

Correlates of bitter, sweet, salty and umami taste sensitivity in European children: Role of sex, age and weight status - The IDEFICS study

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1 Correlates of bitter, sweet, salty and umami taste sensitivity in European children: Role

- 2 of sex, age and weight status the IDEFICS Study
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- 40
- 41 **Conflict of interest**
- 42 All the authors declare that they have no conflict of interest.

43

44 Abstract

We aimed to describe differences in taste sensitivity in children according to age across 7- to 45 11-year-old children from eight European countries. We further compared taste sensitivity 46 47 between boys vs. girls and under-/normal weight vs. overweight/obese children. Within the European multicentre IDEFICS (Identification and prevention of dietary and lifestyle-induced 48 health effects in children and infants) study, 1,938 school children participated in sweet, 49 bitter, salty and umami detection threshold tests between 2007 and 2010, using the paired 50 comparison staircase method. The lowest concentration at which the child was able to detect a 51 difference to water was determined as taste detection threshold as a proxy of taste sensitivity. 52 Mean taste thresholds were calculated stratified for sex, age groups, weight groups and 53 country. BMI was calculated using measured height and weight; socio-demographic 54 information was collected using questionnaires. Ordinal logistic regressions were conducted 55 to investigate the association between sex, weight status (as categorical exposure variable) 56 and age (as continuous exposure variable) and the taste sensitivity for the four taste modalities 57 (as outcome), separately. Older children were more taste sensitive for sweet and salty and less 58 taste sensitive for umami and bitter than younger children. Girls were more sensitive to sweet 59 60 taste than boys. Overweight or obese children were less sensitive to sweet and salty taste compared to normal weight children This was the first study comparing taste sensitivity by 61 62 measuring taste thresholds in children across different European countries. We conclude that taste thresholds are associated with weight status, children become more sensitive to sweet 63 and salty tastes with increasing age, and girls might be more sensitive to sweet than boys. 64

65

66 **1. Introduction**

Overweight and obesity among children continue to be a major public health concern in Europe and worldwide. About 22 million children in Europe are overweight or obese (Watson, 2008). 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 childhood, when other aspects such as healthiness and prices of foods are not yet considered (Birch, 1979, 1998).

73 One dimension that contributes to sensory taste perception is taste sensitivity. Sensory taste sensitivity can be measured through the assessment of taste thresholds where the lowest 74 concentration of a taste modality that can be detected is considered as detection threshold. 75 Sensory taste sensitivity differs substantially between individuals and changes during the 76 developmental stages of infancy and childhood (Anliker, Bartoshuk, Ferris, & Hooks, 1991). 77 78 Although infants and children have up to five times more taste buds than adults, they do not 79 seem to be more taste sensitive, probably because the innervation of taste papillae in infants is not yet fully developed and functional (Plattig, 1984). Nevertheless, studies on taste 80 81 thresholds in children, show inconsistent results. Whereas Anliker et al. observed that children aged 5 to 6 years and adults have similar bitter taste thresholds when determining the PROP 82 83 taster status (Anliker et al., 1991), other studies showed that children and infants have higher taste thresholds; Glanville, Kaplan and Fischer showed this by measuring detection thresholds 84 85 for bitter with different tastants like PROP and quinine sulphate (Glanville, Kaplan, & Fischer, 1964; James, Laing, & Oram, 1997) or lower recognition thresholds for bitter taste 86 87 than adults (Whissell-Buechy, 1990). James et al. measured detection thresholds for sweet (using sucrose), salty (using sodium chloride) sour (using citric acid) and bitter (using 88 caffeine) and reported that 8-9 year old boys had higher thresholds for sweet, bitter and salty 89 tastes than adults and higher sweet and salty thresholds than girls, while girls' taste thresholds 90 were similar to those of adults (James et al., 1997). The results of James et al. indicate that 91 there might also be sex differences with regard to taste sensitivity during childhood. Overberg 92 93 at al. reported that older children showed a higher overall taste sensitivity (sweet, salty, sour, 94 bitter and umami) than younger children assessing taste sensitivity using taste strips with different concentrations of sucrose (for sweet), citric acid (for sour), sodium chloride (for 95 salty), monosodium glutamate (for umami) and quinine hydrochloride (for bitter) (Overberg, 96 Hummel, Krude, & Wiegand, 2012). In contrast, Vennerød et al. found in a longitudinal study 97

that children between 4 to 6 years became less sweet sensitive, more sour and salty sensitive
and remained stable with regard to bitter and umami sensitivity when measuring detection
thresholds with different concentrations of sucrose (for sweet), citric acid (for sour),
monosodium glutamate (for umami) and quinine hydrochloride dehydrate (for bitter)
(Vennerod, Nicklaus, Lien, & Almli, 2018).

Results of studies investigating associations between taste sensitivity and weight status 103 are also contradictory. Overberg et al. found that children and adolescents with obesity had 104 105 higher salty, umami and bitter thresholds than children and adolescents without obesity. In contrast, another study compared taste sensitivity of 39 adolescents with obesity versus 48 106 107 adolescents without obesity and found that those with obesity were more sensitive for sweet and salty taste (Pasquet, Frelut, Simmen, Hladik, & Monneuse, 2007). Further results on the 108 109 other hand showed no associations between salty taste sensitivity in 421 adolescents (Kirsten & Wagner, 2014) nor in 72 children and adolescents (Alexy et al., 2010) and Fernández-110 Aranda et al. did not find any association between taste perception of any taste modality and 111 extreme weight/eating conditions in adults (Fernandez-Aranda et al., 2016). 112

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 may not be applicable in children. Besides age and sex, which may influence individual taste thresholds, taste sensitivity itself may be associated with overweight and obesity as described above.

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. Further, the mentioned studies that investigated weight status differed in their sample size, ranging from 72 to 421. This could be another reason for inconsistent results.

For the first time the present study describes taste sensitivity by measuring taste detection thresholds for sweet, bitter, umami and salty taste in 1,938 boys and girls using a standardised study protocol across 8 European countries. Further, this study analysed the hypotheses that sex, age and weight status are associated with sweet, salty, bitter and umami taste thresholds.

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128 **2. Methods**

129 2.1 Study design and participants

The study sample group is a sub-sample of the IDEFICS (Identification and prevention of 130 dietary and lifestyle-induced health effects in children and infants) study, a prospective 131 European multicentre cohort study whose aim was to investigate the aetiology of lifestyle-132 and nutrition- related disorders such as childhood overweight and obesity. The overall aim 133 and design of the IDEFICS study has been described previously (Ahrens et al., 2011). 134 Between September 2007 and May 2008, a baseline survey (T0) was conducted in 16 228 135 children from 8 European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, 136 Spain and Sweden) aged 2 to 9.9 years. Two years later, between September 2009 and May 137 2010, a follow up (T1) examination was conducted. Between T0 and T1, interventions to 138 improve health behaviour took place in one region in each country, with one other region in 139 each country serving as a control region. A sub-sample of children from the age of 6 years 140 141 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) 142 resulted in a final study sample for this cross-sectional analysis of 1,938 children aged 143 144 between 7 and 11 years old that participated in sweet, salty, bitter and umami threshold tests either at T0 or T1. 145

146 All centres obtained ethical approval from their local institutional review board (e.g. Ethics Committee, University Hospital, Gent, Belgium; Cyprus National Bioethics 147 Committee, Nicosia, Cyprus; Tallinn Medical Research Ethics Committee, Tallinn, Estonia; 148 Ethics Committee of the University of Bremen, Bremen, Germany; Egeszsegugyi 149 Tudomanyos Tanacs, Pecs, Hungary; Comitato Etico dell'Azienda Sanitaria Locale di 150 Avellino, Italy; Regionala Etikprovningsnamnden i Göteborg, Gothenburg, Sweden; Comite 151 Etico de Investigacion Clínica de Aragon, Zaragoza, Spain). Parents gave their written 152 informed consent and children were first informed orally, after which they gave their oral 153 consent to participate in our study. 154

155 2.2 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 (Cole & Lobstein, 2012). Children

were classified into underweight/normal weight and overweight/obese (weight status) using
age- and sex-specific cut-offs published by Cole and Lobstein. The cut-offs for overweight
were for boys the 90.5th and for girls the 89.3rd percentile curve (Cole & Lobstein, 2012).

163 **2.3 Taste threshold tests**

For the taste threshold test, a paired comparison staircase taste threshold test to assess the 164 sweet, salty, umami and bitter detection threshold was arranged as a board game as described 165 by Knof et al. (Knof et al., 2011). In brief, 5 watery solutions (see Table 1) prepared with 166 distilled water, with ascending concentrations of sucrose (8.8-46.7 mmol/l, sweet), sodium 167 chloride (3.4-27.4 mmol/l, salty), monosodium glutamate (0.6-9.5 mmol/l, umami) or caffeine 168 (0.26-1.3 mmol/l, bitter) were presented to the participant in 20 ml cups at room temperature. 169 To prepare the solutions, sucrose from Applichem GmbH, Darmstadt, Germany was used for 170 sweet, sodium chloride from Applichem GmbH, Darmstadt, Germany for salty, caffeine from 171 Carl Roth GmbH & Co. KG, Karlsruhe, Germany for bitter and sodium glutamate from 172 173 Merck KGaA, Darmstadt, Germany for umami. Cups were placed at the bottom of the game 174 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 175 176 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. Thus, the child was only asked to indicate 177 if it tasted something and not which taste they perceived. After the decision was made, the 178 child was not allowed to try again. The first concentration at which the participant could taste 179 180 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 181 modality. After each taste modality, participants recovered for 2 minutes, during which they 182 neutralised their palate with distilled water, and the field staff prepared the next test sequence. 183 According to the examination protocol, the children should not have eaten for at least an hour 184 before the examinations, but should not be hungry either. Adherence to this stipulation was 185 ensured through the fact that the children participated in another examination module of the 186 IDEFICS study prior to the first lesson in the morning. As the children had to be in fasting 187 status for this examination, they received something to drink and eat afterwards. They then 188 joined their classes from where they were taken individually to the sensory taste perception 189 tests. These were solely conducted during the morning hours, in the school setting. The order 190 of presentation of tested taste modalities was fixed as follows: sweet, salty, bitter and umami. 191

The concentrations were chosen based on the DIN (German Institute for 192 Standardisation, www.DIN.de) 10959, which defines concentrations of test samples and 193 procedures for adults. It works with ten aqueous solutions with increasing concentrations of 194 the corresponding test substance. For our purpose the test design had to be adapted to the 195 physical and psychological development of children. First, the number of test solutions was 196 reduced to five. Furthermore, an additional cup of distilled water was provided to the child to 197 compare the test solutions with a neutral taste. Between the different taste modalities children 198 199 rinsed their mouth with water and waited for two minutes before they moved on to the next taste modality. The adapted concentrations and procedures were adjusted after being pre-200 tested in all survey centres (Suling et al., 2011). During the development of the taste threshold 201 tests 40 children were selected randomly to be included into the test-retest procedure. This 202 subsampled consisted of 22 boys and 18 girls aged between 5 and 7 years. The test-retest 203 204 analysis of the taste threshold tests revealed a kappa coefficient of 0.81 (sweet), 0.75 (salty), 0.68 (bitter) and 0.77 (umami) (Knof et al., 2011). Thus, the analysis of test-retest results 205 show a strength of agreement that is rated to be "almost perfect" for sweet and "substantial" 206 for the detection of salty, bitter, and umami (Landis & Koch, 1977). The results of the test re-207 test procedure in the sub-sample were assumed to be applicable to the full sample. We 208 conducted extensive pre-tests and found the test procedures to be only suitable for children 209 from the age of 6 years onwards but not for pre-schoolers (Suling et al., 2011). Schoolchildren 210 were in general able to understand the task and to deliver meaningful results. Therefore, the 211 minimum age was set to 6 years for the actual taste threshold tests. Thus, the test procedure 212 was developed in a way that was easy to understand for children from the ages of 6 years 213 214 (Suling et al., 2011). Training for the implementation of the standardised testing protocol was organised centrally for all 8 countries and testing materials were prepared centrally and then 215 shipped to the survey centres. On the day of testing the survey centres only needed to prepare 216 the solutions with distilled water according to standard operation procedure (SOP). The 217 218 adherence to the testing protocol was monitored via site visits to ensure a maximum degree of 219 standardisation.

220 **2.4 Taste sensitivity**

For our study taste sensitivity was determined measuring taste thresholds. Taste thresholds were measured as described above resulting in five categories of taste sensitivity. Having the lowest taste threshold means being very sensitive to the specific taste modality. Having the highest taste threshold in contrast means being very insensitive to the specific taste modality. The five taste threshold levels were used as categorical variables for each taste modality in the statistical analysis as the main outcome and can be found in table 2.

227 **2.5 Statistical analyses**

Mean taste thresholds (mmol/l) and corresponding standard deviations (SDs) were calculated by age, sex and country. For the calculation of mean taste thresholds (mmol/l) the category 'no threshold' was excluded.

Ordinal logistic regressions were conducted to investigate the associations between sex (as 231 dichotomous exposure), weight status (as dichotomous exposure), age (as continuous 232 exposure) and taste sensitivity for the four taste modalities (as outcome), separately. The 233 234 outcome variable therefore had 6 categories ranging from 'threshold 1' to 'threshold 5' and 'no threshold'. The odds ratios (ORs) associated with one level lower taste threshold (i.e. 235 being one threshold level more sensitive) for girls, overweight/obese children and children 236 being one year older and the corresponding 95% CI were calculated. To prevent for 237 confounding by sex, country, residing in control or intervention region and weight status, the 238 ordinal logistic regression analyses were repeated with adjustment for these variables in 239 240 multivariate model. To exemplify the age trend, raw and adjusted ORs were also calculated for an increase in age of three years. 241

Furthermore, the k-means algorithm (Hartigan & Wong, 1979) was applied to the 242 standardised taste threshold variables (i.e. z-scores) to identify clusters of children with 243 similar taste patterns. In this popular data-driven cluster approach (Lo Siou, Yasui, Csizmadi, 244 McGregor, & Robson, 2011) the within-cluster variance is minimized and children are 245 partitioned into k distinct clusters, in which each child is assigned to the cluster with the 246 closest cluster mean, i.e., with respect to the standardized taste threshold children in the same 247 cluster are close to each other and far apart from children in the other clusters. Standardisation 248 is intended to prevent the k-means algorithm from weighting the variables differently due to 249 different variances. To decide on the appropriate number of clusters the so-called elbow 250 method was used taking into account the explained variances of all two- to eight -cluster 251 252 solutions. The five-cluster solution was favoured which explained 56 % of variance. The different clusters are explained in detail in the results section and can be found in tables 5 and 253 6. A multinomial logistic regression model was used to analyse the association between the 254 derived clusters as dependent variable and the demographic variables (sex, weight status and 255 256 age) as independent variables.

All analyses were carried out with SAS, version 9.3 (Statistical Analysis System, SAS

258 Institute Inc., Cary, USA).

3. Results

The study population included children between 7 and 11 years. In total, 466 children were 7 260 years old, 444 were 8 years old, 328 were 9 years old, 498 children were 10 years old and 202 261 were 11 years old. On average the children were 8.2 (SD 1.3) years old and the proportion of 262 girls and boys was evenly balanced. In total, 25.1% of the children were overweight or had 263 obesity. Children were most likely from Belgium (18.1%) and less likely from Sweden and 264 Germany (8.4% each). For the full sample, the mean BMI z-score was 0.5 (SD 1.2) (Table 1). 265 Mean (SD) taste thresholds of the full sample were 0.7 (0.3) mmol caffeine/l for bitter, 18.7 266 (8.9) mmol sucrose/l for sweet, 10.7 (6.1) mmol sodium chloride/l for salty and 2.9 (2.3) 267 mmol monosodium glutamate/l for umami. 268

269 Table 1: Characteristics of the study sample (total number and percentages or mean and standard deviation (SD))

270	given	by	sex	groups	
	0	~ _	~	0	

	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)
Śweden	80 (8.3)	83 (8.6)	163 (8.4)
Weight status ¹			
Under-/ normal weight	732 (75.5)	719 (74.3)	1451 (74.9)
Overweight/Obese	238 (24.5)	249 (25.7)	487 (25.1)
e	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 (Cole & Lobstein, 2012)

272 ²: BMI z-scores according to Cole and Lobstein (Cole & Lobstein, 2012)

Table 2 shows the concentrations of the different test solutions used. The concentration ranges have been described in the methods section. Further, the table shows the distribution of children assigned to the different taste thresholds. About 2/3 of the children belonged to the first or second sweet taste threshold whereas for bitter the children were more equally distributed across all taste thresholds including 'no threshold'. For salty, 2/3 of the children

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- belonged to the second or third taste threshold and for umami, most of the children belonged 279
- to the first, second or third threshold. 280

	1 (mmol/l)	2 (mmol/l)	3 (mmol/l)	4 (mmol/l)	5 (mmol/l)	No
	n (%)	Threshold n (%)				
Sucrose (sweet)	8.76	17.53	26.29	35.06	46.74	
	520 (26.8)	821 (42.4)	350 (18.1)	123 (6.4)	53 (2.7)	71 (3.7)
Caffeine (bitter)	0.26	0.51	0.77	1.03	1.29	
	340 (17.5)	372 (19.2)	313 (16.2)	220 (11.4)	196 (10.1)	497 (25.6)
Sodiumchloride (salty)	3.42	6.85	13.68	20.51	27.35	
	318 (16.4)	673 (34.7)	622 (32.1)	205 (10.6)	64 (3.3)	56 (2.9)
Monosodiumglutamate	0.59	1.77	3.55	7.10	8.87	
(umami)	441 (22.8)	615 (31.7)	483 (24.9)	248 (12.8)	58 (3.0)	93 (4.8)

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Table 3 shows further descriptive results. It shows the mean taste thresholds of 283 children that were included in our sample according to different groups (sex, weight status, 284 country and age). In our study sample higher sweet sensitivity was observed for girls than for 285 boys. Further, boys needed a slightly higher concentration of salt in water to detect a taste. 286 Overweight children/children with obesity of our sample were less sensitive to the salty taste 287 than under-/normal weight children and needed also a slightly higher sugar concentration in 288 water to detect a taste. For bitter and umami taste sensitivity there was no difference between 289 under-/normal weight children and overweight children/children with obesity. Further details 290 of the country, sex, weight and age group comparisons are presented in table 3. 291

292 Table 3: Means and standard deviations of concentrations to measure taste sensitivity for sex-groups, weight status, countries and age-groups 293

	Sweet threshold (mmol sucrose/l)	Salty threshold (mmol sodium chloride/l)	Umami threshold (mmol monosodium	Bitter threshold (mmol caffeine/l)
			glutamate/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)
Weight status				
Under-/ normal weight	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)
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)
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)

294 295

The results of the unadjusted ordinal logistic regression analysis revealed the 296 following. Odds ratios for the sex comparison - not statistically significant - showed that girls 297 were more sensitive to sweet and salty tastes than boys. (OR (95 % CI): 1.17 (0.99; 1.38) and 298 1.16 (0.99; 1.36), respectively). Statistically not significant odds ratios for overweight/obese 299 children compared to under-/normal weight children showed that overweight/obese children 300 were less taste sensitive towards sweet and salty (OR (95 % CI): 0.88 (0.73; 1.06) and 0.80 301 (0.66; 0.96), respectively). Older children were more sensitive for sweet and salty: Per year of 302 age increase, the statistically significant ORs (95 % CI) for a one level higher sensitivity for 303 sweet and salty were 1.12 (1.06; 1.20) and 1.18 (1.11; 1.26), respectively. Older children were 304 less sensitive for umami and bitter: Per year of age increase, the OR (95 % CI) for a one level 305 lower sensitivity for bitter and umami were 0.95 (0.89; 1.01) and 0.90 (0.84; 0.96), 306 respectively. Here the CIs indicated that the difference for bitter was not and for umami was 307 statistically significant. After adjustment the ORs changed slightly (Table 4). Overweight and 308 309 obese children were more sensitive to bitter than underweight and normal weight children after adjustment. 310

	Sw	veet	Sa	ılty	Bit	ter	Un	nami
	OR^1	OR^2	OR^1	OR^2	OR^1	OR^2	OR^1	OR^2
	95% CI							
Boys	Refe	rence	Refe	rence	Refei	rence	Refe	rence
Girls	1.17	1.16	1.16	1.16	0.96	0.95	0.94	0.93
Gills	0.99;1.38	0.98;1.36	0.99;1.36	0.99;1.37	0.82;1.26	0.81;1.11	0.80;1.11	0.79;1.09
Under-/normal weight	Refe	rence	Refe	rence	Refe	rence	Refe	rence
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
$\Lambda q_2 + 1^3$	1.12	1.16	1.18	1.22	0.95	0.98	0.90	0.88
Age+1 ³	1.06;1.20	1.09;1.23	1.11;1.26	1.15;1.30	0.89;1.01	0.93;1.05	0.84;0.96	0.83;0.94
1 00 1 24	1.43	1.55	1.66	1.83	0.85	0.95	0.73	0.68
Age+3 ⁴	1.18;1.72	1.28;1.88	1.38;1.99	1.52;2.20	0.71;1.02	0.79;1.15	0.61;0.88	0.57;0.83

311	Table 4: Odds ratios f	for being more	sensitive for sex	age and weight status
511		for being more	sensitive for sex,	age and weight status

312 ¹: Unadjusted odds ratios

²: Odds ratios from the full model including sex, country, residing in control or intervention region, age and
 weight status as independent variables

³: Odds ratio to have a one level higher taste sensitivity for an increase in age of one year

⁴: Odds ratio to have a one level higher taste sensitivity for an increase in age of three years

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Based on standardised taste sensitivity data five taste patterns were derived and the following labels were assigned: "Salty and bitter sensitive" (N=166), "Mild taste insensitive" (N=173), "Taste insensitive" (N=148), "Taste sensitive" (N=679) and "Mild taste sensitive" 321 (N=360). Table 5 represents the means and standard deviations of the taste threshold z-scores

322 for each taste pattern.

Taste pattern	Sweet taste z-score	Salty taste z-score	Umami taste z-score	Bitter taste z-score
(N ¹)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Salty and bitter sensitive (166)	0.30 (0.74)	-0.55 (0.55)	0.84 (0.91)	-0.67 (0.60)
Mild taste insensitive (173)	1.57 (0.89)	0.64 (1.04)	-0.13 (0.58)	0.65 (0.85)
Taste insensitive for all taste modalities (148)	0.46 (1.09)	0.87 (1.04)	1.77 (0.65)	0.95 (0.89)
Taste sensitive for all taste modalities (679)	-0.49 (0.62)	-0.84 (0.27)	-0.58 (0.44)	-0.20 (0.96)
Mild taste sensitive (360)	-0.38 (0.60)	0.75 (0.58)	-0.45 (0.46)	-0.05 (0.89)

323 Table 5: Means and standard deviation (SD) of the taste threshold z-scores for each taste pattern

¹: Since children with 'No taste threshold' were excluded the sample size is reduced.
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The five clusters included children (1) who were salty and bitter sensitive but not sweet and umami sensitive, (2) who were insensitive for sweet, salty and bitter but mild sensitive for umami, (3) who were insensitive for all taste modalities, (4) who were taste sensitive for all taste modalities and (5) who were rather sensitive for all taste modalities except for the salty taste.

The results of the multinomial logistic regression analysis can be found in Table 6. 331 Older children had a lower chance to belong to the salty and bitter sensitive, mild taste 332 insensitive, taste insensitive or mild taste sensitive pattern compared to the taste sensitive 333 pattern (OR: 0.83-0.94). In relation to under-/ normal weight children, overweight/obese 334 children were more likely to belong to the salty and bitter sensitive, mild taste insensitive, 335 336 taste insensitive or mild taste sensitive pattern compared to the taste sensitive pattern (OR: 1.34-1.94). Girls had a higher probability than boys to be assigned to the salty and bitter 337 sensitive patterns (OR: 1.38) but were (slightly) less likely to belong to the mild taste 338 insensitive, taste insensitive or mild taste sensitive compared to the taste sensitive pattern 339 (OR: 0.75-0.98). 340

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Table 6: Odds ratios for belonging to the taste patterns for sex, age, and weight status compared to the reference category 'Taste sensitive for all taste modalities¹

	Salty and bitter sensitive	Mild taste insensitive	Taste insensitive for all modalities	Mild taste sensitive for all modalities		
	Odds Ratio ¹ (95% - Confidence interval)					
Boys	Reference	Reference	Reference	Reference		

Girls	1.38	0.75	0.98	0.85
	(0.98; 1.94)	(0.53; 1.05)	(0.69; 1.40)	(0.65; 1.09)
Non- overweight/non-	Reference	Reference	Reference	Reference
obese	1.34	1.94	1.40	1.76
Overweight/obese	(0.90; 1.98)	(1.35; 2.80)	(0.93; 2.11)	(1.32; 2.35)
Age	(0.90, 1.98)	(1.33, 2.80)	(0.33, 2.11)	(1.32, 2.33)
	0.88	0.94	0.83	0.93
	(0.77; 1.00)	(0.83; 1.06)	(0.72; 0.95)	(0.85; 1.03)

¹: Odds ratios from the model including sex, age and weight status as independent variables

345

346 **4. Discussion**

347 4.1 Main results and previous studies

To our knowledge this is the first study that investigated sweet, salty, bitter and umami taste 348 sensitivity in a population-based sample of primary school children from different European 349 countries, following a standardised study protocol. In a previous study of this cohort (Lanfer 350 et al., 2012), we observed that preferences for sweetened over non-sweetened juice were 351 associated with overweight and obesity in both girls and boys. The current study provides 352 complementary data on taste sensitivity for sweet as well as 3 additional taste modalities that 353 354 were not included in the previous preference test, namely bitter, salty and umami. Our 355 analysis revealed that umami and bitter sensitivity did not differ between boys and girls aged 7 to 11 years. Taste sensitivity for sweet and salty differed; girls may have a slightly higher 356 357 sweet and salty taste sensitivity than boys. In line with our results, two studies observed higher sweet but not higher salty taste sensitivity in girls compared to boys (Bobowski & 358 359 Mennella, 2015; Joseph, Reed, & Mennella, 2016). Our results support the recent results, that 11 year old girls have a higher sweet taste sensitivity than boys (Ervina, Berget, & V, 2020), 360 whereas in this population also the bitter sensitivity was higher in girls compared to boys, 361 different than in IDEFICS. An Italian study in contrast did not find any sex differences 362 regarding sweet, bitter, salty and sour taste sensitivity between boys and girls between 5 and 363 12 years of age (Italian Study Group on taste et al., 2012). Also, in very young children 364 between 3 and 6 years, no sex differences in sweet and bitter sensitivity were found (Visser, 365 Kroeze, Kamps, & Bijleveld, 2000). Another study in adults observed that women were more 366 bitter-sensitive than men (Bartoshuk, Duffy, & Miller, 1994; Hyde & Feller, 1981) while a 367 Mexican study found that women were more sensitive to sweet taste than men (Martinez-368 Cordero, Malacara-Hernandez, & Martinez-Cordero, 2015). It seems likely that sex 369 370 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 were less sweet and salty sensitive and more bitter 373 374 sensitive. Additionally, the cluster analysis revealed that overweight children/children with obesity were more likely to belong to any less taste sensitive pattern (e.g. mild taste 375 insensitive and mild taste sensitive) compared to the taste sensitive pattern than under-/ 376 normal weight. A possible explanation for this finding is that children with lower sweet and 377 salty taste sensitivity might consume more foods high in sugar or salt because they perceive 378 these tastes as less intense and may need more sugar and salt to experience the same sweet 379 and salty sensations. Many sugar- or salt rich foods are considered to be highly processed and 380 energy dense, such as snack foods. Their augmented consumption may thus contribute to the 381 382 development of overweight and obesity if the physiological status of the child does not demand the supply of increased nutritional energy. Bitter foods in contrast often belong to the 383 more favourable food groups like many vegetables. Therefore, if overweight and obese 384 children are more sensitive to the bitter taste they might avoid these healthy food or tend to 385 consume them combined with sweet/fatty (energy dense foods) to mask the bitter taste. As 386 stated above this is a possible explanation for our results. The association between taste 387 sensitivity, perceived suprathreshold intensity and actual food intake is still not yet fully 388 understood and needs to be investigated in future studies. A better understanding will also 389 help to explain the association between taste sensitivity and weight status. Previous research 390 did not find any differences neither between overweight/obese and normal weight children 391 regarding salty and umami taste sensitivity (Bobowski & Mennella, 2015) nor between 392 393 sensitivity for the salty taste and body composition (Kirsten & Wagner, 2014). As mentioned before previous studies differed in methods used as well as ages and numbers of children 394 395 included. This may have led to contradicting results. Until the development of our study (2006), no studies had been detected (based on a literature research) describing associations 396 between sour taste and obesity in children. With respect to the demanding examination 397 protocol for this young age group, sour taste was therefore not included in our investigation. 398 399 Nevertheless, it would be of interest for future studies to investigate also sour taste. Sauer et al. discovered that obese adolescents had a poorer ability to identify sour taste (Sauer et al., 400 401 2017). Further, our results show that taste sensitivity in younger children was lower for sweet 402 and salty than in older children and that older children were less likely to belong to the less taste sensitive taste pattern. In accordance with our findings, Visser et al. found that children 403 between 3 and 6 years of age seem to become more sweet sensitive with age (Visser et al., 404

2000). A recent study also found that children were less sweet sensitive than adolescence and 405 adolescence in turn less sweet sensitive than adults (Petty, Salame, Mennella, & Pepino, 406 2020). This may happen because of physiological changes during the development of the taste 407 apparatus and the fact that the innervation of taste papillae is not yet fully developed and 408 functional (Correa, Hutchinson, Laing, & Jinks, 2013). For bitter, it may even be reverse; 409 younger children may be more bitter sensitive than older children. The observed age trend of 410 sweet and bitter sensitivity may be evolutionary meaningful. Sweet sensitivity may be 411 412 lowered during early childhood to ensure sufficient energy intake, and bitter sensitivity in contrast is possibly elevated to ensure the detection of possible toxins (Drewnowski, 2000; 413 Ventura & Mennella, 2011). Additionally, bitter taste perception may change across the 414 lifespan due to repeated exposure to bitter tasting foods. Thus, the bitter taste sensitivity may 415 not only decrease due to evolutionary reasons but also as a result of learning and adaptation 416 processes. With regards to bitter sensitivity we observed that 1/4 of the children indicated to 417 have no threshold. This might appear very high considering that children have an innate 418 aversion towards bitter (Steiner, 1979). This might be due to the concentration range of 419 caffeine that we used in our study. James et al. for example used much higher concentrations 420 of caffeine when testing children than we used (James et al., 1997). Our highest concentration 421 was 0.00139 mol/l vs. 0.01277 mol/l in study of James et al.. The concentration range we used 422 was based on the DIN norm and comparable to other studies (Ervina et al., 2021). 423

We observed higher sweet taste thresholds (18.7 mmol/l) compared to previous 424 investigations in children (girls: 7.2 mmol/l, boys: 17.0 mmol/l ((James et al., 1997) and girls 425 and boys: 12.0 mmol/l (Joseph et al., 2016)). We also observed higher salty taste thresholds 426 (10.7 mmol/l) compared to previous investigations in children (girls: 2.7 mmol/l, boys: 6.1 427 mmol/l ((James et al., 1997) and girls and boys: 3.6 mmol/l (Bobowski & Mennella, 2015)). 428 Caffeine thresholds for children reported in previous studies were higher (girls: 1.1 mol/l, 429 boys: 2.0 mol/l (James et al., 1997)) than observed in the present study (girls and boys: 0.70 430 mmol/l), while those reported for monosodium glutamate were similar to our results (2.4 431 mmol/l vs. 2.9 mmol/l (Bobowski & Mennella, 2015)). The use of different methodologies in 432 the studies mentioned could have led to the contrasting results. The methods could have led to 433 diverse responses due to their specific cognitive demands. Other studies used Propylthiouracil 434 (PROP) to assess the bitter perception. We used caffeine instead of PROP, as PROP was 435 classified as potentially carcinogenic (National Toxicology Program; Organisation, 2001) and 436 was therefore ethically not safe to use in children. 437

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439 **4.2 Strengths and limitations**

There are some limitations to our study that need to be discussed. Compared to 440 441 previous studies that assessed taste sensitivity in children (Italian Study Group on taste et al., 2012; Joseph et al., 2016; Kirsten & Wagner, 2014; Visser et al., 2000), we used a smaller 442 number of test-solutions per taste modality. Generally, the studies referenced here performed 443 training sessions with participating children before conducting the actual test series, thus 444 445 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 446 447 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 448 449 study participants, we measured only detection thresholds instead of identification thresholds for the basic tastes. Furthermore, the order of presentation of all tested taste modalities was 450 fixed and not randomised. This may have led to the positional bias due to the tendency to 451 answer depending on the order of presentation. Umami was tested in the last position because 452 during test development we observed that the umami taste remains on the taste buds for a long 453 time and would thus affect subsequent taste experiments. Again, a further reason was to 454 facilitate the test procedure for all survey centres in order to ensure a standardised test 455 protocol and to minimise the error-proneness. However, in Table 2 it can be seen that the taste 456 sensitivity levels are distributed over the different concentration levels we tested. This implies 457 that there was no general tendency to choose always the first or the last sample in our study 458 population. 459

460 Due to the cross-sectional design of our study we cannot draw any conclusions about 461 the temporality of the associations between sweet and salty taste sensitivity and weight status. 462 Therefore, further longitudinal analyses are needed to further explain this association.

Beside these limitations our study has several strengths, one of them being that we 463 were able to investigate taste sensitivity of isolated taste modalities. In real foods, umami taste 464 is often accompanied by salty or fatty tastes in foods that are often energy-dense. Umami is 465 however also characteristic for foods low in energy, e.g. tomatoes. Therefore, the 466 physiological regulation of umami sensitivity might work differently than for salty or fatty as 467 our results indicate. Our method to assess detection thresholds was adapted from the DIN 468 10959. Our cross-cultural standardised study design resulted in a large sample of children 469 from the general population. Substantial differences in taste sensitivity could be seen between 470 countries. These differences might be culturally determined. National diets may pose a 471

472 particular exposure to their population and may shape taste sensitivity over the long term. 473 Genetic differences across Europe may also explain part of this phenotypic variability. 474 Previous studies in different countries showed inconsistent results and are not comparable due 475 to different substrates or study designs (Bobowski & Mennella, 2015; Italian Study Group on 476 taste et al., 2012; Joseph et al., 2016; Overberg et al., 2012). Our study was able to show that 477 taste sensitivity indeed varied substantially between children from different countries.

478

479 **5.** Conclusion

This is the first study to compare taste sensitivity in children across different European 480 countries. We conclude that taste sensitivity might be associated with weight status and 481 overweight/obese children are often less taste sensitive to sweet and salt than under- and 482 normal weight children. Further, taste sensitivity might increase due to maturation in children 483 as they get older. We observed large differences in taste sensitivity between children from 484 different countries, which can possibly be explained by cultural and/or genetic influences. 485 Further research is needed to explore the impact of cultural factors on taste perception. 486 Cultural traditions and taboos, food preparation and storage aspects and parenting styles, as 487 well as beliefs may play a role. Also the physiological mechanisms occurring during 488 489 maturation in childhood that may influence children's taste perception need to be investigated.

490

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- 494
- 495

496 Author's contribution

HJ: Writing - Original Draft, Data Curation, Formal analysis; TI: Formal analysis, Writing -497 Review & Editing; KB: Conceptualization, Methodology, Writing - Review & Editing; HC: 498 Investigation; FG: Conceptualization, Writing - Review & Editing; SDH: Conceptualization, 499 Investigation, Writing - Review & Editing; FL: Conceptualization, Writing - Review & 500 Editing; DM: Conceptualization, Investigation, Writing - Review & Editing; LAM: 501 Conceptualization, Investigation, Writing - Review & Editing; LL: Conceptualization, 502 Methodology, Investigation, Writing - Review & Editing; VP: Methodology, Writing -503 Review & Editing; AS: Conceptualization, Project administration, Writing - Review & 504 Editing; TV: Investigation, Writing - Review & Editing; WA: Conceptualization, 505

506 Methodology, Writing - Review & Editing, Supervision, Project administration, Funding

507 acquisition; AH: Methodology, Investigation, Writing - Review & Editing, Supervision.

508 All authors have approved the final article.

509 Data Availability

Due to the sensitive nature of data collected, ethical restrictions prohibit the authors from 510 making the minimal data set publicly available. Each cohort centre received approval of the 511 corresponding local Ethical Commission and participants did not provide consent for data 512 sharing. Additionally, all co-authors are members of the IDEFICS consortium who have 513 514 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 accessed by registered scientists who are authorised 515 to access the data with an individual account and an individual password. Statistical analyses 516 are done on the CDS. It is strictly forbidden to copy or download any data from the CDS. 517 518 Data are available on request and all requests need approval by the study's Steering Committee. Interested researchers **IDEFICS** consortium 519 can contact the (http://www.ideficsstudy.eu) or the study co-ordinator (Ahrens@leibniz-bips.de) to request 520 data access. All requests for accessing data of the IDEFICS/I.Family cohort are discussed on a 521 case-by-case basis by the Steering Committee. For this, interested parties are asked to provide 522 details (e.g. for testing reproducibility of results) on the purpose of their request. 523

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