Placing sensory science in health research: Correlates of sensory taste perception & preferences, healthy eating and health outcomes in European Children

Dissertation

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Verteidigt am:

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Hiermit erkläre ich, Hannah Jilani, geboren am 29. 12. 1982 in Hannover, dass für das Verfassen der vorliegenden Dissertation ‚Placing sensory science in health research: Correlates of sensory taste perception & preferences, healthy eating and health outcomes in European Children' folgende drei Aussagen zutreffen:

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Bremen,

Hannah Jilani

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Summary

Overweight and related non-communicable diseases often occur in childhood and persist into adolescence or even adulthood. About 22 million children in Europe are overweight or obese. Multiple dietary factors were identified to be associated with the development of overweight and overweight, such as sensory taste perception and preference. Both sensory taste perception and taste preferences play an important role in food choice and eating behaviour and thus in related health outcomes. But there are large individual differences with respect to the sensory pleasure caused by food and drinks that originate in genetic factors and their interplay with the food environment in which children grow up.

Interventions aiming to promote a healthy food choice may thus benefit from a better understanding of how individual sensory taste perception and taste preferences are shaped in childhood and adolescence. To what degree sensory taste perception and taste preferences are genetically determined or influenced by the environment is not yet fully understood. It is also not clear to what extend individual food choices can be explained by differences in sensory taste perception.

Based on unique data from a large-scale pan-European cohort study in children, adolescents and their families, the present thesis investigated for the first time factors that may influence sensory taste perception and taste preferences in childhood. This thesis took advantage of the great diversity of food environments across Europe and considered biological/ physiological factors (sex, age, number of fungiform papillae) as well as genetic and familial factors (biological parents, parental consumer attitudes/ education level and dietary habits) simultaneously.

Since it is very challenging to obtain valid results about sensory taste perception from children, instruments and test systems were adapted to be used in children with various cultural backgrounds. This thesis analysed sensory test methods to investigate their feasibility and suitability for future large-scale observational studies in young populations. Connecting sensory taste perception with health outcomes presents an innovative research field. The thesis placed sensory taste research in a public health context investigating the relationship between sensory taste perception and health in a large scale epidemiological study.

The thesis was conducted as part of the Europe-wide IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS) study aiming to identify the risks for overweight and obesity and to develop effective intervention strategies in children aged 2-9 years from eight European countries (www.idefics.eu) as well as the follow-up study I.Family aiming to identify the determinants of food choice,

lifestyle and health in European children, adolescents and their parents (www.ifamilystudy.eu). Inclusion of data from the whole family allowed the investigation of the association between parental influence and familial aggregation of sensory taste perception.

Sensory test methods included counting the number of fungiform papillae, a two alternative forced-choice taste threshold test, a paired comparison forced-choice taste preference test and a food and beverage preference questionnaire.

Harmonised data obtained through standardised methods allowed the inclusion of data from all eight participating European countries. The presented analyses include different subgroups of the IDEFICS and I.Family cohort with sample sizes ranging from 83 (publication 1) to 13,165 individuals (publication 4).

Elaborate, state-of-the-art statistical methods were used to investigate the research questions and to account for the structure of the data and for the specific research questions focusing either on descriptive methods or on multivariable regression models.

The thesis is a cumulative dissertation. In total, four scientific articles were drafted and submitted to international peer-reviewed journals (publication 1 to publication 4). Additionally, a book chapter describing all sensory test methods applied in IDEFICS and I.Family was drafted and published (publication 5).

In a sub-sample of the German IDEFICS cohort, the number of fungiform papillae was measured within a defined 6 diameter circle on the tip of the tongue of 83 children between 6 and 9 years. It was analysed whether this objective measurement may be suitable for future large-scale epidemiological studies and whether it can be used to complement subjective measurements of sensory taste perception and preference. Indeed, the results showed that an association between the number of fungiform papillae and fat preference is likely. Therefore, the number of fungiform papillae may be a suitable marker for fat perception (publication 1).

To better understand the development of sensory taste perception and to gain insight into the possibilities of changing sensory taste perception it is necessary to investigate factors that influence and determine sensory taste perception. Publication 2 presents internationally comparable results of sensory sweet, salty, bitter and umami taste thresholds as one measure of sensory taste perception. Sensory taste thresholds vary across European countries, from Spain in the West to Estonia in the East, from Sweden in the North to Cyprus in the South. Sex differences seemed to be rather small during childhood. Age differences were present and possibly resulting from differences in children’s physiological development and maturity. In childhood, higher sweet and salty thresholds were associated with a higher risk for being overweight or obese throughout Europe (publication 2).

During childhood parents have a great influence on what children eat at home. Which food products parents buy and make available for their children may depend partly on their consumer attitudes towards foods and beverages. As part of this thesis it could be shown that children from parents with unfavourable consumer attitudes, in terms of trusting in advertisements and not avoiding food additives, had less healthy food choices in terms of sweet and fatty food as well as overall diet quality. Parents with a lower education level had more unfavourable consumer attitudes. This fact has implications for the education system as well as for food labelling systems and restrictions of food advertisements (publication 3).

Publication 4 describes the resemblance of taste preferences among family members. Inter- and intra-class correlations for all relative pairs of a family showed that correlations were strongest among younger siblings (<12 years) than among older siblings (≥12 years), stronger among girls than among boys and weakest between parents and their children.

The results emphasise the importance of sensory taste perception and taste preferences with regard to health outcomes. This underlines the relevance of sensory science in epidemiological health research. Results also shed light on factors associated with sensory taste perception and taste preferences in children and adolescents. Recommendations for young consumers and future interventions as well as prevention measures aiming at improving dietary behaviours of children should consider involving the whole family. Incorporation of better nutrition and consumer education into elementary education systems as well as healthy nutrition campaigns targeted at less educated parents may beneficially influence dietary habits of families.

CONTENTS

ORIGINAL PUBLICATIONS

1. Hannah Jilani, Wolfgang Ahrens, Kirsten Buchecker, Paula Russo, Antje Hebestreit on behalf of the IDEFICS consortium. Association between the number of fungiform papillae on the tip of the tongue and sensory taste perception in children. Food Nutr Res. 2017 Jul 26;61(1)
2. Hannah Jilani, Timm Intemann, Kirsten Buchecker, Hadjigeorgiou Charalambos, Francesco Gianfagna, Stefaan De Henauw, Fabio Lauria, Dénes Molnar, Luis A. Moreno, Lauren Lissner, Valeria Pala, Alfonso Siani, Toomas Veidebaum, Antje Hebestreit, Wolfgang Ahrens on behalf of the IDFEFICS consortium. Correlates of bitter, sweet, salty and umami taste thresholds in European children: Role of sex, age, country and weight status - the IDEFICS Study. Submitted to the British Journal of Nutrition
3. Hannah Jilani, Hermann Pohlabeln, Kirsten Buchecker, Wencke Gwozdz, Stefaan De Henauw, Gabriele Eiben, Dénes Molnar, Luis A. Moreno, Valeria Pala, Lucia Reisch, Paola Russo, Toomas Veidebaum, Wolfgang Ahrens, Antje Hebestreit on behalf of the IDFEFICS consortium. Association between parental consumer attitudes with their children’s sensory taste preferences as well as their food choice. PLoS One, 2018. 13(8): p. e0200413.
4. Hannah Jilani, Timm Intemann, Leonie Helen Bogl, Gabriele Eiben, Denés Molnar, Luis Moreno, Valeria Pala, Paula Russo, Alfonso Siani, Antonia Solea, Toomas Veidebaum, Wolfgang Ahrens, Antje Hebestreit on behalf of the I.Family consortium. Familial aggregation and socio-demographic correlates of taste preferences in European children. BMC Nutrition 2017 3;87
5. Hannah Jilani, Jenny Peplies, Kirsten Buchecker on behalf of the IDEFICS and I.Family consortia. Assessment of sensory taste perception in children. In: Bammann K, Lissner L, Pigeot I, Ahrens W, editors. Instruments for health surveys in children and adolescents. Heidelberg: Springer Publisher; 2019. p. 257-275.

FIGURES

TABLES

ABBREVIATIONS

IDEFICS: IDentification and prevention of dietary- and lifestyle-induced health EFfects In Children and infantS

FFQ: Food frequency questionnaire

FP: Fungiform papillae

BMI: Body mass index

DAWE: diacetyl tartaric ester

HDAS: Healthy diet adherence score

ISCED: International standard classification of education

MSG: Monosodiumglutamate

SOP: Standard operating procedures

T0/T1/T3/CG: Baseline examinations/ 1st follow-up/ 2nd follow-up/ 3rd follow-up

UNESCO: United Nations Educational, Scientific and Cultural Organization

1 INTRODUCTION

'The true laboratory is the mind, where behind illusions we uncover the laws of truth.'

Jagadish Chandra Bose (1858-1937)

Sensory taste research presents a research field that has entered health research in the last decades. Still, most of the studies conducted in this field take place in laboratory settings using test solutions that do not appear in real life. We aimed to place sensory testing outside the laboratory into settings close to real-life conditions using real foods to obtain results more reflective of decisions and conditions of daily life.

1.1 Development of sensory taste perception and preferences

One substantial lifestyle-related determinant influencing the onset of overweight is dietary behaviour [1]. One major factor influencing dietary behaviour is food choice. During childhood food choice is mostly determined by the sensory perception and liking of foods [2, 3], as children consider other dietary aspects such as health or food prices less. Sensory taste perception and preferences formed during childhood might persist until later life [4]. To which degree taste perception and preferences are learned or genetically defined is not yet fully understood. Sensory taste perception and preferences can be innate, learned, culturally or genetically defined [5]. Furthermore, they can be influenced by food neophobia (fear/refusal of new/unknown foods) or acceptance for new foods [3] (Figure 1). Already infant feeding practices (e.g. breast milk or bottle feeding) may influence the acceptance of foods later in childhood [6, 7]. But also later feeding practices have a large impact on the development of children's preferences [4]. Also, social factors have been found to influence the formation of taste preferences. Role models, for example, may influence children's preferences through their own dietary behaviour [3] as watching parents eat raises children's awareness of their parents' eating behaviour and increases their readiness to taste the same foods [8, 9]. Situations in which children encounter a new taste for the first time influence its acceptance: A friendly situation like a family meal or using foods as rewards may influence food acceptance positively [10]. Yet other factors reported to influence the formation of taste preferences are nutrition literacy, media and food availability [11].

INTRODUCTION

In conclusion, according to current studies sensory taste perception and preferences may be determined by the genetic predisposition and an innate preference for the sweet taste and rejection for the sour and bitter taste [12-14]. But they are also influenced by individual environmental factors like perinatal conditions, role models and social context (Figure 1).

Former studies investigating taste perception and preferences were conducted in a laboratory setting with small study populations [15-20]. Mostly aqueous test solutions instead of real foods were used as test samples for sensory taste perception and preference tests. Therefore, study results were not applicable to daily life situations. Furthermore, in order to achieve findings relevant for everyday life conditions, sensory taste tests should be conducted with real foods children know instead of aqueous test solutions.

To influence food choice of young children it is essential to understand how taste perception and preferences are formed. In a first step, population-based epidemiological studies are needed to study determinants of taste perception and preferences and the impact of taste perception and preferences on food choice and health. Data from young and healthy study subjects from the general population are most informative. To study the named associations in this young target population and to obtain valid results, appropriate methods have to be developed and tested.

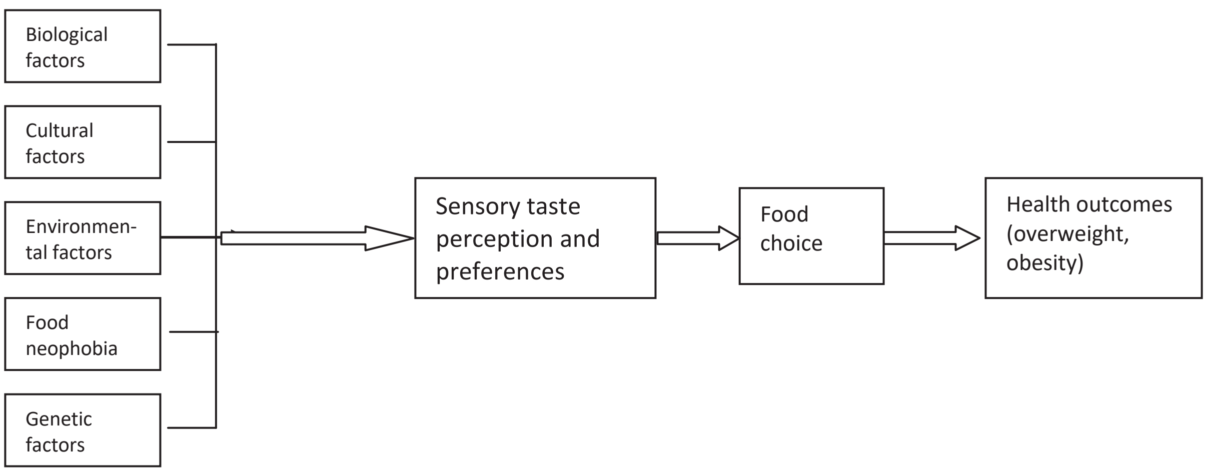


Figure 1: Multifactorial influence on the origin of sensory taste perception and preferences

2 AIMS OF THE THESIS AND RESEARCH QUESTIONS

Overweight belongs to the main public health issues among European children. The onset of overweight often occurs during childhood and tracks into adolescence or even adulthood [21-23]. In Europe, 21.1% of girls and 18.6% of boys between 2 and 9 years are overweight or obese [24]. This illustrates the importance of studying physiological, genetic and environmental determinants that influence or favour the onset of childhood overweight. Overweight represents a risk factor for cardiovascular diseases. One relevant lifestyle-related determinant of overweight is food choice [1]. Food choice is partly determined by sensory taste perception and preferences [3].

Previous cross-sectional analyses of the first survey (2007/2008) of the IDEFICS (IDentification and prevention of dietary- and lifestyle-induced health EFfects In Children and infantS) study (see methods) confirmed an association of sweet and fat preference with overweight in European children [25]. The formation, development and influencing factors of taste perception and preferences could not be fully explained. Meanwhile, data from the second and third survey of the IDEFICS study and the follow-up study I.Family (see methods) are available allowing the conduct of further cross-sectional but also longitudinal investigations. Furthermore, the results of I.Family permit to analyse the parental influence as well as familial aggregation of taste perception and preferences.

The connection of sensory taste perception and preferences with health aspects presents an innovative research field. Contrary to previous research in sensory science that focused rather on the needs of the industry, this thesis places sensory science in a public health context to objectively study the association between taste perception as well as preferences and dietary behaviour which in turn influences health. Additionally, the results of this thesis may contribute to the understanding of the formation, development and influencing factors of taste perception and preferences. This knowledge is essential to develop adequate recommendations for young consumers and successful health programmes aiming to support children and adolescents to develop a healthy dietary behaviour.

2.1 Aims of the thesis

Aims of the thesis were to investigate the development of taste perception and preferences in children and to identify factors influencing sensory taste perception and preferences as well as their impact on overweight/obesity. Geographic, physiological and familial factors but also parental educational level as well as parental consumer attitudes were considered for the subsequently described investigations. In the course of this thesis, new methods were developed and evaluated to assess taste perception and preferences in children in a simple and valid way outside the laboratory setting.

2.2 Research Questions

The thesis is a cumulative dissertation. In total, four scientific articles were prepared and submitted to peer-reviewed journals. The analysis of current gaps and need led to the following research questions which were investigated in this cumulative dissertation:

1. Is measuring the number of fungiform papillae on the tip of the tongue a suitable objective method to complement sensory taste perception tests in children?
    a. Is the number of fungiform papillae associated with sensory taste perception and preferences in children (publication 1)?
2. Are sensory taste thresholds for bitter, sweet, salty and umami correlated with age, sex, country of residence and weight status (publication 2)?
3. Are parental consumer attitudes associated with their children's sweet, fat and umami preferences as well as with their sweet, fatty and processed food choices?
    a. Do parents with a lower education level more often have unfavourable consumer attitudes in terms of trusting in foods and beverages known from advertisements and not trying to avoid additives in food (publication 3)?
4. Is there a familial aggregation of taste preferences for sweet, salty, fatty and bitter in European families (publication 4)?

Additionally, one book chapter was prepared and published describing all sensory taste perception tests applied in IDEFICS and I.Family.

From these research questions concrete hypotheses were derived and tested statistically as described in chapter 3.7.

3 METHODOLOGICAL CONSIDERATIONS AND MAIN FINDINGS

This thesis evaluated four sensory test methods with regard to their application in children and adolescents and in large-scale, multi-cultural epidemiological studies. Further, the thesis aimed at investigating associations between sensory taste perception, food choice and childhood overweight/obesity. The data used to answer the research questions originate from the IDEFICS (www.idefics.eu) [26] as well as from the I.Family (www.ifamilystudy.eu) [27] study.

Previously, the majority of methods which were commonly applied to assess sensory perception and preferences, e.g. during the evaluation of newly developed foods by industry, were designed for adults. Studies on sensory testing in children are still rare. Some studies, however, have provided insight into requirements for sensory assessment in children. Guinard et al., for instance, published a review on sensory and consumer testing in children in 2000 [28] and Popper & Kroll reviewed the current state of knowledge in 2005 [29]. These reviews, including their recommendations, were summarised in the international standard guidelines on sensory evaluation by children and minors and complemented by some very general examples [30]. Nevertheless, at the time the IDEFICS study started the baseline examinations, there was a lack of validated methods for sensory testing with children, especially for the assessment of taste thresholds. Consequently, specific test procedures, tailored for the application in young children, were developed: During the course of the IDEFICS study 2 methods (paired forced choice taste preference test and forced choice taste sensitivity test) to measure sensory taste perception and preferences of preschool and school children were adapted for the use in children [31].

During the I.Family follow-up examinations the method to assess taste preferences was adapted from food based test matrices towards a self-administered questionnaire for children and adolescents. It was particularly challenging to design the test for application in different European countries.

3.1 Study design

The IDEFICS study was a European, multicentre cohort study in which 16,228 children from Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden participated at baseline [26]. Aims of the study were to identify risks for overweight and obesity and to develop effective intervention strategies in children aged 2-9 years in a population-based approach. All methods and procedures were standardised, pre-tested and corrected if needed [32].

The first examination (T0) was conducted between September 2007 and May 2008. After this, standardised interventions were applied in an intervention region of each country. Interventions aimed to improve children's lifestyle behaviour and to achieve structural improvements in the school-/ kindergarten settings [33].

Two years later, a second examination (T1) was conducted in each participating country between September 2009 and May 2010. To evaluate the effect of the intervention, the participants were interviewed again in 2011 (T2).

The I.Family study was the follow-up study of the IDEFICS cohort. All children were invited to participate in the examinations between March 2013 and May 2014 (T3). Additionally to all children, their parents and siblings were invited to participate as well. The aim was to identify determinants of food choice, lifestyle and health taking into account genetic, familial, social, environmental and behavioural factors.

Another survey (CG; contrasting group examination) conducted in those children whose weight status had changed between the IDEFICS study and I.Family took place between October 2014 and May 2016.

3.2 Study groups

Harmonised data obtained through standardised methods allowed the inclusion of data from all eight participating European countries. The presented analyses included different subgroups of the IDEFICS and I.Family cohort with sample sizes ranging from 83 (publication 1) to 13,165 (publication 4) individuals (Table 1).

Table 1: Sample sizes included in publication 1 to publication 4

Number	Article	Study group (n)
1	Association between the number of fungiform papillae on the tip of the tongue and sensory taste perception in children	Fungiform Papillae: 83 Bitter taste threshold and taste preferences: 56
2	Correlates of bitter, sweet, salty and umami taste thresholds in European children: Role of sex, age, country and weight status – the IDEFICS study	1,938
3	Association between parental consumer attitudes with their children's sensory taste preferences as well as their food choice	1,407
4	Familial aggregation and socio-demographic correlates of taste preferences in European children	13,165

METHODOLOGICAL CONSIDERATIONS AND MAIN FINDING

In the IDEFICS study 16.228 children between 2 and 9 years participated in T0. A sub-sample of 20% of the primary school children, aged between 6 and 9 years, was invited to participate in the sensory taste perception module. The IDEFICS study pre-test results had revealed that obtaining reliable answers from preschool children was difficult; thus it was decided to only include children six years and above (see chapter 3.6). These measurements were repeated during T1.

In T3, where the children were invited together with their siblings and parents, the complete dataset consisted of 16,458 participants at the time of data analysis, including 9,617 children and 6,841 parents. Figure 2 shows an overview of the study groups included in the publications of this dissertation.

Measuring the number of fungiform papillae on the tip of the tongue (Publication 1)

Publication 1 was based on a pilot study conducted during T1 in a sub-sample of the German children that participated in the sensory taste perception module of 83 children between 8 and 11 years in 2010. Out of these, 56 children completed also sensory taste threshold and taste preference tests.

Correlates of sensory taste thresholds for sweet, salty, bitter and umami (Publication 2)

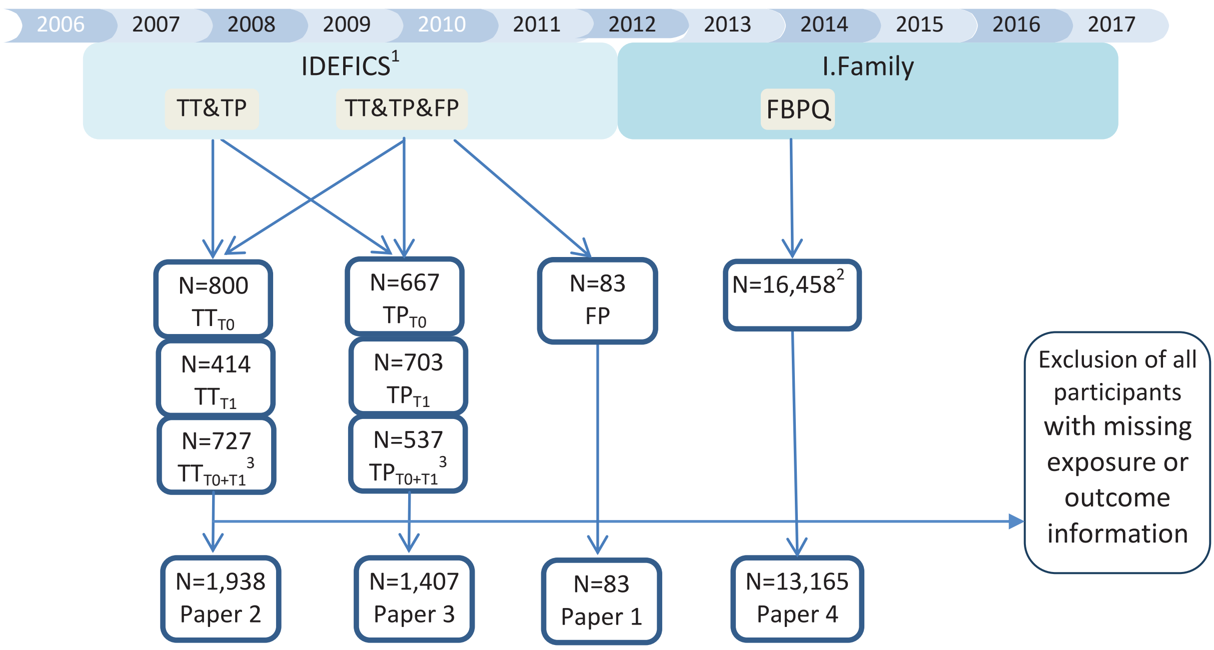
Publication 2 was based on data of children who participated in the taste threshold tests (TT). 800 children participated only at T0 in TT whereas 414 children participated only at T1 in TT. 727 children participated at T0 and T1 in TT. From the children who participated at T0 and T1 only one measurement was included in the analysis. Those children were allocated according to their age at the time of the measurements. The aim was to achieve an even age-distribution within the study group. After including only children with all covariates available this resulted in a study group of 1,938 children between 7 and 11 years from Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden.

Parental consumer attitudes and children's taste preferences as well as food choices (publication 3)

Publication 3 was based on data of those children who participated in the taste preference tests (TP). 667 children participated only at T0 in TP whereas 703 children participated only at T1 in TP. 537 children participated at T0 and T1 in TP. From the children who participated at T0 and T1 only one measurement was included in the analysis. Those children were allocated according to their age at the time of the measurements. The aim was to achieve an even age-distribution within the study group. Further, we excluded children where the parents answered ‘I don’t know’ for the exposure variables (‘trusting in foods and drinks they know from advertisements’ and ‘not avoiding additives in foods’) to receive more distinct results. After including only children with all covariates available this resulted in a study group of 1,938 children between 6 and 11 years from Belgium, Estonia, Germany, Hungary, Italy, Spain and Sweden.

Familial aggregation and socio-demographic correlates of taste preferences (Publication 4)

Publication 4 is based on T3 data. In T3, all children and parents from Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden were invited to complete the food and beverage preference questionnaire (see publication 4). After excluding participants that had more than 20 missing or “Never tried/ Don’t know” answers, we obtained a study sample of 13,165 participants: 2,230 boys <12 years, 2,110 girls <12 years, 1,682 boys ≥12 years, 1,744 girls ≥12 years and 5,388 parents.



Abbreviations: FBPQ: Food and Beverage Preference Questionnaire, TT_{T0}: Taste threshold tests at T0, TT_{T1}: Taste thresholds at T1, TT_{T0+T1}: Taste thresholds at T0 and T1, TP_{T0}: Taste preference tests at T0, TP_{T1}: Taste preference tests at T1, TP_{T0+T1}: Taste preference tests at T0 and T1, FP: Fungiform papillae

[1]: The whole study sample of IDEFICS T0 comprised 16,228 children between 2 and 9 years. Out of these, 20% of the school-aged children were eligible for taste threshold and taste preference tests conducted in T0 and T1.

[2]: Whole study sample at the time of analysis and all participants from the age of 6 years were eligible to answer the questionnaire.

[3]: One measurement of those children that participated at T0 and T1 was included into the analysis. Children were allocated according to their age at the time of the measurements. The aim was to achieve an even age-distribution.

Figure 2: Number of participants included in the publications

3.3 Sensory testing with children

The physical and cognitive abilities of children are less developed than those of adults [34] and thus feasibility needs to be considered when planning sensory testing with children. Tests have to be easy to understand and quick to conduct as children have short attention spans. The whole test procedure must not take too long and the way of explaining the test to the children has to motivate them to participate and to complete the whole test [31]. Furthermore, the decision making process of children is strongly influenced by adult approval and reaction. Commonly, children tend to respond affirmatively to positively phrased questions or change their opinion on enquiry by an adult immediately [28]. A simple question like, “Are you sure?” can easily turn a “yes” into a “no”.

In addition, the test environment should be bright and cosy in a child friendly manner.

Besides the psychological development, there are also differences in the physiological development: Children have about five times more taste buds, and their foliate papillae are larger and more abundant compared to those of adults. Nevertheless, this does not consequently lead to higher taste sensitivity, due to the fact that children’s innervation of taste papillae is not fully developed. The development of the taste apparatus carries on through childhood [35]. A small number of studies reported thresholds for children to be similar to those of adults [16]. Most publications, however, found young children’s sensitivity about a magnitude lower than the one reported for adults [17, 36, 37].

3.4 General considerations

Each study centre obtained ethical approval from its local responsible institutional review board. Parents gave written informed consent for themselves and for their children. Adolescents 12 years and older gave their own written informed consent. All children were informed orally and gave their oral consent to participate in the study.

Food intolerances in terms of food allergies and food sensitivities are highly prevalent in Europe. Especially sensitivity to monosodium glutamate and gluten (celiac disease) is widely spread in Europe. Therefore, parents needed to be asked for particular food allergies of their children before the tests.

Performing examiners were not allowed to use fragrance or perfumed hand cream or to consume cigarettes, coffee, or bubble gum prior to or during the tests. SOP for testing included counselling to ensure that participating children were neither hungry nor full.

Ideally, participants had their last meal one hour before to the test sessions; peppermint chewing gum or sweets with a strong taste were not allowed one hour prior to the tests.

Test environments were most suitable when they were familiar to the children. Ideally, the tests were performed in preschool or school settings. In addition, test environments were supposed to be bright, friendly and colourful rooms where children would feel comfortable but decoration should not be too colourful in order to avoid distraction. Furthermore, there should be no undesired odours like strong smell from kitchens or disinfectants.

3.5 Performance on the European level: The need for standard operating procedures (SOPs) and standardisation

Procedures for application within the framework of a multicentre study have to provide comparable results on an international level. In the IDEFICS study and in I.Family, all survey teams were trained in a central training session previous to the survey to assure standardised test performance and preparation of test solutions in each survey centre. The training was attended by at least one representative of each participating survey centre. This central training was conducted by experts and included lessons on behaviour, used vocabulary and phrasing of questions, as well as lessons on handling the equipment for preparation of test solutions and setting up the test environment. Detailed SOPs were provided to each survey centre.

The test substances, even if they appeared simple such as sucrose or sodium chloride were standardised. A central supply was established for all test material: Food samples, test substances and equipment for preparation of test-solutions, as well as equipment such as drinking cups were purchased centrally and shipped to the survey centres. As an example, to avoid anti-caking and flow-regulating agents which are commonly applied by industry, food samples were purchased centrally without additives and provided to the survey centres pre-packaged and 'ready-to-use' for the preparation of the test solutions.

Taking into consideration that tap water quality differed substantially among the European countries, demineralised water was used for all test samples and procedures. Accordingly, the selection of appropriate food samples on a cross-national level was also very challenging. For example, apple juice which was well accepted in most countries except Cyprus. Even in common foods the food industry is known to adapt national food recipes according to the population's preferences; thus a standardised apple juice recipe was needed for this food sample in all study centres.

3.6 Test designs of sensory taste perception tests

After taking into consideration the aforementioned challenges, five different test procedures were developed to be implemented in the different surveys of the IDEFICS and I.Family study. In this dissertation four out of five methods were analysed. Test procedures 2-5 are described in detail in the book chapter [38]. Here, methods 1-4 are briefly described.

1. In the IDEFICS study, a feasibility study was conducted during T1 to measure the number of fungiform papillae within a defined 6 mm diameter circle on the tip of the tongue using a photographic assessment method.

2. As the taste qualities sweet, salty, bitter, and umami (the taste for monosodium glutamate) appeared to be most promising in the context of overweight and obesity, these qualities were selected for the *taste sensitivity tests* which were conducted in T0 and T1. In the literature, salty and umami tastes have been associated with increased appetite for its stimulation of orosensory receptors and/or improvement of palatability which might increase appetite. A higher consumption of glutamate (umami) has been associated with overweight [39-41]. The sweet taste is predominant in high calorie foods, in particular sweets. The bitter taste on the other hand is characteristic of a variety of vegetables, such as broccoli or Brussels sprouts, which are low in calories and often rejected by young children.

3. Besides taste sensitivity for basic taste qualities, *taste preferences* for sweet, apple flavour (using apple juice), salty, umami and fat (using crackers) were tested experimentally during T0 and T1. Additionally, the sweet (juice) and fat (crackers) taste preference tests were repeated during the CG examinations. Flavour enhancement has recently been discussed in the literature to be linked to increased appetite and thus to contribute to overweight and obesity [42]. The role of fat regarding the development of overweight and obesity appears to be even clearer: Fat is the macronutrient with the highest energy density. More recently, the literature suggested that 'fatty' could be even the sixth primary taste [43, 44].

4. Furthermore, a food and beverage preference questionnaire has been recommended for the use in epidemiological studies to associate sensory taste perception

with health outcomes [45]. For this purpose, a *food and beverage preference questionnaire* assessing preferences for sweet, bitter, salty and fatty was developed for I.Family.

Generally, children from the age of 6 years and older were invited to participate. Pre-tests had shown that children under the age of 6 years often did not comply with the test procedure and that it was not possible to obtain reliable answers from them [32].

The following section describes the characteristics of the applied methods and methodological issues that occurred during the study and should be considered in future research.

3.6.1 Fungiform papillae

The number of fungiform papillae (FP) was measured in a sub-sample of the German study population (n=83) of the IDEFICS study.

3.6.1.1 Test procedure

The test procedure was described in more detail in publication 1. In brief, a photographic method was adapted according to Shabake et al. [46] to measure the number of FP. A defined 6 mm diameter circle on the children's' tongue was coloured and photographed (Figure 3). The procedure took ten to fifteen minutes for the participants. The number of FP was counted on the pictures after they were transferred to a computer. The number of FP in the defined circle of each participant was counted twice by two independent examiners and the mean number of FP was calculated in order to obtain the number of FP on the circle with a 6 mm diameter.

Figure 3: Photos of the stained tongue without (left) and with (right) papillae counted

3.6.1.2 Methodological considerations

Measuring the number of FP within a defined circle on the tip of the tongue with the help of a digital camera has been validated by Shabake et al. (2005) who also applied this method in children. It delivered the same results as the formerly applied method using a video camera and results were meaningful for the number of FP of the whole tongue [46].

Advantages of this method were that this method did not demand high cognitive skills of the children nor did it require a long attention span. Furthermore, the results were not susceptible to external factors. For the study participants this method did not present a great challenge.

Disadvantages of this method were that the post-processing of the obtained pictures was rather laborious for the researchers compared to other methods to assess sensory taste perception. Suitable pictures had to be selected and edited manually with a computer program. The counting had to be done by two researchers independently to reduce errors. An improvement would be the development of a special computer program to facilitate preparation of the pictures and counting the FP. Such a program should be used to prevent errors, e.g. double counting of FP.

In conclusion, measuring the number of FP is a suitable method to objectively measure characteristics of taste perception (e.g. fat perception) in children, in which objective measurements are preferable. In the present study we used a digital camera instead of a reflex camera. Using a reflex camera with macro mode and ring flash to achieve a higher resolution as well as having an appropriate computer program for counting the number of FP are important prerequisites.

3.6.2 Taste threshold tests

As a measure of taste sensitivity, the detection thresholds for the taste modalities sweet, salty, bitter and umami were assessed as the lowest concentration of a respective stimulus that must be exceeded in order to have any taste effect [47].

3.6.2.1 Test procedure

The threshold tests were arranged as a board game (Figure 4) to enhance the children’s motivation. For each basic taste, a row of five test solutions with increasing concentrations of stimuli were presented to the children in small cups with a volume of 20 ml at the lower end of the board.

Figure 4: Game board for taste threshold testing. The board was printed in DIN A3 and laminated

The experimenter introduced the board game with a small background story: The children were told to be “taste detectives”. They learned that some cups on the board may contain pure water whereas others may taste different and they would have to find the latter cups. The children were advised to compare the test solutions with an extra cup of demineralised water. In case the test solution from the board tasted like the water in the cup, they had to place it on a water icon on the upper left side of the board. If the children tasted a difference they had to place the test solution on a red cross over a water icon on the upper right of the board. Between the different solutions children neutralised the taste in their mouth with demineralised water. The test samples were presented from lowest to highest concentration. The whole test procedure lasted no longer than 15 minutes.

3.6.2.2 Test samples

The test solutions were prepared by the survey centres on site. Therefore, for each basic taste quality, a stock solution had to be prepared from the corresponding test substance. From this stock solution, the survey centres had to prepare the five test solutions by dilution in demineralised water. The survey centres received small packages with the test substances in the correct amounts needed to prepare the stock solution. This was done in order to minimise the work load for the survey centres and avoid mistakes.

To assure that the stimuli were adequate for children, the range of concentrations of each test substance was about an order of magnitude above the threshold level reported for adults. The concentration ranges of the test substances were (in the order of assessment):

1. Sucrose (8.8 mmol/l – 46.7 mmol/l) for the sweet taste threshold,
2. Sodium chloride (3.4 mmol/l – 27.4 mmol/l) for the salty taste threshold,
3. Caffeine (0.26 mmol/l to 1.3 mmol/l) for the bitter taste threshold,
4. MSG (0.6 mmol/l to 9.5 mmol/l) for the umami taste threshold.

3.6.2.3 Methodological considerations

The procedure of this method was adapted to facilitate assessment in children and to enhance suitability for epidemiological studies, e.g. in terms of standardising data assessment across different cultures and survey sites. In order to enhance the feasibility to conduct a standardised test procedure all materials were purchased, stored and shipped centrally to all participating countries.

Test-retest reliability was calculated for a German sub-sample and delivered satisfying results. COHEN's κ for test-retest reliability for sensory taste threshold testing was between 0.68 (for bitter) and 0.81 (for sweet) [31] which is classified as 'substantial' (for bitter, salty and umami) to 'almost perfect' (for sweet) [48].

On the other hand, for this threshold test, it was not possible to use real foods. The concentrations used to measure taste thresholds were much lower than concentrations present in real foods. Therefore, taste thresholds, in general, may not reflect the real world taste experiences children face in their daily life and that have an impact on food choice or dietary behaviour [49]. Another circumstance that may influence the outcomes of threshold testing is the order of presentation which was not randomised but fixed in our study (sweet, salty, bitter, umami), in order to minimise the complexity of the test design.

Compared to previous studies in children, we used a smaller number of test-solutions per taste modality for assessing taste thresholds [18, 19, 50, 51]. Generally, these studies performed testing sessions with participating children before conducting the actual test series, thus children were familiar with the test procedures once data collection for the different taste modalities started. Due to our cross-cultural and large-scale study design we chose a simpler study protocol but assured a standardised procedure across all countries and centres. To minimise measurement errors due to the lack of practice and limited cognitive abilities of study participants we measured only detection thresholds instead of identification thresholds for the basic tastes; i.e. the children had to indicate whether the respective solution tasted different from water but they did not have to indicate which taste modality they perceived.

3.6.3 Taste preference tests

Preference testing with children is by far less complicated than threshold testing. To test sweet, flavour, salty, fatty and umami preference, a paired comparison test according to the DIN–Norm 10959 was used in the IDEFICS study. This test allowed a direct comparison between two samples and is a simple, very common technique to evaluate taste preferences.

3.6.3.1 Test procedure

The preference test procedure was described and analysed in publication 3. To create a very easy, child-friendly test design, again a simple game board was developed (Figure 5). During the test the game board was placed on the table in front of the children. In each test sequence, the children had to compare the sensory pleasure of a basic sample and a modification of it. Each pair was presented inside the two circles on the lower end of the board. The children were asked to place their preferred sample on the happy smiley on the top end of the board. Between each test sequence the children rinsed their mouth with demineralised water to avoid adaptation.

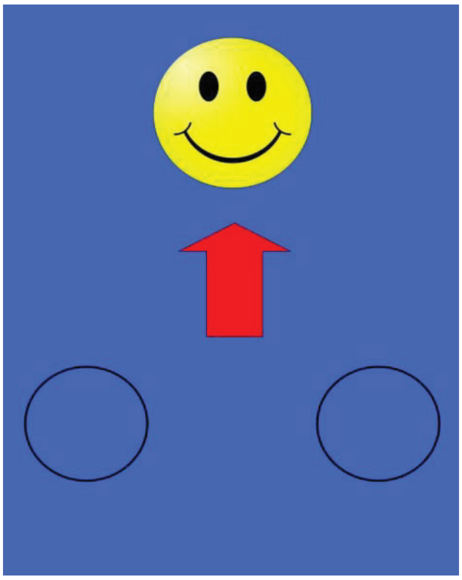


Figure 5: Game board used for preference testing. The board was printed in DIN A4 format and laminated.

3.6.3.2 Food samples

The food samples were selected according to two main criteria: The sensory acceptance of young children in the young age of 6 years and older in all participating countries and the representativeness of the food samples for the evaluated taste quality.

Crackers were used to test salty, fatty and umami taste preferences and apple juice was used to assess the preference for sweet taste and flavour.

3.6.3.3 Food samples for sweet and apple flavour

As reference for the sweet and flavour taste preferences a clear natural apple juice was used with standardised sugar content of 0.53 % to ensure proper acceptance of the juice. To evaluate the taste preference for sweetness, the amount of sucrose in the reference juice was increased to 3.11 %. To evaluate the taste preferences for flavour, 0.05 % apple flavour (nature identical, SENSIENT®) were added to the basic apple juice (Table 2).

Table 2: Overview on recipes for apple juices applied to assess preferences for the sweet taste and flavour

	Apple juice (%)	Sucrose (%)	Flavour (%)
Reference	99.47	0.53	--
Sweet taste	96.89	3.11	--
Flavour taste	99.42	0.53	0.05

All juices were presented on the board (Figure 4) in 20 ml cups. The small sample quantities were chosen in order to prevent the children from becoming full. All survey centres were instructed to serve the juices at 18°C +/- 2°C. The order of assessment was sweet followed by flavour.

3.6.3.4 Food samples for salty, fatty and umami

To evaluate the preferences for salt, fat and umami, crackers were developed. To increase the attractiveness for young children, the crackers were heart-shaped (Figure 6).

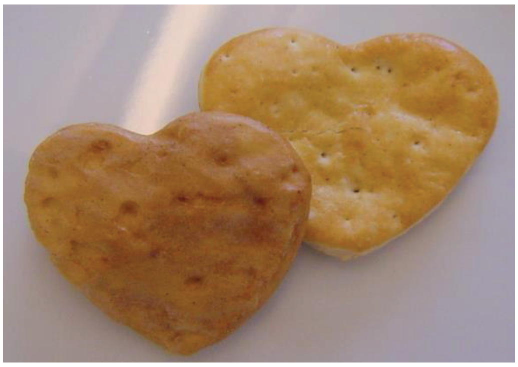


Figure 6: Heart-shaped crackers for the assessment of taste preferences for salt, fat and umami

The reference crackers for all tests were produced according to a basic recipe containing wheat flour, water, 0.7 % salt and 8% fat, finished with soda lye (0.5 % aqueous solution, purchased from Carl Roth Chemicals). To evaluate the taste preference for fat, the amount of fat within the basic recipe was increased from 8% to 18%. To evaluate the taste preference for salt, the amount of salt was increased from 0.7% to 1.6%. Additionally, 1% of an emulsifying agent (DAWE, diacetyl tartaric ester) was added. To evaluate the taste preference for umami, 1% of monosodium glutamate and 1% emulsifier were added to the basic recipe (Table 3).

Table 3: Overview on recipes for crackers applied to assess preferences for the salty, fatty and umami taste

	Flour/water (%)	Salt (%)	Fat (%)	MSG (%)	DAWE (%)
Reference	91.3	0.7	8	0	0
Salt	89.4	**1.6**	8	0	1
Fat	81.3	0.7	**18**	0	0
Umami	89.3	0.7	8	**1**	1

Abbreviations: DAWE: diacetyl tartaric ester, MSG: monosodium glutamate

The order of test assessment was first fatty, then salty and umami last. This order was selected due to the fact that the umami taste remains on the taste buds for a long time and would thus affect subsequent taste experiments.

3.6.3.5 Methodological considerations

The advantages of this method were that the test design of the paired comparison forced-choice preference tests was rather easy to apply as well as to understand and conduct by the children. Furthermore, the test-retest reliability that was calculated for a German sub-sample delivered satisfying results. COHEN's κ for test-retest reliability for sensory taste preference testing was between 0.77 (for sweet and fatty) and 0.86 (for salty) [31] which is classified as 'substantial' (for sweet, fatty and umami) to 'almost perfect' (for salty) [48]. Taste preference tests were accepted by the participants. In general, tests were quick and robust and as they have been conducted with real food samples familiar to the participants. Producing real foods like crackers and juices in a standardised way is more difficult than simply producing standardised aqueous solutions. Nevertheless, we achieved this by using industrial equipment (e.g. oven and filling system).

The disadvantages were that the high standardisation included a rather high effort of finding industrial producers that would produce our different batches of food samples, calculation of the right amount including break and loss, purchasing the raw material, transportation to industrial producers and back to BIPS and to the study centres. Although this method had the advantage of real foods as test samples only one matrix for each taste modality was used. Perception of taste may differ according to the matrix delivering the taste in the oral cavity. For example, fat in more liquid foods might be more ready to diffuse out compared to solid food matrices like crackers. Therefore, liquid foods could be more suitable to measure sensory fat perception. Furthermore, same as for the taste threshold test, there was a fixed order of taste modalities for the taste preference test (sweet, apple flavour, salty, fatty, umami) and the basic sample was always presented first. This could have influenced the results by introducing a *positional bias;* given there was a general tendency to select a sample based on when and where it was presented. A previous analysis showed that single preference tests were not mutually correlated indicating that the IDEFICS children did not have the tendency to always choose the sample presented first or second [52].

3.6.4 Taste preference questionnaire

The instrument to measure sweet, salty, fatty and bitter taste preferences was described and analysed in publication 4. In I.Family, a food and beverage preference questionnaire was developed to assess taste preferences that could be applied in children/adolescents as well as in adults according to existing tools [53, 54]. It consisted mainly of pictures of relevant food and drink items (Figure 7). In total, the questionnaire consisted of 63 items including single foods (e.g. banana, spinach), mixed foods (e.g. hot dog, kebab), condiments (e.g. jam, mayonnaise) and drinks (e.g. cola, lemonade).

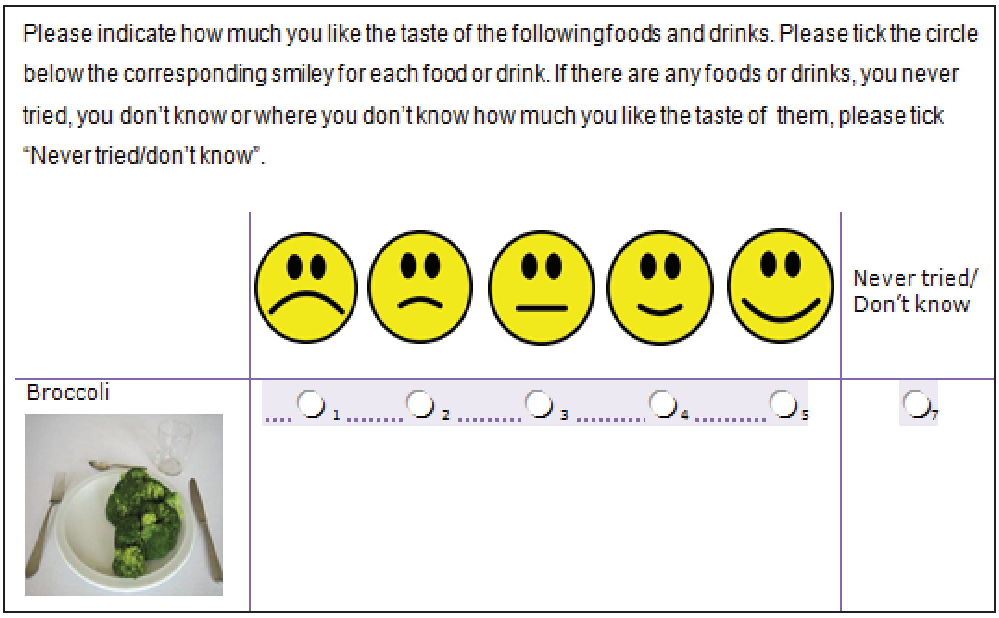


Figure 7: Example (screen shot) from the food and beverage preference questionnaire

3.6.4.1 Test procedure

Participants were asked to indicate how much they liked the taste of the food presented on the pictures using a 5 point likert (smiley-)scale, ranging from disliking to liking. Additionally, participants could indicate that they did not know or had never tasted the specific food item.

3.6.4.2 Taste preference scores

Foods ranked by at least 75% of the participants were used for further analyses. Participants were excluded if they had more than 1/3 missing or "Never tried/ Don't know" answers. To obtain taste preference scores a factor analysis was conducted [55]. This factor analysis was sex- and age-specific to gain more accurate information about the factorial structure of food preference. The sex- and age-strata were: younger boys (<12 years), younger girls (<12 years), older boys (≥12 years), older girls (≥12 years), their mothers and fathers. A food or drink item was considered to belong to a particular factor if the factor loading was higher than 0.30 on that factor. The obtained factors were used to conduct a content analysis in order to assign the factors to the taste modalities sweet, salty, fatty and bitter. Food and drink items with no load on one of the factors were not included in further analyses.

Scores for liking of the specific taste quality were computed by calculating the mean liking of the foods and drinks included in each of the four taste modalities. Scores were calculated individually for younger boys, younger girls, older boys, older girls, their mothers and fathers. The sum of the ratings for the foods and drinks was calculated and divided by the number of foods and drinks included in the specific taste modality group.

3.6.4.3 Methodological considerations

The advantages of this instrument were that it is quick to complete, does not require high cognitive abilities and allows to investigate the association between sensory taste preferences and health outcomes [45]. Thus, it is very inexpensive and suitable for large-scale studies. Using a food and beverage preference questionnaire to assess taste preferences seemed feasible in a large-scale epidemiological study. Asking for preferences for different foods and drinks with different tastes considers multiple sensory factors that have an influence on actual preferences relevant for real life, such as taste sensitivity, taste intensity, social factors and environmental factors as claimed by Hayes and Keast [56]. Additionally, it was well accepted by the study participants as it did not take long (approximately 5 minutes) to complete this questionnaire and no equipment was necessary to assess taste preferences.

Nevertheless, it is self-reported and can be susceptible to misreporting due to social desirability. This tool can be also less exciting among other questionnaires than a practical sensory taste test.

Interestingly, on the one hand, a few foods used in the literature for a specific taste modality did not load on the specific factor for this taste modality in our analysis in any age-group. Especially foods used to represent the bitter taste [57] but also broth for salty [58] had to be excluded in our study. On the other hand, we identified also foods for specific taste modalities that loaded on the corresponding factor in every age-group (Table 4). It may help to consider this (e.g. not using these foods) when planning to use a taste preference questionnaire in future cross-national studies with participants of different age-groups.

Table 4: Foods that did not belong vs. belonged to specific taste modalities according to the factor analysis in all age-groups

	Foods and beverages that did not load on any factor in any age-group	Foods and beverages that loaded on a specific factor in each age-group
Sweet	Wine gum	Milk chocolate Chocolate bar Cola Honey Cornflakes Chocolate spread Fruit yoghurt Fruit juice
Fatty	Avocado	Hamburger Hot dog Sausage Butter
Salty	Broth	Salted nuts Savoury biscuits Salty pistachios Salty sticks
Bitter	Dark chocolate Beer Coffee Wholemeal bread	Broccoli Spinach

3.7 Hypotheses and statistical analysis

Statistical analyses were mainly conducted with SAS (Statistical Analysis System, SAS Institute Inc., Cary, USA), Version 9.3. Parts of the analysis for the investigation on familial aggregation and socio-demographic correlates of taste preferences in European children were conducted with software R, version 3.1.0 [59] (publication 4). Familial correlations in publication 4 were conducted using the software package SAGE (Statistical Analysis for Genetic Epidemiology software), version 6.3 [60]. The significance level was set at α= 5%. Elaborate, state-of-the-art statistical methods were used to investigate the research questions (Chapter 2.2) and to account for the structure of the data and for the specific research questions focusing either on descriptive methods or on multivariable regression models.

<u>Measuring the number of fungiform papillae on the tip of the tongue (Publication 1)</u>

Our research question was whether measuring the number of fungiform papillae on the tip of the tongue is a suitable objective method to complement sensory taste perception tests in children. To answer this question we analysed whether the number of fungiform papillae was associated with sensory taste perception and preferences in children. We tested the following hypotheses:

1. The number of fungiform papillae differs between children with different bitter taste thresholds.
2. The number of fungiform papillae differs between children having a higher sweet, salty, umami or fat taste preference and children not having a higher sweet, salty, umami or fat taste preference.

We tested the hypotheses as follows. The continuous variable number of FP present in categories of every bitter taste threshold (threshold 1 – 5) was pictured in a scatterplot to analyse the association of bitter taste threshold and the number of FP. To test whether those children who reported different taste preferences (dichotomous variable, exposure variable) had a different number of FP on the tip of their tongue (outcome Variable) a Mann-Whitney U-test was calculated. The same test was applied to test differences of the number of FP between different exposure groups, namely the sexes (dichotomous variable), weight status (dichotomous variable) and age-groups (categorical variable). Because of our relatively small sample size we conducted this non-parametric test and no further regression analysis.

<u>Correlates of sensory taste thresholds for sweet, salty, bitter and umami (Publication 2)</u>

We aimed at investigating whether sensory taste thresholds for bitter, sweet, salty and umami are correlated with age, sex, country of residence and weight status in children. From this research question we derived the following hypotheses:

1. The mean sensory taste thresholds for sweet, salty, bitter and umami differ between boys and girls.
2. The mean sensory taste thresholds for sweet, salty, bitter and umami differ between overweight/obese and under-/normalweight children.
3. The mean sensory taste thresholds for sweet, salty, bitter and umami differ between children from different countries.

To test these hypotheses the mean taste thresholds (mmol/l) and corresponding standard deviations (SDs) were calculated by age, sex and country. One-way ANOVAs and *t*-tests were used to examine threshold differences of all taste modalities between sexes, countries, age and weight (according to Cole and Lobstein (2012)[61]) groups.

To be able to analyse trend effects (for age) and the size of effects (for sex, weight status and age) we conducted ordinal logistic regressions to investigate the association between sex (as dichotomous exposure), weight status (as dichotomous exposure), age (as continuous exposure) and the four taste modalities (as outcome), separately. We chose the ordinal logistic regression because our outcome (taste sensitivity) was operationalised as an ordinal scaled variable. Naturally, taste sensitivity is a linear function but we were only able to test several concentrations to determine the individual taste threshold. Still, conducting a ordinal logistic regression provides more information than conducting a logistic regression analysis by categorising the outcome into a dichotomous variable. The odds ratios (ORs) to have a one level lower sensory taste threshold (i.e. being one threshold level more sensitive) for girls, overweight/obese children and children being one year older and the corresponding 95% CI were derived. Furthermore, the corresponding ordinal logistic regressions were conducted using interaction terms between sex and country, weight status and country and age and country to test, if the directions of associations differ by country. To derive adjusted ORs the whole ordinal logistic regression analyses were repeated with adjustment for sex, country, residing in control or intervention region and weight status. To emphasise the age trend raw and adjusted ORs were also calculated for an increase in age of three years.

<u>Parental consumer attitudes and children's taste preferences as well as food choices (Publication 3)</u>

We investigated if parental consumer attitudes are associated with their children's sweet, fat and umami preferences as well as with their sweet, fatty and processed food choices. Further, we analysed if parents with a lower education level more often have unfavourable consumer attitudes in terms of trusting in foods and beverages known from advertisements and not trying to avoid additives in food. We derived the following hypotheses from these research questions:

1. Parental consumer attitudes are associated with their children's sensory taste preferences for sweet, salty, umami and fatty.
2. Parental consumer attitudes are associated with their children's sweet and fatty foods consumption propensity, with children's processed food consumption frequency and with children's quality of their diet.
3. Parental consumer attitudes are associated with their own education level.

To test these hypotheses the associations between the two dichotomised variables for parental consumer attitudes (exposure variable) and the dichotomous outcome variables children's sweet, fat and umami taste preferences were estimated using logistic regression models. Although losing some of the information by dichotomising the outcome variable we suggest that this was reasonable for a better interpretation of the results. Models were adjusted for sex (dichotomous variable), age (continuous variable), country (categorical variable), weight status (dichotomous variable) and highest parental education level (categorical variable) as fixed effects. Logistic regression was also used to analyse the association between highest parental education level and parental consumer attitudes. These regressions were adjusted for country.

The associations between parental consumer attitudes (exposure variable) and the continuous outcome variables fatty, sweet as well as processed food consumption scores and the healthy diet adherence score (HDAS) of their children were analysed with linear regression models adjusted for sex, age, country, weight status and highest parental education level as fixed effects.

The scores to describe the quality of the diet (fatty, sweet as well as processed food consumption scores and the HDAS) were continuous variables. Therefore, we estimated a linear regression model. We decided not to categorise the outcome variables since there is no reasonable cut-off. Still, the results were meaningful and interpretable. The calculation of scores instead of using the raw frequency of sweet and fatty foods allowed us to consider the sweet and fatty food consumption in relation to the total food consumption frequency.

Familial aggregation and socio-demographic correlates of taste preferences (Publication 4)

We aimed at investigating whether there is a familial aggregation of taste preferences for sweet, salty, fatty and bitter in European families. We derived the following hypothesis from this research question.

1. Taste preferences for sweet, salty, bitter and fatty are correlated between boys <12 years, girls <12 years, boys ≥12 years, girls ≥12 years and their mothers as well as fathers and between siblings.

The descriptive analyses of study characteristics of the study population was conducted for each stratum (boys <12 years, girls <12 years, boys ≥12 years, girls ≥12 years, their mothers and fathers) as well as for each participating country. The quartiles (median, p25, p75) of sweet, salty, fatty and bitter liking scores (categorical variable) of each stratum were calculated.

To test the hypothesis we first adjusted for the effect of age on taste preferences by obtaining age-standardised residuals from taste preference scores from regression analyses separately for each stratum. Then, the residuals were used to analyse the correlations between taste preferences of parents and children as well as between and among younger and older siblings. Inter- and intra-class correlations for all relative pairs of a family were estimated using the FCOR (family correlations) program in SAGE (Statistical Analysis for Genetic Epidemiology software), version 6.3 [60].

Additionally, for each sex-by-age stratum, the proportion of variance in sweet, salty, fatty and bitter preference scores that could be explained by the mother's, father's, brothers' and sisters' preference scores and country (potential correlates) was estimated. Several linear mixed models were estimated: A null model, including only a random intercept term for family membership and another model, including the random intercept term and each of the potential correlates as exposure variable only. Taste preference scores for each taste modality were the outcome variables. Based on these models, the proportion of variance in children's taste preference scores that could be explained by preference scores of mothers, fathers, brothers and sisters was calculated. Additionally, the proportion of variance in taste preference scores that could be explained by country was calculated. To assess the impact of sex, age (continuous exposure variable) and highest education level on taste preferences, non-stratified taste preference scores (all children and parents) for each taste modality as outcome variables in a linear mixed model were used.

We chose mixed models with the family affiliation as random effect for our analysis because we assume that family members are more similar to each other with respect to the outcome (taste preferences) than individuals not belonging to the same family.

3.8 Practical experiences from the surveys and main findings

This thesis aimed to investigate correlates of sensory taste perception and preference, but also to place sensory research in a public health context by analysing the impact of sensory perception and preference on weight status in a cohort of European children.

The results of the investigations comprised in the publication 1 to publication 4 of this thesis showed associations of sensory taste perception and preference with weight status and described potential correlates of sensory taste perception and preferences in childhood. This chapter will discuss practical experiences from the surveys and the main findings against the background of relevant literature.

3.8.1 Practical experiences from the surveys

Assessing sensory taste perception and preferences in children across different European countries is challenging. All test procedures need to be standardised and age-appropriate protocols have to be developed. In the course of the IDEFICS and I.Family studies, five different test procedures were developed and implemented in order to measure different dimensions of sensory taste in growing children.

When we started sensory taste perception tests in T0, these kinds of measurements were new to most of the field staff. Therefore and to standardise test procedures, field staff from every country participated in a central training that took place at the coordinating centre before starting the field phase. Preparation of the serial dilutions for the taste threshold tests were practised many times and consequently only trained field staff could prepare the dilutions during the field phase. This implicated certain inflexibility regarding the field staff that could conduct the taste threshold tests. To the field staff the sensory taste perception tests seemed to be interesting and exciting. Experience from the field phase revealed that preparing the solutions and test samples was time consuming and sometimes complicated, especially the solutions for the threshold tests.

For the participants the sensory taste perception tests presented an interesting change compared to the other test procedures like questionnaires or body composition measurements. Participants perceived their participation in the sensory taste perception tests rather as being interesting compared to filling questionnaires.

Due to the time-consuming survey protocol, it was planned in T0 to recruit a sub-sample of 20% of the school children of the IDEFICS sample aged between 6 and 9 years to participate in the sensory taste perception tests. Finally, about 11% of the whole IDEFICS sample was examined within the sensory taste perception module. Highest response rates were observed in Estonia (18%) and lowest in Cyprus (4%, only taste threshold tests) [26]. About 1,900 children from eight countries participated in T0 and about 15,000 children and parents participated in T3 in the sensory test modules.

As reported by the survey centres or discovered during the site visits of the central quality control unit of the project, several problems occurred during the measurements. The survey centres could not always guarantee for the best possible test conditions. It was, for example, sometimes difficult to find quiet and friendly rooms in the schools and to guarantee the absence of undesired odours and sources of distraction, as the survey personnel depended on the schools to offer them appropriate rooms for the period of the examination.

Especially as sensory tests were largely unknown to the experimenters the detailed description of test procedures in the general survey manual, the central training, the provision of SOPs and the site visits of the coordinating centre to all survey centres turned out to be very important. Otherwise non-compliance to the SOPs could occur, e.g. terminating the test sequence of the threshold test once the child detected the first test solution deviant from water or conducting the preference tests in the wrong order (1st crackers, 2nd juice).

As mentioned above, the survey centres complained about the time-consuming preparation of test solutions for the threshold tests (despite of the pre-packaged portions of chemicals that were supplied). The workload caused by the preference tests on the other hand was much smaller since all test materials were delivered ‘ready-to-use’ by the coordinating centre. In general, the experiences from the survey underline the beneficial effect of a central supply of consumables. Especially multicentre studies depend on the maximal standardisation of test materials to obtain comparable results from different settings and countries.

Finally, all centres reported that the game-like character of the sensory test module enhanced the acceptance of this survey module and made it quite popular among the participating children. Furthermore, the sensory tests were reported to be the participants’ favourite procedure during the surveys, which also resulted in a high response rate. In general, all test procedures were age appropriate according to the study populations of IDEFICS and I.Family as they never turned out to require too high cognitive abilities.

Chapter 3.6.1 to 3.6.4 and chapter 3.8.1 describe the sensory methods used, methodological considerations as well as practical experiences from the surveys. In conclusion, it can be said that

1. measuring the number of FP in a defined circle on the tip of the tongue is little demanding for the participants but very costly, time-consuming and laborious for the researchers. Our results showed that the number of FP might be associated with fat preference in children. Therefore, this measure can be of interest for future studies focusing on fat perception and preference.
2. assessing taste thresholds for the basic tastes in children in a cross-national study is on the one hand suitable because material can be easily purchased, stored and shipped. But on the other hand pre-packing of the material as well as preparing the test solutions is time-consuming and performing the test procedure still requires a certain level of cognitive skills to obtain reliable results. Our results showed that taste thresholds were associated with weight status.

3. applying a paired comparison forced- choice preference test with real foods in a cohort of children within a cross-national study is quick and easy to conduct, material can be shipped and stored easily and test preparation for the survey centres is not time-consuming. The central and standardised preparation of the food samples can be challenging and should be considered well before planning to implement such a test in future study protocols.
4. implementing a questionnaire to assess taste preferences in a large scale cross-national study presents a suitable alternative to a practical taste preference test. No equipment and time-consuming test preparation is necessary which means it is also rather cheap. For the study participants it is also very quick and easy to complete. Our results suggest taste- specific food items that are suitable to evaluate taste preferences in every age-group and others that are not suitable to assess taste preferences. The suitable foods should be considered in future studies. It is also possible to consider the implementation of such a questionnaire as a computer tool in future studies. This could be even more attractive to the study participants.

3.9 Main findings

Previous studies investigating the number of FP have focused on their association with the ability to perceive PROP (Propylthiouracil) (e.g. taster status) [62-64]. In 1998, Tepper and Nurse showed for the first time an association between the number of FP and fat perception as well as preference in adults. They have found that participants with a higher number of FP could discriminate differences in fat content of salad dressings compared to participants with a lower number of FP, who were not able to do so but simply preferred the salad dressing with higher fat content [65]. Nachtsheim & Schlich have found that adults with a higher number of FP rated the fat content of milk and cream higher compared to participants with a lower number of FP [66]. We observed, for the first time in children, a tendency towards a lower number of FP in children with a higher fat preference. The lower preference for crackers with added fat in children with a higher number of FP may be explained by the higher fat taste intensity of these children who might therefore need less fat to be satisfied. Consequently, we would expect them to choose foods with lower fat content rather than a higher fat content.

Although studies investigating sensory taste perception in children are still rare and results are partly conflicting [16, 17, 36], most studies have reported that children are less taste sensitive [67-69] but have a higher number of FP than adults [70, 71]. Our results showed comparable tendencies and support the assumption of a possible decrease of the number of FP with every additional year of life. A reason for this finding may be the rapid development of the taste apparatus and the growing tongue during this phase of childhood. Due to the growth of the tongue the number of FP on a defined area may decrease during childhood. In our data 7 and 8 year old children had higher sweet, salty and umami thresholds than older children (9 to 11 year old). For bitter taste, the results suggest a reverse association; younger children (7 to 9 year old) had lower bitter thresholds than older children (10 and 11 year old). The observed age trend of sweet and bitter thresholds may be evolutionarily meaningful. Sweet thresholds may be elevated during early childhood to ensure sufficient energy intake and bitter thresholds in contrast are possibly lowered to ensure the detection of possible toxins [72, 73]. Taste preferences also seemed to change with age. In publication 4, where parents were included, sweet preference declined with increasing age. Further, analyses revealed that salty and fatty preference scores decreased, while bitter taste preference scores increased with age (publication 4). Furthermore, we observed that 17%, 16% and 7% of total variance in sweet, fatty and bitter taste preference scores, respectively, could be explained by age. The strong age effect on the development of taste preferences may be explained by evolutionary mechanisms, as described above.

Our study was able to show that taste thresholds varied substantially between children from different countries. These differences seemed to be systematic, e.g. Cypriot and Belgian children had relatively high taste thresholds compared to Hungarian, Estonian and Spanish children. Nationally common diets may result in a particular exposure of their population and may shape taste thresholds over the long term.

We observed that overweight/ obese children had higher sweet and salty thresholds than underweight/ normal weight children. Children with higher sweet and salty taste thresholds might consume more foods high in sugar or salt because they perceive these tastes as less intense and may need more sugar and salt to get a comparable sweet and salty sensation. Foods high in sugar or salt (e.g. sweet and salty snacks) are also more energy dense and may thus contribute to the development of overweight and obesity [1]. A recent study in 7 to 14 year-old children and adolescents did not find an association between BMI z-score and sucrose thresholds and contrary to the expectations, children with a higher percentage of body fat had even lower thresholds for sucrose [18]. Their age range (7 to 14 years vs. 6 to 11 years) as well as mean age (10.4 years vs 8.2 years) were higher than in our sample and growth velocity might be higher than in our younger study sample. As adolescents grow faster which lowers their BMI physiological changes during different phases of maturation may explain these contradicting results. In accordance with earlier research, we did not find any differences between overweight/ obese and underweight/ normal weight children regarding taste thresholds of the other taste modalities [19, 74]. Due to the cross-sectional design of our study, we cannot draw conclusions about the direction of the association between sweet and salty thresholds and weight status. Higher sweet and salty thresholds may lead to higher consumption of sweet and salty foods which may lead to overweight. Or overweight children who consume high amounts of sweet and salty foods get accustomed to these taste modalities leading to higher sweet and salty thresholds.

Further, we found that children from parents who trusted in products they know from advertisements consumed more processed foods and they had the tendency to consume more often fatty and sweet foods and beverages. Previous studies in the USA and in Europe showed that 20% of commercials shown on TV promote food products and 98% of those advertisements promote foods and beverages high in sugar, sodium and/or fat [75-77]. As a result, those parents trusting in foods and beverages they know from advertisements may preferably buy these products, which may explain the tendency towards a lower overall quality of the diet and a higher consumption frequency of sweet and fatty as well as a higher consumption of processed foods and beverages.

We also observed that children of parents who did not avoid additives in their diet had a higher consumption frequency of sweet foods and beverages, fatty foods and processed foods. A recent study from Norway has found a negative association between a consumer pattern labelled 'healthy', which included the importance of absence of additives, and junk/ convenience food consumption [78]. They have concluded that healthy eating promotion of children should focus on parents' health concerns emphasising natural food without additives. Or results showed that parents with a lower education level trusted more often in products they know from advertisement and tried less often to avoid additives in foods.

It has been found that children of lower educated parents had a lower overall diet quality and consumed more sweet and fatty foods [79]. Hence, our results added valuable knowledge about the pathway from a low education level to an unfavourable diet implying that families with a lower socioeconomic status consume unhealthy and more obesogenic foods because they tend to rely on advertisements more often and do not try to avoid additives as our results suggest.

For all taste modalities, familial correlations were highest among young siblings while among older siblings this correlation was only present in girls. The hypothesis that children resemble their parents' food and taste preferences could only be partly confirmed. Nevertheless, these findings indicated that there are similarities among family members.

To sum up, our main findings were

1. Sensory taste perception and preferences were associated with age (younger children were less taste sensitive and had higher sweet and fatty taste preferences) and sex (girls had the tendency to be more taste sensitive) but not passed on from parents to children.
2. Sensory sweet and salty thresholds were associated with weight status (Overweight/obesity increased the chance to be less sweet and salty taste sensitive) in European children.
3. The education level of parents influenced their consumer attitudes and through this the quality of their children's diet (unfavourable consumer attitudes of parents like trusting foods and drinks from advertisement and not avoiding additives in foods and drinks were associated with a lower quality of their children's diet. Unfavourable consumer attitudes in turn were associated with the educational level of parents (less educated parents had more often unfavourable consumer attitudes)).

3.10 Longitudinal consideration of sweet and fat taste preference

As stated above the sweet and fat taste preference tests described in chapter 3.6.3 were conducted during T0, T1 and the CG. In total, 47 Children conducted the fatty taste preference test at all three time-points whereas 53 children conducted the sweet taste preference test at all three time-points. Table 5 and Table 6 show the characteristic of those children. The highest proportion (64%) of children preferring the high fat cracker was found in the youngest age-group at T0 (mean age 7.1 years). During T1 and CG the fat preference remained stable (55%). The highest proportion (60%) of children preferring the high sweet juice was found in the middle age-group at T1 (mean age 9.0 years).

Table 5: Longitudinal assessment of fat taste preference. Characteristics of 47 children who participated in fat taste preference tests during T0, T1 and CG are either mean (standard deviation) or frequency (percentage)

	T0	T1	CG
Age (years)	7.1 (0.76)	9.1 (0.74)	14.5 (0.80)
Girls (n(%))	28 (59.6)		
Country			
Belgium	4 (8.5)		
Cyprus	4 (8.5)		
Estonia	14 (29.8)		
Germany	4 (8.5)		
Italy	9 (19.2)		
Spain	11 (23.4)		
Sweden	1 (2.1)		
Fat preference	30 (63.8)	26 (55.3)	26 (55.3)
BMI category			
Underweight			3 (6.4)
Normalweight	29 (61.7)	27 (57.5)	20 (42.6)
Overweight	10 (21.3)	13 (27.7)	18 (38.3)
Obese	8 (17.0)	7 (14.9)	6 (12.8)
Bodyfat	32.54 (7.42)	34.21 (8.28)	-
Height (cm)	124.89 (8.36)	136.69 (8.08)	165.66 (10.02)
Delta height (cm)		11.79 (2.25)	28.97 (5.10)

Table 6: Longitudinal assessment of sweet taste preference. Characteristics of 53 children who participated in sweet taste preference tests during T0, T1 and CG are either mean (standard deviation) or frequency (percentage)

	T0	T1	CG
Age (years)	6.9 (0.7)	9.0 (0.7)	14.3 (0.78)
Girls (n(%))	30 (56.6)		
Country			
Italy	16 (30.2)		
Estonia	14 (26.4)		
Belgium	6 (11.3)		
Sweden	1 (1.9)		
Germany	4 (7.6)		
Spain	12 (22.6)		
Sweet preference	25 (47.2)	32 (60.4)	23 (43.4)
BMI category			
Underweight			3 (5.7)
Normalweight	38 (71.7)	33 (62.3)	27 (50.9)
Overweight	8 (15.1)	14 (26.4)	18 (34.0)
Obesity	7 (13.2)	6 (11.3)	5 (9.4)
Bodyfat	31.24 (7.26)	32.75 (8.19)	-
Height (cm)	122.82 (7.99)	134.62 (7.57)	163.77 (9.10)
Delta height (cm)		11.80 (2.04)	29.15 (5.17)

As mentioned in chapter 3.8.2 one hypothesis we generated from our results was that children undergoing a growth spurt might have higher nutritional needs and therefore might have a different sensory taste perception (e.g. taste thresholds) and different sensory taste preferences than children who are currently growing more slowly. Therefore, we analysed the mean change of height between T0 and T1 and between T1 and CG. We found that children preferring the high fat cracker or the high sweet juice grew approximately 2 cm more between T1 and CG (Table 7 and Table 8). It could be that children growing faster prefer higher concentrations of fat or sugar to fulfil their higher nutritional needs. This is also in line with a finding from Coldwell et al. indicating that children with a lower sweet preference had lower concentrations of a marker of bone growth (the bone resorption marker type I collagen cross-linked N-telopeptides (NTx)) in urine than children with a higher sweet preference [80].

Table 7: Mean (standard deviation) of growth in cm between either T0 and T1 or T1 and CG in the different fatty taste preference groups

	Deltaheight T1-T0	Deltaheight CG-T1
Basic taste preference	11.6 (1.5) (n=21[1])	27.5 (3.9) (n=21[2])
Fat taste preference	11.9 (2.7) (n=26[1])	30.1 (5.7) (n=26[2])

[1]: Number of children having basic or fat taste preference at T1, respectively
[2]: Number of children having basic or fat taste at CG, respectively

Table 8: Mean (standard deviation) of growth in cm between either T0 and T1 or T1 and CG in the different sweet taste preference groups.

	Deltaheight T1-T0	Deltaheight CG-T1
Basic taste preference	11.8 (1.7) (n=21[1])	28.4 (4.8) (n=30[2])
Sweet taste preference	11.8 (2.3) (n=32[1])	30.1 (5.6) (n=23[2])

[1]: Number of children having basic or fat taste preference at T1, respectively
[2]: Number of children having basic or fat taste at CG, respectively

3.11 External validity

An issue in public health research is validity which can be distinguished in internal and external validity. Internal validity refers to the degree to which the observed associations can be seen as causal relationship and can be achieved by highly controlled study conditions [81]. External validity in contrast refers to the degree to which the observed results can be generalised to different populations [82]. External validity must be assured when translating research into public health practice for example. The extent of external validity of research results can be assessed by replications in different populations at different times and places. To get more insight into the external validity of our results we replicated one part of the analysis of publication 3 in the full T0 IDEFICS sample with all available co-variables which resulted in a sample of 11,786 children.

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The characteristics of the larger sample can be found in Table 9. Although the two samples differed for example in mean age (8.8 years vs. 6.0 years) and distribution across countries the results could be replicated. All directions of effect sizes remained the same (Table 10 and 12). The association between the HDAS and trusting in advertisements as well as the association between parental education level and not avoiding additives was stronger in the larger sample. Whereas the association between the consumption frequencies of processed foods and trusting in advertisements was stronger in the smaller sample. Thus, although the two study samples differed with respect to age and origin (e.g. in the small sample where no children from Cyprus whereas the larger sample consisted of 7.4% Cypriot children) the analyses revealed comparable results. This even strengthens the importance of the results. Table 11 and Table 13 show the original results of publication 3 to facilitate the

Table 9: Characteristics (total number and percentages or mean and standard deviation (SD)) of exposure and outcome variables given by boys and girls

	Boys	Girls	Total
	N=6,001 (50.9%)	N=5,786 (49.1%)	N=11,786
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Age	6.0 (1.8)	6.0 (1.8)	6.0 (1.8)
Sweet propensity score %	25.9 (11.8)	25.0 (11.4)	25.4 (11.6)
Fat propensity score core %	25.8 (9.4)	25.6 (9.2)	25.7 (9.3)
Processed foods/week	6.4 (5.7)	6.3 (5.7)	6.4 (5.7)
Healthy diet adherence score	21.7 (8.9)	22.2 (8.8)	22.0 (8.9)
	N (%)	**N (%)**	**N (%)**
Overweight/obese[1]	1,076 (17.9)	1,185 (20.5)	2,261 (19.2)
Parents who trust in advertisements (n=10,579	1,024 (19.1)	989 (19.0)	2,013 (19.3)
Parents who do not avoid additives	1,272 (21.6)	1,177 (20.8)	2,449 (21.2)
Highest parental education level[2]			
Low	386 (6.4)	344 (6.0)	730 (6.2)
Medium	2,693 (44.8)	2,552 (44.1)	5,245 (44.1)
High	2,922 (48.7)	2,890 (50.0)	2,890 (49.3)
Country			
Belgium	754 (12.6)	691 (11.9)	1,445 (12.3)
Cyprus	453 (7.6)	422 (7.3)	875 (7.4)
Estonia	636 (10.6)	673 (11.6)	1,309 (11.1)
Germany	753 (12.6)	731 (12.6)	1,484 (12.6)
Hungary	1,056 (17.6)	1,045 (18.1)	2,101 (17.8)
Italy	973 (16.2)	895 (15.5)	1,868 (15.9)
Spain	658 (11.0)	634 (11.0)	1,292 (11.0)
Sweden	718 (12.0)	695 (12.0)	1,413 (12.0)

[1]: BMI z-scores according to Cole and Lobstein 2012 [61].

[2]: International Standard Classification of Education Maximum (ISCED) [83]; maximum of both parents (0, 1, 2 = low education; 3, 4 = medium education; 5, 6 = high education).

Table 10: Association between parental consumer attitudes and their children's food consumption[1]

	Parents trust in advertisements[2]		Parents do not avoid additives[3]	
Outcome:	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**
Proportion of sweet food and beverage consumption	1.59 (1.03; 2.16)	<0.0001	2.46 (1.96; 2.95)	<0.0001
Proportion of fatty food consumption	0.77 (0.29; 1.26)	0.002	1.42 (0.99; 1.85)	<0.0001
Processed food consumption frequency	0.33 (0.06; 0.60)	0.02	0.79 (0.52; 1.03)	<0.0001
Healthy Diet Adherence Score	-1.23 (-1.66; -0.81)	<0.0001	-2.66 (-3.04; -2.29)	<0.0001

Abbreviations: ß= ß-coefficient, CI=Confidence interval
[1]: Model is adjusted for sex, age, country, ISCED (International Standard Classification of Education Maximum (ISCED) [83]) and weight status.
[2]: Reference: Parents do not trust in advertisements.
[3]: Reference: Parents avoid additives.

Table 11: Table 3 from publication 3 about the association between parental consumer attitudes and their children's food consumption[1]

	Parents trust in advertisements2		Parents do not avoid additives3	
Outcome:	ß (95% CI)	p-value	ß (95% CI)	p-value
Proportion of sweet food and beverage consumption	0.97 (-0.45; 2.40)	0.18	2.37 (1.03; 3.70)	0.0005
Proportion of fatty food consumption	0.79 (-0.43; 2.00)	0.21	2.27 (1.12; 3.43)	0.0001
Processed food consumption frequency	1.21 (0.49; 1.93)	0.001	0.91 (0.22; 1.59)	0.0093
Healthy Diet Adherence Score	-0.42 (-1.50; 0.67)	0.45	-2.87 (-3.89; -1.85)	<0.0001

Abbreviations: ß= ß-coefficient, CI=Confidence interval
[1]: Model is adjusted for sex, age, country, ISCED (International Standard Classification of Education Maximum (ISCED) [83]) and weight status.
[2]: Reference: Parents do not trust in advertisements.
[3]: Reference: Parents avoid additives.

Table 12: Influence of parental ISCED level on their consumer attitudes[1]

	High ISCED level (Reference)		Medium ISCED level				Low ISCED level			
Outcome:	**N**	**%**	**N**	**%**	**OR (95% CI)**	**p-value**	**N**	**%**	**OR (95% CI)**	**p-value**
Parents trust in advertisement										
No	4624	87.3	3571	76.9	1.00 (reference)		371	58.0	1.00 (reference)	
Yes	672	12.7	1072	23.1	1.34 (1.19;1.51)	<0.0001	269	42.0	1.97 (1.60;2.42)	<0.0001
Parents do not avoid additives										
No	4657	80.9	3966	77.5	1.00 (reference)		472	70.2	1.00 (reference)	
Yes	1097	19.1	1152	22.5	1.75 (1.58;1.94)	<0.0001	200	29.8	3.17 (2.57;3.90)	<0.0001

Abbreviation: OR: Odds ratio, CI: Confidence interval, ISCED: International Standard Classification of Education Maximum (ISCED) [83]; maximum of both parents (0, 1, 2 = low education; 3, 4, 5 = medium education; 6, 7, 8 = high education)

[1]: Model is adjusted for country.

Table 13: Table 4 from publication 3 about the influence of parental ISCED level on their consumer attitudes[1]

	High ISCED level (Reference)		Medium ISCED level				Low ISCED level			
Outcome	**N**	**%**	**N**	**%**	**OR (95% CI)**	**p-value**	**N**	**%**	**OR (95% CI)**	**p-value**
Parents trust in advertisement										
No	600	82.6	456	75.5	1.00 (reference)		44	57.1	1.00 (reference)	
Yes	126	17.4	148	24.5	1.04 (0.77;1.40)	0.81	33	42.9	2.01 (1.15;3.54)	0.02
Parents do not avoid additives										
No	539	76.9	403	70.2	1.00 (reference)		52	73.2	1.00 (reference)	
Yes	162	23.1	171	29.8	1.91 (1.44;2.54)	<0.0001	19	26.8	1.76 (0.96;3.24)	0.07

Abbreviation: OR: Odds ratio, CI: Confidence interval, ISCED: International Standard Classification of Education Maximum (ISCED) [83]; maximum of both parents (0, 1, 2 = low education; 3, 4, 5 = medium education; 6, 7, 8 = high education)

[1]: Model is adjusted for country.

3.12 Strengths and limitations

A variety of strengths and limitations of the methods and conditions of these investigations were discussed in detail at different parts of the current chapter. The conclusions about the sensory taste tests analysed in this thesis can be found in chapter 3.8.1.

In summary, for future investigations we would suggest to use real foods in sensory taste tests instead of using watery solutions. Further, tested concentrations should be in the range of concentrations present in daily life. A taste intensity test using suprathreshold intensities has been proposed as a measure with an impact on everyday life's decisions regarding food choice [49]. Additionally, test samples should be presented in random order instead of fixed order as in our taste preference tests.

Beside these limitations several strengths should be mentioned. To our knowledge this was the first study that analysed sensory taste perception in children in a large population-based cross-European epidemiological study outside the laboratory setting in a real-world condition. We assured a high level of standardisation through central trainings of the field staff, providing SOPs and conducting field visits during surveys. Further, we supplied all materials centrally.

4 PUBLIC HEALTH IMPLICATIONS AND IMPLICATIONS FOR FUTURE RESEARCH

Results of this thesis revealed an association of taste perception and preference with weight status and of parental consumer attitudes with the quality of their children's diet. This underlines the importance and relevance of sensory science in health research. The following paragraph discusses public health implications and suggestions for future research resulting from these findings.

4.1 Public health implications

The main findings of this thesis have implications for future interventions as well as for health promotion programs aiming to improve children's diet and/ or prevent childhood overweight/ obesity.

First, the positive association of sweet and salty taste thresholds with overweight/ obesity in children mainly implies that there might be a link between taste perception and weight status in childhood. Due to the cross-sectional design of the analysis it is not possible to draw a conclusion about true causality. In either case, this should be considered when planning interventions and health programs: Such interventions should incorporate strategies to sensitise children to different taste modalities in conjunction with reduced sugar, fat and salt concentrations and healthy food choices. Further, age differences in sensory taste perception and preferences underline the importance to develop age specific healthy eating guidelines, e.g. emphasise the importance of fruits (sweet taste) when targeting younger children and vegetables (umami and bitter taste) when targeting older children/ adolescents/ adults. Although we found differences regarding taste preference between age-groups, parents still seem to have a substantial influence on their children's taste preferences and quality of their diet. The parental influence on their children's taste preferences seems to be partially determined by genetic factors but also results from family environmental factors, such as making food available to the children, parental role modelling and parental feeding practices like dietary restrictions. Thus, parents transmit their own taste preferences to their children in different ways. For public health actions parents could be an important access point to help children develop favourable taste preferences (e.g. lower sweet, fatty and salty preferences) and better quality of their diet. Parents should be aware of their responsibility but also their ability to help their children form healthy food preferences and make healthy food choices. Previous research showed that parents are aware of health recommendations for children but that they are partly unaware of their role of transmitting these health recommendations to their children [84]. Consequently, children are not aware of health-related rules. Interventions and health programmes should therefore promote the communication between parents (which act also as gatekeeper for the foods made available to the children during childhood) and children and should support parents to follow up the adherence of set rules.

Successful interventions should also consider the cultural background: The design of one intervention for implementation across Europe is barely feasible or reasonable. Culturally determined, children from one country might have extremely high sweet preferences compared to children from another country who might have elevated fat preferences. Thus, the messages of interventions might be the same but the focus needs to be different according to the cultural background, e.g. although all interventions follow the aim to implement a healthy nutrition, one has to focus on the reduction of sugar whereas another has to focus on the reduction of fat. One starting point could be launching all-day schools with implemented health concepts to promote for instance healthy eating concepts. The schools could consider cultural characteristics and their influence on children's behaviour would increase as children spend most of their time there.

Our results showed that unfavourable consumer attitudes of parents like trusting foods and drinks from advertisement and not avoiding additives in foods and drinks were associated with a lower quality of their children's diet. Unfavourable consumer attitudes in turn were associated with the educational level of parents (less educated parents had more often unfavourable consumer attitudes). There are different levels of action where interventions could be successfully implemented; the global level through education as well as political guidelines regarding sugar, fat and salt contents of processed foods and conditions for food advertisements, the family level as well as the individual level. Health enhancing policies should facilitate healthy choices of children and their families; policies should support parents in teaching their children healthy behaviours. They should also implement rules to limit food advertisement for unhealthy foods.

Since childhood overweight/ obesity presents a major public health issue throughout Europe and our suggestions for public health actions aim to reduce the risk of becoming overweight during childhood and to develop a healthy dietary behaviour these results are of great socio-political relevance.

4.2 Suggestions for future research

The presented results add valuable insight on the relationship of sensory taste perception and preferences with the quality of children's diet as well as overweight/obesity in childhood. Still, research is needed to investigate determinants and modifiability of sensory taste perception and preferences. Furthermore, investigations are needed to study the direction and pathways between sensory taste perception and preferences and overweight/ obesity.

The reason for the country-specific differences we have found for sensory taste perception needs to be investigated. Those differences could result from genetic differences or cultural and environmental influences which we did not consider in our analysis so far. Cultural differences that determine taste perception and preferences may be culture-specific customs or country-specific policies, for example. The same holds true for the family context. Apart from genetic determination, family factors like mere exposure, parental feeding practices such as dietary restrictions or home food availability because of parents' preferences as well as nutrition literacy and family income as well as education could be determinants of children's taste perception and preferences which need to be investigated.

We can answer open research questions partly by further analysing the IDEFICS and I.Family data. For example, it remains of great interest to investigate the formation, development and influencing factors of taste preferences longitudinally. In future analyses we plan to consider prospective data from IDEFICS and I.Family most suitably with an intervention and a control group determined in IDEFICS to study the development and modifiability of sensory taste preferences. Lifestyle related interventions were implemented between T0 and T1 in an intervention group [33]. Successful interventions need to be identified to modify taste preferences towards a favourable direction in order to achieve healthy food choices. However, not only complex interventions but also more simple ones that influence the development of taste preferences - and that can be addressed more easily - need to be identified through prospective investigations. Based on such factors it might be possible to modify taste preferences in a favourable direction.

Our results showed age and sex differences regarding the sensory taste perception and preferences which need to be further analysed. These differences might not depend on age or sex per se but rather on physiological differences and physiological needs that change during lifespan and possibly vary between sexes. Investigations of biological markers, like hormones and other metabolic markers, in association with sensory taste perception could reveal differences due to variances in the physiological status. An elevated need for energy due to a growth spurt for example could increase the preference for the sweet taste [80]. The investigation of biological markers simultaneously with sensory taste perception and preference could also reveal insights into the relationship between weight status and taste perception and preferences. The association between urinary cortisol and sweet as well as fat taste preference could give insight into the phenomenon of emotional eating. The association between markers of growth like N-telopeptides of type I collagen (NTx) and taste preferences could explain the association between physiological needs and taste preferences. In prospective studies, the direction of this association could be investigated by considering also biological and genetic markers.

Another factor that surely affects children's food choice is the texture of foods, but this association has not been studied in children across Europe yet. Food characteristics that influence the healthiness of foods, like fibre content, proportion of fruit pieces or fat content, influence the texture of the food. Children's food choice might be influenced by these differences because they are sometimes picky eaters. Apart from the association of texture with healthy food choice, differences in texture of foods children usually consume could also be associated with the development of other health aspects like mouth muscles, jaws and teeth.

Aside from these etiological aspects there are also some methodological aspects that need further investigation. Our methods were designed to be implemented in a cross-national setting and a high degree of standardisation was assured. Still, we did not assess the intra-individual differences in sweet and fat preferences using different food matrices to measure theses preferences. Cross-validation of sweet and fat preference tests in juice/cracker vs. pudding would provide more information about the suitability of these tests for future studies.

Hence, open research questions for future investigations are:

1. Are sensory perception and preferences modifiable through interventions?
    a. Which interventions are suitable to modify sensory taste perception and preferences?
2. Are sensory taste perception and preferences associated with physiological parameters like hormones and other biomarkers?
3. Which sensory taste perception test methods are most suitable to be applied in children?
    a. Do these methods deliver reliable and valid results?

PUBLIC HEALTH IMPLICATIONS AND IMPLICATIONS FOR FUTURE RESEARCH

This ample range of open research questions again highlights the importance of considering sensory taste perception and preference in health research. Sensory taste research is an innovative field which should be considered when aiming to improve health through healthy eating and reducing overweight and obesity.

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6 SUPPLEMENT

6.1 Publication 1 – publication 5

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ARTICLE

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Association between the number of fungiform papillae on the tip of the tongue and sensory taste perception in children

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ABSTRACT

Background: To measure sensory taste perception in children with an accurate and reproducible method is challenging and objective measurement methods are scarce.
Objective: Aim was to characterize sensory taste perception, by measuring the number of fungiform papillae (FP) and to investigate whether the number of FP is associated with sensitivity for bitter taste and with taste preferences for sweet, salty, fatty or umami in children between 8 and 11 years of age.
Design: Number of FP was measured with a digital camera in 83 children in a German subsample of the IDEFICS study. Among those 56 children performed a taste threshold test for bitter and taste preference tests for sweet, salty, fatty and umami. The association between the number of FP and sensory taste perception was analysed.
Results: There is a tendency towards a lower number of FP in children with a higher fat preference (30 vs. 25 papillae, p=0.06). Results show no association between the number of FP and neither the bitter taste thresholds nor taste preferences for sweet, salty and umami.
Conclusion: Bitter taste threshold might be independent of the number of FP, while the perception of fat was associated with the number of FP.

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Introduction

Dietary behavior is currently considered as one of the factors potentially affecting the development of overweight and obesity, one of the major public health problems among children in Europe. Food choices and dietary behavior in children have been found to be influenced by their taste preferences [1], which differ largely between individuals. Taste preferences might be influenced by individual taste sensitivity [2], which can be examined with sensory taste threshold tests. Conducting these tests with children has been found to be very challenging because the concentration span of children was rather short and their ratings were easily influenced by the examiner [3]. Taste sensitivity has also been evaluated by measuring and counting the number of fungiform papillae (FP). The FP are one of four types of papillae that are located on the tongue and contain multiple taste buds [4]. On top of the FP are taste buds that are responsible for the perception of taste. Although the number of taste buds varies from FP to FP the number of taste buds correlates with number of FP [4]. The number of FP differs between subjects and this may have an impact on the taste sensitivity and subsequently on the preference for the basic tastes [5]. The number of FP can be used as an objective marker that may be less influenced by cognitive abilities of participants. Furthermore, the number of FP cannot be influenced by previously eaten foods and drinks, whereas sensory taste preferences can possibly be influenced by the composition of a previously eaten meal [6–8]. This is an important advantage when using objective measurements: collected data are less probably biased and therefore help to avoid subjective reporting bias (induced through cognitive abilities or by influences of the researcher (e.g. wanting to please the researcher)) and short-term variations in sensory perception. The first method to measure the number of FP in living subjects on a defined area on the tongue by videomicroscopy was established in 1990 by Miller [9]. This extensive method

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was only suitable for laboratory settings and requires very special and non-portable equipment like a chin holder used for ocular examinations, oral examination chair, tongue chamber and a microscope. Shabake et al. developed a simplified method to measure the number of FP using a special digital camera. This method was evaluated against the formerly established one in a sample of seven adults aged 25–38 years and nine children aged 8–9 years [10]. Both methods provided comparable results. The number of FP was measured in a defined region on the tip of the tongue as this area was more easily accessible than the whole tongue and offered reliable results for the number of FP on the whole tongue [10,11].

The aforementioned challenges of the taste threshold measurements and the lack of alternative feasible methods to measure taste sensitivity in epidemiological studies was an obstacle to obtaining reliable and comparable results from different populations tested in different environments. A robust method in terms of portability that meets these challenges was needed to measure sensory taste sensitivity in children from the general population in different designs in large-scale epidemiological studies.

In the IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFects In Children and infantS) study we adapted the method of Shahbake and colleagues to examine the number of FP. The aim of the study was to characterize the sensory taste perception, by measuring the number of fungiform papillae (FP) with a photographic method and further to investigate whether the number of FP is associated with sensitivity for the bitter taste and with taste preferences for sweet, salty, fatty or umami in children between 8 and 11 years of age. If the proposed associations would be observed measuring the number of FP could be a suitable method to objectively measure sensory taste perception in epidemiological field studies of children.

Methods

Study design and sample

The measurement of the number of FP was conducted during the follow up in a subsample of 83 children of the German IDEFICS cohort aged 8-11 years [12]. Of the 83 children, 56 participated in the sensory taste perception test module which included the bitter taste threshold as well as the taste preference tests for the sweet, fatty, salty and umami taste. Parents gave their written informed consent on the participation of their children and the children gave their oral consent. Ethical approval was given by the institutional review board of the University of Bremen.

Instruments

Method 1: measuring fungiform papillae

The method to measure the number of FP was developed according to the photographic method described by Shahbake et al. [10].

Testing was performed in a sitting position and starting with cleansing the mouth by a sip of water. The child placed his or her chin on a non-moving fixture standing on a table in front of them that guaranteed a standard height position during all pictures. The child placed the elbows on the table and fixed the head with the hands. Then the child stretched out the tongue and held it firmly with the lips. The tongue was dried with a filter paper (Whatsmann No. 1). To color the tongue, a blue (brilliant blue E133) colored circled filter paper (6 mm diameter) was placed on the left side of the centerline on the tip of the tongue by using tweezers. The colored filter paper was removed after 5 seconds. An uncolored circled filter paper was placed on the right side of the tongue centerline and was used as a reference circle in case the color on the tongue drifted. Additionally, an individual ID number label was attached to the child's chin in order to assign the photo to the participant. After removing the filter paper the colored part of the tongue was pictured with a digital camera (Casio Exilim EX-H10, 12,1 megapixel) in macro mode, which was fixed on a tripod. Ten pictures were taken from each child. The pictures were transferred to a computer and were processed as well as analyzed with the program GIMP version 2 (GNU Image Manipulation Programme for Windows) (Figure 1). The number of FP of each participant was counted twice by two independent examiners and the mean number of FP was calculated in order to obtain the number of FP on a circle with a 6 mm diameter.

Method 2: sensory taste perception tests

Within the IDEFICS study, two methods were developed to measure (a) taste sensitivity and (b) taste preferences. The development of the standardized test procedures has been previously reported [13].

Taste sensitivity test. In the present study we analyzed the bitter taste threshold. A paired comparison staircase test for bitter threshold was applied by testing five aqueous solutions with ascending concentration of caffeine (0.26 to 1.3 mmol l^{-1}) against pure water. The first sample of the test solutions that was recognized as being different from water was assumed as the bitter taste threshold, which ranged from 1 to 5 according to the number of solutions.

Figure 1. Photos of the stained tongue with and without papillae counted.

Taste preference tests. To assess the taste preference for sweet, fatty, salty and umami we applied forced-choice paired preference tests with apple juice (sweet) and with crackers (fatty, salty and umami) [13]. Each child had to choose a preferred sample out of a pair. The pairs consisted of two samples, one was the basic sample and the other one a modified sample. Sweet preference was measured with a pair of apple juice. The basic sample contained 0.53% sucrose and the modified sample 3.11% sucrose. Fatty, salt and umami preference were measured with crackers. Basic crackers contained 8% fat, 0.7% sodium chloride and 0% monosodium glutamate and the modified sample 18% fat, 1.6% sodium chloride and 1% monosodium glutamate, respectively. The outcome variables were coded with 1 or 2 depending on the preferred sample of the test pairs.

Weight, height, BMI z-score and weight status

Children's weight and height was measured in a fasting state using a Tanita BC 420 SMA scale (TANITA, Tokyo, Japan) for weight measurement and a ECA 225 Stadiometer (SECA GmbH & KG, Hamburg, Germany) for height measurement. BMI was calculated and converted to age- and sex-specific *z*-scores [14]. Children were classified into thin/normal weight and overweight/obese using age- and sex-specific cut-points published by Cole et al. [15].

Statistical analysis

The number of FP that were present in categories of every bitter taste threshold was pictured in a scatterplot to analyze the association of bitter taste threshold and the number of FP. To test whether children with different taste preferences also had a different number of FP, a Mann–Whitney *U*-test was calculated. The same test was applied to test differences of the number of FP between the sexes, weight status and age groups. All analyses were carried out with SAS (Statistical Analysis System, SAS Institute Inc., Cary, USA), version 9.2.

Results

We analysed the number of FP in 83 children (46 girls and 37 boys; mean age 9.8) of the German IDEFICS study. The characteristics of this subsample were shown in Table 1.

The median of the number of FP within the defined circle was 29 (interquartile range: 23–35), ranging from 14 to 46. Only 2.4% of the children had less than 15 or more than 40 FP. There were no differences between boys and girls with regard to the number of FP. Normal weight children (n = 72) had a mean number of FP of 29.3 (SD = 6.8) and obese/overweight children

Table 1. Characteristics of the study sample (total numbers and percentages).

	Girls	Boys
***N* (%)**	46 (55.4%)	37 (44.6%)
Age (years)[a]	9.8 (8.8;10.1)	9.9 (9.4;10.4)
BMI *z*-score[a,b]	0.1 (−0.4; 0.9)	0.1 (−0.7; 1.0)
Overweight/obese[c]	6 (13.0)	5 (13.5)
Number of fungiform papillae[a]	29.3 (23.0; 35.0)	28.5 (24.0; 34.0)
Bitter taste threshold[d]		
Threshold 1 (0.26 mmol caffeine/l[a])	8 (23.5)	3 (13.6)
Threshold 2 (0.51 mmol caffeine/l[a])	12 (35.3)	5 (22.7)
Threshold 3 (0.77 mmol caffeine/l[a])	6 (17.7)	4 (18.2)
Threshold 4 (1.03 mmol caffeine/l[a])	5 (14.7)	1 (4.6)
Threshold 5 (1.29 mmol caffeine/l[a])	0 (0.0)	3 (13.6)
Non taster	3 (8.8)	6 (27.3)
Taste preferences[d]		
High sweet preference[e]	15 (44.1)	9 (39.1)
High salt preference[e]	24 (68.6)	14 (63.6)
High umami preference[e]	14 (40.0)	7 (31.8)
High fat preference[e]	15 (42.9)	6 (27.3)

Abbreviations: BMI *z*-score, Body Mass Index *z*-score.
[a]Numbers are median with interquartile range.
[b]BMI *z*-scores according to Cole et al. [14].
[c]BMI cutoffs according to Cole et al. [15]
[d]Sensory tests conducted in a subsample of n = 56 children.
[e]Preference for the food sample with the added ingredient over the basic food sample.

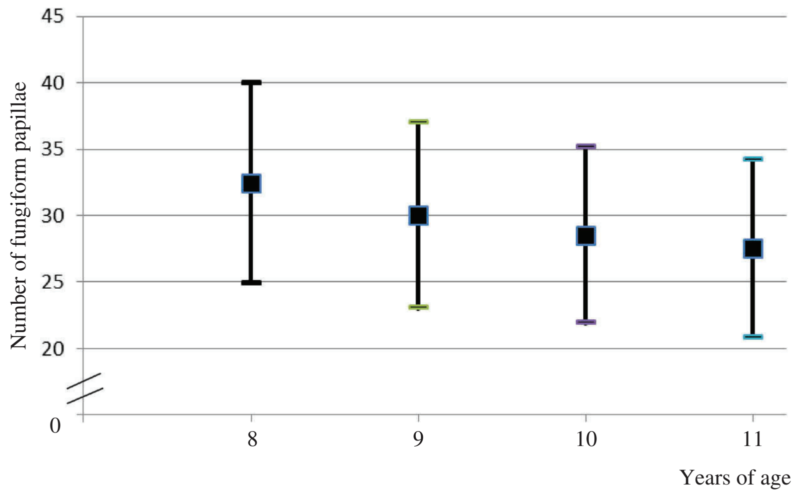

Figure 2. Median (SD) number of fungiform papillae in different age groups.

(n = 11) 26.5 (SD = 7.0). The mean number of FP decreased with increasing age (Figure 2).

There was no association between taste sensitivity for bitter taste and the number of FP. The distribution of the number of FP in categories of the bitter taste threshold is shown in Figure 3.

There was no association between the number of FP and taste preferences for sweet, salty and umami. Only regarding fat preference was there a tendency towards a lower number of FP in the group with the higher fat preference (30 vs. 25 papillae, p = 0.06) (Figure 4).

Discussion

We measured the number of FP in 83 children between 8 and 11 years in a free-living setting (during school hours). The median of the number of FP of this sample was 29. This number was higher than that reported in previous studies [10,16]. Due to our limited sample size we conducted descriptive statistics in the present study rather than inductive statistics and no conclusions about associations can be drawn. Interestingly, in our study, it seems that there is a possible decrease of number of FP with every increasing year of age. This

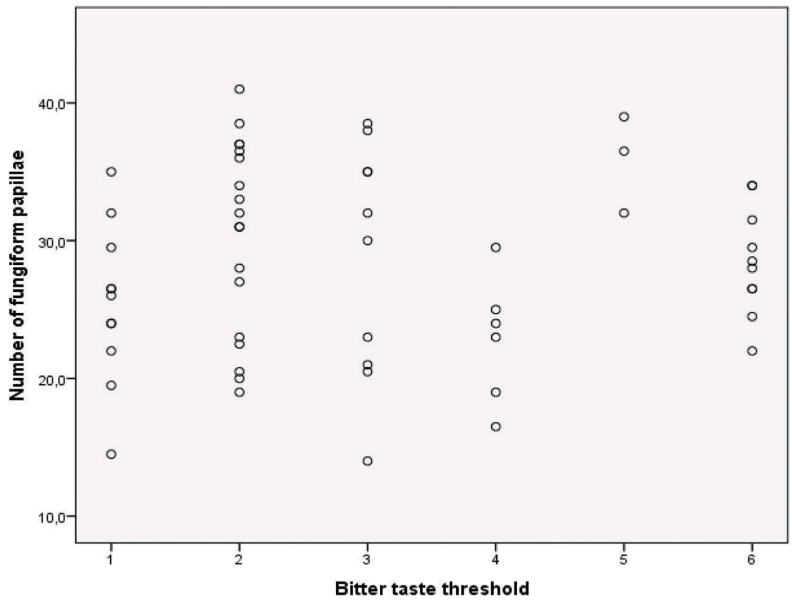

Figure 3. Distribution of fungiform papillae in categories of bitter taste threshold.

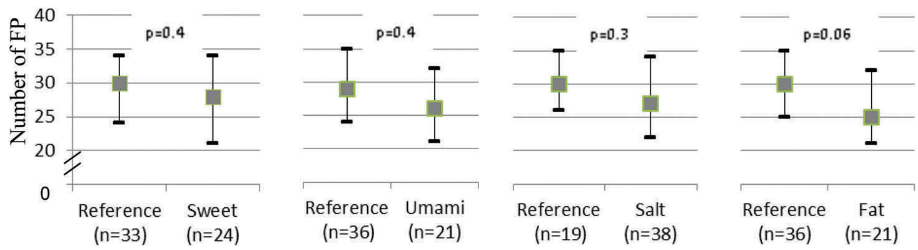

Figure 4. Median (Q1; Q3) number of fungiform papillae in different taste preference groups.

could be due to the rapid development of the taste apparatus that occurs during this phase of childhood. The anterior tongue is growing until approximately 8–10 years [17]. Therefore, the number of FP on a defined area decreases during childhood. This could be confirmed with our results. Our result was also in line with Stein et al. and Segovia et al., who showed a higher density of FP in children than in adults [4,18]. We found no differences between the number of FP for boys and girls. Other studies that compared the number of FP in women and men found higher numbers in women [19,20], suggesting that sex differences might occur later in life due to heterogeneous lifestyles, but this needs to be investigated. Two recent studies that investigated FP in children between 7 and 12 and 5- and 17-year-old children found similar numbers of FP on a 6 mm diameter circle [11,21]. The former found a decrease in number of FP with increasing age and also no differences between male and female participants [11]. In our study, we could not confirm an association between weight status and number of FP. Our result may be explained through the overall smaller sample size and by the small number of overweight or obese children.

A smaller subsample of 56 children additionally accomplished the bitter taste threshold test as well as sweet, salty, umami and fat preference tests. Due to the overall study design, sour taste was not investigated in this large-scale epidemiological study across various European countries. These tests were developed for children from the age of six and older. A pre-test was conducted and test–retest reliability was calculated [13]. We analyzed the association between the number of FP and the results of the taste threshold and taste preference tests. Because all taste tests were measured in the whole mouth we believe that a correlation would have been detected between the number of FP on the tip of the tongue with any of these taste perceptions if there was any. We did not find an association between the number of FP and the bitter taste threshold. Previous studies investigated the association between the number of FP and bitter taste perception using suprathreshold concentrations instead of concentrations around the bitter taste threshold as in the present study [16,22]. These previous studies found positive correlations suggesting that the number of FP might be more relevant for the perceived taste intensity than for the taste threshold. The aforementioned studies used propylthiouracil (PROP) to assess the bitter perception. This could have led to a stronger association between bitter perception and the number of FP because the perception of PROP is genetically determined [23]. We used caffeine instead of PROP, as PROP was classified as potentially carcinogenic [24] and was therefore ethically no alternative for use in children. Hall and Hayes et al. described that the thresholds for caffeine correlate with those for PROP and phenylthiocarbamide (PTC), a substance which was used for bitter perception testing before PROP [19,25].

We observed a tendency towards a lower number of FP in children with a higher fat preference. Tepper & Nurse and Nachtsheim & Schlich also investigated the association between the number of FP and fat perception and liking among adults [22,26]. Tepper & Nurse found that participants with more FP could discriminate differences in the fat content of salad dressings whereas participants with fewer FP were not able to do so but preferred the salad dressing with the higher fat content. Nachtsheim & Schlich found that participants with greater number of FP rated the fat content of milk and cream as higher compared to participants with a lower number of FP. The lower preference for crackers with added fat may be explained by the higher fat taste intensity of children with higher number of FP who might therefore need less fat to be satisfied. Consequently, they would choose foods with lower fat content rather than a higher fat content. The lack of significance of the association regarding the fat preference could be due to matrix effects influencing fat release and consequently fat perception. Fat in liquid foods is more ready to diffuse out compared to solid food matrices like crackers. Therefore, liquid foods could be more suitable to measure sensory fat perception.

In the present study we used a digital camera instead of a reflex camera. This could have led to measurement inaccuracies such as misidentification of papillae (counting filiform papillae as fungiform papillae) due to lower resolution of the obtained pictures. A higher resolution would be achievable by using a reflex camera with macro mode and a ring flash.

Conclusion

We concluded that measuring the number of FP is a suitable method to objectively measure characteristics of taste perception in children, in which objective measurements should be preferred when using a reflex camera with macro mode and ring flash as well as having an appropriate computer program for counting the number of FP. We observed a tendency towards a lower number of FP in children with a higher fat preference. Our results showed no association between the number of FP and the bitter taste thresholds and no association between number of FP and taste preference for sweet, salty and umami.

The issue of using the number of FP as an appropriate marker of taste perception still needs further and more in-depth investigations. In future studies examining children the number of FP should be measured in combination with the perceived taste intensity of different taste qualities.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Publication 2

Correlates of bitter, sweet, salty and umami taste thresholds in European children: Role of sex, age, country and weight status - the IDEFICS Study

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Abstract

We aim to describe sensory taste thresholds in children between 7 and 11 years across eight European countries. We further compare taste thresholds of boys vs. girls and under-/normalweight vs. overweight/obese children. Within the European multicentre IDEFICS (Identification and prevention of dietary and lifestyle-induced health effects in children and infants) study, 1 938 school children participated in sweet, bitter, salty and umami threshold tests between 2007 and 2010, using the paired comparison staircase method. The lowest concentration at which the child was able to identify taste a difference to water was determined as taste threshold. Mean taste thresholds were calculated stratified for sex, age groups, weight groups and country. We measured height as well as weight and collected socio-demographic information using questionnaires. One-way ANOVAs and *t*-tests were used to examine threshold differences of all taste modalities between sexes, countries, age and weight groups. Ordinal logistic regressions were conducted to investigate the association between age (as continuous exposure) and the four taste modalities (as outcome). Older children were more taste sensitive for sweet and salty and less taste sensitive for umami and bitter. Taste threshold levels differed substantially between countries. Girls reported lower sweet thresholds than boys. Overweight or obese children had higher sweet and salty thresholds. This was the first study comparing taste thresholds in children across different European countries. We conclude that taste thresholds are associated with weight status, children become more sweet and salty taste sensitive with increasing age and girls might be more sweet sensitive than boys.

Introduction

Overweight and obesity among children remain to be a major public health concern in Europe and worldwide. About 22 million children in Europe are overweight or obese[34]. One factor that influences an unfavourable weight development is diet. Sensory taste perception is assumed to play a substantial role in food choice, especially in children where other aspects such as healthiness and prices of foods are not yet considered[5,6].

Sensory taste sensitivity differs substantially between individuals and changes during the developmental stages of infancy and childhood[3]. Although infants and children have up to five times more taste buds than adults, they do not seem to be more taste sensitive, probably because the innervation of taste papillae in infants is not yet fully developed and functional[29].

Sensory taste sensitivity can be measured through the assessment of taste thresholds where the lowest concentration of a taste modality that can be detected is considered as detection threshold. Studies on taste thresholds in children however show inconsistent results. Whereas Anliker *et al.* observed that children aged 5 to 6 years and adults have similar bitter taste thresholds[3], other studies showed that children and infants have higher taste thresholds[13,17] or lower thresholds for bitter taste than adults[35]. Sweet thresholds seem to differ between age groups. Most studies suggest that children have a lower sensitivity to perceive sweet taste than adults[11,17,19,37]. James *et al.* reported that 8-9 year old boys had higher thresholds for sweet, bitter and salty tastes than adults and higher sweet and salty thresholds than girls, while girls' taste thresholds were similar to those of adults[17]. Age differences of umami sensitivity have not yet been studied.

Results of studies investigating associations between taste sensitivity and weight status are also contradictory. Overberg *et al.* found that obese children and adolescents had higher salty, umami and bitter thresholds than non-obese children and adolescents[27]. In contrast, Pasquet *et al.* found that obese adolescents had lower thresholds for sweet and salty taste[28]. Kirsten and Wagner as well as Alexy *et al.* on the other hand did not find any association between salty thresholds and body composition in children and adolescents[2,20], and Fernández-Aranda *et al.* did not find any association between taste perception of any taste modality and extreme weight/eating conditions in adults[12].

As it seems most likely that children, due to the development of taste sensitivity during childhood and adolescence, have different taste thresholds than adults, results of studies with an adult population cannot be transferred to children. Besides age and sex, food culture may also influence individual taste thresholds. Taste sensitivity itself may be associated with the development of overweight and obesity.

In general, the inconsistent observations of former studies may possibly result from investigating different age groups and usage of different methodologies to assess taste sensitivity. None of the previous studies investigated the influence cultural differences on sensory taste thresholds by comparing children from diverse countries with a standardised methodology requiring the same cognitive abilities. Up to now, investigations on the effect of sex differences have up to now only been partly analysed systematically(3,14,36).

For the first time the present study describes sensory taste thresholds for sweet, bitter, umami and salty taste in 1 938 boys and girls using a standardised study protocol and compares thresholds between different age and weight groups from eight European countries.

Methods

Study design and participants

The study sample group is a sub-sample of the IDEFICS (Identification and prevention of dietary and lifestyle-induced health effects in children and infants) study, a prospective European multicentre cohort study whose aim was to investigate the aetiology of lifestyle- and nutrition- related disorders such as childhood overweight and obesity. The overall aim and design of the IDEFICS study has been described previously(1). Between September 2007 and May 2008, a baseline survey (T0) was conducted in 16 228 children from 8 European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden) aged 2 to 9.9 years. Two years later, between September 2009 and May 2010, a follow up (T1) examination was conducted. Between T0 and T1, interventions to improve health behaviour took place in one region in each country, with one other region in each country serving as a control region. A sub-sample of children from the age of 6 years onwards was asked to participate in the sensory perception module which consisted of taste threshold as well as taste preference tests. Application of inclusion criteria (see below) resulted in a final study sample for this cross-sectional analysis of 1 938 children aged between 7 and 11 years old that participated in sweet, salty, bitter and umami threshold tests either at T0 or T1.

All centres obtained ethical approval from their local institutional review board (e.g. Ethics Committee, University Hospital, Gent, Belgium; Cyprus National Bioethics Committee, Nicosia, Cyprus; Tallinn Medical Research Ethics Committee, Tallinn, Estonia; Ethics Committee of the University of Bremen, Bremen, Germany; Egeszsegugyi Tudomanyos Tanacs, Pecs, Hungary; Azienda nitaria Locale Avellino Comitato Etico, Avellino, Italy; Regionala Etikprovningsnamnden i Go¨teborg, Gothenburg, Sweden; Comite Etico de Investigacion Clınica de Aragon, Zaragoza, Spain). Parents gave their written informed consent and children were first informed orally, after which they gave their oral consent to participate in our study.

Anthropometric measurements

Children's weight and height were measured in an overnight fasting state using a Tanita BC 420 SMA scale (TANITA, Tokyo, Japan) for weight measurement and a SECA 225 Stadiometer (SECA GmbH & KG, Hamburg, Germany) for height measurement. BMI was calculated and converted to age- and sex-specific z-scores[(8)]. Children were classified into underweight, normal weight and overweight/obese (weight status) using age- and sex-specific cut-points published by Cole and Lobstein[(8)].

Taste threshold tests

As children were very young and the examination protocol was very demanding, we decided to keep the test procedures as simple as possible. Existing literature was consulted to obtain correct concentration ranges and steps. We conducted extensive pre-tests and found the test procedures to be only suitable for children from the age of 6 years onwards but not for pre-schoolers[(30)]. Schoolchildren were in general able to understand the task and to deliver reliable results. Therefore, the minimum age was set to 6 years for the actual taste threshold tests. Still, the procedure needed to be simple and comprehensive. For the actual test, a paired comparison staircase taste threshold test to assess the sweet, salty, umami and bitter detection threshold was arranged as a board game as described by Knof *et al.*[(21)]. In brief, 5 watery solutions prepared with distilled water, with ascending concentrations of sucrose (8.8-46.7 mmol/l, sweet), sodium chloride (3.4-27.4 mmol/l, salty), monosodium glutamate (0.6-9.5 mmol/l, umami) or caffeine (0.26-1.3 mmol/l, bitter) were presented to the participant in 20 ml cups at room temperature. To prepare the solutions, sucrose from Applichem GmbH, Darmstadt, Germany was used for sweet, sodium chloride from Applichem GmbH, Darmstadt, Germany for salty, caffeine from Carl Roth GmbH & Co. KG, Karlsruhe, Germany for bitter and sodium glutamate from Merck KGaA, Darmstadt, Germany for umami. Cups were placed at the bottom of the game board and the child compared each solution against pure distilled water. The observer asked the child if there was any difference between the two tastes. The child indicated if yes or no by placing the cup on the respective field on the game board. If the child was unsure, he/she was allowed to try a second sip of the test solution. After the decision was made, the child was not allowed to try again. The first concentration at which the participant could taste a difference to water was recorded as detection threshold. If the child did not taste a difference to water at any concentration, no taste threshold was assigned for the respective taste modality. After each taste modality, participants recovered for 2 minutes, during which they neutralised their palate with distilled water, and the field staff prepared the next test sequence. According to the examination protocol, the children should not have eaten for at least an hour before the examinations, but should not be hungry either. Adherence to this stipulation was ensured through the fact that the children participated in another examination module of the IDEFICS study prior to the first lesson in the morning. As they had to be in fasting status for this examination, they received something to drink and eat afterwards. They then joined their classes from where they were taken individually to the sensory taste perception tests. These were solely conducted during the morning hours, in the school setting. The order of presentation of tested taste modalities was fixed as follows: sweet, salty, bitter and umami. The five concentration levels were used as categorical variables for each taste modality.

Sensory tests were developed according to the DIN (German Institute for Standardisation, www.DIN.de) 10959, which defines concentrations of test samples and procedures for adults. It works with ten aqueous solutions with increasing concentrations of the corresponding test substance. For our purpose the test design had to be adapted to the physical and psychological development of children. First, the number of test solutions was reduced to five. Furthermore, an additional cup of distilled water was provided to the child to compare the test solutions with a neutral taste. The adapted concentrations and procedures were adjusted after being pre-tested in all survey centres(31). A test-retest analysis of the taste threshold tests in a sub-sample of participating children (n=40) revealed a kappa coefficient of 0.81 (sweet), 0.75 (salty), 0.68 (bitter) and 0.77 (umami)(21). According to Landis and Koch(22), the analysis of test-retest results show a strength of agreement that is rated to be “almost perfect” for sweet and “substantial” for the detection of salty, bitter, and umami. The results of the test re-test procedure in the sub-sample were assumed to be applicable to the full sample. The test procedure was developed in a way that was easy to understand for children from the age of 6 years and older[31]. Training for the implementation of the standardised testing protocol was organised centrally for all 8 countries and testing materials were prepared centrally and then shipped to the survey centres. The adherence to the testing protocol was monitored via site visits to ensure a maximum degree of standardisation.

Statistical analyses

Mean taste thresholds (mmol/l) and corresponding standard deviations (SDs) were calculated by age, sex and country. One-way ANOVAs and *t*-tests were used to examine threshold differences of all taste modalities between sexes, countries, age and weight (according to Cole and Lobstein (2012)[8]) groups. The assumptions for the ANOVA were satisfied (homoscedasticity and normality were checked by comparing SDs between groups and using Q-Q plots). The statistical tests were all conducted with a significance level of $\alpha=0.05$.

Ordinal logistic regressions were conducted to investigate the association between sex (as dichotomous exposure), weight status (as dichotomous exposure), age (as continuous exposure) and the four taste modalities (as outcome), separately. The odds ratios (ORs) to have a one level lower sensory taste threshold (i.e. being one threshold level more sensitive) for girls, overweight/obese children and children being one year older and the corresponding 95% CI were derived. Furthermore, the corresponding ordinal logistic regressions were conducted using an interaction terms between sex and country, weight status and country and age and country to test, if the directions of associations differ by country. To derive adjusted ORs the whole ordinal logistic regression analyses were repeated with adjustment for sex, country, residing in control or intervention region and weight status. To emphasise the age trend raw and adjusted ORs were also calculated for an increase in age of three years.

All analyses were carried out with SAS, version 9.3 (Statistical Analysis System, SAS Institute Inc., Cary, USA).

Results

On average the children were 8.2 (SD 1.3) years old and the proportion of girls and boys was evenly balanced. In total, 25.1% of the children were overweight or obese. Children were most likely from Belgium (18.1%) and less likely from Sweden and Germany (8.4% each). For the full sample, the mean BMI z-score was 0.5 (SD 1.2) **(Table 1)**. Mean taste thresholds of the full sample were 0.7 mmol caffeine/l for bitter, 18.7 mmol sucrose/l for sweet, 10.7 mmol sodium chloride/l for salty and 2.9 mmol monosodium glutamate/l for umami.

Taste thresholds for all taste modalities differed between countries (p-values < 0.0001). Higher sweet thresholds were observed for boys than for girls (p-value = 0.007). There were no statistically significant differences between boys and girls for all other taste thresholds, e.g. boys had slightly higher salty taste thresholds (p-value = 0.07). Overweight/obese children had higher sweet and salty thresholds than under-/normalweight children (p=0.08 and p=0.02, respectively). For bitter and umami taste thresholds there was no difference between under-/normalweight children and overweight/obese children. Detailed results of the country, sex, weight and age group comparisons are presented in **Table 2**.

The ordinal logistic regression analysis revealed the following. Odds ratios for girls to be more taste sensitive compared to boys showed that girls were more taste sensitive towards sweet and salty (OR (95 % CI): 1.17 (0.99;1.38) and 1.16 (0.99;1.36), respectively). Odds ratios for overweight/obese children compared to under-/normalweight children showed that overweight/obese children were less taste sensitive towards sweet and salty (OR (95 % CI):0.88 (0.73;1.06) and 0.80 (0.66;0.96), respectively). Older children were more sensitive for sweet and salty: Per year of age increase, the OR (95 % CI) for a one level lower threshold for sweet and salty were 1.12 (1.06; 1.20) and 1.18 (1.11; 1.26), respectively. Older children were less sensitive for umami and bitter: Per year of age increase, the OR (95 % CI) for a one level higher threshold for bitter and umami were 0.95 (0.89; 1.01) and 0.90 (0.84;0.96) for bitter and umami, respectively. After adjustment the ORs changed slightly (**Table 3**).

Country specific age, weight and sex differences in taste thresholds of all four tested taste modalities are available as supplemental material (Figure S1-S3). Generally, the country-specific results were consistent with the results for all countries in total. Nevertheless, we could also observe some deviant results which showed no consistent pattern.

Discussion

Main results and previous studies

To our knowledge this is the first study that investigated sweet, salty, bitter and umami taste thresholds in a population based sample of primary school children from different countries, following a standardised study protocol. In a previous study of this cohort[23], we observed that preferences for sweetened over non-sweetened juice were associated with overweight and obesity in both girls and boys. The current study provides complementary data on taste thresholds for sweet as well as 3 additional taste modalities that were not included in the previous preference test, namely bitter, salty and umami. Our analysis revealed that umami and bitter thresholds did not differ between boys and girls aged 7 to 11 years. The taste thresholds for sweet and salty differed with girls having slightly lower sweet and salty thresholds than boys. In line with our results, two recent studies observed lower sweet but not lower salty thresholds in girls compared to boys[(7,18)]. Majorana *et al.* in contrast did not find any sex differences regarding sweet, bitter, salty and sour thresholds between boys and girls between 5 and 12 years of age[(16)]. Also, in very young children between 3 and 6 years, no sex differences in sweet and bitter sensitivity were found[(33)]. Bartoshuk *et al.* and Hyde & Feller observed that women were more bitter-sensitive than men[(4,15)] while Martinez-Cordero *et al.* recently found that Mexican women were more sensitive to sweet taste than Mexican men[(24)]. It seems likely that sex differences in taste sensitivity in early childhood are too small to be detectable. However, as taste sensitivity matures until adolescence, it might develop faster in teenage girls than in teenage boys due to the earlier onset of puberty in girls.

Overweight or obese children had higher sweet and salty taste thresholds. A possible explanation for this finding is that children with high sweet and salty taste thresholds might consume more foods high in sugar or salt because they perceive these tastes as less intense and may need more sugar and salt to experience the same sweet and salty sensation. Many sugar- or salt rich foods are considered to be highly processed and energy dense, such as snack foods. Their augmented consumption may thus contribute to the development of overweight and obesity if the physiological status of the child does not demand the supply of increased nutritional energy. Previous research did not find any differences neither between overweight/obese and normal weight children regarding salty and umami taste thresholds[(7)] nor between thresholds for salty and body composition[(20)].Our results show that thresholds in younger children were higher for sweet and salty than in older children. Visser *et al.* found that children between 3 and 6 years of age seem to become more sweet sensitive with age[(33)]. This may happen because of physiological changes during the development of the taste apparatus and the fact that the innervation of taste papillae is not yet fully developed and functional[(9)]. For bitter, it may even be reverse; younger children may have lower bitter thresholds than older children. The observed age trend of sweet and bitter thresholds may be evolutionary meaningful. Sweet thresholds may be elevated during early childhood to ensure sufficient energy intake, and bitter thresholds in contrast are possibly lowered to ensure the detection of possible toxins[(10,32)]. Additionally, bitter taste perception may change across the lifespan due to repeated exposure to bitter tasting foods. Thus, the bitter taste thresholds may not only rise due to evolutionary reasons but also as a result of learning and adaptation processes.

We observed higher sweet taste thresholds (18.7 mmol/l) compared to previous investigations in children (girls: 7.2 mmol/l, boys: 17.0 mmol/l [7] and girls and boys: 12.0 mmol/l [24]). We also observed higher salty taste thresholds (10.7 mmol/l) compared to previous investigations in children (girls: 2.7 mmol/l, boys: 6.1 mmol/l [7] and girls and boys: 3.6 mmol/l [7]). Caffeine thresholds for children reported in previous studies were higher (girls: 1.1 mol/l, boys: 2.0 mol/l [17]) than observed in the present study (girls and boys: 0.70 mmol/l), while those reported for monosodium glutamate were similar to our results (2.4 mmol/l vs. 2.9 mmol/l [7]). The use of different methodologies in the studies mentioned could have led to the contrasting results. The methods could have led to diverse responses due to their specific cognitive demands. Additionally, cross- cultural differences may explain variances for sweet, salty and caffeine thresholds. Other studies used Propylthiouracil (PROP) to assess the bitter perception. We used caffeine instead of PROP, as PROP was classified as potentially carcinogenic[25,26] and was therefore ethically no alternative for a safe use in children.

Strengths and limitations

There are some limitations to our study that need to be discussed. Compared to previous studies that assessed taste thresholds in children[16,18,20,33], we used a smaller number of test-solutions per taste modality. Generally, the studies referenced here performed testing sessions with participating children before conducting the actual test series, thus children were familiar with the test procedures once data collection for the different taste modalities started. Due to our cross-cultural and large-scale study design we chose a simpler study protocol but assured a standardised procedure across all countries and centres. To minimise measurement errors due to the lack of practice and to limited cognitive abilities of study participants, we measured only detection thresholds instead of identification thresholds for the basic tastes. Furthermore, the order of presentation of tested taste modalities was fixed and not randomised. This may have led to the positional bias due to the tendency to answer depending on the order of presentation. This order was selected because the umami taste remains on the taste buds for a long time and would thus affect subsequent taste experiments. A further reason was to facilitate the test procedure for all survey centres in order to ensure a standardised test protocol.

Due to the cross-sectional design of our study we cannot draw any conclusions about the direction of the associations between sweet and salty thresholds and weight status.

Beside these limitations our study has several strengths, one of them being that we were able to investigate thresholds of isolated taste modalities. In real foods, umami taste is often accompanied by salty or fatty tastes in foods that are often energy-dense. Umami is however also characteristic for foods low in energy, e.g. tomatoes. Therefore, the physiological regulation of umami thresholds might work differently than for salty or fatty as our results indicate. Our method to assess detection thresholds was adapted from the DIN 10959. Our cross-cultural standardised study design enabled us to compare the results between different countries and resulted in a large sample of children from the general population. Substantial differences in taste sensitivity could be seen between countries. These differences might be culturally determined. Nationally common diets may pose a particular exposure to their population and may shape taste thresholds over the long term. Genetic differences across Europe may also explain part of this phenotypic variability. Previous studies performed in different countries showed inconsistent results and are not comparable due to different substrates or study designs [7,16,18,27]. Our study was able to show that taste thresholds indeed varied substantially between children from different countries.

This is the first study to compare taste thresholds in children across different European countries. We conclude that taste sensitivity might be associated with weight status and overweight/obese children are often less sweet and salty sensitive. Further, taste thresholds might decrease due to maturation in children when becoming older. We observed large differences in taste sensitivity between children from different countries, which can possibly be explained by cultural or genetic influences.

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Conflict of interest

All the authors declare that they have no conflict of interest.

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Tables

Table 1: Characteristics of the study sample (total number and percentages or mean and standard deviation (SD)) given by sex groups

	Boys	**Girls**	**Total**
	N (%)	**N (%)**	**N (%)**
	970 (50.1)	968 (49.9)	1938 (100.0)
Country			
Belgium	174 (17.9)	176 (18.2)	350 (18.1)
Cyprus	157 (16.2)	136 (14.1)	293 (15.1)
Estonia	102 (10.5)	116 (12.0)	218 (11.3)
Germany	66 (6.8)	96 (9.9)	162 (8.4)
Hungary	122 (12.6)	124 (12.8)	236 (12.2)
Italy	144 (14.9)	126 (13.0)	270 (13.9)
Spain	125 (12.9)	111 (11.5)	246 (12.7)
Sweden	80 (8.3)	83 (8.6)	163 (8.4)
Weight status[1]			
Non-overweight/-obese	732 (75.5)	719 (74.3)	1451 (74.9)
Overweight/Obese	238 (24.5)	249 (25.7)	487 (25.1)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
BMI z-score[2]	0.5 (1.2)	0.5 (1.2)	0.5 (1.2)
Age (years)	8.2 (1.3)	8.2 (1.3)	8.2 (1.3)

[1]: Defined by Cole and Lobstein (19)
[2]: BMI z-scores according to Cole and Lobstein (19)

Table 2: Means and standard deviations of taste thresholds for sex-groups, weight status, countries and age-groups

	Sweet threshold (mmol sucrose/l)	Salty threshold (mmol sodium chloride/l)	Umami threshold (mmol monosodium glutamate/l)	Bitter threshold (mmol caffeine/l)
Sex of the child				
Boys	19.25 (9.43)	10.96 (6.21)	2.86 (2.30)	0.70 (0.35)
Girls	18.16 (8.22)	10.45 (5.96)	2.94 (2.32)	0.70 (0.35)
p-value[1]	0.007	0.07	0.45	0.80
Weight status				
Non-overweight/obese	18.49 (8.53)	10.51 (6.02)	2.92 (2.32)	0.70 (0.35)
Overweight/obese	19.33 (9.75)	11.28 (6.28)	2.85 (2.29)	0.69 (0.34)
p-value[1]	0.08	0.02	0.60	0.70
Country				
Belgium	19.61 (8.06)	12.09 (5.57)	3.21 (2.33)	0.80 (0.35)
Cyprus	23.87 (11.01)	12.21 (7.17)	3.42 (2.43)	0.66 (0.23)
Estonia	15.61 (6.23)	8.94 (4.75)	2.27 (1.83)	0.78 (0.35)
Germany	19.35 (9.07)	10.50 (5.48)	3.56 (2.30)	0.74 (0.33)
Hungary	16.31 (7.13)	10.80 (7.20)	1.96 (1.85)	0.54 (0.30)
Italy	17.62 (9.21)	11.33 (6.29)	3.26 (2.51)	0.73 (0.34)
Spain	17.06 (7.29)	9.21 (5.08)	2.72 (2.33)	0.63 (0.35)
Sweden	18.85 (8.65)	8.67 (4.91)	2.59 (2.20)	0.78 (0.36)
p-value[2]	<.0001	<.0001	<.0001	<.0001
Age				
7 years	19.58 (9.66)	11.33 (6.29)	3.26 (2.56)	0.68 (0.35)
8 years	19.52 (9.50)	11.99 (6.90)	2.81 (2.23)	0.68 (0.34)
9 years	17.76 (8.52)	10.01 (5.96)	2.66 (2.13)	0.64 (0.35)
10 years	17.57 (7.34)	9.66 (5.06)	2.87 (2.25)	0.74 (0.34)
11 years	19.34 (9.21)	10.19 (5.72)	2.77 (2.25)	0.77 (0.36)
p-value[2]	0.0003	<.0001	0.0037	0.0002

[1]: *T*-tests were used to examine differences between sexes and weight status groups
[2]: ANOVA was used to examine differences between countries and age groups

Table 3: Odds ratios for becoming more sensitive for sex, age and weight status

	Sweet		Salty		Bitter		Umami	
	OR 95% CI	OR[1] 95% CI	OR 95% CI	OR[1] 95% CI	OR 95% CI	OR[1] 95% CI	OR 95% CI	OR[1] 95% CI
Boys	Reference		Reference		Reference		Reference	
Girls	1.17 0.99;1.38	1.16 0.98;1.36	1.16 0.99;1.36	1.16 0.99;1.37	0.96 0.82;1.26	0.95 0.81;1.11	0.94 0.80;1.11	0.93 0.79;1.09
Under-/normalweight	Reference		Reference		Reference		Reference	
Overweight/obese	0.88 0.73;1.06	0.83 0.69;1.01	0.80 0.66;0.96	0.77 0.64;0.93	1.13 0.94;1.35	1.20 1.01;1.45	1.03 0.86;1.24	1.04 0.86;1.25
Age[2]	1.12 1.06;1.20	1.16 1.09;1.23	1.18 1.11;1.26	1.22 1.15;1.30	0.95 0.89;1.01	0.98 0.93;1.05	0.90 0.84;0.96	0.88 0.83;0.94
Age[3]	1.43 1.18;1.72	1.55 1.28;1.88	1.66 1.38;1.99	1.83 1.52;2.20	0.85 0.71;1.02	0.95 0.79;1.15	0.73 0.61;0.88	0.68 0.57;0.83

[1]: Odds ratios adjusted for sex, country, residing in control or intervention region and weight status
[2]: Odds ratio to have a one level lower sensory taste threshold (i.e. one level more sensitive) for a difference in age of one year
[3]: Odds ratio to have a one level lower sensory taste threshold (i.e. one level more sensitive) for a difference in age of three years

Publication 3

RESEARCH ARTICLE

Association between parental consumer attitudes with their children's sensory taste preferences as well as their food choice

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Data Availability Statement: Due to the sensitive nature of data collected, ethical restrictions prohibit the authors from making the minimal data set publicly available. Each cohort centre received approval of the corresponding local Ethical Commission and participants did not provide consent for data sharing. Additionally, all co-authors are members of the IDEFICS consortium who have access to the full dataset that is stored on a secured central data server (CDS) that is hosted by the coordinating centre. Data can only be

Abstract

Background

We investigated the association between the consumer attitudes of European parents and their children's taste preferences and food choice. Furthermore, we studied whether the parental consumer attitudes were related to education level.

Methods

This analysis included 1,407 IDEFICS study children aged 6.0 to 11.8 years and from 7 European countries, who participated in the sensory taste perception module between 2007 and 2010. Parental consumer attitude was operationalized as *'trusting in foods known from advertisements' (trusting advertisements)* and as '*not avoiding additives in food' (not avoiding additives)*. Parents reported their educational attainment and completed a food frequency questionnaire for their children. Consumption frequencies of sweet, fatty and processed foods as well as a healthy diet adherence score were calculated. Children performed fat, sweet and umami taste preference tests. Multivariable logistic models were used to analyse the association between *parental consumer attitudes* and their children's taste preference frequencies as well as parental education. Linear regression models were

accessed by registered scientists who are authorised to access the data with an individual account and an individual password. Statistical analyses are done on the CDS. It is strictly forbidden to copy or download any data from the CDS. Data are available on request and all requests need approval by the study's Steering Committee. Interested researchers can contact the IDEFICS consortium (http://www.ideficsstudy.eu) or the study co-ordinator (Ahrens@leibniz-bips.de) to request data access. All requests for accessing data of the IDEFICS/I.Family cohort are discussed on a case-by-case basis by the Steering Committee. For this, interested parties are asked to provide details (e.g. for testing reproducibility of results) on the purpose of their request.

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used to analyse the association between *parental consumer attitudes* and their children's food consumption.

Results

Parental consumer attitudes were not associated with children's fat, sweet and umami taste preferences. Children of parents *trusting advertisements* consumed more frequently processed foods (β = 1.21, 95% CI: 0.49; 1.93). Children of parents *not avoiding additives* consumed more often sweet, fatty and processed foods and had a lower healthy diet adherence score (β = 2.37, 95% CI: 1.03; 3.70; β = 2.27, 95% CI: 1.12; 3.43; β = 0.91, 95% CI: 0.22; 1.59; β = -2.87, 95% CI: -3.89; -1.85, respectively). *Unfavourable parental consumer attitudes* were associated with a lower parental education level across Europe (Compared to high education: Odds Ratio (OR) of *trusting advertisements* with medium education: 1.04, 95% CI: 0.77; 1.40; OR with low education: 2.01, 95% CI: 1.15; 3.54; OR of *not avoiding additives* with medium education: 1.91, 95% CI: 1.44; 2.54; OR with low education: 1.76, 95% CI: 0.96; 3.24).

Conclusions

Across Europe, unfavourable parental consumer attitudes are associated with a lower diet quality of their children. Parental consumer attitudes in turn were associated with their own level of education. This has implications for policy makers, interventions and health promotion programmes that aim to promote healthy eating.

Introduction

A diet high in sugar and fat has been found to contribute to a positive energy balance and unfavourable weight development in children [1]. As parents provide most of the foods children consume, they have a great influence on their children's diet through their own nutritional behaviour and child-feeding practices [2, 3]. Thus the consumer behaviour of the parents, such as buying particular foods and beverages, may determine their children's diet as they act as gatekeepers [3]. Parental consumer behaviour can be described as (i) parental attitudes towards food and beverage products they know from advertisements and (ii) parental efforts to avoid additives in food they purchase for the family. These two factors might influence the taste preferences and food consumption frequencies of their children through their gatekeeping function as already mentioned. Foods and beverages that are heavily marketed are typically high in sugar and fat [4] and are thus more unhealthy and more obesogenic. Furthermore, literature describes that foods containing additives, including taste enhancers, preservatives, and colours might be harmful for children [5].

It is unclear if these described attitudes, hereafter called parental consumer attitudes, are associated with children's taste preferences as well as their sweet, fatty and processed food consumption frequency, and the overall quality of their diet. During childhood food choice is mostly determined by the sensory perception and liking of foods [6, 7]. Sensory taste perception and preferences formed during childhood might persist until later life [8] and affect dietary intake. Hence, to influence food choice of young children it is essential to understand how taste perception and preferences are formed. It is also unclear to what extent the parental consumer attitudes are related to their educational level. If there is no other access to nutritional knowledge, parents with a lower education might be more receptive for what is said in advertisements and might hence put more trust in the information given therein.

To understand what influences the dietary behaviour of children, it is essential to investigate how taste and food preferences are formed. Therefore, we investigated whether parental consumer attitudes are associated with parental educational level as well as with their children's taste preferences and food consumption patterns. Should these associations prove to be present, this could be a pathway from low educational level to an unhealthy diet and thus offer a starting point for interventions.

Methods

Subjects

The data for this study was taken from the IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS) multicentre study, which aimed to investigate the prevalence and aetiology of overweight and obesity of children in Europe and to implement and evaluate intervention programs to reduce childhood overweight and unhealthy lifestyles [9]. Between September 2007 and June 2008, a baseline survey (T0) was conducted on 16,228 aged two to 9.9 year old children from 8 European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden). Two years later, between September 2009 and May 2010 a follow up (T1) examination was conducted on 13,596 children. The children were approached through primary school and kindergarten settings. A sub-sample of 20% of schoolchildren from the age of 6 years onwards was asked to participate in taste preference tests. In total, 1,407 children between 6.0 and 11.8 years from Belgium, Estonia, Germany, Hungary, Italy, Spain and Sweden who participated in the preference tests either at T0 or T1 were included in this cross-sectional analysis.

All centres obtained ethical approval from their respective local institutional review board (e.g. Ethics Committee, University Hospital, Gent, Belgium; Tallinn Medical Research Ethics Committee, Tallinn, Estonia; Ethics Committee of the University of Bremen, Bremen, Germany; Egészségügyi Tudományos Tanács, Pécs, Hungary; Azienda nitaria Locale Avellino Comitato Etico, Avellino, Italy; Regionala Etikprövningsnämnden i Göteborg, Gothenburg, Sweden; Comité Ético de Investigación Clínica de Aragón, Zaragoza, Spain). All parents gave informed written consent for their children's participation. The children were informed orally about the study procedures and gave their oral consent before participating. All instruments, questionnaires and recording sheets were developed in English, translated to the national language and then back-translated to check for translation errors. National examples of foods or other items were provided where deemed necessary.

Parental consumer attitudes

The children's dietary behaviour and the parental consumer attitudes regarding nutrition were assessed using the Children's Eating Habit Questionnaire (CEHQ) [10, 11]. Among others, the parents were asked whether they *trust in products that they know from advertisements* and whether they *try to avoid additives in foods and beverages*. They could answer 5 'agree', 4 'agree moderately', 3 'unsure', 2 'disagree moderately' or 1 'disagree'. Parents that answered with 'unsure' were excluded from the present analysis. The answering categories of these two variables were dichotomised as: 'Do not trust in products from advertisements' (answers: disagree and moderately disagree) vs. 'Trust in products from advertisements' (answers: agree and agree moderately) and 'Do not avoid additives in foods and beverages' (answers: disagree and moderately disagree) vs. 'Avoid additives in foods and beverages' (answers: agree and agree moderately). To ease reading of the text, the following shorter terms will be used henceforth in the text: 'trust in products from advertisements' will be stated as 'trusting advertisements' and 'do not avoid additives in foods and beverages' will be stated as 'not avoiding additives'

Parental education

Self-reported educational attainment was classified according to the International Standard Classification of Education (ISCED). The ISCED classification comprises levels 0–8 (ISCED level 0: Early childhood education, ISCED level 1: Primary education, ISCED level 2: Lower secondary education, ISCED level 3: Upper secondary education, ISCED level 4: Post-secondary non-tertiary education, ISCED level 5: Short-cycle tertiary education, ISCED level 6: Bachelor's or equivalent level, ISCED level 7: Master's or equivalent level, ISCED level 8: Doctoral or equivalent level) [12]. For the present analysis, highest education level of the household was clustered into three groups; 'Low' (ISCED level 0–2), 'medium' (ISCED level 3–5) and 'high' (ISCED level 6–8).

Sensory taste preferences

As children were very young and the examination protocol was very demanding, we decided to keep the test procedures as simple as possible. Thus, the whole test procedure was standardised and adapted for use in very young children. We conducted extensive pre-tests and found that the test procedures were only suitable for children from the age of 6 years and not for preschoolers [13]. Therefore, the minimum age was set to 6 years during the actual taste threshold tests. Children from the age of 6 years and older were in general able to understand the task and to deliver reliable results. Nevertheless, the procedure needed to be simple and comprehensive. A paired comparison forced choice taste preference test to assess the sweet, salty, umami, aroma and fatty taste preference was arranged as a board game as described in Knof et al. [14]. In brief, for every taste modality, two food samples were presented to the child at room temperature. Apple juice was used to assess sweet and aroma preference and crackers were used to assess salty, fatty and umami preference. One sample represented the basic taste and the other the modified taste (e.g. 3.11% sucrose, 0.05% apple aroma, 1.16% sodium chloride, 18% fat or 1% monosodium glutamate were added). Samples were placed at the bottom of the game board and the child compared the basic sample against the modified one. The interviewer asked the child which of the two samples he/she liked best. The child indicated this by placing the preferred sample on the respective field on the game board. The child had to choose the preferred sample and was then classified as preferring either the basic or the modified taste. The interviewer recorded the answer on paper recording sheets. After each taste modality, participants had a 2-minute break during which they neutralised their palate with distilled water, and the interviewer prepared the next test sequence. According to the examination protocol, at least 1 hour had passed after having eaten but children were not hungry either. The adapted procedures were adjusted after being pre-tested in all survey centres [13]. A test-retest analysis of the taste preference tests in a sub-sample of participating children revealed a kappa coefficient of 0.77 (sweet), 0.86 (salty), 0.77 (fatty), 0.78 (aroma) and 0.80 (umami). The results of the test re-test procedure in the sub-sample were assumed to be applicable to the full sample. The samples were shipped centrally for examination and standard operation protocols (SOP) as well as a central training of field staff were provided to all centres. To ensure a high degree of standardisation, the adherence to the SOPs was monitored during site visits.

In this analysis we investigated the sweet, fat and umami taste preferences as dichotomous variables (basic taste preference vs. modified taste preference).

Food choices and healthy diet score

As part of the CEHQ, parents were asked to complete a proxy food frequency questionnaire (FFQ) for their children's diet during the previous month. It was tested for reliability and validity [11, 15]. The questionnaire had 43 items including processed, fatty and sweet, as well as healthy foods and beverages. The answer possibilities were 'never/less than once a week', '1–3 times a week', '4–6 times a week', '1 time/day', '2 times a day', '3 times a day' and 'I have no idea'. Based on these answers, the children's weekly consumption frequencies were calculated. The relative consumption frequency of fatty (fried potatoes, whole fat milk, whole fat yoghurt, fried fish, cold cuts/sausages, fried meat, fried eggs, mayonnaise, cheese, chocolate- or nut-based spread, butter/margarine on bread, nuts/seed/dried fruits, salty snacks, savoury pastries, chocolate-based candies, cake/pudding/cookies and ice cream) as well as sweet (fresh fruits with added sugar, fruit juice, sugar-sweetened drinks, sweetened breakfast cereals, sweetened milk, sweetened yoghurt, jam/honey, chocolate- or nut-based spread, chocolate-based candies, non-fat candies, cake/pudding/cookies and ice cream) foods and beverages per week over all foods were calculated, resulting in the continuous variables sweet propensity score and fat propensity score ranging from 0% to 100% [16]. The consumption frequency of processed foods was calculated as the sum of weekly intake of processed meat, hamburgers, hot dogs, kebab and snacks such as savoury pastries and fritters reported for the previous month.

Based on weekly consumption frequencies, an a priori healthy diet adherence score (HDAS) was calculated [17]. The HDAS aimed to reflect the adherence of the children to food-based dietary guidelines common in all participating countries. In particular: limited intake of refined sugar, reduced fat intake, choosing whole meal foods, high consumption of fruits and vegetables and consuming 2–3 times fish per week. Hence, the HDAS consisted of 5 dimensions including weekly consumption frequency of foods high in sugar, of foods low in fat, of whole meal foods, of fruits and vegetables as well as of fish. Depending on the consumption frequency derived from the FFQ for each dimension, a score between 0 and 10 could be reached. To calculate this, the consumption frequency of foods high in sugar, of foods low in fat, of whole meal foods, of fruits and vegetables as well as of fish, were considered either as total consumption frequency or as the proportion of all consumed food items as follows: To obtain a score for each dimension

1. The weekly consumption frequency of sugar containing foods was divided by the weekly consumption frequency of all consumed foods. The following food items were considered as sugar containing foods: sweetened breakfast cereals, sweetened drinks, fruit juices, sweetened milk, sweet yoghurt and fermented milk beverages, fruits with added sugar, jam and honey, chocolate or nut based chocolate, candy bars, loose candies, marshmallows, biscuits, cakes, pastries, puddings, ice cream, milk or fruit based bars. A proportion of 10% or less of sugar containing foods was assigned to the score of 10, and a proportion of 15.7% or more was assigned to 0. The scores of 1 to 9 were equally distributed in steps of 0.6 percentage points.

2. The weekly consumption frequency of foods generally low in fat (cooked vegetables, eggs, fish, meat and low fat dairy products and spread) was divided by the intake of both cooked and fried vegetables, eggs, fish, meat and low and high fat dairy products and spread. The proportion of foods low in fat over all fat containing food items was assigned to scores of 1 to 10 by assigning 100% low fat foods to 10 and 10% or less to 0. The scores 1 to 9 were equally distributed in steps of 10%.

3. The proportion of whole meal bread ('whole meal bread, dark roll, dark crispbread') over all kinds of bread ('white bread, white roll, white crispbread' + 'whole meal bread, dark roll,

dark crispbread') was calculated. A proportion of 100% whole meal bread was assigned to 10 and a proportion of 10% or less to 0. The scores 1 to 9 were equally distributed in steps of 10%.

4. The total consumption frequency of fruits and vegetables (fresh fruits, cooked vegetables, potatoes and beans, legumes and raw vegetables) of 35 portions per week (5 a day) was assigned to a score of 10. Less than 1 portion per week was assigned to 0. The scores 1 to 9 were equally distributed in equidistant categories.

5. The total consumption frequency of fish of 2.5 times per week was assigned to a score of 10. No fish at all was assigned to 0. The scores 1 to 9 were equally distributed in steps of 0.3 times per week.

For the total HDAS, the scores of all dimensions were summed up. Thus, the HDAS ranged from 0 to 50. A higher score represented a higher adherence to the food-based dietary guidelines, presumed to be a healthier diet.

Potential confounders

Children's age, sex and country were assessed via parental questionnaires. Children's weight and height were measured in an overnight fasting state using a Tanita BC 420 SMA scale (TANITA, Tokyo, Japan) for weight measurement and a SECA 225 Stadiometer (SECA GmbH & KG, Hamburg, Germany) for height measurement. BMI was calculated and converted to age- and sex-specific z-scores. Children were classified into thin/normal weight and overweight/obese (weight status) using age- and sex-specific cut-points published by Cole and Lobstein [18]. Children's age, sex, country and weight status were considered as possible confounders.

Statistical analysis

The associations between the two dichotomised variables for parental consumer attitudes and the dichotomous outcome variables children's sweet, fat and umami taste preferences were estimated using logistic regression models. Models were adjusted for sex, age, country, weight status and highest parental education level as fixed effects.

The associations between parental consumer attitudes and the continuous outcome variables fatty, sweet as well as processed food consumption and HDAS of their children were analysed using linear regression models adjusted for sex, age, country, weight status and highest parental education level as fixed effects. Sample sizes for these analyses varied due to missing values for the avoiding additives variable (missing: n = 61).

Further, the association between highest parental education level and parental consumer attitudes was analysed by means of a logistic regression (adjusted only for country but not for child dependent variables).

We used SAS® (Statisitcal Analysis System, SAS Institute Inc., Cary, USA), version 9.3 for all analyses and set the level of significance at $p<0.05$.

Results

Study characteristics

The study sample comprised 689 girls (49.0%) and 718 boys (51.0%). The age ranged from 6.0 to 11.8 years (mean 8.8 years). 22.4% of the children were overweight or obese. 5.5% of the children had parents with low education, 42.9% with medium education and 51.6% with high education.

About 56% and 58.6% of all children preferred the test sample with the added fat and added sugar over the basic taste sample, respectively, and 45% of all children preferred the sample with added monosodium glutamate over the sample without added monosodium glutamate. Regarding parents, 21.8% stated that they trust in foods and beverages they know from advertisements and 26.2% answered that they do not avoid foods and beverages with additives. Children consumed processed food products on average 6.4 times per week and had fatty and sweet food and beverage propensity scores of 26.4% and 25.4%, respectively. There were no substantial differences between boys and girls with regard to the study characteristics (Table 1).

Parental consumer attitudes and children's taste preferences

In general, country, sex, age, weight status and parental education were significant confounders ($p<0.05$) in almost all analyses (data not shown). For example, boys consumed sweet foods more frequently ($p<0.05$) and increasing age was associated with increasing consumption frequency of processed foods ($p<0.05$). However, since these influences are beyond the scope of the present analysis, we will focus our attention on the results presented hereafter which are relevant for our study aim.

The logistic regression analysis revealed no association between trusting advertisements and children's sweet, fat or umami preferences (Table 2). Further, we did not observe an association between not avoiding additives and children's sweet, fat or umami preferences.

Parental consumer attitudes and children's food choice

We observed a non-significant but positive association between parental trusting advertisements and children's sweet (ß = 0.97, 95% CI = -0.45; 2.40) and fatty (ß = 0.79, 95% CI = -0.43; 2.00) food and beverage consumption. Further, we found a significantly higher consumption frequency of processed foods (ß = 1.21, 95% CI = 0.49; 1.93) in children whose parents trust advertisements than in those whose parents do not trust advertisements. Children of parents not avoiding additives consumed sweet and fatty foods and beverages over all foods more often and also consumed processed foods more often per week (ß = 2.37, 95% CI = 1.03; 3.70; ß = 2.27, 95% CI = 1.12; 3.43; ß = 0.91, 95% CI = 0.22; 1.59, respectively) compared to children whose parents avoided additives. Children whose parents did not avoid additives also had a significantly lower HDAS (ß = -2.87, 95% CI = -3.89; -1.85) compared to those whose parents avoided additives (Table 3).

Parental education level and consumer attitudes

Highest parental education level was associated with parental consumer attitudes. Parents with low education level had significant higher odds (OR = 2.01, 95% CI = 1.15; 3.54) of trusting advertisements compared to parents with high education level. Further, parents with medium or low education level had higher odds (OR = 1.91, 95% CI = 1.44; 2.54 and OR = 1.76 95% CI = 0.96; 3.24, respectively) of not avoiding additives compared to parents with high education level (Table 4).

Discussion

Our analyses revealed that parental consumer attitudes are associated with their children's (parental reported) food intake but not with their taste preferences, and that the parental attitudes depend on parental education level across Europe.

Table 1. Characteristics (total number and percentages or mean and standard deviation (SD)) of exposure and outcome variables given by boys and girls.

	Boys	Girls	Total
	N = 718 (51.0%)	N = 689 (49.0%)	N = 1407
	Mean (SD)	Mean (SD)	Mean (SD)
Age	8.8 (1.2)	8.8 (1.1)	8.8 (1.1)
Sweet propensity score %	26.0 (11.4)	24.8 (10.4)	25.4 (11.0)
Fat propensity score core %	26.3 (9.4)	26.5 (9.6)	26.4 (9.5)
Processed foods/week	6.4 (5.6)	6.4 (6.1)	6.4 (5.8)
Healthy diet adherence score	21.7 (8.5)	21.9 (8.4)	21.8 (8.5)
	N (%)	N (%)	N (%)
Overweight/obese[1]	147 (20.5)	168 (24.4)	315 (22.4)
Parents who trust in advertisements	153 (21.3)	154 (22.4)	307 (21.8)
Parents who do not avoid additives	172 (24.9)	180 (27.5)	352 (26.2)
Highest parental education level[2]			
Low	44 (6.1)	33 (4.8)	77 (5.5)
Medium	294 (41.0)	310 (45.0)	604 (42.9)
High	380 (52.9)	346 (50.2)	726 (51.6)
Sweet preference	427 (59.5)	398 (57.8)	825 (58.6)
Fat preference	399 (55.6)	387 (56.2)	786 (55.9)
Umami preference	332 (46.2)	301 (43.7)	633 (45.0)
Country			
Belgium	106 (14.8)	94 (13.6)	200 (14.2)
Estonia	66 (9.2)	72 (10.5)	138 (9.8)
Germany	205 (28.6)	221 (32.1)	426 (30.3)
Hungary	96 (13.4)	92 (13.4)	188 (13.4)
Italy	84 (11.7)	71 (10.3)	155 (11.0)
Spain	98 (13.7)	76 (13.4)	174 (12.4)
Sweden	63 (8.8)	63 (9.1)	126 (9.0)

[1]: BMI z-scores according to Cole and Lobstein 2012 [18].

[2]: International Standard Classification of Education Maximum (ISCED) [12]; maximum of both parents (0, 1, 2 = low education; 3, 4 = medium education; 5, 6 = high education).

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We did not find any association between parental consumer attitudes and general preferences for sweet, fat and umami. Therefore, the diet that is associated with the parental consumer attitudes did not appear to be related to taste preferences. Results of a previous study, also suggested that taste preferences might not be associated with dietary habits [16]. We presume that taste preferences might be determined by other factors and that children do not necessarily prefer what parents provide. Furthermore, using food matrices for the taste preference tests limited the textual conditions of foods to only juice and crackers, compared to the variety of textures children were exposed normally. This may have caused the non-significant association between parental consumer attitudes and taste preferences. The perception of taste may differ according to the matrix delivering the taste in the oral cavity. For example, fat in more liquid foods might be more ready to diffuse out compared to solid food matrices like crackers. Accordingly, a semi-fluid matrix possibly allows the sweet taste to remain longer in the oral cavity compared to apple juice. This should be considered in future studies. We observed that children from parents trusting advertisements tended to consume fatty and sweet foods and beverages more often and also consumed more processed foods. These children had a non-significant tendency to have a lower diet quality indicated by a lower HDAS. Previous studies in

Table 2. Association between parental consumer attitudes and their children's taste preferences[1].

Outcome:	Parents trust in advertisements[2]							Parents do not avoid additives[3]					
	No		Yes					No		Yes			
	N	%	N	%	OR (95% CI)	p-value		N	%	N	%	OR (95% CI)	p-value
Sweet preference													
Low	462	42.0	120	39.1	1.00 (reference)			163	46.3	395	39.7	1.00 (reference)	
High	638	58.0	187	60.9	0.94 (0.71; 1.25)	0.69		189	53.7	599	60.3	0.85 (0.65; 1.11)	0.23
Fat preference													
Low	495	45.0	126	41.0	1.00 (reference)			442	44.5	156	44.3	1.00 (reference)	
High	605	55.0	181	59.0	1.07 (0.81; 1.42)	0.64		552	55.5	196	55.7	1.01 (0.78; 1.32)	0.92
Umami preference													
Low	609	55.4	165	53.8	1.00 (reference)			545	54.8	193	54.8	1.00 (reference)	
High	491	44.6	142	46.2	1.02 (0.77; 1.35)	0.89		449	45.2	159	45.2	1.04 (0.80; 1.36)	0.78
All children	1407							1346					

Abbreviations: OR = Odds Ratio, CI = Confidence interval

[1]: Logistic regression model, adjusted for sex, age, country, ISCED (International Standard Classification of Education Maximum (ISCED) [12]) and weight status [18]

[2]: Reference: Parents do not trust in advertisement

[3]: Reference: Parents avoid additives

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the USA and in Europe showed that 20% of commercials shown on TV promote food products and 98% of these advertisements promote foods and beverages high in sugar, sodium and/or fat [19–21]. As a result, the parents trusting advertisements may prefer to buy the advertised products, which may explain the tendency towards a lower overall quality of the diet and a higher consumption frequency of sweet and fatty as well as processed foods and beverages.

Table 3. Association between parental consumer attitudes and their children's food consumption[1].

	Parents trust in advertisements[2]		Parents do not avoid additives[3]	
Outcome:	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**
Proportion of sweet food and beverage consumption	0.97 (-0.45; 2.40)	0.18	2.37 (1.03; 3.70)	0.0005
Proportion of fatty food consumption	0.79 (-0.43; 2.00)	0.21	2.27 (1.12; 3.43)	0.0001
Processed food consumption frequency	1.21 (0.49; 1.93)	0.001	0.91 (0.22; 1.59)	0.0093
Healthy Diet Adherence Score	-0.42 (-1.50; 0.67)	0.45	-2.87 (-3.89; -1.85)	<0.0001

Abbreviations: ß = ß-coefficient, CI = Confidence interval

[1]: Model is adjusted for sex, age, country, ISCED (International Standard Classification of Education Maximum (ISCED) [12]) and weight status.

[2]: Reference: Parents do not trust in advertisements.

[3]: Reference: Parents avoid additives.

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Table 4. Influence of parental ISCED level on their consumer attitudes[1].

	High ISCED level (Reference)		Medium ISCED level				Low ISCED level			
Outcome	N	%	N	%	OR (95% CI)	p-value	N	%	OR (95% CI)	p-value
Parents trust in advertisement										
No	600	82.6	456	75.5	1.00 (reference)		44	57.1	1.00 (reference)	
Yes	126	17.4	148	24.5	1.04 (0.77;1.40)	0.81	33	42.9	2.01 (1.15;3.54)	0.02
Parents do not avoid additives										
No	539	76.9	403	70.2	1.00 (reference)		52	73.2	1.00 (reference)	
Yes	162	23.1	171	29.8	1.91 (1.44;2.54)	<0.0001	19	26.8	1.76 (0.96;3.24)	0.07

Abbreviation: OR: Odds ratio, CI: Confidence interval, ISCED: International Standard Classification of Education Maximum (ISCED) [12]; maximum of both parents (0, 1, 2 = low education; 3, 4, 5 = medium education; 6, 7, 8 = high education)

[1]: Model is adjusted for country.

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Even more pronounced was the effect of parental consumer attitude on their children's dietary behaviour when considering the analyses of the associations of not avoiding additives. Children of parents not avoiding additives had a lower overall diet quality, consumed sweet foods and beverages more often, and also consumed more fatty foods as well as more processed foods. A recent study from Norway found a negative association between a consumer pattern labelled as 'healthy' which included the importance of absence of additives and junk/convenience food consumption [22]. They concluded that healthy eating promotion of children should focus on parents' health concerns, emphasising on natural food without additives.

We found that a lower parental education (from high over medium to low) is associated with unfavourable consumer attitudes (trusting advertisements and not avoiding). Since children with less educated parents have a lower overall diet quality and consume more sweet and fatty foods [23], our results add valuable knowledge about the pathway from low education to an unfavourable diet, indicating that families with a lower socioeconomic status consume unhealthy and more obesogenic food, because they tend to rely on advertisements more often and do not try to avoid additives.

Because early learned dietary behaviour and taste preferences can persist until later on in life [8], it very important to ensure a healthy and adequate diet during childhood, that is particularly low in sugar and fat, so as to prevent an early onset of overweight. To be able to do so, parents need information about a healthy diet for their children.

This study has some strengths and limitations that need to be addressed. As parents reported the dietary intake of their children, underreporting could have occurred due to social desirability and to missing details on their children's outside home eating. Nevertheless, self-reported food group consumption is more robust against misreporting compared to energy reporting [24]. The described underreporting would have led to a decrease of the association between parental consumer attitudes and children's food intake. The same holds true for the misreporting of consumer attitudes due to social desirability. Should parents have reported more favourable consumer attitudes, this could have also led to an attenuation of the association we investigated. Apart from these limitations our study has several strengths. All measurements including the taste preference tests were developed especially for children. A high standardisation between countries was assured through the central training sessions offered to all study personnel and the central provision of all study materials. Site visits further guaranteed high quality of data collection. All methods were pre-tested and test-retest reliability was calculated with 'almost perfect' results [13, 14]. Our study sample consists of a large number of children and we were able to account for relevant possible confounders.

To our knowledge, this is the first study that investigated the impact of parental consumer attitudes on their children's taste preferences and dietary habits in different European countries using standardised methods. Our results can possibly to some extent explain the higher prevalence of overweight and obesity [25–27] as well as the higher consumption of sweet and fatty foods [23, 28] in children from families with a lower socioeconomic status.

Conclusion

Our results show that parents' consumer attitudes are associated with their children's food intake. Additionally, we showed that parents with a low education background have less favourable consumer attitudes. This has implications for policy makers, interventions and health promotion programmes that aim to promote healthy eating. Additionally, our results underline the importance of an incorporation of nutrition and consumer education into school education to help develop a healthier dietary as well as consumer behaviour among children.

Supporting information

S1 File. Full author list of IDEFICS consortium.
(DOCX)

Acknowledgments

We gratefully acknowledge the participation of all children and parents in our study. All named authors are members of the IDEFICS consortium. Every institution of the consortium that contributed data or methods relevant for this manuscript is represented by at least one co-author, including affiliations. One lead author and senior author including a contact email address are indicated in the list of authors.

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Familial aggregation and socio-demographic correlates of taste preferences in European children

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Abstract

Background: Studies on aggregation of taste preferences among children and their siblings as well as their parents are scarce. We investigated the familial aggregation of taste preferences as well as the effect of sex, age, country of residence and education on variation in taste preferences in the pan- European I.Family cohort.

Method: Thirteen thousand one hundred sixty-five participants from 7 European countries, comprising 2,230 boys <12 years, 2,110 girls <12 years, 1,682 boys ≥12 years, 1,744 girls ≥12 years and 5,388 parents, completed a Food and Beverage Preference Questionnaire containing 63 food items representing the taste modalities sweet, bitter, salty and fatty. We identified food items that represent the different taste qualities using factor analysis. On the basis of preference ratings for these food and drink items, a preference score for each taste was calculated for children and parents individually. Sibling and parent-child correlations for taste preference scores were calculated. The proportion of variance in children's preference scores that could be explained by their parents' preference scores and potential correlates including sex, age and parental educational was explored.

Results: Mean taste preferences for sweet, salty and fatty decreased and for bitter increased with age. Taste preference scores correlated stronger between siblings than between children and parents. Children's salty preference scores could be better explained by country than by family members. Children's fatty preference scores could be better explained by family members than by country. Age explained 17% of the variance in sweet and 16% of the variance in fatty taste preference. Sex and education were not associated with taste preference scores.

Conclusion: Taste preferences are correlated between siblings. Country could explain part of the variance of salty preference scores in children which points to a cultural influence on salt preference. Further, age also explained a relevant proportion of variance in sweet and fatty preference scores.

Keywords: Familial aggregation, Taste preferences, Children, Cross-cultural, Europe

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Background

Taste preferences are the main food choice driver, especially in children for whom aspects such as healthiness and economics, e.g. food prices, generally play a minor role [1, 2].

If taste and food preferences derive from mere exposure, availability and familiarity, then the taste and food preferences of children should resemble that of their parents because they share meals and parents influence the availability of foods and drinks in the home [3, 4]. Further, it can also be assumed that the shared genetic information, environmental factors, as well as close personal interaction lead to similar taste and food preferences.

Previous studies from the 1980s however only observed weak positive correlations between taste and food preferences of children and their parents [5–9]. No differences were observed between the correlation of mothers and fathers with preferences of either boys or girls. The largest correlations were observed between spouses and between siblings as described in a review by Rozin [9]. Rozin then argued that the observed relationship between taste and food preferences should have been stronger for parents and their children due to the close relationship and the shared genetic characteristics (family paradox) [9]. Birch on the other hand concluded that weak correlations within families occurred due to communalities of a cultural group and underlined the need for cross-cultural research in this field [6]. Studies reported in a review by Reed et al. analysing the heritability of fat preference measured through fat intake in family and twin studies described a narrow sense heritability between 0 and 0.48 [10]. Previous studies in twin children based on questionnaires show a moderate genetic basis for food preferences in children [11, 12] and adolescence [13]. The shared family environment influences food preference of young children, but this influence disappears already in adolescence [13] and is also absent in adults [14]. Studies in Finnish adult twins that evaluated taste preferences by taste tests confirm a moderate heritability for individual differences in sweet taste preferences (41% for the strongest sucrose solution) [15] and further show that 34-50% of the variation in pleasantness of sour foods [16] and 18-58% of the variation in the pleasantness of oral pungency and spicy foods [17] can be attributed to genetic factors.

Preferences for sweet and fatty as well as aversion to bitter are innate [18, 19] and change during childhood. The age of the child can therefore also influence taste preferences. Further, children's diet is associated with their parents' educational level [20], presenting another possible influencing factor on children's taste preferences.

Taste and food preferences develop during childhood and the process may persist until later in life [21]. Therefore, it is of great importance to understand how these preferences develop and how they can be influenced to support healthy food choices. Thus it is of interest to study the hypothesis that the taste preference of children resembles that of their parents.

The aim of this study was to assess food preferences of children and their parents, to identify foods representing the sweet, salty, fatty and bitter taste, and to investigate the association between sweet, salty, fatty and bitter taste preferences of children from different age-groups, their siblings and their parents from seven European countries. Further, the effect of sex, age, parental education and country of residence on taste preferences was investigated.

Methods

Study group

I.Family is a European multi-centre longitudinal study that presents the follow-up of the IDEFICS (**I**dentification and prevention of Dietary- and lifestyle-induced health EFects In Children and infantS) cohort [22, 23]. Between March 2013 and April 2014, all children that participated in the IDEFICS study were invited to take part in I.Family. Additionally, their siblings and parents were invited to the follow-up examinations. For the taste preference analysis, we included all participants from the age of 6 years onwards. From this sub-group, 13,165 participants (2,230 boys <12 years (also referred to as younger boys), 2,110 girls <12 years (also referred to as younger girls), 1,682 boys ≥12 years (also referred to as older boys), 1,744 girls ≥12 years (also referred to as older girls) and 5,399 parents) who fulfilled the inclusion criteria (age, sex, measured height, weight and biological relationship) completed the Food and Beverage Preference Questionnaire. Our study group comprises 5,128 child-mother dyads (with 3,588 mothers) and 3,223 child-father dyads (with 1,811 fathers) from 7 European countries (Cyprus, Estonia, Germany, Italy, Hungary, Spain and Sweden).

The large sample size of the I.Family study allowed conducting age-group specific analyses. Therefore, for the analysis the children were divided in boys <12 years, girls <12 years, boys ≥12 years, girls ≥12 years. The cut-off of 12 years was chosen because children 12 years and older are entering adolescence and therefore other factors like peers and growing independency might influence taste preferences whereas smaller children are more dependent on their parents with regard to food availability. The cut-off of 12 years seems reasonable not only for these social aspects but also for biological aspects. In a sub-sample of children (n=7123 children) information on breaking of the voice (for boys) and onset of menarche (for girls) was available. According to these characteristics a proportion of 84% of children classified as pubertal were ≥12 years old and 11% of children classified as pubertal were <12 years. In an even smaller sub-sample (n=5286) information for Tanner stages according to pubic hair (for boys) and breast development (for girls) was available. According to these characteristics 97% of prepubertal children were <12 years and 95% of pubertal children were ≥12 years old.

Each study centre obtained ethical approval from its local responsible institutional review board. Parents gave written informed consent for themselves and for their children. Adolescents 12 years and older gave their own written informed consent. All children were informed orally and gave their oral consent to participate in our study.

Questionnaire and anthropometric measurements

We obtained information on sex, age and highest level of education for each participant using self-completion questionnaires. Parents completed their own questionnaire as well as for their children under twelve years old. Adolescents twelve years and older completed the questionnaire on their own. For each parent we categorised the highest educational level acquired according to the International Standard Classification of Education (ISCED) ranging from 1 (low education) to 8 (high education) [24]. For the present analysis the education level was grouped into three categories; 'low education' (ISCED level 0-2), 'medium education' (ISCED level 3-5) and 'high education' (ISCED level 6-8).

The height and weight of all participants were measured in a fasting state. The body mass index (BMI) was calculated for all participants and for all children it was converted into age- and sex-specific z-scores [25]. Participants were classified as thin/normal weight and overweight/obese (weight status) using age- and sex-specific cut-points published [25] for children. For adults, the cut off of 25 kg/m^2 was chosen to classify parents as overweight/obese [26].

Food and Beverage Preference Questionnaire

We developed a questionnaire that assessed preferences for sweet, salty, fatty and bitter and could be applied in children/adolescents as well as in adults. Duffy et al. described a preference questionnaire as useful for epidemiological studies to connect chemosensation with health outcomes [27]. Previously, a preference questionnaire for French adults was tested for reliability and collected data showed associations between assessed preferences and health outcomes as well as dietary intake [28–30].

We mainly compiled foods and drinks that were included in earlier food and beverage preference questionnaires [28, 31]. The questionnaire contained food photographs that were appropriate to be used in all age groups (Figure 1). In total, the questionnaire consisted of 63 items including single foods (e.g. banana, spinach), mixed foods (e.g. hot dog, kebab), condiments (e.g. jam, mayonnaise) and drinks (e.g. coke, lemonade).

Participants were asked to indicate how much they liked the taste of the food presented on the pictures using a 5 point likert (smiley-)scale, ranging from disliking to liking. Thus the variable of liking for each food and drink item ranged from 1 to 5, with 1 meaning 'do not like at all' and 5 meaning 'like very much'. Additionally, participants could indicate that they do not know or have never tasted the specific food item. A pre-test was conducted in every country to ensure the feasibility of all food items across countries.

Sensory taste preference score

Only foods that were ranked by at least 75% of the participants were included in this analysis. Participants were excluded when they had more than 20 missing or "Never tried/ Don't know" answers. To assess the associations between foods and beverages, a latent variable exploratory factor analysis was conducted [32]. Further, a sex and age specific factor analysis was conducted to gain more accurate information about the factorial structure of food preference. The strata were boys <12 years, girls <12 years, boys ≥12 years, girls ≥12 years, and their mothers and fathers. We used the oblimin transformation, which allowed an analysis using non-orthogonal factors [33]. Different diagnostic tools were applied to identify an appropriate number of factors including Horn's parallel test, Wayne Velicer's Minimum Average Partial criterion and the optimal coordinates index [34]. We chose a 13 factor solution for every age and sex specific group. A food or drink item was considered to belong to a particular factor if the factor loading was greater than 0.30 on that factor. The factor analysis explained between 32% and 41% of the overall variance in the variables (fathers 41%, mothers 39%, older girls 36%, older boys 38%, younger boys 37% and younger girls 32%). We then used the obtained factors to conduct a content analysis in order to assign the factors to the taste modalities sweet, salty, fatty and bitter (Table 1). Food and drink items with no load on one of the factors were not included in further analyses.

We computed scores for liking of the specific taste modality by calculating the mean liking of the foods and drinks included in each of the 4 categories. Scores were calculated individually for younger boys, younger girls, older boys, older girls well as their mothers and fathers. To this end we calculated the sum of the ratings for the foods and drinks and divided the sum by the number of foods and drinks that were included in the specific taste modality group.

Statistical analysis

Descriptive analysis of study characteristics of the study population were conducted by each stratum (boys <12 years, girls <12 years, boys ≥12 years, girls ≥12 years

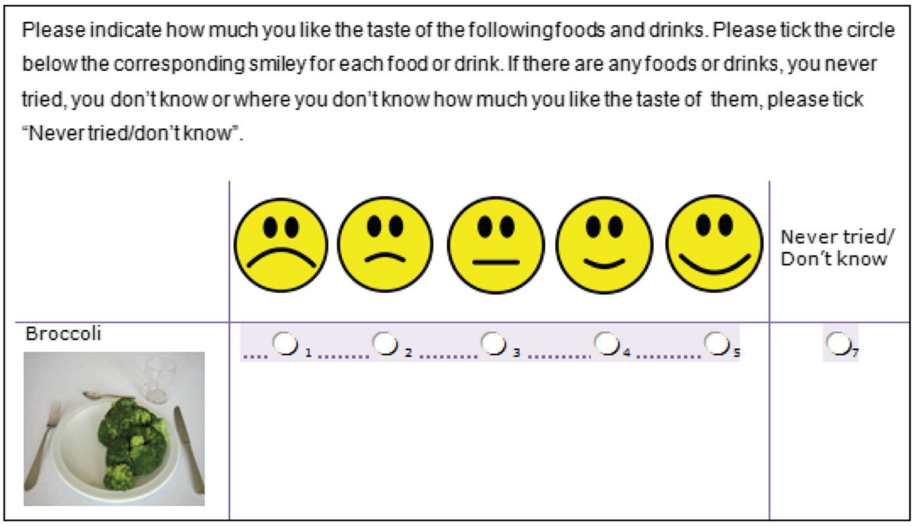

Fig. 1 Example (screen shot) from the food and beverage preference questionnaire

their mothers and fathers) as well as by each participating country. We also calculated the quartiles (median, p25, p75) of sweet, salty, fatty and bitter liking scores of each stratum.

To adjust for the effect of age on taste preferences, age standardised residuals from taste preference scores were obtained from regression analyses separately for each stratum. The residuals were used to analyse the associations between taste preferences of parents and children as well as between and among younger and older siblings. We estimated inter- and intraclass correlations for all relative pairs of a family using the FCOR (family correlations) program in SAGE (Statistical Analysis for Genetic Epidemiology software), version 6.3 [35].

In a sub-group analysis we analysed the correlations of taste preferences between parents and their children as well as between siblings separately for those children whose father and mother had similar preferences (difference between mother's and father's preference score between -1 and 1) vs. those children whose father and mother had different preferences (difference between mother's and father's preference score below -1 or above 1). Rozin supposed that children from parents with incongruent preferences might receive a 'mixed message', which might lead to a disappearance of the familial aggregation effect [9].

Additionally, for each sex-by-age stratum, we estimated the proportion of variance in sweet, salty, fatty and bitter preference scores that could be explained by mother's, father's, brothers' and sisters' preference scores and country (potential correlates). We estimated several linear mixed models: a null model, including only a random intercept term for family membership and another model, including the random intercept term and each of the potential correlate only. Based on these models we calculated the proportion of variance in children's taste preference scores that could be explained by preference scores of mothers, fathers, brothers and sisters. Additionally, we calculated the proportion of variance in taste preference scores that could be explained by country. To assess the impact of sex, age and highest education level on taste preferences, we used non-stratified taste preference scores (all children and parents) for each taste modality as dependent variables in a linear mixed model. Sample sizes for these analyses varied due to missing values for particular covariates (e.g. parent or sibling information).

The factor analysis was conducted using statistical software R, version 3.1.0 [36]. Familial correlations were conducted using SAGE. All other analyses were carried out using the statistical software SAS (Statistical Analysis System, SAS Institute Inc., Cary, USA), version 9.3.

Results

Study characteristics

Thirteen thousand one hundred sixty-five participants from 7 European countries, comprising 7,766 children and 5,399 parents participated in our study. 49.6% of the children and 66.5% of the parents were female. 28.1% of the children and 56.6% (48% of mothers and 74% of fathers) of the parents were overweight or obese and 53.6% of the families had at least one parent with a high education. More detailed characteristics can be found in Table 2. Country-specific characteristics can be found as Additional file 1: Table S1.

The median (p25;p75) family size was 3.0 (2.0;4.0), ranging from 1 to 7. Numbers of different family types can be found in Table 3. The most abundant family type

Table 1 Foods and drinks representing four taste modalities

	Boys <12 years	Girls <12 years	Boys ≥12 years	Girls ≥12 years	Fathers	Mothers
Sweet						
Milk chocolate	X	X	X	X	X	X
Chocolate bar	X	X	X	X	X	X
Lemonade	X	X		X	X	X
Coke	X	X	X	X	X	X
Diet coke	X		X	X	X	X
Donut	X		X	X	X	X
Jam	X	X	X	X		X
Honey	X	X	X	X	X	X
Plain croissant		X	X	X	X	X
Chocolate croissant	X	X		X	X	X
Cornflakes	X	X	X	X	X	X
Chocolate crispies		X	X	X	X	X
Chocolate spread	X	X	X	X	X	X
Banana	X	X		X	X	
Fruit yoghurt	X	X	X	X	X	X
Yoghurt	X		X	X	X	X
Fruit juice	X	X	X	X	X	X
Chocolate pudding		X	X			
Gateau			X		X	X
Ice tea					X	
Ice cream					X	X
Water					X	
Wholemeal bread					X	
Salty						
Salt			X			
Salted nuts	X	X	X	X	X	X
Salted pistachios	X	X	X	X	X	X
Savoury biscuits	X	X	X	X	X	X
Salty sticks	X	x	X	X	X	X
Olives					X	
Feta					X	
Fatty						
Hamburger	X	X	X	X	X	X
Hot Dog	X	X	X	X	X	X
Fried chicken	X		X	X	X	X
Steak	X			X	X	
French fries	X	X	X	X		X
Chips	X	X	X	X		X
Sausage	X	X	X	X	X	X
Salami	X			X	X	X
Butter	X	X	X	X	X	X
Mayonnaise	X		X	X	X	X
Milk		X		X	X	

Table 1 Foods and drinks representing four taste modalities *(Continued)*

	Boys <12 years	Girls <12 years	Boys ≥12 years	Girls ≥12 years	Fathers	Mothers
Cream			X	X	X	X
Mashed potatoes			X			
Kebab				X	X	X
Nachos				X		X
Chili sauce				X		X
Bitter						
Broccoli	X	X	X	X	X	X
Spinach	X	X	X	X	X	X
Lettuce	X		X			
Olives	X		X			X
Lasagne			X			
Red cabbage					X	X
Sprouts					X	X
Asparagus					X	X
Grapefruit						X
Steak						X

Foods that did not load on any factor: For boys <12 years: whole meal bread, lasagne, cream, whole milk skimmed milk, mashed potatoes, sausage, broth, salt, nachos, choco crispies, wine gum, dark chocolate, water, donut, ice cream, ice tea, plain croissant, cream gateau. For girls <12years: whole meal bread, lasagne, lettuce, cream, mayonnaise, mashed potatoes, fried chicken, steak, broth, olives, salami, salt, nachos, wine gum, dark chocolate, yoghurt, water, donut, ice cream, ice tea. For older boys ≥12 years: whole meal bread, chili, grape fruit, whole milk skimmed milk, steak, broth, salami, kebab, nachos, banana, lemonade, wine gum, dark chocolate, water. For older girls ≥12 years: whole meal bread, lasagne, lettuce, grape fruit, skimmed milk, mashed potatoes, broth, olives, salt, wine gum, dark chocolate water, chocolate pudding, ice cream, ice tea, cream gateau. For fathers: whole meal bread, coffee, lasagne, chili, lettuce, beet, grape fruit, skimmed milk, mashed potatoes, avocado, broth, french fries, Crisps, nachos, wine gum, dark chocolate, chocolate pudding, jam. For mothers: whole meal bread, coffee, lasagne, lettuce, beer, whole milk, skimmed milk, avocado, broth, feta, salt, banana, wine gum, dark chocolate, water, chocolate pudding, ice tea.

Table 2 Characteristics of the study sample

	Mothers	Fathers	Boys <12y	Girls <12y	Boys ≥12y	Girls ≥12y
	N = 3588	*N* = 1811	*N* = 2230	*N* = 2110	*N* = 1682	*N* = 1744
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age	41.4 (5.3)	44.5 (5.9)	9.6 (1.5)	9.7 (1.5)	13.6 (1.1)	13.6 (1.1)
BMI	25.8 (5.3)	27.9 (4.4)	18.3 (3.7)	18.3 (3.6)	21.0 (4.3)	21.2 (4.2)
BMI z-score[a]	-	-	0.6 (1.2)	0.5 (1.1)	0.7 (1.2)	0.6 (1.1)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Overweight/obese	1698 (47.9)	1316 (74.0)	616 (27.8)	596 (28.3)	505 (30.1)	457 (26.3)
Low education[b]	162 (4.7)	43 (2.5)	-	-	-	-
Medium education[b]	1540 (44.3)	670 (38.6)	-	-	-	-
High education[b]	1772 (51.0)	1023 (58.9)	-	-	-	-
Italy	591 (16.5)	179 (9.9)	400 (17.9)	352 (16.7)	323 (19.2)	321 (18.4)
Estonia	520 (14.5)	224 (12.3)	311 (14.0)	288 (13.7)	227 (13.5)	275 (15.8)
Cyprus	726 (20.2)	466 (25.7)	529 (23.7)	518 (24.6)	413 (24.6)	393 (22.5)
Sweden	335 (9.3)	163 (9.0)	242 (10.9)	215 (10.2)	143 (8.5)	147 (8.4)
Germany	561 (15.6)	247 (13.6)	311 (14.0)	293 (13.9)	262 (15.6)	281 (16.1)
Hungary	632 (17.6)	377 (20.8)	268 (12.0)	277 (13.1)	230 (13.7)	227 (13.0)
Spain	223 (6.2)	155 (8.6)	169 (7.6)	167 (7.9)	84 (5.0)	100 (5.7)

Abbreviations: *y* year, *BMI* Body mass index
[a]:BMI z-scores according to Cole and Lobstein 2012 [25]
[b]:International Standard Classification of Education Maximum (ISCED); maximum of both parents (0, 1, 2 = low education; 3, 4 = medium education; 5, 6 = high education) [24]

Table 3 Numbers of family types of the study sample

Family types	No. of families	%
Mother only	57	1.0
Mother – 1 child	1343	24.4
Mother – 2 children	632	11.5
Mother – 3 children	83	1.5
Mother – 4 children	14	0.3
Mother – 5 children	1	0.0
Fathers only	23	0.4
Father – 1 child	203	3.7
Father – 2 children	112	2.0
Father – 3 children	15	0.3
Father – 4 children	2	0.0
Father and Mother only	21	0.4
Father and Mother – 1 child	694	12.6
Father and Mother – 2 children	618	11.2
Father and Mother – 3 children	110	2.0
Father and Mother – 4 children	10	0.2
Father and Mother – 5 children	2	0.0
1 child only	1143	20.7
2 children	380	6.9
3 children	41	0.7
4 children	8	0.1
5 children	1	0.0
Total	5513	100

was a mother with 1 child (24.4%). But also mother and father with 1 or 2 children represented together 23.8%.

Excluding food items which were known to or tasted by less than 75% of the participants led to the following exclusions in the stratum of younger boys: Asparagus, brussels sprouts, beer, black coffee, chili sauce, grapefruit, red cabbage, avocado, feta and kebab. In the stratum of younger girls: Asparagus, brussels sprouts, beer, black coffee, chili sauce, grapefruit, red cabbage, diet coke, avocado, feta and kebab. In the stratum of older boys: Asparagus, brussels sprouts, beer, black coffee, red cabbage, avocado and feta. In the stratum of older girls: Asparagus, brussels sprouts, beer, black coffee, red cabbage, avocado and feta. In the groups of mothers and fathers no food or drink items were excluded.

Quartiles (median, p25, p75) of the subsequently calculated scores are displayed in Table 4. Highest scores were achieved for the sweet and fatty score in young children. Lowest scores were observed in children for the bitter score. Parents had higher bitter scores than children.

Correlations of taste preferences among family members

Table 5 shows the results for interclass correlations between children from different age and sex strata as well as mothers and fathers and intraclass correlations among siblings for residuals of sweet, bitter, salty and fatty scores.

Correlations showed significant but weak family aggregation for almost all taste modalities and types of relative pairs.

Among all types of parent-offspring pairs, correlations were highest (r=0.20) between fathers and daughters ≥12 years old for sweet and between fathers and sons ≥12 years old for fatty.

Sibling-sibling correlations (independent of sex) were highest (0.20 to 0.26) among siblings <12 years of age for all taste modalities, while those among ≥12 year old siblings ranged from 0.08 to 0.15. Brother-brother correlations ranged from -0.01 to 0.35 and were significant only for those <12 years of age for all taste modalities. Correlations among ≥12 year old brothers were not significant. Correlations between <12 year old brothers and ≥12 year old brothers ranged from 0.18 to 0.34 and were significant for sweet and fatty.

Sister-sister correlations for sisters <12 years of age ranged from 0.21 to 0.32 and were significant for all taste modalities. Correlations among ≥12 year old sisters ranged from 0.09 to 0.37 and were significant for sweet, salty and bitter. Correlations between <12 year old sisters and ≥12 year old sisters ranged from 0.15 to 0.26 and were significant for sweet and bitter.

Brother-sister correlations for brothers and sisters <12 years of age ranged from 0.11 to 0.22 and were significant for sweet, salty and fatty. Correlations between ≥12 year old brothers and sisters ranged from 0.03 to 0.12 and were not significant. Correlations between sisters <12 years of age and brothers ≥12 years of age ranged from 0.16 to 0.22 and were significant for salty and fatty. Correlations between brothers <12 years of age and sisters ≥12 years of age ranged from 0.15 to 0.25 and were significant for sweet and bitter.

The comparison of taste preferences between children whose parents both had similar preferences and those whose parents had different preferences showed that taste preferences of children from parents with same preferences correlated stronger to their parents' preferences than those of children from parents with incongruent taste preferences (data not shown). The highest correlations were seen for sweet preference scores between <12 year old girls with ≥12 year old sisters (r=0.44) and between mothers and sons ≥12 years old (r=0.27) as well as for fatty preference scores between mothers and sons ≥12years old (r=0.33) and between fathers and sons ≥12 years old (r=0.34).

Explanation of variance

Table 6 shows the proportion of variance in children's taste preference scores that could be explained by their mother's, father's, brothers' and sisters' taste preference

Table 4 Age and sex specific distribution of sweet, salty, fatty and bitter taste preference scores (median, p25, p75)

	Boys (< 12 years)	Girls (< 12 years)	Boys (≥12)	Girls (≥12)	Fathers	Mothers
Sweet score						
N	2227	2106	1681	1743	1810	3587
Median(p25;p75)	4.2 (3.8;4.6)	4.2 (3.7;4.6)	4.1 (3.7;4.5)	4.1 (3.6;4.4)	3.6 (3.1;4.0)	3.5 (3.0;3.9)
Salty score						
N	2175	2073	1663	1734	1803	3568
Median(p25;p75)	4.0 (3.3;4.8)	4.0 (3.3;4.8)	3.8 (3.0;4.3)	3.8 (3.0;4.5)	3.7 (3.0;4.2)	3.5 (2.8;4.0)
Fatty score						
N	2228	2109	1682	1743	1809	3587
Median(p25;p75)	4.3 (3.8;4.6)	4.3 (3.7;4.6)	4.2 (3.8;4.5)	3.9 (3.5;4.3)	3.7 (3.3;4.2)	3.4 (2.9;3.9)
Bitter score						
N	2197	1939	1677	1658	1809	3587
Median(p25;p75)	3.0 (2.3;4.0)	3.0 (2.0;4.0)	3.4 (2.8;4.0)	3.0 (2.0;4.0)	3.6 (3.0;4.2)	3.8 (3.3;4.3)

and country. The proportion of variance that could be explained by parents was highest for fat preference (between 4.3% and 8.3%). For girls and boys ≥12 years of age, 6.4% and 5.8%, respectively, of sweet taste preference score could be explained by their parents' sweet taste preference score. The bitter taste preference score of <12 year old girls 6% of variance could be explained by parents' bitter taste preference score. The proportions of variance in children's taste preference score that could be explained by country were under 4% for all age and sex strata except for salt taste preference scores, where proportions of explained variance by country were between 5.4% and 7.5% for all age and sex strata.

Table 7 shows the proportions of variance in non-stratified taste preference scores that could be explained by sex, age and highest education level. Age explained 17%, 16% and 7% of sweet, fat and bitter preference, respectively. All other proportions of explained variance by sex and highest education level were below 5%.

Discussion

In our study we analysed sweet, salty, bitter and fatty taste preferences among European families. We observed a decrease with age in sweet, salty and fatty preference scores, while bitter taste preference scores increased with age. Further, taste preference scores correlated stronger among siblings than between children and their parents. For all taste modalities correlations were highest among younger siblings and among older siblings only present in girls. Nevertheless, these age- and sex-group specific correlations need to be interpreted with more caution since they were not as powered as the overall correlations. Furthermore, we observed that 17%, 16% and 7% of total variance in the non-stratified sweet, fatty and bitter taste preference scores, respectively, were explained by age. The strong age effect on taste preferences indicated by these results might be evolutionary meaningful, similar to the innate preference for sweet as well as fatty and the aversion for bitter [37]. Another explanation might be the matured taste perception of parents; children have about five times more taste buds, and their foliate papillae are larger and more abundant compared to those of adults. Nevertheless, this does not consequently lead to higher taste sensitivity, due to the fact that children's innervation of taste papillae is not fully developed. The development of the taste apparatus carries on through childhood [38, 39]. These age-related differences could explain stronger correlations among siblings compared to correlations of parents' taste preferences with those of their children.

Our data confirm earlier observations of a stronger correlation of food preferences between siblings than between children and their parents [8]. As an explanation for these findings, Pliner and Pelchat suggested that siblings share more genetic information than children share with their parents [8]. In contrast to parents and children, siblings share 25% of the dominant genetic effects. Siblings may share more similar experiences (e.g. school, peers) as compared to their parents as they are closer in age. Additionally, if gene expression is age dependent, gene expression of siblings closer in age should is expected to be more similar.

Another factor that influences children's taste and food preference is food neophobia, the rejection of new and unknown foods [37]. This phenomenon is reported to decrease with increasing age from childhood to adulthood [40]. Our observations when looking at the number of food and drink items excluded because of missing values before conducting the factor analysis are in line with this. The number of excluded items decreased with increasing age suggesting that with increasing age the participants get familiar with a greater variety of foods and drinks. This was supported by our factor analysis that showed an increasing number of items per taste modality with increasing age of participants.

Table 5 Familial correlations (r), standard errors (SE) of the mean and p-values for residuals of sweet, salty, fatty and bitter taste preference scores

	Relationship		Sweet	Salty	Fatty	Bitter
Parent-offspring		No. of pairs	7838	7623	7849	7451
		r ± SE	0.16 ± 0.01	0.08 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
		p-value	<0.0001	<0.0001	<0.0001	<0.0001
Parent-offspring	<12 years	No. of pairs	4501	4358	4500	4215
		r ± SE	0.14 ± 0.02	0.08 ± 0.02	0.15 ± 0.02	0.13 ± 0.02
		p-value	<0.0001	<0.0001	<0.0001	<0.0001
	≥12 years	No. of pairs	3337	3265	3349	3236
		r ± SE	0.18 ± 0.02	0.09 ± 0.02	0.15 ± 0.02	0.14 ± 0.02
		p-value	<0.0001	<0.0001	<0.0001	<0.0001
Mother-daughter	<12 years	No. of pairs	1453	1411	1453	1336
		r ± SE	0.14 ± 0.03	0.10 ± 0.03	0.18 ± 0.03	0.18 ± 0.03
		p-value	<0.0001	0.0003	<0.0001	<0.0001
	≥12 years	No. of pairs	1085	1064	1091	1039
		r ± SE	0.19 ± 0.03	0.09 ± 0.03	0.19 ± 0.03	0.12 ± 0.03
		p-value	<0.0001	0.0026	<0.0001	0.0002
Mother-son	<12 years	No. of pairs	1486	1433	1487	1434
		r ± SE	0.16 ± 0.03	0.08 ± 0.03	0.13 ± 0.03	0.10 ± 0.03
		p-value	<0.0001	0.0029	<0.0001	0.0001
	≥12 years	No. of pairs	1095	1074	1097	1085
		r ± SE	0.19 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.19 ± 0.03
		p-value	<0.0001	0.0007	0.0002	<0.0001
Father-daughter	<12 years	No. of pairs	755	735	753	680
		r ± SE	0.10 ± 0.04	0.05 ± 0.04	0.14 ± 0.04	0.18 ± 0.04
		p-value	0.0056	0.1781	0.0001	<0.0001
	≥12 years	No. of pairs	584	571	585	548
		r ± SE	0.20 ± 0.04	0.09 ± 0.40	0.10 ± 0.04	0.16 ± 0.04
		p-value	<0.0001	0.0269	0.0147	0.0002
Father-son	<12 years	No. of pairs	807	779	807	765
		r ± SE	0.15 ± 0.04	0.01 ± 0.04	0.15 ± 0.04	0.07 ± 0.04
		p-value	<0.0001	0.7291	0.0001	0.0527
	≥12 years	No. of pairs	573	556	576	564
		r ± SE	0.15 ± 0.04	0.06 ± 0.04	0.20 ± 0.04	0.10 ± 0.04
		p-value	0.0003	0.1851	0.0002	0.0258
Sibling-sibling	<12 years	No. of pairs	965	922	967	848
		r ± SE	0.26 ± 0.03	0.23 ± 0.03	0.26 ± 0.03	0.20 ± 0.03
		p-value	<0.0001	<0.0001	<0.0001	<0.0001
	≥12 years	No. of pairs	434	417	434	401
		r ± SE	0.15 ± 0.05	0.10 ± 0.05	0.08 ± 0.05	0.18 ± 0.05
		p-value	0.0026	0.0399	0.0953	0.0006
Brother-brother	<12 years	No. of pairs	287	274	287	264
		r ± SE	0.34 ± 0.05	0.20 ± 0.06	0.32 ± 0.06	0.35 ± 0.06
		p-value	<0.0001	0.0009	<0.0001	<0.0001
	≥12 years	No. of pairs	123	120	123	121

Table 5 Familial correlations (r), standard errors (SE) of the mean and p-values for residuals of sweet, salty, fatty and bitter taste preference scores *(Continued)*

	Relationship		Sweet	Salty	Fatty	Bitter
		r ± SE	0.17 ± 0.09	0.10 ± 0.09	0.15 ± 0.09	-0.01 ± 0.09
		p-value	0.0584	0.2824	0.1004	0.9035
	<12 years-≥12 years	No. of pairs	264	255	265	261
		r ± SE	0.34 ± 0.05	0.18 ± 0.06	0.30 ± 0.06	0.16 ± 0.06
		p-value	<0.0001	0.0035	<0.0001	0.0074
Sister-sister	<12 years	No. of pairs	237	225	238	195
		r ± SE	0.24 ± 0.06	0.32 ± 0.06	0.26 ± 0.06	0.21 ± 0.07
		p-value	0.0002	<0.0001	0.0001	0.0028
	≥12 years	No. of pairs	107	104	107	95
		r ± SE	0.24 ± 0.09	0.22 ± 0.10	0.09 ± 0.10	0.37 ± 0.09
		p-value	0.0128	0.0250	0.3802	0.0003
	<12 years-≥12 years	No. of pairs	248	244	248	226
		r ± SE	0.25 ± 0.06	0.15 ± 0.06	0.18 ± 0.06	0.26 ± 0.06
		p-value	<0.0001	0.0172	0.0028	0.0001
Sister-brother	<12 years	No. of pairs	441	423	442	389
		r ± SE	0.20 ± 0.05	0.20 ± 0.05	0.22 ± 0.05	0.11 ± 0.05
		p-value	<0.0001	<0.0001	<0.0001	0.0336
	≥12 years	No. of pairs	204	193	204	185
		r ± SE	0.09 ± 0.07	0.04 ± 0.07	0.03 ± 0.07	0.12 ± 0.07
		p-value	0.2121	0.6198	0.6526	0.1084
	<12 years-≥12 years	No. of pairs	233	226	233	212
		r ± SE	0.16 ± 0.06	0.21 ± 0.06	0.22 ± 0.06	0.19 ± 0.07
		p-value	0.0125	0.0012	0.0007	0.0048
	≥12 years-<12 years	No. of pairs	255	255	255	244
		r ± SE	0.25 ± 0.06	0.15 ± 0.06	0.17 ± 0.06	0.21 ± 0.06
		p-value	<0.0001	0.0155	0.0058	0.0011

Beside these biological relationships, social factors may also account for our findings. As children grow older, their attitudes towards foods and drinks change [41] and the influences of peers become stronger [42]. This could be an explanation for the low correlation among older boys. Older female siblings in our study still resembled each other.

Furthermore, parental encouragement and family rules have been reported to affect the eating habits of children [43, 44]. Parents may tend to offer a healthier diet to younger children compared to adolescents. Especially mothers are more aware and adhere more to dietary guidelines also when feeding their children [45]. These facts may lead to different exposure for younger children than older children which may be another explanation for the stronger correlations among younger children compared to older children or children-parent correlations. Fathers in contrast have been found to have a high influence on a child's sweet and fatty food choice, including all types of sugar, sweets, unhealthy drinks such as soft drinks and unhealthy fats [46]. This is in line with our results showing that in particular the sweet preference scores of fathers could partly explain their children's sweet preference scores. Further, the correlations between fathers and daughters were observed to be high for sweet and between fathers and sons high in fat preference.

While our study has the strength that it includes data from more than 7,000 children and 5,000 parents from 7 European countries, some methodological aspects need to be addressed. Logue et al. stated 6 conditions that must be fulfilled to investigate familial aggregation in food preferences. *1. The range of examined foods must be ample enough and should not include only commonly liked or disliked foods. Additionally, the used scale must be wide enough.* In the present study we chose a wide variety of foods and drinks that produced a broad range of answers on likes and dislikes. It is however still

Table 6 Percentage of children's variance in preference scores that could be explained by preference scores of their family members and country

		% Variance by mother	% Variance by father	% Variance by parents	% Variance by male siblings <12 y	% Variance by female siblings <12 y	% Variance by male siblings ≥12 y	% Variance by female siblings ≥12 y	% Variance by country
Sweet score	Boys <12 y.	2.7	2.4	4.1	-	4.2	8.2	4.1	1.3
	Girls <12 y.	2.2	1.2	2.2	4.2	-	1.5	7.0	3.7
	Boys ≥12 y.	3.5	2.9	5.8	8.3	1.5	-	0.5	3.1
	Girls ≥12 y.	4.4	3.4	6.4	4.2	6.6	0.6	-	3.0
Salty score	Boys <12 y.	0.6	0[a]	1.0	-	4.3	3.7	1.2	5.4
	Girls <12 y.	1.1	0.6	0.7	4.4	-	4.9	1.3	7.5
	Boys ≥12 y.	1.4	0.4	1.9	3.9	4.1	-	0.2	5.8
	Girls ≥12 y.	1.0	0.6	1.3	1.2	1.3	0.3	-	6.4
Fatty score	Boys <12 y.	2.3	2.4	4.3	-	5.2	5.1	1.9	2.2
	Girls <12 y.	3.4	2.1	5.5	5.1	-	2.8	1.7	1.4
	Boys ≥12 y.	1.7	5.1	8.3	5.1	2.7	-	0[a]	3.8
	Girls ≥12 y.	3.8	0.8	6.1	1.9	1.6	0[a]	-	1.5
Bitter score	Boys <12 y.	0.7	0.2	0.7	-	0.7	4.0	4.1	2.7
	Girls <12 y.	3.1	2.8	6.0	0.9	-	3.4	8.5	2.4
	Boys ≥12 y.	3.0	1.1	2.2	4.1	3.4	-	0.8	1.6
	Girls ≥12 y.	1.3	2.9	3.1	4.8	7.9	0.9	-	2.7

[a]Due to model misspecification or lack of power negative variance percentage were estimated and therefore set to zero

possible that the number of food items that were chosen influenced the factorial structure that we obtained. A study conducted by Skinner et al. included 194 food items, whereas other studies included 59, 47, 32, 94 [5, 7, 9, 32, 47]. Since we included children as young as 6 years old, we needed a scale simple enough to be understood and answered also in that age range. According to the 'ASTM Guide for sensory evaluation of products by children and minors', six year old children are able to answer simple liking scales [48]. *2. The sample size should be large enough.* A main strength of our study is the large sample size including a large number of children, adolescents and parents. To our knowledge there is only one study that included more participants, but the study was conducted only in adults [28]. *3. Sex differences should be taken into account and 4. The preferences should be reported by each participant him/herself and no proxy should be used.* It has been discussed in the literature that parents' reports about their children's preferences in the context of comparing children's and parents' preferences might pull the answers in the direction of parents' preferences [1, 5, 49]. In conformity with Logue et al. we stratified our analysis by sex and every participant completed the questionnaire by him/herself. *5. The biological relationship between children and parents should be taken into account and lastly, the participating children should be living together with their parents.* We included only biological parents and assumed that they were living together with their children because they participated in the study as a family and children were rather young. Another strength of this study is the availability of other additional correlates of taste preferences such as parental educational and country of residence.

Using a food and beverage preference questionnaire to assess taste preferences seemed feasible in a large-scale epidemiological study. Asking for preferences for different foods and drinks with different tastes considers multiple sensory factors that have an influence on actual preferences which are relevant for real life, such as taste sensitivity, taste intensity, social factors, and environmental factors as claimed by Hayes and Keast [50].

Table 7 Percentage of variance in preference scores that could be explained by sex, age and highest education

	% Variance sweet core (n)	% Variance salty score (n)	% Variance fatty score (n)	% Variance bitter score (n)
Sex	1.2 (13,173)	0.4 (13,035)	4.4 (13,177)	0.0 (12,886)
Age	17.4 (13,173)	3.4 (13,035)	16.3 (13,177)	7.2 (12,886)
ISCED[a]	0.0 (12,679)	0.0 (12,549)	0.4 (12,684)	0.0 (12,405)

[a]International Standard Classification of Education [24]

Conclusion

To our knowledge this is the first European multicentre epidemiological study investigating the familial aggregation of taste preferences in a high number of participants from seven European countries, following a standardized study design. We conclude that the family paradox stated by Rozin still remains partly unsolved [9]. The hypothesis that children resemble their parents' food and taste preferences could only be partly confirmed. Nevertheless, we found a correlation of taste preferences among siblings. This finding does indicate that there are similarities among family members. Age could explain part of the variance in sweet and fatty preference scores. Country could explain part of the variance of salty preference scores in children which points to a cultural influence on salt preference. No other studied correlate was associated with taste preference scores.

Additional file

Additional file 1: Table S1. Country specific characteristics of the full study sample. (DOCX 15 kb)

Abbreviations

BMI: Body Mass Index; IDEFICS Study: Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS Study; ISCED: International Standard Classification of Education; SAS: Statistical Analysis System

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Availability of data and materials

Interested researchers can contact the I.Family consortium (http://www.ifamilystudy.eu) to discuss possibilities for data access. However, due to the high sensitive data collected in children and adolescents, ethical restrictions prohibit the authors from making the minimal data set publicly available.

Authors' contributions

HJ had the idea of the analysis and wrote the paper and had primary responsibility for final content, TI and LHB supported data analysis, GE, DM, LM, PR, AS, ASo, TV and WA conducted field work and research, WA and AS coordinated the study, VP and LHB conducted research, AH and WA supervised this analysis. All authors were responsible for critical revisions and final approval of the manuscript.

Ethics approval and consent to participate

All centres obtained ethical approval from their local institutional review board (e.g. Cyprus National Bioethics Committee, Nicosia, Cyprus; Tallinn Medical Research Ethics Committee, Tallinn, Estonia; Ethics Committee of the University of Bremen, Bremen, Germany; Egészségügyi Tudományos Tanács, Pécs, Hungary; Azienda Sanitaria Locale Avellino Comitato Etico, Avellino, Italy; Regionala Etikprövningsnämnden i Göteborg, Gothenburg, Sweden; Comité Ético de Investigación Clínica de Aragón, Zaragoza, Spain.). All parents gave informed written consent and children were informed orally and gave their consent for participating in this study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Publication 5

12 Assessment of Sensory Taste Perception in Children

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on behalf of the IDEFICS and I.Family consortia

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Abstract In health research, sensory taste perception and preference have rarely been examined. Especially in children, it is challenging to conduct sensory taste perception and preference tests due to their still developing cognitive and physiological abilities.

In the IDEFICS and I.Family studies, four different instruments were developed to assess sensory taste perception and preference in children, adolescents and their parents across Europe. A taste threshold test and a taste preference test were adapted to be used in children from the age of 6 years onwards in the IDEFICS study. A food and beverage preference questionnaire and a taste intensity test were adapted to be used in I.Family. The taste preference test and the taste intensity test were conducted with standardised real foods to obtain results that are relevant for real life conditions.

The taste threshold test was conducted with standardised solutions.

All test procedures were standardised across the participating countries, all materials were supplied centrally and tested for suitability.

Experiences in the surveys showed that these four instruments were suitable to be used in children and adolescents. The game-like character of the sensory test module enhanced the acceptance of this survey module and made it quite popular among the participating children. Furthermore, the sensory tests were reported to be the participants' favourite procedure during the surveys. In conclusion, these tests were feasible to be conducted in large-scale studies across Europe.

12.1 Assessment of Sensory Taste Perception

Sensory taste perception differs substantially between subjects. But it is evident that how people perceive the taste of foods influences their food choice and how much they eat (Sorensen et al. 2003). Therefore, the global aims of the assessment of sensory perception in the IDEFICS and I.Family studies (Ahrens et al. 2011, 2017) were to investigate the variation of sensory taste perception in European children and adolescents and its association with overweight, obesity, metabolic syndrome and food choice. Relating sensory taste perception with health outcomes presented an innovative research field resulting in novel insights in this area.

Previously, the majority of methods commonly applied to assess sensory perception, e.g. during the evaluation of newly developed foods by industry, were designed to work with adults. Studies on sensory testing in children are still rare. Some studies, however, provided an insight into requirements for sensory work with children. Guinard, for instance, published in 2001 a review on sensory and consumer testing with children (Guinard 2001) and Popper and Kroll reviewed the current state of knowledge in 2004 (Popper and Kroll 2005). These reviews, including their recommendations, are summarised in the international standard guidelines on sensory evaluation by children and minors and some very general examples are given (ASTM 2013). Nevertheless, before the start of the IDEFICS and I.Family studies, there was a lack of validated methods for sensory testing with children, especially for the assessment of taste thresholds. Consequently, specific test procedures, tailor-made for the application in young children, had to be developed. During the course of the IDEFICS and I.Family studies, four methods to assess sensory taste perception and preference were adapted for the use in children and adolescents.

Previous publications using sensory data from the IDEFICS and I.Family studies have investigated associations between taste sensitivity/preferences and food intake as well as weight status or have assessed correlates of taste preferences and have described the development of the methods (Knof et al. 2011; Lanfer et al. 2012, 2013; Lissner et al. 2012). A special challenge was to design the test for application in different European countries. Since gold standards were missing, the newly developed tests needed to be validated in terms of reliability and repeatability.

12.2 Implications for Sensory Testing with Children

During childhood, the physical and cognitive abilities are still less developed than those of adults (Doty and Shah 2008) and thus different approaches are needed when planning sensory testing with children. Tests have to be easy for the investigator to conduct and for the children to understand. Additionally, children have a short attention span, implying that the whole test procedure must not take too long. The way of explaining the test to the children has to be motivating to get the consent of the children and keep them attending until they completed the whole test (Knof et al. 2011).

Furthermore, the decision making process of children is strongly influenced by adult approval and reaction. Commonly, children tend to respond affirmatively to positively phrased questions or change their opinion on enquiry by an adult immediately (Guinard 2001). A simple question like "are you sure?" can easily turn a "yes" into a "no".

Besides the psychological development, there are also differences in the physiological development: children have about five times more taste buds, and their foliate papillae are larger and more abundant compared to those of adults. Nevertheless, this does not consequently lead to higher taste sensitivity, due to the fact that children's innervation of taste papillae is not fully developed. The development of the taste apparatus carries on through childhood (Plattig 1984). A small number of studies reported thresholds for children to be similar to those of adults (Anliker et al. 1991). Most publications, however, found young children's sensitivity to be about a magnitude lower than the one reported for adults (Glanville et al. 1964; James et al. 1997).

It can be summarised that for sensory testing with children, there are four main challenges:

1. To attract and motivate children to participate in the test procedure and to keep the motivation up throughout the whole test duration. Therefore, it works best to arrange the test procedure as

a (board) game and introduce it to the children with a small story. This offers the possibility for direct interaction and involvement. Due to the game-like character of the procedure, children hardly notice taking part in a test, which prevents them from pretending or cheating in order to achieve better results.

2. To cope with the cognitive abilities of children, most notably reflected in a short attention span, distractibility, as well as difficulties in task comprehension. Thus, the procedure needs to be easily understandable and attractive for the participating children. The test should therefore last no longer than 15 minutes. This is crucial in order to receive good results. If children get bored, their answers will be influenced by their impatience to finish the game as soon as possible.
3. To eliminate any influence of the performing experimenter as children of this age are easily led by adults. The experimenter's relationship with the child is of great importance. In particular, special attention of the performing experimenter has to be paid on the language and phrasing of questions. This should be done in a way that avoids influencing the child's decisions. Furthermore, appropriate tone and body language is of high importance to help the children to make them feel comfortable. To exclude distraction from parents, guardians, or caregivers, these should ideally wait in another room.
4. To account for the limited physiological development of children, i.e. the less developed taste apparatus and the smaller hands compared to those of adults. Children are accustomed to kid-sized

furniture and materials at home as well as their school environments. Suitable equipment, for instance small cups, will arouse the children's interest and thus increase willingness to participate. To account for the children's underdeveloped taste apparatus, the range of concentrations of applied test solutions needs to be adapted.

Test environments are most suitable when they are familiar to the children. Ideally the tests are performed in pre-school or school setting. In addition, the test environment should be bright and decorated in a child-friendly manner which, however, should not be too colourful in order to avoid distraction.

12.3 Performance on European Level – The Need for Standard Operating Procedures (SOPs) and Standardisation

Procedures for application within the framework of a multi-centre study have to provide comparable results on a cross-national level. In the IDEFICS and I.Family studies all survey teams were trained at a central training session previous to the survey to assure standardised test performance and preparation of test solutions in each survey centre (for access see Section 12.8). This central training was conducted by experts and included lessons on behaviour, the used vocabulary and the phrasing of questions, as well as lessons on handling the equipment for preparation of test solutions and setting up the test environment. Detailed SOPs were provided to each survey centre.

The test substances, even if they appear simple such as sucrose or sodium chloride, were also standardised. A central supply was established for all test material: food samples, test substances and equipment for preparation of test solutions, as well as equipment such as drinking cups were purchased centrally and shipped to the survey centres. As an example, to avoid anti-caking and flow-regulating agents which are commonly applied by industry, food samples were purchased centrally without additives and provided to the survey centres pre-packaged and 'ready-to-use' for the preparation of the test solutions. Taking into consideration that tap water quality differs substantially among the European countries, demineralised water was used for all test samples and procedures.

Accordingly, the selection of appropriate food samples is also very challenging on a cross-national level. For example, a juice which is well accepted in one country can be broadly rejected in other countries and recipes may differ by country.

12.4 General Considerations

For children as minors parental or guardian consent is required in addition to an oral consent given by the children to participate in the test procedures. Food intolerances in terms of food allergies and food sensitivities are highly prevalent in Europe. Especially, the sensitivity for monosodium glutamate and gluten (celiac disease) is widely spread in Europe. Therefore, parents needed to be asked for particular food allergies of their children before the tests.

Performing experimenters were not allowed to use fragrance or perfumed hand cream and to consume cigarettes, coffee, or bubble gum prior to the tests. It was assured that participating children were neither hungry nor saturated. Ideally, they had had their last meal one hour previous to the test sessions. Peppermint chewing gums or sweets with a strong taste were not allowed one hour prior to the tests.

Test environments were instructed to be bright, friendly decorated and colourful rooms, where children feel comfortable. It was pointed out that there should be no undesired odours like strong smell from kitchens or disinfectants.

12.5 Test Designs of Sensory Taste Perception Tests

After taking into consideration the aforementioned challenges, four different test procedures were developed to be implemented in the different surveys of the IDEFICS and I.Family studies.

1. As the taste qualities sweet, salty, bitter, and umami (the taste for monosodium glutamate) appeared to be most important in the context of overweight and obesity, these qualities were selected for the *taste sensitivity tests*. In the literature, salty and umami tastes have been shown to be associated with increased appetite (He et al.

2008; Bellisle 1999; Rogers and Blundell 1990) and the sweet taste characterises many calorically dense foods. The bitter taste, however, is characteristic for a variety of vegetables, such as broccoli or Brussels sprouts, which are low in calories and often rejected by young children.

2. Besides taste sensitivity for basic taste qualities, *taste preferences* for sweet, salty, umami, aroma and fat were assessed. Aromas have recently been discussed in literature to be linked to increased appetite and thus to contribute to overweight and obesity (Azadbakht and Esmaillzadeh 2008). The role of fat appears to be even clearer: fat is the macronutrient with the highest energy density.

3. Furthermore, a food and beverage preference questionnaire was recommended for the use in epidemiological studies as a proxy for sensory taste perception (Duffy et al. 2009). For this purpose a *food and beverage preference questionnaire* that assesses preferences for sweet, bitter, salty and fatty was developed for I.Family.

4. In a subsample of the I.Family children, a *taste intensity test* for sweet and fatty was conducted to assess the perceived taste intensity, taste preference and taste sensitivity. The use of suprathreshold intensities was recommended due to its impact on every day life's decisions regarding food choice (Snyder and Bartoshuk 2009).

Generally, for all four test procedures, children from the age of 6 years and older were invited to participate. Pre-testing showed that children under the age of 6 years often did not comply with the test procedure and that it was not possible to obtain reliable answers from them (Suling et al. 2011).

12.5.1 Threshold Testing

As a measure of taste sensitivity, the detection threshold was assessed as the lowest concentration of a stimulus that must be exceeded, in order to have any taste effect (Pfaffmann et al. 1971). Reliance on cognitive resources are minimised for this methodology. In addition, to deal with response bias in terms of experience and expectation, the paired comparison forced-choice technique was used (Linschoten et al. 2001).

12.5.1.1 Test Procedure

The basic test design for the sensory threshold tests was based on the Deutsche Industrie Norm (DIN, German Industry Standard) 10959 for adults which works with ten aqueous solutions with increasing concentrations of the corresponding test substance. For our purpose, the test design had to be adapted to the physical and psychological development of children. First, the number of test solutions was reduced to five. Furthermore, an additional cup of demineralised water was provided and the children were instructed to compare each test solution with this cup to identify a deviant taste.

The threshold tests were arranged as a board game (Fig. 12.1) to attract and motivate the children to take part in the test procedure. For each basic taste a row of five test solutions with increasing concentrations of stimuli were presented in small cups, with a volume of 20 ml to the children at the lower end of the board.

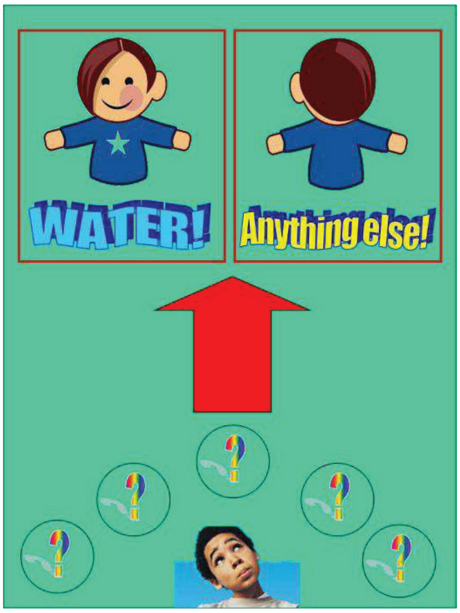

Fig. 12.1 Game board for taste threshold testing. The board was printed in A3 and laminated (source: Ahrens 2015)

The experimenter introduced the board game with a small background story: the children were told to be “taste detectives”. They learned that some cups on the board may contain pure water whereas some other may taste different and they would have to find the deviant cups. The children were advised to compare the test solutions with an extra cup of demineralised water. In case that the test solution from the board tasted like the water in the cup, they had to place it on a water icon on the upper left side of the board. If the child tasted a difference they had to place the test solution on the crossed water icon on the upper right of the board. Between the different solutions the children neutralised the taste in their mouth with demineralised water. The whole test procedure did not exceed 15 minutes.

12.5.1.2 Test Samples

The test solutions were prepared by the survey centres on-site. Therefore, for each basic taste quality a concentrated stock solution had to be prepared from the corresponding test substance. Using this stock solution the survey centres had to prepare the five test solutions by dilution in demineralised water. The survey centres received small packages with the test substances in the correct amounts needed to prepare the stock solution. This was done in order to minimise the efforts for the survey centres and avoid mistakes.

To assure that the stimuli were adequate for children the range of concentration of each test substance was about an order of magnitude above the threshold level reported for adults. The concentration ranges of the test substances were (in the order of assessment):

1. sucrose (8.8 mmol/l – 46.7 mmol/l) for the sweet taste threshold,
2. sodium chloride (3.4 mmol/l – 27.4 mmol/l) for the salty taste threshold,
3. caffeine (0.26 mmol/l – 1.3 mmol/l) for the bitter taste threshold,
4. monosodium glutamate (0.6 mmol/l – 9.5 mmol/l) for the umami taste threshold.

12.5.2 Preference Testing

Preference testing with children is by far less complicated than threshold testing. For the preference tests, a paired comparison test according to the DIN standard 10959 was used in the IDEFICS study. This test allowed a direct comparison between two samples and is a simple, very common technique to evaluate taste preferences. Several studies showed that children 6 years and older were capable of performing paired preference tests (Guinard 2001; Popper and Kroll 2005; Kimmel et al. 1994; Kroll 1990; Léon et al. 1999; Liem et al. 2004).

A preference test is an affective test and evaluates the higher degree of liking for one over at least one other sample. Comparing the preference of two samples is called a paired preference test. It is one of the most important approaches in sensory testing, as the test design is easy and thus allows focusing on the presented samples. It goes well with the sensory abilities of young children to discriminate the preferences in the offered food pairs. Furthermore, the paired comparison forced choice technique was used: the children had to make a preference decision between the two samples. If children could or would not make a choice no result was reported to avoid additional stress on the children.

12.5.2.1 Test Procedure

To create a very easy, child-friendly test design, a simple game board was developed for the preference test (Fig. 12.2). During the test, it was placed on a table in front of the children. In each test sequence, the children had to compare the sensory pleasure of a basic sample and a modification of it. Each pair was presented inside the two circles at the bottom of the board. The children were asked to place their preferred sample on the happy smiley at the top of the board. Between each test sequence the children rinsed their mouth with demineralised water to avoid adaptation.

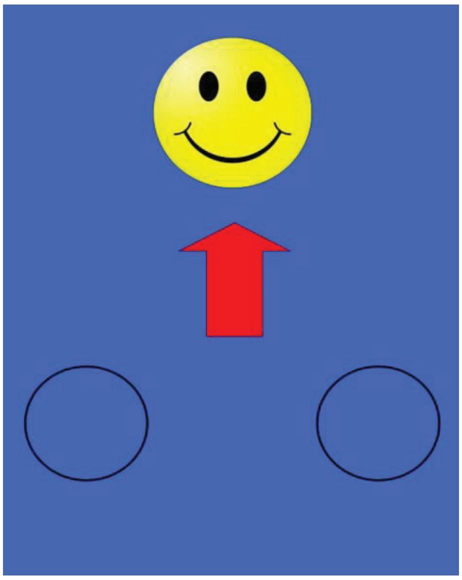

Fig. 12.2 Game board used for preference testing. The board was printed in A4 format and laminated (source: Ahrens 2015)

12.5.2.2 Food Samples

The food samples were selected according to two main criteria: the sensory acceptance by school children aged 6 years and above in all participating countries and the representativeness of the food samples for the evaluated taste quality. Crackers were used to test salty, fatty and umami taste preferences and apple juice was used to assess the preference for sweet taste and aroma.

12.5.2.2.1 Food Samples for Sweet and Aroma

As reference for the sweet and aroma taste preferences, a clear natural apple juice was selected, with an addition of 0.53% sucrose to ensure proper acceptance of the juice. To evaluate the taste preference for sweetness, the amount of sucrose in the reference juice was increased to 3.11%. To evaluate the taste preferences for aroma, 0.05% apple aroma (nature identical, SENSIENT®) was added to the basic apple juice (Table 12.1).

Table 12.1 Overview on recipes for apple juices applied to assess preferences for the sweet taste and aroma

	Apple juice (%)	Sucrose (%)	Aroma (%)
Reference	99.47	0.53	--
Sweet taste	96.89	3.11	--
Aroma taste	99.42	0.53	0.05

All juices were presented on the board (Fig. 12.2) in 20 ml cups. The small sample quantities were chosen in order to prevent the children from becoming saturated. All survey centres were instructed to serve the juices at 18°C +/- 2°C. The order of assessment was sweet followed by aroma.

12.5.2.2.2 Food Samples for Salty, Fatty and Umami

To evaluate the preferences for salt, fat and umami, a cracker was developed. To increase the attractiveness for young children the crackers were heart-shaped (Fig. 12.3).

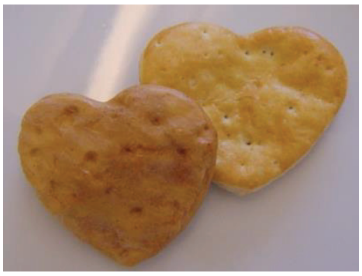

Fig. 12.3 Heart-shaped crackers for the assessment of taste preferences for salt, fat and umami

The reference crackers for all tests were produced according to a basic recipe containing wheat flour, water, 0.7% salt and 8% fat, finished with soda lye (0.5% aqueous solution, purchased from Carl Roth Chemicals). To evaluate the taste preference for fat, the amount of fat included in the basic recipe was increased from 8% to 18%. To evaluate the taste preference for salt, the amount of salt was increased from 0.7% to 1.6%. Additionally, 1% of an emulsifying agent (DAWE, diacetyl tartaric ester) was added. To evaluate the taste preference for umami, 1% of monosodium glutamate and 1% emulsifier were added to the basic recipe (Table 12.2).

Table 12.2 Overview on recipes for crackers applied to assess preferences for the salty, fatty and umami taste

	Flour/water (%)	Salt (%)	Fat (%)	MSG (%)	DAWE (%)
Reference	91.3	0.7	8	0	0
Salt	89.4	**1.6**	8	0	1
Fat	81.3	0.7	**18**	0	0
Umami	89.3	0.7	8	**1**	1

DAWE = diacetyl tartaric ester; MSG = monosodium glutamate (source: Knof et al. 2011)

The order of test assessment was first fat then salt and umami last. This order was selected due to the fact that the umami taste remains on the taste buds for a long time and would thus affect subsequent taste experiments.

12.5.3 Food and Beverage Preference Questionnaire

In I.Family a food and beverage preference questionnaire was developed to assess preferences for sweet, bitter, salty and fatty that could be applied in children/adolescents as well as in adults. This questionnaire did not require high cognitive abilities from the participants. The food and beverage preference questionnaire was compiled based on existing tools (Deglaire et al. 2012; Vereecken et al. 2013). Additionally, bitter food items were compiled from studies that investigated the bitter taste perception by reviewing the literature. Thus, the questionnaire assessed preferences for the sweet, fatty, bitter and salty taste. It contains pictures of relevant food and drink items to be used in all age groups (Fig. 12.4). In total, the questionnaire consisted of 63 items including single foods (e.g. banana, spinach), mixed foods (e.g. hot dog, kebab), condiments (e.g. jam, mayonnaise) and drinks (e.g. coke, lemonade).

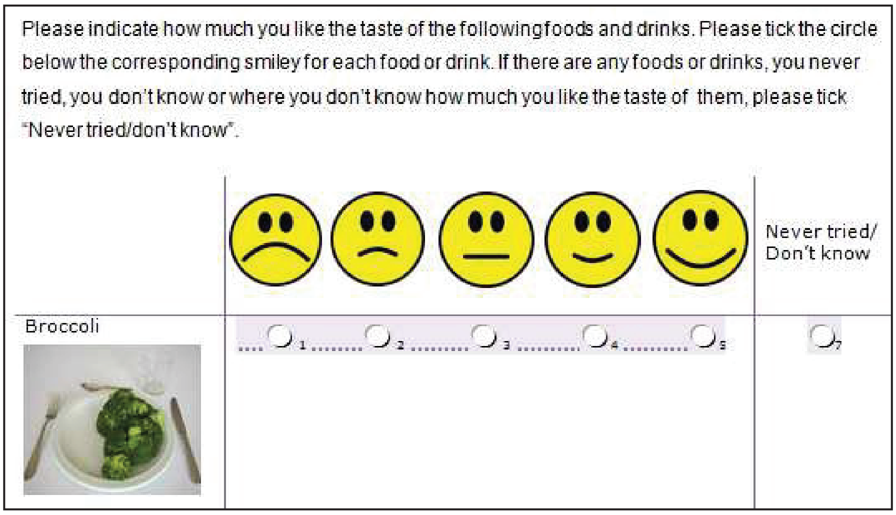

Fig. 12.4 Example (screen shot) from the food and beverage preference questionnaire (source: Jilani et al. 2017)

12.5.3.1 Test Procedure

Participants were asked to indicate how much they liked the taste of the food presented on the pictures using a 5-point Smiley Face Likert scale, ranging from disliking to liking. Thus the variable of liking for each food and drink item ranged from 1 to 5, with 1 meaning 'do not like at all' and 5 meaning 'like very much'.

Additionally, participants could indicate that they do not know or have never tasted the specific food item. A pre-test was conducted in every country to ensure the feasibility of all food items across countries.

12.5.3.2 Taste Preference Scores

Foods that were ranked by at least 75% of the participants (e.g. less than 25% of the participants never tried or did not know the food) were used for further analyses. Participants were excluded when they had more than 20 items missing or “Never tried / Don’t know” answers. Furthermore, a sex- and age-specific factor analysis was conducted to gain more accurate information about the factorial structure of food preference. The strata were younger boys (<12 years), younger girls (<12 years), older boys (≥12 years), older girls (≥12 years), and their mothers and fathers. A food or drink item was considered to belong to a particular factor if the factor loading was greater than 0.30 on that factor. The factor analysis explained between 32% and 41% of the overall variance of the variables (fathers 41%, mothers 39%, older girls 36%, older boys 38%, younger boys 37% and younger girls 32%). The obtained factors were used to conduct a content analysis in order to assign the factors to the taste modalities sweet, salty, fatty and bitter. Food and drink items with no load on one of the factors were not included in further analyses.

Scores for liking of the specific taste quality were computed by calculating the mean liking of the foods and drinks included in each of the four categories. Scores were calculated individually for younger boys, younger girls, older boys, older girls as well as their mothers and fathers. The sum of the ratings for the foods and drinks was calculated and divided by the number of foods and drinks that were included in the specific taste modality group.

12.5.4 Taste Intensity Test

For the last survey of I.Family a new sensory taste perception test was applied. Perceived taste intensity for different concentrations of sugar and fat were assessed in a smaller subsample in every participating country. At that time a more advanced technique could be implemented because all participating children were at least 8 years old. Cold whipped vanilla pudding was chosen as matrix to measure perceived taste intensity (Mennella et al. 2012). Children are in most cases familiar with pudding, which increased the acceptance and adherence to the test procedures. It can be varied easily in sugar and fat concentrations, and due to its fluidity the sweet and fatty taste can diffuse easily in the oral cavity. Children with at least one parent present participated in this test.

12.5.4.1 Test Procedure

The test was organised in two different blocks (fatty/sweet). It started either with sweet or fat in a randomised order. Participants had to rate three cups of pudding with different sugar concentrations and three cups of pudding with different fat concentrations on a 9-point-scale (Fig. 12.5) and on a 'generalised Labelled Magnitude Scale' (gLMS) developed by Green et al. (1996) and modified by Bartoshuk et al. (2004). Finally, they were asked to indicate their favourite pudding of the fat and the sweet block placing it on a happy smiley (Fig. 12.5). The three samples were presented in counterbalanced order in 20 ml plastic cups to level out potential confounding effects (Zandstra and de Graaf 1998). To test fatty taste perception the term 'creamy' was used to avoid negative associations to fat.

Between each test sequence the participants rinsed their mouth with demineralised water to avoid adaptation and took a 2 to 3 minutes break. The test continued with the second block following the same procedure. The pudding samples were presented under a red light in order to mask colour differences.

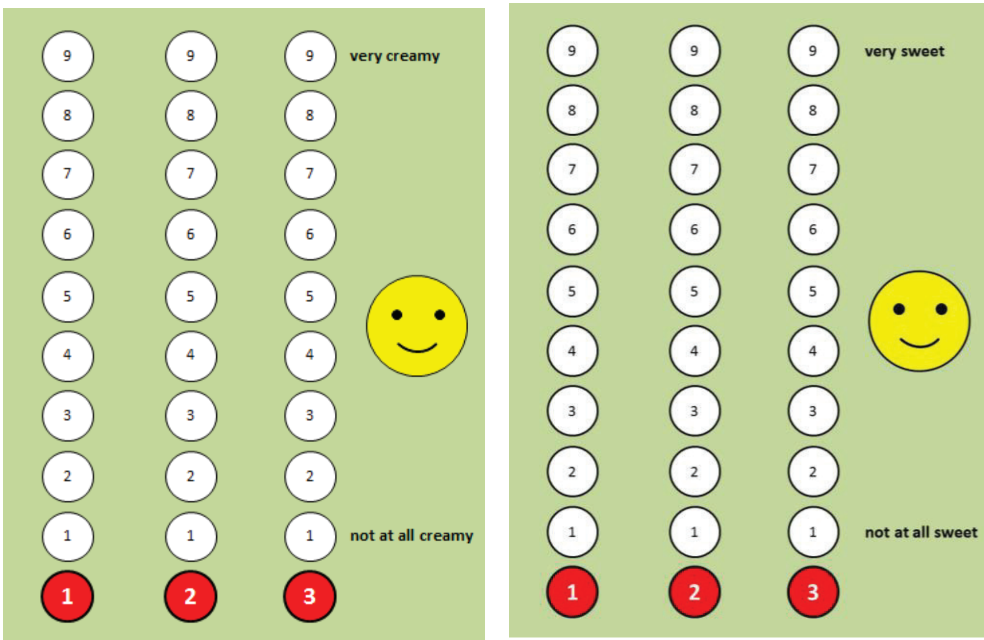

Fig. 12.5 Game board used for intensity testing. The board was printed in A3 format and laminated

12.5.4.2 Test Samples

Cold whipped vanilla pudding (RUF Schlemmer Crème, vanilla flavour) was chosen as flavour carrier with different concentrations of sugar and fat. The first concentration step was the same for both test blocks (sweet/fatty) and contained 14.5% sugar and 3.1% fat. For testing sweet taste perception the proportion of fat was kept stable and the amount of sugar was modified to 24.1% in a second concentration step and to 36.2% in a third concentration step (Table 12.3). Sucrose was used to modify the sugar concentration. For testing fatty taste perception the proportion of sugar was kept stable and the amount of fat was modified to 6.8% in a second concentration step and to 14.1% in a third concentration step (Table 12.3). Centrally purchased and shipped cream was used to modify the fat concentration.

Table 12.3 Overview of recipes for vanilla pudding applied to assess preferences for the sweet and fatty taste

	Sucrose[1] %	Fat[2] %
Reference	14.5	3.1
2nd concentration step	24.1	6.8
3rd concentration step	36.2	14.1

[1] Fat concentration was kept stable at 3.1%
[2] Sugar concentration was kept stable at 14.5%

12.6 Test-retest Reliability

To assess the test-retest reliability the preference and threshold tests were performed over two consecutive mornings under identical conditions, partially with different experimenters. For threshold testing, it was assessed if the threshold levels, measured on the second day, were lower, higher or matched exactly to the threshold values measured on the previous day.

For the preference tests, it was assessed if the children's choice from the consecutive session matched their choice from the previous session. In total, 40 children (aged 6 to 10 years) participated in the retest session for threshold testing and 20 (aged 6 to 10 years) for preference testing. These groups were homogenous in terms of age and sex.

Test-retest reliability was determined by comparing the two scores of the consecutive sessions. To assess intra-rater reliability of the developed methods, weighted Cohen's Kappa (κ) coefficients were calculated.

Table 12.4 Test-retest correlation for the threshold test procedure (N=40)

Taste quality	COHEN's κ
Sucrose	0.81
Sodium chloride	0.75
Caffeine	0.68
Monosodium glutamate	0.77

(Source: excerpt from Table 3 in Knof et al. 2011)

Table 12.5 Test-retest correlation for the taste preference test procedure (N=40)

Preference for	COHEN's κ
Sweet taste	0.77
Aroma taste	0.78
Fatty taste	0.77
Salty taste	0.86
Umami taste	0.80

(Source: excerpt from Table 4 in Knof et al. 2011)

Analysis of test-retest results for threshold testing shows a strength of agreement that is rated to be "almost perfect" for the detection of sucrose, according to Landis and Koch (1977). For the detection of sodium chloride, caffeine, and MSG the strength of agreement is rated to be "substantial" (Table 12.4). For preference testing, the degree of stability between the sessions is rated to be "substantial", for the sweet, aroma-, fatty, and umami taste. For the salty taste preference, the strength of agreement again is “almost perfect” (Table 12.5).

This shows that sensory testing with children is reliable, provided that the applied methods are adapted to the special requirements.

12.7 Experiences from the Surveys

A subsample of 20% of the school children who participated in the IDEFICS study was selected to perform the battery of sensory tests (threshold and preference tests) in the baseline survey. Sensory testing was restricted to children 6 years of age and older because experiences in the pre-test showed that pre-school children often did not comply with the test procedure and that it was not possible to obtain reliable answers from them (Suling et al. 2011). Altogether, 1,861 children from eight countries participated in the taste threshold tests and 1,718 in the taste preference tests in the IDEFICS study (Ahrens et al. 2011). In I.Family, 7,841 children and 5,938 parents filled in the food and beverage preference questionnaire as well as 985 children and 788 parents participated in the taste intensity test. In addition, 1,046 children and 58 parents participated in the taste preference test.

As reported by the survey centres or discovered during the site visits performed by the central quality control unit of the project, several problems occurred during the examinations. The survey centres could not always guarantee for the best possible test conditions. It was, for example, sometimes difficult to find bright, colourful and friendly decorated rooms in the schools and to guarantee the absence of undesired odours and sources of distraction, as the survey personnel depended on the schools to offer them appropriate rooms for the examination.

Since sensory tests were largely unknown to the experimenters the detailed description of test procedures in the general survey manual, the central training, the provision of SOPs and the site visits of the coordinating centre to all survey centres turned out to be very important. Otherwise non-compliance to the SOPs would have occurred, e.g. terminating the test sequence of the threshold test once the child detected the first test solution deviant from water or conducting the preference tests in the wrong order (1^{st} crackers, 2^{nd} juice).

The survey centres complained about the time-consuming preparation of test solutions for the threshold and intensity tests (despite of the pre-packaged portions of chemicals that were supplied). The workload attributed to the preference tests was much smaller since all test materials were delivered ‘ready-to-use’ by the coordinating centre. In general, the experiences from the survey underline the beneficial effect of a central supply of consumables. Especially multi-centre studies depend on careful standardisation of test materials to obtain comparable results from different settings and countries.

Finally, all centres reported that the game-like character of the sensory test module enhanced the acceptance of this survey module and made it quite popular among the participating children. Furthermore, the sensory tests were reported to be the participants' favourite procedure during the surveys. In general, all four test procedures were age-appropriate according to the study populations of the IDEFICS and I.Family studies as they never turned out to require too high cognitive abilities.

12.8 Provision of Instruments and Standard Operating Procedures to Third Parties

All instruments described in this chapter including the General Survey Manuals that provide among other all standard operating procedures can be accessed on the following website: www.leibniz-bips.de/ifhs after registration.

Each third partner using the instruments provided in this chapter is kindly requested to cite this chapter as follows:

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follow-up including the development of new instruments and the adaptation of previously used instruments was conducted in the framework of the I.Family study (www.ifamilystudy.eu) which was funded by the European Commission within the Seventh RTD Framework Programme Contract No. 266044 (KBBE 2010-14).
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