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Research report

A step-by-step introduction to vegetables at the beginning of complementary feeding. The effects of early and repeated exposure [☆]



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ABSTRACT

Breastfeeding (BF) is associated with willingness to accept vegetables. This may be due to the variety of flavours delivered via breast milk. Some mothers add vegetables to milk during complementary feeding (CF) to enhance acceptance. The present study tested a step-by-step exposure to vegetables in milk then rice during CF, on intake and liking of vegetables. Just before CF, enrolled mothers were randomised to an intervention (IG, $n = 18$; 6 BF) or control group (CG, $n = 18$; 6 BF). IG infants received 12 daily exposures to vegetable puree added to milk (days 1–12), then 12×2 daily exposures to vegetable puree added to rice at home (days 13–24). Plain milk and rice were given to CG. Then both received 11 daily exposures to vegetable puree. Intake was weighed and liking rated on days 25–26 and 33–35 after the start of CF in the laboratory, supplemented by the same data recorded at home. Vegetables were rotated daily (carrots, green beans, spinach, broccoli). Intake, liking and pace of eating were greater for IG than CG infants. Intake and liking of carrots were greater than green beans. However, at 6m then 18m follow up, vegetable (carrot > green beans) but not group differences were observed. Mothers reported appreciation of the structure and guidance of this systematic approach. Early exposure to vegetables in a step-by-step method could be included in CF guidelines and longer term benefits assessed by extending the exposure period.

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Introduction

Despite the known health benefits of consuming diets which are rich in plant sources, fruit and vegetable intakes are generally lower than recommended across Europe (Yngve et al., 2005). In the UK, children are encouraged to eat vegetables as part of their five-a-day fruit and vegetables, but only around 20% manage to meet this recommendation. Most children aged 5–15 years eat just one portion of vegetables each day and 7% of children eat no fruit or vegetable (National Obesity Observatory, 2012). A meta-analysis of school based interventions to promote intake of fruit and vegetables revealed a low success rate with an average of only 0.07 g of additional vegetable eaten (Anderson et al., 2005; Evans, Christian, Cleghorn,

Greenwood, & Cade, 2012; Ransley et al., 2007). This suggests that it is challenging to persuade school-aged children to eat more vegetables through interventions. In any case, it seems particularly difficult to increase the intake of vegetables in children (Zeinstra, Koelen, Kok, & de Graaf, 2009). Vegetables may be rejected for a number of different reasons, from their bitter taste, unfamiliar texture, their relatively low energy content to simple lack of access in many families (Krolner et al., 2011; Mennella & Ventura, 2011). Food learning starts very early; especially the first two years of life seem important for the development of healthy eating habits (Cashdan, 1994) as this is a period in which new foods are relatively easily accepted (Lange et al., 2013; Schwartz, Chabanet, Lange, Issanchou, & Nicklaus, 2011). Once food habits are established they tend to be stable. Various studies (see Nicklaus & Remy, 2013) for review) have shown that food preferences which are developed at an early age, have a long-lasting influence.

Methods which mothers use to promote the intake of vegetables in their children are numerous, since it is recognised that these foods are less appealing than others (see Schwartz et al., 2013). Strategies include adding ketchup or seasoning and even hiding vegetables (Caton, Ahern, & Hetherington, 2011). Providing vegetables

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by “stealth” delivers the benefits of consuming vegetables but with a non-recognisable form and the taste of vegetables is masked by other more familiar and liked flavours. However, this strategy probably does not allow for familiarisation with the visual, olfactory, gustatory or textural properties of vegetables to increase familiarity and willingness to eat (Aldridge, Dovey, & Halford, 2009; Dazeley, Houston-Price, & Hill, 2012), visual cues being particularly important for children in sensory-decision making in deciding to eat a novel fruit (Dovey et al., 2012). In other words, this approach leads to vegetable intake but not to learning to like, eat and recognise vegetables.

Adding flavours which are already liked promotes intake of vegetables through flavour-flavour learning (FFL). Systematic studies of FFL reveal that this improves the likelihood of trying a vegetable but does not increase liking or intake of the unmodified vegetable relative to mere exposure in pre-school children (Anzman-Frasca, Savage, Marini, Fisher, & Birch, 2012; De Wild, De Graaf, & Jager, 2013). The most successful strategy to promote vegetable intake is mere or repeated exposure (Ahern, Caton, Blundell, & Hetherington, 2013; Hausner, Olsen, & Møller, 2012; Remy, Issanchou, Chabanet, & Nicklaus, 2013). Mere exposure (Zajonc, 1968) produces a favourable response to a novel stimulus if experience with that stimulus produces no negative consequence, through the mechanisms of ‘familiarity’ (Zajonc, 1968) and ‘learned safety’ (Kalat & Rozin, 1973). Successful vegetable introduction includes not only repeated exposure but also applying daily variety in 3–5 day rotation schemes (Nicklaus, 2011).

Given that vegetables are typically eaten within a composite meal and that they are generally disliked by children, it is important to understand whether pairing vegetables with other flavours can enhance their acceptance or whether children learn to prefer the composite rather than the pure vegetable flavour. In particular, during CF there is a sudden transition from milk feeding to the strong taste of pure vegetables, but would liking and intake be facilitated by a more gradual approach? In France, as recommended in specific national CF guidelines (Comité National de l'enfance, 2014; INPES, 2004, 2005), some mothers add water from cooking vegetables or vegetable puree to milk as a means of developing their child's taste for vegetables (Schwartz et al., 2013). The amount added is increased gradually over time. Thus French mothers recognise that special strategies may be needed to develop acceptance of foods such as vegetables which are generally disliked (Schwartz et al., 2013). If infants learn to like the flavour of a new vegetable by its association with milk or another familiar food, then this practice could limit acceptance of vegetables given in a pure form since acceptance might be context-dependent, namely, liked only in combination with the familiar, liked flavour. On the other hand, vegetables offered systematically and gradually with the aim to replace the composite with only the pure vegetable may assist in promoting acceptance. The practice adopted by French mothers has not been tested systematically to our knowledge and in any case it is not known whether this practice can be generalised beyond the French context.

Flavouring milk, either breast or formula milks, by adding vegetable cooking water or puree might seem unusual in some cultures. However, breastfed infants are naturally exposed to flavour varia-

tions in breast milk because mother's exposure to dietary flavour cues is transmitted through breast milk (Hausner, Bredie, Mølgaard, Petersen, & Møller, 2008). This phenomenon, called a ‘flavour bridge’, transmits to the weanlings the flavours of the mother's diet before starting the CF process and it increases compliance and acceptance of new foods (Hausner, Nicklaus, Issanchou, Mølgaard, & Møller, 2010; Mennella, Jagnow, & Beauchamp, 2001).

Therefore, the aims of the current intervention were to test the effects of providing vegetables step-by-step in milk and then in cereal during CF on intake and liking of pure vegetables, and to investigate the acceptability of this strategy among mothers. The hypotheses were that: (a) the gradual introduction to vegetables will increase intake and liking of those vegetables (carrot, green bean, broccoli and spinach); (b) this exposure effect would generalise to another, unfamiliar vegetable (parsnip); and (c) that any differences found between the two groups would endure until 6 months and 18 months after the intervention.

Method

Participants

Mothers were recruited from the local community using widespread advertising within mother and baby groups and a recruitment agency between September 2011 and May 2012. Infants under the age of 12 weeks were not able to participate but could be part of the study after this time. Infants suffering from a chronic health condition requiring medication, born prematurely before 37 weeks of gestation (Migraine et al., 2013), fed hydrolysed-protein formula (Mennella & Beauchamp, 2002; Mennella, Forestell, Morgan, & Beauchamp, 2009; Mennella, Kennedy, & Beauchamp, 2006) or with a known food allergy were not eligible to participate. In total, the research team made contact with 48 mothers and from this initial contact 40 mothers were screened and accepted into the study. Mothers were randomised to either the intervention (n = 20) or control group (n = 20) after they had consented to the study and before they had completed any questionnaires. Written informed consent was obtained from all mothers of the participating infants. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institute of Psychological Sciences (University of Leeds) ethics committee who adheres to the principles set down by the British Psychological Society (Ethics Ref No: #11-0031). Of the original 40 mother–infant dyads who agreed to take part in the study, complete data were obtained from 36 mothers (18 in each group). Reasons for non-completion included missed appointments (n = 2), relocation (n = 1) and return to work (n = 1).

Procedure

Each group followed a 35 day CF intervention (see Fig. 1). IG infants received 12 daily exposures to vegetable puree added to milk (days 1–12), then 12 × 2 daily exposures to vegetable puree added to baby rice at home (days 13–24). Plain milk and cereal were given

Day Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Phase	Milk: 12 days												X1 a day				Baby Rice: 12 days				X2 a day				Purees: 11 days					X1 a day					
Control Group	Plain milk												usual quantity				Plain Baby Rice				max~77g				Vegetable puree					max~260g					
Intervention Group	Flavoured milk												max~50g				Flavoured Baby Rice				max~72g				Vegetable puree					max~260g					
	Ca	GB	Sp	Br	Ca	GB	Sp	Br	Ca	GB	Sp	Br	Ca	GB	Sp	Br	Ca	GB	Sp	Br	Ca	GB	Sp	Br	Ca	GB	Sp	Br	Ca	GB	Sp	Br	Ca	GB	Pa

Fig. 1. Timeline of the study from Day 1 to Day 35 (laboratory sessions are indicated in red).

to the control group. Then both groups received 11 daily exposures to vegetable puree. The rationale for exposing the infants to vegetables added to milk then to cereal then as a puree was to mimic the progression that mothers use in building up gradually to a stronger, distinctive taste. Also the reason for choosing puree rather than cooking water from vegetables was to standardise the amount and intensity of vegetable flavour.

Mothers were invited to the Human Appetite Research Unit (HARU) Infant Laboratory and given a full explanation of all study procedures. They were each given a pack containing a 35 day diary and all of the equipment and foodstuffs they would need to complete the study. They were informed that breast or formula feeding should continue as normal. They were free to start the 35 day CF plan whenever they decided so that they retained control of when to start the weaning process.

Both groups were asked not to introduce any other new foods or flavours to the infants during the study.¹ They were told that apart from the complementary study feeds prescribed, they should only offer the infants their usual milk and water. Mothers were also reminded that milk should remain the main food for the infants and that the study feeds should not be considered as a substitute for any milk feeds during the study, but additional to their normal practice.

The 35 day diary asked mothers to record everything the infant consumed each day and to state the start and end time of each feed, then the amount where known of each milk feed (formula only), water or complementary feed. They were also asked to make a note of any observations immediately after each feed. Over the course of each day, they were asked to observe and record their infant's reaction to a feed using a 9-point scale (1 = dislikes extremely through 9 = likes extremely). The groups were instructed to do this as follows – for the Control Group – responses after one milk feed each day from Days 1–12; then after two baby rice meals each day from Days 13–24; then each pureed vegetable feed from Days 25–35. For the Intervention Group, responses were recorded to one vegetable flavoured milk feed each day from Days 1–12; then after two vegetable flavoured baby rice meals each day from Days 13–24; then each pureed vegetable feed from Days 25–35. Mothers were informed that they could give the infant the complementary study feeds at any time of day to fit in with their own individual schedule. They were given full instructions on making up each of the feeds. Each page in the 35 day diary clearly indicated the milk/food/vegetable to be offered each day. They were instructed to give the infant as much or as little as they wanted of the study feed each day. They were told to stop if the infant showed 3 clear refusals of the feed (as they had been for the laboratory day) and if they had not tasted any at all to try again later in the day with a new batch of the feed. Refusals were explained to the mothers as behaviours such as head turning, being fussy or playing. Full instructions were given on the use of a small set of portable digital pocket scales (MYCO MZ-100, Dalman) to weigh accurately intakes (i.e. by weighing bottles or bowls before and after each feed) of all feeds consumed across the day.

Laboratory sessions took place on Days 25, 26, 33, 34 and 35. For these sessions mothers were invited to the HARU infant laboratory and they gave the infants the vegetable puree under controlled conditions. The room was prepared with the infant high chair and video camera in the same location for each visit. Window blinds were pulled down to prevent external distractions. Mothers were asked to describe the baby's well-being and any issues such as illness, sleep problems and changes to appetite were recorded. Mothers were then briefed on the procedure for the session. Mothers were shown

a power point presentation on how to feed the infants during the test session and they were able to ask questions. They were instructed to feed the baby at their own pace, avoid verbal interaction (and use a neutral tone of voice with any verbal interaction), avoid contacting the spoon with the lips after the first few spoons and to let the baby lead by letting them move forward to the spoon when feeding. This was done to limit variability in presenting the food during the filming (see Madrelle, Barends, Weenen, & Hetherington, 2013). They were instructed to stop feeding after the infant showed 3 consecutive refusals. To standardise the situation for all infants as much as possible, the mothers were shown still photos and videos demonstrating infant food refusal behaviours such as pulling away, back arching, grabbing the spoon or pushing it away, looking away and down, spitting food out, yawning, becoming playful and losing interest, fussing and crying. The mothers were instructed to replace the spoon in the bowl after each refusal and to wait a few seconds before trying again. The researcher and mother made a joint decision on when 3 refusals had been reached. The duration of the feed was then recorded and rate of eating estimated by dividing amount eaten by time taken to complete the meal.

The sessions were organised to be relaxed and informal. The researcher prepared a bowl containing 2 jars of the target vegetables which was warmed, weighed and given to the mother (this was based on the study conducted by Maier, Chabanet, Schaal, Leathwood, & Issanchou (2008) for which the content of 3 jars were prepared and offered in laboratory sessions). The mother fed the infant at each session with the researcher sitting on a chair behind the infant (watching the infant in a strategically placed mirror) to ensure an optimum feeding environment with limited distractions. The feeding session was filmed from just before the mother was given the bowl until 3 refusals had been reached. Any remaining vegetable on the infant's face, hands and on the chair was collected and returned to the bowl. The remaining puree was re-weighed, the time taken to eat recorded and vegetable intake calculated.

At the end of the feeding session, both the mother and the researcher independently rated the infant's liking of the food. This was done using the same 9 point liking scale used in the 35 day diary. All the laboratory sessions were video recorded to enable the analysis of the infants' liking of the food by the researchers.

Study materials – foods

We had to obtain single vegetable purees from mainland Europe since these were not available in the UK. All jars of vegetables contained baby-food grade ingredients which meet the European regulation (Directive 2006/125/CE). All food products were appropriate for infants aged 4–6 months. Prior to the start of the study, 5 vegetables were chosen as the target vegetables (green beans, carrot, spinach, broccoli and parsnip) (Table 1). This was based on evidence of common use in the UK taken from Ahern et al. (2013). The vegetables represented a selection of vegetables which are

Table 1
Foods used in the study.

Food	Flavour	Brand
Pure Baby rice	Pure Baby rice	Baby rice, pack 100 g from Cow & Gate, 4–6 months
Vegetable puree	Carrots	"Mon 1er petit pot" from Bledina, in a 130 mL jar, 4–6 months
Vegetable puree	Green beans	"Mon 1er petit pot" from Bledina, in a 130 mL jar, 4–6 months
Vegetable puree	Spinach	"Mon 1er petit pot" from Bledina, in a 130 mL jar, 4–6 months
Vegetable puree	Broccoli	From Nutricia, in a 125 mL jar, 5 months
Vegetable puree	Parsnip	Creamy parsnip & potato from Cow & Gate, in a 130 mL jar, 4–6 months

¹ However, one mother from the IG did introduce other foods in the period that the vegetables were introduced.

Table 2
Sensory ratings of vegetable purees.

	Intensity taste	Taste recognisability	Metallic/iron taste	Sweet taste	Sour taste	Bitter taste
Broccoli	69.7 ^a	50.8 ^b	24.5 ^b	21.6 ^c	46.8 ^b	19.8 ^c
Carrots	79.0 ^a	86.9 ^a	23.0 ^b	89.9 ^a	74.6 ^a	59.3 ^{ab}
Green beans	63.2 ^a	79.2 ^a	15.3 ^b	15.0 ^c	52.2 ^{ab}	35.7 ^{bc}
Parsnip	40.0 ^b	47.5 ^b	3.0 ^b	64.9 ^b	45.1 ^b	11.2 ^c
potato						
Spinach	83.5 ^a	84.9 ^a	82.9 ^a	10.0 ^c	56.8 ^{ab}	70.9 ^{ab}

Numbers in the same column with different letters are significantly different.

generally liked (root vegetables) and disliked (cruciferous, leafy green vegetables) by children.

Mothers were offered bottles, wide teats and manual breast pumps as needed. They were also given CF spoons, small containers for measuring quantities of milk/rice/puree and a set of small digital scales to ensure the feeds were accurately prepared and recorded.

Sensory profiles

A sensory description of vegetables added to milk, rice and in pure form was conducted by a trained panel to ensure that vegetable flavour was delivered with noticeable (at least to adults) intensity across forms.

The trained panel consisted of 10 panelists, complete data were obtained for 7 panelists, and only these were used for further analysis. They received more than 8 sessions of training on taste description of the study products and on the way to use the scoring scales between August and November 2011. First, the panellists were asked to score in a semi-monadic presentation general taste intensity, vegetable taste recognisability and intensity of basic taste attributes (bitter, sour and sweet) and metallic/iron taste of all five vegetable purees. Secondly, they were asked to do rank-ratings of the same attributes for each of the vegetable + milk/vegetable + cereal/vegetable puree clusters; each vegetable cluster being evaluated separately. Products were prepared under a standardised method and served blind with a Latin square design to ensure balanced order. Perceived vegetable intensities of each variant were scored on a 100 mm line scale, from 'weak' (left anchor, converted into 0) to 'strong' (right anchor, converted into 100) for intensity ratings and from 'not at all' to 'very recognisable' for recognisability ratings. Two replications were performed in accordance with procedures used in our previous research (Caton et al., 2013). Results of the sensory profiling are presented in Table 2 and Fig. 2.

It was agreed that adding puree 40% by weight to milk and 70% by weight to cereal provided the best results to discriminate flavour without overwhelming the flavour of milk or cereal. These concentrations enabled a gradual transition of flavour strength from milk to cereal within the intervention group (Fig. 2).

Study materials – questionnaires

At the first visit to the laboratory questionnaires were given to the mothers after giving consent. These included a general demographics questionnaire to record general information about the family (number of children, parental ages, education, employment, salary, etc.) and specific questions about parents (weight, height, health) and infants (date of birth, weight at birth, length at birth, and mode of feeding, feeding routine). Then a series of validated questionnaires were included to examine maternal diet (Food Frequency Questionnaire; FFQ (Hammond, Nelson, Chinn, & Rona, 1993) and their level of anxiety (State and Trait Anxiety Inventory; STAI

(Spielberger, 1999)), infant's feeding behaviour (Baby Eating Behaviour Questionnaire; BEBQ (Llewellyn, van Jaarsveld, Johnson, Carnell, & Wardle, 2011)). The height and weight of the infants were also measured at this visit.

De-brief questionnaire

On completion of the 35 day intervention study, mothers were asked to complete an extensive questionnaire designed to gain insights into current CF practices in the UK, problems associated with CF and the best practices advice/information which would be of most use to them. Mothers were asked about CF knowledge prior to taking part in the study (sources of information used, concerns about CF and factors that influenced their decision on when to start CF), CF knowledge having completed the study (advice given, new knowledge gained and confidence in feeding their infant), and the influence the study had on their plans for feeding their infant in the future (factors that would influence their decision on foods to give, products they would look for and purchase). They were also asked about the feasibility of following a step by step approach using single vegetables as first CF foods. The mothers were paid £120 upon completion of the study in recognition of travel expenses, inconvenience and time spent on diary data collection and filming.

Follow-up visits at 6 months and 18 months

To evaluate the duration of the effects of the intervention, follow-up measurements were carried out 6 months and 18 months after the completion of the study. This means the follow up took place when the children were aged 12 months and 24 months approximately. Intake and liking for the same purees carrot and green beans were conducted at home following the same procedure during two separate feeding sessions at 6 months. Although the infants were aged 12 months by this stage and it would have been more age-appropriate to provide finger foods, the purees were offered to be consistent with the intervention. However, at 18 months mothers were simply asked to rate how often these vegetables were offered and how much they were liked by their infant in whatever form these were given using a questionnaire.

Before these measurements, mothers were reminded of the original procedure. At 6 months follow up, 15 mothers in the IG completed the two feeding sessions, while 16 mothers completed them in the CG (86% return rate). At 18 months follow up, 11 mothers in the IG and 3 mothers in the CG completed questionnaires and the 3 day diary (39% return rate). We compared responders and non-responders on a number of maternal and infant characteristics to seek a possible explanation for the low engagement of the mothers at 18 months. We found that mothers who responded were older and were more likely to have been breastfeeding or mixed breast/formula feeding at screening than those who did not respond. There were no other differences found (such as maternal BMI, education, STAI scores or infant characteristics) between responders and non-responders.

A series of validated questionnaires were included to examine infants' feeding behaviour including their habitual vegetable intake through an adapted FFQ (Hammond et al., 1993) and Child Eating Behaviour Questionnaire (CEBQ; Wardle, Guthrie, Sanderson, & Rapoport, 2001).

Statistical analysis

Data are presented as means (\pm SEM, unless otherwise stated). Maternal characteristics were compared using analysis of variance (ANOVA). Repeated measures ANOVA were performed on laboratory based intake data (absolute weight in grams) with veg-

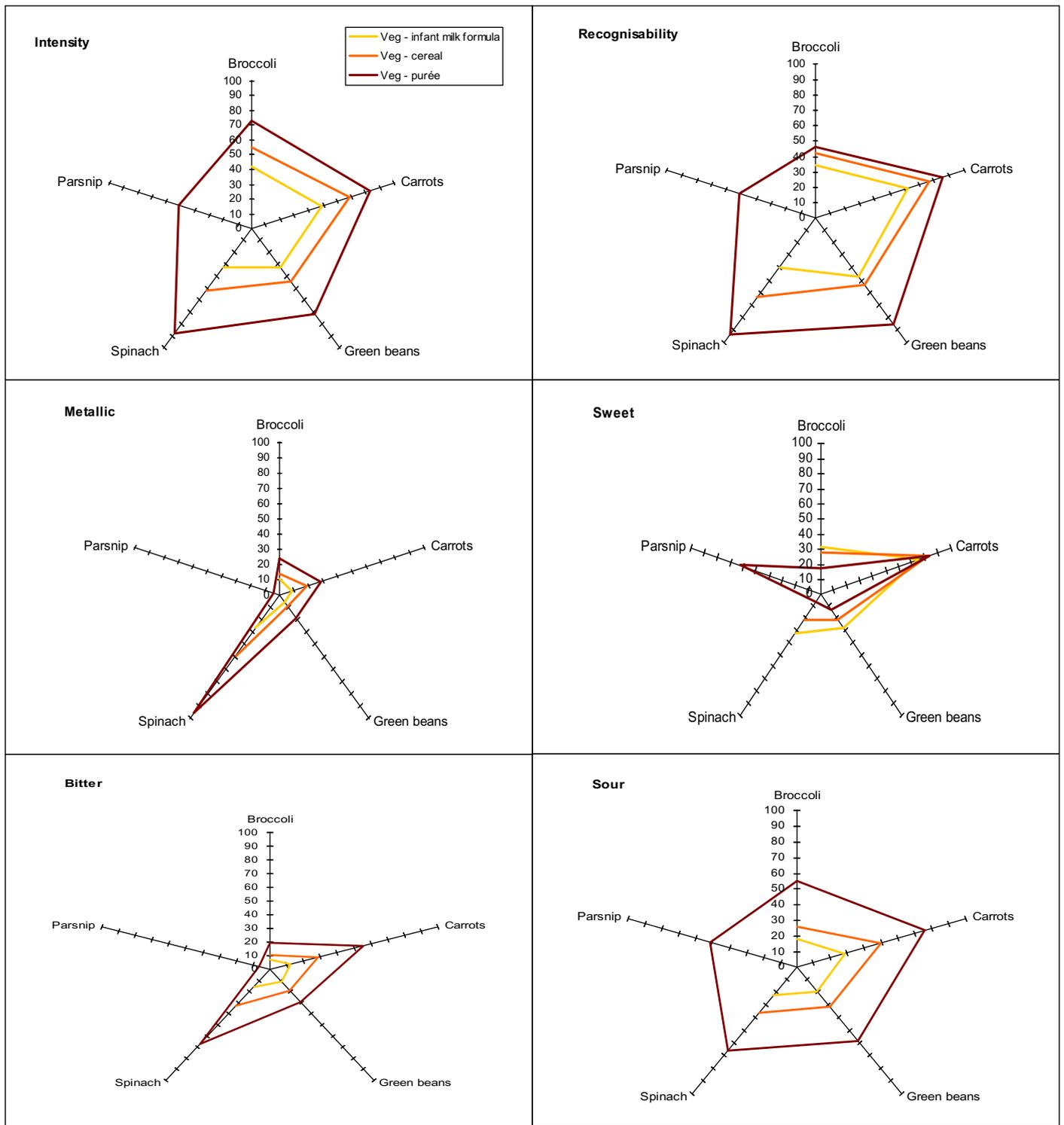


Fig. 2. Results of the sensory profile of the products: vegetable + infant milk formula, vegetable + baby cereal and vegetable in puree (mean ratings).

etable (carrot, green bean) and time (laboratory sessions 1 and 2) and group (intervention, control) as main effects. Also, vegetable intake with both at home and laboratory based measures were analysed with vegetable (carrot, green bean, spinach, broccoli) and group (intervention, control) and time (first, second sessions) as main factors. Intakes were also analysed each day by group. Rate of eating (pace) was calculated as amount eaten, divided by time taken to eat and then analysed using repeated measures ANOVA.

Sphericity was not assumed in the analysis and the Greenhaus-Geisser correction was applied. To test for group effects by time, intakes were also compared using planned comparison t-tests and follow-up data from diaries were also analysed using ANOVA.

Post hoc comparisons were performed using Bonferroni adjustments for multiple comparisons or t-tests for planned comparisons. Variables of interest were tested for correlation, such as between liking and intake and between researcher and maternal ratings of

liking, and these were performed using Spearman product-moment correlations.

The same analyses were carried out for liking data from the laboratory sessions and for the follow up data at 6 months (but not at 18 months since the sample size was too small).

All children tested were included in the analysis, but cell size varied according to data available.

For example, where data were outliers as detected by boxplots, these were removed and where infants were unwell on the day of testing, their data were excluded.

Sensory data were analysed using ANOVA, using a mixed model with random assessors, main effects were tested against interaction and significances between samples tested with Fisher's LSD for multiple comparisons. For all attributes sample effects were found.

SPSS (v20, Chicago, USA) was used for all statistical analyses except the sensory data for which Senpaq (v5.0, QI Statistics, Theale, UK) was used. Alpha value chosen was 0.05.

Results

Participants

Forty parents provided informed consent for their infants to take part in the study; however, complete data were collected on 36 mother–infant dyads. Descriptive data are summarised in Table 3 indicating that maternal characteristics across the groups were very similar. The only significant difference detected was on the averaged State Trait Anxiety Inventory (STAI) across three measurement days showing that mothers randomised to the intervention group (29.15 ± 6.6) were more anxious in comparison to the control group (24.62 ± 4.4 ; $F(1, 29) = 4.97$, $p < 0.05$). To check if the intervention group became anxious over time, repeated measures ANOVA showed no interaction between group and time, suggesting that it was not participation in the intervention which increased anxiety but that this group was more anxious overall.

Descriptive data related to children's characteristics are presented in Table 4. Also, data relative to children's mode of feeding at birth, during screening at the time of study are summarised in Table 5. No significant differences in children's characteristics and eating behaviours across the two groups were observed.

Intake data – laboratory sessions

Vegetable intake recorded on Days 25, 26, 33 and 34 (i.e. carrot, green bean) was significantly higher overall in the IG (81.7 ± 9 g) compared to the CG (44.13 ± 8 g) [main effect of group: $F(1, 31) = 9.6$, $p < 0.001$; Cohen's $d = 4.41$, observed power = 0.85]. Vegetable intake increased over time from the first (55 ± 6 g) to the second exposure (70.8 ± 8 g) [main effect of time: $F(1, 31) = 4.526$, $p = 0.04$]. The

Table 4

Characteristics of the children who completed the study. Data are presented as frequency (percentage) and means (\pm SD).

Children's characteristics	Control (n = 18)	Intervention (n = 17)	Total (n = 35)	P value (group differences)
Gender (male, %)	8 (44.4%)	8 (44.4%)	16 (42.9%)	$p = 1.00$
Birth weight (kg)	$3.57 \pm (0.64)$	$3.68 \pm (0.71)$	$3.63 \pm (0.67)$	$p = 0.65$
Age on day 1 (months)	$4.88 \pm (0.63)$	$4.78 \pm (0.53)$	$4.83 \pm (0.57)$	$p = 0.59$
BMI for age (z-score)	$0.48 \pm (1.24)$	$-0.03 \pm (1.05)$	$0.23 \pm (1.16)$	$p = 0.19$
Weight for length (z-score)	$0.54 \pm (1.22)$	$0.02 \pm (1.05)$	$0.29 \pm (1.16)$	$p = 0.19$
BEBQ EF	$3.84 \pm (0.43)$	$3.82 \pm (0.37)$	$3.83 \pm (0.40)$	$p = 0.92$
BEBQ FR	$2.05 \pm (0.76)$	$2.17 \pm (0.67)$	$2.11 \pm (0.71)$	$p = 0.65$
BEBQ SR	$2.27 \pm (0.84)$	$2.31 \pm (0.78)$	$2.29 \pm (0.79)$	$p = 0.88$
BEBQ SE	$2.29 \pm (0.84)$	$2.34 \pm (0.56)$	$2.32 \pm (0.70)$	$p = 0.86$
BEBQ GA	$2.35 \pm (0.70)$	$2.47 \pm (0.80)$	$2.41 \pm (0.74)$	$p = 0.65$

BEBQ, Baby Eating Behaviour Questionnaire; EF, enjoyment of food; SR, satiety responsiveness; SE, slowness in eating; GA, general appetite.

main effect of vegetable type was highly significant [$F(1, 31) = 31.99$, $p < 0.001$] indicating that on both laboratory days more carrot (83.1 ± 9 g) was eaten compared to green beans (42.7 ± 5 g). No interaction effects were significant. Therefore, the intervention increased vegetable intake overall, intake increased with time in both groups and carrots were eaten in greater amounts than green beans (see Fig. 3).

The rate of eating also differed by group [$F(1, 27) = 12.99$, $p < 0.01$] by time [$F(1, 27) = 13.6$, $p < 0.001$] and by vegetable [$F(1, 27) = 13.17$, $p < 0.01$] but the interaction effect between time and vegetable just failed to reach significance [$F(1, 27) = 3.55$, $p = 0.07$; see Fig. 4]. Thus infants in the IG consumed the vegetable puree at a faster rate (7.5 ± 0.6 g/min) than the controls (4.7 ± 0.55 g/min); rate of eating increased from the first (5.2 ± 0.4 g/min) to the second exposure (7 ± 0.55 g/min); and carrot (6.7 ± 0.55 g/min) was eaten more rapidly than green bean (5.5 ± 0.4 g/min).

Intake data – at home

First of all, intakes recorded at home did not differ from those measured in the laboratory; therefore, data are presented with both measures included to examine any changes with time (see Fig. 3). Data are presented in two ways: first of all, intake of carrot (Days 25, 29, 33) and green bean (Days 26, 30, 34) were measured on three occasions, so effects of time could be tested (Fig. 3); secondly intake of 4 vegetables (carrot, green bean, broccoli and spinach) were

Table 3

Characteristics of the mothers who completed the study. Data are presented as means (\pm SD).

Maternal characteristics	Control (n = 18)	Intervention (n = 17)	Total (n = 35)	P value (group differences)
Maternal age (years)	30.88 ± 4.44	33.65 ± 5.43	32.20 ± 5.02	$p = 0.10$
Maternal height (m)	1.68 ± 0.06	1.67 ± 0.08	1.67 ± 0.07	$p = 0.55$
Maternal weight (kg)	73.98 ± 24.23	69.08 ± 18.17	71.60 ± 21.33	$p = 0.51$
Maternal BMI (kg m^{-2})	26.01 ± 7.71	24.69 ± 5.13	25.37 ± 6.53	$p = 0.56$
Maternal education, n (%)				$p = 0.90$
Below university	7 (38.9%)	8 (47.1%)	15 (42.9%)	
University+	11 (61.1%)	9 (52.9%)	19 (57.1%)	
Primiparous (first time mothers yes, %)	13 (72.2%)	8 (47.1%)	21 (60.0%)	$p = 0.96$
State_Trait_Anxiety Day 1	25.78 ± 6.37	30.94 ± 7.55	28.56 ± 7.28	$p = 0.64$
State_Trait_Anxiety Day 25	23.58 ± 5.39	27.17 ± 6.20	26.47 ± 5.77	$p = 0.48$
State_Trait_Anxiety Day 35	24.62 ± 5.03	28.89 ± 9.88	26.31 ± 8.24	$p = 0.56$
Follow-up daily veg intake ^a	3.04 ± 0.82	3.30 ± 1.51	3.18 ± 1.21	$p = 0.55$

^a Portions per day from the Hammond et al. FFQ.

Table 5
Mode of feeding at birth, during screening and at the time of study. Data are presented as frequency (percentage).

Maternal feeding practice	Control (n = 18)	Intervention (n = 17)	Total (n = 35)	P value (group differences)
Feeding philosophy, n (%)				<i>p</i> = 0.78
On demand	13 (76.5%)	13 (76.5%)	26 (76.5%)	
Schedule	4 (23.5%)	4 (23.5%)	8 (23.5%)	
Feeding method birth, n (%)				<i>p</i> = 0.26
Entirely formula	2 (11.1%)	0 (0%)	2 (5.7%)	
Almost entirely formula	0 (0%)	1 (5.9%)	1 (2.9%)	
Mostly formula some breast	1 (5.6%)	0 (0%)	1 (2.9%)	
Mostly breast with some formula	2 (11.1%)	1 (5.9%)	3 (8.6%)	
Entirely breastfeeding	13 (72.2%)	15 (88.2%)	27 (77.1%)	
Feeding method screening, n (%)				<i>p</i> = 0.62
Entirely formula	12 (70.6%)	8 (66.7%)	20 (69.0%)	
Almost entirely formula	1 (5.9%)	0 (0%)	1 (3.4%)	
Equally breast and formula	1 (5.9%)	0 (0%)	1 (3.4%)	
Mostly breast with some formula	0 (0%)	1 (8.3%)	1 (3.4%)	
Entirely breastfeeding	3 (17.6%)	3 (25.0%)	6 (20.8%)	
Feeding method current, n (%)				<i>p</i> = 0.18
Entirely formula	9 (50.0%)	6 (35.3%)	15 (42.9%)	
Almost entirely formula	5 (27.8%)	3 (17.7%)	8 (22.8%)	
Mostly breast with some formula	1 (5.5%)	4 (23.5%)	5 (14.3%)	
Entirely breastfeeding	3 (16.7%)	4 (23.5%)	7 (20.0%)	

recorded twice through the rotation so differences by vegetable over time were also tested (Fig. 4).

Across the three days of carrot and green bean intakes, there was no main effect of time but once again a significant effect of group [$F(1, 29) = 10.58, p < 0.01$] and vegetable [$F(1, 57.75) = 46.77, p < 0.001$]. Thus the IG ate more overall and carrot was eaten in greater amounts than green bean (see Fig. 3).

Intakes of the four different vegetables also confirmed that the IG consumed more than the CG [$F(1, 29) = 10.677, p < 0.01$], and the main effect of vegetable was also confirmed [$F(2.32, 79.8) = 14.3, p < 0.001$]. Post hoc tests revealed that carrot (76.7 ± 8.7 g) was consumed more than all other vegetables except broccoli ($p < 0.001$). Green bean (41 ± 6 g) was consumed less than all other vegetables ($p < 0.05$). Spinach (52.4 ± 6 g) was consumed less than broccoli (67.8 ± 6 g; $p = 0.054$).

Examining intakes each day by group reveals that differences were found from the first day of measurement but that by D35 when parsnip was offered in the laboratory no differences in intake were found (intervention = 66 ± 8.6 g; control = 49 ± 11.7 g; Fig. 3). It is not clear whether this was due to time (and therefore experience) or attributable to the vegetable offered.

At 6 months follow up the effect of vegetable was still apparent [$F(1, 28) = 4.39, p < 0.05$]: carrot was eaten more than green bean. At 6 months vegetable intake was significantly different by time as expected, since number and portion size of vegetables eaten increased, but no main effect of group was found. At 18 months follow up further analyses of group effects were not possible due to the low CG response rate.

Liking

Maternal ratings of liking did not differ by group nor by time, but by vegetable [$F(1, 32) = 40.7, p < 0.001$], indicating that mothers reported that their infants liked carrots (7.0 ± 0.3) more than green

bean (4.9 ± 0.34) (see Fig. 5). However, ratings of liking made by the investigators were significant for group [$F(1, 28) = 6.78, p < 0.05$], marginally significant for time [$F(1, 28) = 3.55, p = 0.07$] and for vegetable [$F(1, 28) = 27.63, p < 0.001$]. Investigator ratings indicated that infants differed by group assignment (intervention = 6.7 ± 0.3 ; control = 5.5 ± 0.3), that these ratings improved marginally with time (first visit = 5.8 ± 0.3 ; second visit = 6.4 ± 0.3) as well as by vegetable (carrot = 7.0 ± 0.3 ; green bean = 5.1 ± 0.3). Despite differences in ratings by mothers and researchers revealed within the ANOVA, correlations between maternal and researcher ratings of liking were significant for all vegetables and all time points ($r = 0.65 - 0.81, p < 0.01$ in all cases). However, researchers were not blind to the group assignment and this may have affected judgements. Video analyses of behaviours and facial expressions in response to the vegetables have been coded and reported elsewhere (Madrelle et al., 2013). These analyses conducted by raters blind to the condition assignment revealed significant differences by group for affective reactions with fewer avoidance/dislike behaviours (e.g. looking away, arching back, upper lip raising nose wrinkling) reported in the IG.

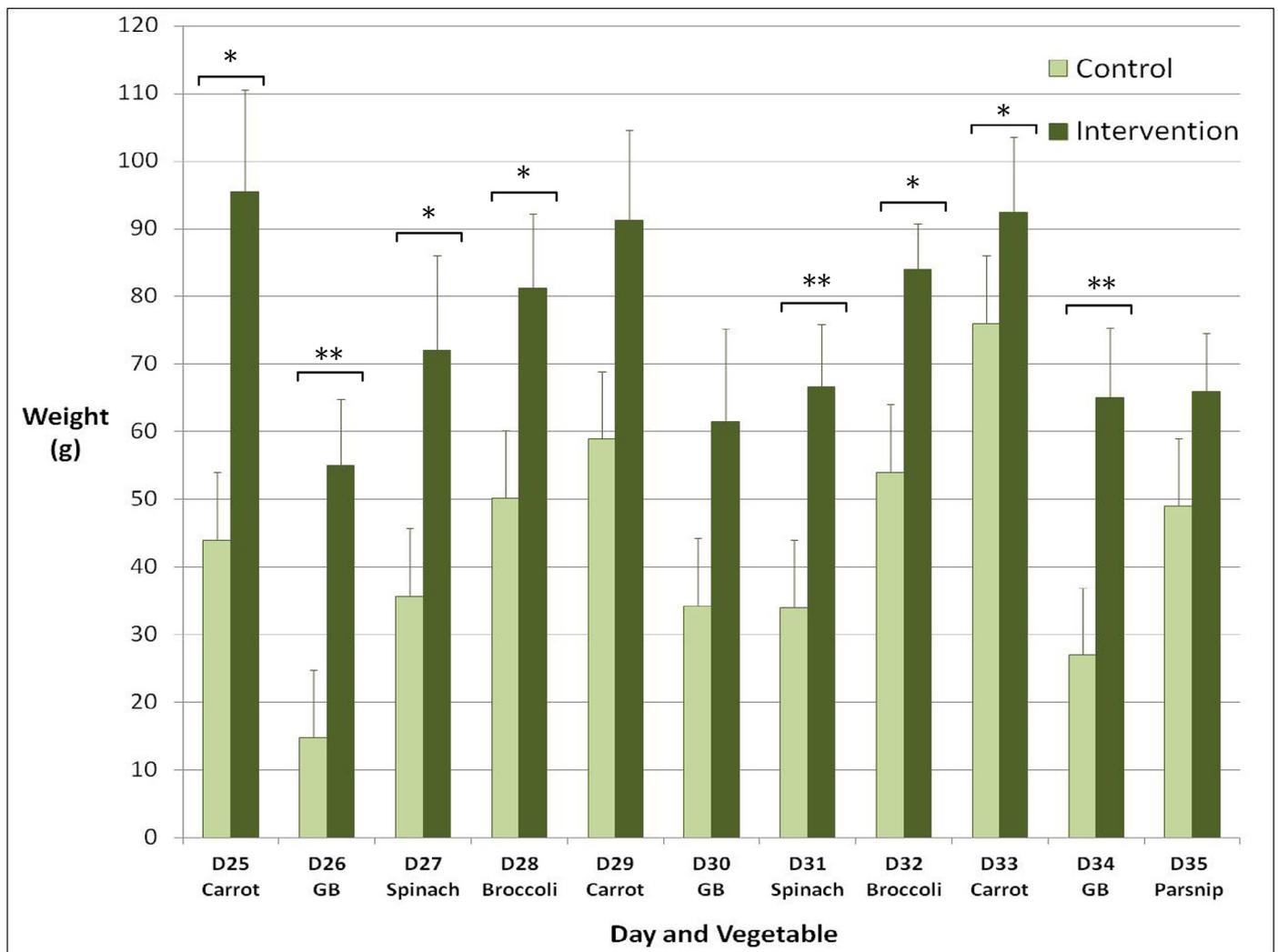
At 6 months follow-up, liking for vegetables was significantly different across groups [$F(1, 28) = 5.27, p = 0.029$], t-test indicated significant difference for carrot (intervention = 7.14 ± 0.53 ; control = $5.69 \pm 0.49, t(28) = -2.01, p = 0.05$) and marginally significant difference for green bean (intervention = 6.14 ± 0.62 ; control = $4.56 \pm 0.58, t(28) = -1.86, p = 0.07$). By 6 months significant differences in liking for carrots were found compared to green bean in both groups [$F(1, 28) = 5.27, p = 0.029$]. At 18 months follow up further analyses of group effects were not possible due to the low CG response rate.

De-brief

Reports from the mothers regarding the ease or difficulty of following the 35 Day CF guide were recorded during the de-brief session. Overall, reports were positive and the systematic nature of the procedure with day by day instructions on CF was appreciated by mothers. In all, 82.6% reported that they found the procedure easy or very easy to follow. Of the mothers assigned to the intervention group almost half (48.2%) reported concerns about using jars of pureed vegetables since they would have preferred to use fresh vegetables. However, they found that having the jars available for the sake of the study saved time and effort. An unexpected finding of the de-brief session was that the majority of mothers (80.5%) in this study found the instructions on recognising food refusal (delivered by slide presentation to enable standardisation across conditions and sessions) extremely useful. Mothers suggested that the CF guide could be offered by health visitors and that cues of food refusal could be illustrated within such a guide. Most mothers (93.1%) reported that a benefit of participating in the study was gaining confidence (either more confident or extremely confident) during CF about the types and amounts of foods to provide.

Discussion

The present investigation demonstrated that early exposure to a rotation of vegetable flavours first added to milk then to cereal increased intake and liking of these vegetables during CF. Infants assigned to the intervention ate more of the target vegetables (carrots and green beans) in the laboratory and at home than those assigned to the control group. The intervention group consumed these foods more rapidly than the control group, and liked these foods more according to investigator ratings though not according to maternal ratings. The effects were specific to the exposed vegetables, thus no evidence of generalisation was found by Day 35 when infants were offered a new vegetable, namely parsnip.



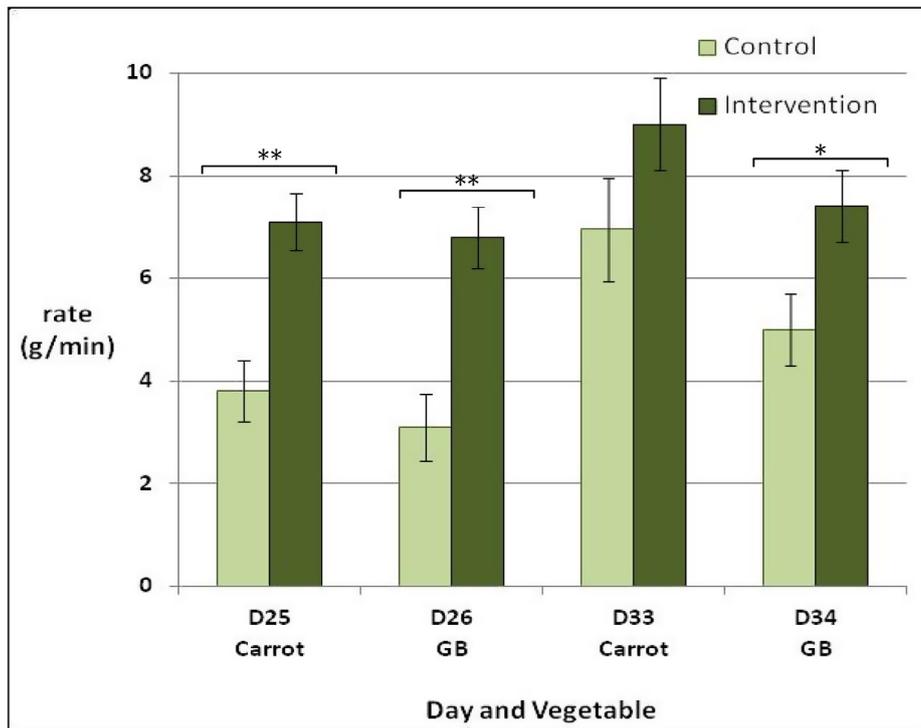
* $p < 0.05$; ** $p < 0.01$

Fig. 3. Mean (SEM) vegetable intakes recorded each day in the laboratory (D25, 26, 33, 34, 35) and at home (D27, 28, 29, 30, 31, 32).

A previous study showed that infants exposed to caraway via breast milk had a higher acceptance of a caraway flavoured potato puree than formula fed children (Hausner et al., 2010). However, the positive flavour exposure effect through breastfeeding on acceptance of the caraway flavoured potato puree was no longer significant after exposure to the target flavoured puree. Indeed, it was shown that the difference in relative intake in caraway potato puree between breast- and formula-fed groups disappeared after an exposure period of 10 days for all infants to the caraway potato (Hausner et al., 2010). Based on these findings, adding vegetable purees to milk and to baby cereal was expected to produce a more intense vegetable flavour than the natural transmission of flavours through maternal dietary intakes to breast milk, which in turn might generate higher or quicker acceptance of the target vegetables. Interestingly, this step-by-step exposure improved green bean acceptance even though previously proven difficult to promote acceptance at CF (Mennella, Nicklaus, Jagolino, & Yourshaw, 2008; Nicklaus, 2011). Previous research has shown that increasing exposure to variety within the meal rather than from day-to-day was needed to reach good acceptance of green beans (Mennella et al., 2008). In our study, the observed effects were specific to the offered vegetables, and no evidence of generalisation was found by Day 35 when infants were offered parsnip. It is not clear what accounts for

this; perhaps acceptance of parsnip was relatively easy therefore the effect of the intervention relatively small. Indeed the sensory profile shows that the parsnip puree had low overall taste intensity, is sweet but not sour or metallic/iron and is least bitter of all study vegetables, in contrast to green beans (low in sweetness but sour and bitter) or spinach purees (low in sweetness and most metallic/iron and bitter; Table 2). Stimulus generalisation predicts that food cues similar to an original cue in a learning paradigm will produce a response comparable to that elicited during learning, i.e. liking of the target will generalise to other similar stimuli, in this case other 'similar' vegetables. However, the present study failed to demonstrate this effect, perhaps due in part to how different the new vegetable (parsnip) was to the target vegetables. It is nevertheless important to test the limits of stimulus generalisation in the food context since this could have useful applications in encouraging healthy eating in young children.

The impact of early, repeated and varied exposure to vegetables had a longer term influence on liking ratings than on intake. At 6 months follow-up group differences for liking of carrot remained and showed a trend for group, however, differences in intake were not seen for group, only for vegetable (carrot eaten more than green bean). The lack of effect on intake may be attributable to the fact that the same purees were used for follow up

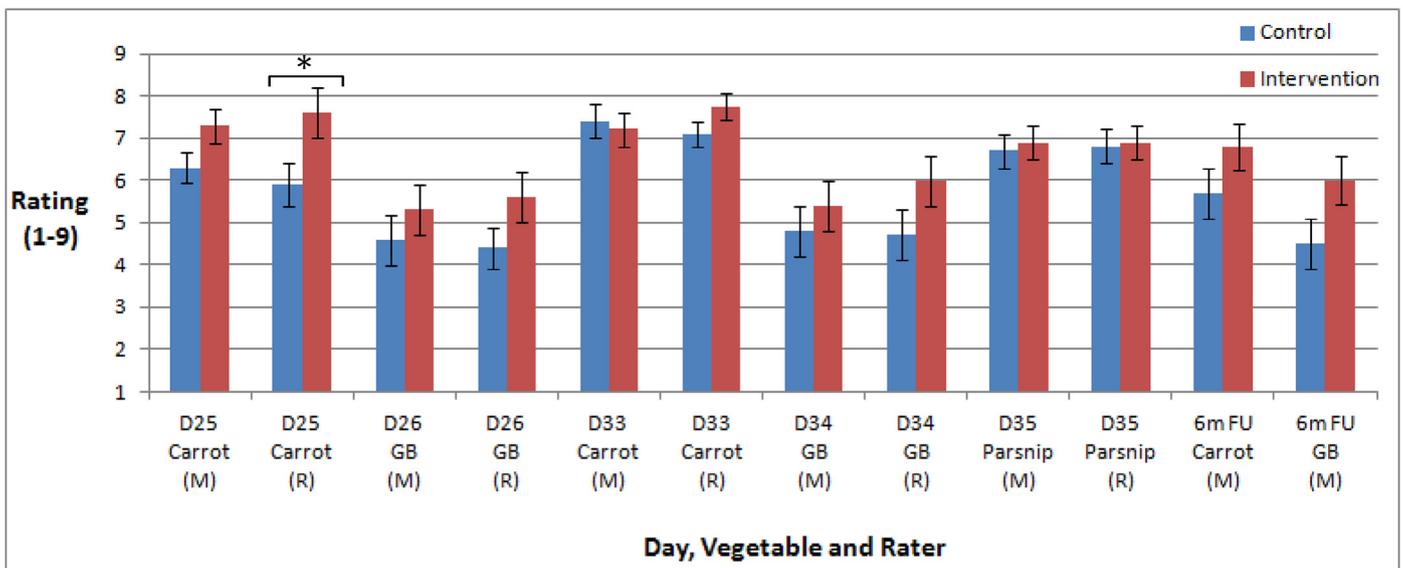


* $p < 0.05$; ** $p < 0.01$

Fig. 4. Mean (SEM) rate of eating for each vegetable (laboratory sessions only).

assessments and these purees were too smooth and liquid to be well accepted by older children. However, differences in intake and liking of green beans compared to carrots were observed consistently from the period of the intervention to 6 months follow-up. Since liking is linked to food intake, the intervention was moderately successful in establishing greater liking for a generally disliked vegetable, namely green bean.

Several studies have now shown that CF with vegetables facilitates liking and intake of vegetables compared to CF with fruit (Barends, de Vries, Mojet, & de Graaf, 2013; Fildes & Cooke, 2012; Remy et al., 2013). Timing of introduction of vegetables is also of importance: “the earlier vegetables were introduced, the higher the acceptance of new vegetables was” (Lange et al., 2013, p. 89). The present study confirms and extends these findings by



* $p < 0.05$

Fig. 5. Mean (SEM) ratings of liking by mothers (M) and researchers (R) during laboratory visits and home visits (follow-up measurements).

demonstrating that gradual introduction of vegetable taste by adding vegetables to already familiar and liked foods (milk or cereal) facilitates both intake and liking. Repeated exposure is known to enhance children's acceptance of a novel vegetable from weanlings to pre-schoolers and this seems sufficient to promote intake compared to adding sweetness or energy to the novel vegetable (Ahern et al., 2013; Hausner et al., 2012; Remy et al., 2013). In the present study, the flavour of vegetable was paired with milk (breast or formula) then with baby cereal, thus mechanisms of flavour–flavour learning and associative conditioning may have been invoked. However, the relative efficacy of each of these was not examined.

Havermans and Jansen (Havermans & Jansen, 2007) reported increased preference for a target vegetable that had previously been paired with dextrose compared to an unsweetened vegetable in a group of school children. Milk is naturally sweet – the sweetness of breast milk has been estimated as equivalent to a 2.12% solution of sucrose (McDaniel, Barker, & Lederer, 1989) and the sweetness of standard formula milks is similar (Schwartz et al., 2010) – therefore a potential mechanism contributing to the present outcome is that any unpleasant, bitter or sour notes of the vegetables were reduced not only by dilution but also by the sweetness of milk, as observed within the results of the sensory profile (Fig. 2).

The intervention produced differences in intake and liking of the vegetables when served alone (i.e. without milk or cereal) thus this strategy generalised from pairing the flavour (within a composite) to the pure flavour. It may be hypothesised that the smooth transition to an increase in flavour strength is a way to limit context dependent learning. Thus infants did not appear to like only the composite, but rather the novel flavour itself. If infants had only learned to like the blended stimulus (e.g. milk + vegetable flavour, or cereal + vegetable flavour) then generalisation would not have been found.

Mode of feeding (mostly breastfed, mostly formula fed) did not predict intake of vegetables nor did frequency of vegetable intake by the mother. Two previous papers that addressed this issue showed there is a positive effect of breast feeding (Burnier, Dubois, & Girard, 2011; Grieger, Scott, & Cobiac, 2011) on vegetable acceptance; however, Lange et al. did not find an effect of the length of exclusive breast feeding on new vegetable acceptance. More research is warranted to elucidate this issue.

Limitations of the present study include the use of a small sample size per condition which might have weakened the strength of the effects observed. Indeed effect sizes tended to be small to medium but the observed power was high for the main effect of group. Therefore, the intervention was sufficiently powered to detect differences between the groups. Also introducing the pure vegetables to both groups at Day 25 meant that the duration of the intervention was only 24 days; therefore, a longer period of repeated exposure might be needed to produce more durable effects. Another possible limitation of the study was that most of the intervention was conducted at home. It is then difficult to ensure that instructions were strictly followed. However, guidance was precise, mothers reported what they did in a daily diary and intakes recorded at home did not differ from those measured in the laboratory. The advantage of doing an in home intervention is of course that it is more ecologically valid. By the time of the follow up measurements, both the IG and CG had been repeatedly offered a daily rotation of vegetables during the intervention, as this condition was applied to both groups. In an ideal study set up, a third group should be added, in which this repeated exposure condition was not applied and only follow-up measurements are done. Since mothers were free to offer foods from the family diet after the intervention, a further limitation is that this was not recorded in detail. In summary, future studies could increase the exposure time and sample size to increase power to detect longer term differences with more confidence and could include

more measures of the family diet, including what was offered to infants between the intervention and follow-up.

Despite these limitations, the present study confirms findings elsewhere that early and repeated experience with vegetables serves to increase acceptance (Anzman-Frasca et al., 2012; Birch, Gunder, Grimm-Thomas, & Laing, 1998; Birch, McPhee, Steinberg, & Sullivan, 1990; Gerrish & Mennella, 2001; Loewen & Pliner, 1999; Pliner & Stallberg-White, 2000; Sullivan & Birch, 1994; Wardle et al., 2003; Wardle, Herrera, Cooke, & Gibson, 2003). This investigation has extended the period of exposure to the very first stages of CF and has provided experimental evidence supporting a cultural practice already advised in France to encourage vegetable acceptance (Comité National de l'enfance, 2014; INPES, 2004, 2005). French mothers claim that early exposure is necessary since vegetables are generally disliked and therefore need additional measures to encourage acceptance (Schwartz et al., 2013). Results of the present study confirm that some benefits can be derived from this way of early and repeated exposure during CF.

The mothers enrolled in this study provided positive feedback on the format of the diary with daily instructions on what to offer to their baby, they enjoyed watching their infant reacting to pure vegetables given in the laboratory and appreciated the instructions on how to recognise when their infants had eaten enough. Practical tips and clear examples of behaviours are sparse in CF guidelines (Schwartz, Scholtens, Lalanne, Weenen, & Nicklaus, 2011). One of the obvious implications of this research, therefore, is that mothers would benefit from the development of a CF guide which combines the strategy of a step-by-step introduction to vegetables with guidance on how to identify food refusal and infant satiety cues, and advice on how to deal with food refusal in the early stages of food learning. Another practical implication of this research is that providing mothers with vegetable purees or at least with instructions on how to prepare them easily would encourage mothers to feed their children with vegetables in the CF period.

This study showed that a simple intervention can make a significant difference in the extent to which infants accept and consume vegetables. It is clear that children and adults at present consume too few vegetables and that this plays a role in the development of diet-related diseases. The approach taken in this study was simple and well-structured and mothers provide positive feedback on its utility. This suggests that advice based on a step-by-step approach may be adopted by mothers and may then contribute in a simple and pragmatic way to enhance intake of vegetables.

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