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CLINICAL TRIAL DESIGN

WILEY

The Obese Taste Bud study: Objectives and study design

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Abstract

Aims: Taste modifies eating behaviour, impacting body weight and potentially obesity development. The Obese Taste Bud (OTB) Study is a prospective cohort study launched in 2020 at the University of Leipzig Obesity Centre in cooperation with the HI-MAG Institute. OTB will test the hypothesis that taste cell homeostasis and taste perception are linked to obesity. Here, we provide the study design, data collection process and baseline characteristics.

Materials and Methods: Participants presenting overweight, obesity or normal weight undergo taste and smell tests, anthropometric, and taste bud density (TBD) assessment on Day 1. Information on physical and mental health, eating behaviour, physical activity, and dental hygiene are obtained, while biomaterial (saliva, tongue swap, blood) is collected in the fasted state. Further blood samples are taken during a glucose tolerance test. A stool sample is collected at home prior to Day 2, on which a taste bud biopsy follows dental examination. A subsample undergoes functional magnetic resonance imaging while exposed to eating-related cognitive tasks. Follow-up

Kerstin Rohde-Zimmermann and Imke Schamarek have equal contributions.

investigations after conventional weight loss interventions and bariatric surgery will be included.

Results: Initial results show that glycated haemoglobin levels and age are negatively associated with TBD, while an unfavourable metabolic profile, current dieting, and vegan diet are related to taste perception. Olfactory function negatively correlates with age and high-density lipoprotein cholesterol.

Conclusion: Initial findings suggest that metabolic alterations are relevant for taste and smell function and TBD. By combining omics data from collected biomaterial with physiological, metabolic and psychological data related to taste perception and eating behaviour, the OTB study aims to strengthen our understanding of taste perception in obesity.

KEYWORDS

inflammation, metabolic disease, obesity, oral microbiome, taste cell homeostasis, taste perception

1 | INTRODUCTION

Obesity and associated metabolic diseases have reached pandemic level and are still on the rise.[1] Taste perception can contribute to increased caloric intake and ultimately weight gain, hence the sense of taste might be of greater relevance in the context of obesity than is reflected in the level of attention it has so far been granted. Indeed, changes in taste perception can be observed in people with obesity.[2] While recent research focuses on uncovering the mechanisms of taste sensation, understanding alterations in taste cell homeostasis in obesity is highly relevant to solving the obesity puzzle. Increasing evidence indicates that the sense of taste not only contributes to obesity, but is also affected by excessive fat storage, potentially resulting in a vicious circle that enhances obesity development.[3] Interestingly, hormones such as leptin and adiponectin, but also insulin have been shown to affect taste bud physiology with consequences on taste sensation.[4,5] In addition, an increasing number of molecules have been found to be of relevance for taste bud physiology.[2] However, mechanisms involved in taste modulation and taste perception in the context of obesity are largely unknown. Accumulating research puts lingual taste buds into the spotlight as these might represent a new target for interventions when treating patients for obesity and associated metabolic disease. Thus, taste cell homeostasis is influenced by a multitude of factors which, in the context of obesity, can contribute to extensive changes with substantial consequences for taste perception, and ultimately food preference and consumption and therefore weight regulation.[2,6]

The aim of the Obese Taste Bud (OTB) study is to identify mechanisms causing changes in the sense of taste in obesity and body weight regulation. We hypothesize that obesity is linked to an altered taste bud transcriptome driven by the affected metabolic status, which translates to taste-related eating habits. Therefore, the OTB study is investigating a wide range of factors that potentially contribute to an altered lingual taste bud cell (TBC) biology in obesity compared to normal weight, including (epi)genetic factors, salivary compounds, the oral and the gut microbiome, metabolic parameters, and behavioural and psychological variables. Focusing on lingual taste cells in obesity, the OTB study covers a wide range of factors, from subjective taste perception, through functional and structural changes, to the molecular and (epi)genetic mechanisms involved. Above and beyond detailed investigations of the lingual taste cell itself, the study aims to achieve a comprehensive understanding of the complex interplay of lingual taste bud cells as an endocrine organ with other tissues as well as the central processes in the regulation of food preference and eating behaviour and how this potentially translates into the development of obesity and metabolic disease. It aims to understand interactions of metabolic imbalances and food compounds with the molecular basics of taste cell renewal, cell fate decisions and consequent taste signalling.

Speaking of obesity as a pandemic health issue, targeting lingual taste buds, given their life-long turnover, easy accessibility and the fact they are a one-of-a-kind organ influenced by numerous internal and external stimuli, could be a powerful tool in the search for potent treatment and prevention strategies for this disease.

2 | STUDY DESIGN AND METHODS

The OTB study is an ongoing cross-sectional observational study initiated in 2020 and being conducted at the University Hospital of Leipzig Obesity Centre in cooperation with the Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG), Helmholtz Centre Munich at the University Leipzig and the University Hospital Leipzig. The study is being performed in accordance with the 1964 Helsinki Declaration and later versions, was approved by the Ethics Committee of the University of Leipzig (011/20-ek) and is registered at Clinicaltrials.gov (NCT04633109) and the German Registry for Clinical Studies (DRKS00022950). Participants are recruited via flyer and poster advertisements within the campus of the University Hospital Leipzig. They are screened for eligibility via a telephone interview and included in the study based on strict inclusion and exclusion criteria (Table 1). Data collection in a study population with obesity and in a normal weight control group occurs on two separate days. Day 1 starts at 7:30 AM after an overnight fast, after informed written consent has

TABLE 1 Study inclusion and exclusion criteria.

Inclusion criteria
Men and women
Age between 18 and 69 years
Exclusion criteria
BMI < 18 kg/m^2
Anaemia, Hb <5 mmol/L (male) and <4 mmol/L (female)
Current pregnancy or lactation
Current or history of malignant disease
Current or history of radiation therapy of the head and/or neck region
Current or history of chemotherapy
Severe psychiatric disease
Neurological disease
Current drug abuse
Current steroid treatment
Current immune suppressive therapy
Severe cardiac, renal or liver disease
Known dysfunction of the gustatory system
Known coagulation disorders

Abbreviations: BMI, body mass index, Hb, haemoglobin.

been given by each participant, and includes a structured medical interview, a fasting blood draw, a standardized taste and smell test, anthropometric measures, saliva sampling, tongue swap, taste bud density (TBD) assessment, and an oral glucose tolerance test (OGTT), if no contraindications apply. Thereafter, participants are given breakfast. Participants are further asked to collect a stool sample at home, preferably on the morning of test Day 2 or the day before. In addition, they are asked to provide questionnaire-based information on health and lifestyle factors (Table 2). On Day 2, a dental examination is followed by the extraction of 5–8 fungiform papillae from the tongue's surface for molecular analyses. A subsample, currently comprising 26 participants with overweight or obesity, has further undergone functional magnetic resonance imaging (MRI) as part of a separate intervention study MIFOOD (#NCT05353504 at Clinicaltrials.gov, in cooperation with the Max Planck Institute for Cognitive and Brain Sciences, Leipzig, Germany). Inclusion criteria for the MIFOOD study are body mass index (BMI) ≥25 kg/m^2 and no MRI contraindications. To avoid a prolonged fasting period, these participants did not undergo an OGTT. The study design is summarized in Figure 1.

3 | MATERIALS AND METHODS

3.1 | Data acquisition for lifestyle and health factors, eating behaviour and sociodemographic factors

To assess factors regarding physical and mental health, eating behaviour, food choices and food preferences, physical activity, and other health behaviours, a total of 11 validated questionnaires are applied (Table 2). The participants completed the questionnaires during the

TABLE 2 Description of the questionnaires applied in the OTB study.

Questionnaire (Abbreviation)	Assessed construct	Reference
Three Factor Eating Questionnaire (TFEQ)	Trait eating behaviour: Disinhibition, restraint, restraint eating, cognitive restraint, uncontrolled eating, and emotional eating	Stunkard and Messick[41] de Lauzon et al.[42]
The German Leeds Food Preference questionnaire (LFPQ-G)	Computer based assessment of explicit liking, explicit wanting and implicit wanting for four food classes of high versus low fat and sweet versus savoury foods	Wardle and Marsland[43] Schamarek et al.[44]
Food Frequency Questionnaire (FFQ)	Frequency of consumption of different food items	Willett et al.[45]
Power of Food Scale (POFS)	Individual sensitivity to external food-related stimuli and hedonic eating behaviour	Lowe et al.[46]
International Physical Activity Questionnaire (IPAQ)	Physical activity evaluated with 3 activity levels and a continuous outcome measure based on metabolic equivalent of task (MET) minutes	Craig et al.[47]
Yale Food Addiction Scale (YFAS)	Characteristics of food addiction based on the Diagnostic and Statistical Manual of Mental Disorders criteria	Gearhardt et al.[48]
General Depression Scale (ADS-L)	Level of depressive mood	Radloff[49]
Food Craving Questionnaire (FCQ)	Extent of craving for specific foods	Cepeda-Benito et al.[50]
Perceived Stress Scale (PSS)	Perceived stress or perceived stress levels.	Cohen et al.[51]
36-Item Short Form Health Survey (SF-36)	Health-related quality of life. (referring to the last 4 weeks).	Ware Jr and Sherbourne[52]
Alcohol use Disorder Identification Test (AUDIT)	Drinking habits and alcohol consumption	Saunders et al.[53]
Bristol Stool Scale (BSS)	Assessment of human stool characteristics	Lewis and Heaton[54]

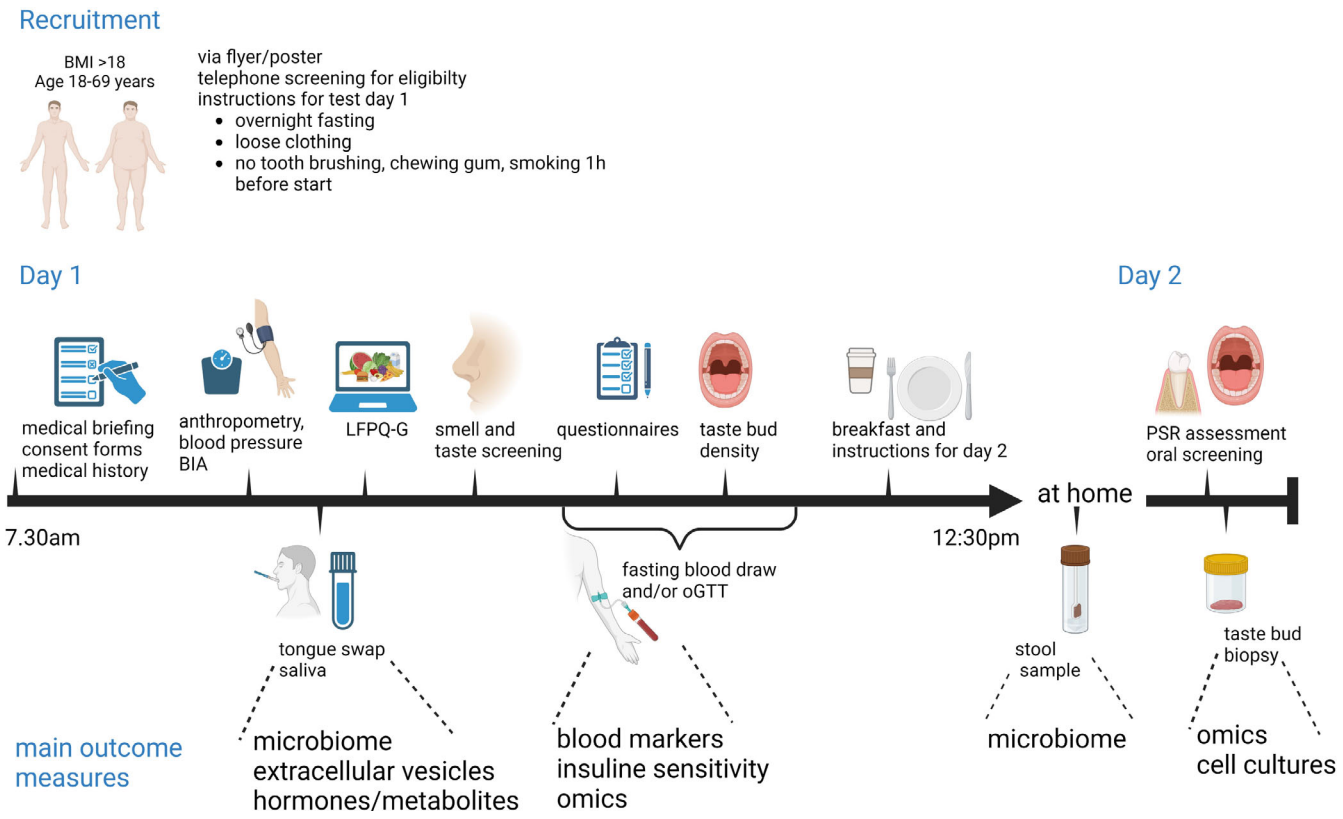

FIGURE 1 Data acquisition and planned main outcome measures of the Obese Taste Bud study. Created with BioRender.com. BIA, bioelectrical impedance analyses; BMI, body mass index; LFPQ-G, German version of Leeds Food Preference Questionnaire; PSR, periodontal screening and recording.

OGTT or at home using Lime Survey.[7] In addition, sociodemographic data, data on smoking behaviour, dieting, dental hygiene, potential smell and taste dysfunction, medical history, and for women, information regarding menstrual cycle, were obtained via a standardized questionnaire on Day 1. As microbiome analyses are conducted, the self-reported frequency of antibiotic treatment during the past 5 years was also assessed. On Day 2, a short questionnaire was used to obtain data on dental hygiene, cigarette smoking, and meal times on the day of taste bud biopsy.

In the context of the MIFOOD study, eligible participants have additionally undergone structural and functional 3-Tesla MRI. Briefly, in addition to the anatomical and diffusion-weighted imaging sequences, participants were asked to rate wanting of food and art images in a semi-satiated state using a task-based event-related approach. This approach allows the evaluation of differences in wanting scores and brain region activity for food compared to art stimuli. For further details on the design and analysis plan of this decision-making task, see Medawar et al.[8]

3.2 | Blood sampling and GTT

On Day 1, blood samples were collected in the fasted state for immediate analysis of a panel of clinical and metabolic parameters, which was carried out by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the University of Leipzig Medical Centre (Table 3). Additional samples were obtained for the preparation of serum and plasma (with and without protease inhibitors) and stored at −80°C for later assessment of hormone and adipokine concentrations and inflammatory biomarkers, as well as whole blood for RNA (Tempus blood RNA Tubes, Thermo Fisher Scientific), DNA (DNA exact Monovette, Sarstedt), and genetic and proteome analyses (K_3 EDTA Monovette, Sarstedt). In addition, an OGTT was performed with consecutive blood draws through an indwelling venous cannula at the following time points with respect to 75-g oral glucose administration: −15, 0, 15, 30, 90 and 120 min. Blood samples collected during the OGTT were sent to the hospital laboratory for immediate analyses of insulin, glucose and C-peptide levels, and taken to the HI-MAG laboratory for the preparation of serum and plasma, before storing at −80°C.

3.3 | Anthropometric data

Anthropometric measures included body height (m) and weight (kg), waist and hip circumference, and arm, lower leg and upper leg circumferences (all in cm). The latter three were measured by defining the middle of the arm or lower/upper leg to assess measures for their respective muscle or fat content. Waist-to-hip ratio and BMI were calculated by dividing waist (cm) by hip (cm) and body weight (kg) by body height (m^2), respectively. Body height and weight were

TABLE 3 Fasting blood parameters.

Blood parameters	Unit	Blood material
Haematology		
Blood count		EDTA-whole blood
Complete blood count		EDTA-whole blood
Liver/pancreas		
Alanine aminotransferase	μkat/L	Serum
Aspartate aminotransferase	μkat/L	Serum
Alcalic phosphatase	μkat/L	Serum
Gamma-glutamyl transferase	μkat/L	Serum
Bilirubin, total	μmol/L	Serum
Bilirubin, direct	μmol/L	Serum
Carbohydrate metabolism/ diabetes mellitus		
Fasting blood glucose	mmol/mol	Fluoride EDTA-plasma
HbA1c	%	EDTA-whole blood
HbA1c	mmol/mol	EDTA-whole blood
Fasting insulin	pmol/L	Serum
C-peptide	nmol/L	Serum
Kidney		
Creatinine (enzymatic)	μmol/L	Serum
Glomerular filtration rate, calculated from creatinine	mL/ min/1.73 m^2	Serum
Lipid diagnostic		
Triglycerides	mmol/L	Serum
Total cholesterol	mmol/L	Serum
HDL cholesterol	mmol/L	Serum
LDL cholesterol	mmol/L	Serum
Free fatty acids	mmol/L	Serum
Thyroid gland		
Thyrotropin	mU/L	Serum
Free triiodothyronine (T3)	pmol/L	Serum
Free thyroxine (T4)	nmol/L	Serum
Adrenal gland		
Cortisol	nmol/L	Serum

Abbreviations: HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

measured using a calibrated scale (stadiometer seca764, SECA), with the participants instructed to wear light clothes and no shoes during measurement. Anthropometrics were complemented by a bioelectrical impedance analysis, which provides detailed information on body composition including body fat (%), lean mass (%), body water, body cell mass, and extracellular mass. Briefly, bioelectrical impedance analysis (without shoes and socks) was performed after participants lay down for 10 min before actual measurement, and electrodes were placed on both arms and legs according to the manufacturer's instructions (BIACORPUS RX 4004 M, Medical healthcare). Trained study assistants ensured there was no contact between the legs and arms and upper body so that optimal current flow could be obtained, and also ensured a quiet environment before and during measurement to avoid increased movement of body fluids, which would influence output measures.

3.4 | Clinical and metabolic variables

Obesity was defined according to the criteria proposed by the World Health Organization as follows: BMI <18.5 kg/m^2 defined underweight and BMI ≥25.0 kg/m^2 overweight, while BMI ≥30.0 kg/m^2 was defined as obese category I, BMI ≥35.0 kg/m^2 as obese category II and BMI ≥40.0 kg/m^2 as obese category III.[9] Abdominal obesity was defined as waist circumference >102 cm in men and >88 cm in women. Systolic and diastolic blood pressure (BP) were measured with a BP monitor from Omron Healthcare prior to the fasting blood draw with the participant lying down. Three independent BP measures were taken, and the mean value of these was calculated for subsequent use. Hypertension was defined as systolic BP >130 mmHg or diastolic BP >85 mmHg or the use of antihypertensive medication. Participants with fasting plasma glucose (FPG) levels between 5.56 and 6.9 mmol/L and/or glycated haemoglobin (HbA1c) between 5.7% and 6.4% and/or OGTT 2-h values between 7.8 and 11.0 mmol/L were defined as prediabetes.[10] An FPG level ≥7.0 mmol/L and/or an OGTT 2-h value ≥11.1 mmol/L and/or an HbA1c level ≥6.5% defined diabetes mellitus.[10] Components of metabolic syndrome were defined as impaired FPG (FPG >5.56 mmol/L or treatment), elevated BP (systolic BP >130 mmHg or diastolic BP >85 mmHg or treatment), elevated triglycerides (>1.7 mmol/L or treatment), low high-density lipoprotein (HDL) cholesterol (<1.0 mmol/L in men, <1.3 mmol/L in women or treatment), and abdominal obesity (waist circumference >94 cm in men, >80 cm in women). Components of metabolic syndrome were transformed to dichotomous variables before analysis according to Alberti et al.[11] On Day 2, a dental examination was performed to exclude any severe dental conditions or severe pathologies within the oral cavity, and to characterize clinically the tongue and mouth mucosa. Periodontal health was assessed with the Periodontal Screening and Recording (PSR) score using a dental probe, with the highest score (0 = periodontally healthy to 4 = suspected severe periodontitis) among all sextants indicating the highest risk of periodontitis, according to standardized clinical practice.

3.5 | Taste and smell perception including assessment of current hunger

Taste perception was assessed with commercially available taste strips (Burghart ODOFIN Taste Strips, Medisense). These are filter papers

impregnated with different solutions creating each of the four basic tastes: sweet (sucrose), sour (citric acid), salty (sodium chloride) and bitter (quinine hydrochloride). Additional taste strips impregnated with L-glutamate were used to assess the ability to taste umami. Taste strips are placed in the centre of the participant's tongue, and the participant is asked to identify the presented taste according to a forced choice paradigm. Eighteen taste strips are presented according to a standardized protocol during which each taste quality is presented in four increasing concentrations in a randomized order. Two non-impregnated control strips are included, which are not considered in the final taste score. The participants evaluated each presented taste strip using a computer-based survey via Lime Survey.[7] After each trial, the participants were asked to rinse their mouth with water, and were instructed to spit the water into a container to prevent them from swallowing. Subscales for each taste quality were calculated based on the number of correctly identified taste strips varying from 0 to 4. A sum score was calculated as the sum of all correctly identified taste strips of the four basic taste qualities, sweet, sour, salty and bitter (0-16). Based on the manufacturer's recommendations, umami was not included in the taste sum score. Taste sensation was further defined as normo- (≥2 correct), hypo- (1 correct or false) or ageusia (0 correct) per taste quality of sweet, sour, salty or umami. Normogeusia for bitter taste was defined if ≥1 taste strips were correctly identified, hypogeusia was defined as present if the participant falsely identified the taste, and ageusia was defined if 0 strips were identified correctly. A total score <9 including all taste qualities, except umami, was defined as age-independent ageusia.

Participants were further asked to evaluate taste liking (anchored by 'not at all' and 'very much') for each taste quality and intensity on a 100-mm visual analogue scale and to indicate, using a forced choice paradigm, how sure they were ('I couldn't taste anything and needed to guess': 50% sure; 'I could taste something, but am not sure which taste': 75% sure; 'I could taste something and am almost sure which taste': 90%; 'absolutely sure': 100%) of their evaluation. Prior to the taste test, subjective hunger was assessed on a 100-mm visual analogue scale anchored with 'no hunger' to 'greatest possible hunger'.

A Sniffin' Sticks test battery (identification test, blue. Burghart [ODOFIN] Sniffin' Sticks, Germany) was used to test smell perception. In this test, participants must identify the odour of 16 different pencil-like samples of smells, which are moved and waggled in close proximity to their nose for 5 s. Participants must select from the four odour options presented on one standardized card per Sniffin' Stick. A sum score was calculated based on the number of correctly identified odours (ranging from 0 to 16), while hypo-, hyper- or normosmia were defined according to the manufacturer's instructions (revised version 01.06.2021). Briefly, based on norm data obtained within a test population of healthy subjects with normal smelling ability, the classification of normosmia and hyposmia was based on the age group presenting the best performance during the smell test.[12] Accordingly, based on the number of correctly identified odours, people reaching the 10th percentile present normosmia and people above the 90th percentile present hyperosmia or are called 'supersmellers'. A definition of anosmia would only be possible with an extended smell test, which was not included in the present study.

3.6 | TBD and fungiform papillae sampling

The TBD of fungiform papillae was assessed by trained study assistants following a standardized procedure. Commercially available blue food dye was applied evenly across the tongue's surface; this increases the visibility of individual papillae. A circular piece of filter paper (VWR International BVBR), 15 mm in diameter, was placed on the tip of the anterior part of the left side of the tongue closest to the sulcus medianus of the tongue (Figure 2). A digital single lens reflex camera (Canon EOS RP, RF 24-105 mm 4.0–7.1 IS STM objective) was used to take a photograph of the tongue and the digital images were analysed using ImageJ software (https://imagej.nih.gov/ij/download.html). To quantify taste buds, a standardized protocol was followed by which the filter paper placed on the tongue was used as a reference scale to standardize the area used for TBD assessment. The circle tool of the software was used to draw and copy the filter paper area to the other side of the tongue at a defined place (adjacent to the sulcus medianus and 5 mm proximal from the tip of the tongue; Figure 2). Fungiform papillae within that reference area were counted separately by two trained study assistants and according to the Denver papillae protocol.[13] The interrater coefficient was 0.914 (95% confidence interval 0.862–0.946; $p < 0.001$), indicating excellent reliability. The mean score of both counts was calculated and used to quantify TBD in further analysis.

Five to eight fungiform papillae were removed from the dorsal surface of the anterior tongue by trained dentists at the Department of Prosthodontics and Materials Science at the University Clinic of Leipzig following a standardized protocol described in greater detail elsewhere.[14] Briefly, participants were asked to refrain from taking painkillers, food supplements and spices known to potentially enhance bleeding risk, for 2 weeks prior the procedure. On the day of papillae removal, participants were seated in a dental chair and local

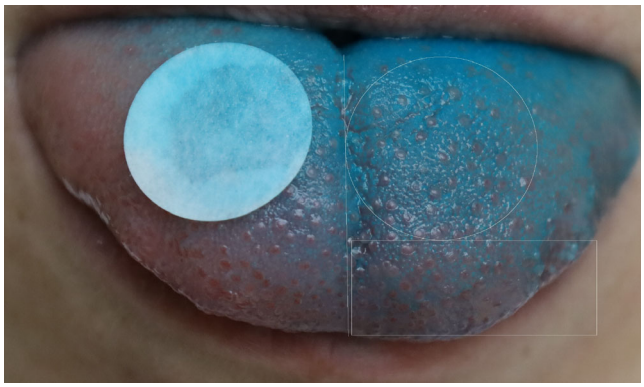

FIGURE 2 Evaluation of taste bud density (TBD). Papillae density is calculated using Image J software according to the Denver Papillae Protocol by analazing a reference area (shown as circle) obtained using a standardized procedure.[40]

anaesthesia was applied upon request, using Xylocain Pumpspray dental with 10 mg lidocaine per use. Before papillae removal, a thorough inspection of the oral cavity was performed to assess general tissue health and includes dental status and standardized assessment of periodontitis using a dental probe to perform the periodontal screening. The dentist held the anterior quarter of the tongue as it extended from the oral cavity, and sterile, curved spring micro-scissors (Fine Science Tools, Europe) were used to remove fungiform papillae. Any capillary bleeding was treated by blotting with sterile gauze and usually stopped within minutes. The removed fungiform papillae were placed directly or via a dental probe (Fine Science Tools, Europe), without pinching the papilla, into RNAlater (Sigma-Aldrich) to protect nucleic acids from degradation at room temperature. Samples were washed with RNase free water before storage at −80°C and will ultimately be subjected to either RNAsequencing or epigenetic analyses. In addition, samples from a limited number of participants will be placed in isolation buffer (26 mM NaHCO3, 2.5 mM NaH2PO4, 20 mM Glucose, 65 mM NaCl, 20 mM KCL, 1 mM EDTA) instead of RNAlater for the immediate preparation of primary cell cultures according to the protocol described elsewhere.[13] The procedure of papillae removal takes 10–15 min. After papillae removal the participant remains in the chair for visual monitoring by trained staff for 15 min. There is no noticeable effect on taste perception and new papillae are formed within 3–5 weeks and regain full function.[14] This procedure removes, at most, 20 to 25 of the approximately 5000 taste buds in humans (3.5 to 4 taste buds per fungiform papillae) or 0.4%. Removing this tissue is not usually perceived as painful. Occasionally mild discomfort is reported which lasts less than a day. Participants were instructed about after care for the rest of the day to avoid infections or inflammation. This included the avoidance of very hot/cold and very spicy foods, strongly carbonated water, as well as mechanically touching the site of sample collection.

3.7 | Saliva, tongue swap, and stool sampling

Saliva samples and tongue swaps were collected in fasted participants, who were further asked to refrain from drinking, smoking, chewing gum, or brushing their teeth for at least 30 min prior to sampling. To eliminate any acute oral pathologies, a brief inspection of the oral cavity is performed before samples are collected. To obtain a sample of the tongues surface for microbiome analysis a sterile swap is used, which was immediately stabilized in DNA/RNA Shield (Zymo Research). To collect saliva, the participants are asked to passively drool into tubes, one of which contains stabilizing fluid, for microbiome analysis (DNA Genotek Inc.). A total of 5 mL of saliva is collected. Samples are stored either on ice or at room temperature (microbiome samples) before further pre-processing and final storage at −80°C until analyses. Samples will be used for hormone and protein analysis, the isolation of extracellular vesicles, the extraction of DNA for genetic screening, and microbiome analysis. Saliva pH is measured after samples reached room temperature and using indicator paper (pH-Fix, Whatman, VWR). A cell count is performed using a burker chamber.

On Day 1, participants are further provided with the materials needed to collect a stool sample (DNA/RNA Shield Faecal Collection Tubes, Zymo research). The stool sample is to be returned on the second visit and stored at −80°C after pre-processing and until further use.

3.8 | Statistical analysis

Statistical analyses were carried out using SPSS version 27 (IBM, Ehningen, Germany). Descriptive analysis included proportions (%) for categorical variables and median values (25th and 75th percentiles) for continuous variables with non-Gaussian distribution, after the Kolmogorov–Smirnov test was applied to test for Gaussian distribution. The Mann–Whitney *U*-test, the chi-squared or Fisher's exact test and Student's *t*-tests were used to analyse group differences. Correlations were assessed using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient if not normally distributed. The calculation of interrater coefficient for TBD assessment was based on mean-rating (k = 2), consistency and a two-way mixed-effects model.[15] A p value of <0.05 was considered statistically significant.

4 | RESULTS: FINDINGS TO DATE

The characteristics of the study population recruited between January 2020 and December 2022 are summarized in Table 4, stratified by weight status. Participants were mostly women (63.8%), which is a self-selection bias commonly found in human research and comparable with other European cohorts.[16–19] Nevertheless, there were no gender differences between participants with overweight/obesity and participants with normal weight. Although this does not reflect the occurrence of overweight and obesity in the German population, in which men are more overweight or obese than women,[16] this study does not claim to represent the German population as it was not designed as a population-based study. Nevertheless, the similar gender disparities in the two subgroups provides a suitable sample to investigate the study aims outlined above. The mean age was 36 years, with age ranging from 19 to 68 years. The relatively young median age most likely results from a higher willingness of particularly young individuals to participate in studies, which are usually time-consuming, as in this case where participants had to visit the study facility twice. Similarly, our sample was characterized by a high percentage of individuals with a high educational level, which may lead to greater motivation to participate in studies. Significant differences in age between lean participants and those with overweight and obesity appear to be the result of a higher percentage of students within the lean control group, given that recruitment was closely tied to the University Hospital of Leipzig. The majority of participants reported that

TABLE 4 Baseline characteristics of the study population: participants recruited between 2020 and 2022, stratified by weight status.

Characteristic	Total cohort ($N = 105$)	Overweight + obese ($N = 62$)	Normal weight ($N = 43$)	*p* value
Age (years)	36 (26.5 / 48.5)	41 (30 / 50.5)	30 (24 / 46)	**0.007**
Age range (years)	19–68	19–68	20–67	
Sex: female | male (%)	63.8 | 36.2	61.3 | 38.7	67.4 | 32.6	0.519
Nationality				0.402
German (%)	78.1	71	88.4	
Others (%)	3.8	4.8	2.3	
n.r. (%)	18.1	24.2	9.3	
Marital status				0.623
Single (%)	52.4	43.5	65.1	
Married | registered partnership | living together (%)	21.9	25.8	16.3	
Divorced (%)	6.7	6.5	7	
n.r. (%)	19	24.2	11.6	
Education				0.173
Up to class 8 (%)	1.9	3.2	0	
Junior high school (%)	7.6	9.7	4.7	
Up to class 10 (%)	13.3	16.1	9.3	
Advanced technical college (%)	2.9	1.6	4.7	
Secondary school examination (%)	56.2	45.2	72.1	
n.r. (%)	18.1	24.2	9.3	
Employment				**0.004**
Currently employed (%)	67.6	69.4	65.1	
Currently not employed (%)	1	1.6	0	
n.r. (%)	21	27.4	11.6	
Student (%)	10.5	1.6	23.3	**0.001**
Dentist visit frequency				0.059
Once in 6 month (%)	29.5	33.9	23.3	
Once a year (%)	49.5	40.3	62.8	
Less than once a year (%)	1.9	1.6	2.3	
Only in case of acute dental pain (%)	1	0	2.3	
n.r. (%)	18.1	24.4	9.3	
Special eating habits				
Vegetarian: yes | no | n.r. (%)	14.3 | 82.9 | 2.9	6.5 | 88.7 | 4.8	25.6 | 74.4 | 0	**0.008**
Vegan: yes | no | n.r. (%)	6.7 | 90.5 | 2.9	1.6 | 93.5 | 4.8	14.0 | 86.0 | 0	**0.016**
Current dieting: yes | no | n.r. (%)	12.4 | 78.1 | 9.5	19.4 | 64.5 | 16.1	2.3 | 97.7 | 0	**0.003**
Smoking: yes | no | n.r. (%)	10 |5 | 80 | 9.5	11.4 | 72.5 | 16.1	9.3 | 90.7	0.528
Alcohol consumption				0.267
Never (%)	4.8	0	2.3	
Once a month (%)	13.3	6.5	14	
2–4 times a month (%)	28.6	12.9	46.5	
2–3 times per week (%)	18.1	16.1	20.9	
≥ 4 times per week (%)	1.9	16.1	4.7	
n.r. (%)	33.3	48.4	11.6	

Note: Data are presented as median (25th / 75th percentile) or in percent (%). *p* values were calculated using the Mann–Whitney *U*-test, *t*-test or chi-squared test. *p* values <0.05 were considered as statistically significant and are highlighted in bold. Smoking was defined as smoking at least one cigarette per day. 9.5% of all participants did not state their smoking habit.

Abbreviations: n.r., no response; *N*, number.

they were currently employed and were single. With regard to smoking and alcohol consumption, both of which have been linked to taste perception in previous studies, only a few individuals reported current smoking on a regular basis, and there were no differences between individuals with overweight or obesity and normal weight.[20–22] Similarly, no group differences were found for alcohol consumption. Further, individuals with overweight or obesity and normal weight did not show significant differences with regard to frequency of dentist visits. Interestingly, normal-weight individuals significantly more often reported eating a vegan or vegetarian diet than individuals with overweight or obesity, who in turn reported current dieting significantly more often. As the study was conducted in Germany, most participants were of German nationality and only a minority reported a different nationality.

4.1 | Association analyses of BMI groups with clinical and metabolic parameters

Given the stratification by BMI, the overweight and obesity subgroup displayed a significantly higher body weight, waist and hip circumference, higher percentage of fat mass and significantly greater waist-to-hip ratio than the lean subgroup. As expected, individuals with overweight or obesity had a less favourable metabolic profile. Thus, they showed significantly increased triglycerides, low-density lipoprotein (LDL) cholesterol, HbA1c, FPG and insulin, despite a significantly lower HDL cholesterol level compared to the control group. Significantly higher systolic BP became evident in the subsample of individuals with overweight or obesity. This is reflected in the higher percentage of diagnosed hypertension (40.3%) and type 2 diabetes mellitus cases (11.3%) in the overweight and obesity subgroup compared to lean individuals (23.3% [$p = 0.068$] and 0% [$p = 0.023$], respectively). However, only one participant met the criteria for metabolic syndrome. Results are summarized in Table 5.

4.2 | Association analyses of BMI groups with TBD and taste and smell perception

Participants showed a median TBD of 26.8 taste buds within the standardized area of the tongue surface, which is in line with previous findings in comparable samples.[23,24] However, we did not find significant differences between lean and overweight or obese individuals in this sample consisting of the first 105 participants of the OTB study (data not shown). Although Kaufman et al. showed that a reduced number of taste buds was associated with increased neck circumference as a marker of human obesity, this result is in line with the study by Archer et al. who reported no differences in TBD between obesity and normal weight groups.[23,25] These diverging results might be a consequence of a wide variance of TBD in general and decreased statistical power due to sample size.

We further addressed the prevalence of participants among functional categories of gustation and olfaction within the total cohort but also in the lean and the overweight/obese subgroup. As expected, most participants presented normogeusia and normosmia. We could not identify differences in the distribution of ageusia, hypo- or

TABLE 5 Main clinical and metabolic parameters.

Clinical/ metabolic parameter	*N*	Total cohort	*N*	Overweight + obese	*N*	Normal weight	*p* value
Height (m)	105	1.71 (164 / 1.77)	62	1.71 (1.64 / 1.76)	43	1.73 (1.64 / 1.79)	0.588
Weight (kg)	105	80.0 (64.9 / 101.6)	62	91.7 (82.8 / 113.4)	43	63.2 (57.9 / 75.3)	**<0.001**
BMI (kg/m^2)	105	26.7 (22.7 / 34.3)	62	31.1 (27.8 / 37.6)	43	22.3 (20.8 / 23.5)	**<0.001**
Waist (cm)	105	90 (75.0 / 103.8)	62	100.8 (89.8 / 116.3)	43	75.0 (71.0 / 82.0)	**<0.001**
Hip (cm)	105	102 (91.3 / 114.0)	62	110.3 (102.8 / 126.0)	43	91.0 (87.0 / 95.0)	**<0.001**
WHR	105	0.86 (0.80 / 0.93)	62	0.88 (0.82 / 0.93)	43	0.82 (0.77 / 0.88)	**0.006**
Fat mass (%)	85	50.9 (45.60 / 62.20)	45	34.7 (23.8 / 53.5)	40	15.2 (12.2 / 17.9)	**<0.001**
Systolic BP (mmHg)	56	120 (113 / 130)	41	122 (115 / 132)	15	114 (107 / 122)	**0.028**
Diastolic BP (mmHg)	56	79.5 (74 / 85)	41	80 (76 / 86)	15	76 (67 / 81)	0.064
FPG (mmol/mol)	65	4.62 (4.25 / 4.8)	25	4.7 (4.5 / 5.0)	40	4.4 (4.1 / 4.7)	**0.005**
FPI (pmol/L)	72	40.9 (28.6 / 84.7)	47	70.8 (35.3 / 110.0)	25	28.3 (22.8 / 39.1)	**<0.001**
HbA1c (%)	101	5.5 (5.2 / 5.8)	59	5.5 (5.2 / 5.8)	42	5.4 (5.2 / 5.7)	**0.047**
Total cholesterol (mmol/L)	74	4.7 (4.0 / 5.7)	49	4.8 (4.3 / 5.7)	25	4.2 (3.8 / 5.6)	0.102
HDL cholesterol (mmol/L)	74	1.5 (1.2 / 1.9)	49	1.4 (1.2 / 1.7)	25	1.9 (1.5 / 2.2)	**<0.001**
LDL cholesterol (mmol/L)	74	2.9 (2.1 / 3.7)	49	3.0 (2.7 / 4.0)	25	2.3 (1.9 / 3.6)	**0.006**
Triglycerides (mmol/L)	74	1.0 (0.8 / 1.6)	49	1.2 (1.0 / 1.8)	25	0.8 (0.7 / 1.0)	**<0.001**

Note: Data are presented as median (25th / 75th percentile). A p <0.05 were considered statistically significant and were calculated using the Mann–Whitney test.

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; *N*, number; WHR, waist-to-hip ratio.

normogeusia for the taste sum score nor in single taste qualities between the BMI groups. The same held true for hyper-, hypo- or normosmia, where no differences in case distribution among lean and overweight/obese groups were obtained. Data are presented in Figure 3.

4.3 | Associations of TBD, taste, and olfactory function with taste perception and clinical and metabolic parameters

In the first recruited subcohort, exploratory analysis showed that TBD did not translate into taste perception of the basic taste qualities, as no association was found between TBD and taste perception of any of the taste qualities (Table 6). This indicates that qualitative rather than quantitative factors are of relevance in the complex process of perceiving taste. It further shifts the focus towards a deeper understanding of taste bud cell physiology, which can be achieved by analysing transcriptomic or (epi)genetic data of taste cells. This approach may help to elucidate how obesity potentially affects the complex sense of taste. However, age was previously found to negatively affect taste function, although a loss of taste buds is less important than the reduced functionality of signal transduction within taste cells. In the current subcohort, TBD decreased with age, but no associations between age and taste perception were found (Table 6 and Figure 4).[26–29] Likewise, TBD was negatively correlated with HbA1c level, although this did not withstand adjustment for age (Figure 4). As several studies point towards an association between diabetes and poorer quality of taste perception,[30–32] there is a need not only for a larger sample set to confirm these preliminary results, but also for more in-depth data analyses including more aspects of taste bud biology as functionality seems at least partially to be independent of TBD. Diverse metabolic parameters were found to be associated with perception of differential taste qualities in the present preliminary cohort.

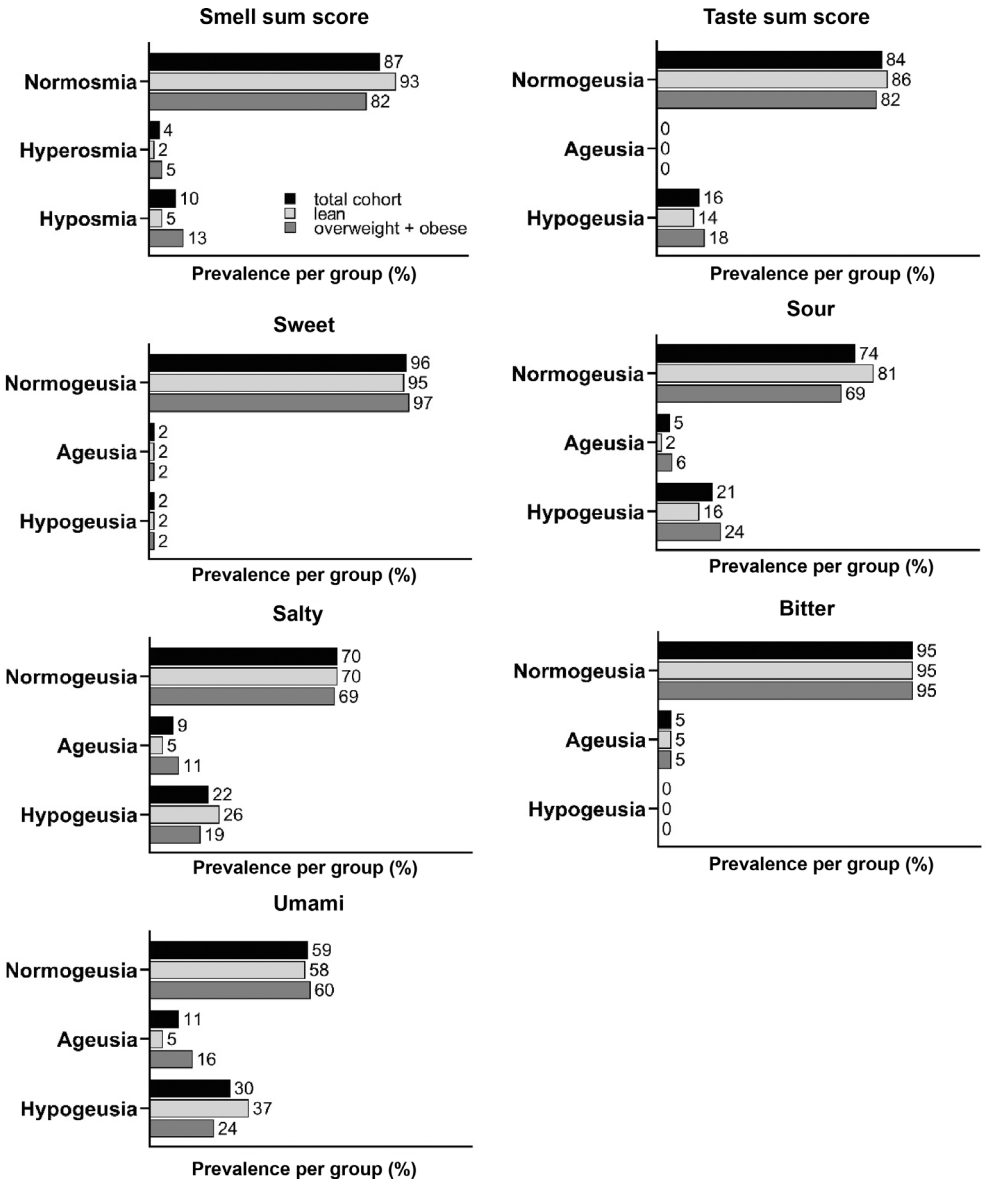

FIGURE 3 Prevalence of gustatory and olfactory performance of study participants. Presented is the group prevalence of taste and smell function within the total cohort ($N = 105$) and stratified by BMI (lean $N = 43$, overweight/obese $N = 62$). A chi-squared test or Fisher's exact test was used to address group differences while a p value <0.05 was taken to indicate statistical significance.

TABLE 6 Exploratory analyses of taste and smell perception with clinical and metabolic factors.

Clinical/metabolic parameter	Sweet	Sour	Salty	Bitter	Umami	Taste sum score	Smell sum score
Age (years)	−0.120 [0.230]	−0.085 [0.397]	−0.119 [0.233]	−0.123 [0.218]	−0.041 [0.682]	−0.152 [0.128]	**−0.216 [0.029]**
Sex	−0.063 [0.528]	−0.047 [0.641]	0.039 [0.697]	−0.189 [0.058]	0.010 [0.920]	−0.121 [0.226]	0.169 [0.089]
Weight (kg)	−0.018 [0.858]	−0.146 [0.144]	−0.072 [0.473]	**−0.255 [0.01]**	−0.063 [0.531]	−0.189 [0.058]	0.098 [0.327]
BMI (kg/m^2)	0.005 [0.957]	−0.153 [0.126]	−0.062 [0.534]	**−0.229 [0.020]**	−0.046 [0.644]	−0.161 [0.106]	0.025 [0.799]
Waist (cm)	0.023 [0.816]	−0.094 [0.348]	−0.026 [0.796]	−0.170 [0.088]	0.030 [0.765]	−0.091 [0.362]	0.036 [0.717]
Hip (cm)	0.031 [0.755]	−0.088 [0.377]	−0.044 [0.663]	−0.158 [0.114]	0.012 [0.908]	−0.09 [0.371]	0.021 [0.831]
WHR	0.006 [0.956]	−0.050 [0.616]	0.087 [0.385]	−0.121 [0.224]	0.030 [0.765]	−0.016 [0.869]	0.049 [0.625]
Fat mass (%)	0.014 [0.899]	**−0.218 [0.049]**	−0.057 [0.610]	−0.114 [0.306]	0.044 [0.697]	−0.140 [0.210]	−0.060 [0.590]
Systolic BP (mmHg)	0.013 [0.926]	**−0.365 [0.007]**	−0.067 [0.634]	−0.115 [0.413]	0.126 [0.367]	−0.223 [0.109]	−0.067 [0.633]
Diastolic BP (mmHg)	0.058 [0.678]	**−0.254 [0.067]**	0.026 [0.851]	0.025 [0.858]	−0.064 [0.65]	−0.056 [0.692]	−0.098 [0.483]
FPG (mmol/mol)	0.011 [0.935]	−0.100 [0.437]	−0.063 [0.627]	0.061 [0.639]	−0.013 [0.920]	−0.006 [0.965]	−0.034 [0.793]
FPI (pmol/L)	−0.104 [0.396]	−0.039 [0.749]	0.034 [0.784]	−0.127 [0.297]	−0.046 [0.705]	−0.031 [0.802]	0.073 [0.553]
HbA1c (%)	**−0.286 [0.004]**	−0.136 [0.18]	−0.018 [0.861]	−0.183 [0.071]	−0.2 [0.048]	−0.175 [0.084]	−0.031 [0.764]
Total cholesterol (mmol/L)	**−0.395 [0.001]**	−0.047 [0.696]	−0.110 [0.363]	−0.213 [0074]	−0.067 [0.578]	−0.204 [0.089]	−0.226 [0.058]
HDL (mmol/L)	0.032 [0.788]	0.134 [0.265]	−0.018 [0.883]	0.232 [0.052]	0.041 [0.735]	0.130 [0.278]	**−0.422 [<0.001]**
LDL (mmol/L)	**−0.453 [<0.001]**	−0.064 [0.598]	−0.091 [0.450]	**−0.284 [0.016]**	−0.084 [0.484]	**−0.236 [0.047]**	−0.156 [0.195]
Triglycerides (mmol/L)	−0.211 [0.078]	−0.089 [0.461]	0.118 [0.327]	−0.134 [0.267]	−0.100 [0.408]	−0.018 [0.880]	−0.007 [0.951]
Alcohol intake	−0.057 [0.640]	0.207 [0.085]	0.085 [0.485]	0.069 [0.572]	0.049 [0.687]	0.115 [0.344]	−0.064 [0.598]
Smoking	−0.157 [0.135]	0.011 [0.916]	0.009 [0.932]	−0.048 [0.651]	−0.169 [0.108]	−0.018 [0.866]	0.129 [0.222]
Current dieting	0.118 [0.262]	0.180 [0.087]	0.11 [0.294]	0.197 [0.060]	**0.212 [0.042]**	0.192 [0.067]	0.076 [0.473]
Vegan diet	0.030 [0.767]	**0.235 [0.019]**	−0.082 [0.422]	0.042 [0.677]	0.067 [0.512]	0.080 [0.432]	−0.041 [0.685]
Vegetarian diet	0.006 [0.952]	0.132 [0.192]	0.031 [0.758]	0.177 [0.248]	−0.043 [0.669]	0.124 [0.221]	0.072 [0.478]
Number of dentist visit	−0.056 [0.609]	−0.043 [0.692]	−0.077 [0.483]	−0.015 [0.891]	−0.017 [0.873]	−0.090 [0.411]	0.184 [0.090]
TBD	0.072 [0.481]	0.092 [0.372]	0.018 [0.857]	0.165 [0.107]	−0.027 [0.796]	0.154 [0.132]	0.054 [0.602]

Note: Data are presented as ß [*p*-value] and were calculated using Spearman's rank correlation coefficient. *p* values <0.05 were considered as statistically significant and are highlighted in bold. Alcohol intake was defined as never, once per month, 2–4 times per month, 2–3 times per week or ≥4 times per week. Smoking (yes, if at least 1 cigarette per day), current dieting, vegan and vegetarian diet were defined as yes, no or no response.
Abbreviations: BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; *N*, number; TBD, taste bud density; WHR, waist to hip ratio.

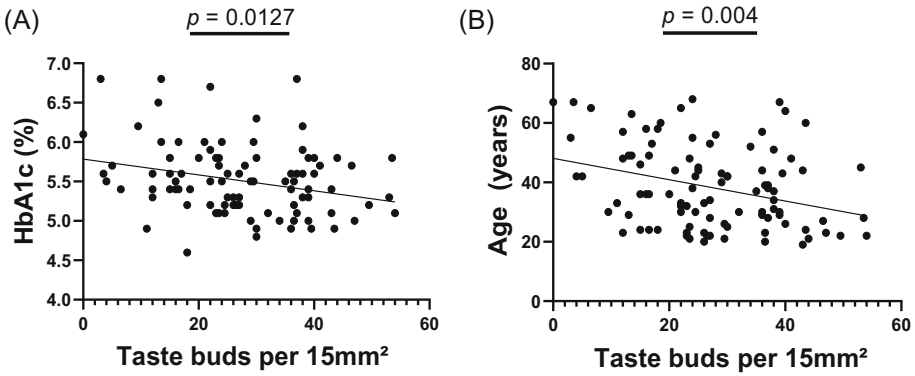

FIGURE 4 Correlation of glycated haemoglobin (HbA1c) and age with taste bud density. Data were analysed using spearman's rank correlation coefficient and presented without adjustment for confounders age, sex, and body mass index.

Thus, HbA1c, total cholesterol and LDL cholesterol showed inverse associations with different taste qualities. These exploratory results might indicate that a less favourable metabolic state is associated with reduced taste perception. Interestingly, current dieting and a vegan diet were positively associated with taste perception of differential taste qualities, suggesting that diet per se impacts taste perception. This is in line with previous findings showing that a vegetarian diet is associated with an increased sensitivity towards bitter taste as compared with other diets.[33] Moreover it was shown that metabolic syndrome is associated with salty taste perception and that a lower sensitivity to salty taste is associated with a Mediterranean diet.[34] Beside the basic taste qualities, a study by Liu et al. showed that the consumption of a low-fat diet decreased the threshold for detection of oleaic acid in lean subjects, while no change in obese individuals was shown.[35] These results indicate, on the one hand, that taste perception is indeed associated with a person's metabolic state and type of diet, while, on the other hand, a profound understanding of complex taste qualities is necessary rather than a focus only on basic taste qualities. Further behavioural variables, such as smoking, alcohol consumption or frequency of dentist visits, were not associated with taste perception, which contrasts with previous results in which smoking was associated with a reduced sucrose detection threshold in women and current smokers reported lower bitter and salt intensities on the tip of their tongue.[36,37] Because no effects of smoking and alcohol consumption were found in the current subpopulation regarding taste perception, it might be of interest to understand the impact of dosage of alcohol consumption and frequency of smoking. In addition to taste perception, the current preliminary analyses suggest an association not only between smell sum score and age but also with HDL cholesterol level (Table 6). Indeed, it is reported that elderly people lose their sense of smell and also present limited odour discrimination abilities.[38] Moreover, an association between total cholesterol level and smell and taste dysfunction was shown in a Chinese cohort, despite several studies pointing towards metabolic alterations being linked with smell dysfunction.[39] Future analyses in the complete sample set of the OTB cohort are necessary to address these questions more closely and with respect to further data obtained. Hence, the current data provide new hypotheses which need to be proven in the final dataset and in further, larger cohorts. Results are summarized in Table 6.

4.4 | Strength and limitations

Strengths of the OTB study include its unique human cohort sample of subjects with normal weight and obesity, where a broad range of measures allow a complex investigation of taste perception and alterations in taste bud homeostasis in obesity. A wide range of omics data from diverse biomaterial, such as taste buds, but also blood, saliva and stool, as well as physiological and psychological aspects are included. Further MRI data are available for a subsample. This wide range of data allows us to investigate the association of taste perception with eating behaviour at different levels of processing, from taste cell function to cognitive evaluation. This thorough data assessment involving taste and eating behaviour may contribute to a deeper understanding of the role of taste in food preference and eating habits in humans. In addition, extensive control of potential confounding variables was achieved through the application of strict inclusion and exclusion criteria and the endeavour to minimize measurement errors.

A limitation arises from potential selection bias due to advertising the study within the campus of the University Hospital of Leipzig, which resulted in a high percentage of students, who are enrolled in health-associated education programmes, comprising the lean control group in this study. Therefore, participants of the control group are relatively young, have a higher educational level and are interested in health behaviours, which may impact their lifestyle, for example, choosing a healthy diet. However, the proximity to the obesity treatment facilities of the University Clinic made it possible to broaden the study population and include participants with a wide range of BMI. In addition, the OTB study only includes people living in Germany and therefore does not represent all ethnicities. Furthermore, we did not assess actual food intake and solely relied on self-reported eating habits, which may not accurately reflect a person's eating behaviour. In addition, in terms of human cohort studies, a relatively small sample size will limit the power of analyses and may result in a potential loss of information or overestimation of results obtained. However, to date, research involving the deep phenotyping and characterization of a study population with regard to taste bud biology, taste sensation, and eating behaviour, especially in obesity, is scarce; therefore, this study will help to fill the gap in knowledge about alterations of taste sensation among people with obesity.

5 | DISCUSSION AND OUTLOOK

To date, 105 participants have been recruited within the OTB study and were phenotyped and characterized with regard to physiological and psychological factors of taste perception and eating behaviour.

Further participants will be enrolled, and the analyses presented here will be expanded by data acquisition on two primary outcome measures, the first of which is identification of differential gene and protein regulation in isolated human taste cells in normal weight and obese individuals by obtaining transcriptomics data using RNA sequencing. The combination of these data with epigenomics data derived from isolated papillae fungiformes will broaden our understanding of taste cell gene regulation and potential alterations in obesity. However, because the limited amount of taste bud biomaterial does not allow paired analyses, subgroups will be defined and subjected to either RNA sequencing or epigenetic analyses. The subgroups will be defined to allow the comparison of different BMI groups. Additionally, transcriptomic and epigenomic data will be analysed in the blood of all study participants, further strengthening our understanding of taste cell gene regulation. The combination of omics data is of special interest due to the missing link between TBD and taste sensation seen so far in the first participants of the OTB study. This missing link may point to the need to understand how qualitative rather than quantitative analyses of taste buds might be more relevant to unravel mechanisms of taste perception in obesity. In addition, among all participants, further analyses of not only blood, but also the saliva level of adipokines and cytokines for instance, allow a broad view of the metabolic feedback to taste cells. Analyses of in vitro primary cell cultures from taste cell biopsy are planned for a third limited subset of study participants and will shed further light on physiological mechanisms influencing taste perception. The second primary outcome is the identification of associations between taste cell biology and not only parameters of the presented interventions but also omics data, which will help us understand the complexity of eating behaviour and taste alteration in obesity.

In addition, two secondary outcome measures will be addressed. The first is the assessment of differences in salivary, tongue, and gut microbiome in obesity in contrast to lean individuals, which will be obtained by 16S ribosomal RNA sequencing and, with the combination of further data obtained, will allow us to draw conclusions on potential influences on taste cell homeostasis, taste perception, and eating patterns. The composition of the oral microbiome and metabolomics might be a candidate implicated in the association found in the preliminary dataset between frequency of dentists visits and TBD. Therefore, a closer look at dental health, which was assessed as a PSR score, and a thorough oral inspection by the dentist, will be included in the future analysis of the final sample size. The second is the evaluation of the content of extracellular vesicles from saliva samples, which will be analysed and compared between the study groups as well as related to other parameters of this study. Extracellular vesicles may directly sense nutritional signals from the oral cavity towards the brain and therefore might be involved in central eating regulation, while their origin contributes to our understanding of cell–cell communication. Analyses of extracellular vesicles may allow us to identify further factors of relevance for taste cell homeostasis, which may translate into taste perception and eating behaviour. We plan to analyse these secondary endpoints in the total cohort.

To further investigate the effect of weight loss on taste perception, longitudinal approaches are currently being established including participants who underwent bariatric surgery or completed a conservative weight loss programme. Data collection, as described above, occurs before bariatric surgery and at 6 months, 1 year and 2 years after bariatric surgery. This approach also includes the investigation of visceral and subcutaneous adipose tissue samples, which are collected during bariatric surgery if study subjects are also participating in the Leipzig Obese BioBank cohort (approval no: 159-12-21052012). This allows a closer investigation of potential effects of adipose tissue derived hormones or inflammatory parameters on taste perception or the potential effects the oral microbiome exerts on extraoral sides with fat tissue, in particular.

In summary, the OTB study includes a broad range of data (transcriptomics, [epi]genomics, proteomics, microbiome data) derived from diverse biomaterial (taste buds, blood, stool, saliva) which will be analysed with state-of-the-art applications, such as next-generation sequencing, and combined with physiological (e.g., blood markers, anthropometrics, taste, and smell screening, medical history, brain MRI) and psychological (e.g., overall health, lifestyle, and eating questionnaires) factors, allowing deep characterization of taste bud homeostasis, taste perception and eating behaviour in obesity and related comorbidities.

AUTHOR CONTRIBUTIONS

Kerstin Rohde-Zimmermann and Imke Schamarek designed the study, enrolled participants, acquired the data and wrote the manuscript. Imke Schamarek and Alexander Kersten performed data analyses and wrote the draft of the manuscript. Andrea Lorenz, Cita Nottmeier, Michael Schmidt, Anuschka Roesner, Sebastian Hahnel and Till Koehne are responsible for dental screening and taste bud biopsy. Florian Christoph Richter supported in the recruitment process. Kristin Röhrborn was involved in sample preparation. A. Veronica Witte supported with performing brain MRI imaging. Matthias Blüher and Michael Stumvoll supported study setup. All authors contributed to discussions and finalizing the manuscript draft.

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CONFLICT OF INTEREST STATEMENT

Matthias Blüher has received honoraria as a consultant and speaker from Amgen, AstraZeneca, Bayer, Boehringer-Ingelheim, Daiich-Sankyo, Lilly, Novo Nordisk, Novartis, Pfizer and Sanofi. The remaining authors have no conflict of interest to declare.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15563.

DATA AVAILABILITY STATEMENT

The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Blüher M. Adipose tissue inflammation: a cause or consequence of obesity-related insulin resistance? *Clin Sci (Lond)*. 2016;130(18):1603-1614.
2. Rohde K, Schamarek I, Blüher M. Consequences of obesity on the sense of taste: taste buds as treatment targets? *Diabetes Metab J*. 2020;44(4):509-528.
3. Lin F, Liu Y, Rudeski-Rohr T, Dahir N, Calder A, Gilbertson TA. Adiponectin enhances fatty acid signaling in human taste cells by increasing surface expression of CD36. *Int J Mol Sci*. 2023;24(6):5801.
4. Yoshida R, Noguchi K, Shigemura N, et al. Leptin suppresses mouse taste cell responses to sweet compounds. *Diabetes*. 2015;64(11):3751-3762.
5. Crosson SM, Marques A, Dib P, Dotson CD, Munger SD, Zolotukhin S. Taste receptor cells in mice express receptors for the hormone adiponectin. *Chem Senses*. 2019;44(6):409-422.
6. Schamarek I, Anders L, Chakaroun RM, Kovacs P, Rohde-Zimmermann K. The role of the oral microbiome in obesity and metabolic disease: potential systemic implications and effects on taste perception. *Nutr J*. 2023;22(1):28.
7. LimeSurvey GmbH. LimeSurvey: an open source survey tool/LimeSurvey GmbH, Hamburg, Germany. http://www.limesurvey.org
8. Medawar E, Beyer F, Thieleking R, et al. Prebiotic diet changes neural correlates of food decision-making in overweight adults: a randomised controlled within-subject cross-over trial. *Gut*. 2024;73(2):298-310.
9. https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle-who-recommendations.
10. American Diabetes Association Professional Practice Committee. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2022. *Diabetes Care*. 2022;45(Suppl 1):S17-S38.
11. Alberti KGMM, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120(16):1640-1645.
12. Oleszkiewicz A, Schriever VA, Croy I, Hähner A, Hummel T. Updated Sniffin' sticks normative data based on an extended sample of 9139 subjects. *Eur Arch Otorhinolaryngol*. 2019;276(3):719-728.
13. Ozdener MH, Rawson NE. Primary culture of mammalian taste epithelium. *Methods Mol Biol*. 2013;945:95-107.
14. Spielman AI, Pepino MY, Feldman R, Brand JG. Technique to collect fungiform (taste) papillae from human tongue. *J Vis Exp*. 2010;42:2201. doi:10.3791/2201
15. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med*. 2016;15(2):155-163.
16. Schienkiewitz A, Kuhnert R, Blume M, Mensink GBM. Overweight and obesity among adults in Germany—results from GEDA 2019/-2020-EHIS. *J Health Monit*. 2022;7(3):21-28.
17. Szendroedi J, Saxena A, Weber KS, et al. Cohort profile: the German Diabetes Study (GDS). *Cardiovasc Diabetol*. 2016;15:59.
18. Reedijk M, Lenters V, Slottje P, et al. Cohort profile: LIFEWORK, a prospective cohort study on occupational and environmental risk factors and health in The Netherlands. *BMJ Open*. 2018;8(2):e018504.
19. Locke AE, Kahali B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197-206.
20. Risso D, Drayna D, Morini G. Alteration, reduction and taste loss: main causes and potential implications on dietary habits. *Nutrients*. 2020;12(11):3284.
21. Nyberg ST, Heikkilä K, Fransson EI, et al. Job strain in relation to body mass index: pooled analysis of 160 000 adults from 13 cohort studies. *J Intern Med*. 2012;272(1):65-73.
22. Kurshed AAM, Ádány R, Diószegi J. The impact of taste preference-related gene polymorphisms on alcohol consumption behavior: a systematic review. *Int J Mol Sci*. 2022;23(24):15989.
23. Kaufman A, Kim J, Noel C, Dando R. Taste loss with obesity in mice and men. *Int J Obes (Lond)*. 2020;44(3):739-743.
24. Miller IJ. Human taste bud density across adult age groups. *J Gerontol*. 1988;43(1):B26-B30.
25. Archer N, Shaw J, Cochet-Broch M, et al. Obesity is associated with altered gene expression in human tastebuds. *Int J Obes (Lond)*. 2019;43(7):1475-1484.
26. Barragán R, Coltell O, Portolés O, et al. Bitter, sweet, salty, sour, and umami taste perception decreases with age: sex-specific analysis, modulation by genetic variants and taste-preference associations in 18 to 80 year-old subjects. *Nutrients*. 2018;10(10):1539.
27. Pavlidis P, Gouveris H, Anogeianaki A, Koutsonikolas D, Anogianakis G, Kekes G. Age-related changes in electrogustometry thresholds, tongue tip vascularization, density, and form of the fungiform papillae in humans. *Chem Senses*. 2013;38(1):35-43.
28. Takeuchi K, Yoshii K, Ohtubo Y. Age-related electrophysiological changes in mouse taste receptor cells. *Exp Physiol*. 2021;106(2):519-531.
29. Shin Y-K, Cong W-n, Cai H, et al. Age-related changes in mouse taste bud morphology, hormone expression, and taste responsivity. *J Gerontol A Biol Sci Med Sci*. 2012;67(4):336-344.
30. Rohani B. Oral manifestations in patients with diabetes mellitus. *World J Diabetes*. 2019;10(9):485-489.
31. Bhandare NN, Keny MS, Nevrekar RP, Bhandare PN. Diabetic tongue—could it be a diagnostic criterion? *J Family Med Prim Care*. 2014;3(3):290-291.

32. Hardy SL, Brennand CP, Wyse BW. Taste thresholds of individuals with diabetes mellitus and of control subjects. *J Am Diet Assoc*. 1981;79(3):286-289.
33. Jalil Mozhdehi F, Abeywickrema S, Bremer PJ, Peng M. Comparing taste detection thresholds across individuals following vegan, vegetarian, or omnivore diets. *Foods*. 2021;10(11):2704.
34. Veček NN, Mucalo L, Dragun R, et al. The association between salt taste perception, mediterranean diet and metabolic syndrome: a cross-sectional study. *Nutrients*. 2020;12(4):1164.
35. Liu D, Archer N, Duesing K, Hannan G, Keast R. Mechanism of fat taste perception: association with diet and obesity. *Prog Lipid Res*. 2016;63:41-49.
36. Pepino MY, Mennella JA. Effects of cigarette smoking and family history of alcoholism on sweet taste perception and food cravings in women. *Alcohol Clin Exp Res*. 2007;31(11):1891-1899.
37. Berube L, Duffy VB, Hayes JE, Hoffman HJ, Rawal S. Associations between chronic cigarette smoking and taste function: results from the 2013-2014 national health and nutrition examination survey. *Physiol Behav*. 2021;240:113554.
38. Boyce JM, Shone GR. Effects of ageing on smell and taste. *Postgrad Med J*. 2006;82(966):239-241.
39. Huang Z, Huang S, Cong H, et al. Smell and taste dysfunction is associated with higher serum total cholesterol concentrations in Chinese adults. *J Nutr*. 2017;147(8):1546-1551.
40. Nuessle TM, Garneau NL, Sloan MM, Santorico SA. Denver papillae protocol for objective analysis of fungiform papillae. *J Vis Exp*. 2015;100:e52860.
41. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*. 1985;29(1):71-83.
42. de Lauzon B, Romon M, Deschamps V, et al. The three-factor eating questionnaire-R18 is able to distinguish among different eating patterns in a general population. *J Nutr*. 2004;134(9):2372-2380.
43. Wardle J, Marsland L. The leeds food preference questionnaire: development of a measure of food preferences for use in dietary studies. 1990;20(4):825-833.
44. Schamarek I, Richter F, Tönjes A, et al. The German Leeds food preference questionnaire (LFPQ-G): a validation study. *Food Qual Prefer*. 2023;112:105035.
45. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*. 1985;122(1):51-65.
46. Lowe MR, Butryn ML, Didie ER, et al. The power of food scale. A new measure of the psychological influence of the food environment. *Appetite*. 2009;53(1):114-118.
47. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381-1395.
48. Gearhardt AN, Corbin WR, Brownell KD. Development of the Yale food addiction scale version 2.0. *Psychol Addict Behav*. 2016;30(1):113-121.
49. Radloff LS. The CES-D scale. *Appl Psychol Measur*. 1977;1(3):385-401.
50. Cepeda-Benito A, Gleaves DH, Fernández MC, Vila J, Williams TL, Reynoso J. The development and validation of Spanish versions of the state and trait food cravings questionnaires. *Behav Res Ther*. 2000;38(11):1125-1138.
51. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983;24(4):385.
52. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Med Care*. 1992;30(6):473-483.
53. Saunders JB, Aasland OG, Babor TF, La Fuente JR d, Grant M. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction*. 1993;88(6):791-804.
54. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol*. 1997;32(9):920-924.

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