly more frequent consumption of salty baked products, cured meats, sweets and desserts, alcoholic beverages, and soft drinks than did women. On the other hand, women were more likely to consume fish, fruit and nuts. Macronutrients intake, in terms of percentage of total energy intake, differed between female and male subjects. Clearly, as expected, men consumed higher total energy and carbohydrates compared to women.

In order to provide further insights into the complexities of human eating behavior, the present study focused the attention on less investigated nongenetic factors potentially influencing food preference and habits. In particular, we considered the oral microbiota composition since, recently, a relationship between reduced taste perception and specific oral bacteria's growth has been reported [35], in agreement with previous findings [33,34]. Solemdal and colleagues [34] investigated variables related to taste ability and oral health in acutely hospitalized elderly, showing that taste perception, particularly for sour taste, was reduced in acutely hospitalized elderly with high lactobacilli growth. Besnard and colleagues [33] tested the hypothesis that obese and normal-weight adults could be characterized by an impaired fat taste perception, which could be also linked to a change in the microbial composition. This study showed no difference in the fat taste perception and composition of oral microbiota between normal-weight and obese subjects. Otherwise, specific bacterial composition was found in lipid non-tasters, irrespectively of nutritional status. Moreover, in our previous study, we found that subjects who were characterized by a greater responsiveness to PROP presented differences in the relative abundance of some taxa compared to subjects who were less responsive to the PROP compound. In particular, five bacterial genera, including the Gram-positive genera *Actinomyces*,

Oribacterium, *Solobacterium*, and *Catonella*, and the Gram-negative *Campylobacter*, were overrepresented in the most responsiveness group.

In the present study, interesting further correlations between the relative abundance of bacterial taxa on tongue dorsum and gustatory functions were found. The present results showed that a number of taxa were inversely correlated with salt and sour thresholds, showing that a great salty and sour sensitivity may be linked to specific taxa, mainly attributed to Clostridiales and Bacteroidales order. Given the diversity of genera and species within the oral microbiome [57], it is overall difficult to propose systematic explanations of such links. However, a hypothesis may reside in bacterial modulatory ability as suggested by Alcock and colleagues [58], who described a potential involvement of microbes in the manipulation of eating behavior by altering the host preferences through a modulation of receptor expression, as in vivo animal model studies on gut microbiota showed [59,60]. Another plausible explanation may lie in bacterial ability to degrade carbohydrates into disaccharides, monosaccharides, and organic acids, used as “building material” for biofilms [61]. The physical barrier between tastants and taste receptors would, as a consequence, be less or more efficient, thus influencing sensitivity. According to our results, also Feng and colleagues [62] reported that a higher proportion of Actinobacteria was linked to lower taste sensitivity, while a higher proportion of Bacteroidetes increased sensitivity.

Nonetheless, a more detailed characterization of microbial communities and their metabolic feature would be of interest, but this study supports that the oral microbiota composition deserves to be considered as an influencing variable when investigating peri-receptor events involved in chemosensory processes.

The role of diet in shaping the gut microbiota is widely recognized [63,64]. However, until recently, only a few studies have considered the association between habitual diet and oral microbiota.

In the present study, interesting correlations between the relative abundance of bacterial taxa on tongue dorsum and dietary intake were found. Indeed,

Clostridia class was positively associated with total energy, fat, and protein intake but negatively associated with fiber intake, whereas Proteobacteria phylum and *Prevotella* genus showed the opposite association. Since it has been found that oral cavity and stool bacteria overlapped in nearly half (45%) of the subjects in recent studies [65,66], it is possible to hypothesize that dietary habits could affect both oral and gut microbiota in a similar way. Indeed, our results are in line with the general assumption that some gut microbial taxa are positively associated with vegetable-rich (*Prevotella*) or protein/fat-rich diets (*Clostridia*) [67,68]. However, further studies are warranted to clarify whether observations from the gut microbiome are transferrable to the oral microbiome. In this context, the oral microbiome could be further investigated as potential marker of long-term consumption of healthy or unhealthy diets.

The strengths of the present study include an investigation of the relationship between taste sensitivity for all the four tastes with a range of parameters of food consumption frequency and food intake. In particular, dietary intake was investigated through assessment of actual food intakes (seven-day food records) and habitual intakes of different categories of foods and beverages (FB-FQ), capturing different aspects of eating habits. Finally, the multidisciplinary approach applied in the present study offers new insights into the reciprocal impact between taste perception, food intake, and oral microbiota composition.

The present study has also several limitations. Firstly, participants involved were a small sample of Italian women and men of similar age (young) and BMI (normal range). Therefore, the findings of this study cannot be generalized to other ethnicities, ages, or BMI groups. Secondly, the study design was cross-sectional and the findings represent only relationships among variables under study while no causations can be ascertained. Thirdly, limitations to the study include validity of food intake measurements. Reported intakes may be inaccurate due to memory recall, interviewer and subject bias,

and responder fatigue, all of which contribute to underestimating or overestimating food intake measures [69].

In conclusion, the present study shows a link between taste sensitivity and dietary measurements in a group of young healthy women and men with normal BMI and food intake. Moreover, significant relationships between taste sensitivity and dietary measurements, but also with oral microbiota composition, were found.

These findings have implications for eating behavior, as perceived sensory properties of foods and beverages clearly influence preferences and the type and amount of food consumed [1]. Moreover, this study provides further support that nongenetic factors, such as the oral bacteria lining the tongue, should be adequately considered in order to gain new insights into taste-related eating habits that may influence long-term health outcomes. The impact of genetic and nongenetic characteristics, including the complex interactions among multiple factors related with food cues and exposure, can affect food choices and dietary intake. For this reason, this topic remains an important research area to be further investigated, since all these aspects reciprocally influence each other, driving towards individual eating behavior.

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Project administration, PR and EP; Supervision, EP; Visualization, GG; Writing—original draft, CC; Writing—review & editing, PR, ML, GG, and EP. All authors read and approved the final manuscript.

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CHAPTER 4

TASTE PERCEPTION, ORAL MICROBIOTA AND NUTRITIONAL STATUS

Taste perception and oral microbiota are associated with obesity in children and adolescents

Mameli C, **Cattaneo C**, Panelli S, Comandatore F, Sangiorgio A, Bedogni G, Bandi C, Zuccotti G, Pagliarini E. Taste perception and oral microbiota are associated with obesity in children and adolescents. ***PLOS One***. 2019 14(9), e0221656

Taste perception and oral microbiota are associated with obesity in children and adolescents

Abstract

Obesity in childhood and adolescence is considered the most prevalent nutritional disorder, in which eating behaviours represent one important factors of influence. Many aspects influence eating behaviours, but taste is considered the main predictor. However, data concerning correlations of obesity, taste sensitivity and behavioural attitudes, such as food neophobia, in children and adolescents are inconsistent. Moreover, it has been suggested that oral bacteria could have a possible role in obesity development and, also, in taste perception. In this context, the present study focused on host related factors with a proposed link to weight gain. To this purpose, taste sensitivity, salivary microbiota composition and food neophobia were compared between children and adolescents with and without obesity in a cross-sectional study. Results showed that children with obesity presented a significantly lower ability in correctly identifying taste qualities and were characterized by a lesser number of Fungiform Papillae (reported as FP/cm^2) compared to normal-weight subjects. Differences in the ecological indexes of microbial alpha-diversity was found between subjects with obesity and normal-weight ones. Moreover, independently from nutritional status, some bacterial genera seemed to differ between subjects with different sensitivity. The potentiality of this multidisciplinary approach could help to better understand and deepen the sensory-driven and microbiological factors related to weight gain.

Introduction

Obesity is one of the most serious international health concerns. Prevalence of childhood obesity is increasing worldwide and its rates in Italy are among the highest (36% for boys and 34% for girls) [1, 2]. Obesity is considered a

multifactorial aetiology disease, which seems to be genetically based, but requires environmental, psychological and social influences to exhibit [3]. An important portion of such environmental influences is represented by diet and related eating behaviours [4]. Although many factors contribute to eating behaviours, taste is considered one of the main predictor in determining children's food acceptance and choices [5].

It is well known that sensitivity for taste qualities differs between individuals and polymorphisms of the genes coding for taste are supposed to be one of the multifactor causes of these inter-individual differences [for a review see: 6]. Moreover, many researchers reported differences in taste sensitivity between obese and non-obese adults [7-11] as well as children [12, 13]. In particular, individuals with a higher body mass index (BMI) are characterized by lower taste sensitivity for all the basic taste and were significantly less responsive to the bitterness of the 6-n-propylthiuracil (PROP) compound, which is considered a phenotypic marker of genetic variation in taste and the most studied one. Consequently, obese subjects need to consume more to have the same stimulation of taste and oral somatosensory system in order to compensate their impaired sensitivity. This lacking sensitivity is hypothesised to have relationships with food intake and body weight variation with implication on long-term health outcomes [14].

However, data concerning correlations between taste sensitivity and obesity are inconsistent [15] and centered mainly on the PROP responsiveness, whereas little is currently known about other taste qualities, especially in children.

Besides individual variation in chemosensory perception, food neophobia (literally the reluctance to eat novel foods) is another aspect to be considered as an important trait in shaping food habits [16]. It has been argued that increased food neophobia may lead children to limit their food choices largely to palatable, high in calories-fat-sugars foods [17], which in turn could represent a risk for excess weight gain. However, studies that have systematically examined the relationship between food neophobia, taste

perception and children weight status are scarce and still under investigation [18].

Interestingly, recent research on the enormously complex and vast microbial community in the gastrointestinal tract has provided new insights into the mechanisms of obesity and obesity- related diseases [19]. The majority of studies on the human microbiome focused the attention on the distal gut [20, 21], but recently, it has been suggested that oral bacteria could have a potential direct role in development of obesity [22]. However, rather surprisingly, oral microbiota has been poorly investigated in relation to this pathology. Goodson and colleagues [23] reported differences in abundances of salivary bacteria in overweight women compared with normal weight women, suggesting that some taxa could be biomarkers for excess adiposity. In addition, a relationship between sensitivity and oral bacteria was proposed, associating taste perception with the growth of specific oral bacteria [24-27]. Since the composition of oral microbiota appears to have an important but still unclear role in obesity development and to affect sensitivity, an approach to inquiry into the relationship between obesity, taste sensitivity and oral microbiota composition seems required.

In this context, the aim of the present study was to focus on host related factors with a proposed link to weight gain. To this purpose, taste sensitivity, salivary microbiota composition and food neophobia were compared between children and adolescents with and without obesity in a cross-sectional study.

Material and methods

Subjects

Participants were recruited at the Obesity Clinic of the V. Buzzi Children's Hospital (Milan, Italy) from January 31, 2018 to May 31, 2018. The inclusion criteria were: essential obesity with body mass index (BMI) ≥ 2 standard deviations (SD) according to WHO charts [28], age ≥6 and ≤14 years and Caucasian ethnic group. The exclusion criteria were genetic/syndromic

obesity and history of any psychiatric diseases diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSMV) [28]. Moreover, we excluded patients with acute or chronic diseases disturbing smell or taste function, with diseases affecting weight or those treated with medications affecting weight (e.g. corticosteroids), patients taking drugs that are known to affect smell or taste and subjects who consumed any antibiotics two months before the study. Healthy sex- and aged-matched controls were recruited as control group from other departments of the clinic applying the same exclusion criteria.

Informed consent has been obtained from all subjects' parents and/or legal guardians. The study was approved by the Ethics Committee of ASST-FBF-Sacco (Milan, Italy), conducted in accordance with the Declaration of Helsinki and all methods were performed in accordance with the relevant guidelines and regulations.

Each subject was subjected to the anthropometric evaluation, to the collection of saliva samples and to the screening of gustatory functions as well as the evaluation of his/her attitude and preferences towards foods, as described in detail below.

General procedure

Participants were asked not to eat, to drink nothing but water and not to chew chewing gum at least 2 h before testing. Participants were subjected to 4 successive sessions. Session 1 included a medical exploration, in which children and adolescents were screened by the medical team and measured for their height and weight to identify the condition of normal-weight or obesity. During Session 2 the oral samplings of saliva were collected. The Session 3 was devoted to the assessment of taste sensitivity (Gustatory function screening and Fungiform Papillae count). During the Session 4, children and adolescents were asked to complete a questionnaire concerning Food Neophobia.

Anthropometric measurements

Body weight was measured using a medical-certified scale (SECA, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a children's medical-certified stadiometer (SECA, Hamburg, Germany). BMI was calculated as body mass (W, kg) divided by height (H, m) squared. The BMI values were transformed into BMI z scores using WHO reference values for paediatric BMI [29]. Obesity was defined by BMI z score ≥ 2 SD (i.e., at least 2 standard deviations above the age- and sex-specific expected value) and normal-weight was defined by BMI between – 2 and 1 SD, in accordance to using WHO reference values for paediatric BMI [29].

Oral sample collection and DNA Extraction

Subjects were restricted for at least 2 hours (h) of food intake prior to sample collection as mentioned previously. Unstimulated whole saliva samples were collected by direct spitting into a sterile plastic tube in a time span not exceeding 10 minutes (min). Samples were immediately frozen until analysis. DNA was extracted from 1 ml saliva using the QIAamp DNA Blood Mini Kit, Qiagen (Hilden, DE) and following the protocol suggested by the manufacturer to assure an unbiased representation of bacterial taxa [30]. The DNA concentration of extracted samples was assessed fluorometrically.

PCR Production of 16S rRNA amplicons (V3-V4 regions) and sequencing

For amplicon production, the V3-V4 hypervariable regions of the prokariotic 16S rRNA gene were targeted [31]. PCR was performed in a 50-ml volume containing template DNA, 1x HiFi HotStart Ready Mix (Kapa Biosystems, Wilmington, MA), 0.5 mM of each primer. The cycling program, performed on a Bio-Rad T100 thermal cycler (Bio-Rad, Hercules, CA) included an initial denaturation (95°C for 3 min), followed by 30 cycles at 94°C for 30 seconds (s), 55°C for 30 s, 72°C for 30 s, and a final extension (72°C for 5 min). Clean-up of amplicons was performed using Agencourt AMPure XP SPRI magnetic beads (ThermoFisher Scientific). Illumina sequencing libraries were finally constructed through the link of indexes (Nextera XT Index Kit, Illumina, San

Diego, CA), quantified using a Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA), normalized and pooled. Libraries were subjected to paired-end sequencing (2 x 300 bp format) on an Illumina MiSeq platform at BMR Genomics (Padova, Italy). Two amplicons were produced and sequenced for each subject enrolled in the study.

Bioinformatics and community analyses

The bioinformatic treatment of sequencing data was based on the Mothur software [32]. Briefly, raw FASTQ files were quality-filtered using Trimmomatic [33]. High-quality reads were then analysed following the SOP mothur procedure [32]. Chimeric sequences were identified using UCHIME [34] and then removed. The selected sequenced were clustered into operational taxonomic units (OTUs) at 97% similarity using VSEARCH [35]. OTUs were finally annotated, and taxonomy was assigned, against the reference database SILVA [36].

The main ecological indexes of a-diversity Shannon and Chao were computed using Mothur [32]. Diversity in composition among samples (b-diversity) was evaluated at all taxonomic ranks (from phyla to genera) by plotting the relative heatmap using the function heatmap.2 of the Gplots [37] R library, and the relative Principal Component Analysis (PCA) using the R library Ade4 [38].

Gustatory function screening

The protocol used is fully described elsewhere [13, 39]. Gustatory screening was performed applying the 'Taste Strips' method [40, 41], in which prefabricated filter papers impregnated with different taste solutions were used. The 'Taste Strips' method is reported to have a good test-retest-reliability [40], a good acceptance by children and adolescents and has been applied in several research and clinical contexts [41-44]. According to previous studies [13, 39, 45, 46] a total number of 18 paper strips were used, four different concentrations for each taste qualities (sweet, sour, salty and bitter) and two blank strips. The taste strips were presented in increasing concentrations, randomising the taste quality order at each level of

concentration. Taste strips were placed on the tongue and subjects were asked to identify the taste quality and to select one of five possible answers (sweet, sour, salty, bitter, no taste) on a form. Before the session started, taste qualities were explained to the participants. In order to control for carry-over effect subjects were asked to rinse their mouth with water before assessment of each taste strip.

Fungiform Papillae count assessment

The protocol used is fully described elsewhere [9]. The fungiform papillae (FP) count was measured according to Nachtsheim and Schlich [47]. Testing was performed in a sitting position and starting with cleansing the mouth by a sip of water. The child placed the elbows on the table and fixed the head with the hands. The tongue was dried with filter paper and stained with a blue food colorant (F.lli Rebecchi, Color Dolci). A circle of filter paper of 6 mm diameter was used as a template and placed on the left side of the tongue, approximately 1-2 cm from the tip. Several photos of the tongue were taken using a 16-megapixel digital camera (NIKON Corporation, Japan) in macro mode with no flash. After selecting the best photo Adobe Photoshop software was used and three circles were drawn in the front of the anterior tongue using the template. The number of FP was counted inside each marked circles, according to Bakke and Vickers [48] and was counted twice by two independent examiners, and therefore the mean of the two counts was calculated.

Food Neophobia assessment

To investigate Food Neophobia, participants received the Italian Children Food Neophobia Scale (ICFNS), validated by Laureati and colleagues [49] in a large cohort of school-aged children. The ICFNS consists of eight items, four related to neophobic and four related to neophilic attitudes. In order to aid younger subjects to better understand the level of agreement/disagreement for each item a facial expression is used to exemplify the 5-point scale (‘very false to me’ – ‘very true for me’). This resulted in a food neophobia score

ranging from 8 to 40, which was calculated for each child (neophilic item scores were reversed). Higher scores denote greater food neophobia.

Statistical analysis

Coarsened exact matching (CEM) was used to match cases with obesity and non-obese controls on the basis of sex (same) and age (within 2 years) [50]. Most continuous variables were not Gaussian-distributed and are all reported as 50th percentile (median) and 25th and 75th percentiles (interquartile range, IQR). Discrete variables are reported as the number and proportion of subjects with the characteristic of interest. Descriptive statistics took CEM into account by means of CEM-related weights [51].

To answer the main study question, i.e. whether there is any difference in gustatory functions between controls and cases, all correctly identified taste strips of the qualities sweet, sour, salty, and bitter were summarised in a Total Taste Score (TTS) giving a maximum score of 16 points. Differences between controls and cases was evaluated by means of a linear regression model (LRM) using TTS as outcome and obesity (0 = no; 1 = yes) as predictor. The LRM took CEM into account by using CEM-related weights and robust 95% confidence intervals [51]. Age and gender were then added to the LRM as covariables to evaluate their potential confounding effect on the relationship between TTS and nutritional status.

The secondary study question, i.e. whether there is a difference in the density of FP between controls and cases, was tested by means of a Poisson regression model (PRM) using the density of fungiform papillae as outcome and nutritional status (0 = control; 1 = case) as predictor. The PRM took CEM into account by using CEM-related weights and robust 95% confidence intervals [51]. Age and gender were then added to the PRM as covariables to evaluate their potential confounding effect on the relationship between FP density and nutritional status. The third study question involved the characterization of the salivary microbiota composition in cases and controls. This point, descriptive in nature, was addressed at the taxonomic level of

phyla and classes by plotting distributions of cases and controls on dot charts [52].

Statistical analysis was performed using Stata 15.1 (Stata Corporation, College Station, TX) together with the user-written CEM command [51].

Results

From January 31st, 2018 to May 31st, 2018 we recruited 34 subjects affected by obesity and 33 controls, who fulfilled the inclusion criteria. Because of CEM, 45% of the children were female among controls (n = 15) and 56% among cases (n = 19). The median (IQR) age was the same in controls and cases again as an effect of CEM. The characteristic of cases and controls are detailed in Table 1.

Table 1. Characteristics of cases and controls.

	Controls (n=33)			Cases (n=34)		
	P_{50}	P_{25}	P_{75}	P_{50}	P_{25}	P_{75}
Anthropometric measurements						
Age (years)	10	8	12	10	8	12
Weight (kg)	31.7	27.0	44.0	53.8	40.5	65.7
Weight (SD WHO)	0.20	-0.24	0.87	2.88	1.90	3.48
Height (m)	1.40	1.27	1.58	1.50	1.33	1.60
Height (SD WHO)	0.42	-0.72	1.05	1.12	0.52	2.29
BMI (kg/m^2)	17.5	15.3	17.9	24.2	21.2	26.3
BMI (SD WHO)	-0.16	-0.54	0.66	2.30	2.02	2.68
Gustatory functions						
Total Taste Score	14	12	15	12	9	13
Sweet taste score	4	3	4	4	3	4
Sour taste score	3	3	3	2	2	3
Salted taste score	4	3	4	3	2	4
Bitter taste score	4	3	4	3	2	4
Fungiform papillae (n/cm^2)	26	21	28	18	14	22
Neophobia	20	20	26	19	16	24

Because of CEM, 45% of the children were female among controls (n = 15) and 56% among cases (n = 19). The median (IQR) age was the same in controls and cases again as an effect of CEM.

Difference in gustatory functions between cases and controls

A Total Taste Score (TTS) of 16 was the possible maximum score achieved by subjects, obtained by calculating the sum of all four taste qualities presented in the four different concentrations. The correct answers given by subjects in identifying the two blank strips (no taste) have been not been considered for the calculation of the TTS. The TTS obtained in the present study ranged between 5 and 16. As expected, sweet and salty strips were the most often correctly identified, while bitter was the most difficult taste quality to recognize. The mean difference in TTS between cases and controls was -2.3 (95% CI -3.2 to -1.4, $p < 0.001$, LRM). Such effect size was virtually unmodified after correction for age and gender (mean = -2.4, 95% CI -3.2 to -1.6, $p < 0.001$, LRM). In general, cases presented significantly more difficulties in correctly identifying the different taste qualities compared to controls, resulting in a lower TTS. Moreover, when considering taste qualities separately, some of them were identified less often by cases. Indeed, the mean difference in sweet score was -0.4 (95% CI -0.7 to 0.0, $p < 0.05$, LRM), in sour score was -0.7 (95%CI -1.0 to -0.3, $p < 0.001$, LRM) and in bitter score was -0.7 (95% CI -1.2 to -0.2, $p < 0.05$, LRM). The components of the main outcome (TTS) are reported for descriptive purposes.

The mean difference in the density of fungiform papillae between cases and controls was -6 FP/cm^2 (95% CI -8 to -4, $p < 0.001$, PRM). Such effect size was unmodified after correction for age and gender. In general, controls showed a greater FP density compared to cases.

The regression lines representing the neophobia = f(fungiform papillae) had common intercepts (test for common intercepts) and slopes (test for common slopes) so that a single regression can be used to show the association in the whole sample (Fig 1).

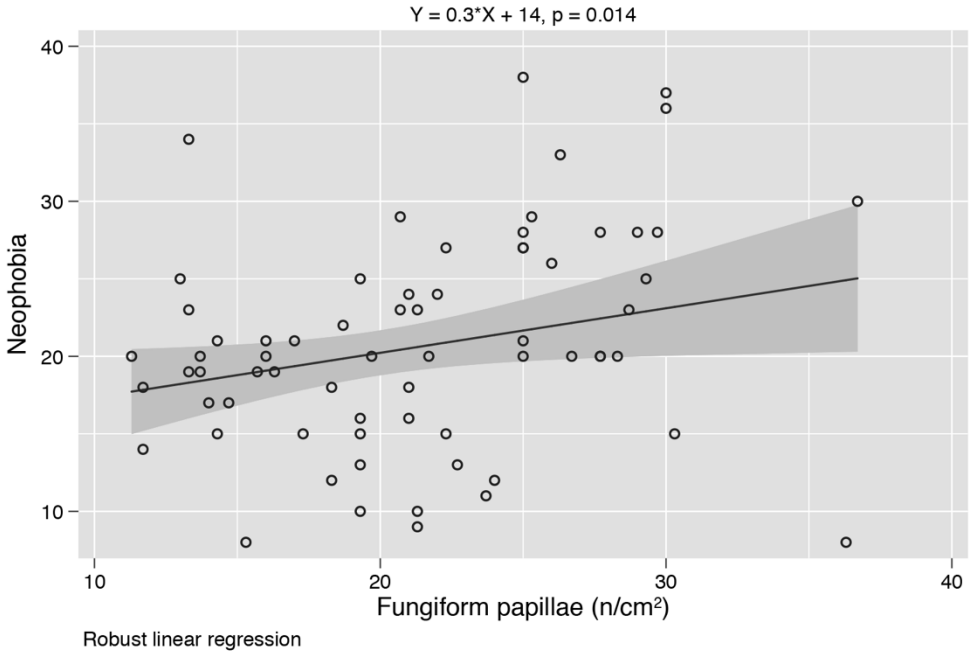


Fig 1. Association between Food neophobia and Fungiform Papillae density. Regression line representing the association between Food neophobia and FP density in the whole sample.

Difference in oral microbiota composition between cases and controls

Two 16S rRNA amplicons were obtained, sequenced and analysed for each subject. After quality filtering, a total of 11,384,103 high-quality reads were obtained and classified into a total of 76,163 OTUs at 97% similarity level, representing 17 phyla, 32 classes, 61 orders, 120 familiae and 252 genera. The average number of OTUs per sample was 576.9, ranging from a minimum of 322 to a maximum of 1133 (S1 Table).

The median Chao index measured for case samples resulted significantly higher than in control samples (Wilcoxon test, p-value < 0.05, median in case samples 861.7, in control 757.8) (Fig 2). Moreover, for each sample, a rarefaction curve (or individual sample-based rarefaction curves) was drawn by sequentially computing the number of OTUs for an increasing number of reads (S1 Fig).

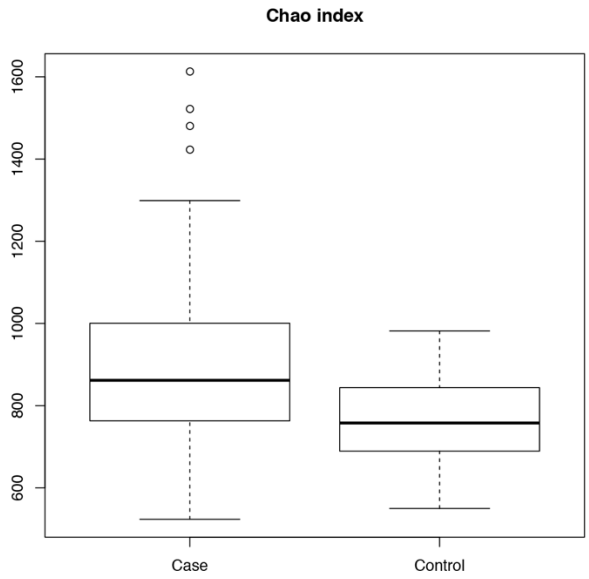


Fig 2. α-diversity (observed species) of the saliva microbiota composition. Box-plots representing the α-diversity (observed species) of the saliva microbiota composition in case and control groups through Chao index.

The dot chart of the distribution of the salivary microbiota at the phylum level in cases and controls is displayed in Fig 3. The means plotted in the graph are CEM-weighted and, thus, take the case-control matching into account. The composition at the phylum level resulted very similar in cases and controls. The largest difference was seen for Proteobacteria (22% in controls vs. 17% in cases, Wilcoxon test, p-value < 0.05).

The composition of the salivary microbiota in cases and controls at the taxonomic level of bacterial classes is reported in Fig 4. The means plotted in the graph are CEM-weighted and, thus, take the case-control matching into account.

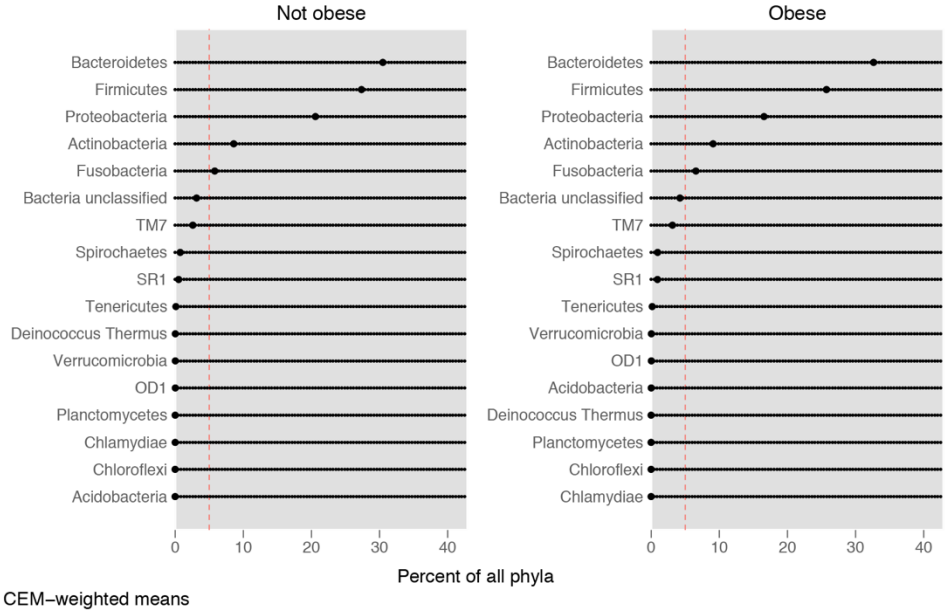


Fig 3. Distribution of the salivary microbiota at the phylum level. Dot chart of the distribution of the salivary microbiota at the phylum level in cases and controls.

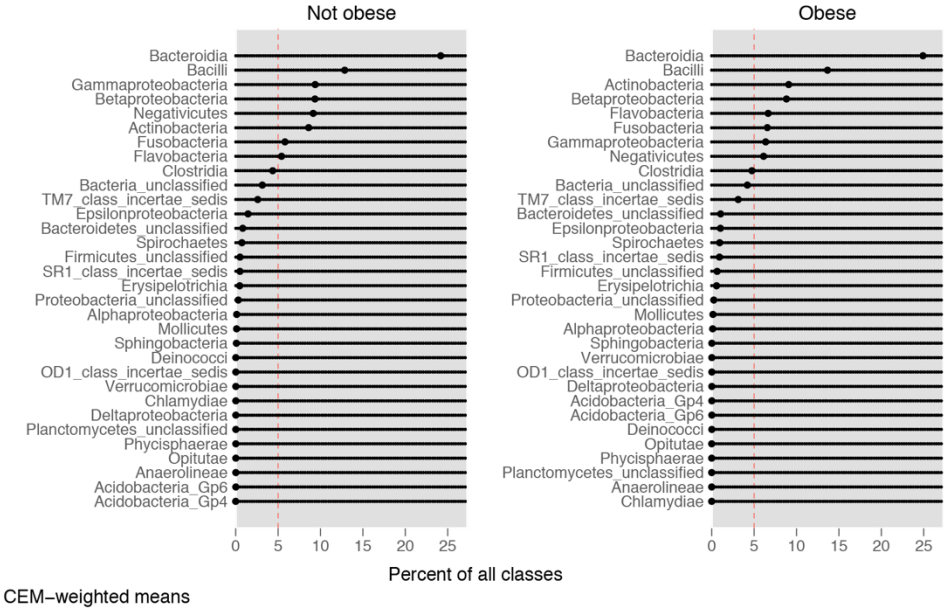
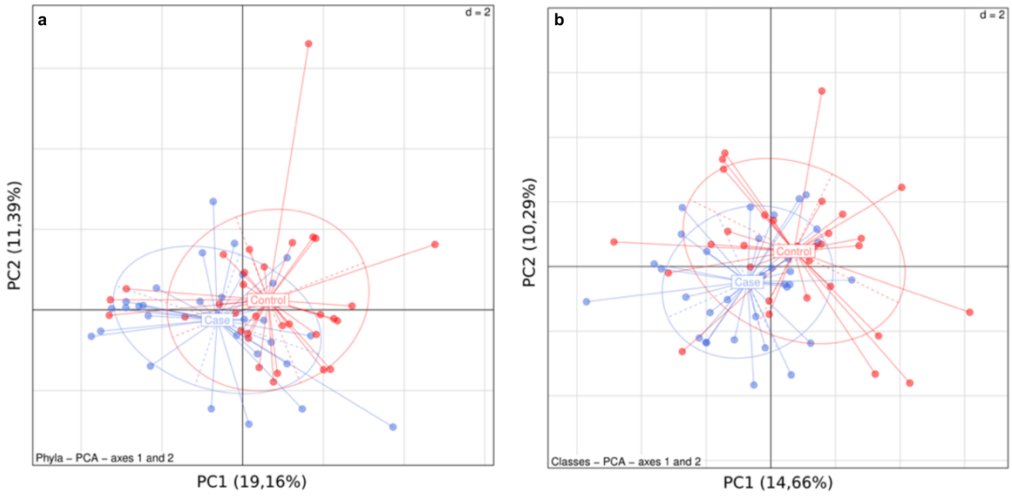


Fig 4. Distribution of the salivary microbiota at the class level. Dot chart of the distribution of the salivary microbiota at the class level in cases and controls.

Overall, despite minor differences in relative rankings between cases and controls, the composition in bacterial classes resulted similar in cases and controls. The largest difference was observed for Gammaproteobacteria and Negativicutes (9% in controls vs. 6% in cases for both classes).

The Principal Component Analysis (Figs 5a and 5b) confirmed that the bacterial consortia presented similar structures in cases and controls, either at phylum (Fig 5a) and class (Fig 5b) taxonomic levels.



Figs 5a and 5b. Principal Component Analysis of the microbiota profile. Principal Component Analysis of the microbiota profile in both groups (Controls in red vs Cases in blue) at a) phylum level and at b) class level.

In Fig 6 is shown the heatmap based on the Euclidean distance of the most abundant bacterial phyla and on the dendrogram produced by the clustering analysis.

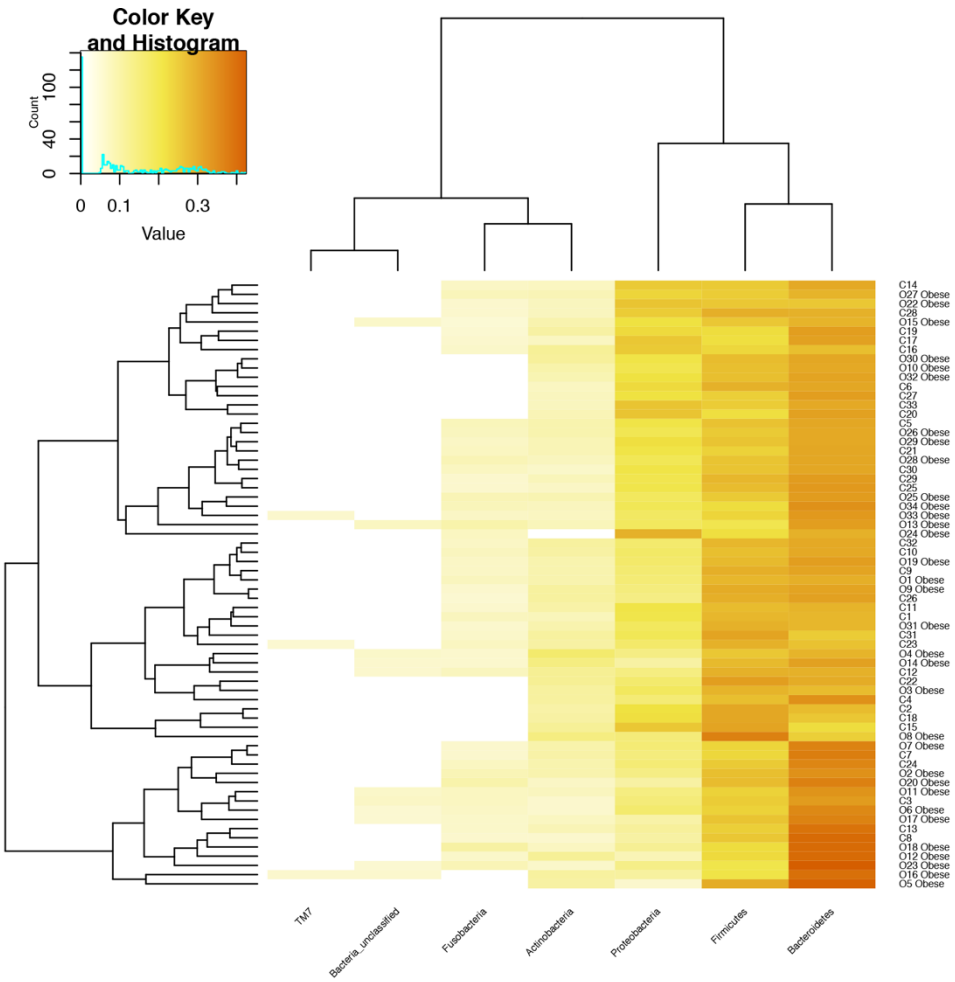


Fig 6. Correlations between the subjects and the abundance levels of selected phyla. Heatmap representing the correlations between the subjects and the abundance levels of selected phyla that were represented in the microbiota samples.

From this analysis it emerges that subjects do not seem to cluster based on their nutritional status. Instead, a cluster characterized by a higher representation in Bacteroidetes, which comprised either cases and controls, seemed the only one to emerge from this analysis. It seemed, thus, interesting to compare subjects chosen for being at the 'extremes' of Bacteroidetes abundance, independently from their nutritional status. Within the study cohort, we selected 7 subjects characterized by the highest Bacteroidetes abundance (dark orange colour in Fig 6) and 7 subjects characterized by the

lowest Bacteroidetes abundance (light yellow colour in Fig 6), forming 2 clusters, named respectively 'Group 1' and 'Group 2', the characteristics of which are given in S2 Table. These groups seem to differ for the ability of subjects in correctly identifying the different taste qualities, and especially the bitter taste. In particular, Group 1 presented a general lower TTS (11.43 ± 3.15 vs 14.00 ± 0.00) and, especially, a lower ability in identifying bitter taste compared to Group 2 (2.43 ± 1.13 vs 4.00 ± 0.00).

Discussion

It is becoming clear that the origin of obesity is multifactorial disease and the purpose of this study was to deepen the investigation on the host related factors proposed as potential causes affecting weight gain. In recent years, compelling evidence has been accumulated on the relations between taste perception and body mass index, suggesting that individuals with a higher BMI showed reduced taste sensitivity [for a review see 15]. However, the majority of studies have focused on the relation between nutritional status and taste perception in adults. Moreover, bitter sensitivity in relation to BMI was mainly examined for the PROP compound. Data in literature about the sensitivity of children towards all taste qualities appear to be incomplete and, in particular, little is currently known about the perception of other bitter compounds [12-14]. Indeed, only Overberg and colleagues [13] investigated the relationship between taste sensitivity for all five taste qualities and nutritional status in children and adolescents with and without obesity, showing a higher sensitivity for all tastes in the former. Accordingly, the hypothesis that children and adolescents characterized by a different nutritional status, presented differences in their taste sensitivity was confirmed in the present study, with subjects with obesity showing a lower ability in correctly identifying taste qualities compared to the group of controls. Taste sensitivity has also been evaluated by measuring and counting the number of FP/cm^2. Because FPs contain the taste buds of the anterior tongue, many literature data suggest that

individual differences in their density and size (e.g. diameter) could be responsible of different chemosensory perception among individuals [53-55]. Moreover, a negative correlation between FP density and obesity was suggested in adults [9, 10]. Our findings seem to be in agreement with this hypothesis, showing that normal-weight controls presented a greater density of FP and were also more sensitive to basic tastes than subjects with obesity. This impaired taste perception in children and adolescents with obesity supports the assumption that the taste system is impaired in subjects affected by this disease [9, 10-13]. We can presume that, as a results of low taste sensitivity, high amounts of tastants would be required to elicit a response within taste receptor cells, which in turn may affect eating behaviour, contributing to excess energy intake and perhaps increasing obesity.

As previously reported, obesity is considered a disease with a multifactor aetiology, thus, other factors not strictly related to taste perception could be involved in weight gaining. Indeed, previous studies showed that body weight could be associated with some traits related to personality, such as food neophobia [9, 18, 56]. Quite surprisingly, there has been very little research carried out to ascertain the relationship between food neophobia, taste perception and nutritional status. Food neophobia is considered a maladaptive behaviour, which can lead to decreased dietary variety and quality. Food neophobics may choose to eat familiar food, normally more energy-dense than healthier food, which could clearly affect their nutritional status leading them to a greater prevalence of overweight [10].

However, our results did not highlight any relationship between nutritional status and food neophobia, accordingly to previous studies already conducted with children [49] and young adults [57]. Concerning about the relationship between food neophobia and gustatory functions, the present study showed that, independently of nutritional status, children and adolescents who present an higher FP density are significantly more neophobic than less sensitive individuals, suggesting that neophobic reactions could be associated with higher sensitivity. Our results are in agreement with literature data reporting

that children, who are more sensitive to taste or tactile sensations, have fewer positive consequences when trying new foods, particularly those characterized by strong sensory properties, leading to greater neophobic attitudes [54, 58]. However, it is still unclear whether the food rejection shown by neophobic subjects is facilitated by higher arousal levels when approaching new foods or by an actual physiological predispositions to taste hypersensitivity [59].

In this study, we also focus on the link between oral bacterial community and obesity. The analysis of the salivary bacterial consortia revealed that people with obesity have a higher bacterial richness than normal weight controls. This result is in contrast with the current literature which normally reports decreases in ecological indexes of bacterial richness and diversity as a trademark of many dysbiotic states, characterizing a variety of pathological conditions, among which obesity. Indeed, decreased gut microbiome diversity has been linked to obesity [60, 61]. Similar associations between the altered microbial diversity and unhealthy or inflammatory states in the host have been found with the oral microbiota [62, 63].

Data in literature reported that significant differences in the gut microbiome has been found between people with obesity and controls [20]. Data from animal models and human studies have shown correlations between alteration in gut phyla and obesity disease, but results are inconsistent [20, 22, 64]. In the present study, very few significant variations in relative abundances of some taxa were noted between cases and controls at higher taxonomic levels in the salivary microbiome. The lack of greater variation between the salivary microbiota of these groups may be due to the relatively small sample size of each group. It is also possible that the young age of the subjects involved and the relatively shorter duration of their disease do not allow to highlight a clear microbial flora variation. It is notable that past researches on the relationship between the oral microbiota composition and obesity, have yielded contradictory results. For example, it has been reported that levels of many bacteria differed in the saliva of overweight women when

compared with healthy individuals [23]. Specifically, these authors found that *Prevotella spp.* (belonging to Bacteroidetes) was more abundant in the overweight while *Selenomonas spp.* was present only in the overweight individuals, suggesting that these taxa could be biomarkers for excess adiposity. Moreover, Ziegler and colleagues [65] suggested an association between obesity and bacterial cellular abundance of Firmicutes and Actinobacteria in oral biofilm. However, recent studies reported no differences in oral microbiota composition according to BMI [26, 66].

The role oral microbiota plays in influencing taste perception is a novel field of investigation. Recently, a relationship between taste sensitivity and oral bacteria was suggested, associating taste perception with the growth of specific taxa. Solemdal and colleagues [25] found that sour taste was particularly impaired in children with high *lactobacilli* growth. They suggested that the acids produced by the bacteria may cause an adaption in sour taste perception, thus increasing their sour taste threshold. In addition, non-taster children, who presented a decreased taste sensitivity for PROP, were associated with higher *mutans streptococci* counts [67]. However, these assumptions were not supported by microbiomic or predicted metagenome analyses. Our previous studies, which investigated both taste perception and oral microbiota, applying reliable and sensitive methods and using microbiomic analysis of tongue microbial ecosystem, supported the hypothesis that oral bacteria may have a role in influencing and/or modulating taste perception [24, 27]. Indeed, we reported that young adults with a reduced taste responsiveness are characterized by different oral microbiota composition, in agreement with previous findings [26, 68]. In the present study, due to the selection of few samples belong to the two groups accordingly to heatmap, we conducted descriptive statistics rather than inductive statistics and no conclusions about associations can be drawn. Interestingly, however, it seems that there is an increase in the proportion of Bacteroidetes and Bacteroidia and a decrease in the proportion of Proteobacteria in Group 1, which includes subjects characterized by a general

lower ability to perceive all the taste qualities and, especially bitter taste. In conclusion, the results of this study support the hypothesis that children and adolescents with a different nutritional status differ in their taste sensitivity. However, these cross-sectional results are required to be confirmed through longitudinal studies. No relations were found between nutritional status and food neophobia, however, independently of nutritional status, children and adolescents who present a greater FP density are significantly more neophobic than less sensitive individuals. We report that our obese and normal-weight subjects differ for the ecological indexes of microbial alpha-diversity. Some minor differences in taxa composition were also noticed, (e.g. for Proteobacteria). Moreover, independently from nutritional status, some bacterial genera seemed to differ among subjects with different ability in perceiving taste qualities. Further exploration of the oral microbiome in relation to taste perception and nutritional status will enhance our understanding of the host related factors that are proposed as potential causes affecting weight gain. This multidisciplinary approach offer new insights into the reciprocal impact between host related factors and obesity, and could open new strategy lines for obesity prevention and therapy in childhood.

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Author Contributions: CC, CM, GVZ, EP designed the study. AS recruited patients and controls. CC carried out the experiment and collected samples. CC and SP assayed samples. CC, SP, FC, GB performed statistical analysis. CC wrote the first draft of the manuscript. CC, CM, SP, FC finalized the manuscript. CM, CC, SP, FC, CB, GZ, EP regularly discussed the

experiments, analysed the results, and provided useful suggestion during the project. All authors read and approved the final manuscript.

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Supplemental Figures and Tables

S1 Tab. Number of reads per 35 sample after filtering.

Sample	nr. of reads
C1_repeat1	92305
C1_repeat2	88168
C10_repeat1	94210
C10_repeat2	84205
C11_repeat1	76577
C11_repeat2	106399
C12_repeat1	102343
C12_repeat2	83016
C13_repeat1	107531
C13_repeat2	102039
C14_repeat1	111308
C14_repeat2	104019
C15_repeat1	108268
C15_repeat2	122074
C16_repeat1	98132
C17_repeat1	96435
C17_repeat2	89789
C18_repeat1	117087
C18_repeat2	96490
C19_repeat1	84574
C19_repeat2	102132
C2_repeat1	96387
C2_repeat2	93567
C20_repeat1	82885
C20_repeat2	113782
C21_repeat1	116695
C21_repeat2	118034
C22_repeat1	126141
C22_repeat2	93869
C23_repeat1	75043

C23_repeat2	73903
C24_repeat1	91381
C24_repeat2	80473
C25_repeat1	76784
C25_repeat2	86707
C26_repeat1	84962
C26_repeat2	94676
C27_repeat2	110746
C28_repeat1	77215
C28_repeat2	74817
C29_repeat1	83194
C29_repeat2	82165
C3_repeat1	80131
C3_repeat2	77578
C30_repeat1	66930
C30_repeat2	84503
C31_repeat1	79761
C31_repeat2	75737
C32_repeat1	86335
C32_repeat2	75615
C33_repeat1	83292
C33_repeat2	88312
C4_repeat1	79941
C4_repeat2	87314
C5_repeat1	67315
C5_repeat2	94438
C6_repeat1	74668
C6_repeat2	76488
C7_repeat1	83148
C7_repeat2	80756
C8_repeat1	103642
C8_repeat2	82985
C9_repeat1	88831
C9_repeat2	75607

OB1_repeat1	114139
OB1_repeat2	92166
OB10_repeat1	89937
OB10_repeat2	55579
OB11_repeat1	69522
OB11_repeat2	71730
OB12_repeat1	79056
OB12_repeat2	119528
OB13_repeat1	66981
OB13_repeat2	75604
OB14_repeat1	88635
OB14_repeat2	41131
OB15_repeat1	46569
OB15_repeat2	67806
OB16_repeat1	34295
OB16_repeat2	90679
OB17_repeat1	58481
OB17_repeat2	68829
OB18_repeat1	82286
OB19_repeat1	79842
OB19_repeat2	84145
OB2_repeat1	95978
OB2_repeat2	56131
OB20_repeat1	109112
OB20_repeat2	103267
OB22_repeat1	91715
OB22_repeat2	89636
OB23_repeat1	103325
OB23_repeat2	108016
OB24_repeat1	88377
OB24_repeat2	95520
OB25_repeat1	97936
OB25_repeat2	78828
OB26_repeat1	89449

OB26_repeat2	81828
OB27_repeat1	94477
OB27_repeat2	79616
OB28_repeat2	86107
OB29_repeat1	93720
OB29_repeat2	86279
OB3_repeat1	103306
OB3_repeat2	95465
OB30_repeat1	122241
OB30_repeat2	90231
OB31_repeat1	87822
OB31_repeat2	82477
OB32_repeat1	69230
OB32_repeat2	86538
OB33_repeat1	73869
OB33_repeat2	89069
OB34_repeat1	87530
OB34_repeat2	73822
OB4_repeat1	118773
OB4_repeat2	103181
OB5_repeat1	97589
OB5_repeat2	135662
OB6_repeat1	111152
OB6_repeat2	53249
OB7_repeat1	102638
OB7_repeat2	122062
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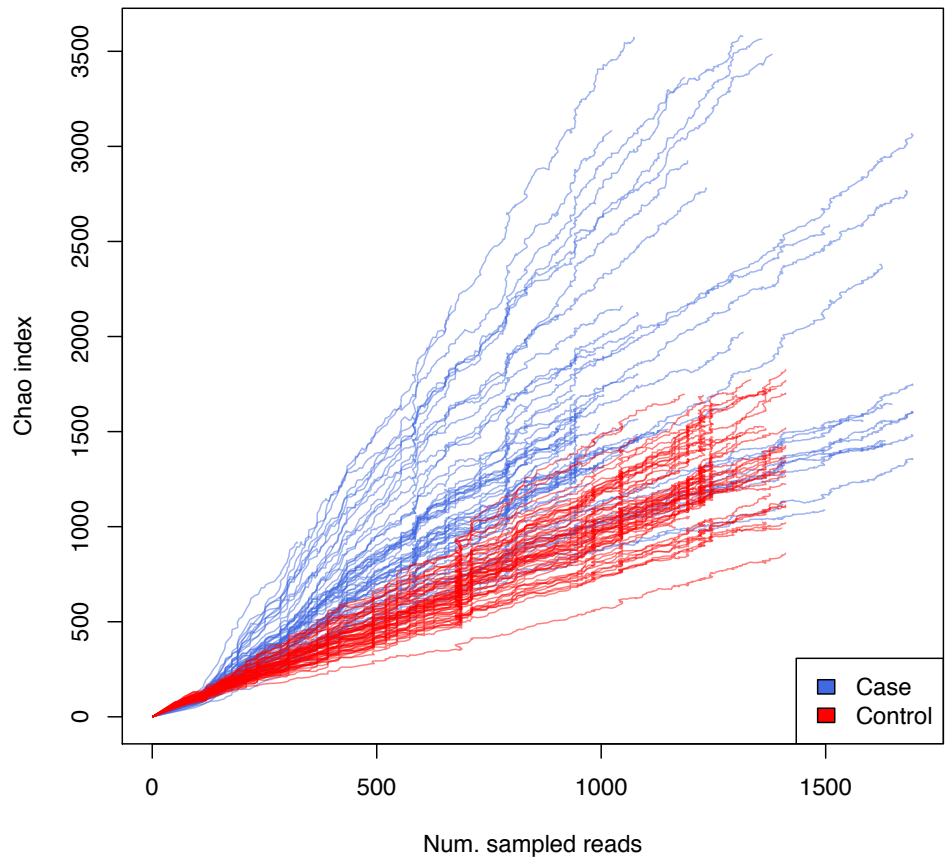
OB8_repeat2	65293
OB9_repeat1	85329
OB9_repeat2	116180



S1 Fig. Rarefaction curves within samples.

S2 Tab. Characteristics of study participants belong to Group 1 and Group 2.

Group	Participant ID	Status	BMI (SD)	Age (SD)	Gender F:M	TTS (SD)	Sweet TS (SD)	Sour TS (SD)	Bitter TS (SD)	Salty TS (SD)
1	O 7	Case	21.89 (1.72)	9.57 (2.29)	4:3	11.43 (3.15)	3.29 (0.76)	2.71 (0.76)	2.43 (1.13)	3.00 (1.15)
	C 13	Control								
	O 18	Case								
	C 8	Control								
	O 12	Case								
	O 5	Case								
	O 23	Case								
2	C 15	Control	19.77 (1.19)	9.57 (1.19)	3:4	14.00 (0.82)	3.86 (0.38)	2.53 (0.53)	4.00 (0.00)	3.57 (0.53)
	O 8	Case								
	C 31	Control								
	C 18	Control								
	O 22	Case								
	C 23	Control								
	C 16	Control								

CHAPTER 5

CROSS-CULTURAL DIFFERENCES IN TASTE AND TEXTURE PERCEPTION AND PREFERENCE

Cross-cultural differences in lingual tactile acuity, taste sensitivity phenotypical markers, and preferred oral processing behaviors

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Cross-cultural differences in lingual tactile acuity, taste sensitivity phenotypical markers, and preferred oral processing behaviors

Abstract

Cultural and genetic differences in consumer populations across the world are important determinants for food preferences. The present study investigated differences in preferred oral processing behaviors between Chinese Asian and Danish Caucasian consumers and the possible relationship to lingual tactile acuity and the two most well-researched phenotypic markers of taste sensitivity, such as 6-n-propylthiouracil (PROP) responsiveness and Fungiform Papillae Density (FPD). A total of 152 consumers (75 Chinese, 77 Danish) were enrolled in the study and categorized by their preferred oral processing behaviors. Lingual tactile acuity was assessed according to responses to stimulation with Von Frey filaments. The responsiveness to PROP and the FPD were also determined. Cross-population differences were found in preferred food oral processing behaviors in these two cohorts, as Chinese consumers were characterized by a larger number of 'Soft processing likers' (77% of the population) who preferred soft food processing in the mouth. Contrarily, Danish consumers mostly belonged to the 'Firm processing likers' group (73% of the population) who had preferences for foods that needed firm processing on biting and chewing. Moreover, the group of 'Firm processing likers' were shown to be more sensitive to touch at the apex of the tongue compared with the 'Soft processing likers' in both population cohorts. Cross-population differences in lingual tactile acuity were not significant. Differences in FPD and PROP responsiveness were found between these two population cohorts, with Chinese consumers generally characterized by greater FPD and PROP responsiveness compared to the Danish subjects.

This study provides evidence on cross-cultural differences in preferred oral processing behaviors and in the two phenotypic marker of taste sensitivity. However, further studies are needed to draw conclusive relationships between preferred oral processing behavior and oral tactile acuity, PROP responsiveness and tongue anatomy.

Introduction

The variation in oral texture perception of foods across consumer populations is supposed to depend on individual differences in tactile acuity and processing behaviors in the mouth. Tactile acuity has been widely studied at the surface of the skin (for a review: Abraira & Ginty, 2013) and four mechanoreceptors have been identified. These specialized nerve endings convey specific sensations such as light pressure and touch as well as stretch and high-frequency vibration. In the anterior tongue, neuroanatomical studies have shown that somatosensory trigeminal neurons terminate as a network of fibers in the peri-gemmal tissue (des Gachons et al., 2011; Suemune et al. 1992; Whitehead, Beeman, & Kinsella, 1985). Gairns and Garven (1952) were the first to find anatomical evidence in humans that somatosensory endings from the trigeminal nerve (V) innervate Fungiform Papillae (FP). Later research confirmed these findings and showed that twenty-five percent of FP innervation arise from the chorda tympani nerve (taste), and seventy-five percent from the trigeminal nerve (pain, touch and temperature) (Silver & Finger, 1991). Mechanical stimuli are likely to activate some receptors of the trigeminal nerve endings, which surround taste buds in the FP and terminate in the papilla apex (des Gachons et al., 2011). Considering FP as a common anatomical unit of the sense of taste and the somatosensory system, these anatomical structures are assumed to act as an ‘array of sensors for detecting oral touch sensations’ (Bartoshuk et al. 1994; Prescott, Soo, Campbell, & Roberts, 2004; Prutkin et al., 2000) and predict tactile acuity and discrimination (Bangcuyo & Simons, 2017; Engelen & Van der Bilt, 2008; Prescott, Soo, Campbell, & Roberts,

2004). This anatomical colocation can explain the positive correlations between FP and trigeminally mediated oral somatosensations such as the tongue spatial resolution acuity (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone, 2003), the textural aspects of creaminess (Hayes & Duffy, 2007; 2008; Nachtsheim & Schlich, 2013; Proserpio et al., 2016) and roughness perception (Bakke & Vickers, 2008).

In addition to the relationship between FP and lingual tactile acuity the responsiveness to the bitter tastant 6-n-propylthiouracil (PROP) has also been proposed has another factor involved in texture perception. Indeed, several studies have associated PROP taster status with the perceived intensity and the ability to discriminate trigeminal sensations and textures (Bakke & Vickers, 2011; Bartoshuk et al., 1994; de Wijk et al., 2007; Pickering, Simunkova, & Di Battista, 2004; Pickering & Robert, 2006; Tepper & Nurse, 1997; Yackinous & Guinard, 2001). However, results on association between PROP status and texture are still contradictory and some studies could not find associations between PROP status and oral texture perception (Drewnowski et al. 1998; Lim, Urban, & Green, 2008).

There are some indications that Asian and Caucasian consumers have different oral chemosensory abilities (Guo & Reed, 2001; Tepper, 2008). In Caucasian populations, 20 to 25% is estimated to be PROP non-taster (less responsive to PROP). Whereas the estimated proportion of non-tasters in Asian populations in China and Japan is between 10 and 20% (Guo & Reed, 2001). Additionally, Essick and colleagues (2003) found that PROP sensitivity seems to covary among Asian and Caucasian females, reflecting individual differences in the density and diameter of FP on the anterior tongue. However, very few studies investigated possible ethnicity differences in food texture perception and no differences in oral tactile acuity between Caucasian and Asian subjects could be detected by the letter recognition method (Essick, Chopra, Guest, & McGlone, 2003).

This letter recognition method has been popular for measuring oral touch sensitivity across subjects (Essick, Chen, & Kelly, 1999; Essick, Chopra, Guest, & McGlone 2003; Lukasewycz & Mennella 2012; Steele, Hill, Stokely, & Peladeau-Pigeon, 2014). In this test subjects are asked to use their tongues to identify letters of the alphabet of varying sizes embossed onto Teflon strips (Essick et al., 1999). A challenge with the oral letter test is that subjects across cultures may differ in their recognition ability due to different alphabets and symbols in their languages, thus making this method possibly less suitable for cross-cultural studies. Other methods for oral touch acuity include a two-point discrimination task (Engelen, Van der Bilt, & Bosman, 2004), grating orientation discrimination (Van Boven & Johnson, 1994), and other physiological measures (Bangcuyo & Simons, 2017; Linne & Simons, 2017). The majority of studies on oral tactile acuity utilized static or moving two-point discrimination or grating recognition tasks, which may have limited reliability as tools for determining touch detection and punctate pressure (Miles et al., 2018). An alternative method concerns a localized one point touch testing with von Frey fibers (Semmes-Weinstein monofilaments). This method concerns a touch detection task, where subjects report presence or absence of the stimulus. This method is reported to be repeatable, accurate, and most reliable for measuring light touch–deep pressure sensibility of the tongue and the hard palate (Bell-Krotoski & Tomancik, 1987; Bodin, Jäghagen, & Isberg, 2004; Cordeiro, Schwartz, Neves, & Tuma, 1997; Henkin & Banks, 1967). When studying cross-cultural differences in oral touch acuity, methods aiming at point touch detection may be more suitable as they provide localized absolute detection thresholds. Thus preventing biases from cultural differences in object recognition.

Besides the phenotypic markers of taste sensitivity and lingual tactile acuity, there is a growing body of research on oral processing behavior, i.e. the way to manipulate and manage a food in the mouth (de Wijk, van Gemert, Terpstra, & Wilkinson, 2003; Jeltema, Beckley, & Vahalik 2014; 2015; 2016; Yackinous & Guinard, 2001). It has been suggested that differences in food manipulation and mastication could affect sensory sensations (Lassauzay et al., 2000; Po et

al., 2011). Brown and Braxton (2000) identified four different groups of people based on their efficiency in reducing the size of foods (i.e. almonds and chewing gum) and suggested that individual differences in the mouth ability to manipulate and handle the product may be an important driver of liking and preferences. More recently, Jeltema and collaborators (2014) suggested the existence of Mouth Behavior (MB) groups and showed that consumers can be typified by the way they manipulate food in their mouths. Their scheme categorized consumers into so-called (a) Smooshers, (b) Suckers, (c) Chewers, and (d) Crunchers. These groups fell into two major mouth processing styles. The first one, represented by Suckers and Smooshers, preferred to process food between the mouth's roof and tongue. They diverged principally in the hardness of preferred foods. Suckers preferred harder foods that could be sucked on for a long time, such hard candies and foods that they could hold in their mouths. Smooshers preferred soft foods, such as puddings or creamy candies that would spread throughout the mouth and could be held in for a longer time, not requiring much mouth activity. The second one, represented by Crunchers and Chewers, preferred to use their teeth to break down foods. In particular, Crunchers were more forceful in their bite, preferring foods that broke up on biting. Chewers liked foods that did not fracture on biting and could be chewed. If such consumer MB groups exist, one would expect a possible cultural dimension, as different populations have different habits on how to prepare and consume foods. However, such differences in oral MB may also be related to fundamental differences in mouth anatomy and texture perception. Moreover, other factors such as salivary flow, mouth size, dental bite, dental status and health could play a role in defining subjects' MB, affecting chewing and mastication performance (Chen, 2009; Jeltema, Beckley, & Vahalik, 2016).

Thus, there are evidences to suggest that PROP phenotypical and population cultural factors may play a role in texture perception and preferences. PROP tasters appear to have a better lingual tactile acuity than other taster groups and may more readily detect small particles and granularity in foods. It seems

plausible that PROP tasters will be more sensitive to gritty contaminants in foods and may more readily reject such foods (Essick, Chopra, Guest, & McGlone, 2003). Thus, a high responsiveness to PROP and variation in FPD are possibly involved in choices of some food textures. Likewise, other influences such as personality traits, cultural habits and societal factors are most certainly important in texture preferences. As reported above, it has well been established that Asian and Caucasian populations differ in PROP responsiveness, which is most definitely seen in the higher proportion of supertaster-tasters in the Asian population. It is less certain that Asian and Caucasian populations differ in lingual tactile acuity beyond differences in their PROP status and FPD counts. The two populations have not been shown to differ in the letter recognition task on the anterior dorsal part of the tongue, but no studies have reported differences on touch detection ability on this part of the tongue.

The present study aimed at investigating whether differences exist in PROP responsiveness, FPD and touch detection ability on the anterior dorsal part of the tongue among Asian and Caucasian adults. Furthermore, the importance of such sensory differences for preferred oral food processing behaviors in these populations has been explored. The objectives were to (i) find relationships between PROP taste sensitivity, FPD and touch detection ability among Asian and Caucasian population cohorts, (ii) classify Asian and Caucasian according to their preferred food oral processing behaviors, and (iii) explore lingual touch detection ability in relation to oral texture preferences.

Material and methods

Subjects

One hundred and fifty-two healthy, non-smoking subjects between the ages of 18 and 55 years were recruited to attend the consumer test. Two cohorts were recruited from the greater Copenhagen area and included seventy-five

of the subjects, the Asian cohort (56 F, 19 M; mean age= 26.9 ±2.9; age distribution: 56% aged 18–30 years and 44% aged 31–55 years; BMI= 21.6 ±3.6) and another seventy-seven subjects, the Caucasian cohort (52 F, 15 M; mean age= 29.8 ±9.1; age distribution: 61% aged 18–30 years and 39% aged 31–55 years; BMI= 24.1 ±5.0). Seventy-two percentage of Chinese subjects had been living in Denmark for less than two years at the moment of the test. Informed, written consent was obtained from all subjects on the first test day. The present study was performed according to the principles established by the Declaration of Helsinki and the protocol was approved by the Institutional Ethics Committee of the University of Copenhagen.

General procedure

Participants attended one study session lasting 1 h and completed 4 different tasks: 1) a first questionnaire to collect general demographic information followed by a second questionnaire to identify subjects' mouth behavior; 2) Lingual tactile acuity task using three von Frey filaments; 3) tongue pictures for the estimation of FPD; 4) a screening procedure for PROP responsiveness.

Questionnaire to assess mouth behavior

Participants' mouth behavior was assessed through a questionnaire, which was derived from the work of Jeltema and collaborators (Jeltema, Beckley, & Vahalik 2014; 2015; 2016). There were 20 text-based questions where subjects were asked to respond to a variety of statements aimed at understanding how they preferred to manipulate food in their mouths. Additionally, 4 picture-based questions were used to further evaluate the subjects' liking of a group of products from a mouth processing perspective. These products were carefully chosen to represent those that would best differentiate between groups. A Likert 6-point agree/disagree scale anchored 'strongly disagree' (1) to 'strongly agree' (6) was used for all statements. The reader is referred to Table

S1 (Supplementary material) for the presentation of questions that were used to type individuals for MB.

Lingual tactile acuity evaluation

During the lingual tactile acuity evaluation blindfolded subjects were seated in an upright position and their tongue stimulated or not with three von Frey filaments (no. 1.65, 2.36 and 2.44), one at a time. The number of the filaments corresponds to a logarithmic function of the equivalent forces of 0.008, 0.02 and 0.04 g, respectively, according to the manufacturer (Aesthesio®: Precise Tactile Sensory Evaluator, DanMic Global, LLC, San Jose, California, USA). For each filament, the subjects were given 5 true and 5 mock touch exposures on the tongue's apex. The stimulation order was counterbalanced for the three filaments. The true touch with a filament was defined as 'signal' and the corresponding response as either 'hit' or 'not detected'. The responses from the mock exposures were defined as 'correct rejection' or 'false positive'. The subjects also rated their degree of certainty in their response (either signal sure, signal not sure, no signal not sure, or no signal sure). From the subjects' responses and certainty ratings *R-index values* (%) were calculated (O'Mahony, 1992). As reported by Lee and Van Hout (2009), 'the *R-Index* is an estimated probability of correctly identifying a target stimulus (the signal) when presented pairwise with a 2nd stimulus (the noise). As frequently happens with difference test, the R-Index values could range from 50% to 100%. If the subject cannot discriminate between the 2 stimuli, the judge will have to guess and the chances of correctly identifying the signal the *R-Index* will be 50%, otherwise the R-Index will be 100% if the judge can discriminate perfectly between the 2 stimuli. Thus, the better the discrimination, the higher the value will be'.

Fungiform Papillae Density (FPD)

The area to count individual FPD was selected following the procedure adapted from Bakke and Vickers (2011) and previously described by Proserpio and colleagues (2016). Tongue pictures were collected with blue

staining, which was obtained by swabbing with blue food coloring, using a cotton-tipped applicator. This fungiform papillae easily visible on the anterior portion of the dorsal surface of the tongue. Digital pictures were recorded using a Canon digital camera (Canon EOS 700D) in a brightly light room using the camera's macro mode with no flash. The best photograph of each blue stained tongue was selected to measure the FPD, and ImageJ software was used to mark the area in which papillae were to be counted. A set of three 0.6 cm diameter circles was drawn on the front of the anterior tongue, according to Bakke and Vickers, (2011). One operator, blind to any data concerning subjects and with 3-year experience, counted FP in two different moment (at least 3 weeks between the first and second count). The counts were submitted to 1-way fixed ANOVA. Counts were considered valid if the operator effect was not significant ($p > 0.05$). FP were counted inside the three marked circles and the average count over the three circles was used for each subject (Proserpio et al., 2016). The Denver Papillae Protocol (Nuessle, Garneau, Sloan, & Santorico, 2015) was followed to determinate FP according to shape, color, size and recession. The individual FPD was then calculated by reporting the number of FP to a common unit area of 1 cm^2.

Taste responsiveness to PROP

A method proposed by Prescott and colleagues (2004) was used for evaluating participants' PROP status. The intensity of bitterness of a supra-threshold 0.0032 M solution of PROP (European Pharmacopoeia Reference Standard, Sigma-Aldrich) was rated using the Generalized Labeled Magnitude Scale, gLMS (Bartoshuk et al., 2004), anchored at the top with the descriptor 'strongest imaginable sensation of any kind', which was defined in the context of all sensations, including painful ones. Practice on the use of the gLMS was provided to ensure that participants understood the scale and examples of the intensities of an array of ordinary sensory experiences (e.g., loudness of whispers, brightness of the sun) were provided. Then, subjects were presented with two identical samples (10 ml) and were instructed to hold

each sample in their mouth for 10 s, then to expectorate the solution. After 20 s they were asked to evaluate the bitterness intensity. To control for carry-over effect, a 90s break was given to the subjects to rinse their mouths with water after the first sample evaluation (Laureati et al., 2018). The average of bitterness scores was used for each subject and respondents were grouped according to their PROP status based on arbitrary cut-offs. Non-tasters (NT) were 17.8% of total sample (arbitrary cut-off gLMS ≤17, moderate), whereas Super-tasters (ST) were 36.2% (arbitrary cut-off gLMS ≥53, very strong). The remainder of the respondents were considered as Medium-tasters (MTs) (Fischer et al., 2013; Hayes et al., 2010).

Data Analysis

In order to identify the two major different Mouth Behavior styles among consumers, a latent class analysis with two classes was performed on the scores (6-point scale) for the 24 evaluated items of the questionnaire. The differences across clusters are identified by Wald test (χ^2) along with p-values and R^2.

The association between population cohort, Mouth Behavior and tactile acuity (expressed as *R-index value*) was analysed by a Generalized Linear Model considering *Population cohort* (Danish and Chinese), *Mouth Behavior* groups ('Firm processing likers' and 'Soft processing likers') and *Filament thickness* (no. 1.65, 2.36, 2.44) and their 2-way interactions as independent variables. Data were further analyzed separately for Danish and Chinese considering *Mouth Behavior* groups ('Firm processing likers' and 'Soft processing likers') and *Filament thickness* (no. 1.65, 2.36, 2.44) and the respective interaction as independent variables in order to have better insights on the relative contribution of these factors on dependent variables. Post-hoc comparisons using the Bonferroni test adjusted for multiple comparison were conducted when appropriate.

The relationship between tactile acuity (*R-index* for the thinner filament no. 1.65), PROP responsiveness and FPD was evaluated graphically and with Pearson's correlation r.

To determine the cross-cultural relationship between Mouth Behaviors and FPD, a Generalized Linear Model was constructed with the FPD as dependent factor, and *Population cohort* (Danish and Chinese), *Mouth Behavior* groups ('Firm processing likers' and 'Soft processing likers') and the respective interaction as independent factors. To check for possible confounding or modulating effects, the analysis was performed by adding *R-index values*, as covariate to the model. Additionally, the same model was run using the mean intensity ratings of PROP responsiveness as dependent factor. Both models have been run separately on Chinese and Danish subjects.

For all the analyses, a p-value of 0.05 was considered as threshold for statistical significance. Data are presented as means with standard errors (SEM). Statistical analysis was performed using IBM SPSS statistical software version 25 (SPSS Inc, Chicago, IL, USA). Latent class analysis was performed in Latent Gold 5.1 (Statistical Innovations, Belmont, USA).

Results

Mouth Behavior mapping

Two distinct clusters were identified: Cluster 1 'Soft processing likers' with 79 participants and 'Firm processing likers' composed of 73 participants. The number of participants in each cluster is listed in **Table 1**.

Table 1. Numbers of participants in each MB group by population cohort.

MB Group	Description	Chinese	Danish	Total
		Number (%)	Number (%)	Number (%)
Firm processing likers (FPL)	Prefer foods that require to use the incisors and/or molars to break down rapidly or deform the food matrix	17 (23%)	56 (73%)	73 (48%)
Soft processing likers (SPL)	Prefer foods that could be held in the mouth for a longer time and manipulate them between the tongue and roof of the mouth	58 (77%)	21 (27%)	79 (52%)
Total		75	77	152

For the two clusters significant differences were identified for 14 of the 24 questions used for the classification, results shown in Figures 1a-b, with the questions sorted according to size of the difference.

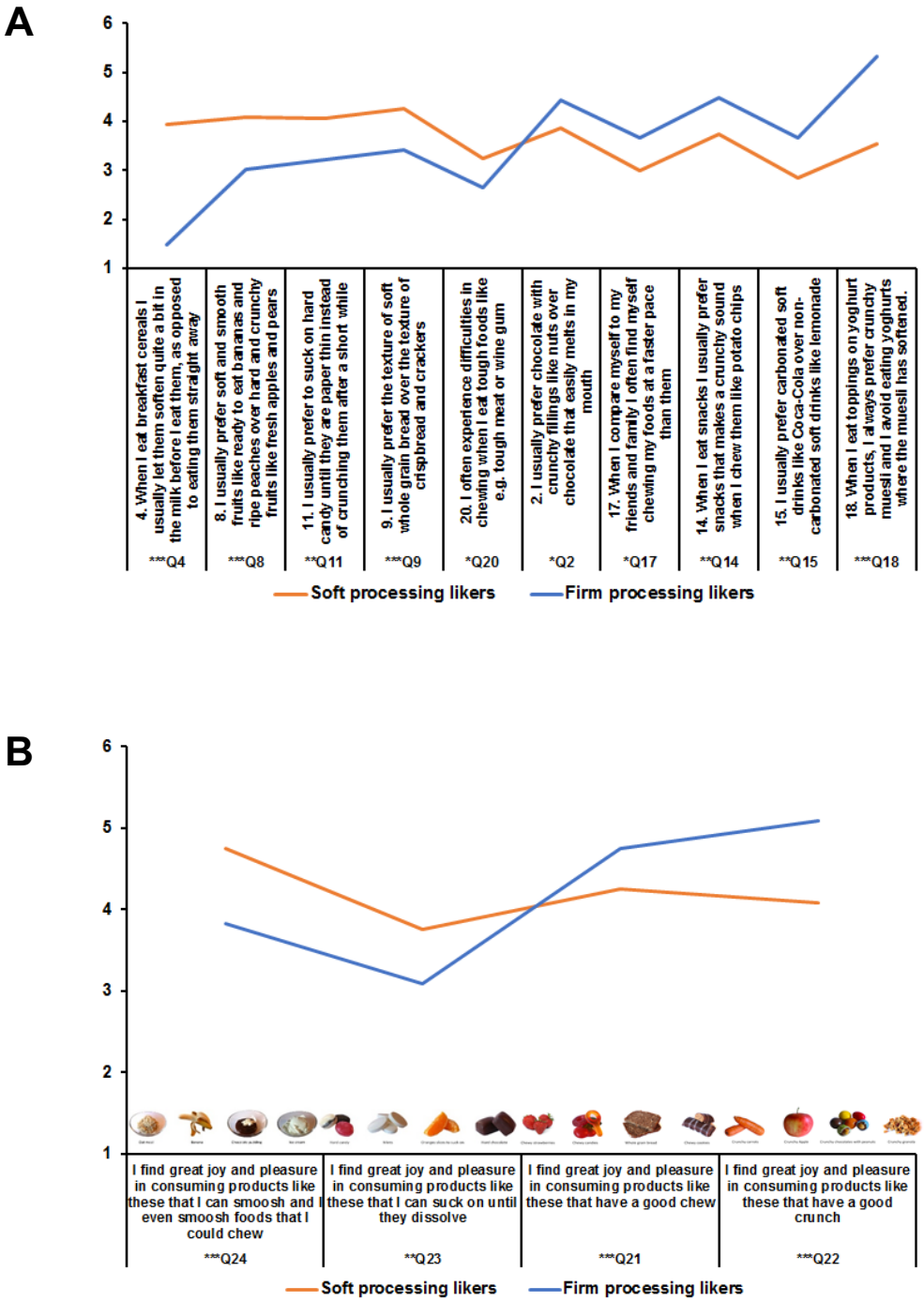

Figure 1a-b. Latent Class Analysis output regarding (a) significant text-based questions and (b) significant picture-based questions for Soft and Firm processing likers.

The most discriminating questions are Q8, Q18 and Q22 ($p<0.0001$) and regards soft smooth (banana/ripe peaches) versus more crunchy fruits (apples/pears), yogurt with crunchy versus softened muesli and finding or joy and pleasure consuming crunchy food as raw carrots, apples, peanuts coated with chocolate, crunchy granola or not. Questions that are best explained by the cluster are Q4 and Q18 ($R^2 = 0.462$ and 0.427) followed by Q22, Q8 and Q24. The 10 non-significant questions may be either not discriminating, were difficult to understand, or be more relevant for further subgrouping in larger population samples.

In Table 2 are reported Cluster means and statistical Wald, p-values and R^2 for the 24 questions.

Table 2. Cluster means and statistical Wald, p-values and R^2 for the 24 indicators (questions).

		Means				
	Items	Cluster 1 - Soft processing likers	Cluster 2 - Firm processing likers	Wald	p-value	R^2
Q1	I usually prefer a chewy piece of candy like e.g. wine gum over a hard piece of candy.	3.79	3.88	0.13	0.72	0.001
Q2	I usually prefer chocolate with crunchy fillings like nuts over chocolate that easily melts in my mouth	3.87	4.44	4.80	0.03	0.038
Q3	When I eat oranges I enjoy to put the slices into my mouth and suck the orange juice out of the slices instead of	2.96	2.47	2.88	0.09	0.024

	just chewing the slices right away					
Q4	When I eat breakfast cereals I usually let them soften quite a bit in the milk before I eat them, as opposed to eating them straight away	3.95	1.48	13.11	0.001	0.462
Q5	When I eat chocolate I usually prefer chocolate with a good chewing texture over chocolate that easily melts in the mouth	3.65	3.72	0.07	0.78	0.001
Q6	When I eat fruits I usually prefer crunchy fruits like fresh apples over more chewy fruits that I can chew on like pineapple or strawberries	3.10	3.47	2.32	0.13	0.020
Q7	When it comes to chocolate I usually prefer chocolate that is hard enough to suck on over chocolate that quickly melts in my mouth	3.35	3.44	0.17	0.68	0.001
Q8	I usually prefer soft and smooth fruits like ready to eat bananas and ripe peaches over hard and crunchy fruits like fresh apples and pears	4.09	3.01	16.77	< 0.0001	0.152

Q9	I usually prefer the texture of soft whole grain bread over the texture of crispbread and crackers	4.27	3.43	11.37	0.001	0.102
Q10	When I eat ice cream I eat it right out of the freezer instead of letting it thaw a little	3.43	3.71	0.90	0.34	0.007
Q11	I usually prefer to suck on hard candy until they are paper thin instead of crunching them after a short while	4.07	3.21	7.79	0.005	0.076
Q12	I enjoy to eat foods that are smooth and easily spreads in my mouth like puddings and ice cream	4.86	4.52	3.63	0.06	0.030
Q13	When I eat cake I usually prefer a chewy cake like brownie instead of a crunchy cake like biscuits	4.40	4.33	0.10	0.75	0.001
Q14	When I eat snacks I usually prefer snacks that makes a crunchy sound when I chew them like potato chips	3.73	4.49	10.27	0.002	0.089
Q15	I usually prefer carbonated soft drinks like Coca-Cola over non-carbonated soft drinks like lemonade	2.84	3.66	7.21	0.007	0.061
Q16	When I eat sweets I usually prefer chocolate that easily melts in my mouth over hard candy that I would need to suck on	4.20	3.88	1.83	0.18	0.015

Q17	When I compare myself to my friends and family I often find myself chewing my foods at a faster pace than them	3.00	3.66	5.82	0.02	0.046
Q18	When I eat toppings on yoghurt products, I always prefer crunchy muesli and I avoid eating yoghurts where the muesli has softened	3.53	5.33	18.11	< 0.0001	0.427
Q19	I often find myself chewing foods on one side of the mouth only	3.89	3.49	2.72	0.09	0.021
Q20	I often experience difficulties in chewing when I eat tough foods like e.g. tough meat or wine gum	3.25	2.66	5.43	0.02	0.046
Q21	I find great joy and pleasure in consuming products like these that have a good chew (examples are chewy strawberry, jelly gums, whole grain bread, chewy biscuits, illustrated with photos)	4.25	4.75	6.91	0.01	0.058
Q22	I find great joy and pleasure in consuming products like these that have a good crunch (examples are raw carrots, apples, peanuts coated with chocolate, crunchy granola, illustrated with photos)	4.08	5.09	17.20	< 0.0001	0.159

Q23	I find great joy and pleasure in consuming products like these that I can suck on until they dissolve (examples are oat meal, banana, chocolate pudding, ice cream, illustrated with photos)	3.76	3.09	9.54	0.002	0.085
Q24	I find great joy and pleasure in consuming products like these that I can smoosh and I even smoosh foods that I could chew (examples are orange slices, hard candy, mints, chocolates without nuts and pieces, illustrated with photos)	4.75	3.83	13.58	0.001	0.124

Relationship between tactile acuity, population cohort and Mouth Behavior

The Generalized linear model for tactile acuity showed that the main factors *Filament thickness* and *Mouth Behavior* were highly significant sources of variation (Wald χ^2 =172.50, p <0.0001; *Mouth Behavior*: Wald χ^2 =12.02, p <0.001, respectively). The main factor *Population cohort* presented a tendency toward significance (Wald χ^2 =3.01, p =0.08). *Post-hoc* tests revealed significant higher *R-index values* when the tongue was stimulated with the thicker filament no. 2.44 (*R-index* = $91.7^a \pm 1.8$), as compared to stimulation with the filament no. 2.36 (*R-index*= $86.8^b \pm 1.8$), and the thinnest filament no. 1.65 (*R-index*= $71.6^c \pm 1.8$).

The *R-index values* were higher, although not significant, in Chinese (84.6 $\pm$ 1.1) compared to the Danish population cohort (82.0 $\pm$ 1.0). Similar results were found when analyzing the data according to signal detection theory (d-prime values).

Subjects characterized as 'Firm processing likers' obtained significant higher *R-index values* (85.9 ± 1.1) compared to subjects characterized as 'Soft processing likers' (80.7 ± 1.0).

The same model, conducted separately on Chinese and Danish subjects, indicated that the main factor *Filament Thickness* was a significant source of variation in both populations (Chinese: Wald χ^2 =69.40, p <0.0001; Danish: Wald χ^2 =71.38, p <0.0001). The main factor *Mouth Behavior* was a significant source of variation for both Chinese (Wald χ^2 =5.22, p <0.05) and Danish consumers (Wald χ^2 =7.17, p <0.01). Indeed, post-hoc tests revealed that 'Soft processing likers' obtained significantly lower *R-index values* compared to 'Firm processing likers' in both population cohorts (Chinese: SPL: 82.2 ± 1.9 *vs*. HPL: 87.1 ± 1.0 and Danish: SPL: 79.3 ± 1.7 *vs*. HPL: 84.7 ± 1.1). None of the 2-way interactions were significant.

Relationship between tactile acuity, PROP status and Fungiform Papillae Density

The characteristics of participants in each population cohort are listed in Table 3.

Table 3. Subjects' characteristics according to PROP status and FPD in Chinese and Danish population cohorts.

PROP status	Chinese		Danish		Total	
	n	FP/cm^2 (mean ±SEM)	n	FP/cm^2 (mean ±SEM)	n	FP/cm^2 (mean ±SEM)
Supertaster (ST)	36	69.7 ±2.9	19	62.5 ±3.8	55	67.2 ±2.4
Medium taster (MT)	29	57.4 ±3.9	41	57.7 ±2.6	70	57.6 ±2.1
No taster (NT)	10	66.1 ±5.4	17	46.1 ±4.1	27	53.5 ±3.4

No correlations were found between the tactile acuity (R-index) and the other two variables considered. Additionally, the subject's ratings of the bitterness of the PROP solutions was positively correlated with the FPD ($r = 0.28$; $p < 0.001$; $R^2 = 0.08$), although with a very low Pearson's correlation. Notably, no significant correlation in the Chinese population cohort was found ($r = 0.17$, $p = 0.15$; $R^2 = 0.03$).

Cross-cultural differences in Mouth Behaviors in relation to FPD and to PROP status

A summary of the main results obtained through the Generalized Linear Model to determine the cross-cultural relationship between Mouth Behaviors and FPD and PROP has been reported in Table 3.

Table 3. Summary of the main results obtained through the Generalized Linear Model to determine the cross-cultural relationship between Mouth Behaviors and FPD and PROP.

Phenotypical marker	Chinese	Danish	p-value
	FP/cm^2 (mean ±SEM)	FP/cm^2 (mean ±SEM)	
FPD	62.9 ±1.4	56.6 ±1.3	< 0.001
PROP responsiveness	51.4 ±2.0	38.3 ±1.9	< 0.0001

A significant effect of *Population cohort* on subjects' FPD (Wald $\chi^2 = 10.67$; $p < 0.001$) and PROP responsiveness (Wald $\chi^2 = 21.78$; $p < 0.0001$) was found, with Chinese population cohort characterized by a greater FPD and a greater responsiveness to PROP compared to Danish consumers.

The main factor *Mouth Behavior* and the interaction factor *Population cohort* x *Mouth Behavior* were not a significant source of variation on FPD and PROP responsiveness.

Interestingly, when the relationship between Mouth Behaviors and FPD was analyzed separately on Chinese and Danish subjects, the main factor *Mouth Behavior* have been found as significant source of variation only in Chinese population cohort (Wald χ^2 =4.25, p <0.05), with Chinese 'Soft processing likers' characterized by a greater FPD (65.8 $\pm$1.4 FP/cm^2) than 'Firm processing likers' (59.9 $\pm$2.5 FP/cm^2). No significant effect has been highlighted running the same model considering the main factor *Mouth Behavior* in relation to PROP responsiveness.

Discussion

With focus on the two major groups of MB, quantitative latent class analyses (McCutcheon, 1987; Magidson & Vermunt, 2004; Vermunt & Magidson, 2005) was used for the classification of preferred mouth behavior. This approach deviated from that proposed by Jeltema and colleagues (2014), in which subjects were forced to choose the type of mouth behavior most desirable to them based on a pictorial presentation of different foods and, at the same time, to indicate what mouth behavior they rejected most. This approach was reported to be more accurate in separating behavioral groups than the standard surveys (Jeltema, Beckley, & Vahalik, 2015). However, it is important to note that just because a person claims to prefer a specific mouth behavior, does not mean that the others are rejected. Indeed, even though a person may generally like soft textures, and often chose foods that could be smooshed or sucked, it could be possible that (s)he may also prefer hard texture foods for other reasons, making the classification in the subgroups of Jeltema and colleagues difficult. Moreover, texture preferences could also be potentially affected by characteristics related to food product itself, such as flavor, particle size, matrix type, fat content and microstructure, and/or related to consumers psychological and physiological factors, such as consumer's familiarity, expectations and sensitivity.

To segment our subjects in relation to their preferred mouth behavior, the present study used a quantitative alternative, the latent class analysis which has several advantages: it is a statistical method and probability based, all relevant information about the respondents perception of mouth behavior can be taken into account (in this case the scoring of the 24 items on a 6 point ordinal scale), the output includes for each respondent the probability to belong to each of the clusters and the classification is based on the highest probability. Furthermore, the relationship between the items and the clusters is also based on probabilities and the level of significance for each of the 24 items is included.

In the present study, with two clusters the broad categorization of preferred oral food processing into 'Firm processing likers' and 'Soft processing likers' was confirmed. Besides, the two classes of preferred MB showed, that the Danish Caucasian population with 73% 'Firm processing likers' had similarity to the North American population reported by Jeltema and colleagues (2014). In their study among 500 participants 76% had preferences for foods that needed firm processing on biting and chewing (33% Crunchers and 43% Chewers). The two populations of Chinese and Danish subjects showed distinct differences in preferred food oral processing behaviors. The Chinese population was characterized by a larger number of 'Soft processing likers' (77% of the population), who preferred foods that could be held in the mouth for a longer time and manipulated between the tongue and roof of the mouth. On the contrary, Danish consumers mostly belonged to the 'Firm processing likers' group, who preferred foods that require using the incisors and/or molars to break down or deform the food in the mouth. A further classification of participants in subgroups of respectively 'smooshers' and 'suckers' and 'chewers' and 'crunchers', as suggested by Jeltema and colleagues (2014), was not feasible due to the limited sample size.

Chinese population in the present study deviated significantly from the Caucasian with much higher preferences for soft food processing in the mouth. These results may indicate that cultural and dietary habits in food

consumption (e.g. cooked, refined (noodles) foods vs less cooked, less refined (rye bread) foods) may influence the preferred oral texture processing between Asians and Caucasians. The large differences also suggest that Chinese participants, living in Denmark for less than two years, did not adapt towards similar texture preferences as the Danish consumers (who had been resident since birth). Other studies have indeed shown that Chinese beliefs and food preferences persist up to twenty years after moving to a foreign country and continue even in subsequent generations (Murray, Easton, & Best, 2001). It should be noted that preferred MB does not necessarily relate to preferred oral texture perception.

Differences in tactile acuity as measured with the von Frey filaments showed that Chinese subjects are equally sensitive across the range of fibers as Danish subjects, confirming previous results reported by Essick and colleagues (2003). Moreover, the group of 'Firm processing likers' were shown to be more sensitive than the 'Soft processing likers' in both population cohorts. Thus results, suggested that lingual acuity did not play a role in subjects' decreased preferences of foods rich in texture. Perhaps other aspects than tactile acuity, such as culturally-driven experience and familiarity with foods as discussed above, have a more influencing role in establishing food texture preferences.

Previous studies found that lingual tactile thresholds were significantly associated with FPD, such that higher densities resulted in greater tactile acuity (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone, 2003). Moreover, several studies have found correlations between PROP intensity and texture perception (Bakke & Vickers, 2011; de Wijk et al., 2007; Hayes & Duffy, 2007; Pickering, Simunkova, & Di Battista, 2004; Pickering & Robert, 2006). A reasonable explanation has been that PROP intensity is related to FPD, which in turn is related to trigeminal innervation. However, to our knowledge, no direct association has been shown to exist between the density of trigeminal (tactile) innervation and the density of taste buds and/or fungiform papillae. Indeed, the areas between fungiform papillae are also innervated and could

conceivably be more densely innervated in subjects with a lower density of papillae. Nevertheless, our study failed to establish direct correlation between tactile acuity and PROP responsiveness or FPD, as previously suggested (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone; 2003). This could be due to the different tasks used to measure tactile acuity in this study (von Frey Filaments) compared to the previous ones (letter-recognition tasks). Moreover, in the present study we only measured mechanical stimulation and not orientation, which perhaps more likely could highlight differences related to morphological variables. As previously reported, the relationship between FPD and PROP has been extensively studied, since both measures have been used as indices of taste sensitivity in general. Many studies have reported a positive relationship between these two measures, but the magnitude of this association has shown considerable variation, ranging from relatively high Pearson's r values > 0.8, to moderate ($r \leq 0.5$) and low ($r \leq 0.3$) (see Piochi, Dinnella, Prescott, & Monteleone, 2018 for a review). Consistent with some of these studies, we found that FPD and perceived PROP intensity were correlated with each other, but presented a low Pearson's r value. No association was found in the Chinese population cohort, with some overlap among the three PROP status groups. Moreover, looking at the subjects' characteristics according to PROP status and FPD in Chinese and Danish population cohorts, it is possible to observe that Chinese presented a great variability in FPD, with NT subjects characterized by a similar density as the STs. A possible explanation for these unexpected findings is that populations with different genetic admixtures were studied, and the presence of more extreme phenotypes in some populations relative to others (e.g. a greater number of ST in Asians compared to the Caucasians) may be driving the observed effects (Barbarossa et al., 2015; Tepper, 2008). Moreover, several recent studies have failed to find a significant relationship between FPD and PROP phenotype (Dinnella et al., 2018; Fisher et al. 2013; Garneau et al. 2014), and other factors than polymorphism of TAS2R38 have been hypothesized as possible variables involved in FPD variation (e.g. polymorphisms of gene

controlling for gustin functionality) (Barbarossa et al., 2015; Calò et al., 2011; Melis et al., 2013; Padiglia et al., 2010).

As far as we know, this is the first study to investigate the relationship between morphological and phenotypical data (FPD and PROP responsiveness) and preferred oral processing behavior. Chinese consumers generally presented a greater responsiveness to PROP and were characterized by a greater FPD compared to Danish consumers. However, our results suggest that the cross-cultural differences found in preferred Mouth Behaviors seem not to be associated with FPD and PROP responsiveness. Thus, we conclude that oral processing behaviors appear to involve other perceptual mechanisms that are unrelated to morphological or phenotypical subject characteristics. For this reason, due to the complex nature of texture perception and preferences, it is essential to identify other relevant factors and define characteristics that govern the processes involved.

While research findings from this work are significant, limitations of the study should also be noted. The first limitation of our study is the relatively small sample size, not balance for gender. While there were several statistically significant observations, the findings may not be generalized to the entire Chinese and Danish populations, and it is recommended to extend the number of subjects involved in the experiment in order to avoid 'false positive' associations and obtain a more robust and generalizable outcome. Another issue to be noted is that the evaluation of taste sensitivity is limited to PROP responsiveness and FPD evaluation and these two general markers for taste sensitivity still present contradictory relationship between each other and between perception of basic tastes (see Piochi, Dinnella, Prescott, & Monteleone, 2018 for a review). Thus, a combination of taste perception measurements, such as data related to fundamental taste thresholds, should be included to better characterize the overall subjects' perception. Moreover, the use of the von-Frey filaments for detection threshold could be insufficient for this task due to the fact that the lowest available force (0.008 g) could not be sensible enough to establish the real detection threshold of the subjects. Thus, different

tongue sensitivity methods (e.g. Luneau Cochet-Bonnet aesthesiometers) (Miles et al., 2018) could be employed to measure touch detection threshold since these aesthesiometers had the benefit of providing an increased number of extremely lower-force stimuli than the filaments. Additionally, two-point discrimination task and stereognostic letter-recognition task could be used to evaluate the roughness sensitivity and the point-and-edge sensitivity, respectively, in order to include discrimination of size and orientation. However, it should be noted that the latter method could not be suitable for cross-cultural studies among populations with very different handwritten characters (e.g. Latin *vs* Chinese alphabet characters).

It would be necessary in future researches to investigate whether lingual tactile acuity is related to sensitivity toward, rather than preference for, textural aspects of foods. For example, particle detection could be associated to lingual tactile acuity and, therefore, could highlight more evidence about how subjects perceive and prefer foods.

Conclusion

Cross-cultural differences in preferred oral processing behavior were found, as Chinese subjects predominantly preferred to manipulate foods between the tongue and roof of the mouth. On the contrary, Danish subjects mostly preferred to use the teeth to break down foods in the mouth. Chinese subjects presented differences in oral tongue anatomy as shown by greater FPD and PROP responsiveness compared to Caucasian Danish subjects.

A significant but low correlation was found between PROP status and FPD, while no direct correlation between tactile acuity and PROP responsiveness or FPD were found. The reason of having no direct correlation between subjects' ability in touch detection and morphological and phenotypical data (FPD and PROP status) is still not certain. These observations suggest that thoughtfulness should be applied in studying texture perception, since focusing solely on PROP and FPD evaluation may be not sufficient to

understand the variability and complexities of phenomena raised from the interaction between food and mechanoreceptors in the oral cavity. Thus, the physiological parameters that we investigated should be examined in more detail (e.g. PF size and relevant distributions, more sensitive method to valuate touch detection). Moreover, this study provides evidence that cultural background could represent a strong influence in the oral process preference for texture.

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CHAPTER 6

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

General conclusions and future perspectives

Research has proven that food choices and eating habits are a complex behavior mediated by a number of biological and environmental factors. Indeed, food consumption is mainly driven by food preferences, which largely depend on taste and sensory perception. Since differences exist between individuals' sensitivity to oral stimuli, and these differences could modulates our response to food preferences and consequently diet, the present thesis focuses on understanding how and, to what extent, individual variability can contribute to explain food preferences and behaviors.

In summary, we confirmed that PROP responsiveness could be used as an reliable index for general taste sensitivity in water solutions. Moreover, interindividual differences in taste perception were found to influence habitual food consumption and intake. For instance, subjects who were orally hypersensitive to salty or sweet tastes seem to increase the frequency consumption of less healthy foods, like bakery and salty baked products and sweets and desserts, than the hypersensitive groups.

Cross-cultural differences have been found in oral tongue anatomy and taste sensitivity as shown by greater FPD and PROP responsiveness of Asian subjects compared to Caucasian subjects. Moreover, oral processing behaviors seem to distinguish the two population cohorts involved, with Chinese subjects predominantly preferred to manipulate foods between the tongue and roof of the mouth, while Danish subjects mostly preferred to use the teeth to break down foods.

The present thesis focuses also in identifying the factors that could predispose individuals to obesity disease by influencing their dietary decisions. The results showed that taste sensitivity occurred differently accordingly to subjects' nutritional status. In particular, obese children and adolescents involved in the study presented a lower ability in correctly identifying taste qualities compared to the group of normal-weight. This impaired taste perception in obese children and adolescents supports the assumption that

this reduced sensitivity in obese subjects could lead them to require a higher amounts of tastants to elicit a response within taste receptor cells and be more satisfied.

Given the hypothesis that microbes in the gastrointestinal tract could affect individuals' eating behaviors and food preferences, and the composition of microbiota appears to have an important but still unclear role in obesity development, the present thesis focuses on a novel field of investigation. In particular, the unexplored relationships among oral microbiota, taste perception, eating behaviors and nutritional status were investigated.
Altogether, the results suggest that the oral microbiota composition deserves to be considered as an influencing variable when investigating taste perception.
Moreover, interesting correlations between the relative abundance of oral bacterial taxa and dietary intake were highlighted. In particular, *Clostridia* class was positively associated with total energy, fat, and protein intake but negatively associated with fiber intake, whereas *Proteobacteria* phylum and *Prevotella* genus showed the opposite association, supporting the general assumption that some microbial taxa are positively associated with vegetable-rich (*Prevotella*) or protein/fat-rich diets (*Clostridia*).
In addition, also in children and adolescents we found that some bacterial genera seemed to differ among subjects with different ability in perceiving taste qualities, independently from nutritional status.

In conclusion, findings from the present thesis could help to shed light on the complexities of human eating behavior, understanding how and which host-related factors could affect people food choices and habits. Clearly, there are still more questions than answers. Nevertheless, the potential implications of this novel field of investigation are intriguing. The identification of individuals who may be sensory predisposed to an unhealthy dietary pattern and the evaluation of multiple aspects of individuality, including the microbiota, could be a first step in targeted strategies to improve individuals' nutritional status

and health, and may contribute to the development and implementation of microbiome-taste-tailored diets. Moreover, these outcomes could be used as a starting point to understand the driving force of food preferences and help food industries in developing food products that match different consumers' needs and could help develop further strategies for obesity prevention and therapy.

Lastly, the potentiality of this multidisciplinary approach opens new avenues of research by highlighting associations between sensory and consumer science, food technology and nutrition.

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APPENDICES

Papers with Impact Factor

- Laureati, M., **Cattaneo, C.**, Bergamaschi, V., Proserpio, C., Pagliarini, E., 2016. School children preferences for fish formulations: the impact of child and parental food neophobia. *J. Sens. Stud.*, *31*(5), 408-415.
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- **Cattaneo, C.**, Gargari, G., Koirala, R., Laureati, M., Riso, P., Guglielmetti, S., Pagliarini, E., 2019. New insights into the relationship between taste perception and oral microbiota composition. *Sci. Rep.*, 9(1), 3549.
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Oral communications

- Proserpio, C., Invitti, C., Laureati, M., **Cattaneo, C.**, Pagliarini, E. Esiste una relazione tra sensibilità gustativa, neofobia e obesità?. VI Convegno Nazionale Società Italiana di Scienze Sensoriali (SISS), Bologna, Italy, November 30th - December 2nd, 2015.
- **Cattaneo, C.** New perspective on taste perception: does oral microbiota composition play a role? 8th E3S & SISS Symposium "*Tasting The Future In Sensory And Consumer Science*" Milan, Italy, May 28th, 2019.
- **Cattaneo, C.**, Liu, J., Sporring, J., Bech, A.C., Pagliarini, E., Bredie, W. L. P. Mapping the tongue: a novel approach to explore cross-cultural differences in chemosensory perception. 13th Pangborn Sensory Science Symposium, Edinburgh, UK, July 28th – August 1st, 2019.
- **Cattaneo, C.** New perspective on taste: exploring association among oral perception, tongue physiology and oral microbiota composition. XXIV Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology. Firenze, Italy, September, 11th - 13th, 2019.
- **Cattaneo, C.**, Pagliarini, E. Obesità: un questiona di gusto. Ottava Edizione del Congresso 'La Pediatria nella Pratica Clinica'. Milano, Italy, February, 6th - 8th, 2020.

Posters

- Laureati, M., **Cattaneo, C.**, Proserpio, C., Pagliarini, E. Sensory profiling of fibre-enriched apple purées by using the Check-All-That-Apply method with school aged children. EuroSense, Djion, 11th-14th September, 2016.

- Laureati, M., **Cattaneo, C.**, Proserpio, C., Pagliarini, E. Do children like fish? Perceptive and behavioral factors related to children's acceptance of fish school formulations. EuroSense, Djion, 11th-14th September, 2016.

- Laureati, M., Pagliarini, E., **Cattaneo, C.**, et al., Associations between food neophobia and responsiveness to "warning" sensations in real food products in a large population sample. 12th Pangborn Sensory Science Symposium, Providence, Rhode Island, USA, August 20th-24th, 2017.

- **Cattaneo, C.** New insights into the obesity phenomenon: perceptive, behavioural and microbiological determinants of weight gain. XXII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology. Bolzano, Italy, September, 20th-22nd, 2017.

- **Cattaneo, C.**, Lavelli, V., Proserpio, C., Gallotti, F., Laureati, M., Pagliarini, E. Consumers' attitude towards food by-products and novel technologies. 8th European Conference on Sensory and Consumer Research, Verona, Italy, September 2nd-5th, 2018.

- **Cattaneo, C.**, Guglielmetti, S., Laureati, M., Pagliarini, E. Shaping individuals' eating behavior: do taste perception and oral microbiota have a role? 8th European Conference on Sensory and Consumer Research, Verona, Italy, September 2nd-5th, 2018.

- **Cattaneo, C.**, Liu, J., Sporring, J., Bech, A.C., Pagliarini, E., Bredie, W. L. P. Mapping the tongue: a novel approach to explore cross-cultural differences in chemosensory perception. 13th Pangborn Sensory Science Symposium, Edinburgh, UK, July 28th – August 1st, 2019.

- **Cattaneo, C.**, Mameli, C., Zuccotti, G.V., Panelli, S., Comandatore, F., Pagliarini, E. Relationship between taste sensitivity and oral microbiota composition: exploring their role in obesity development. 13th Pangborn Sensory Science Symposium, Edinburgh, UK, July 28th – August 1st, 2019.

Awards

- Marie Pangborn Sensory Science scholarship (15000$) provided by Purdue University (Indiana USA) for the high quality research on a sensory topic under the guidance of a sensory scientist.
- Pangborn Symposium Student bursary (500€) provided by the 13th Pangborn Sensory Science Symposium Chairs 2019.
- Rick Bell Memorial Scholarship (1500€) provided by the selection committee of the 13th Pangborn Sensory Science Symposium.
- Premio Giovani Ricercatori (500€) provided by SISS and awarded at 8th European Conference on Sensory and Consumer Research (September 2nd-5th, 2018, Verona) for the best poster.

Teaching assistant in tutoring activities

Co-supervisor of 7 theses (Faculty of Agricultural and Food Science, University of Milan): 4 Bachelor Degree theses whose 2 in Food Science & Technology and 2 in Food Services Science & Technology, 3 Master Degree thesis whose 2 in Human Nutrition & Food Science and 1 in Food Science & Technology.

Overview of completed training activities

Discipline specific course and activities	Organizer and location	
6th Convegno Nazionale SISS – Società Italiana Di Scienze Sensoriali	SISS, Bologna (IT)	2016
Course 'FIZZ - Software solutions for Sensory Analysis and Consumer Tests. What's new?'	SISS, Bologna (IT)	2016
1st Congress Woman in Olfactory Science (WIOS)	SISSA, Trieste (IT)	2017
Seminar 'Taste physiology and food choice'	UNIMI, Milano (IT)	2017
Seminar 'Communicating for food waste reduction: investigation of different messages and strategies'	UNIMI, Milano (IT)	2017
Seminar 'Food preferences and taste genetics'	UNIMI, Milano (IT)	2017
Seminar 'Interventions to increase vegetable intake in early childhood; results from different European studies'	UNIMI, Milano (IT)	2017
Post Graduate Course: 'Sensory Perception & Food Preference: The role of context'	Graduate school VLAG, Wageningen (NL)	2018
Workshop SISS	SISS, Bologna (IT)	2018
8th European Conference on Sensor Sensory and Consumer Research	Elsevier, Verona (IT)	2018
Visiting PhD student at Department of Food Science, Section for Food Design and Consumer Behaviour (six months)	KU, Copenhagen (DK)	2018
8th E3S & SISS Symposium	E3S - SISS, Milano (IT)	2019
13th Pangborn Sensory Science Symposium	Elsevier, Edinburgh (UK)	2019

PhD courses and activities	Organizer and location	
PhD opening academic years (XXII - XXXIII cycles)	UNIMI, Milano (IT)	2016 - 2017
PhD final exams and ceremonies (XXIX and XXX cycles)	UNIMI, Milano (IT)	2016 - 2018
Food Systems PhD Journal clubs	UNIMI, Milano (IT)	2017-2019
XXII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology	UNIBZ, Bolzano (IT)	2017
XXIV Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology	UNIFI, Firenze (IT)	2019
Course 'Sustainability concepts in food technology – methodological approaches and case studies'	UNIMI, Milano (IT)	2018
Course 'Introduction to statistical analysis of ecological and environmental data'	UNIMI, Milano (IT)	2019
Course 'The intestinal microbiota: interactions with host and diet'	UNIMI, Milano (IT)	2019

Transferable skills	Organizer and location	
Open access – open data e il mondo delle pubblicazioni	UNIMI, Milano (IT)	2017
La valutazione della ricerca: indicatori bibliometrici e peer review	UNIMI, Milano (IT)	2018
Come scrivere un progetto di ricerca: parte 1. Le 100 cose che avrei voluto sapere quando ero un dottorando	UNIMI, Milano (IT)	2018
Come scrivere un progetto di ricerca: parte 2. Le 100 cose che avrei voluto sapere quando ero un dottorando	UNIMI, Milano (IT)	2019