Past exposure to fruit and vegetable variety moderates the link between fungiform papillae density and current variety of FV consumed by children.

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Abstract

Higher fungiform papillae density (FPD) has been associated with lower taste sensitivity thresholds and greater perceived taste intensity along with consumption of fewer fruit and vegetables (FV). Children exposed to greater variety of FV tend to habitually consume more FV, however, it is unknown whether exposure effects are attenuated by individual differences in FPD or whether these effects vary according to sensory properties of FV. This study examined the links between children's FPD, current variety of FV consumed, and past experiences with variety of fruit and vegetables. FPD counts were obtained from 61 children between 5 and 9 years old, in schools from affluent areas of Birmingham (UK). Parents completed food frequency questionnaires indicating the variety of FV consumed by children in the last 7 days. Parents also indicated the number of different FV types the children had tasted in their lifetime. FV were subdivided to reflect differences in their sensory properties. The results showed that children with higher FPD who in their lifetime had tasted a greater variety of FV ate a larger variety of FV compared to children with higher FPD, but with lower past exposure. When examining effects within specific subcategories of fruits and vegetables, this pattern held for non-astringent fruit and showed a trend for non-bitter vegetables. Children with lower FPD consumed similar variety of FV irrespective of past experiences with variety of FV. The results suggest that when strong or irritant sensory food properties are not a barrier to intake, higher FPD in the presence of supportive home food environment may be beneficial for FV intake. Individual phenotypic differences may affect responsiveness to environmental factors in children's intake of FV.

Keywords Fungiform papillae, dietary exposure, fruit, vegetables, astringency, bitterness

1.0 Introduction

Fruit and vegetable (FV) consumption in children is universally poor [1, 2] and in the UK, fewer than 1 in 4 children eat the recommended numbers as reported in Health Survey for England [3]. There are numerous inherent and environmental barriers to FV intake (for a review see Fogel & Blissett [4]) and low caloric density of FV compared to energy dense food options does not aid the natural mechanisms by which we learn to like foods. FV are the most commonly rejected group of foods [5], but even within the broad category of FV, there is variation in rejection rates depending on the sensory properties of the specific FV. For example, among vegetables, the Brassica genus (e.g. broccoli, Brussels sprouts), which are higher in bitter polyphenols, typically show low intake rates [6] and among fruits, astringent fruits (e.g. berries) show lower intake rates [7].

The best predictor of children's dietary intake is what their parents eat (e.g.[8] [9] [10]. Parental dietary habits will shape home availability and accessibility to various foods [11] and as such parents will determine children's early exposure to FV. Skinner and colleagues [12] showed that exposure to a wide variety of fruit during early childhood was predictive of consumption of a wide variety of fruit during late childhood. Similarly, Resnicow and colleagues [13] found that lifetime exposure to variety of FV was correlated with children's current FV intake in a 7-day recall paradigm. Reinaerts et al. [14] measured children's lifetime exposure to variety of FV and their FV intake and demonstrated that lifetime exposure to more fruit was a significant predictor of higher current fruit intake, and lifetime exposure to more vegetables was a significant predictor of higher current vegetable intake. This study measured exposure based only on a small number of the most common FV, and as such could not account for the potential effects of exposure to a wide variety of less common products. Together, these studies suggest that a higher variety food environment promotes intake of a wider variety of FV. However, whether this effect is true for all subcategories of FV, particularly the ones most often rejected by children, requires further investigation.

Environmental factors affect children’s opportunities to consume FV, but there are also a number of intrinsic predispositions that in the past have been shown to affect children’s responsiveness to environmental stimuli. It has been previously suggested that fungiform papillae (FP) located on the tongue may play a role in sensory evaluation of foods. The tongue is covered with three types of projecting papillae which carry taste buds: FP are located on the anterior tongue, foliate papillae at the back edges and circumvallate papillae are arranged in a half circle shape at the back of the tongue [15]. FP resemble button mushrooms and are concentrated at the tip of the tongue. Each one carries between 0 to 15 taste buds [16]. Density of FP (FPD) has been associated with sensitivity to the bitter tastant PROP [17] and perceived bitterness of quinine [18]. People with greater taste bud density on FP have also been shown to perceive greater taste intensity from sugar, salt and PROP [19]. Hayes and Duffy [20] also found that greater FPD was associated with greater perceived creaminess, which points to the importance of FP for both taste and tactile evaluation of stimuli. FPD has also been linked to intake of FV, but the nature of this association is complex. Duffy et al. [18] reported that among PROP non-tasters, those with higher FPD ate more vegetables of all types, compared to non-tasters with lower FPD or PROP tasters, which they interpreted as facilitation of vegetable intake by FP when bitterness of vegetables is not a barrier. The same pattern was reported by Feeney et al. [21] in a sample of 7-13 year olds, who also found a positive association between vegetable intake and FPD in PROP non-tasters, which suggests that FPD may in fact be a separate contributor to vegetable consumption independent of PROP status The relationship between FPD, taste function, and avoidance of bitter vegetables is not unequivocal, however; other studies have reported no links between FPD and taste function, which points to the importance of further research in this area [22] [23].

Whether benefits from growing up in a variety rich environment are equal for all children, independently of their inherent predispositions, and whether they generalise to all FV subcategories, is at present not well understood. For example, children who are more sensitive to taste or tactile sensations are also more neophobic [24]. The greater taste acuity associated with higher FPD may mean that these children have fewer positive and greater negative consequences when trying new foods, particularly ones with strong sensory properties, leading to greater reluctance to try new foods. Given that more neophobic children are less responsive to exposure based interventions [25] and that parents often do not purchase or serve their children previously rejected foods [26], children with greater taste sensitivity may decrease their own exposure to FV. Therefore we may see weaker effects of past exposure on current variety of FV acceptance in those children with higher FPD. However, it is also possible that greater taste sensitivity may facilitate acceptance of foods that have lower levels of palatable tastants (such as sugar, salt, fat), and therefore we may see stronger effects of past exposure on current variety of non-astringent and non-cruciferous FV accepted in children with greater FPD. Astringency is both a taste and tactile sensation as it is a combination of acidic properties interpreted by taste receptors and ‘puckering’ sensation interpreted by tactile mechanoreceptors [27]. Therefore, we may see differences in the relationship between exposure, FPD and current variety of FV acceptance dependent on the subtype of FV examined.

To address these gaps in the literature, this study looked at the relationship between total past exposure to the types of FV that children had experienced in their lifetime, FPD and variety of FV consumed by children in the past 7 days. This was investigated across different types of FV, which have been shown to differ in sensory properties, including cruciferous vegetables and astringent fruit. We hypothesised that children with greater past exposure to variety of FV will currently consume larger variety of FV. We also hypothesised that effects of past exposure to variety of FV will be moderated by children's FPD and would be exclusive to non-cruciferous vegetables and non-astringent fruit.

2.0 Method

2.1 Participants

The participants of this study were a subsample of a larger study of children's taste processing, which included 99 children (50 boys, 49 girls) between 5-9 years old (M=7.1± SD=1.4). Of these, 61 children underwent successful FP testing (29 boys, 32 girls). A small number of children did not assent to the FP testing (n=2) and data of some children were not included in the analyses due to poor image quality (n=36), which was caused by excessive head movement, poor lighting conditions and/or inability to remain still with a protruded tongue for sufficient amount of time that would allow image capturing. The majority of children were within healthy weight limits for their gender adjusted height and age according to WHO cut-offs [26], with mean BMI z-score of BMI_z=.20±1.0, and the majority were of white British descent (n=55; 3 children of Asian background, 3 mixed). Children were tested in a designated room in one of four schools which participated in the study. Whilst many diverse schools were contacted to participate in the study, all four schools who consented to take part were of high socioeconomic status: Index of Multiple Deprivation Rank [29] indicated that all schools were located in the top 5% of the most affluent areas in the UK. The food diaries and questionnaires were completed by either mothers (n=56), fathers (n=4), or the grandparent (n=1). Parents were on average 38.6 (SD=7.9) years old. The University of Birmingham Ethics Committee granted permission for this study (Reference ERN_11-0780).

2.2 Measures and procedures

2.2.1 Demographics & anthropometrics

Participants' age, gender and ethnicity were collected by parental report. Children were weighed in light clothing without shoes using standard bathroom scales (accurate to 0.1 kg) and height was measured using a stadiometer (Seca Leicester Portable height measure). Children's weight and height were later converted to BMI z-scores, corrected for age and gender using British 1990 Child Growth Reference Chart (UK90). Parents gave informed consent and verbal assent was gained from each child prior to participation.

2.2.2 FV consumption

Fruit and vegetable consumption was reported by the parents, who completed a FV frequency questionnaire [30]. The fruit and vegetables in the questionnaire were chosen on the basis of their availability in the local supermarkets and were a comprehensive list of all available FV in the locality (63 fruits, 59 vegetables). The parents were asked to report which of the FV the child and themselves consumed in the previous 7 days as discrete food items or as part of a dish/recipe.

Current variety of FV was defined as the count of all the different FV the child had eaten in the previous week, independent of the portion serving. Fruits were split into astringent and non-astringent fruit and vegetables into cruciferous and non-cruciferous groups. Fruit juice was not included in the FV count. Astringent fruit contained fruit with astringent and irritant properties due tohigher content of tannins (berries, sharon fruit, pomegranate), naringin and hesperidin (lemons and limes) and ascorbic acid (kiwi and pineapple) [7] [31-33]. Yoghurts were not counted. Potatoes were not included in the vegetable count. Cruciferous vegetables included: cabbage, Brussels sprouts, broccoli, cauliflower, bok choy, Chinese cabbage, kohlrabi, kale, turnip, rocket, garden cress, watercress and radish. The remaining vegetables were defined as non-cruciferous [34].

2.2.3. Past Exposure

In a separate column on the same FFQ, parents were also asked to put a letter 'N' next to any FV that the child had never tried. The quantity of the products the child had never tried was used as a measure of child's past exposure to variety of FV, with larger numbers of products never tried indicating lower exposure. The sum of products the child has never tried was an indicator of their past exposure to FV. The data was next transposed to aid interpretation, so that larger numbers would indicate greater past exposure. Two parents did not place any mark in the column for their child's or their own exposure, and these parents were excluded from the analyses because it could not be established whether they had a veryhigh exposure, or whether they mistakenly not marked any products. Subdivision to astringent fruit and cruciferous vegetables was not used when examining past exposure to variety of FV, given the small sample size and resultant limited power.

2.2.4 Fungiform papillae density

FPD was measured using a standardised procedure after Shahbake and colleagues [33]. Children rinsed their mouth with water and their tongue was dried with the filter paper. Children were then instructed to sit down, protrude their chin forward and place hands under the chin. Children were asked to stick their tongue out and stabilize it by pressing the upper lip against the tongue. The behaviour was first demonstrated by the researcher and the child was asked to mimic the researcher for practise. Next, a 1cm square of filter paper with a safe blue food dye was placed on the anterior part of the tongue, close to midline. The dyed filter paper was placed on the tongue for 3s and removed, followed by another drying of the tongue. Next, a white strip 3 x 0.5 cm of a filter paper was placed to the right side of the tongue, as close to midline as possible (as a reference to calculate the scale of magnification). The time to obtain images was approximately 3 minutes. Three images were taken and the best quality ones were chosen for analyses (for example, see Figure 1).

Using the scale indicated by the paper strip and Inkscape 4.0, a 1 cm^2 area was superimposed over the photo and the number of FP were counted for each image three times. Criteria for identification of fungiform papillae were adapted after Shahbake et al. [35]. The rater was blind to FV consumption data. A two-way mixed single measures intra-class correlation was conducted between the three counts of FPD. Consistency was seen with a high level of 90.7% agreement across counts (ICC, (3,1) = .907). The mean of the three counts was used in analyses.

Fig 1. Example photograph with image adjustments to ease analysis.

3.0 Analysis

Given the small sample size and non-normally distributed data, bootstrapping was performed drawing 1000 bootstrapped samples for each test. The analyses were reported with the significance values and 95% CIs for bootstrapped samples. Alpha level of 0.05 was used as a cut-off. CIs not including zero were taken as measure of reliability of the results. To test the hypothesis that there would be an interaction between FPD and FV exposure level on variety of FV consumed by children in the previous 7 days, children were split across the median into those with higher and lower FPD and higher and lower past exposure to FV. Next, 2x2 ANOVAs were performed to test these interactions with outcome variables, which included the overall FV variety, variety of fruit (further split into astringent and non-astringent) and variety of vegetables (further split into cruciferous and non-cruciferous). All statistical analyses were conducted in SPSS version 21.0 (IBM).

4.0 Results

4.1 Fungiform papillae density

The mean number of Fungiform Papillae (FP) counted was M= 37.3±9.9 with density ranging between 23-67/cm^2. The data were marginally skewed ($M_{skewness}$=1.01, SE= 0.31). Children were subsequently split into lower (n=31) and higher FPD (n=30), based on the median split (Mdn=36.7). Children with higher FPD (M=7.5±1.4) were slightly older than children with lower FPD (M=6.8±1.3). No other differences were observed.

4.2 Past Exposure to Fruit and Vegetables

Reported FV exposure levels for children showed normal distribution (skewness and kurtosis within acceptable limits), while parental reported exposure was negatively skewed ($Z_{skewness}$>1.96). Parents reported higher exposure to FV for themselves (M= 114.0±9.4) than their children (M=92.3±19.7). Higher parental and child exposure to FV a showed moderate positive relationship (Pearson's r= 0.46, p<0.001; 95% CI [0.26-0.67]). Children were next divided into Lower Exposure (LE; n=29) and Higher Exposure group (HE; n= 30), based on the median split (Mdn= 88.5). Children in HE and LE groups did not differ in age, gender, parental age or BMI.

4.3 Current variety of fruit and vegetables consumed

Parents reported high levels of FV variety for themselves and their children in the past 7 days, and these were moderately correlated (see Table 1). There were no child gender differences in the reported variety of FV consumed (p>0.05) and no associations with children's BMI_z (r=-.10, p=.47, 95% CI [-0.34, 0.14]). Older children were reported to consume a larger variety of FV but 95% CIs showed marginal effects (r=0.25, p=0.049; 95% CI [0.002, 0.48]).

Table 1. Mean (±SD) and range of reported variety of FV consumed in the previous 7 days and their subcategories, for children and their parents, as well as correlation coefficient (Pearson's r) and bootstrapped 95% confidence intervals.

	Child	Parent	r	p	95%CIs
FV	15.6±7.5 (1-35)	39.9±8.7 (1-40)	0.59	<0.001	0.34, 0.76
Fruit	6.6±3.9 (1-20)	15.1±3.7 (1-17)	0.42	0.001	0.16, 0.63
Astringent F.	1.5±1.3 (0-6)	3.0±1.4 (0-6)	0.57	<0.001	0.33, 0.76
Non-astringent F.	5.1±3.1 (1-16)	12.2±2.9 (1-14)	0.37	0.004	0.10, 0.58
Vegetables	9.1±5.2 (0-22)	24.8±6.6 (0-28)	0.59	<0.001	0.39, 0.76
Cruciferous V.	1.3±0.9 (0-3)	2.5±1.1 (0-5)	0.53	<0.001	0.35, 0.71
Non-cruciferous V.	7.8±4.6 (0-21)	22.3±5.8 (0-23)	0.59	<0.001	0.39, 0.76

4.4 Interaction between past exposure to FV and fungiform papillae density on current variety of FV consumed

Mean counts of currently consumed variety of FV among children with lower and higher levels of FPD, and children with lower and higher levels of past exposure to FV are reported in Table 2. Children with higher FPD were reported to have marginally higher variety of all FV types currently consumed, except for cruciferous vegetables. Also children who were in the past exposed to a larger variety of FV were reported to consume higher levels of all FV types.

Interactions between children's past exposure to FV and their FPD, on current reported variety of FV consumed in the past 7 days are reported in Table 3. There was a main effect of past exposure on all FV types, except for cruciferous vegetables. There were significant interactions between past exposure to FV and FPD, on current consumed variety of FV, particularly for non-astringent fruit, and a trend for variety of non-cruciferous vegetables consumed. Effect sizes were small. Pairwise comparisons are depicted in Fig 2. Children who in the past had been exposed to a wider variety of FV, were reported to consume higher FV variety in the past 7 days, and this effect was larger for children with higher FPD. Children with higher FPD who have been exposed to lower FV variety in the past, tended to consume lower variety of FV, as reported by their parents.

Table 2. Mean variety (±SD) of FV consumed in the last 7 days by children characterised as lower and higher in FPD and Lower and Higher Past Exposure group (reported by mothers).

	FPD		Past Exposure	
	High	Low	High	Low
FV	16.4± 7.6	14.8±7.3	18.3±7.4	13.4±6.4
Fruit	6.6±4.1	6.5±3.7	7.7±3.9	5.4±3.5
Astringent	1.6±1.3	1.4±1.4	1.9±1.4	0.9±0.9

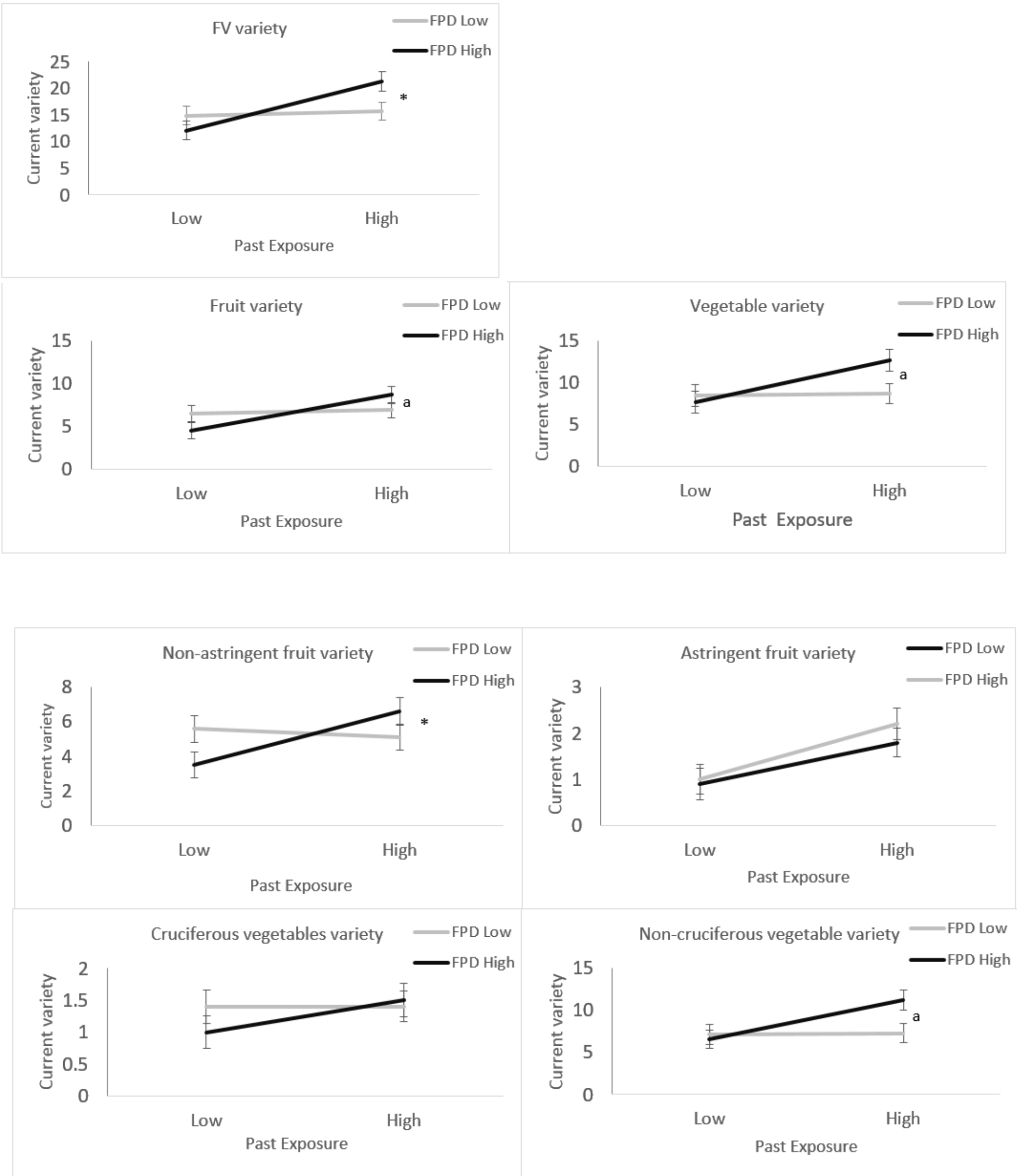
Non-astringent	5.0±3.1	5.1±3.1	5.8±3.0	4.5±2.9
Vegetables	9.8±5.4	8.3±4.9	10.5±5.6	8.0±4.4
Cruciferous	1.1±0.9	1.3±1.0	1.4±1.0	1.2±0.9
Non-cruciferous	8.6±4.8	7.0±4.3	9.1±5.1	6.8±3.7

Table 3. Bootstrapped 2x2 ANOVAs representing main effects and interactions between FPD and past exposure level, on children's intake of variety of FV and their subcategories.

Current FV variety	Source of variation	F-value	p	$_{p}\eta^2$
FV	FPD	0.64	0.43	0.012
	Exposure	8.06	0.006	.13
	FPD x Exposure	5.78	0.02	.10
Fruit	FPD	0.01	0.92	<0.01
	Exposure	5.71	0.02	.10
	FPD x Exposure	3.76	0.058	.06
Astringent	FPD	0.36	0.55	.01
	Exposure	9.12	0.004	.14
	FPD x Exposure	0.15	0.70	.01
Non-astringent	FPD	0.15	0.70	0.01
	Exposure	2.85	0.097	.05
	FPD x Exposure	5.15	0.027	.09
Vegetables	FPD	1.36	0.25	0.02
	Exposure	4.26	0.044	.07
	FPD x Exposure	3.29	0.075	.06
Cruciferous	FPD	0.36	0.55	<0.01
	Exposure	0.77	0.38	.01
	FPD x Exposure	0.65	0.42	.01
Non-cruciferous	FPD	2.11	0.15	.08

	Exposure	4.56	0.037	.04
	FPD x Exposure	3.49	0.067	.06

Fig.2. Interactions between the FPD and past exposure to FV on variety of fruit and vegetables currently consumed, as reported by the parents. *p<0.05, [a] p<0.08



5.0 Discussion

We hypothesised that children who in the past were exposed to greater variety of FV would be currently reported to consume a greater variety of FV, compared to children with lower past exposure. We predicted that effects of past exposure would be moderated by children's FPD. In line with previous literature, we also hypothesised that these effects would be differentiated in non-cruciferous vegetables and non-astringent fruit, which do not have bitter and/or irritant properties. The results of this study show partial support for the hypotheses. Children who in the past were exposed to a larger variety of FV were reported to consume a larger variety of FV in the preceding 7 days, compared to children with lower past exposure. There were no main effects of FPD, but FPD showed an interaction with past exposure to FV on parental report of current variety. The same trends were reflected in all FV sub-types and as predicted were significantly different for non-astringent fruit and marginally significantly different for non-cruciferous vegetables, albeit with a small effect size. These results suggest that higher FPD may facilitate intake of greater variety of non-irritant FV in the presence of a variety rich environment.

The results of this study show support for the link between FPD and FV variety, but add to the literature by showing that this relationship is moderated by environmental exposure and may also vary depending on food type. Past research linked FPD to bitter taste blindness, with reports showing that supertasters have higher FPD compared to tasters and non-tasters [17] and other reports indicating that while detecting bitter compounds is programmed by TAS2R38 receptors, the intensity of those sensory sensations is moderated by taste bud densities on FP [19]. In the current study we saw facilitating effects of FPD on current variety of FV consumed only in the presence of variety rich environment, with effects limited to FV with non-bitter and non-astringent properties. Those results support previous research by Duffy et al. [18] and Feeney et al. [21] who suggested that higher FPD facilitates FV intake, when bitterness of foods is not a barrier, among PROP non-tasters. In adults, higher FPD is associated with greater threshold and intensity ratings for tastants [17-20] as well as facilitation of pleasantness from tactile stimulation [36]. We speculate that children with higher FPD may be more able to perceive reinforcing tastes (sweetness, saltiness) and perhaps pleasant textures (e.g. crispness, crunch) in non-astringent fruit and non-bitter vegetables; and in combination with greater environmental exposure, learn to accept a greater variety of these into the diet. It is also possible that children with greater FPD reject greater numbers of cruciferous vegetables and astringent fruit, thus encouraging some parents to offer a greater variety of non-astringent fruit and non-cruciferous vegetables in compensation, which may facilitate their acceptance. We can speculate that in children with higher FPD but less exposure to variety of FV in their past, the opportunities to learn about the pleasant tastes and/or textures of some FV are limited, neophobia persists, and fewer varieties of FV are consequently accepted. Further prospective research is required to explore these mechanisms.

In the current study variety of cruciferous vegetables and astringent fruit was very low, however this is not surprising given their strong sensory properties, and other barriers to intake, which in the case of astringent fruit could also include their price and seasonality. Past reports suggest that repeated exposure increases preference for previously disliked vegetables (e.g. [37] [38], and our findings suggest that parents of children with higher FPD may be able to mitigate against potentially negative effects of associated taste sensitivity on FV variety acceptance by ensuring high levels of exposure to FV across the early years of life.

Nonetheless, further studies are necessary to establish whether repeated exposure effects would work for highly disliked FV with strong sensory properties, particularly in children with inherent barriers to intake, such as bitter taste sensitivity. Therefore, the focus of exposure may be best placed with non-astringent fruit and non-cruciferous vegetables in children with higher FPD. Further studies with larger sample sizes and experimental paradigms are necessary to further investigate this.

The results indicate that individual phenotypic characteristics may exert influence on behavioural outcomes however these may be altered by positive feeding practices such as facilitating exposure to a wide variety of FV and increasing their home accessibility. We need to consider the reciprocal relationship between child's individual characteristics and home environment; children who experience greater sensory intensity may make it more difficult to introduce greater variety of FV, particularly those with richer sensory characteristics, thus making it more difficult to successfully and regularly offer a greater variety of foods.

Limitations

There were several limitations of this study which need to be addressed. The sample size of this study was small, as a number of tongue photographs were of poor quality, which limits the power of the results and may affect the small effect sizes which were detected. Bootstrapping methods have been used to aid interpretation and have been reported here; non-bootstrapped analyses showed the same patterns. Socioeconomic status of the areas from which data were collected were very high; this is likely to have affected the variety of FV both offered to the child in the past and current quality of diet, given the strong links between socioeconomic status and FV consumption [39]. Parental self-reports of FV intake and exposure must be interpreted with caution, given ecological validity issues associated with FFQs. Furthermore, parental report of past exposure did not indicate the degree or frequency of exposure and may not take into account exposure that occurs in school or other settings where parents are not present to monitor food choice. Furthermore, we have focused here on simple counts of the variety of FV accepted, rather than portions or amounts consumed. Thus, the findings can only be applied to understanding of dietary variety; it is possible that children with high FV variety may still not be consuming sufficient portions of FV each day. Further work is required to examine whether similar effects are found when examining number of portions of FV consumed.

Conclusions

This is the first study to demonstrate that children's FPD is an important factor in the relationship between their past FV exposure and current FV variety. The results indicate that among children with low FPD, exposure to FV does not appear to greatly affect intake. However, children who have higher FPD are reported to consume greater variety of FV if they have been exposed to larger variety of FV in the past, compared to children with higher FPD with lower past exposure. Interestingly, this pattern was only seen for FV without strong sensory properties, such as astringency or bitterness. The variety of astringent fruit and bitter vegetables consumed was low across all children, irrespective of their FP phenotype or past FV exposure. The results suggest that future studies which look at intake of FV in children must consider both inherent and environmental influences on dietary outcomes, and individual differences in responsiveness to feeding practices and home environment.

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