

*The effects of taste sensitivity and repeated taste exposure on children's intake and liking of turnip (*Brassica rapa* subsp. *rapa*); a bitter *Brassica* vegetable*

Article

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1 The effects of taste sensitivity and repeated taste exposure on children's intake and liking of
2 turnip (*Brassica* rapa subsp. *rapa*); a bitter *Brassica* vegetable

3

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51 **Abstract**

52 Low consumption of vegetables in children is a concern around the world, hence approaches
53 aimed at increasing intake are highly relevant. Previous studies have shown that repeated taste
54 exposure is an effective strategy to increase vegetable acceptance. However, few studies have
55 examined the effect of repeated taste exposure on children varying in bitter taste sensitivity.
56 This study investigated the influence of taste genotypes and phenotypes on the effects of
57 repeated taste exposure to a *Brassica* vegetable. 172 preschool children aged 3 to 5 years were
58 recruited into this study. Turnip was selected as the target vegetable and parents completed a
59 questionnaire to ensure unfamiliarity. During the intervention, children were exposed to
60 steamed-pureed turnip for 10 days (once/day). Intake and liking were measured before, during
61 and after the intervention, and a follow-up was done 3 months post-intervention. Taste
62 genotypes (*TAS2R38* and gustin (*CA6*) genotypes) and taste phenotypes (PROP taster status
63 and fungiform papillae density) were determined. There was a significant effect of exposure
64 shown by significant increases in intake ($p<0.001$) and liking ($p=0.008$) post-intervention;
65 however, there were no significant effects of taste genotypes or phenotypes on intake and
66 liking. In summary, repeated taste exposure is confirmed to be a good strategy to increase
67 vegetable acceptance in children, regardless of bitter taste sensitivity.

68
69 **Keywords:** repeated taste exposure, bitter taste sensitivity, *Brassica*, turnip, children,
70 *TAS2R38*, gustin

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101 **Introduction**

102
103 Adequate consumption of vegetables has been shown to be associated with positive health
104 outcomes and may provide protection against chronic diseases such as heart disease, stroke,
105 diabetes and cancers (Dias, 2012). Phytochemicals such as carotenoids, flavonoids,
106 glucosinolates, vitamins and minerals are potential anticarcinogenic compounds found in
107 vegetables (Van Duyn & Pivonka, 2000). Despite these health benefits, vegetable intake in
108 both children and adults is reported to be below recommendation in the UK (Bates et al., 2014;
109 Bates et al., 2016) as well as in other countries globally (Micha et al., 2015). One serious
110 concern for children being that eating habits in childhood are a determinant of adult diet
111 (Mikkilä, Räsänen, Raitakari, Pietinen, & Viikari, 2004).

112
113 Many researchers have suggested that low consumption or avoidance of certain foods is due to
114 food neophobia, a condition defined as a reluctance to try unfamiliar foods (Pelchat & Pliner,
115 1995). Cooke, Wardle, & Gibson (2003) found that greater food neophobia in 2- to 6-year-old
116 children was related to lower consumption of vegetables, fruits and meat. They suggested that
117 these foods (especially vegetables) are avoided because they may contain toxins; food
118 neophobia serves to protect humans from ingesting these potentially dangerous foods. Similar
119 results were found in a study by Russell & Worsley (2008), which revealed that food neophobia
120 in 2- to 5-year-old children has the strongest impact on intake of vegetables followed by meat
121 and fruits. These studies suggest that food neophobia is crucial in determining children's
122 dietary intake and food preferences.

123
124 Innate preferences pose another challenge to promoting vegetable consumption. Humans are
125 born with an innate preference for sweet tastes and a tendency to reject bitter tastes (Galindo,
126 Schneider, Stähler, Töle, & Meyerhof, 2012), which leads to children eating sweet foods but
127 avoiding vegetables, particularly the bitter ones (Wardle, Sanderson, Gibson, & Rapoport,
128 2001). Furthermore, taste sensitivity could also be a barrier, as studies show that individuals
129 who are more sensitive to bitter taste consume fewer vegetables than less sensitive individuals
130 (Duffy et al., 2010; Sacerdote et al., 2007; Sandell et al., 2014), although this effect has not
131 been confirmed in all studies (Feeney, O'Brien, Scannell, Markey, & Gibney, 2014).

132
133 Studies of bitter taste sensitivity often use 6-n-propylthiouracil (PROP) or
134 phenylthiocarbamide (PTC), bitter compounds that have a thiourea group. Although PROP and
135 PTC are synthetic compounds, the thiourea moiety is found within glucosinolate compounds
136 present in *Brassica* vegetables (Keller & Adise, 2016). The ability to taste PROP/PTC is
137 genetically determined (Barajas-Ramírez, Quintana-Castro, Oliart-Ros, & Angulo-Guerrero,
138 2016) where the *TAS2R38* gene which encodes a bitter taste receptor is predominantly
139 responsible for the taste detection of the thiourea group (Bufe et al., 2005). There are 3 common
140 single nucleotide polymorphisms (SNPs) (*rs713598*, *rs1726866* and *rs10246939*) that can be
141 found within *TAS2R38* genotype which give rise to 3 common haplotypes (PAV/PAV,
142 PAV/AVI and AVI/AVI) (Kim, Wooding, Ricci, Jorde, & Drayna, 2005). Kim et al. (2003)
143 discovered that individuals with PAV/PAV genotype are PTC super-tasters, while those who
144 carry PAV/AVI and AVI/AVI are medium-tasters and non-tasters, respectively. Previous
145 studies have concluded that PAV/PAV individuals perceive greater bitterness from *Brassica*
146 vegetables than AVI/AVI individuals, and that this can influence their liking (Sandell &
147 Breslin, 2006; Shen, Kennedy, & Methven, 2016). In contrast, Duffy et al., (2010) reported
148 that the AVI/AVI individuals had a lower consumption of vegetables (regardless of vegetable
149 type) compared to the other two common genotypes.

150

151 In addition to this specific bitter genotype, sensitivity to all tastes is often associated with
152 fungiform papillae density (FPD) (Hayes, Sullivan, & Duffy, 2010; Yackinous & Guinard,
153 2002). Duffy et al. (2010) found that individuals with high FPD perceived PROP as more bitter
154 than low FPD individuals, which might then influence the high FPD individuals to consume
155 fewer bitter vegetables. However the association between these two factors remain
156 inconclusive as there are studies which report that PROP responsiveness was not related to
157 FPD (Dinnella et al., 2018; Fischer et al., 2013; Garneau et al., 2014; Piochi et al., 2019).

158

159 In relation to FPD, Henkin, Martin and Agarwal (1999) suggested that gustin (CA6) genotype
160 plays an important role in taste bud development and Padiglia et al. (2010) reported that
161 individuals who are PROP tasters carry A/A genotype more frequently, while non-tasters tend
162 to carry G/G genotype on CA6 SNP rs2274333.

163

164 Many strategies have been tested with the intention of encouraging children to eat more
165 vegetables; one of them is repeated taste exposure. Repeated tastings contribute to food
166 familiarity, which is an important determinant of food liking in children (Birch, 1999).
167 Therefore, exposure to vegetables can be effective in increasing vegetable intake and liking in
168 children. Repeated taste exposure has been proposed to be effective for various age ranges;
169 from infants and preschoolers to schoolchildren (Wardle et al., 2003a). Anzman-Frasca,
170 Savage, Marini, Fisher and Birch (2012) and Wardle, Herrera, Cooke and Gibson (2003b)
171 found that 8 exposures of novel and disliked vegetables increased the vegetable acceptance in
172 children aged 3 to 7 years while Lakkakula, Geaghan, Zanovec, Pierce and Tuuri (2010) found
173 that 10 exposures increased acceptance of disliked vegetables in primary school children. Other
174 studies also reported that 10 exposures are effective to increase intake of a vegetable in
175 preschool children (Caton et al., 2013) and infants (Remy, Issanchou, Chabanet, & Nicklaus,
176 2013). Furthermore, a review by Spill et al. (2019) reported that 8-10 or more exposures can
177 increase fruit and vegetable acceptability in children ages 4 to 24 months. Appleton,
178 Hemingway, Rajska, & Hartwell (2018) reported that multiple exposures to a vegetable can
179 also increase intake of other vegetables.

180

181 However, to date, no study has measured the effectiveness of repeated taste exposure in relation
182 to both taste genotype and phenotype. Thus, the present study aimed to determine the effects
183 of repeated taste exposure on acceptance of an unfamiliar *Brassica* vegetable among children
184 with varying bitter taste sensitivity. Four different methods were used to assess taste sensitivity,
185 two exploring the genotypes known to relate to bitter taste sensitivity and two to explore the
186 behavioural phenotype. We hypothesised that repeated taste exposure would increase vegetable
187 acceptance in all children, with children who are less sensitive to bitter taste showing a greater
188 increase than children who are more sensitive to bitter taste.

189

190 Materials and methods

191

192 **Study design:** The study was given a favourable opinion for conduct by the University of
193 Reading Research Ethics Committee (study number 14/40). Following a pre-intervention test
194 of intake, children received 10 exposures (once/attended school day) of steamed-pureed turnip,
195 after which it was offered once again at a post-intervention test. The primary outcome measure
196 was intake of steamed-pureed turnip and rated liking was the secondary outcome. A follow-up
197 was done 3 months after post-intervention to assess the durability of the effects of repeated
198 taste exposure.

199

200 **Recruitment:** A letter explaining the purpose and protocol of the study was sent to primary
201 schools in Reading and Wokingham (Berkshire, UK). Once permission was granted from the
202 head teacher, parents were given an information sheet explaining the details of the study as
203 well as a consent form for them to sign if they agreed to their child participating.

204
205 **Power calculation:** Data from a previous study was used to estimate the minimum number of
206 children required in this study, assuming a mean difference in intake of 4.9 g after an exposure
207 period, with a standard deviation of 8.16 g (Wardle et al., 2003a), a significance level of $p=0.05$
208 (one sided) and a power of 80%. Enough children were needed in each *TAS2R38* PAV/PAV,
209 PAV/AVI and AVI/AVI group to allow comparisons between genotypes. This power
210 calculation indicated that 44 children (Fig. 1) were needed for each genotype group. Taking
211 into account an expected dropout rate of 10%, the target number of children was 48 per group.
212 The proportion of the population with the 3 common *TAS2R38* genotype groups is
213 approximately 25% of PAV/PAV, 50% of PAV/AVI and 25% of AVI/AVI (Duffy et al., 2004),
214 so to ensure the required number of 48 in each group, the aim was to recruit 200 children.
215

$$n > 2F (\sigma/d)^2$$

$$n > 2(7.85) \times (8.16/4.9)^2$$

$$n > 15.7 \times 2.77$$

$$n > 44$$

216 **Fig. 1:** Power calculation to determine number of participants in this study.

217
218 **Participants:** 172 children (82 males and 90 females) aged between 3 years 1 month to 5 years
219 7 months (mean age: 4 years 9 months) were recruited from 6 schools. The inclusion criterion
220 was that children needed to be unfamiliar with turnip, as reported by their parents. The
221 exclusion criteria were allergy to turnip, prior familiarity with turnip, as reported by parents,
222 and liking of the steamed-pureed turnip given at pre-intervention test. No child met the
223 exclusion criteria.

224
225 **Selection of target vegetable:** Turnip (*Brassica rapa* subsp. *rapa*) was selected as the target
226 vegetable as it is one of the most unfamiliar *Brassica* vegetables in the UK, based on a previous
227 study that used a 'Food Familiarity and Liking Questionnaire' which included fruits and
228 vegetables (Heath, 2012). Samples were prepared either in the primary school's kitchen or the
229 sensory kitchen at the Department of Food and Nutritional Sciences, University of Reading,
230 UK, by identical means. The tuber part was used in the preparation of the samples. Prior to
231 cooking, turnips were peeled and stems and tails removed, then washed and sliced to a
232 thickness of approximately 0.5 cm. Approximately 2.4 kg of sliced turnips were placed into an
233 electric 3-tier steamer (Tefal) (800 g in each tier), with 1 L of water added to the base of the
234 steamer, and steamed initially for 25 min. Subsequently, sliced turnips from tier 1 were
235 transferred to tier 3 and vice versa (to ensure equal heat circulation), water was added again up
236 to 1 L and the turnips were steamed for another 25 min. Turnips were then blended using a
237 hand blender (Russell Hobbs) for approximately 5 min until the texture was smooth. All cooked
238 turnips were then placed into plastic containers, labelled and stored in a freezer at -18°C prior
239 to testing. The sensory profile of the steamed-pureed turnip was described and rated by a trained
240 sensory panel as summarised in Supplementary A (Table S2). This confirmed that the final

241 product, as served to children in this study, had a characteristic bitter taste in addition to sweet
242 taste and green vegetable and earthy flavours.

243
244 **Vegetable serving:** Prior to serving, the steamed-pureed turnip was defrosted, reheated in a
245 microwave (800W) and stirred every 2 min until the temperature reached >75°C. At pre- and
246 post-intervention tests, on Day 5 and 8 of exposure and at follow-up, 100 g of steamed-pureed
247 turnip was served in a 230 ml transparent plastic serving dish and labelled with each
248 participant's code; a plastic teaspoon was provided. On Day 1, 2, 3, 4, 6, 7, 9 and 10 of
249 exposure, approximately 5 g of steamed-pureed turnip was given to the children on a plastic
250 teaspoon. The puree was served warm (approximately 40 to 45°C) in rooms varying in
251 temperature between approximately 20°C and 24°C.

252
253 **Repeated taste exposure test:** Before the study began, researchers attended 2 sessions
254 (minimum 2 hours per session) at each school, so that they were familiar to the children. Parents
255 completed a 'Vegetable preference and familiarity' questionnaire that comprised a list of 46
256 *Brassica* and non-*Brassica* vegetables to determine children's familiarity with and liking of
257 turnip.

258
259 At pre- and post-intervention tests, Day 5, Day 8 of the exposure period and follow-up, children
260 were given one pot of 100 g of steamed-pureed turnip. Children were individually taken out of
261 their classes to a separate room. They were asked to eat as much as or as little as they wanted.
262 No persuasion or force was used. Intake and liking of the puree were measured at these times.
263 For the rest of the exposure days (Day 1, 2, 3, 4, 6, 7, 9 and 10), only 1 teaspoon (approximately
264 5 g) of the puree was given, intake and liking were not measured, but refusal to eat was
265 monitored. At these times, children were taken out of their classes in groups of between 2 and
266 5 children.

267
268 Intake was measured in grams (g) using a digital weighing scale (3 decimal places) (Salter).
269 Liking was assessed using a 3-point hedonic scale. Using hedonic scales with this age group is
270 challenging (Chen, Resurreccion, & Paguio, 1996), and researchers took several steps to
271 increase the reliability of the data. Cartoon faces were used (one with a deep frown, one a
272 neutral face and one with a broad smile) alongside child-friendly descriptors ('yucky', 'just
273 okay' and 'yummy'). These were coded as 1, 2 and 3 respectively for analysis. In addition,
274 children were asked to describe the taste when they completed the scoring. This provided
275 researchers with the opportunity to check that children had understood the scale, for example
276 when a child's facial expression did not appear to align to their score. When this happened,
277 researchers explained the scoring again to ensure the child understood.

278
279 **DNA extraction and genotyping:** Buccal swab samples were collected at schools after the
280 end of the intervention. The DNA samples were collected by rubbing a Isohelix DNA buccal
281 swab on the inside of a child's cheeks and then stored until DNA extraction at room temperature
282 and kept dry through the use of Isohelix Dri-Capsules (Cell Projects Ltd, Kent, UK). The
283 researcher swabbed both cheeks of each child for approximately 1 min on each cheek. The
284 swabs were sent to IDna Genetics Ltd. (Norwich, UK) for extraction and genotyping, with 10%
285 of the swabs sent as blinded replicates to ensure accuracy. DNA were extracted using Isohelix
286 Buccalyse DNA Extraction Kit (Cell Projects, Kent, UK) according to the manufacturer's
287 instructions, then diluted 1:8 with water prior to analysis. Polymorphisms of *TAS2R38*
288 (*rs713598*, *rs1726866* and *rs10246939*) and *CA6* (*rs2274333*) were analysed using the KASP
289 genotyping chemistry (LGC Group, Middlesex, UK). Diluted DNA was dried into 384-well
290 PCR plates (Life Technologies, UK) then 5 µL of KASP Master mix (LGC Group, Middlesex,

291 UK) and primers were added. PCR amplification was performed as follows: 94°C for 15 min,
292 94°C for 15 s, 65°C for 20 s, 94°C for 15 s, 57°C for 20 s (Life Technologies, UK). The
293 fluorescent products were detected in an Applied Biosystems instrument (Life Technologies,
294 UK).

295

296 **PROP taster status:** PROP taster status was determined by using filter papers impregnated
297 with PROP and these were prepared as described in Zhao, Kirkmeyer and Tepper (2003).
298 Approximately 10 g of PROP (HPLC grade) (Sigma-Aldrich) was dissolved in 1000 mL boiled
299 spring water (Harrogate Spring water, UK) on a stirring hotplate to prepare a 50 mmol/L PROP
300 solution. Filter paper disks (Whatman Grade 1, 30 mm in diameter, Sigma-Aldrich Cat No:
301 1001-030) were then placed into the PROP solution for 30 s then taken out. The filter paper
302 disks were then placed on a tray wrapped with aluminium foil and then dried in an oven for 1
303 h at 121°C.

304

305 At the end of all study visits, children were asked to take a sip of water and then the PROP
306 impregnated filter paper was placed on the tip of their tongue for a few seconds until the paper
307 was wet, and removed. A simple forced-choice method was used, adapted from Keller,
308 Steinmann, Nurse and Tepper's (2002) method, which has a high test-retest reliability ($r=0.92$).
309 Children were asked a question 'Did you taste anything?' Those who answered 'no', were
310 categorised as non-tasters. Those who reported the filter paper has a taste were then questioned
311 as to what it tasted like. Responses of 'bad', 'bitter' and 'yucky' were recorded as tasters. Those
312 who did not verbally state the filter paper had a taste but who exhibited rejection signs such as
313 grimacing or frowning were also categorised as tasters.

314

315 **Fungiform papillae counts:** The method to count FPD was adapted from Feeney and Hayes
316 (2014). The tongue was dried and coloured using a blue food colouring (Sainsbury's, UK). A
317 1 cm² paper was cut and paste on a ruler as a marker, then the ruler was placed next to the
318 tongue. Photographic images (tongue including the square on the ruler) were taken using a
319 digital camera (Canon EOS 700D) on macro setting. Approximately 3 to 10 images were taken
320 for each child and the best image was used to count the papillae; the fungiform papillae identify
321 as pink circles against a blue background. Images were viewed in Microsoft Office Power Point
322 2013 where the outer square on the ruler was drawn to enable the square to be moved to middle,
323 left and right areas of the tip of the tongue. The left and right areas have been shown to be
324 reliable measures of FPD (Shahbake, Hutchinson, Laing, & Jinks, 2005). There was a high
325 correlation between mean FPD of left and right area and mean FPD of middle area of the tongue
326 ($r=0.94$, $p<0.001$), hence the middle area was used in this analysis in order to include data from
327 the first 2 schools where only a single "middle" count had been taken. All fungiform papillae
328 in a 1 cm² stained area were counted by 2 researchers to ensure accuracy ($r=0.94$, $p<0.001$).
329 Quartile calculation was used to categorise children into 3 groups (low, medium and high FPD);
330 the upper quartile as the high FPD, the lower quartile as the low FPD and the middle two
331 quartiles as the medium FPD group.

332

333 **Statistical analysis:** Shapiro-Wilk tests showed that the data were not normally distributed.
334 Both parametric and non-parametric tests were used to analyse data, and both sets of analyses
335 revealed the same main effects. Therefore, only parametric tests are reported as these allowed
336 testing of the interactions between main effects. Paired t-tests were used to compare means of
337 intake and liking between 2 time points. One-way repeated measure ANOVAs were used to
338 compare mean intake and liking across 3 or 4 time points. To evaluate the effects of taste
339 sensitivity and time on intake and liking, we used mixed ANOVAs with time as a within-
340 subjects factor and taste sensitivity group (taste genotype group or taste phenotype group) as a

341 between-subjects factor. Bonferroni tests were used for post hoc with a significance value of
342 $p < 0.05$. Associations between groups of categorical data were analysed using Chi-square tests.
343 All analyses were performed using SPSS (version 21, New York, USA).

345 Results

347 Of the 172 children who participated in this study, only 134 children had complete data sets
348 which included data for intake and liking (at pre- and post-intervention), and all taste sensitivity
349 measurements (*TAS2R38*, *CA6*, PROP taster status and FPD). These data were then used for
350 the main analyses. Data analyses by excluding missing data according to individual taste
351 sensitivity measurement were also performed to maximise number of children. However results
352 were consistent with the analyses using complete data sets. Hence, only results of complete
353 data sets are reported. Taste genotype and phenotype characteristics of children are described
354 in Table 1.

355
356 **Table 1:** Taste genotype and phenotype characteristics of participants with complete data
357 (n=134).

Characteristic	n (%)
<i>TAS2R38</i>	PAV/PAV 22 (16.4)
	PAV/AVI 67 (50.0)
	AVI/AVI 33 (24.6)
	PAV/AI 3 (2.2)
	PAV/AAV 2 (1.5)
	AAI/AAI 1 (0.7)
	AAV/AAI 1 (0.7)
	AAV/AVI 1 (0.7)
	AAI/AVI 4 (3.0)
<i>CA6</i>	A/A 62 (46.3)
	A/G 56 (41.8)
	G/G 16 (11.9)
PROP taster status	Taster 108 (80.6)
	Non-taster 26 (19.4)
FPD	High (57 to 113 papillae/cm ²) 33 (24.6)
	Medium (36 to 56 papillae/cm ²) 63 (47.0)
	Low (17 to 35 papillae/cm ²) 38 (28.4)

358
359 16.4% of children had PAV/PAV *TAS2R38* genotype, 50.0% were PAV/AVI, 24.6% were
360 AVI/AVI and 8.8% had a rare genotype (PAV/AAV, PAV/AI, AAI/AVI, AAV/AAI,
361 AAI/AAI and AAV/AVI). 46.3% carried A/A *CA6* genotype, 41.8% carried A/G genotype and
362 11.9% had G/G genotype. For taste phenotype, the majority of participants (80.6%) were
363 categorised as PROP tasters while 19.4% were non-tasters, similar to the proportions reported
364 in previous studies (Bouthoorn et al., 2014; Lumeng, Cardinal, Sitto, & Kannan, 2008). In
365 addition, quartile calculation showed that 24.6% had high FPD, 47.0% had medium FPD and
366 28.4% had low FPD. Ethnicity was known only for 91 children; based on the Office for
367 National Statistics's (2015) ethnicity classification in England, 40 children were white, 27
368 children were Asian/Asian British, 11 children were Black/African/Caribbean/Black British,
369 10 children were mixed/multiple ethnic and 3 children were in 'other' ethnic group.
370

371 **Relationship between taste genotypes and phenotypes:** Distribution of *TAS2R38*, *CA6* genes
 372 and FPD according to PROP taster status are shown in Table 2. The majority of the children
 373 who carried PAV/PAV *TAS2R38* (n=20/22), A/A *CA6* genotypes (n=52/62) or had high FPD
 374 (n=26/33) were PROP tasters. In contrast, 2 PAV/PAV children were non-tasters and 27
 375 AVI/AVI children were tasters, 10 non-tasters had A/A and 9 tasters had G/G *CA6* genotypes.
 376 Additionally, 7 children with high FPD were categorised as non-tasters and 33 children with
 377 low FPD were tasters.

378
 379

Table 2: Relationship between taste genotypes and phenotypes (full data set, n=134).

Genotypes and phenotypes		PROP taster status	
		Taster	Non-taster
<i>TAS2R38</i>	PAV/PAV	20	2
	PAV/AVI	53	14
	AVI/AVI	27	6
	PAV/AI	3	0
	PAV/AAV	2	0
	AAI/AI	1	0
	AAV/AI	0	1
	AAV/AVI	0	1
	AAI/AVI	2	2
<i>CA6</i>	A/A	52	10
	A/G	47	9
	G/G	9	7
FPD	High (57 to 113 papillae/cm ²)	26	7
	Medium (36 to 56 papillae/cm ²)	49	14
	Low (17 to 35 papillae/cm ²)	33	5

380

381 Chi-square tests were used to determine associations between genotypes and phenotypes. To
 382 avoid counts below 5, 2 genotype groups within *TAS2R38* and *CA6* were combined. The
 383 PAV/PAV *TAS2R38* genotype was combined with the PAV/AVI genotype into one group as
 384 both groups have the sensitive PAV haplotype. The PAV/PAV-PAV/AVI group would be
 385 expected to have more tasters than the AVI/AVI group. For *CA6*, the A/G and G/G genotype
 386 were combined as both groups have the recessive allele G, where it would be expected that
 387 children in the A/G-G/G group have less FPD compared to the A/A group (dominant allele).
 388 Results showed that there were no significant associations between *TAS2R38* and PROP taster
 389 status ($\chi^2(1)=0.001$, $p=0.98$), between FPD and PROP taster status ($\chi^2(2)=1.34$, $p=0.51$) or
 390 between *CA6* genotype and PROP taster status ($\chi^2(1)=0.79$, $p=0.37$). There were no other
 391 associations found: *CA6* and FPD ($\chi^2(2)=1.18$, $p=0.55$), *TAS2R38* and *CA6* ($\chi^2(1)=0.59$,
 392 $p=0.44$), *TAS2R38* and FPD ($\chi^2(2)=0.63$, $p=0.73$). These results showed that taste genotypes
 393 and phenotypes were independent of one another in this study.

394

395 **Effects of repeated taste exposure on intake and liking of steamed-pureed turnip:** Results
 396 revealed that overall intake significantly increased post-intervention from 14.8 ± 24.0 g to 29.8 ± 34.9 g ($t(133)=-6.17$, $p<0.001$) (Fig. 2). Overall liking increased significantly from 2.3 ± 0.9
 397 to 2.5 ± 0.8 post-intervention ($t(133)=-2.35$, $p=0.02$) (Fig. 3).

398

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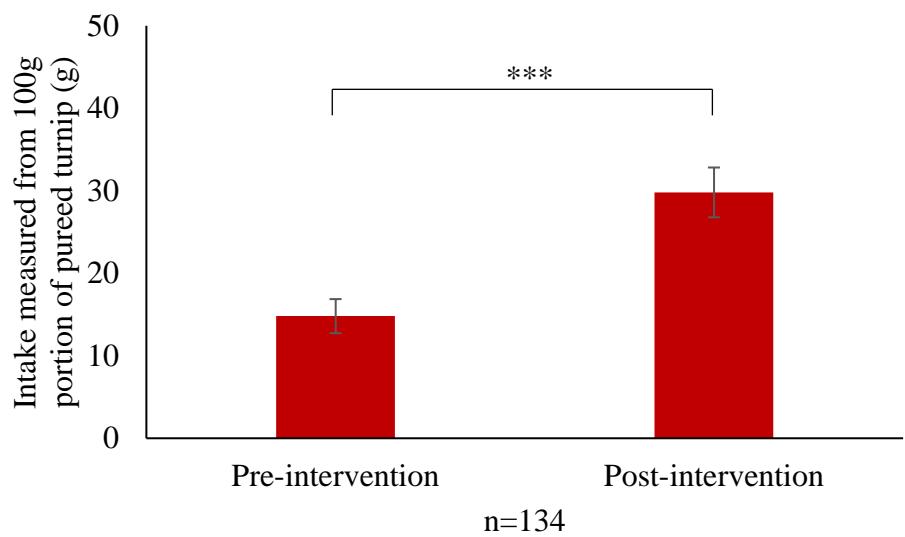


Fig. 2: Overall intake for steamed-pureed turnip at pre- and post-intervention. Values are means \pm SEM. *** $p<0.001$.

403

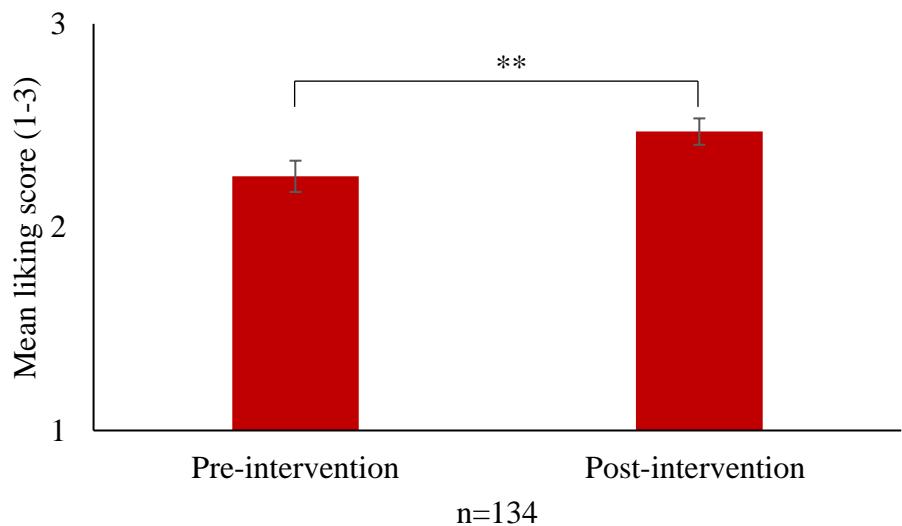


Fig. 3: Overall liking scores for steamed-pureed turnip at pre- and post-intervention. Values are means \pm SEM. ** $p<0.01$.

404 **Vegetable intake pre and post repeated exposure according to taste genotypes and**
 405 **phenotypes:**

406
 407 **TAS2R38:** To investigate the effect of *TAS2R38* genotype on the change in intake with time
 408 (pre- or post-intervention), a mixed model ANOVA (2 (time) \times 3 (genotype)) was conducted.
 409 Results confirmed the significant main effect of time (exposure) on intake ($F(1,119)=31.19$,
 410 $p<0.001$, $\eta_p^2=0.21$) with intake increasing significantly post-intervention; however there was
 411 no significant main effect of *TAS2R38* ($F(2,119)=0.08$, $p=0.93$, $\eta_p^2=0.001$) and no interaction

412 between time and *TAS2R38* ($F(2,119)=0.68$, $p=0.51$, $\eta_p^2=0.01$) (Fig. 4). Similarly, the analysis
 413 confirmed the main effect of time on liking ($F(1,119)=6.12$, $p=0.02$, $\eta_p^2=0.05$) but no
 414 significant main effect of *TAS2R38* was found ($F(2,119)=1.75$, $p=0.18$, $\eta_p^2=0.03$) and no
 415 interaction between time and *TAS2R38* ($F(2,119)=0.37$, $p=0.69$, $\eta_p^2=0.01$).

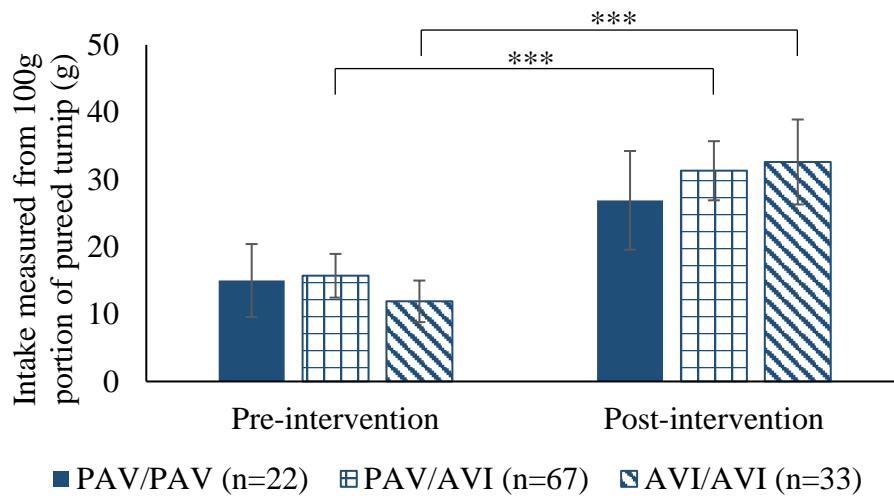


Fig. 4: Intake for steamed-pureed turnip at pre- and post-intervention for participants within each *TAS2R38* genotype group. Values are means \pm SEM. *** $p<0.001$.

416
 417 **Gustin (CA6):** Results from a mixed model ANOVA (2 (time) x 3 (genotype)) confirmed that
 418 there was a significant main effect of time on intake ($F(1,131)=32.55$, $p<0.001$, $\eta_p^2=0.20$) but
 419 there was no significant main effect of *CA6* ($F(2,131)=0.11$, $p=0.90$, $\eta_p^2=0.002$) and no
 420 interaction between time and *CA6* ($F(2,131)=0.89$, $p=0.42$, $\eta_p^2=0.01$) (supplementary Fig. S1).
 421 In the analysis of the effect of the *CA6* genotype and exposure (time) on liking, the main effect
 422 of time was not significant ($F(1,131)=3.65$, $p=0.06$, $\eta_p^2=0.03$). There was no significant effect
 423 of *CA6* ($F(2,131)=0.32$, $p=0.73$, $\eta_p^2=0.01$) and no interaction ($F(2,131)=0.54$, $p=0.58$, $\eta_p^2=0.01$).
 424

425
 426 **PROP taster status:** Analysis of a mixed model ANOVA (2 (time) x 2 (PROP taster status))
 427 again confirmed the main effect of time on both intake ($F(1,132)=29.19$, $p<0.001$, $\eta_p^2=0.18$)
 428 and liking ($F(1,132)=4.49$, $p=0.04$, $\eta_p^2=0.03$) but with no significant main effect of PROP taster
 429 status ($F(1,132)=1.47$, $p=0.23$, $\eta_p^2=0.01$; $F(1,132)=0.92$, $p=0.34$, $\eta_p^2=0.01$, respectively) and
 430 no significant interaction between time and PROP taster status ($F(1,132)=0.75$, $p=0.39$, $\eta_p^2=0.01$;
 431 $F(1,132)=0.19$, $p=0.67$, $\eta_p^2=0.001$, respectively) (supplementary Fig. S2).

432
 433 **Fungiform papillae density (FPD):** Analysis of a mixed model ANOVA (2 (time) x 3 (FPD
 434 group)) again confirmed the significant main effect of time on intake ($F(1,131)=35.51$,

435 p<0.001, $\eta_p^2=0.21$) but there was no significant main effect of FPD ($F(2,131)=1.18$, $p=0.31$,
 436 $\eta_p^2=0.02$) and no interaction ($F(2,131)=2.40$, $p=0.10$, $\eta_p^2=0.04$) (supplementary Fig. S3). For
 437 liking, the significant main effect of time was confirmed ($F(1,131)=4.84$, $p=0.03$, $\eta_p^2=0.04$) but
 438 there was no significant main effect of FPD ($F(2,131)=0.54$, $p=0.59$, $\eta_p^2=0.01$) and no
 439 interaction ($F(2,131)=0.03$, $p=0.97$, $\eta_p^2<0.001$). Overall liking significantly increased post-
 440 intervention.

441

442 These analyses demonstrate that there were significant increases in intake and liking of
 443 steamed-pureed turnip from pre- to post-intervention, irrespective of taste genotypes and
 444 phenotypes.

445

446 **Vegetable acceptance during the exposure days:** In these analyses, data at Day 5 and 8 of
 447 exposure were included to compare mean intake and liking at 4 different time points. Out of
 448 134 children used for previous analyses, only 132 children had intake and liking data at all 4
 449 time points (pre-intervention, Day 5, Day 8 and post-intervention). 4-point one way repeated
 450 measures ANOVA again confirm the significant main effect of time on intake ($F(2.4, 319.3)=20.37$,
 451 p<0.001, $\eta_p^2=0.14$). Intake significantly increased from pre-intervention (15.0 ± 24.1 g) to Day 5 (21.6 ± 28.9 g, $p=0.002$), remained constant at Day 8 (22.7 ± 30.6 g, $p=1.00$)
 452 and increased again at post-intervention (30.3 ± 35.0 g, $p<0.001$) (Fig. 5).

453

454 For liking, the significant main effect of time was again confirmed ($F(2.5, 320.6)=5.25$,
 455 p=0.003, $\eta_p^2=0.04$) where liking significantly increased from pre-intervention (2.3 ± 0.9) to
 456 Day 5 (2.6 ± 0.7 , $p=0.004$) and remained stable until post-intervention.

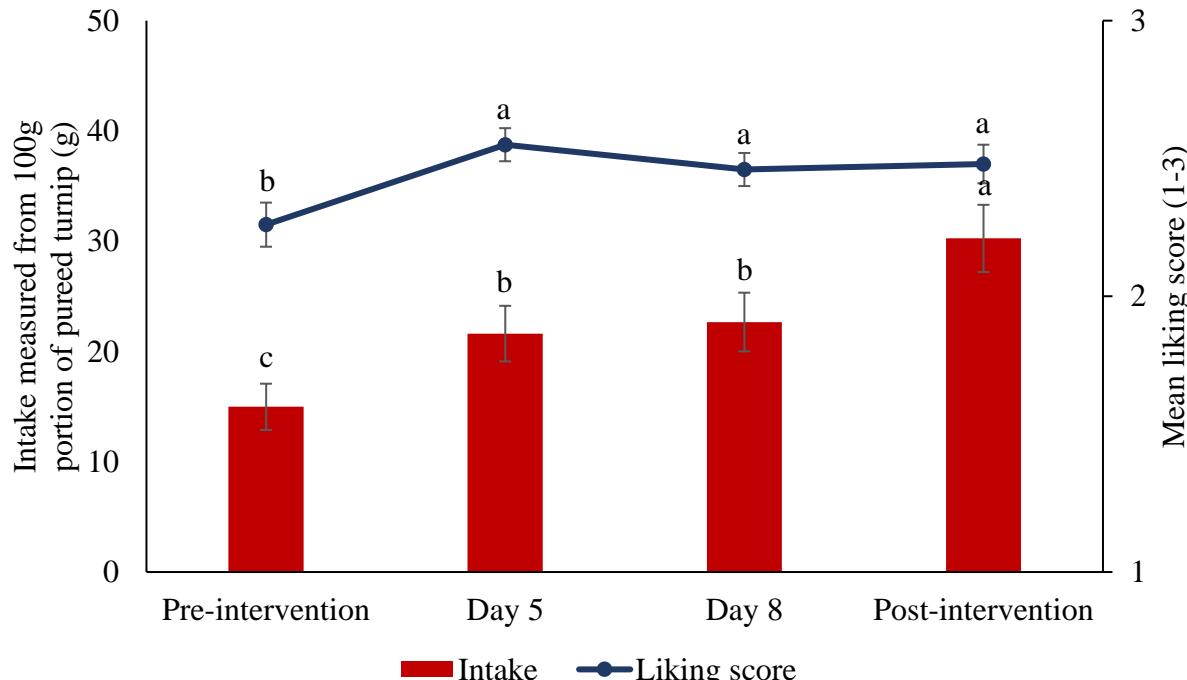


Fig. 5: Change in intake and liking scores for steamed-pureed turnip from pre-intervention, Day 5 and 8 of exposure to post-intervention. Values are means \pm SEM. Differences in letters indicate significant differences between time points.

458
459 **Vegetable acceptance during exposure days according to taste genotypes and phenotypes:**
460 Taste genotypes and phenotypes were incorporated into the analyses to determine whether
461 these factors interact with time (pre-intervention, Day 5, Day 8 or post-intervention) to
462 determine turnip intake and liking. The significant main effect of time on intake and liking was
463 confirmed in each analysis; however there were no significant main effects of any taste
464 genotype nor phenotype and no interactions between these factors and time (data not shown).
465
466 **Effects of repeated taste exposure at follow-up:** Of 134 children, 121 children participated
467 in the 3 month follow-up. 3-point one-way repeated-measures ANOVA tests were carried out
468 to determine any lasting effect of repeated taste exposure. Results revealed a significant effect
469 of time on intake ($F(1.7, 206.1)=42.13, p<0.001, \eta^2_p=0.26$). Intake increased significantly from
470 both pre-intervention (15.5 ± 25.1 g, $p<0.001$) and post-intervention (31.4 ± 35.9 g, $p=0.002$)
471 to follow-up (38.3 ± 37.7 g) (Fig. 6).
472
473 For liking, there was a significant main effect of time ($F(1.9, 222.8)=7.54, p=0.001, \eta^2_p=0.06$).
474 Liking increased significantly from pre-intervention (2.2 ± 0.9) to follow-up (2.5 ± 0.8 ,
475 $p=0.001$); however, there was no difference in liking from post-intervention to follow-up
476 ($p=1.00$).
477

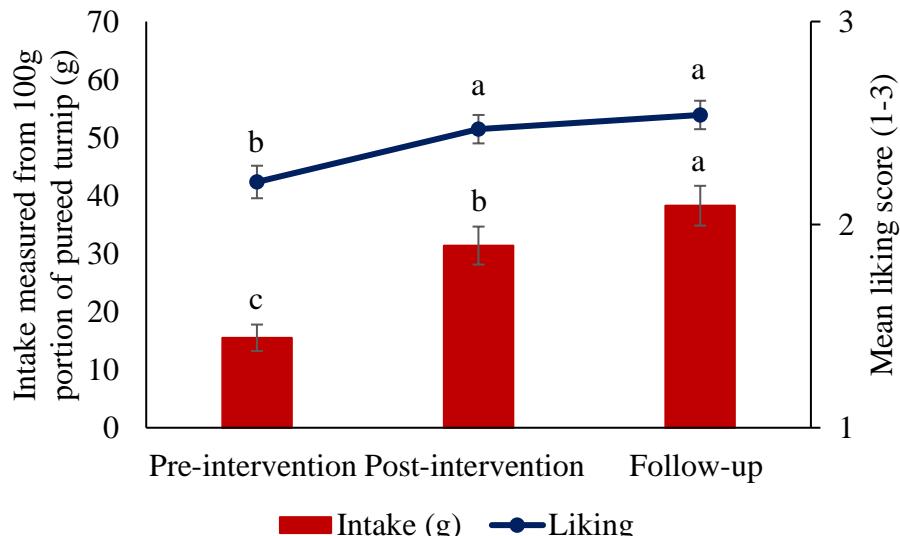


Fig. 6: Intake and liking scores for steamed-pureed turnip at pre-, post-intervention and follow-up. Values are means \pm SEM. Differences in letters at the top of each bar indicate significant differences ($p<0.05$).

478
479 **Effects of repeated taste exposure at follow-up according to taste genotypes and**
480 **phenotypes:** Taste genotypes and phenotypes were incorporated into the analyses to determine
481 whether these factors interact with time (pre-intervention, post intervention or follow-up) on
482 turnip intake and liking. The significant main effect of time on intake and liking was confirmed
483 in each analysis; however there were no significant main effects of any taste genotype nor
484 phenotype and no interactions between these factors and time (data not shown).
485

486 **Discussion**

487

488 The findings of this study show that there was a significant increase in overall intake and liking
489 of steamed-pureed turnip over repeated taste exposure. Other studies have found the same
490 effects of repeated taste exposure; for example Ahern, Caton, Blundell and Hetherington
491 (2014) reported that intake of novel vegetables (swede, turnip and celeriac) increased after
492 repeated exposure in preschool children (15 to 56 months). Hausner, Olsen, et al. (2012)
493 described that repeated taste exposure is a powerful strategy to enhance vegetable acceptance
494 as it was found that intake of a novel vegetable (artichoke) increased after 10 exposures in 2-
495 to 3-year-old children. Similarly, repeated taste exposure increased the acceptance of initially
496 disliked vegetables (red bell pepper and yellow squash) in 3- to 6-year-old children (Anzman-
497 Frasca et al., 2012). These findings also show that children can learn to like bitter tastes over
498 time if they are given opportunity to taste them repeatedly, even though children are born with
499 a tendency to dislike bitter tastes. However, as our study did not include a non-bitter vegetable
500 as a comparator food, we cannot confirm how the increase in liking of turnip compares to the
501 changes previously reported for less bitter vegetables. In future research it would be interesting
502 to compare the effects of repeated taste exposure between different types of vegetables.

503

504 In this study, it was observed that overall intake and liking significantly increased after 5
505 exposures and that intake continued to increase significantly post-intervention, while liking
506 remained stable. In agreement with previous studies, results indicate that 5 exposures might be
507 sufficient to increase acceptance of a novel vegetable (Caton et al., 2013; Hausner, Olsen, et
508 al., 2012). It was also found that intake and liking increased significantly from pre-intervention
509 to follow-up, which indicates a long-term effect of repeated taste exposure. This result is
510 supported by Caton et al. (2013) and Hausner, Olsen, et al. (2012) who report that repeated
511 taste exposure could increase vegetable acceptance up to 5 weeks and 6 months, respectively.

512

513 When intake was evaluated separately according to taste genotypes (*TAS2R38* and *CA6*) and
514 phenotypes (PROP taster status and FPD), no significant effects were found for any taste
515 genotype/phenotype. It is possible that the effects of exposure obscured genuine effects of taste
516 genotypes and phenotypes. This current study is underpowered to conclude a null effect of taste
517 sensitivity on repeated taste exposure as the original sample size calculation was based on
518 effect sizes in studies where no information on taste sensitivity was available. Based on the
519 data from our study, a sample size calculation with 90% power indicates that 770 children are
520 needed in a future study to conclude whether taste genotypes and phenotypes could
521 significantly affect intake of this bitter vegetable after exposure.

522

523 To our knowledge, this is the first study that examines the role of both taste genotype and
524 phenotype on the effects of repeated taste exposure. A previous study by Fisher et al. (2012)
525 investigated both bitter phenotype and repeated taste exposure on liking of broccoli by Hispanic
526 children in the US. In agreement with our study they reported that liking of broccoli increased
527 after 7 weeks of exposure among children, with no difference in rated liking due to PROP
528 sensitivity. The Fisher study used a more thorough PROP phenotype procedure than used in
529 our own study, each child evaluating three concentrations of PROP. They concluded that 30%
530 of the children were bitter insensitive whereas we found 20% did not taste the PROP taste
531 papers in our own study. However, the 30% PROP insensitive number from the more accurate
532 method does fit very well with the 30% of children with the bitter insensitive AVI/AVI
533 genotype found in our own study. Moving forward we consider that there are a number of
534 advantages to taking the genotype rather than the phenotype measurement approach. We were
535 able to readily determine which children had the “super-sensitive” PAV/PAV genotype (16%)

536 and which had the “average sensitivity” PAV/AVI genotype (50%). In addition, bitter sensitive
537 children do not like the taste of PROP, whereas the buccal swab taken for genotyping is quick
538 to administer and has no unpleasant taste or side-effect. In contrast to our own results, the
539 Fisher study reported a decrease of broccoli intake following exposure which the authors
540 suggested could be caused by a monotony effect. Several studies have investigated the effects
541 of taste genotype and phenotype on vegetable intake; for example Bell and Tepper (2006)
542 found that PROP non-taster children consumed more vegetables than tasters. This is also
543 supported by Dinehart, Hayes, Bartoshuk, Lanier and Duffy (2006) who reported that PROP
544 sensitive individuals consumed fewer vegetables, while the same research group found that
545 adults with AVI/AVI *TAS2R38* genotype consumed more vegetables (Duffy et al., 2010).
546 Sandell et al. (2014) also found that the less bitter sensitive adults consumed more vegetables
547 than adults with heightened bitter sensitivity.

548
549 Although liking increased across the whole sample post-intervention, there were no significant
550 differences according to taste genotype or phenotype group. It is possible that the 3-point
551 hedonic scale that was used in this study was insufficiently sensitive to detect differences in
552 children’s liking and that a scale with more than 3-points would have been better. However, it
553 was selected because young children (below 6 years) might have difficulty interpreting wider
554 hedonic scales (e.g. 5- or 7-point scales) (Stone & Sidel, 2004). Chen, Resurreccion and Paguio
555 (1996) have demonstrated that a 9-point hedonic scale is not suitable for 3- to 5-year-old
556 children, and that 3-, 5- and 7-point scales work best with 3-, 4- and 5-year-old children,
557 respectively. Despite the steps undertaken to ensure children understood how to complete the
558 scale, on a few occasions children rated high liking despite displaying a facial dislike
559 expression on tasting the steamed-pureed turnip. When this happened, researchers re-explained
560 the scale. Future researchers may consider taking additional steps to ensure the reliability of
561 hedonic scales with this age group, for example training children on how to use the scale in
562 advance until their scores are reliable.

563
564 Considering the relationship between taste genotypes and phenotypes, our results did not find
565 associations between *TAS2R38*, FPD, *CA6* and PROP taster status. It was expected that
566 children with high FPD, PAV/PAV *TAS2R38* and A/A *CA6* would be PROP tasters, and those
567 with low FPD, AVI/AVI *TAS2R38* and G/G *CA6* would be non-tasters, but there were
568 anomalies. It was found that the number of children categorised as PROP tasters/non-tasters
569 was not always consistent with the expected PAV/PAV or AVI/AVI *TAS2R38* genotype. These
570 unexpected results are thought to be due to the simplified method used to identify PROP taster
571 status in this study. Children were categorised into either PROP tasters or non-tasters by tasting
572 just one concentrated level of PROP impregnated into a filter paper, whilst other studies have
573 used a more complex method to separate adult participants into 3 categories (PROP super-,
574 medium- or non-tasters). This method requires participants to taste different concentrations of
575 PROP solutions and sodium chloride (NaCl) solutions and then rate the intensity of the
576 solutions using a labelled magnitude scale (LMS) (Tepper, Christensen, & Cao, 2001; Shen,
577 Kennedy, & Methven, 2016). However, Keller and Adise (2016) argued that young children
578 (under 7 years old) would struggle to use more complex scales, and most studies involving
579 children have used a simple forced-choice screening method to categorise them into either
580 tasters or non-tasters, the method selected for the current study. Turnbull and Matisoo-Smith
581 (2002) determined PROP taster status in 3- to 6-year-old children using a more sensitive
582 procedure, in which PROP thresholds and suprathresholds of the children were measured on
583 simple categorical scales. Despite its sensitivity, the method is not practical for a large field-
584 based study such as ours as it involves tasting multiple solutions. The relationship between
585 taste genotype and phenotype is complex; as Hayes, Bartoshuk, Kidd and Duffy (2008)

586 explained, PROP sensitivity is not entirely dependent on taste genotypes and phenotypes and
587 there might be more than just one receptor (ie: *TAS2R38*) or mechanism that explains PROP
588 bitter taste sensitivity. Furthermore, Piochi, Dinnella, Prescott, & Monteleone (2018)
589 concluded that the association between PROP bitter taste sensitivity and FPD is not
590 straightforward as there may be other factors contributing to differences in findings such as
591 age, gender and method variability. In addition, most studies did not consider the quantification
592 of taste buds to provide information about fungiform papillae functionality. It is possible that
593 it is the interactions between genotype and phenotype that have an impact on vegetable intake
594 and liking, rather than taste genotype or phenotype alone; however the number of participants
595 was insufficient to sub-divide groups further in order to investigate these interactions in this
596 study.

597

598 **Conclusion**

599

600 This study confirms that repeated taste exposure is a good method to enhance acceptance of an
601 unfamiliar vegetable in children regardless of their bitter taste sensitivity. Repeated taste
602 exposure is simple and easy for parents to implement in a home-setting environment to
603 encourage children to eat bitter-tasting vegetables. This study also demonstrates that repeated
604 taste exposure is not only effective in the short-term, but remains effective 3 months after
605 exposure.

606

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608

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611

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