# Children and vegetables:

Strategies to increase children's liking
and intake of vegetables

**Victoire W.T. de Wild**

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**To Chris, Floris en Eva**

"Children are like little scientist actively trying to make sense of the world rather than soaking up information passively."

*Jean Piaget* (1896-1980)

## Abstract

### Background and aim

Children's vegetable intake is far below that recommended. Despite increased awareness of the importance of vegetable consumption for health, it remains challenging to improve children's vegetable intake. Since food preferences are central to food intake, it is important to understand how they are shaped and which factors play a role in this. So far, research on the formation of vegetable preferences has focused mainly on infants and school age children but is not elaborately investigated in toddlers/pre-schoolers. Therefore the aim of this thesis was to investigate the underlying mechanisms and modifying factors that play a role in developing 2–5-year-old children's acceptance of vegetables. Effects of different learning mechanisms, strategies, and modifying factors were explored by diverse studies, including four intervention studies in ecological settings (day-care centres and at home). In another study, we compared 10 intervention studies across Europe.

### Methods

We conducted a series of day-care and in-home interventions. Healthy toddlers and pre-school children participated in the studies. Vegetable liking was measured by relative preference, and consumption was measured by (ad libitum) intake. First, we studied the underlying mechanisms – flavour–nutrient learning, flavour–flavour learning, and repeated exposure – involved in the development toddlers' food preferences in the short and long term. Novel products like green vegetable soups and vegetable crisps were used as test products, using within-subject designs. The soups differed in energy density to test flavour–nutrient learning (n=28), and the crisps were offered with different dips to test flavour–flavour learning (n=39). Next, we investigated the efficacy of other strategies like taste modification (n=103) and choice-offering (n=70) on 2–5-year-old children's vegetable liking and intake, using between-subjects designs. Children consumed different vegetable products at home at dinnertime and therefore we used more familiar vegetables as test products. Finally, we combined the results of 10 intervention studies across Europe to explore the influence of individual child characteristics such as breastfeeding history and breastfeeding duration, age, gender, and food neophobia on 2– 6-year-old children's (n=750) actual vegetable intake.

### Results

We found a clear and persistent effect of repeatedly offering novel and/or disliked vegetables on 2–5-year-old children's *intake*. Results for *preferences* were inconsistent across the studies. We found no strong evidence that strategies such as flavour–

flavour learning, flavour–nutrient learning, diluting/hiding a vegetable were more effective in changing vegetable preference than repeated exposure alone. We observed a small positive effect of choice-offering; this strategy could possibly be effective in somewhat older children who already like vegetables, to increase their consumption volume. Factors like breastfeeding duration, vegetable liking, and food neophobia were important for children's vegetable intake. Children who were more reluctant to try novel food had lower vegetable intake and were not responsive to strategies like repeated exposure, blending, mixing, or hiding vegetables. Longer breastfeeding duration was positively associated with a higher vegetable intake by 2–6-year-old children across three European countries. Gender and age had no influence.

## Conclusions

This thesis demonstrates that repeatedly offering a novel or disliked vegetable in a trusted positive environment is highly effective in promoting toddlers' and pre-school children's vegetable intake. Repeated exposure seems to be the way to teach young children to accept novel or disliked foods. Other strategies such as flavouring, adding energy, or taste modification may be helpful in promoting young children's willingness to try and taste vegetables. Additional strategies such as choice-offering are needed to promote intake of already liked/familiar vegetables when children get older. Individual differences in child characteristics such as food neophobia, breastfeeding duration, and age play a role in shaping food preferences and therefore should get more attention in strategies to promote children's vegetable acceptance. These results can be used by parents, caregivers, and public health organizations to stimulate children's vegetable consumption to maintain a more balanced diet.

# Contents

# Chapter 1

**General introduction**

Children's vegetable intake is far below that recommended [1]. Despite increased awareness of the importance of vegetable consumption for health, it remains challenging to improve children's vegetable intake [2-5]. Therefore, it is important to understand more about the reasons why children do not eat sufficient vegetables. Since food preferences are central to food intake [6-10], it is important to understand how food preferences are shaped in children and which factors play a role in this. Once we understand the processes at work here, we can gain better insight into the development of food preferences – with a special interest in vegetable preferences. This knowledge can be used to encourage vegetable intake in children.

In this thesis, the focus is on children aged 2 to 5 years. There are several reasons for this. First, food preferences are already established early in life, remain stable, and track into adulthood [11-14]. Second, children's food preferences have been studied mainly in infants and school-age children, yet the 'overlooked' group of 2–5-year-olds is going through a unique development stage that impacts on the formation of dietary habits. In this age group, increasing autonomy plays an important role in children's development [15]. For example, toddlers are getting more mobile, exploring food by putting it into their mouth by themselves, and deciding what to eat or not to eat. On the other hand, food neophobia (i.e. fear of trying new food) occurs at the age of 1.5 to 2 years and peaks between 2 and 6 years of age. Consequently, we do not know precisely how food preferences develop in this toddlerhood, although this sensitive period in childhood is an important timeframe for shaping food preferences. In sum, it is unclear whether research on food preferences with infants and school-age children is applicable to toddlers. It seems that especially in this age group we have a window of opportunity to stimulate healthy food habits such as acquiring vegetable preferences.

Therefore, in this thesis we studied how food preferences for vegetables develop in 2–5-year-old children, thereby investigating the underlying mechanisms and modifying factors that play a role in developing food preferences. The research was carried out within the HabEat project (www.habeat.eu): an EU/FP7-funded multi-disciplinary project (psychology, epidemiology, behavioural science, nutrition, and sensory science) on understanding how infants and children develop and form food habits and on exploring strategies to change these habits within early childhood (< 5 years). The project is conducted by scientists from six European countries (Denmark, France, Greece, Portugal, the Netherlands, and the United Kingdom) and focuses mainly on the improvement of young children's vegetable acceptance.

In this introductory chapter, we provide a background to children's vegetable intake and its impact on health, how food preferences are formed, how to measure preferences in children, and which learning mechanisms play a role in establishing food preferences and intake, and describe other relevant factors involved in vegetable preference and intake. Finally, the aim and the outline of this thesis are presented.

**1**

### Children's vegetable intake and its impact on health

Trends in the four last Dutch National Food Consumption Surveys show that vegetable consumption in 1–3-year-old children has declined by more than 40% in 20 years [1, 16]. Average vegetable consumption for this age group was still 70 grams per day in 1987/1988, whereas in 2005/2006 the amount had declined to 38 grams. The Dutch recommendation for children is 50–100 grams of vegetables per day for 1–3-year-old children and 100–150 grams of vegetables per day for 4–8-year-old children. Only a small proportion of Dutch children eat sufficient vegetables: just one in five 2–3-year-old children meets the recommended vegetable intake and almost none of the 4–6-year-old age group do so [1].

Vegetables are rich sources of micronutrients, minerals, and bioactive compounds such as fibres, vitamins, iron, and anti-oxidants [17-19]. There is evidence that a high consumption of fruit and vegetables may prevent several diseases (e.g. diabetes, obesity, cardiovascular diseases, and some types of cancer) [2-5, 20-24]. Green leafy vegetables like spinach and brassica vegetables like cabbage and broccoli contain anti-oxidants and minerals rich in e.g. iron, and thus play an important role for optimal growth and development in children. Iron deficiency is the most common nutritional deficiency in children. It affects 9% of American children aged 1–3 years and ranges from 10% in the Netherlands up to 50% in Austria, Finland, and the United Kingdom [25-27]. Iron is needed for the cognitive process and motor development; a shortage can lead to impairments in concentration and learning capabilities in young children.

Another advantage of consuming vegetables is that they are low in energy. Diets that are high in vegetables tend to be lower in fat and vice versa; therefore, increasing vegetable intake may lower fat intake [28, 29]. The diets of young children in many European countries are unbalanced, in particular because they contain too many lipids, too many simple carbohydrates, and not enough fruit and vegetables [28, 30, 31]. These unbalanced dietary patterns in young children have been associated with a high prevalence of childhood obesity in developed countries [8, 32]. The worldwide prevalence of obesity has nearly doubled in the last three decades [33]. In the Netherlands, around 15% of children between the ages of 2 and 21 years are overweight, of which 2% tend towards obesity [34]. Having a tendency towards obesity young in life has been shown to be an important predictor of enduring obesity later in life [35-37]. Childhood obesity is one of the biggest challenges facing public health now and in future.

### Development of food preferences in children

It is important to understand how food preferences are formed in childhood since preference is one of the most important predictors of intake [6-10]. Food preferences are already formed early in life. Even during pregnancy, flavours from the mother's diet

transmit to the amniotic fluid and can affect an infant's early flavour experience [9, 38-42]. Children are born with a predisposition to prefer sweet tastes and to avoid bitter and sour tastes such as green leafy vegetables and unripe fruit. These innate preferences may have an evolutionary origin, as poisonous substances are often bitter or sour but almost never sweet. Within the first year, infants develop a preference for salt taste [8].

Food selection is not guided by this innate preference alone; children develop preferences over time and learn via experiences with food and eating [43]. Infants and toddlers are learning what, how much, when, and how to eat, and food likes and dislikes are formed as early as the age of 2–3 years [44, 45]. The first years of life play an important role in the process of shaping these preferences. Consequently, this transitional preschool period may be an effective stage for shaping healthy eating habits.

Food preferences are not based only on taste; other sensory properties such as colour, appearance, and texture are important determinants of food acceptance among children. In general, children dislike vegetables. One possible reason is their appearance and bitter taste [8, 46, 47]. Vegetables are low in calories and therefore lack the reinforcement of post-ingestive consequences. Additionally, the green colour, the structure, the texture, and mouth feeling of vegetables are often not appealing for children [47-49].

## Measuring preference/liking and intake

As already stated, food preference is seen as one of the most important predictors for food intake [10, 50-52]. Preference and liking are different constructs that are both used in relation to food intake. Preference refers to the selection of one food item over another, whereas liking refers to a hedonic evaluation of a food and is often graded on a hedonic scale (e.g. 9-point Likert scale). Preference/liking is used in relation to intake or consumption of a certain food. The more one likes a food, the more one will eat it. Consumption is often expressed as the amount of food eaten, and intake is often measured in quantities, frequencies, or grams.

Sensory properties of foods are important determinants of their acceptance among adult consumers, and this holds true for children as well. Sensory tests such as paired-comparison, ranking, and discrimination tests are used to rate subjective pleasantness or liking of certain sensory characteristics of food using hedonic scales or visual analogue scales to evaluate liking for test foods. Measuring food preferences in children is different from measuring preferences in adults. Measuring liking in young children is challenging because of their limited concentration span and cognitive capabilities, and especially in infants (0–1 year old) and toddlers (1–3 years old) because of their limited verbal skills. Preschool children (3–5 years old) start to develop early language skills, observe facial expressions, and respond to questions and pictures. Children over the age of 2 years can reliably perform a paired-preference test, whereas more complex tasks such as hedonic

scaling are more suitable for children older than 4 years [53]. Children of 4 years of age have difficulty ranking different solutions in a discrimination test but are able to prefer one item over another, whereas 5-year-olds succeed in performing both types of tests (i.e. discrimination and preference test) [54]. Children aged 4–5 years focus more on the appearance and texture of the product and can tell whether they like or dislike its taste, but it is hard for them to recognize the specific taste [47].

Currently, preference measured by a paired-preference test is the most suitable test for measuring vegetable liking in 2–4-year-old children. In this thesis, our main outcome measures are preferences and intake. We use the term 'preference' for liking, and consumption is measured as actual vegetable intake (in grams).

## Learning mechanisms in relation to food preferences

It is important to understand how children acquire food preferences. Different learning mechanisms such as conditioning, exposure, imitation, and social interactions in particular within the family are known to be involved in food preference forming [8]. Repeated exposure, flavour–flavour learning, and flavour–nutrient learning are potential learning mechanisms, among others, used to acquire/learn acceptance of unknown or disliked tastes. However, these mechanisms have not been studied extensively in toddlers. Therefore, these mechanisms are a central focus of this thesis.

### Repeated exposure

Repeated exposure is effective in creating acceptance of a novel food as it permits learned safety to be reached by the repeated offering of small amounts of food [55]. Kalat and Rozin's (1971) learned safety theory suggests that a unique learning process is responsible for flavour-aversion learning. For example, animals experience ingestional neophobia when a novel food is given. If illness does not follow the ingestion of the new food, the animal learns that the novel food is safe. Therefore, learned safety counters the natural reluctance to consume new foods. By repeatedly offering a child an unfamiliar taste or food, we can teach the child to accept it when a positive change appears in the child's appreciation of that food by experiences of learned safety. Repeated exposure has been studied as a strategy to teach children to accept novel tastes including vegetables. Although results have shown that repeated exposure leads to increased acceptance of food, these experiments were not always related to vegetable tastes [8, 10, 39, 56-60].

### Flavour–nutrient and flavour–flavour learning

In Pavlovian conditioning, a neutral stimulus (NS) or an unconditioned stimulus (UCS) is associated with a conditioned stimulus (CS). After conditioning, the CS has become associated with the UCS or the NS to create a new conditioned response (CR). This conditioning or associative learning seems to be a key mechanism by which

food preferences are established. For example, the flavour of a food product (UCS) is associated with a familiar taste (CS) (i.e. flavour–flavour conditioning), or with the post-ingestive consequences (CS) (i.e. flavour–nutrient learning), or with the atmosphere of eating (i.e. social learning) [7, 50, 61].

Flavour–nutrient learning is assumed to be an important mechanism that predisposes children to prefer energy-dense foods. It is based on associations between a flavour and an effect of ingestion: the experience of a flavour followed by a positive post-ingestive experience, for example providing nutrients (fats or carbohydrates) leads to satiation [62-64]. Flavour–nutrient learning has been convincingly demonstrated in animal studies [65, 66]. In humans, both adults and children, the evidence for flavour–nutrient learning as an effective learning mechanism is less conclusive [63, 67-71].

One of the conditions for changes in liking induced by positive post-ingestive consequences is the extent to which the person is hungry [64]. Furthermore, it has been proposed that learning occurs during a critical developmental window, and that stimuli used in food preference learning studies need to be sufficiently novel and neutral [72, 73]. Young children still have to learn most aspects of normal dietary behaviour and how satiating various foods are meant to be. Hence, flavour–nutrient learning may be an effective learning mechanism at this young age. So far, in studies testing flavour–nutrient learning conducted with children, products such as unfamiliar drinks and yogurts with carbohydrates or dietary fats as energy source were used in experiments with 4–8-year-old children. It is not clear whether flavour–nutrient learning can be effective as a strategy to increase vegetable liking or/and intake [68]. To our best knowledge, its long-term effects – more than within a few weeks following the experiment – have not yet been studied.

The concept of flavour–flavour learning depends on the association between a novel flavour and a familiar or already liked flavour, resulting in a positive shift in preference for the initially neutral or disliked flavour [7, 50, 61]. One of the advantages of using flavour–flavour learning over flavour–nutrient learning is that it does not require substantial ingestion of vegetables, only tasting or sipping is necessary. Whereas in flavour–nutrient learning a feeling of satiation has to occur to make the mechanism effective, in flavour–flavour learning ingestion of small amounts of the product is enough. Havermans reported a significant increase in vegetable liking specifically for a vegetable taste paired with the sweet taste of dextrose in school-age children, thereby facilitating the acceptance of a specific vegetable within a short period of time [74]. Capaldi also showed that adding sucrose to a fruit or a vegetable decreased dislike for fruits in children and vegetables in adults [75]. Both studies reported on liking scores, and the question remains as to whether the observed positive shift in liking indeed actually leads to higher vegetable intake. It also remains unclear whether these positive shifts in preferences are stable over a longer period of time.

In summary, the above-mentioned learning mechanisms are known to be involved in shaping children's food preferences, but less understood in relation to young children's vegetable preferences and intake. Studies so far have focused mainly on infants and school-age children, despite the fact that toddlerhood is seen as an important transition period for the development of food experiences. The impact of these learning mechanisms has only been examined in the short term, within a few weeks following an experiment, and this does not provide a real understanding as to the long-term impact (e.g. within months) of these mechanisms on food preference development.

Some of the studies performed in this thesis required the use of special products. When testing the mechanism of flavour–nutrient learning and flavour–flavour learning, the target vegetables had to be neutral or disliked and relatively novel, but suitable for processing, cooking, and still appealing for children. Some vegetable products were developed especially for this study after consultation with a Dutch chef (Pierre Wind) specialized in working with school-aged children.

## Strategies in relation to food preferences

As already stated, it is during toddlerhood that a child learns what, how much, and when to eat, and it is in this time period that children have to learn to appreciate and eat a variety of initially disliked vegetables. The strategies already discussed – repeated exposure, flavour–flavour and flavour–nutrient learning – are known to be involved in the acceptance of novel foods including vegetables. However, not many other strategies that can help to stimulate vegetable intake have yet been investigated in toddlers/pre-schoolers. Therefore, we look more closely at two other strategies to enhance young children's vegetable liking and intake: taste modification and choice offering.

### Modifying flavours

Adjusting the taste or the texture of a product is another way to modify children's food preferences. Techniques such as blending, mixing, diluting, or hiding vegetables are known to be involved in enhancing and stimulating children's vegetable liking and intake [32, 76-78]. The idea here is that the relative strong or bitter disliked taste of a vegetable is modified by using one of these techniques, which in turn can make it easier for the child to accept the initially disliked vegetable taste. A few studies have investigated whether incorporating vegetables in meals not in their original form but in ways of which children are unaware (e.g. blending/mixing in sauces, soups, or by hiding the vegetables in other food items) actually increases vegetable liking and intake. Mothers have mentioned in interviews that this is a popular method for trying to encourage children to eat vegetables; however, liking and/or intake were not actually measured here [76]. Spill showed that incorporation of pureed vegetables into other foods seems to be an effective strategy to increase children's vegetable intake and decrease their energy

intake [78]. Intake was measured in this study, but it remains unclear whether using this technique can modify food preferences. As young children have to learn to appreciate and eat a variety of initially disliked vegetables, it is of interest to investigate whether strategies such as hiding, mixing, or blending can contribute to stimulating vegetable liking and intake.

### Choice-offering

Another example of a strategy to stimulate food intake is choice offering. Toddlers and preschool children are in the early stages of experiences with adults' foods and developing their own likes and dislikes. This transition preschool period in which the child develops his/her own likes and dislikes presents an opportunity to explore food choices. Offering a choice leads to feelings of autonomy and a sense of personal control, and this in turn increases intrinsic motivation, according to self-determination theory [79-81]. More intrinsic motivation has been related to psychological and behavioural benefits, such as healthy eating behaviour and physical exercise [82-85]. Little research has been done on this topic in relation to vegetables, and it remains unclear whether choice-offering works for young children's vegetable intake [86].

## Individual variations/differences in relation to food preferences

There are several characteristics and traits for which children show inter-individual variation and differences that may affect food preferences differentially. In addition, some factors in children's environments and/or feeding history may play a role. Factors addressed in this thesis are the role of breastfeeding history, age and gender, parents' social and educational status, and child eating characteristics such as neophobia.

Food preferences have been shown to occur through pre-natal experience and breastfeeding [87-89]. Breastfed children are more likely than formula-fed children to accept novel tastes/foods – including vegetables – later in life [9, 41, 59, 90-93], but findings are inconsistent across studies [9, 32, 41, 59, 90-93]. It is therefore important to investigate whether breastfeeding has indeed the potential to influence children's vegetable preferences later in life (chapter 6).

As children grow older, they become more reluctant to try novel tastes/foods (i.e. food neophobia), a response seen in most children. Food neophobia develops around 1.5–2 years of age, rises dramatically around 2 years of age, and decreases gradually [94]. Food neophobia  is associated with food intake, with neophobic children trying and liking fewer foods. Although food neophobia may occur for all types of food, some studies have suggested that it is highest for vegetables [95, 96]. Consequently, this individual trait can influence the forming of food preferences in this time period when young children have to learn to appreciate and eat a variety of initially disliked vegetables. It remains unclear

whether it is possible, and what strategy is likely, to increase neophobic children's liking for, and intake of, vegetables.

Although preference is seen as one of the most important predictors for food intake, other factors play a major role in intake and food choice [50, 52]. Demographic factors such as age and gender are also known to be related to eating habits. The most important determinants of children's vegetable intake are gender, age, socio-economic position, preferences, parental intake, and home availability/accessibility [14, 32, 52]. Girls tend to have a higher or more frequent intake than boys; and age, socio-economic status, preferences, parental intake, and home availability/accessibility are all positively related to intake.

These individual differences can affect the processes involved in shaping food preferences. Therefore, we investigate the influence of the demographic variables gender and age, breastfeeding duration, and food neophobia on children's vegetable preference and intake, in order to have a better insight into whether the described mechanisms involved in forming food preferences are equally effective across children (chapters 4, 5, and 6).

## Rationale and thesis outline

Although studies have reported on strategies which can help to improve children's vegetable liking and intake, outcomes are not always conclusive, and the experiments are often performed on infants and school-age children. The underlying mechanisms involved in vegetable preferences are not elaborately investigated in toddlers. Learning mechanisms such as flavour–flavour learning (FFL) and flavour–nutrient learning (FNL) in relation to children's vegetable preference and intake have not been tested extensively, and it is even harder to investigate FNL in children. Few studies have investigated the relative effectiveness of the different learning strategies in promoting vegetable intake in toddlers/pre-schoolers. This knowledge could be useful to better target interventions aiming to modify food preferences. Since preferences are already formed early in life and may result from experiences with various nutrients during a sensitive period from an early age and can track into adulthood, it is important to understand the underlying mechanisms playing a role in young children's vegetable preference and acceptance. So far, little research on the formation of preferences for vegetables has been performed on toddlers (2–3-year-olds).

Furthermore, the impact of these learning strategies has only been examined within a few weeks following the experiment, and this does not provide a real understanding as to the long-term impact of these strategies on food preference development. In this thesis, we study the underlying learning mechanisms and modifying factors to gain a better understanding of toddlers/pre-schoolers vegetable preference and intake (chapters 2, 3, 4, 5, and 6). The experiments are conducted in ecologic conditions, i.e. at meal times in an environment where children usually have their meals, and with real foods.

Therefore, the following specific objectives were formulated:

- To assess the efficacy of different strategies like repeated exposure and flavour–nutrient and flavour–flavour learning on young children's vegetable preference and intake in the short and long term.
- To examine whether the use of different taste modifications like masking, diluting, or hiding a vegetable can improve young children's vegetable preference and intake.
- To study the effect of offering a choice to children on increasing vegetable intake.
- To explore the influence of modifying factors such as age, gender, breastfeeding duration, and food neophobia on children's vegetable intake.

Figure 1.1 gives an overview of the empirical chapters in this thesis and shows the learning mechanisms and modifying factors involved in children's vegetable preferences as studied in this thesis.
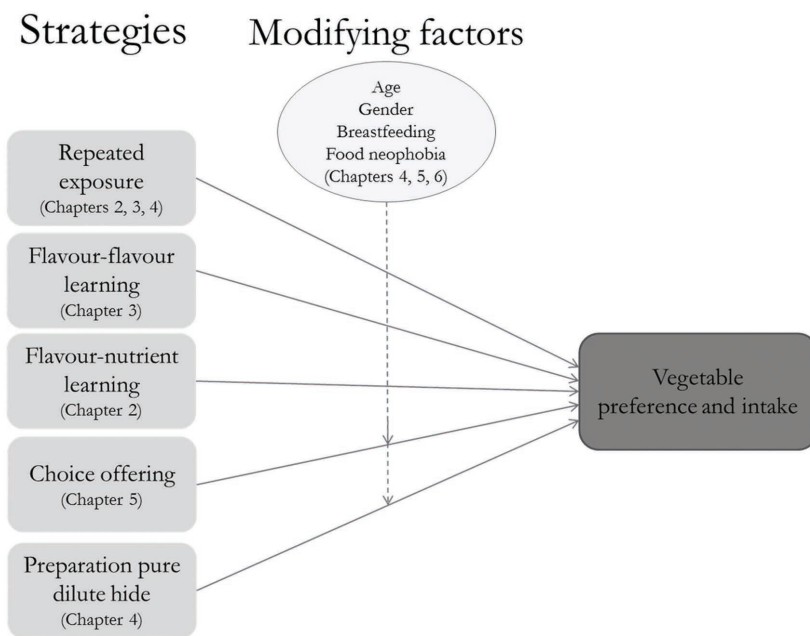


**Figure 1.1** Schematic overview of the learning mechanisms and modifying factors involved in children's vegetable liking and intake as described in this thesis.

Chapter 2 describes the effect of flavour–nutrient learning and repeated exposure as mechanisms to increase toddlers' preference for, and intake of, green vegetables. The efficacy of repeated exposure and flavour–flavour learning as strategies to increase toddlers' vegetable preference and intake are compared in a study as described in chapter 3. Chapter 4 describes a study designed to evaluate the effect of offering vegetables in

different taste sensations (i.e. pure, dilute, hide) on its acceptance by toddlers. Chapter 5 investigates the influence of the strategy of offering a choice of vegetables. The relation between breastfeeding and later vegetable intake in 2–6-year-old children is described in chapter 6. Here, the results of 10 intervention studies performed under the EU research programme HabEat across Europe are investigated and discussed. Finally, chapter 7 discusses the main outcomes and the methodological considerations of this thesis, and implications including recommendations for further research are presented.

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# Chapter 2

**Effectiveness of flavour nutrient learning and mere exposure as mechanisms to increase toddler's intake and preference for green vegetables**

Promoting children's intake of vegetables

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## Abstract

Children's consumption of vegetables is still below recommendations. Since preference is the most important predictor of children's intake and most children dislike vegetables, new strategies are needed to increase their preferences for vegetables. Flavour nutrient learning (FNL) could be an effective mechanism to change preferences. Forty healthy toddlers were included in a randomized intervention study. During an intervention period of 7 weeks, they consumed vegetable soups (endive and spinach) twice per week. Half of the group received a high-energy variant of one soup (e.g. HE spinach) and a low energy variant of the other (LE endive), whereas for the other half the order was reversed (HE endive, LE spinach). Primary outcome measures were preference and ad libitum consumption (with a maximum of 200 g) of both vegetable products (LE), measured before, shortly after the intervention period, and 2 and 6 months following conditioning to assess longer-term effects. After completion of the intervention period, 28 children (14 girls and 14 boys, age 35 months; SD ± 8.3) met criteria for FNL to occur, and were included in further data analysis. Results showed a significant increase (~58 g) in ad libitum intake for both vegetable soups (stable over time), but irrespective of the energy content. This indicates a robust effect of mere exposure on intake, but no FNL. For preference, however, results showed a significant shift in liking for the vegetable soup consistently paired with high energy, supporting FNL.

Keywords: Flavour-nutrient learning; Young children; Learning; Food preference; Healthy dietary habits.

## Introduction

Recent surveys show that the diets of young children in many European countries contain too many lipids, too many carbohydrates and not enough fruit and vegetables [1, 2]. In The Netherlands vegetable consumption in young children has declined with more than 40% in 19 years and the daily recommendation, 50-100 g/day up to the age of four, is not met [3, 4].

Studies so far have shown the importance of the early years in food experience in later food behaviour [5-9]. During the first years of life, infants and toddlers are learning what, how much, when and how to eat and different studies have shown that food likes and dislikes are formed as early as the age of 2-3 years [10, 11]. From previous interventions we see that, compared to fruits, especially promoting vegetable intake in children seems to be challenging [12-14]. One explanation is that many children do not like vegetables because of their appearance and bitter taste [8, 15, 16]. Also, vegetables are low in calories and therefore lack reinforcing post-ingestive consequences.

Multiple learning mechanisms, e.g. mere exposure, Pavlovian conditioning, evaluative learning, and social modelling, are known to be involved in food preference formation [15]. One of these learning models, flavour nutrient learning (FNL), is the focus of the present study and is assumed to be an important mechanism that predisposes children to prefer energy-dense foods [17, 18]. FNL depends on the association between a flavour and an effect of ingestion, that is, energy or nutrients that lead to satiation.

FNL has been convincingly demonstrated in animal studies [19, 20]. In humans, both adults and children, the evidence for FNL as an effective learning mechanism is less conclusive. Studies from [18], Johnson et al. (1991), Kern et al. [21] revealed findings supportive for the occurrence of FNL in 2-5-year-old children by pairing unfamiliar drinks and yogurts with carbohydrates or with dietary fats. But others have encountered difficulty to replicate these findings [22-24].

In order to explain the variability in outcomes of studies based on FNL mechanisms in humans, several factors have been proposed that appear to determine when FNL (and other types of associative learning) is likely to occur. A critical component for changes in liking induced by positive post-ingestive consequences is the extent to which the subject was hungry [25]. Furthermore, it has been proposed that learning occurs during a critical developmental window, and that stimuli used in food preference learning studies need to be sufficiently novel and neutral [26, 27]. Young children still have to learn most aspects of normal dietary behaviour and how satiating various foods are meant to be. Hence, changes are favourable for FNL to be an effective learning mechanism at this young age. In addition, young children are relatively insensitive to higher-level beliefs and attitudes (i.e. dietary restraint, considering certain foods (sweet, energy-dense) as

'forbidden' foods) that may reduce the ability of post-ingestive consequences to shape food preferences.

In this study we, therefore, investigated the efficacy of FNL as a key learning mechanism to improve the intake and preference of (novel) vegetables in toddlers (2-4 years). Our second aim was to establish whether potential effects of FNL on vegetable acceptance and intake were of longer-term duration, that is, does increased vegetable acceptance and intake remain stable over a 2 and 6 months follow up period. We assessed this by measuring vegetable intake and preference before and after a conditioning period using vegetable soups in two flavours and applying energy manipulation. During this conditioning period toddlers were exposed fourteen times from which seven times, a vegetable soup high in energy density (HE) was offered and seven times a vegetable soup low in energy density (LE) was offered. We expected that after the conditioning period, the vegetable flavour which was repeatedly paired with the high-energy content (experimental condition), was consumed more and preferred over the flavour that was repeatedly paired with a low-energy content (control condition). In addition, we hypothesised that this mechanism stayed stable in time (2 and 6 months after the conditioning period).

## Materials and methods

The present study is part of the HabEat project 'Determining factors and critical periods in food Habit formation and breaking in Early childhood'. The aim was to investigate the efficacy of flavour-nutrient learning (FNL) as a strategy to increase acceptance of novel vegetables. The study was approved by the medical Ethical Committee of Wageningen University (NL 34553.081.10) and registered by the Dutch Trial Registration (NTR, TC = 2793).

### Sample size

Power calculation (see below) indicated that we needed 12 children per condition (flavour A, HE; flavour B, LE and vice versa), resulting in a total of 24 children. We took into account a dropout rate of ~15% and also anticipated on the removal of data of a number of children (~15%) from data analysis, as a consequence of their refusal to eat the soups (in which case FNL cannot possibly occur). Therefore, we aimed to recruit 40 children.

Power calculation:

$$N = \frac{(Z\,alpha - Z\,beta)^2\,\sigma^2}{(\mu1 - \mu2)^2} = \frac{(1.6449 + 0.8416)^{2*}\,34^2}{25^2} = 12$$

With $\sigma$ of 34 g [4], $\mu1 - \mu2$ of 25 g (=0.75 SD) and a significance level of $p = 0.05$ one sided and a power of 80%.

## Subjects

A total of 40 healthy children aged 2–4 years was recruited from 2 day care-centres in Wageningen, The Netherlands. Participation was voluntary and parents and day care-centres were thoroughly informed about the study. Written parental consent was given for the participating children. Inclusion into the study required presence of the child at the day care-centre for at least 2 days per week. Subjects were screened for food allergies and health problems (as reported by the parents). Table 2.1 shows the main subject characteristics.

**Table 2.1** Subject characteristics.

| | Total (n=40) | Analysis (n=28) | Girls (n=14) | Boys (n=14) | SPIN-HE (n=15) | ENDI-HE (n=13) |
|---|---|---|---|---|---|---|
| *Age* (Months) | | | | | | |
| Mean (SD) | 36 (7.6) | 35 (8.3) | 37.8 (9.5) | 33 (6.7) | 33 (8.1) | 37 (8.7) |
| Range | 21 - 46 | 21 - 46 | 21 - 46 | 22 - 43 | 21 – 46 | 22 - 44 |
| | | | | | | |
| *Weight* (kg) | | | | | | |
| Mean (SD) | 14.9 (2.4) | 14.8 (2.7) | 14.7 (3.2) | 14.8 (2.3) | 14.0 (2.4) | 15.5 (2.9) |
| Range | 10.0 – 19.0 | 10.0 -19.0 | 10.0 -18.5 | 11.0 -19.0 | 10.0 -18.5 | 11.0-19.0 |
| | | | | | | |
| *Height* (cm) | | | | | | |
| Mean (SD) | 96.1 (7.4) | 95.2 (8.1) | 96.2 (9.4) | 94.1 (6.8) | 91.5 (6.3) | 98.7 (8.3) |
| Range | 79.0 – 111.5 | 79.0 -111.5 | 79.0 -111.5 | 83.0-108.0 | 79.0 – 100.0 | 83.0-111.5 |

## Study design

In this crossover intervention, study subjects were randomly assigned to one out of two conditions using vegetable soups that differed in flavour and energy content as test products. During the intervention period, half of the participants (n = 20) received vegetable soup flavour A low in energy content (LE) consistently paired with vegetable soup flavour B high in energy content (HE), whereas the other half of the participants received the reverse (i.e. flavour A HE + flavour B LE). The intervention took place at

2-day care-centres in Wageningen, The Netherlands. The study consisted of five parts: a pre-test, a conditioning period, a post-test following the conditioning period, and 2 and 6 months follow-up. The total length of the study was 8 months (see Figure 2.1).

Children's preference and intake of both vegetable products were measured before the conditioning trials started ($T = 0$). Here, we offered the low energy variant of the vegetable products. During the conditioning trials (14 exposures, twice a week over a period of 7 weeks), children were offered repeatedly a fixed amount (125 g) of vegetable product that differed in flavour and energy content. Per subject, one flavour was consequently paired with a high energy (experimental condition) content (malto-dextrin and sunflower oil added) whereas the other flavour was consequently paired with a low energy (control condition) content (no malto-dextrin and sunflower oil added).
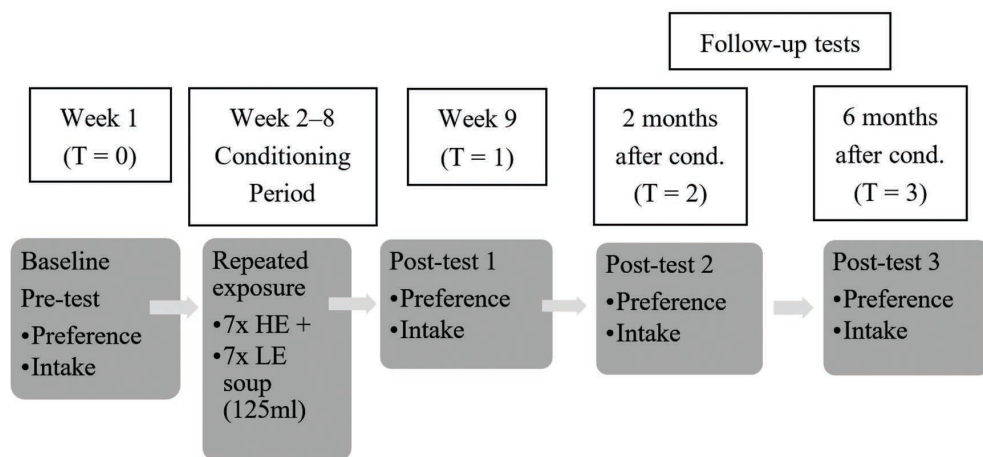


**Figure 2.1** Schematic outline of the study in time. A detailed description of pre- and post-tests, the conditioning period, and measurements of intake and preference, can be found in the text of the Methods section.

The vegetable products were consumed just before lunchtime as an entrée, followed by the regular lunch. Directly after the conditioning period, the first post-test was conducted. Here, children's preference and intake of both vegetable products (the low energy variants) were measured again ($T = 1$). Additionally, two follow-up tests, at 2 months ($T = 2$) and 6 months after the conditioning period ($T = 3$), were done to assess longer-term effects, again with the low energy products. The children were not aware that intake was measured, nor which vegetable flavour had been coupled to a high or low energy content during the intervention period. They were told to consume as much as they liked. After the study the parents were debriefed. Used products, procedures and measurements are described in detail below.

## Products used

The vegetable soups were developed especially for this study after consultation of a Dutch chef (Pierre Wind) specialised in working with school-aged children. The recipes were developed to meet the criteria for successful FNL to be able to occur [25]. First, target vegetables had to be neutral or disliked and relatively novel. We chose two vegetables, endive and spinach, that are typical green leafy, bitter, and hence, unpopular vegetables in young children. Also, endive and spinach are not present in the top five of the list of most commonly consumed vegetables in Dutch children at the age 2-4 years [4, 28]. The form in which the vegetables were presented, i.e. soups, added to the unfamiliarity as Dutch children are not used to eating green pure vegetable soups. Second, the FNL variants should have equal sensory characteristics, but different energy densities, preferable 100 kcal/100 g energy difference between LE and HE variant. The low energy variant should contain ideally as little kcal as possible. The endive and spinach soup recipes consisted primarily of pure vegetables (35%). Sunflower oil and malto-dextrin (Fantomalt, Nutricia) were added to increase the energy density of the HE variants. Details of the composition are described in Appendix A. The recipes resulted in two energy densities per flavour: low (LE: 19 kcal/125 g) and high (HE: 138 kcal/125 g), with an energy difference of 119 kcal between the HE and LE vegetable soup.

## Preparation

Fresh vegetables were used to prepare the soups. The soups were produced according to the Good Manufacturing Practices (GMPs) guidelines. To get a smooth texture the soups were blended in a Hamilton blender (model UBB 250 S, Beach Brands INC., NC USA). The soups were stored cold (±4 °C) and warmed to 85 °C before serving to the toddlers.

## Procedure

Prior to the start of the study, the participating day care centres and their staff were thoroughly informed on the aim of the study and instructed on the procedures to be followed during the intervention stage. Children were stimulated to eat as much as they liked from the vegetable soups served for that day. The children and the day care leaders were blinded to the treatment that is they were unaware which product was high or low in energy. The products were served in small soup bowls. All children received a placemat and a spoon. Test sessions were held 10-15 min before lunch time at the same days of the week and were part of the normal lunch routine at the day care centre. Children were seated at tables in small groups (5- 10 children) together with their day care leaders. After serving the soups, the researchers left the room, to minimise interference with the normal lunch routine and facilitate that children were

at ease and display their normal eating behaviour as much as possible. Children that were not included in the study but present at the day care during the conditioning days were offered a commercial available green vegetable soup (Knorr). The day care leaders received randomly one of the soups as offered to the subjects. See Figure 1 for a schematic overview of the experiment.

## Baseline measurements

### Preference

Preference during baseline was assessed using a paired preference test. Each child was taken apart and was invited to taste the two target vegetable products (LE variants presented in randomized order across participants in small cups of 20 ml). Children were asked by the researcher to indicate the product/flavour they preferred. If unable or unwilling to make a choice, children were encouraged by the researcher by asking the children the following two questions: "Which soup would you like to finish?" and "Which soup would you give to your friend?" If this did not result in a choice, the child's preference was scored as 'neutral'.

### Intake

After the preference test was conducted, ad libitum intake was measured, by offering 200 g of one of the two target vegetable products (A or B; LE versions) as a starter, 10 – 15 min before regular lunch. The children were invited to eat as much as they liked. The setting during lunchtime was the same as described above. At a different day in the same week ad libitum intake of the other flavour (A or B, depending on what the child was presented with on the previous day) was measured.

## Conditioning period

During a 7-week period of repeated exposure, the subjects consumed LE and HE soups alternately on two weekdays. The order of the LE and HE soups was semi-randomized within subjects and with the restriction that subjects did not receive the HE or LE soup more than twice in a row. Intake of the soups was closely monitored for each child individually by weighing the soup bowls before and after lunch. Children were assigned to one out of two conditions: either spinach HE/endive LE; n = 20, or endive HE/spinach LE; n = 20 and received only that combination of flavour energy pairing throughout the whole conditioning period.

## Post-test

One week following the conditioning period we repeated preference and intake measurements conform the procedure as described for the baseline measurements.

## Follow up tests

In order to test the sustainability of potential FNL effects, two follow-up measurements were included at 2 and 6 months respectively after completion of the conditioning period. Follow-up measurements involved preference and intake measurements of the LE soups as described under baseline session.

## Sensory evaluations

A separate panel of 20 adults (mean age 22.1 (SD 1.8), 18 female and 2 male) was invited to the sensory lab to perform sensory profiling of the target samples. Soups were rated on the following attributes: aroma, sweetness, saltiness, bitterness, sourness, creaminess, thickness, satiation and taste intensity. A 100-mm visual analogue scale (VAS-Scale) with labelled ends ranging from "not at all" (0 mm) to "very" (100 mm) was used to rate the intensity of each attribute, using FIZZ software (Biosystemes, Couternon, France). The soups were served in a randomized order. Sensory profiling revealed a difference between the HE and LE variants of the soups in "sweetness" ($F(1,47) = 32.15$; P <0.0001); "bitterness"($F (1,47) = 5.92$; P = 0.019) and "taste intensity" ($F(1,47) = 5.47$; P <0.024). As expected, HE soups were perceived as sweeter, less bitter, and stronger in taste intensity than the LE soups. All other attributes (aroma, sourness, saltiness, creaminess, viscosity, and satiation) did not differ between HE and LE soups.

## Measurements

The main outcomes of the study were ad libitum soup LE intake (with a maximum of 200 g) across repeated exposures and preference ratings. Consumption was measured by pre– and post-weighing on a digital scale with a precision of 0.1 g (model S-4001, Denver Instruments, NY, USA). Consumption was used as an indicator of preference. In addition, preference ratings were obtained by performing two paired preference tests at baseline and during post-tests. During the intervention period, apart from soup consumption we also registered intake during regular lunch (i.e. number of sandwiches consumed), as an indicator of children's responsiveness to the caloric density manipulation via the soups. Food neophobia scores were assessed with a child and parents food neophobia questionnaire [29, 30].

## Statistics

Data are presented as mean values with standard deviation unless otherwise specified. The statistical program PAWS Statistics was used (version 18; SPSS Inc. Chicago, USA) to perform a GLM (General Linear Model) analysis and Chi-square tests of the following hypotheses:

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Hypothesis 1: to investigate an effect of FNL on intake we tested the following main effects: exposure (baseline versus post-tests), energy conditioning (energy+ and energy-), and their interactions in a 2-factor ANOVA model (GLM for repeated measures).

Hypothesis 2: to investigate an effect of FNL on preference we used the chi-square test to test for shifts in preference over time (baseline versus post-tests). Tests were performed 2-sided, and P values <0.05 were considered significant.

## Results

Of the 40 children eligible to participate, 12 were excluded from data analysis due to low intake levels during the conditioning period. For FNL to occur, sufficient intake of the products is required. Based on previous studies on FNL with children [21, 31], we set our 'threshold' for successful conditioning at an intake level of minimally 70% of the volume offered per serving (i.e. 70% of 125 g = 87.5 g) for at least 10 out of 14 exposures. Hence, all data reported are based on the sample of 28 children (14 girls and 14 boys) that met the criteria for successful conditioning (see Table 2.1 for subject characteristics and distribution across conditions (n = 15 in the SPIN-HE/ENDI-LE condition and n = 13 in the ENDI-HE/SPIN-LE condition). Of these 28 children, 19 took part in the 2 months follow-up and 17 in the 6 months follow-up. The most important reason for drop-out during the follow-up measurements was that children reached the age of four and left the day care-centre. Under the Dutch school system, children leave day care and enter primary school, soon after their fourth birthday.
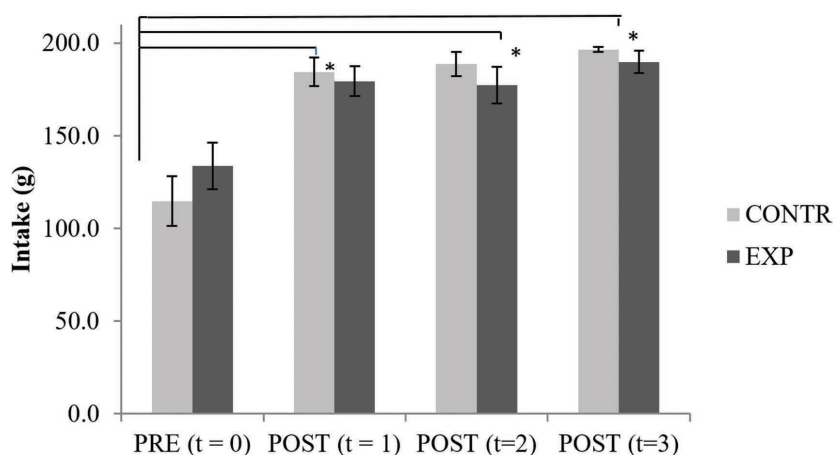
### Intake

*Intake-baseline versus T1*

GLM repeated measures analysis with exposure (baseline, $T = 1$) and condition (experimental, control) as within subjects variable, revealed a significant main effect of exposure ($F(1, 27) = 21.0$, $p < 0.0001$) on ad libitum intake. No main effect of energy was found, nor an interaction effect. This indicated that after the conditioning period intake of the vegetable soups was significantly increased, but this was regardless of energy density (see Figure 2.2). Average intake increased with 69.6 g (SD 88.1) in the control condition (i.e. low energy soup previously paired with no added energy) and with 45.7 g (SD 72.5) in the experimental condition (i.e. low energy soups previously paired with high energy), at T1 (1 week after the conditioning period).

*Compensation: number of sandwiches following soup consumption*

As an indication of children's responsiveness to the caloric density manipulation, we tested whether FNL affected the consumption during regular lunch (number of

sandwiches) after the children finished the soups. Figure 2.3 depicts the average number of sandwiches (±SEM) eaten after serving of the high-energy soup (HE) versus the low energy soup (LE) per week during the conditioning period (week 2 – 8 of the study). GLM repeated measures showed a significant main effect of energy ($F(1, 22) = 23.2$, $P < 0.0001$) on sandwich intake. On average, children ate less sandwiches (-0.3 (± 0.3 SD)) after having consumed the high energy soup. This reduction in sandwich intake corresponds to approx. 60 kcal, which is half of the energy difference between the low and the high energy content soup.



**Figure 2.2** Ad libitum intake (mean ± SEM) of soup previously paired with low energy (control condition, CONTR; no energy added) versus soup previously paired with high energy (experimental condition, EXP; added energy), at baseline (t = 0), after conditioning (t = 1), and two (t = 2) and six months (t = 3) following the end of the conditioning period. * P < 0.05.
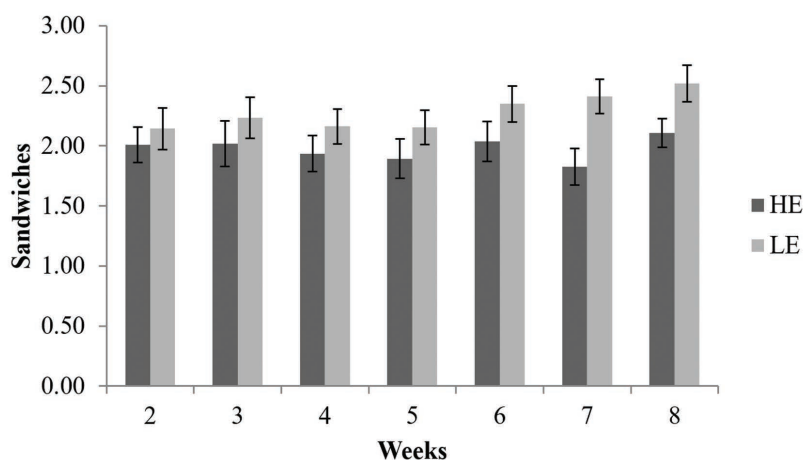


**Figure 2.3** Average number of sandwiches (+/- SEM) eaten after serving of the high-energy soup (HE) versus the low energy soup (LE) per week during the conditioning period (week 2 – 8 of the study). There is a significant main effect of energy (p < 0.0001), indicating that after the HE soup children ate on average less sandwiches than after the LE soup.

*Follow-up tests: Intake-baseline versus T2 and baseline versus T3*

To test sustainability, intake at baseline ($T = 0$) was compared with intake 2 months ($T = 2$) and 6 months ($T = 3$) after the conditioning period. GLM repeated measures analysis with exposure (baseline, $T = 2$) and condition (experimental, control) as within subjects variable, revealed a significant main effect of exposure ($F(1, 18) = 32.1$, $p < 0.0001$) on intake 2 months after the conditioning period, indicating that the initial increase in intake after conditioning had remained stable. This was also confirmed for the 6 months follow-up measurement, as GLM repeated measures analysis with exposure (baseline, $T = 3$) and condition (experimental, control) as within subjects variable, yielded again a significant main effect of exposure ($F(1, 16) = 26.3$, $p<0.0001$) on intake 6 months after the conditioning period (see Figure 2.2). Taken together, the intake of vegetable soup remained stable in time; respectively in the control condition 188 ± 35 g; 197 ± 6 g and in the experimental condition respectively 177 ± 52 g; 190 ± 25 g.

## Preference

*Preference – baseline versus T1*
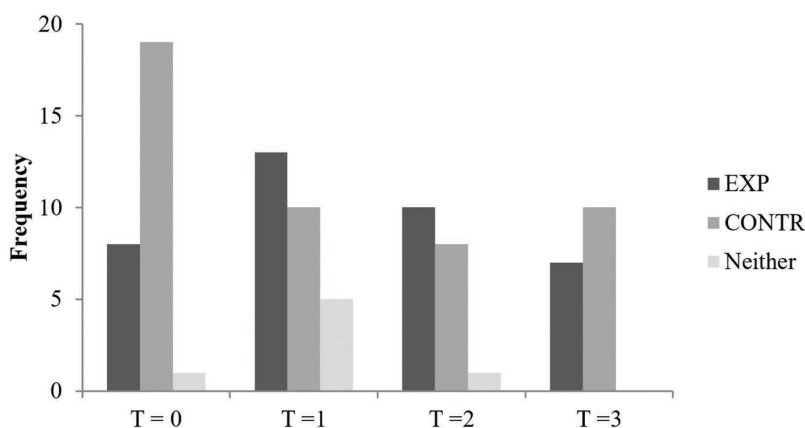


**Figure 2.4** Results of preference test at baseline (t = 0), after conditioning (t = 1), two months after conditioning (t = 2) and six months after conditioning (t = 3). On the Y-axis the number of participants that preferred one flavour above the other. In case a child was unable or unwilling to make a choice, it scored 'neutral'. The labels 'control (CONTR) vs. experimental (EXP)' indicate the soup flavour (either endive or spinach) that was coupled to low (no energy added) vs. high energy (energy added) during the conditioning period.

The distribution of preference for one vegetable flavour versus the other was compared between baseline and the first post-test (1 week after the conditioning period). A chi-squared test showed a significant shift in preference ($\chi^2 (2) = 6.7$; $P = 0.036$) in the expected direction, that is, a shift in preference towards the vegetable flavour that during

conditioning had been consistently coupled with high energy density (see Figure 2.4). Figure 2.4 showed us the preference for the flavour (measured with low energy variants of both soups) which was repeatedly paired with high energy (experimental condition) versus the flavour, which was paired with the low energy (no added energy; control condition) during the conditioning period.

### Follow-up tests

*Preference-baseline versus T2 and baseline versus T3*

To test sustainability, the distribution of preference for one vegetable flavour versus the other was compared over time (baseline, 2 months since the conditioning period, and after 6 months respectively). Chi squared tests showed a marginally significant effect across time ($\chi^2$ (6) = 11.1; P = 0.085), see Figure 2.4. Post hoc testing in which we compared baseline with T2, baseline with T3, and T2 en T3 showed that this effect largely dependent on the shift in preference from baseline to T1, but disappeared after time and lacked significance during the follow-up measurements (all p > 0.20).

In summary, whereas results for intake were negative, the preference data were tentatively supportive for FNL, in particular on the short-term (i.e. immediately after the conditioning period).

## Discussion

The objective of this study was to investigate whether flavour nutrient learning (FNL) can be an effective mechanism to increase young children's intake of and appreciation for novel vegetable products. As outcome parameters for FNL, we measured both intake and preference. We observed that intake increased significantly after a conditioning period, but this was independent from energy condition. This is indicative for a robust mere exposure effect but does not support the occurrence of FNL. The increase in intake remained stable in time and was still present at 2 and 6 months after conditioning took place. With regard to preference, our findings on FNL were more promising, as our data revealed a significant shift in preference from baseline to post-conditioning where the soup flavour that was consistently paired with high energy levels, became more preferred. The effect disappeared in the follow-up measurements. Taken together, these findings reflect moderate support for FNL to play a role in the development of young children's acceptance of novel vegetables into their diets. Mere exposure, on the other hand, proved to be a powerful mechanism to promote vegetable consumption in young children, with longer-term potential.

The present findings on mere exposure effects are consistent with previous research. Several earlier studies conducted with young children have reported similar results [18, 23, 32-35]. Four recent studies reported comparable findings confirming that mere exposure is a strong mechanism that facilitates acceptance in young children of a novel vegetable taste but found no support for FNL [22, 23, 33, 36].

Nevertheless, some studies have shown that children develop increased preference for foods and drinks temporally paired with caloric density, thereby supporting the efficacy of FNL [18, 21, 31]. However, this was observed for novel-flavoured drinks (orange–chocolate and bubble gum flavour [18]), or novel-flavoured yoghurts (high-fat and low-fat [21, 31]). These products, even in low energy variants, probably have a stronger appeal to children than bitter tasting vegetables. This is relevant because for FNL to occur, sufficiently high intake levels of the test products are needed during conditioning. Previous failures to demonstrate FNL for vegetables in children could be due to rejection of the test products resulting in extremely low intake levels [23, 24]. In contrast to other studies, we do find some support for FNL as a mechanism that may contribute to young children's acceptance and liking of novel vegetables. While acknowledging that the present findings on FNL are not very robust, one can speculate about more than one learning strategy being active at a time. Hence, FNL may have an additive or even synergistic effects that strengthens the effect of mere exposure. Alternatively, sensitivity to detect an effect of FNL on intake might be compromised by the relative short interval (approx. 15 min) between serving the soups and regular lunch (sandwiches). Studies in animals have shown that FNL can even occur when the reinforcer (carbohydrate, protein or fat) is delayed with respect to the consumption of the flavour [37]. It is not known whether this can be translated to humans. If this is the case, however, then flavours of both HE and LE soups might have been associated with nutrients (regular lunch). Another explanation for the lack of an effect of FNL on intake could be the high initial intake levels in a subgroup of children. In developing the test products (vegetable soups) and the procedure, we aimed at optimising both product and serving context in order to encourage children to consume the soups. Apart from a subgroup of non-eaters/non-learners who rejected the soups almost completely (and were removed from data-analysis), there was also a subgroup of children (N = 12) that finished nearly the complete serving (200 g) at baseline and continued to eat most of the servings during conditioning and post-tests. This may have resulted in a ceiling effect for intake.

There are several strengths and weaknesses in the present study that are worthwhile to discuss. First, we consider the test products and the context as strengths of this study. Despite the fact that we served pure green vegetable soups in endive and spinach flavour, the products were well accepted by most of the children. According to Lucas and Sclafani (1999) [38] carbohydrates are more effective than fat in FNL in rats. Our HE products contained both carbohydrate (malto dextrin) and fat (sunflower oil), whereas

other studies on vegetables used only carbohydrates [24] or only fat to manipulate energy density [23]. However, adding carbohydrates may have a drawback in human studies, as sensory profiling showed that adults perceived the HE variants of the soups as sweeter and more intense tasting than the LE variants, irrespective of the fact that the HE and LE soups did not differ in ingredients except for malto dextrin and sunflower oil content. It remains to be determined whether these sensory differences in sweetness, bitterness and taste intensity was perceived in a similar way by the toddlers, and whether this may have influenced their preference for either the HE or LE energy. This cannot be judged based on the current findings, as during baseline and post-test we tested intake and preference for the low energy soups only. With regard to context, we feel that the familiar and trusted environment of the day care-centre has contributed to the willingness of the children to accept the novel vegetable soups. In addition, turning the soup eating twice a week into a special event (with attractive soup bowls and placemats) also stimulated the children to eat the soups, as well as the group setting with children seated for lunch in small groups with their regular day care leader. Studies on social facilitation show that individuals eat more in presence of others than they do when they are alone and that intake increases as the number of co-eaters increases [39]. Also, conditions for FNL to occur were favourable in terms of children being hungry when soups were served and the use of vegetable flavours (endive, spinach) that are novel to most toddlers in The Netherlands [25-27].

A second strength is the inclusion of both intake and preference as outcome measures signifying increased acceptance. With children this young, it is obviously a challenge to measure preference due to their cognitive and linguistic abilities. For this reason, researchers may refrain from preference testing and focus on intake [23]. In the present study, however, combined measurement of intake and preference resulted in more differentiated conclusion with regard to the evidence for mere exposure and/or FNL.

Finally, assessment of longer-term effects up till 6 months after the end of the conditioning period is a clear strength of the present study. Follow-up measurements suggest that at least mere exposure effects can be quite stable over time, which is obviously a promising finding in light of the development of public health strategies to increase childrens' consumption and acceptance of vegetables. The drawback of long-term assessments in this particular age-group (2-4 year old toddlers) was that we faced a drop-out of participants over time.

The current study has some limitations as well. Although we favour the combination of intake and preference as measures reflecting whether FNL takes place, the methods available for preference testing that generate reliable and replicable results in young children are limited. However, according to a review paper on sensory and consumer testing with children [40], toddlers can make decisive "yes / no" distinctions , although

they have difficulty making complex decisions and have a limited attention span. Therefore, we feel that paired preference testing, the method of choice in this study, can be considered as sufficiently valid. Related to this, sensory profiling of the target products was done by an independent sample of adults, as young children lack the cognitive, evaluative and language skills to perform this type of quantitative descriptive analysis. It is not clear to what extent sensory evaluations made by adults can be extrapolated to young children.

A possible limitation already mentioned is that the maximum serving at baseline and post-conditioning testing was 200 g of soup, which we expected to be quite a substantial serving for these young children. Against our expectations, part of the children finished the complete serving at baseline, which may have induced a ceiling effect reducing our sensitivity for detecting FNL effects on intake. Finally, after baseline preference testing and independent from the outcome, children were randomly assigned to one out of two conditions. Half of the participants received consistently flavour A in the HE version and flavour B in the LE version. For the remaining subjects, this order was reversed. The initial data (n = 40) indicated an even distribution in baseline preference for endive or spinach flavoured soup. However, after the conditioning period twelve subjects were excluded from further data analysis as they did not meet the minimal intake criteria for FNL to be able to occur. In retrospect, it turned out that randomization for the remaining 28 subjects had resulted in a skewed distribution of preference ratings for the flavours (endive and spinach) coupled to high and low energy. As can be observed in Figure 2.4 relatively few children indicated a preference during baseline for the flavour that was coupled to high energy during conditioning. We cannot exclude the possibility that this may have biased our results by enabling regression to the mean.

Taken together, the present study clearly demonstrated mere exposure and possibly FNL to be effective learning strategies to increase the intake and acceptance of novel green vegetable soups in young children. Future studies should explore the specificity of these effects, as we cannot conclude from the current results that they will transfer to other vegetables or vegetables in their pure form (i.e. cooked endive and spinach as part of dinner).

In conclusion, toddlers substantially increased their intake (~47% increase compared to baseline) of endive and spinach vegetable soups independent from energy manipulation, indicating strong support for mere exposure. With regard to preference, we found tentative support for FNL to add to the effect of mere exposure. The positive effects on intake remained stable for at least 6 months after conditioning. The findings of the present study are promising with regard to promoting vegetable intake and liking, and show that mere exposure is an efficient learning mechanism in young children and relatively easy to implement in daily life, at home or at day care-centres.

## Acknowledgements

## Appendix A

**Table A1.** Energy, ingredients of low energy (LE) and high-energy (HE) soups.

|  | Spinach soup LE | Spinach soup HE | Endive soup LE | Endive soup HE |
|---|---|---|---|---|
| *Energy* (kcal/125g) | 20 | 138 | 19 | 137 |
| *Vegetable* | 44 | 44 | 44 | 44 |
| *Water* | 75 | 53 | 75 | 53 |
| *Malto- dextrin* | 0 | 15.7 | 0 | 15.7 |
| *Sunflower oil* | 0.8 | 7.2 | 0.8 | 7.2 |
| *Rice flour* | 1.4 | 1.4 | 1.4 | 1.4 |
| *Boiled potato* | 2.9 | 2.9 | 2.9 | 2.9 |
| *Salt* | 1 | 1 | 1 | 1 |

**2**

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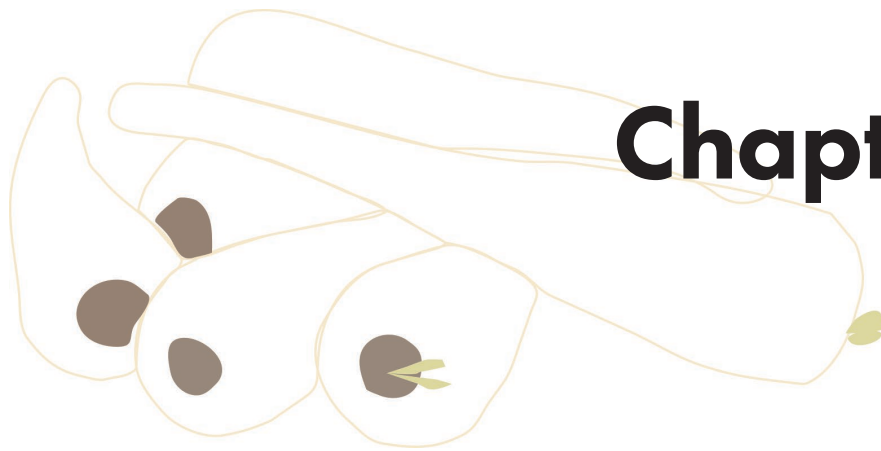
# Chapter 3

**Efficacy of repeated exposure and flavour-flavour learning as mechanisms to increase preschooler's vegetable intake and acceptance.**

Strategies to promote vegetable intake

Victoire W.T. de Wild
Cees de Graaf
Gerry Jager

## Abstract

### Background

Dutch children's diets, like the diets of many children in Europe and the US are not balanced, do not contain enough vegetables and have been associated with a high prevalence of childhood obesity. Promoting children's vegetable intake is challenging.

### Objective

We investigated the relative effectiveness of repeated exposure (RE) and Flavour-Flavour-Learning (FFL) in increasing vegetable intake and acceptance in pre-schoolers.

### Methods

During an intervention period of seven weeks, 39 toddlers (aged 1.5 to 4 years) consumed red beet and parsnip crisps at day-care centres in Wageningen, The Netherlands. Half of the group received red beet crisps with a dip of tomato ketchup (Conditioned (C)), and parsnip with a neutral white sauce (Unconditioned, (UC)), whereas for the other half the order was reversed (red beet (UC), parsnip (C)). Preference and ad libitum consumption of vegetable crisps were measured once before and three times after the intervention over the course of a 6-months follow-up period, to assess longer-term effects.

### Results

Intake increased significantly after the intervention for both vegetables (on average with 8 g; an increase of approximately 300%), and this effect was persistent even six months afterwards. The increase was irrespective of crisps being offered with C or UC dip sauce.

### Conclusions

These results suggest a robust and persistent effect of RE but no effect of FFL. Offering pure vegetable tastes repeatedly is sufficient to increase intake.

Keywords: Exposure; FFL; Preschoolers; Vegetable liking and intake.

## Introduction

Dutch children's diets, like the diets of many children in Europe and the US do not contain enough fruit and vegetables [1-4]. These dietary patterns in young children have been associated with a high prevalence of childhood obesity in developed countries [5, 6]. Dietary interventions in children should focus on decreasing energy density of meals [7], e.g. substitution of more energy-dense meal components by low energy-dense components like vegetables. However, a recent review of school based interventions to improve daily fruit and vegetable intake, showed that these interventions moderately improve fruit intake but have minimal impact on vegetable intake [8]. Liking of vegetables does not come naturally or easy to most children and this has been linked to vegetables' intrinsic bitter taste and texture characteristics [6, 9]. A British survey study confirms this discrepancy in liking between vegetables and fruits. When children were asked to rate different food categories, the ten lowest rated foods included six vegetables, whereas fruit was the second best-liked food [10].

Food likes and dislikes are formed as early as the age of 2-3 years [11-13] and are predictive for eating behaviours in later life [14-16]. Children eat what they like and children's food preferences are strongly related to their intake [17, 18]. Several learning mechanisms, for example, repeated exposure (RE), Pavlovian conditioning (associative learning) and social modelling are known to be involved in forming food preference in children [6, 19, 20]. Repeated exposure involves repeated encounters with the sensory characteristics of a food product and a positive change in the individual's appreciation of that food (i.e. offering a child repeatedly an unfamiliar taste or food can teach the child to accept it). Flavour-flavour learning (FFL) depends on the association between a novel flavour and a familiar or already liked flavour, resulting in a positive shift in preference for the initially neutral flavour [20-22]. Havermans (2007) reported a significant increase in vegetable liking specifically for the vegetable taste being paired with the sweet taste of dextrose, and so facilitating the acceptance of a specific vegetable [23]. For example, repeatedly pairing Brussels sprouts with applesauce can facilitate the acceptance of initially disliked or unknown Brussels sprouts.

There is growing evidence for repeated exposure as the most robust and powerful mechanism to enhance vegetable consumption in children [24-28]. For flavour-flavour learning (FFL), however, findings are inconsistent, with some studies reporting additive positive effects on children's liking of vegetables [23, 27, 29], whereas other studies fail to find an effect of FFL on vegetable liking and intake [24, 25].

The present study investigates whether the reported positive effects of FFL in addition to RE can be replicated and to investigate the relative strength of RE and FFL effects on enhancing vegetable intake and preference in young children. Our second aim was

to establish whether potential effects of RE and FFL on vegetable preference and intake remains stable over a 2- and 6-months follow-up.

## Methods

### Participants

Forty-five children aged 1.5–4 years were recruited from two day-care centres in Wageningen, The Netherlands. Parents signed an informed consent for their child's participation. Participants were screened for food allergies and health problems (as reported by the parents). The study was approved by the IRB of Wageningen University (NL38107.081.11) and registered by the Dutch Trial Registration (NTR, TC = 3253). Of the 45 children eligible to participate, six were excluded from data analysis because they had no intake at all of the dip sauces. Hence, all data analyses reported are based on the remaining sample of 39 children who met the criteria for successful conditioning (i.e., at least 7 times exposure of 1 gram consumption of dip sauce). Table 3.1 shows the main characteristics of the participating children. Of these 39 children, 37 participated in the 2-months follow-up and 26 in the 6-months follow-up. The most important reason for dropout was leaving day-care to attend primary school.

**Table 3.1** Subject and group characteristics. Characteristics (Age, Weight, Height) of children included in the data analysis (n=39) including group characteristics.

| | Total Included in data-analysis (n =39) | Girls (n=19) | Boys (n=20) | Participants PN-TK/ RB-WS (n = 19) | Participants RB-TK/ PN-WS (n = 20) |
|---|---|---|---|---|---|
| *Age* (Months) | | | | | |
| Mean (SD) | 32.6 (8.4) | 33.3 (7.9) | 31.9 (9.0) | 32.8 (8.1) | 32.4 (8.9) |
| Range | 18 - 45 | 22 - 44 | 18 - 45 | 22- 44 | 18 - 45 |
| *Weight* (kg) | | | | | |
| Mean (SD) | 14.2 (2.8) | 13.9 (1.9) | 14.5 (3.4) | 14.0 (2.3) | 14.4 (3.1) |
| Range | 9.6 – 21 | 9.6 – 17 | 11 - 21 | 11 - 19 | 9.6 - 21 |
| *Height* (cm) | | | | | |
| Mean (SD) | 95.5 (7.3) | 95.9 (5.6) | 95.2 (8.7) | 95.1 (4.8) | 95.8 (8.9) |
| Range | 83 - 113 | 83 - 104 | 84 - 113 | 87 - 103 | 83 – 113 |

TK, tomato ketchup; PN, parsnip; RB, red beet; SD, standard deviation; WS, white sauce

## Design

A within subject design, semi crossover was used to evaluate effects of repeated exposure (RE) and flavour-flavour learning (FFL). Participants were randomly assigned to one of two conditions offering freeze-dried vegetable crisps with a dip. Half of the participants received red beet crisps combined with tomato ketchup (TK(C)) consistently paired with parsnip crisps combined with white sauce (WS(UC)). The other half of the participants received the reverse, i.e., red beet crisps + WS(UC) and parsnip crisps + TK(C). During the intervention, the order of the C and UC crisps was semi-randomized within subjects, with the restriction that subjects did not receive the C or UC crisps more than twice in a row. Children were assigned to one out of two conditions: either parsnip TK(C)/ red beet WS(UC); n = 22, or red beet TK(C)/parsnip WS(UC); n = 23.

Notably, both conditions represent RE by repeatedly offering the same two vegetables. In addition, an effect of FFL was investigated by offering two different dip sauces together with the vegetables. Figure 3.1 shows a schematic outline of the study in time.

## Test foods

The vegetable crisps were developed especially for this study after consultation of a Dutch chef (Pierre Wind) specialized in working with school-aged children. Target vegetables had to be neutral or disliked and relatively novel, but suitable for processing into freeze-dried crisps. We chose two vegetables, parsnip and red beet, that are not present in the top five of the list of most commonly consumed vegetables in Dutch children at the age 2-4 years [3, 30]. The form in which the vegetables were presented added to the unfamiliarity, as Dutch children are not used to eat vegetables in this format. As a consequence of freeze-drying (i.e. all water is extracted) the vegetables are more intense in their specific taste. Tomato ketchup (TK) was selected as a liked and familiar dip. Preference for TK was confirmed by performing preference tests with toddlers: 63% liked TK best, compared to 13% WS, and 24% had no preference at T = 0. Preference remained stable in time with rated preferences for TK varying from 73% to 79% (T =1, T =2 and T =3). Used products during conditioning varied from 23 to 33 kcal per serving, in which 6 g crisps and 15 g dip were offered (see Table 3.2). Children not participating in the study were offered rice waffle (Nutricia, Bambix, Zoetermeer, The Netherlands) and dip (WS or TK).

Raw, fresh vegetables were used to prepare vegetable crisps. Vegetables were blanched and further processed in a professional freeze-dryer (model Lyofast S08 and model super modulyo freeze-dryer, Edwards, West Sussex, UK). Products were sealed in bags of either 6 grams each (used in conditioning period) or bags of 30 gram each (used in pre-tests and all post-tests). These amounts are comparable to a small (mini) bag of regular crisps vs. a normal sized (family) bag of crisps. Dips were offered in small cups containing 15 gram (approx. one tablespoon).
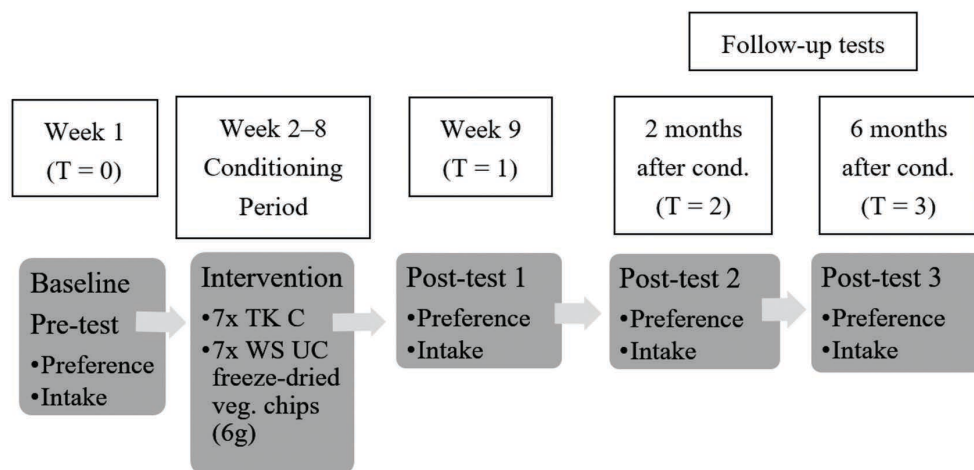
**Figure 3.1** Schematic outline of the study in time. The study consisted of five parts: a pre-test, a conditioning period, a post-test following the conditioning period, and 2- and 6-months post-tests. Intake and preference were the outcome measures.

**Table 3.2** Energy density of the different vegetable crisps and dip sauces used during the intervention, calculated for each ingredient separately (kcal/portion) and calculated for the combination (kcal/serving) as offered to the toddlers before lunch as a starter.

| | Tomato ketchup (TK) (C) | White sauce (WS) (UC) | Red beet (RB) (freeze-dried) | Parsnip (PN) (freeze-dried) |
|---|---|---|---|---|
| *Kcal per 100g* | 87 | 30 | 304 | 341 |
| *Kcal per portion* | 13 | 5 | 18 | 20 |
| | RB_WS | PN_TK | PN_WS | RB_TK |
| *Kcal per serving* | 23 | 33 | 25 | 31 |

## Measures

Before and after the intervention took place, ad libitum vegetable crisps intake and relative ranking of preference was assessed. Ad libitum intake was measured by offering 30 gram of one of the two target vegetable products offered by itself without dip (i.e. plain crisps). Children were invited to eat as much as they liked. At a different day in the same week, ad libitum intake of the other vegetable was measured. Preference was assessed using a paired preference test. Children performed the test separately and were invited to taste the two target vegetable products. Children were asked to indicate the product/flavour they preferred above the other. This procedure was repeated for the dip sauces separately.

During the intervention (14 exposures, twice a week), intake of crisps (offered in 6-gram bags) and dip sauce (offered in cups of 15g) were monitored separately for each child

individually. Children were encouraged by the day-care leaders to dip the crisps in the sauce (served in a small cup). Consumption of crisps and dip sauces was measured by pre- and post-weighing on a digital scale with a precision of 0.1g (model S-4001, Denver Instruments, Bohemia, NY, USA).

Two follow-up measurements were included at two and six months respectively after completion of the intervention, involving relative preference ranking and ad libitum intake of the vegetable crisps without dip (plain crisps) as described above.

All sessions were held 10-15 minutes before lunchtime at the same days of the week and were part of the normal lunch routine at the day-care centre, to minimize interference with the daily routine. Children were seated at tables in small groups (5-10 children) together with their day-care leaders. The children were not aware their intake was measured or which condition they participated in.

## Statistical Analysis

Data are presented as mean values with standard deviation unless otherwise specified. Statistical analyses were carried out using SPSS (version 19; SPSS Inc., Chicago, IL, USA). To examine the effect of RE and FFL on vegetable intake GLM (General Linear Model) analysis were carried out with exposure and condition as main factors and their interactions in a 2-factor ANOVA model (GLM for repeated measures). A chi-square test was used to test for shifts in preference over time (baseline vs. post-tests). Tests were performed 2-sided, and P-values < 0.05 were considered significant.

## Results

### Intake

*Intake - baseline versus T1*

Figure 3.2 shows that intake of the vegetable crisps significantly increased after the conditioning period, but this was regardless of the coupling to either tomato ketchup or white sauce. GLM repeated measures analysis revealed a significant main effect of exposure ($F_{(1,38)}$ = 74, $p < 0.0001$) on ad libitum intake, but no main effect of condition ($p > 0.4$) or interaction effect ($p > 0.2$) was found. Average intake increased by 278% (7 g (0.03 cups); SD 7) for the unconditioned crisps (i.e. plain crisps previously paired with white sauce) and by 315% (9 g (0.04 cups); SD 7) for the conditioned crisps (i.e. plain crisps previously paired with tomato ketchup) since baseline measurement.
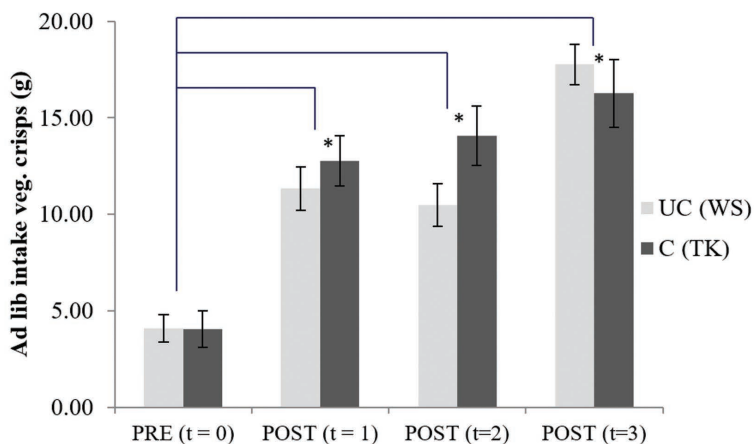
**Figure 3.2** Ad libitum intake (mean ± SEM) of crisps previously paired with tomato ketchup (conditioned condition; C) versus crisps previously paired with white sauce (unconditioned condition; UC) at baseline (t = 0), after conditioning (t = 1), and 2- (t = 2) and 6-months (t = 3) following the end of the conditioning period. * P < 0.05

*Intake – baseline versus follow-up, T2 and T3*

GLM repeated measures analysis with exposure (baseline T = 0, T = 2, T = 3) and conditioning (C,UC) as within subjects variable, revealed a significant main effect of exposure ($F(2,24) = 65$, $p < 0.0001$) on ad libitum intake, no main effect of conditioning ($p > 0.5$) or interaction effect ($p > 0.15$) was found. This indicates that the positive effect of exposure during the intervention on intake was still there two and six months after the intervention (see Figure 3.2). Taken together, the intake of vegetable crisps remained stable in time; respectively in the unconditioned condition 11 +/- 7 g (T2); 18 +/- 5 g (T3) and in the conditioned condition 14 +/- 9 g (T2); 16 +/- 9 g (T3).

*Intake during the conditioning period*

Intake of vegetables crisps and dip sauces was separately monitored during the conditioning period. GLM repeated measures analysis showed that the average intake of crisps paired with tomato ketchup was higher than the average intake of crisps paired with white sauce during the conditioning period; respectively 5 g (SD 1) and 4 g (SD 2) (See Figure 3.3a). In addition, intake increased significantly over time (main effect of exposure, $p < 0.001$). No interaction effects were found ($p > 0.05$). During conditioning, tomato ketchup was preferred above white sauce. The average intake of tomato ketchup was 8 g (SD 4) compared to 4 g (SD 3) for white sauce (see Figure 3.3b).
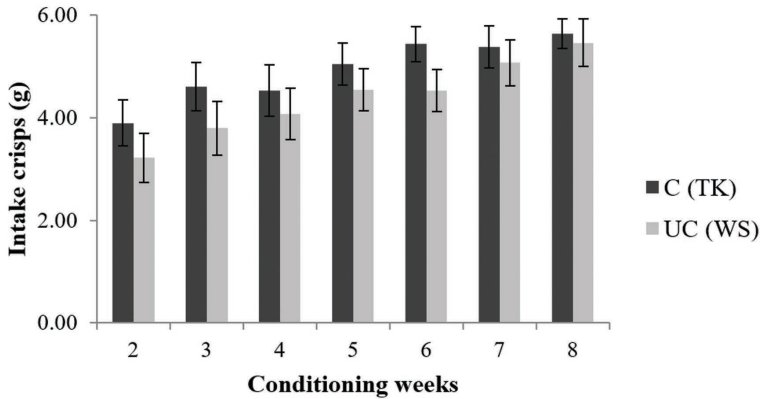
**Figure 3.3a** Average intake of freeze-dried vegetable crisps (+/- SEM) during the conditioning period (week 2 – 8 of the study). Average intake of crisps paired with tomato ketchup (C) was higher than average intake of crisps paired with white sauce (UC), respectively 4.9 g (SD 1.5) and 4.3 g (SD 1.8).
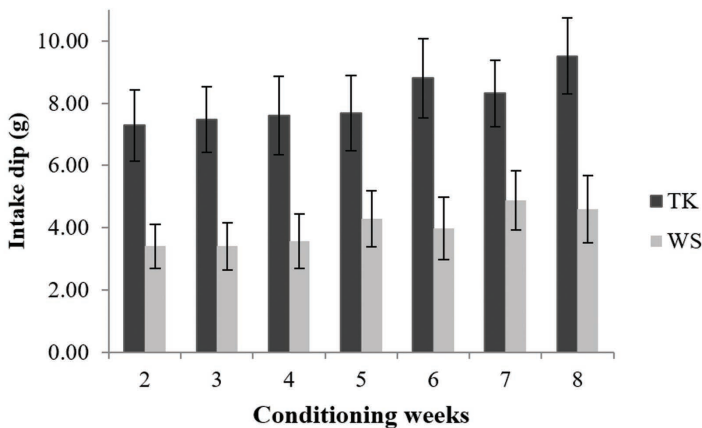


**Figure 3.3b** Average intake of dip sauces (+/- SEM) during the conditioning period (week 2 – 8 of the study). Dip sauces were monitored separately from the crisps intake. Average intake of tomato ketchup was higher than average intake of white sauce, respectively 8.0 g (SD 4.2) and 4.0 g (SD 2.9).

## Preference

### Preferences

The distribution of preference for one vegetable flavour versus the other was compared between baseline and the first post-test using a chi-squared test. No significant shift in preference became apparent, when comparing preference before, and after conditioning (p > 0.25), see Figure 3.4. This indicates no effect of FFL. To test persistence, the distribution of preference for one vegetable flavour versus the other was compared over time (baseline, two months since the conditioning period, and after six months

respectively). Chi squared tests showed no significant effect across time (p > 0.25), see Figure 3.4.
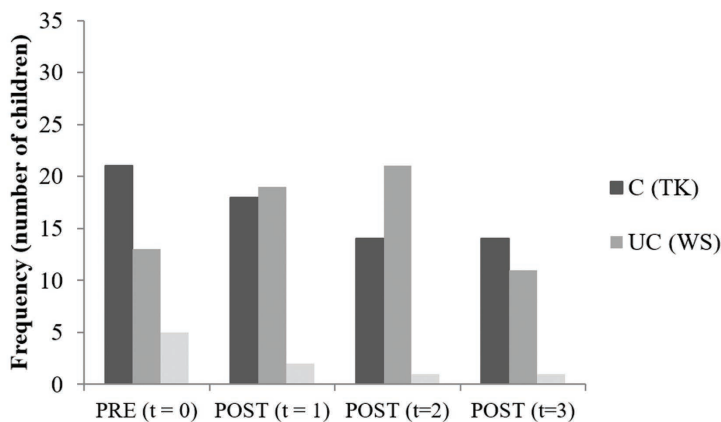


**Figure 3.4** Results of preference test at baseline (t = 0), after conditioning (t = 1), two months after conditioning (t = 2) and six months after conditioning (t = 3). On the Y-axis the number of participants that preferred one flavour above the other. In case a child was unable or unwilling to make a choice, it scored 'non preference'. The labels 'conditioned (C) vs. unconditioned (UC)' indicate the vegetable crisps flavour (either red beet or parsnip, measured with crisps no dip sauces added) that was previously coupled to tomato ketchup (C) vs. white sauce (UC) during the conditioning period.

## Discussion

This study investigated the relative efficacy of repeated exposure (RE) and flavour-flavour learning (FFL) as mechanisms to increase vegetable intake and acceptance in young children. Vegetable intake increased significantly by approximately 300% (from baseline) after the intervention, but regardless of condition. The increase in intake persisted over time and was still present two and six months afterwards. We observed no shifts in preference for one vegetable over the other as a consequence of FFL. These findings reflect that RE but not FFL proved to be the primary mechanism explaining increased vegetable consumption in young children and, notably, one with longer-term potential.

The present findings on RE effects are consistent with previous research. Several earlier studies conducted in young children reported similar results [31-34]. Five recent studies confirmed that RE is a strong learning mechanism to promote vegetable acceptance in children [24-28]. Contrary to our expectations, we found no additive positive effects of FFL on vegetable intake and preference. This lack of result is, however, in concordance with findings from recent studies performed by Anzman-Frasca et al. [24] and Savage et al. [35]. Here, children repeatedly tasted small portions of vegetables that were initially disliked, presented either alone (RE) or with a liked dip. Liking scores and vegetable intake increased similarly in both treatment conditions, which are indicative for RE. But,

comparable to our present findings, tasting the vegetables with dip did not lead to greater intake of that vegetable. The authors suggest, however, that offering vegetables with liked dips facilitates the willingness to taste and therefore could be used to encourage initial tasting of vegetables.

Nonetheless, a number of studies reported positive effects of FFL on the acceptance and intake of vegetables in liking [23, 25, 27, 36]. There are several possible explanations for the discrepancy in findings between these studies and the present one. For one, it could be related to the products used. Both Caton et al. [25] and Hausner et al. [27] used a vegetable puree in their studies, which is a familiar product to young children, but possibly less appealing once they reach an age of two years and older. In the present study, much thought and effort was spent on developing a novel vegetable product that was attractive to toddlers. Hence, the attraction of the vegetable crisps, served in a familiar and trusted environment of the day-care centre and with their peers enjoying the crisps as well could have contributed towards intake in both conditions regardless of the dip sauces (C, UC).

A second explanation could be the procedure applied. Johnston and colleagues compared an exposure only condition with a condition in which vegetables were paired to a preferred taste (i.e. peanut butter) [36]. Pairing to a preferred taste significantly increased intake of vegetables. Interestingly, we also found that average intake of crisps paired with tomato ketchup was higher than intake of crisps paired with white sauce during conditioning. During baseline and post-test measurements intake of and preference for the two flavours (red beet and parsnip) of vegetable crisps were presented without dip. Then, the potential FFL effect observed during the conditioning period was no longer present. The increased intake of vegetables during conditioning is conform the findings of Anzman & Savage [24, 35] and suggests that FFL might have added value used as a strategy to promote initial tasting of vegetables in young children.

Strengths of this study include the assessment of longer-term effects after a 2- and 6-months follow-up period. Follow-up measurements indicate that mere exposure effects are quite stable over time, which is obviously a promising finding in light of the development of public health strategies in prevention of obesity to increase children's consumption and acceptance of vegetables. Another strength involves the appealing vegetable products that were well accepted and consumed by the toddlers. This is relevant as a recent study of Howard et al., investigating toddler's food preferences, showed that lower maternal liking and food neophobia (usually increasing from age two and reaching its peak at approx. 5 years of age) were associated with a greater proportion of vegetables never tried by toddlers [37]. However, the choice for vegetable crisps also has a downside. In general, children are familiar with crisps and also like its texture, and this may have positively influenced their willingness to try the vegetable crisps. It remains to be determined to what extent exposure to vegetable tastes via crisps will transfer to a better acceptance and

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intake of vegetables cooked in a regular way, that is, vegetable tastes accompanied by textures that are often less appealing to young children. This is a matter of future study.

The study has some other limitations as well. Besides intake, we focused on self-reported preference. It is a matter of debate whether children in this age group are capable to perform complex preference tests that generate reliable and replicable results, but they seem capable of making "yes / no" distinctions [38]. Therefore, we considered the paired preference testing method as sufficiently valid. Related to this, sensory profiling of the target products was done by an independent sample of adults, as young children lack the cognitive, evaluative and language skills to perform this type of quantitative descriptive analysis. It is not clear to what extent sensory evaluations made by adults can be extrapolated to young children. The test environment is another issue of consideration. The familiar and trusted environment of the day-care setting together with peer pressure, likely contributed to the high acceptance and consumption of the vegetable crisps, and it remains to be seen whether this strategy will be equally effective in an at home situation.

In conclusion, repeated exposure is an effective method of promoting vegetable intake in young children [24-28, 31-34]. This conclusion is reassuring insofar that simply offering pure vegetable tastes repeatedly seems sufficient to increase intake over time, rather than adding energy or flavour to the food. Facilitating children's acceptance of pure vegetable tastes by repeated exposure may lead to a reduction in the daily energy intake by substitution of energy-dense meal components, such as starches or meat, and therefore contribute to a healthier balanced diet. This research translates into an easy to follow message for public health, parents and caregivers, and its implementation clearly deserves more attention.

## Acknowledgements

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# Chapter 4

**Efficacy of offering vegetables in different taste experiences on its acceptance in toddlers**

Offering spinach pure, diluted or hidden, all seems to work well

Victoire W.T. de Wild

Cees de Graaf

Gerry Jager

## Abstract

Children's consumption of vegetables is far below recommendations. The present study compared three strategies on their effectiveness to increase intake; repeatedly offering a pure or a diluted vegetable taste, or 'hiding' the vegetable, and taking food neophobia into account. Children ($M_{age}$ 3.0; SD 0.6; n = 103) were randomly assigned to one of the four groups: pure (pure spinach), diluted (spinach a la crème), hidden (spinach ravioli) and control (green beans). Parents assessed their child's neophobia using the Child Food Neophobia Scale. During the intervention, children consumed their vegetable products six times at home during the main meal. Mean outcome measure was ad libitum intake of plain cooked spinach, measured pre- and post-intervention. GLM repeated measures analysis with intake (pre, post) as within-subjects, group as between-subjects factor, and neophobia score as covariate yielded a significant effect of exposure (p<0.001) and an interaction of intake*neophobia (p=0.008). All groups increased their spinach intake from pre- (53.4 g +/- 56.7) to post-exposure (90.6 g +/- 75.0) by an average increase of 70% (+/- 40 g; p < 0.001), irrespectively of the strategy. There was no interaction effect between group and exposure. To conclude, toddlers increased their ad libitum intake of plain cooked spinach post-intervention, irrespective from whether they were exposed to the pure spinach taste, or to a diluted taste, or whether the vegetable was 'hidden'. This important finding makes it easier for parents and caregivers to stimulate children's vegetable consumption. However, the effect on intake did depend on the child's neophobia status, with neophobic children being less responsive to the intervention. This indicates that difficult eaters need another approach to stimulate vegetable intake.

Keywords: Taste modification; Vegetable consumption; Young children; Food neophobia

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## Introduction

Children's vegetable consumption is far below recommendation. The recommendation of the daily vegetable intake for Dutch toddlers is 50 to 100 gram (one to two serving spoons per day), but average vegetable consumption in this age group is 38 gram per day, which is less than one serving spoon per day [1]. It is a universal concern how to get children to eat sufficient vegetables. Parents want to be ensured that their children have a healthy varied diet and vegetables are viewed as an essential part. However, it remains a challenge to stimulate the intake of a variety of vegetables in children, especially, because vegetables are often initially disliked by children due to their appearance and bitterness [2, 3]. Therefore, it is of interest to study if strategies like masking or covering the original often disliked vegetable taste is an effective technique in stimulating children's vegetable intake.

Different preparation techniques like blending, mixing, mashing, pureeing or seasoning are used in practice to improve the vegetable intake in children [4, 5]. Many of these techniques have in common that vegetables are (covertly) incorporated in other foods so the pure often not liked vegetable taste and texture is somewhat diluted and therefore more acceptable to children. An intervention study of Zeinstra showed that vegetables offered in it their pure form were rejected by children due to their bitter, strong intrinsic taste [6]. Few studies investigated whether incorporating vegetables in meals not in their original form but in ways children are not aware of it (e.g. blending/mixing in sauces, soups, or by hidden the vegetables in other food items), actually increases vegetable liking and intake. One study interviewed and questioned mothers in the U.K. and they reported that several techniques such as modelling, modifying the taste or texture, masking the taste, or presenting the vegetable in a different form, were popular methods to stimulate vegetable intake in children. One technique, the so called vegetables by stealth (i.e. presenting the vegetable in a form where it was not obvious) was a popular method how to deal with vegetable and children [5]. Another study confirmed that indeed children ate more vegetables when these were incorporated in foods served as entrées over a day (breakfast, lunch and dinner) to children (3-5 years of age) at the day-care [7].

Apart from a dislike of vegetables because of their inherent sensory properties (taste, texture), food neophobia (i.e. the fear of trying/testing a novel food) also influences vegetable intake in a negative way in children. This is strengthened by the fact that the age when neophobia peaks (toddlerhood, pre-schoolers), co-occurs with the period when young children have to learn to appreciate and eat a variety of initially disliked vegetables. This is reflected by findings showing that, although not every child has the same level of food neophopbia, the highest occurrence is between two and six years old and during this age period food neophobia is strongest for vegetables compared to all food product types [8, 9]. It remains unclear if and what strategy to increase liking for and

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intake of vegetables is most effective for this difficult group of eaters.

The aim of the current study was to investigate the efficacy of offering vegetables in different taste experiences on its taste perception (i.e. preference) and intake in pre-schoolers. The target vegetable, spinach, was offered overtly in two forms, cooked in its pure form (pure taste), diluted with cream (softened taste), and covertly as filling of ravioli (mixed taste). The second objective was to investigate how food neophobia mediates the efficacy of the different presentation strategies.

## Materials and methods

### Participants

One hundred and four children aged two to four years were recruited from six day-cares in Wageningen, a small city in the middle of the Netherlands. Participation was voluntary. Written informed consent was obtained from the parents of the participating children. Parents and day-cares employees were thoroughly informed about the study by an information booklet and information session prior the intervention. Participants were screened for food allergies and health problems (as reported by the parents), which resulted in 103 participants that were included in our sample. The study protocol was approved by the Institutional Review Board of Wageningen University and registered at the Dutch Trial Registration (NTR, TC = 4755). Sample size calculation was based on the standard deviation of the mean vegetable consumption of Dutch children aged 2 to 4 years (= 34 g) and a meaningful expected increase set at half a SD (= 17 g = one tablespoon of cooked vegetables) [1]. To detect a significant difference between the groups, with alpha set at 0.05 and a power of 0.80, at least 25 children were needed per group. Table 4.1 shows the main subject and group characteristics.

### Study design

A between subjects design was used to identify potential group differences in plain spinach intake after an intervention (for products descriptions see subparagraph Study foods). Groups differed from each other on how the target vegetable was offered during the intervention. Children were randomly assigned to one of the four groups: 'Control' (no spinach but green beans), 'Pure' (pure cooked spinach), 'Dilute' (spinach à la crème), and 'Hide' (spinach ravioli). Pre and post exposure measurements took place of ad libitum intake and liking for plain spinach one week before and one week after the intervention at the day-cares. Each child was exposed once per week to the vegetable product at home during the main meal in the evening, with a total length of six weeks. Depending on the assigned group, children and their families received only one of the

four vegetable products (i.e. green beans; pure spinach; spinach à la crème; or spinach ravioli) during the intervention. Figure 4.1 shows a schematic outline of the study in time.

**Table 4.1.** Characteristics of the children overall and per group included in the study. Data are presented as means (SD) or frequency (%).

| | All (n = 103) | Control (n = 26) | Pure (n = 26) | Dilute (n= 25) | Hide (n= 26) |
|---|---|---|---|---|---|
| Age (months) | 35.5 (6.8) | 35.8 (7.2) | 34.5 (6.9) | 36.1 (6.7) | 35.4 (6.7) |
| Gender (boys, %) | 54 (52.4) | 15 (57.7) | 13 (50.0) | 12 (48.0) | 14 (53.8) |
| Weight (kg) | 14.1 (2.2) | 14.6 (2.1) | 14.2 (2.5) | 14.1 (1.9) | 13.3 (2.0) |
| Height (cm) | 94.4 (6.6) | 94.5 (6.6) | 94.9 (5.3) | 96.5 (7.1) | 92.0 (7.0) |
| Breastfeeding duration (months) | 8.5[*] (7.6) | 9.6[a] (8.7) | 8.8[a] (5.9) | 11.1[a] (8.5) | 4.3[b] (4.9) |
| Consumption Green beans[1] (g) | 59.3 (39.2) | 55.0 (32.4) | 66.7 (38.1) | 52.6 (36.1) | 63.1 (49.5) |
| Consumption Spinach [1] (g) | 47.3 (38.8) | 42.9 (38.8) | 58.9 (41.2) | 43.9 (31.3) | 44.2 (43.0) |
| Consumption Carrots [1] (g) | 54.4 (37.7) | 53.1 (37.7) | 67.4 (45.9) | 47.4 (28.8) | 50.2 (35.8) |
| Baseline vegetable liking (1-5 score) | 3.3 (1.3) | 3.1 (1.3) | 3.5 (1.3) | 3.2 (1.3) | 3.3 (1.1) |
| Liking Green beans[2] (1-5 score) | 3.9 (1.3) | 3.7 (1.4) | 3.9 (1.3) | 3.9 (1.5) | 3.9 (1.2) |
| Liking Spinach [2] (1-5 score) | 3.3 (1.6) | 3.1 (1.5) | 3.5 (1.4) | 3.2 (1.5) | 3.5 (2.2) |

[*] P < .01; [a, b] Means with different superscript letters are significantly different
[1] Consumption at home as reported by the parents before the intervention started
[2] Liking scores from the children asked and reported by the parents before the intervention started

## Study products

Spinach was chosen as target vegetable as it is a green leafy vegetable and generally not liked by children. Green beans were chosen as a control vegetable since children generally like this vegetable and is one of the most consumed vegetables. Both vegetables fit into a typical Dutch main meal of potatoes/rice/pasta, vegetables and meat/fish/vegetarian product [1].
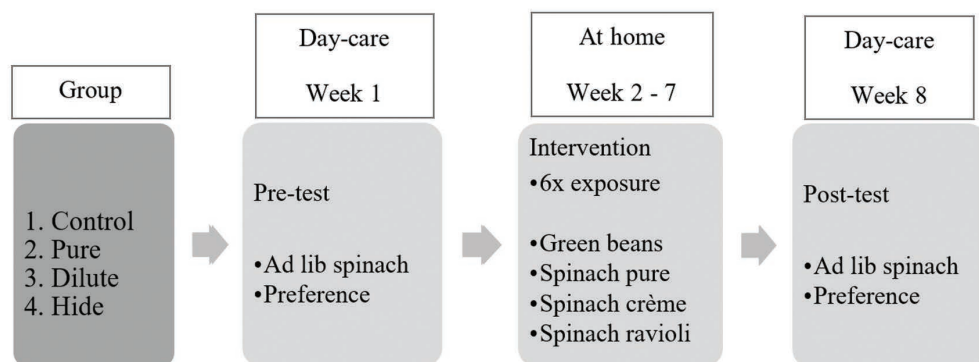
**Figure 4.1** Schematic outline of the study in time. The study consisted three parts: a pre-test, an intervention period, and a post-test following the intervention period. Intake and preference obtained by the pre and post-tests were the mean outcome measures.

In order to create different taste experiences the target vegetable (i.e. spinach) was offered in three different forms: 'Pure', 'Dilute', and 'Hide'. 'Pure' meant pure spinach without flavourings; 'Dilute' was pure spinach diluted with some cream (spinach a la crème) to soften the stringent spinach taste; and 'Hide' was pure spinach covered in an envelope of pasta (ravioli), so that the child was less aware of consuming spinach. The products in the 'Control', 'Pure' and 'Dilute' group were commercially available (Iglo frozen green beans, 2.5 kg; Iglo frozen chopped spinach, 2.5 k; and Albert Hein frozen spinach a la crème, 1kg) and were repacked in family portions and delivered frozen via the day-cares on a weekly basis. The packages contained the recommended daily amount (RDA) of vegetables for the whole family, to ensure that every member of the family could comply with the RDA. The spinach ravioli ('Hide' group) was developed especially for this study after consultation of an Italian caterer. Each ravioli contained 70% spinach of the total weight.

The spinach used for the ad libitum intake during the pre-and post-tests at the day-cares contained primarily pure spinach (98%; Iglo frozen chopped spinach). Small amounts of sunflower oil (0.6%), salt (0.1%) and rice flour (1%) were added to increase the willingness to taste the products during the pre and post-tests (all amounts in concordance with infant food industry regulations and the European regulation (Directive 2006/125/CE)). The spinach used during the pre and post-test is called "plain spinach".

## Measures & procedures

*Prior the intervention (demographics & child characteristics)*
Data collected prior to the intervention included demographics such as the child's date of birth, gender, weight, height, total breastfeeding duration. Additionally, data was collected concerning vegetable liking (for vegetables in general and specific questions for

green beans and spinach), previous exposure to vegetables (number of vegetables, specific green beans and spinach), and vegetable intake (green beans, spinach) as reported by the parents. Parents were asked to rate their child's vegetable liking in response to the question "My child likes most of the vegetables" on a 5-point scale ranging from "1 = totally disagree" to "5 = totally agree". This question was repeated for green beans and spinach respectively. The frequency of children's exposure to green beans and spinach at home (i.e. "1 = once/week", "2 = once/month", "3 = sometimes", and "4 = never") was asked from the parents by questionnaires. Parents were also asked to estimate their children's average consumption of green beans and spinach in numbers of (table) spoon(s) or they had to indicate the consumption in grams. When the consumption was reported in number of (table) spoon(s) it was converted in grams. Food neophobia scores were assessed with a child food neophobia 6-item questionnaire [10, 11]. The six statements of the CFNS (Child Food Neophobia Score) were scored on a 5-point scale from "1 = strongly disagree" to "5 = strongly agree". Higher scores indicate stronger food neophobia (i.e. fear of trying/testing new products).

### Pre-test (preference & intake)

*Preference* was assessed using a paired preference test that was conducted before the ad libitum intake was measured. Children performed the test separately and were invited to taste two vegetables (i.e. spinach and green beans), offered to the children in a random order. Children were asked to indicate which vegetable they preferred above the other. If a child was unwilling or unable to choose, children were encouraged by the researcher by asking the children the following two questions: "Which vegetable would you like to finish?" and "Which vegetable would you give to your friend?" If this did not result in a choice but the child indicated that he/she liked both vegetables, preference was scored as 'neutral positive'. When the child indicated that he/she disliked both vegetables, preference was scored as 'neutral negative'. In all other cases, the child's preference was scored as 'not tasted/refused/shy'.

*Ad libitum intake* was measured by offering 200 gram of plain spinach served warm (+/- 80 $^0$C) in small bowls, 10 – 15 minutes before the regular lunch time to minimize interference with the daily routine. Children were invited to eat as much as they liked and were seated at tables in small groups (5- 10 children) together with their day-care leaders. Spinach intake was measured by weighing their bowls before and after lunch (left overs) on a digital scale with a precision of 0.1g (model S-4001, Denver Instruments, USA; model Kern-572, Kern & Sohn, Germany).

### Intervention

Families received weekly a vegetable parcel including their vegetable product for one meal, cooking instructions, and a food diary. A standardized weighing scale with a precision of 1g (type Fiesta, Soehnle, Germany) was supplied to all participating

families together with the first delivery of the vegetable parcel. Cooking instructions involved how to prepare the vegetables and cooking duration. Other instructions related to the minimum amount of serving (i.e. 50 g green beans/spinach/spinach à la crème or 3 pieces ravioli) to comply with the daily recommended vegetable intake, and the instruction not to serve any other vegetable(s) than the provided ones. After the main meal parents had to fill out the food diary by providing the following information: any deviation from the prescribed instructions; start and end time of the dinner; the consumed vegetables and other meal components (e.g. meat/fish, pasta/potatoes); the weight of the vegetables served to the child before and after the meal in gram (i.e. weighing their plates before and after the meal (left overs)); the child's liking of the vegetables (parent's perception and rated on a 9-point scale from "1 = extremely disgusting" to "9 = extremely delicious"); and whether the child had physical complaints that day that might have affected appetite.

### Post-test (intake & preference)

One week following the intervention we repeated the preference test and the ad libitum intake at the day-cares as described above in the pre-test section. The same procedures were followed.

### Statistics

The statistical programme PAWS Statistics was used (version 22; SPSS Inc. Chicago, USA) to analyse the data. Spearman's Rho correlation coefficients were used to investigate the relationship between specific vegetable liking, vegetable exposure and specific vegetable intake before, during and after the intervention. General linear model (GLM) repeated measures analysis was performed to test a possible effect of group with ad libitum plain spinach intake as dependent variable and condition/group (i.e. Control, Pure, Dilute, Hide) as between-subjects variable and food neophobia score of the child as covariate. To investigate possible differences in intake food neophobia scores were averaged across the items and were converted in three categories: neophilic (1 – 2.7), average (2.8 – 4.0), and neophobic (4.1 – 5) [12]. Chi-square tests were used to investigate possible differences in preference before and after the intervention. A mixed linear model was used to test for significant differences in intake and in liking during the intervention between the four groups (i.e. Control, Pure, Dilute, and Hide). We used a random intercept model that takes into account the individual variation in intercepts between participants and the correlation between observations in the same participant. Intake respectively liking during the six exposures was the dependent variable and group was the fixed covariate.

## Results

Table 4.1 showed no differences in child characteristics between the groups (all p > 0.4), except for breastfeeding duration ($\chi^2$ (3) = 12.3; p = 0.006). Children from the 'Hide' group were shorter breastfed (4.3 months +/- 4.9) compared to children from the 'Control', 'Pure', and 'Dilute' group (9.6 months +/- 8.7; 8.8 months +/- 5.9; and 11.1 months +/- 8.5 respectively).
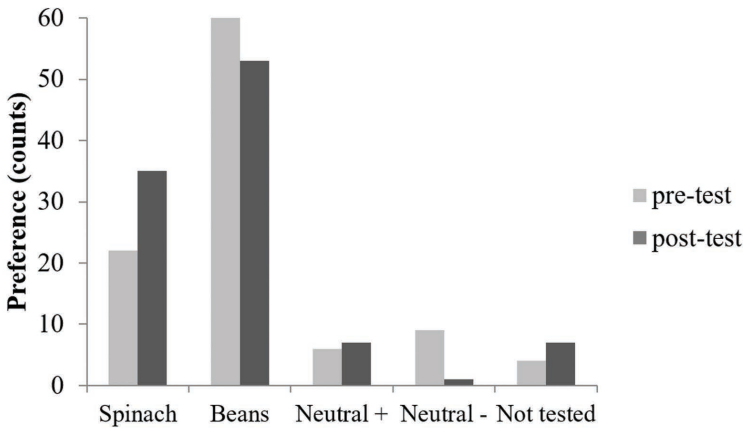
### Vegetable data

Prior to the intervention, children consumed on average 47 +/- 39 g spinach and 59 +/- 39 g green beans as reported by the parents. At home, more children were weekly exposed to green beans than to spinach, 54% and 19% respectively. Additionally, more children were seldom or never exposed to spinach compared to green beans, 29% and 11% respectively. Also children liked green beans more than they liked spinach as reported by the parents prior to the intervention (liking scores 3.9 +/- 1.3 and 3.3 +/- 1.7, respectively). Spearman's correlation coefficients yielded a positive relation between liking scores for spinach prior the intervention and spinach consumption before, during and after the intervention (all p < 0.001). There was also a positive relation between spinach consumption at home and spinach consumption during pre- and post-tests (all p < 0.001). Exposure to spinach (lower number corresponds with more exposure -see subparagraph measures & procedures) was negatively related to spinach consumption during the pre- and post-tests (all p < 0.01). This indicates that children who liked spinach more before the intervention, and consumed already more spinach at home and were exposed more often to spinach at home, consumed more spinach at the day-cares during the pre- and post-tests.

### Preference

The distribution of preference for one vegetable over the other was compared between pre- and post-test. A chi-squared test showed a significant shift in preference ($\chi^2$ (4) = 10.7; p = 0.03) in the expected direction. That is, the number of children who preferred spinach over green beans increased with 60% from before to after the intervention (from 22 children before the intervention to 35 children after the intervention). However, overall green beans were liked more than spinach (53 children vs. 35 for spinach; see figure 4.2). Chi-squared test performed per group showed no significant shifts towards preference for one vegetable over the other from before to after the intervention.

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**Intake**

GLM repeated measures analysis with exposure (pre, post) and group (Control, Pure, Dilute, Hide) as within subjects variable and food neophobia as covariate, revealed a significant main effect of exposure ($F(1, 102) = 23.3$; $p < 0.001$) on ad libitum plain spinach intake. No main effect of group was found, nor an interaction effect. This indicated that after the intervention period all groups significantly increased their spinach intake from pre- (53.4 g +/- 56.7) to post-exposure (90.6 g +/- 75.0), regardless the type of strategy used. Figure 4.3 shows that the average intake increased with 37.8 g (+/- 68.9) in the 'Control' group, with 46.7 g (+/- 68.9) in the 'Pure' group, with 40.4 g (+/- 61.8) in the 'Dilute' group, and with 24.1 g (+/- 55.0) in the 'Hide' group.



**Figure 4.2** Results of preference before (pre) and after (post) intervention. On the Y-axis the number of participants that preferred one flavour above the other (i.e. spinach or beans). When the child indicated that he/she liked both vegetables, preference was scored as 'neutral positive'. When the child indicated that he/she disliked both vegetables, preference was scored as 'neutral negative'. In case a child was unable or unwilling to make a choice, it scored 'not-tested'. Overall, there is a significant shift in preference towards the spinach flavour ($p = 0.03$).

In addition, a significant effect of food neophobia was found ($F(1, 102) = 21.1$; $p < 0.001$) on plain spinach intake and an interaction effect of food neophobia and exposure ($F(1, 102) = 7.4$; $p = 0.008$). No interaction effect was reported for group and food neophobia. This indicates that food neophobia did affect intake in the expected direction (i.e. higher food neophobia score corresponded with lower spinach intake), whereas used strategy had no influence on intake. Figure 4.4 shows the differences in plain spinach intake in time by neophobia category (i.e. neophobic (n = 12), average (n = 29), neophilic (n = 57)). The differences in intake between the pre and post-test were significant among the neophobia categories ($\chi^2 (2) = 11.7$; $p = 0.003$) and Mann-Whitney yielded significant differences between all categories (all $p < 0.03$). The food neophilic group increased their intake from pre to post-test by 54 g (82%), the average

group increased their intake by 26 g (74%) and the intake of the food neophobic group increased only with 6 g (14%).
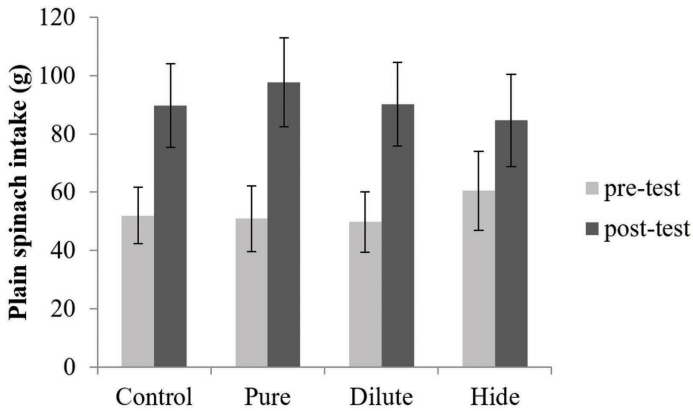


**Figure 4.3** Ad libitum intake (mean ± SEM) of plain spinach intake before (pre) and after (post) intervention by group (i.e. 'Control'; 'Pure'; 'Dilute'; and 'Hide'). GLM yielded an effect of exposure (p < 0.001), but neither group nor interaction effect were found.
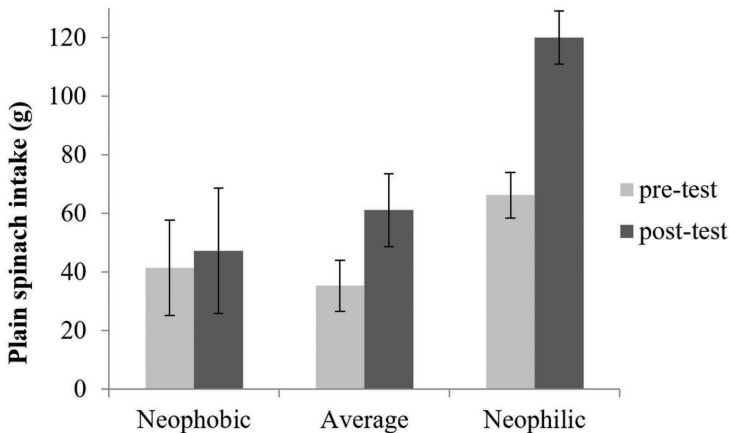


**Figure 4.4** Ad libitum plain spinach intake before (pre) and after (post) the intervention by food neophobia category (i.e. 'Neophobic'; 'Average'; and 'Neophilic'). Differences in intake between all categories were found (all p < 0.03), with an increase of 54 g (82%) in the neophilic group, an increase of 26 g (74%) in the average group, and an increase of only 6 g (14%) in the neophobic group.

## Intervention (intake & liking)

Intake (measured in g) and liking (as rated by the parents) were monitored during the intervention period. Mixed models yielded no significant effect of group, that is, there was no difference in vegetable intake during the intervention between the groups (see figure 4.5). Average intake in the 'Control' group was 48.0 g (+/- 22.7), 67.5 g (37.8)

in the 'Pure' group, 68.6 g (+/- 32.9) in the 'Dilute' group, and 52.7 g (+/- 50.5) in the 'Hide' group. For liking, results showed a significant effect for group (F = 8.2; p = 0.005), with lower liking scores in the 'Hide' group compared to the other groups (liking scores 'Hide' = 4.5 +/- 2.2; 'Control' = 6.2 +/- 1.3; 'Pure' = 5.6 +/- 2.1; 'Dilute'= 6.1 +/- 1.4). This indicates that children in the 'Hide' group liked their vegetable product (i.e. spinach ravioli) less compared to children who received pure spinach, spinach à la crème or green beans during the intervention period.
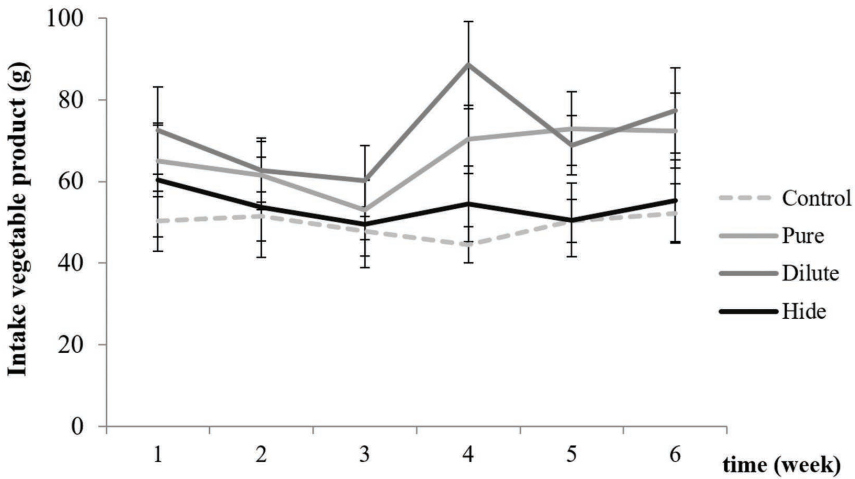


**Figure 4.5** Average intake of the vegetable product ±SEM (i.e. green beans, pure spinach, spinach a la crème, spinach ravioli) during the conditioning period (week 2–7 of the study). There were no differences in intake across the four groups. Average intake in the 'Control' group was 48.0 g (+/- 22.7), 67.5 g (37.8) in the 'Pure' group, 68.6 g (+/- 32.9) in the 'Dilute' group, and 52.7 g (+/- 50.5) in the 'Hide' group.

## Discussion

The aim of current study was to compare different taste experiences on their effectiveness to increase vegetable preference and intake (i.e. repeatedly offering a pure vegetable taste, or a diluted vegetable taste, or 'hiding' the vegetable) hereby taking food neophobia into account. For preference, the number of children who preferred spinach over green beans increased but overall green beans was still preferred most after the intervention. Here, type of used strategy had no influence. For intake, all groups increased spinach intake over time by approx. 40 g. Thus, toddlers increased their cooked plain spinach intake after the intervention, but irrespective from whether they were exposed to the pure vegetable taste, the diluted vegetable taste, or whether the vegetable was hidden. Children who were exposed to a control vegetable (i.e. green beans) also increased their spinach intake after the intervention. This suggests that repeatedly offering (any)

vegetable works to stimulate vegetable intake. The effect on intake did, however, depend on the child's neophobia score, with neophobic children being less responsive to the intervention. This indicates that difficult eaters need another approach to stimulate their vegetable intake.

The data in the current study support the interpretation that repeated exposure is sufficient to increase vegetable liking and intake. In the current study vegetable intake increased in all the groups after the intervention, but no differences were found across the groups. This indicates that a strategy such as hiding a vegetable was not more effective than the other strategies. Some studies reported positive effects of hiding a vegetable on acceptance and/or intake of vegetables [5, 7]. The discrepancies in findings may be explained by the products used in the studies. Spill et al. (2011) used different vegetable purees (i.e. broccoli, cauliflower, tomato, squash, and zucchini) which were covertly added to familiar entrees such as zucchini bread, pasta and noodles served over a day [7]. In addition to the manipulated entrees, unmodified side dishes were served from which the child could choose. In the current study, the target vegetable (i.e. spinach) was covered in a pasta dish and was specially developed for the study. The ravioli contained 70% spinach to ensure that children when served three ravioli's could meet the daily recommended amount of vegetable intake. Consequently, the spinach was more visible while cutting this relative big ravioli and therefore the vegetable was less covert while eating, which in turn could have influenced our outcome. Caton et al. [5] investigated how mothers introduce vegetables into the diets of their infants and reported that among other strategies for promoting vegetable intake, offering vegetables by stealth (i.e. masking the taste or presenting the vegetables so that the child was not aware of them) was one of the most commonly identified strategies. However, in this survey study, actual intake, as a reflection of the effectiveness of covertly offering vegetables, was not reported. Hence, it could be that although offering vegetables by stealth is a popular method applied by parents, whereas it is not necessarily more effective than other strategies, as our current findings indicate.

The results of this study are in line with other recent studies who also reported on repeated exposure as being a powerful and effective strategy to increase vegetable acceptance in children[13-18]. In most of these studies a novel vegetable product (e.g. artichoke puree; parsnip and red beet crisps; green vegetable soup; salsify) was served and accepted over time by the children through repeatedly offering the vegetables. Studies in which more common vegetables (e.g. broccoli, cauliflower, sugar snaps, carrots, peas, red bell pepper and tomatoes) were used reported that repeated tastings of initially disliked vegetables increased liking and intake in a school setting [19, 20]. These findings were confirmed in our study, where liking towards the disliked target vegetable improved and ad libitum plain spinach intake increased post-intervention. So, based on current findings repeated exposure is an effective strategy to enhance common vegetables as well in toddlers.

Our results concerning food neophobia are consistent with work from Caton et al. (2014). Caton examined what individual characteristics predict the patterns of vegetable acceptance and showed that children with higher food fussiness scores were more likely to be non-eaters (i.e. children eat less than 10 gram by the fifth exposure during the intervention) [21]. They also reported that younger children were less fussy and enjoyed food more, both characteristics that add to vegetable acceptance. Our current findings showed that neophobic children did not increase their vegetable intake after the intervention and this was irrespectively of the used strategy. This indicates that strategies like repeated exposure, masking or hiding a vegetable are not effective for this specific group and that difficult eaters need another approach to stimulate vegetable intake. Strategies for stimulating food intake in food neophobic children are poorly understood. Parents often use controlling or coercive strategies (e.g. "if you eat your vegetables, you will get your dessert") if children are reluctant to eat. However these pressure to eat strategies are not effective [22]. A strategy to stimulate food intake in 1-3-year-old children, when food neophobia occurs, is to start already early with exposure of a variety of vegetables [21, 23-27]. Still based on current findings, it remains difficult to apply a strategy as repeated exposure to be effective in food neophobic children.

One notable result was that even the children who were exposed to green beans (i.e. control group) during the intervention increased their ad libitum plain spinach intake post-intervention. One possible explanation for finding an effect for the control group is generalisation. Colour is one of the determinants to accept food and/or to make a decision to taste the food [3, 28-30]. For example, when a cherry-flavoured drink was coloured green instead of being red, 37% of participants identified it not as cherry flavour but as lemon or lime-flavour [31]. In general, participants perform very poorly when identifying flavours if they were uncoloured or incorrectly coloured. The green colour of the green beans to which the children in the control group were exposed during the intervention, may have led to a positive effect on acceptance of plain spinach via a carry-over effect. Future research should involve a control group with a different coloured vegetable to investigate if the carry-over effect is limited to only colour or that we can speak of a carry-over effect to other vegetables as well.

An alternative explanation is that children in the control group increased their intake of plain spinach after the intervention due to social context effects such as peer pressure, role modelling by the day-care staff and parental styles. Previous research confirmed that children are responsive to adapt to peers' food choices and intake by eating the same amounts of food [32, 33]. Also interventions aimed to improve fruit and vegetable intake proved to be effective by using peer-modelling [34, 35]. In the current study, children were seated together in small groups (5-10 children) during the pre and post-tests at the day-cares, which could have facilitated intake via peer pressure. In addition, the day-care staff can have an important role in children's dietary intake at the day-care by being a positive

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role model by eating healthy foods together with the children [36, 37]. An intervention based on teacher modelling showed to be effective in encouraging food acceptance in pre-school children [38]. Although the day-care staff in our study was instructed to behave as they usual do, not to alter their daily routine at the day-care and the fact that the researchers were not present while children ate their spinach during lunch, it may still have stimulated the children to eat their plain spinach by seeing the day-care leader consuming the dish as well. Finally, parents of the participants were actively involved in the study, as they had to prepare the vegetables at home and monitor intake and liking during the intervention. Their commitment and higher awareness for the vegetable intake of their child may have stimulated vegetable intake in general.

The increase in spinach intake in this study was substantial, as children changed their intake from pre to post-intervention from the lower limit almost to the upper limit of the daily recommend amount. This 'success' could be used to plead for introduction of a warm lunch including vegetables at the day-care which is not common practice in the Netherlands: children are accustomed to have a warm meal including their main source of vegetables normally served at home in the evening. Our findings imply to make use of different meal times or snack moments to serve children more/sufficient vegetables for stimulating their intake.

The current study has some limitations as well. In current study vegetables were served at home during the intervention and therefore less controlled than performing measurements in a more laboratory setting. However, it concerns here secondary outcome measures and the main outcome measures were assessed at the day-care with more experimental control by the researchers and by the instructed day-care staff. However at home, parental factors like parental style (e.g. positive reinforcement), family composition (e.g. present brother(s)/sister(s), meal composition (e.g. rice/pasta and/or meat/fish/vegetarian product) could have affected the intake during the intervention. However, we assume that variations in these factors would be randomly distributed across the four groups. In addition, parents proved to be committed to the intervention and motivated to comply with the procedures; 95% returned completed food diaries. All together, we belief it is unlikely that factors such as differences in parental style or for example deviations from the procedures caused a systematic effect on intake across the groups. It may have contributed, however, to the overall positive effect, which was detected across all the groups.

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## Conclusion

The findings of this study demonstrate that repeatedly offering a common but generally disliked vegetable is an effective method to stimulate vegetable consumption in young children. Masking or covering vegetables did not have an additional effect. This suggests that just offering pure vegetable tastes repeatedly is sufficient to increase intake. Additionally, all groups increased their vegetable intake over time, including the control group. This indicates that context factors such as a positive ambience and peer pressure mediate the effect and add to the acceptance of vegetables in children. This is an important finding which makes it easier for parents/caregivers and public health to stimulate children's vegetable intake at home and at day-cares. Only neophobic children did not improve their vegetable intake. Probably this specific group needs other strategies to improve their vegetable acceptance for increasing their intake.

## Acknowledgements

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# Chapter 5

**Influence of choice on vegetable intake in children:**

**An in-home study**

The veggies-at-home study

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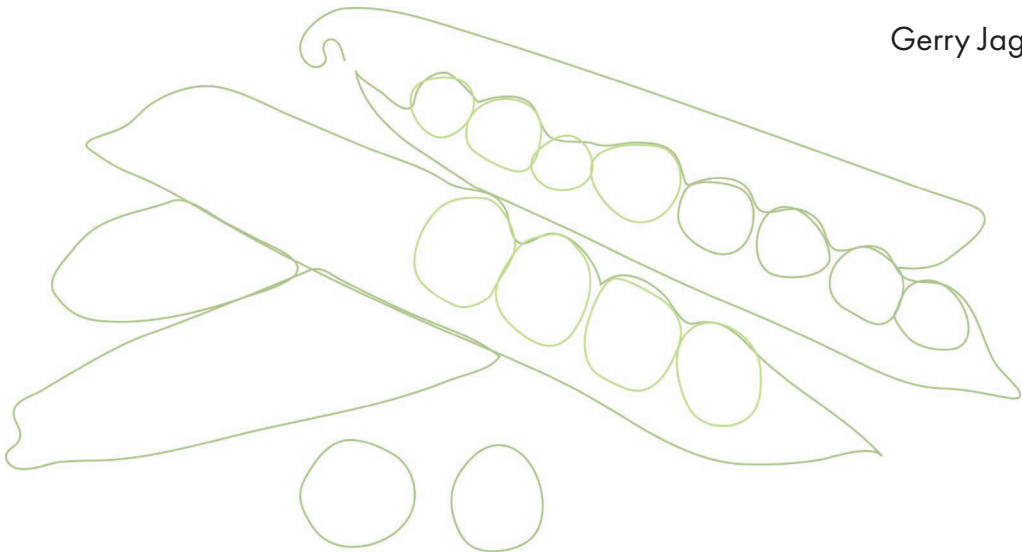
## Abstract

Children's vegetable consumption is still far below that recommended, and stimulating their intake is a challenge for caregivers. The objective of this study was to investigate whether choice-offering is an effective strategy to increase children's vegetable intake in an in-home situation. Seventy children (mean age 3.7; SD 1) randomly assigned to a choice or a no-choice condition, were exposed 12 times to six familiar target vegetables at home during dinner. In the choice group, two selected vegetables were offered each time, whereas the no-choice group only received one vegetable. Vegetable intake was measured by weighing children's plates before and after dinner. A mixed linear model with age, gender, and baseline vegetable liking as covariates was used to compare intake between the choice and the no-choice group. Mixed linear model analysis yielded estimated means for vegetable intake of 48.5g +/- 30 in the no-choice group and 57.7 g +/- 31 for the choice group ($P$ = 0.09). In addition, baseline vegetable liking ($P$ < 0.001) and age ($P$ = 0.06) predicted vegetable intake to be higher when the child liked vegetables better and with older age. These findings suggest that choice-offering has some, but hardly robust, effect on increasing vegetable intake in children. Other factors such as age and liking of vegetables also mediate the effect of offering a choice.

Keywords: Choice as strategy; Vegetable consumption; Young children; Healthy diet; Family dinner

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## Introduction

Poor vegetable intake in children is a persistent problem, despite increased awareness of the health importance of vegetable consumption [1-4]. Parents and caregivers often struggle with putting theory into practice in order to ensure that their children meet the recommended amounts of vegetable intake through meals at home [5-8]. There is a need for strategies that have proven effectiveness and are relatively easy to implement at home by caregivers themselves. One such strategy might be choice-offering. Increasing a person's options and choices has been shown to strengthen people's intrinsic motivation to actually implement life style behaviours, healthy eating behaviour, and physical exercise [9-11]. This holds true for children as well, as illustrated by an observational study where mothers indicated that it is important for 3- and 4-years-old children to have some freedom of choice over personal issues, like which clothes to wear or which game to play, in order to develop a sense of autonomy and individuality [12]. Evidence for the effect of choice-offering on children's vegetable consumption is mixed. Hendy [10] found that choice-offering together with rewarding the child and insisting on trying one bite were the most effective strategies to accept new fruits and vegetables in preschool children. However, actual intake was not measured in this study [10]. Zeinstra and colleagues studied whether offering a pre-meal choice would affect children's vegetable liking, intake and motivation to eat familiar vegetables, but found no effect [13]. Here, ambience characteristics (children were invited to have dinner with one parent in a restaurant and were excited about this) may have overruled the impact of the experimental manipulation (choice/no-choice). A recent study from Rohlfs Domínguez, Gámiz [14] used a modified version of the study design from Zeinstra, Renes [13], now with unfamiliar vegetables and carried out in a school canteen. They reported a positive effect of choice-offering on children's vegetable intake [14]. Hence, choice-offering has potential, but several factors seem to differentially affect its effectiveness. For example, it matters whether novel or familiar vegetables are involved [10, 13, 14]. In addition, consumption context seems to play a role (e.g. day-care, school, dining out) [15-17]. The objective of the present study was to investigate whether choice-offering is effective in promoting young children's vegetable intake of familiar vegetables when applied by caregivers in an in-home situation.

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## Materials and methods

### Participants

Seventy-five children, aged 2–5 years, recruited from 3 day-care centres in Wageningen, The Netherlands, started the intervention. Parents with children in the targeted age range received an information letter and an invitation to register their child(ren) for participation via the day-cares. Participation was voluntary and parents and day care-centres were thoroughly informed about the study. Due to drop out (e.g. relocation), the reported data are based on a sample of 70 children (see Table 5.2, results section). Parents signed an informed consent for their child's participation. Participants were screened for food allergies and health problems (as reported by the parents). The study protocol was approved by the Institutional Review Board of Wageningen University and registered at the Dutch Trial Registration (NTR, TC = 3757). Sample size calculation was based on the mean vegetable consumption of Dutch toddlers (= 34 g) and an expected increase of one tablespoon of cooked vegetables eaten extra in the choice group (one tablespoon = 17 g = ½ SD) [7]. To detect a significant difference between groups, with alpha set at 0.05 and a power of 0.80, at least 25 children were needed per group.

### Experimental design

Participants were randomly assigned to either a 'no-choice' or a 'choice' group using a two-block design (no-choice/choice), stratified for age (two, three and four to five years of age). Each child was exposed 12 times to six familiar target vegetables at home during dinner, which is the traditional hot meal including vegetables in The Netherlands. The no-choice group received only one type of vegetable per dinner session, whereas the choice group received two types of vegetables from which to choose, or they could choose to eat both vegetables during the meal. Table 5.1 gives an example of the total exposure schedule for one participant in each group. The vegetables were served according to a semi-randomized schedule with the restriction that participants in the no-choice group did not receive the same vegetables on two successive days. Although the same six target vegetables were offered in both groups, participants in the choice-group were exposed to a larger variety (i.e. larger number of repeated exposures) of vegetables compared to the no-choice group, as a consequence of the design. To be able to check for a potential effect of variety in addition to choice on vegetable intake, two of the six target vegetables, i.e. peas and string beans, were offered an equal number of times (i.e. twice) during the whole intervention period in both groups (see Table 1).

**Table 5.1** Experimental design showing exposure-schedule of one participant during the study for the no choice and the choice-group. As target vegetables the children received broccoli (bro), carrots (Crt), peas (Pea), cauliflower (Cfl), French beans (FB), and string beans (SB) twice per week at home. Peas (Pea) and String Beans (SB) were offered an equal number of times (i.e. two times) in the choice and the no-choice group.

| Group | day 1 | day 2 | day 3 | day 4 | day 5 | day 6 | day 7 | day 8 | day 9 | day 10 | day 11 | day 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *No choice* | Bro | Crt | SB | Cfl | FB | Pea | Crt | FB | Cfl | Bro | Pea | SB |
| *Choice* | Bro/ SB | Crt/ Cfl | FB/ Cfl | Crt/ Cfl | Cfl/ FB | Pea / Crt | Bro/ FB | Bro/ Crt | SB / Bro | Bro/ Cfl | FB/ Pea | FB/ Crt |

## Procedures

Procedures were explained to the parents in an explanatory session and to accustom the families to these procedures, the week prior to the intervention was used to practice. Families received one vegetable parcel including the vegetables for two meals, cooking instructions, a food diary, and a standardized weighing scale with a precision of 1 g (Soehnle, FIESTA, Nassau, Germany). Cooking instructions involved how to prepare the vegetables, the duration of cooking time, and to use no other vegetable(s) other than the provided ones. After dinner parents had to fill out the food diary by providing the following information: any deviation from the prescribed instructions; start and end time of the dinner; the consumed vegetables and other meal components (e.g. meat/ fish, pasta/potatoes); the weight of the vegetables served to the child before and after the meal; the child's liking of the vegetables (parent's perception); and whether the child had physical complaints that day that might have affected appetite.

## Study foods

Six target vegetables were offered in both groups: peas, carrots, broccoli, French beans, cauliflower, and string beans. Selection of the target vegetables was based on the following criteria: (i) most commonly eaten vegetables by 2- to 5-year-old Dutch children; (ii) vegetables fitting into a typical Dutch dinner [7]. The packages contained the recommended daily intake (RDA) of vegetables (raw, fresh and pre-cut) for the whole family for two dinner sessions per week (see Table 5.2), to ensure that every member of the family could comply with the RDA. However, parents were instructed to serve their children as much as they liked to eat or as much as they were used to eat, to minimize interference with their normal habits/schedule. As a consequence a child could eat more than the RDA of its age group.

**Table 5.2** Amount in grams of vegetables offered per person by group based on RDA.

| | No choice | Choice [*] | |
| --- | --- | --- | --- |
| | Target vegetable | Target vegetable 1 | Target vegetable 2 |
| *Adult* | 200 | 150 | 150 |
| *Child > 4 years* | 150 | 112.5 | 112.5 |
| *Child < 4 years* | 100 | 75 | 75 |

[*] In the choice group, where two vegetables were served per meal, each family member received ¾ of the RDA per vegetable. Thus, total portions of vegetables were 1.5 times the total portions in the no-choice condition. This was done to make sure that portion sizes per vegetable were still sufficient if children choose to eat only one out of the two offered vegetables.

## Measures

The main outcome of the study was the children's intake (in gram) of the vegetables. Vegetable intake was measured by weighing their plates before and after dinner (left overs). Data collected prior to the intervention included the child's date of birth, gender, weight, height, and general vegetable liking. This baseline vegetable liking was rated by the parents in response to the question "My child likes most of the vegetables" on a 5-point scale ranging from "1 = totally disagree" to "5 = totally agree". BMI z-scores were calculated using the WHO anthropometric calculator (v 3.2.2, www.who.int/childgrowth/software/en/).

## Statistics

Statistical programme PAWS Statistics was used (version 19; SPSS Inc. Chicago, USA) to analyse the data. A mixed linear model was used to test for significant differences in intake between the two groups (no-choice/choice). We used a random intercept model that takes into account the individual variation in intercepts between participants and the correlation between observations in the same participant. Intake during the 12 exposures was the dependent variable and group was the fixed covariate (model 1). According to the literature, age, gender, and vegetable liking can influence vegetable intake in children [7, 8, 18-21]. Therefore, in a second step, age, gender, and baseline vegetable liking were entered as fixed covariates (model 2). Model 2 is represented as follows:

$$\text{Intake}_{ij} = b_{0j} + b_1.\text{group}_j + b_2.\text{age}_j + b_3.\text{gender}_j + b_4.\text{baseline vegetable liking}_j + \varepsilon_{ij}$$

where intake$_{ij}$ is the vegetable intake of child $j$ at time $i$ , b$_{0j}$ is the between-subject random effect, and $\varepsilon_{ij}$ is the within-subject error.

Spearman's Rho correlation coefficients were used to investigate the relationship between baseline vegetable liking and vegetable intake during the intervention. General

linear model (GLM) multivariate analysis was performed to test a possible effect of variety with average intake of each vegetable type (i.e. the mean intake of each of the six vegetables across the 12 meals during the intervention) as dependent variable and condition/group (choice, no-choice) as between-subjects variable.

## Results

### Age, gender, and BMI

Table 5.3 shows the demographic characteristics of the population. The groups did not differ on characteristics like age, gender, and BMI.

### Baseline vegetable liking and vegetable consumption during the intervention

Score for baseline vegetable liking was positively correlated with average overall vegetable intake ($\varrho$ = 0.47, $P$ < 0.001). The children who liked vegetables more before the intervention, tended to consume overall more vegetables. Despite randomized assignment of children to either the choice or the no-choice group, children in the no-choice group on average liked vegetables better than children in the choice group (mean score 3.2 (SD 1.0) and 2.5 (SD 1.0) respectively, $P$ < 0.01). Figure 5.1 shows the vegetable intake per group per exposure, which fluctuates (45–66 g). Standard errors indicate high variability between-subjects across the exposures.

**Table 5.3**. Subject and group characteristics of children included in data-analysis.

| | Total in analysis (n= 70) | Girls (n = 35) | Boys (n = 35) | Participants No-choice group (n =36) | Participants Choice group (n = 34) |
|---|---|---|---|---|---|
| *Age* (Months) | | | | | |
| Mean (SD) | 3.7 (1.0) | 3.7 (1.1) | 3.7 (0.9) | 3.7 (1.1) | 3.6 (1.0) |
| Range | 1.9–5.9 | 1.9–5.8 | 2.2-5.9 | 1.9–5.9 | 2.1–5.6 |
| *Weight* (kg) | | | | | |
| Mean (SD) | 15.6 (2.8) | 15.3 (2.9) | 16.0 (2.8) | 15.8 (3) | 15.4 (2.7) |
| Range | 10-22 | 10–20.9 | 10-22 | 10-22 | 11–20.9 |
| *Height* (cm) | | | | | |
| Mean (SD) | 101.0 (9.9) | 100 (11.3) | 102.0 (8.5) | 100.7 (9.7) | 101.3 (10.4) |
| Range | 80-121 | 80-121 | 86-121 | 80-121 | 84–121 |
| **BMI**-z-score | | | | | |
| WHO (SD) | -0.19 (1.1) | -0.26 (1.2) | -0.12 (1.0) | -0.18 (1.1) | -0.2 (1.1) |
| **Baseline vegetable liking** (SD) | 2.9 (1.1) | 2.7 (1.1) | 3.1 (1.0) | 3.2 (1.0) | 2.5 (1.0)[*] |

[*] $P$ < 0.01

## Mixed model: predicting vegetable intake by choice-offering

Table 5.4 shows the summarized results. Mixed model analyses yielded estimated mean vegetable intake figures for the two groups (choice/no-choice). Based on model 1 (crude), the estimated means were 52.1 (SD 35) g for the no-choice group and 54.2 (SD 36) g for the choice group. For model 2 (COV), with gender, age and baseline vegetable liking as covariates, the estimated means for intake for the no-choice group were 48.5 (SD 30) g and 57.7 (SD 31) g for the choice group. Model 2 fitted significantly better than model 1 ($\chi^2$ (3) = 269.6, $P$ < 0.0001). Group (i.e. choice/no-choice) was a marginal significant ($P$ = 0.09) predictor of vegetable intake during the intervention in the expected direction. Baseline vegetable liking significantly predicted vegetable intake ($P$ < 0.001), age had a marginally significant effect ($P$ = 0.06), and gender had no effect ($P$ = 0.52). No significant interaction effects of group with the covariates in the model were found. The effects of group, age and baseline vegetable liking were in the expected direction, with a regression coefficient of $\beta_{choice}$ = 9.26 (5.39 SE), $\beta_{age}$ = 4.84 (2.48 SE) and $\beta_{veg.liking}$ = 12.04 (2.59 SE), respectively. Hence, choice was positively associated with higher vegetable intake, as was age and baseline vegetable liking.
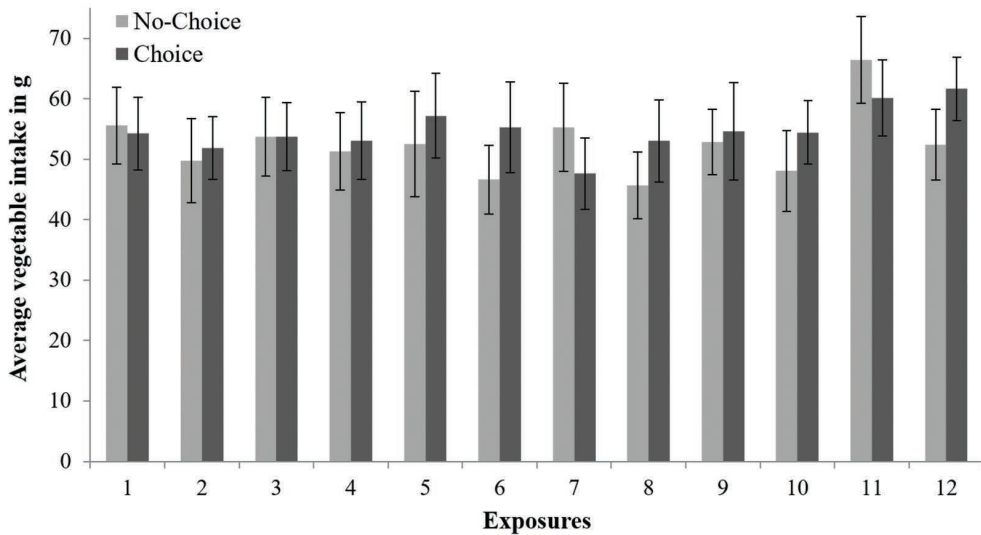


**Figure 5.1** Average vegetable intake in gram per group per exposure and its standard error.

**Table 5.4** Estimated means and corresponding P-values per model (model 1 (crude); model 2 (COV), incorporating covariates age, gender, and baseline vegetable liking).

|  | Estimated means (95% CI) | | *P*-value |
|---|---|---|---|
|  | No Choice | Choice |  |
| **Model 1** | 52.1 [43.6; 60.6] | 54.2 [45.5; 62.9] | 0.73 |
| **Model 2** | 48.5 [41.2; 55.7] | 57.7 [50.2; 65.2] | 0.09 |
| **Age** |  |  | 0.06 |
| **Gender** |  |  | 0.52 |
| **Baseline vegetable liking** |  |  | < 0.001 |

## Variety/type of vegetable

Table 5.5 shows the children's intake of each vegetable (broccoli, carrot, cauliflower, French beans, peas, string beans) during the intervention period per group. The results indicate that some vegetables (cauliflower, carrots) were eaten more than others (peas). However, a GLM multivariate analysis with mean intake of each vegetable as dependent variable and group (choice, no choice) as between-subjects variable, revealed no significant effect of group (F (6, 62) = 0.8; $P$ = 0.6) on vegetable intake per variety, nor an interaction effect ($P$ = 0.4). This indicates that although children in the choice group were exposed to a larger variety (i.e. larger number of repeated exposures) than the children in the no-choice group, this did not affect overall intake of the target vegetables during the intervention.

**Table 5.5** Children's vegetable intake in g (median (SD)) per variety and per group.

|  | Broccoli | Carrots | Peas | String beans | Cauliflower | French beans |
|---|---|---|---|---|---|---|
| **No-choice** Range | 34.5 (31.6) 0-165[a] | 38.0 (35.1) 0-144 | 23.8 (24.2) 0-93 | 23.5 (37.2) 0-149 | 39.7 (41.0) 0-190[a] | 25.8 (25.1) 0-105 |
| **Choice** Range | 30.0 (21.9) 0-80 | 36.0 (33.1) 0-128 | 22.5 (25.6) 0-96 | 27.5 (28.1) 0-95 | 35 (30.9) 0-112 | 35 (42.1) 0-233[a] |

[a] Some children ate more than the daily recommendation since parents were instructed to serve their children as much as they liked to eat/as much as they were used to eat, to minimize interference with their normal routine.

## Discussion

The aim of this study was to investigate whether choice-offering is an effective strategy to increase children's intake of familiar vegetables during the family dinner in an in-home situation. After correcting for potential confounding effects of age, gender, and baseline vegetable liking, the results of this study showed a small, but hardly robust effect of choice-offering on vegetable intake in the expected direction (i.e. choice-offering increases vegetable intake). Age and vegetable liking prior to the intervention also affected consumption; the older the children, and the more children liked vegetables before the intervention (as reported by the parents), the more vegetables they ate during the intervention. Gender made no difference.

The results of this study are partly in line with recent work by Rohlfs Domínguez, Gámiz [14], who reported a positive effect of choice-offering, but not with the findings of Zeinstra, Renes [13], who observed no effect of choice-offering. The discrepancy in findings might be related to ambiance or consumption context factors. In Zeinstra, Renes [13] study, children were randomized over three conditions (i.e. a pre-meal choice, a choice or a no-choice condition) and, as in our study, they were served familiar vegetables (together with potatoes and sausages) but in a restaurant setting (non-familiar context) during dinner. In Rohlfs Domínguez, Gámiz [14] study, children were also randomized over these three conditions but cooked vegetables were presented as a single dish (not as part of the meal; unfamiliar presentation) in a school canteen (familiar context) during lunch. Our study took place in a trusted familiar context at home, where familiar vegetables were served as part of the meal during dinner rather than lunch. In The Netherlands, dinner is the main meal where children eat vegetables, whereas lunch commonly consists of a sandwich meal without vegetable dishes. The current intervention did not interfere with the children's normal routine, a factor that, together with a trusted environment, is thought to be positively related to food acceptance in children [15, 16].

Former studies investigating the effect of choice on fruit and vegetable intake showed that a salad bar, serving four to seven different kinds of vegetables and fruits, did not increase fruit and vegetable consumption amongst elementary school students compared with students who got pre-portioned servings [22]. Fruit and vegetable consumption was positively related to the number of different fruit and vegetable items offered at the salad bar. A larger variety offered made it more likely that the children's preferred fruits and vegetables were amongst the selection from which to choose [22]. The idea that more variety can increase children's vegetable intake was confirmed by recent studies from Bucher, Siegrist [23] and Roe, Meengs [24]. Roe, Meengs [24] showed that providing a variety of vegetables and fruit as a snack led to increased consumption of both, but pre-school children were less likely to select vegetables than fruit. A study from Bucher, Siegrist [23] reported that children (7-10 years old) chose a more balanced lunch when offered a

two-vegetable choice of a fake food buffet (i.e. a method that uses replica food items for experimental investigation of food choice) compared to children who were offered only one vegetable. These studies indicate that serving a variety can be effective in increasing intake. The distinction between 'variety' and 'choice' as an intervention is sometimes not straightforward, because choice-offering implies that more than one vegetable at a time is provided, hence a bigger variety. In our study, two of the six target vegetables were offered an equal number of times in both conditions. As a consequence of the design, the remaining four vegetables were offered more often in the choice group. Although some vegetables (cauliflower, carrots) were eaten more than others (peas), this was regardless of choice status or frequency with which the vegetables were offered during the intervention. Although we acknowledge that offering variety can positively affect intake, it did not explain the outcomes of the present study. Instead, it seems more plausible that 'familiarity' comes into play here. Familiarity of food is an important factor in food acceptance and preferences, and shapes children's eating behaviour [15]. Recent work has confirmed that repeated exposure is one the most effective strategies to increase acceptance of a novel or unfamiliar vegetable taste (i.e. artichoke puree and green vegetable soup) [25-27]. This mainly concerns the initial acceptance of a novel taste, that is, the willingness to try and to start eating unfamiliar vegetables where the amount consumed is of less importance. This is different from the situation where one wants to increase the liking, and subsequently the intake of vegetables with which the child is already familiar, in order to ensure that children meet the recommended amount of daily vegetable intake.

There are several explanations as to why the current study failed to demonstrate a robust effect of choice. Firstly, choice-offering may only work with regard to issues of consequence or of interest to children. Provision of choice is thought to induce feelings of autonomy and a sense of personal control, and in turn this increases intrinsic motivation [28]. Katz and Assor [29] describe how more intrinsic motivation has been related to psychological and behavioural benefits such as higher interest, liking, greater satisfaction, and better health. However, they also propose that choice can only be effective if the topic of choice reflects the needs of the participants. Hence, choices can be encouraging if the participant has an interest in the topic [29]. Because young children often do not like vegetables in the first place, it is conceivable that offering a choice signifies little intrinsic salience or motivation to them. To be motivated to choose vegetables, one may first have to like them. It seems that there are conditions to be met, such as interest in the topic (i.e. vegetable liking), to make a strategy like choice-offering effective in enhancing children's vegetable intake.

Second, as consequence of the design, the total amount of vegetables offered per meal in the choice group was 1.5 times higher than in the no-choice group. This was done to enable the child to eat sufficient amounts of either one of the vegetables offered, or from

both. However, offering larger portions could have influenced the outcome. Bigger portion size is related to higher intake [30-33], at least for palatable foods such as snacks, French fries, popcorn or sweets, but it is less clear whether this holds true for vegetables. In a study from Mathias, Rolls [34] children increased their vegetable and fruit intake when their portions were doubled, but this effect was limited to children who liked these particular foods, whereas no effects were seen in children who disliked vegetables and fruits. Other studies showed that doubling the portion size was only effective for fruits but not for vegetables in children aged 5-6 years [35], or only had a positive effect on vegetable intake in children when combined with other strategies like eating vegetables as a starter or entrée [36, 37]. Taken together, offering a bigger portion of vegetables alone may not be sufficient to increase intake. Future research should focus further on the boundary conditions for portion size to be effective in case of vegetable consumption.

Third, vegetable liking could have influenced the outcome. In our study, vegetable liking was also positively related to intake. The choice and the no-choice group differed in baseline vegetable liking .This difference was unintended and a potential confounder. Vegetable liking was included in the mixed model analysis as a covariate, but in retrospect, it would have been better if we had randomized children into the choice and no-choice group on the basis of their baseline vegetable liking scores so that we would have had an even distribution of this characteristic across groups. Nevertheless, chances are low that the difference in vegetable liking between the groups led to an overestimation of the effect of choice-offering on intake, because vegetable liking was on average higher in the no-choice group. Instead, it may have weakened the observed small effect of choice on intake.

Fourth, it could be that part of the children in this study was too young to be able to appreciate the concept of choice. One can question whether children as young as two years old already mastered the cognitive concepts and skills needed to evaluate and appreciate a choice option in a similar way as 5-year-old children. We found an effect of age on vegetable intake but no interaction effect with choice/no choice. It is therefore, not possible to draw any conclusions based on the present study. Hence, whether a strategy like choice-offering in relation to food and vegetables in particular is better suited for somewhat older children, remains to be determined in future studies.

To our best knowledge, this is the first study to investigate choice-offering as a strategy to increase vegetable intake in a real-life setting. The intervention took place in an in-home situation, with as little interference in the normal dinner routine as possible. We believe this strengthens the face validity of this study. A potential drawback of this approach is of course that, in a naturalistic setting, the researcher has less experimental control. Parental and familial factors may have influenced the intake, such as parental style or the presence/absence of brothers and sisters. And even though the vegetables were prepared

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and offered the same across the families, other components of the meal would have been different (i.e. rice or pasta, type of meat provided). In addition, children may have been more or less hungry at the start of each dinner. Due to randomization, however, we assume that variations in these other factors were randomly distributed across groups. In addition, we strived to have optimal commitment and compliance from the parents to the procedures. Ninety-five percent of the parents returned completed food diaries, indicating high involvement and compliance with the procedures. Taken together, we feel it is unlikely, that other factors such as parental style or deviations from the procedures caused a systematic effect on vegetable intake in one of the conditions (choice/no-choice) selectively. Nonetheless, factors that were not under experimental control act as confounders and result in 'noise' in the outcomes, which makes it harder to detect a significant effect of choice-offering.

Another limitation of the current study is that our design and sample sizes did not allow for splitting the groups into subgroups for different ages. This would have compromised statistical power. We suspect that choice-offering may have been less effective in the younger children, say two and three-years of age, because they may still lack the cognitive development to truly understand and appreciate the concept of choice. Hence, future studies should focus on different age groups to investigate whether there is a minimal age to apply a strategy like choice-offering.

Finally, future research could examine combined strategies of mere exposure, followed by other strategies, for example choice-offering. Especially in young children, repeated exposure can be applied to enhance acceptance and intake of novel or unfamiliar vegetables [25-27]. Once the child has accepted the novel taste and is willing to eat the vegetable, that is, the vegetable has become familiar, choice-offering could be implemented as a strategy to further increase vegetable consumption until the child has reached the recommended daily amounts.

## Conclusion

In summary, the results of this study suggest a small effect of choice-offering on children's vegetable intake during dinner in an in-home situation. Various factors seem to influence the effect of offering a choice, such as consumption context, age, and relatedly, the child's development stage, whether it concerns novel or familiar vegetables, and overall liking of vegetables by the child in the first place. We need future studies to further substantiate these findings. If we can identify the factors that strengthen or weaken the effect of choice-offering, it could be a relatively simple strategy that parents can use at home to stimulate their children's vegetable intake.

## Acknowledgements

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# Chapter 6

**Relation between breastfeeding duration and later vegetable intake in 2-6-year-old children in 10 studies across Europe**

Breastfeeding supports children's vegetable intake
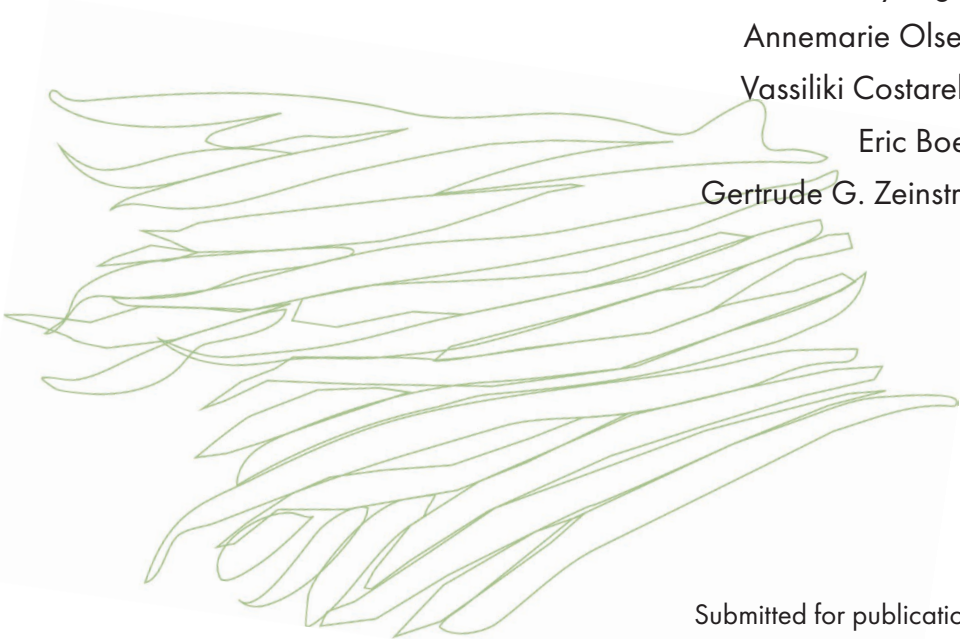
Victoire W.T. de Wild

Gerry Jager

Annemarie Olsen

Vassiliki Costarelli

Eric Boer

Gertrude G. Zeinstra

## Abstract

### Background and objective

Breastfeeding facilitates young children's acceptance of new foods, including vegetables, but this relationship seems inconsistent across studies. Because increasing children's vegetable intake remains challenging, it is important to investigate whether breastfeeding has indeed potential to influence children's vegetable intake at a later age. The aim was –using a unique HabEat dataset from European experiments that measured children's actual vegetable intake– to investigate whether breastfeeding duration predicts vegetable intake in 2–6-year-old children.

### Methods

We used a conceptual model to predict children's vegetable intake based on breastfeeding duration, with gender and age as (co)variates using General Linear Model (GLM) analysis. Vegetable intake was actually measured (in grams) in 10 experimental studies in children aged 2–6 years (N = 750) across three European countries: Denmark, Greece, and The Netherlands. We also investigated the relationships between several child (eating) characteristics and vegetable intake.

### Results

Breastfeeding duration was positively associated with children's vegetable intake in all the three countries. Gender and age had no influence. We noticed differences in the outcome of this relation across and between the countries using different cut-off times of breastfeeding duration, implying that the relationship is not straightforward. All countries showed a negative relation between food neophobia and children's vegetable intake (all $p < 0.01$) and a positive relation between vegetable liking and intake (all $p < 0.01$).

### Conclusions

Breastfeeding duration contributes to vegetable intake in 2–6-year-old children, but the mechanism of this relation and the required period of breastfeeding duration remain unclear. Other factors such as vegetable liking, food neophobia, and enjoyment of food play a role in vegetable intake. Given these findings, total breastfeeding duration and other child characteristics may be more important than exclusive breastfeeding alone.

Keywords: Length of breastfeeding; Actual vegetable consumption; Child (eating) characteristics; Food neophobia; Food enjoyment; Real-life data; Three European countries

## Introduction

According to the World Health Organization's (WHO) guidelines, breastfeeding offers optimal nutrition for an infant, and the WHO recommends that infants should be exclusively breastfed for the first six months of life [1]. Research has shown that breastfeeding may have additional advantages; breastfed children are more likely than formula-fed children to accept novel tastes –including vegetables– later in life [2-7]. However, not all studies show evidence for this relationship [8-10]. The relationship between breastfeeding history and vegetable consumption later in life is particularly relevant because a high vegetable intake is associated with a reduced risk of multiple diseases (e.g. obesity, diabetes, cardiovascular diseases, and some types of cancer) [10-13]. Nevertheless, children's vegetable intake is far below that recommended, and previous studies have shown that increasing children's vegetable intake is a difficult task [14-17]. Therefore, it is important to investigate whether breastfeeding has indeed potential to influence children's vegetable intake at a later age.

The majority of studies investigating the relationship between breastfeeding history and later vegetable consumption are cohort studies involving retrospective research, meaning that children's vegetable and fruit consumption measures are based on food frequency questionnaires filled out by parents or carers about a previous period. These self-reported data can cause an information bias, leading to over- or underreporting of the child's actual vegetable consumption. Using actual intake data is more reliable. Such data have been collected in the EU research programme HabEat, which examined how food habits are formed in infants and young children with a focus on fruit and vegetables. Within this project, 10 intervention studies were performed in different European countries [18-20]. All these studies aimed to increase vegetable intake in 2–6-year-old children and investigated the effectiveness of different (social) learning strategies: e.g. imitation of a child idol, imitation of a teacher, choice-offering, involving the children in vegetable preparation, and repeated exposure in combination with sensory variation. Children's actual vegetable intake was measured, and we used these data in the present paper. Data on demographics, breastfeeding history, and child and parent characteristics were collected across the HabEat studies via questionnaires. This enabled us to study the relationship between breastfeeding history and other relevant factors, and the child's actual vegetable consumption as measured prior to the intervention in these studies.

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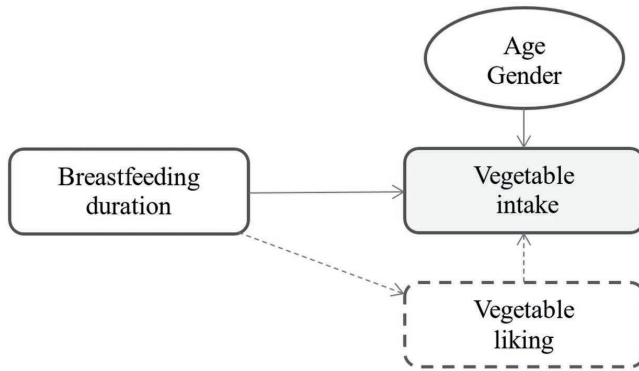
**Figure 6.1** Flowchart of the theoretical model to illustrate the relation between breastfeeding duration and children's vegetable intake. In this model, we predict vegetable intake based on breastfeeding duration, age, and gender. Apart from a direct effect on intake, vegetable liking may be connected to vegetable intake indirectly, via breastfeeding duration. Solid lines demonstrate the relationships investigated in this study. Dotted lines and the dotted line box are added to complete the model, but were not included in the statistical analysis.

We used a conceptual model (see Figure 6.1) to explore the effect of breastfeeding duration on children's vegetable intake. Apart from breastfeeding duration, age and gender were included in the model as separate factors (covariates), on the assumption that age and gender are related to children's vegetable intake (e.g. older children eat more, as do boys) but are causally unrelated to breastfeeding duration [16, 17, 21, 22]. Children's liking of vegetables is also known to be related to vegetable intake [9]. Apart from a direct effect on intake, vegetable liking may be connected to vegetable intake indirectly, via breastfeeding duration. This is reflected in the previously mentioned notion that breastfed children are more likely to accept novel tastes. For this reason, it is difficult to disentangle the effects of breastfeeding duration and vegetable liking on vegetable intake. Therefore, vegetable liking was not included in the main model, but was taken into account together with other factors that are thought to be associated with vegetable intake, such as child eating behaviour characteristics. For example, lower levels of neophobia and a higher enjoyment of food have both been associated with a higher liking and intake of vegetables in 2–6-year-old children [9, 23].

Hence, it was additionally investigated how these child (eating) and parental characteristics, such as vegetable liking, maternal education, food neophobia, enjoyment of food, food fussiness, satiety responsiveness, and food responsiveness, correlate to children's vegetable consumption later in life.

To summarize, in the present study, data for 750 children aged between 2 and 6 years from Denmark, Greece, and The Netherlands were collated and analysed. The aim was to investigate –making use of the unique HabEat dataset from European experiments

that measured children's actual vegetable intake– whether breastfeeding duration, with age and gender as covariates, predicts vegetable intake in 2–6-year-old children. In addition, we investigated the relationships between child (eating) characteristics and children's vegetable intake.

## Subjects and Methods

The analyses were based on data from 10 experimental studies involving 2–6-year-old children, performed between 2011 and 2014 at four institutions in three countries: Denmark (University of Copenhagen: two studies), Greece (Harokopio University: three studies), and The Netherlands (Wageningen University: one study; Wageningen UR Food & Biobased Research: four studies). Vegetable intake was measured before and after the intervention and during the intervention sessions. An overview of the design of each study is presented in Table 6.1 Studies were executed in naturalistic settings such as day-care centres, primary schools, the home environment, and a restaurant. Target vegetables used in the studies were carrots, Chinese radishes, sugar snaps, corn, cucumbers, tomatoes, peas, green beans, broccoli, cauliflower, and string beans.

In addition to collecting study-specific data, all studies collected similar data on demographics and child characteristics –e.g. Child Eating Behaviour Questionnaire (CEBQ) [24]– to enable pooling of the data for joint analyses. Vegetable intake was the main outcome measure in all studies. The first exposure to, and intake of, the target vegetable was used as baseline measure for the present analyses. Henceforth, we refer to baseline or first exposure intake as 'initial vegetable intake'.

### Vegetable intake

Initial vegetable intake was used as the dependent variable in the current data analyses. In all experiments, children were offered a portion of 100g of vegetables, except for two Dutch studies which offered 130g and 150g, respectively. Therefore, initial vegetable intake was converted to a ratio measure (100%) to be able to compare the data from all 10 studies. Vegetables were offered raw as a snack (morning or afternoon), raw just before lunch, or cooked as a part of the main meal (evening), depending on what fitted best in the dietary customs per country (see Table 6.1). Intake in grams was calculated by pre- and post-weighing the servings, and subtracting leftovers from the offered portion.

### Demographic variables

Before the start of the intervention part of the studies, parents completed questionnaires at home on demographics and child eating behaviour characteristics. Parents reported

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their child's date of birth, gender, weight, height, and maternal educational level. Child body mass index (BMI) z-scores were calculated using the WHO anthropometric calculator (WHO v3.2.2, www.who.int/childgrowth/software/en/). For comparison reasons, maternal educational level was rescaled from a 6- or 7-category scale to a 3-category scale: i.e. low, middle, and high ('low' is primary and/or secondary school, 'middle' is vocational education and 'high' is higher vocational education and/or university degree).

## Child eating behaviour characteristics

Breastfeeding history was assessed via two questions: 'breastfed yes/no' and 'duration of breastfeeding'. We chose 'total breastfeeding duration' instead of 'breastfed yes/no' for our model, to have a continuous and a more accurate measure. Breastfeeding duration was ascertained in weeks or months, except in the Greek sample, where seven categories were used (from < 1 week up to > 12 months), which were rescaled to weeks. In addition, parents were asked to report their child's vegetable liking on a 5-point scale ranging from '1 = totally dislikes' to '5 = totally likes'. In Greece, vegetable liking was scored on a 7-point scale and consequently rescaled to a 5-point scale for comparison reasons. To assess the child's eating habits, parents completed the seven dimensions of the validated Child Eating Behaviour Questionnaire (CEBQ) [24] and the 6-item version of the Food Neophobia Scale for Children (CFNS) [25, 26]. For the current data analyses, we used four dimensions of the CEBQ: food fussiness (FF; 6 items), satiety responsiveness (SR; 5 items), food responsiveness (FR; 5 items), and enjoyment of food (EF; 4 items). Items were scored on a 5-point scale ranging from '1 = never' to '5 = always' and per dimension averaged across the items. Higher scores correspond with a stronger expression of the characteristic.

The six statements of the CFNS were scored on a 5-point scale from '1 = strongly disagree' to '5 = strongly agree' and averaged across the items. Higher scores indicate stronger food neophobia. In total, data for 1107 children were initially included in the dataset. After excluding children with missing data for breastfeeding history and/or initial vegetable intake (n = 357), data for 750 children were used for statistical analyses.

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Table 6.1 Overview of the designs of the 10 studies performed in three countries (Denmark, Greece, and The Netherlands) within the European HabEat project with vegetable intake as main outcome.

| Study Type | Country (# studies) | Age (years) | Setting | Timing | Design (n) | Product | # exposures (+ portion) |
|---|---|---|---|---|---|---|---|
| **Repeated exposure + sensory variation** | DK (1) | 3–5 | Kindergarten | Afternoon snack +/-14.00h | Between subject (111) | Raw: Chinese radish sticks or triangles or grated | 7x 100g |
| | NL (1) | 4–6 | Primary school | Morning snack 10–10.30 h | Between subject (54) | Raw: carrots stick & slices | 10x 100g |
| **Imitation Teacher + idol** | GR (2) | 3–5 | Nursery | Before lunchtime 11.00 h | Between subject (167) | Raw: carrot sticks | 8x 100g |
| | NL (2) | 4–6 | Primary school | Morning snack 10–10.30 h | Between subject (177) | Raw: carrot sticks | 8x 100g |
| **Choice** | DK (1) | 3–5 | Kindergarten | Afternoon snack +/-14.00 h | Within subject (33) | Raw: sugar snaps, carrots, baby corn | 6x 100g |
| | GR (1) | 3–5 | Nursery | Before lunchtime 11.00 h | Within subject (46) | Raw: cucumber, tomato, carrot | 6x 100g |
| | NL (1) | 2–6 | At home | Main meal 'Dinner' +/-18.00 h | Between subject (67) | Cooked: peas, carrots, broccoli, green beans, cauliflower, string beans | 12x 100–150g |
| **Self-prepare** | NL (1) | 4–6 | Restaurant | Main meal 'Dinner' +/-18.00 h | Between subject (95) | Cooked: carrots, green beans | 4x 130g |

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## Statistics

### Descriptives

The statistical program PAWS Statistics (version 19; SPSS Inc., Chicago, USA) was used to analyse the data. The alpha level for significance was set at $p < 0.05$. First, means per country were calculated to investigate potential differences between the countries in initial vegetable intake, demographics, and eating behaviour characteristics. The assumption of normal distribution of the data (intake and most independent variables) was often not met. Therefore, non-parametric tests were applied for this analysis. Differences between the countries regarding intake, age, BMI z-score, and the children's eating characteristics were examined using Kruskal-Wallis tests followed by Mann Whitney tests for paired comparisons. Differences between the countries in gender distribution and percentages of children that were breastfed (yes/no) were analysed by using Chi-square tests. Jonckheere-Terpstra was used to test potential differences between countries regarding maternal education (ordinal data).

### Modelling intake by breastfeeding duration

The child's initial vegetable intake was modelled by using breastfeeding duration, gender, and age as predictor variables. The ratio measure of intake (the percentage of the offered vegetables eaten by the child) was not normally distributed as some of the children ate almost nothing (intake close to 0%), whereas others ate almost the maximum quantity offered (intake close to 100%). This type of data can be modelled by logistic regression. However, one of the disadvantages of logistic regression is that it requires a logarithmic transformation of the data and reverse transformation to allow for a meaningful interpretation of the results. We therefore compared a logistic regression approach with a General Linear Model (GLM) univariate procedure, which provides regression analysis and analysis of variance for one dependent variable (initial vegetable intake) by one or more independent variables (breastfeeding duration and age as covariates and gender as fixed variate). The GLM model is expressed as follows:

$$\text{Intake}_i = B_0 + B_{BF} \cdot X_{\text{Breastfeeding}} + B_{Age} \cdot X_{Age} + B_{Gender} \cdot X_{Gender} + \varepsilon_i$$

In this equation, intake is the predicted vegetable intake of child $i$, $B_0$ is the intercept, $B_{BF}$, $B_{Age}$, $B_{Gender}$ the regression coefficients, and $\varepsilon_i$ is the error for the $i$th participant.

Figure 6.2 shows the results based on logistic regression versus GLM for the Danish data where the difference between the outcomes of the two models was largest compared to the other countries. The differences between the models in predicting vegetable intake were minor, and this justified use of GLM instead of logistic regression. Therefore, GLM analysis was used and is reported in the remainder of this paper.

To investigate the influence of observations with a longer duration of breastfeeding on the estimation of the regression coefficients, the GLM analysis was repeated using different cut-off values for breastfeeding duration. As cut-off values, 6 months, 1 year, and 2.5 years were chosen; 6 months because this conforms with the WHO recommendation, 1 year as this was met by a substantial number of children breastfed in our current dataset, and 2.5 years as this represented a long breastfeeding period in the current sample.
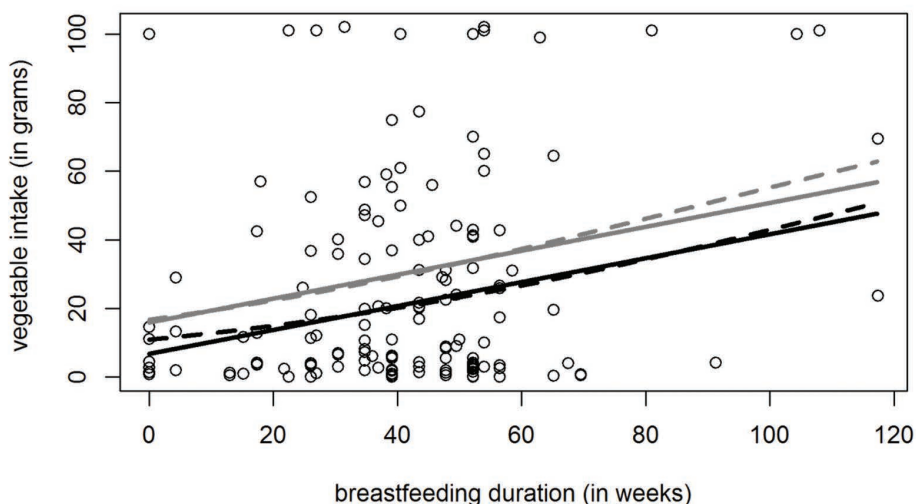


**Figure 6.2** Results of predicting children's vegetable intake based on breastfeeding duration in Denmark using logistic regression (dotted line) versus GLM (continuous line) with black lines for girls and grey lines for boys. The fact that differences between the models in predicting vegetable intake were minor justified the use of GLM.

## Other relevant relationships

Besides breastfeeding duration, age, and gender, other factors that may have affected initial vegetable intake are vegetable liking and relevant child eating behaviour characteristics. Spearman's correlation coefficients were calculated to examine the relationship between these factors.

# Results

## Demographic variables

Table 6.2 describes the characteristics of the study sample overall and per country.

**Table 6.2** Characteristics of participants (Means +/- SD) overall and per country (n in italics).

| n | All<br>*750* | Denmark<br>*144* | Greece<br>*213* | The Netherlands<br>*393* |
|---|---|---|---|---|
| *Demographics* | | | | |
| Age (months) | 54.9 +/- 9.9<br>*747* | 53.1 +/- 9.7 [a]<br>*143* | 51.2 +/- 5.8 [a]<br>*213* | 57.6 +/- 10.9 [b]<br>*391* |
| BMI z-score | -.006 +/- 1.4<br>*673* | .02 +/- 1.1 [a]<br>*121* | .30 +/- 1.6 [a]<br>*208* | -.20 +/- 1.3 [b]<br>*344* |
| Girls % | 47.8<br>*750* | 47.9<br>*144* | 43.7<br>*213* | 50.0<br>*393* |
| Maternal education<br>(1 = low; 2 = middle;<br>3 = high) | 2.5 +/- 0.7<br>*730* | 2.4 +/- 0.8<br>*140* | 2.6 +/- 0.6<br>*204* | 2.6 +/- 0.6<br>*386* |
| *Child eating behaviour characteristics* | | | | |
| Breastfeeding (% yes) | 82.4<br>*750* | 94.4 [a]<br>*144* | 83.0 [b]<br>*213* | 77.6 [b]<br>*393* |
| Breastfeeding duration<br>(weeks) | 23.1 +/- 21.6<br>*750* | 41.1 +/- 20.8 [a]<br>*144* | 15.2 +/- 15.4 [b]<br>*213* | 20.7 +/- 21.2 [c]<br>*393* |
| Vegetable liking<br>(5-point scale) | 3.5 +/- 1.3<br>*616* | 3.2 +/- 1.7 [a]<br>*113* | 3.9 +/- 1.0 [b]<br>*199* | 3.4 +/- 1.3 [a]<br>*304* |
| Child food neophobia<br>(5-point scale) | 3.0 +/- 1.0<br>*706* | 2.7 +/- 0.8 [a]<br>*111* | 3.1 +/- 0.8 [b]<br>*202* | 3.1 +/- 1.1 [b]<br>*393* |
| Enjoyment of food<br>(5-point scale) | 3.5 +/- 0.7<br>*746* | 3.7 +/- 0.7 [a]<br>*144* | 3.7 +/- 0.8 [a]<br>*210* | 3.4 +/- 0.7 [b]<br>*392* |
| Food fussiness<br>(5-point scale) | 3.0 +/- 0.9<br>736 | 2.7 +/- 0.8<br>144 | 3.0 +/- 0.9<br>300 | 3.1 +/- 0.8<br>392 |
| Satiety responsiveness<br>(5-point scale) | 3.1 +/- 0.7<br>*744* | 3.1 +/- 0.7 [a]<br>*144* | 3.2 +/- 0.7 [a]<br>*208* | 3.0 +/- 0.7 [b]<br>*392* |
| Food responsiveness<br>(5-point scale) | 2.2 +/- 0.7<br>*742* | 2.2 +/- 0.8<br>*144* | 2.3 +/- 0.7<br>*206* | 2.2 +/- 0.7<br>*392* |

[a, b, c] Means with different superscript letters are significantly different.

No significant differences between the three countries were found for gender and maternal education. The overall mean of 2.5 +/- 0.7 for maternal education reported in our sample was high (i.e. between a middle level score of 2 and a high level score of 3). Significant differences between countries were found for age ($\chi^2$ (2) = 89.1; $p < 0.001$) and BMI z-score ($\chi^2$ (2) = 17.5; $p < 0.001$). Dutch children were older (57.6 months +/- 10.9 old) than Danish children (53.1 months +/- 9.7 old; $p < 0.001$) and Greek children (51.2 months +/- 5.8 old; $p < 0.001$). BMI z-score was lower in The Netherlands (-0.20 +/- 1.3) compared to 0.02 +/- 1.1 in Denmark ($p = 0.02$) and 0.30 +/- 1.6 in Greece ($p < 0.001$).

### Child eating behaviour characteristics

A significant difference between the three countries was found for percentage of children being breastfed ($\chi^2$ (2) = 20.7; $p < 0.001$). In the Danish population, the percentage of breastfeeding mothers was higher (94.4 %) than in Greece (83.0 %; $p = 0.001$) and in The Netherlands (77.6 %; $p < 0.001$). In addition, breastfeeding duration differed significantly between the three countries ($\chi^2$ (2) = 135.1; $p < 0.001$). Danish children were breastfed for the longest period (41.1 weeks +/- 20.8), followed by Dutch children (20.7 weeks +/ 21.2), and, lastly, Greek children (15.2 weeks +/- 15.4) (all $p < 0.001$). Greek children scored higher on vegetable liking than Dutch and Danish children ($\chi^2$ (2) = 16.3; $p < 0.001$) with an average score of 3.9 +/- 1.0 versus 3.4 +/- 1.3 ($p < 0.001$) and 3.2 +/- 1.7 ($p = 0.01$), respectively. Differences between the countries were observed for food enjoyment ($\chi^2$ (2) = 17.0; $p < 0.001$), satiety responsiveness ($\chi^2$ (2) = 8.8; $p = 0.01$), and food fussiness ($\chi^2$ (2) = 17.8; $p < 0.001$). Dutch children scored lower on food enjoyment (3.4 +/- 0.7) and satiety responsiveness (3.0 +/- 0.7) than Greek (respectively, 3.7 +/- 0.8; $p < 0.001$ and 3.2 +/- 0.7; $p = 0.01$) and Danish children (respectively, 3.7 +/- 0.7; $p < 0.001$ and 3.1 +/- 0.7; $p = 0.04$). Danish children had a lower food fussiness score (2.7 +/- 0.8) than Greek and Dutch children (respectively, 3.0 +/- 0.8 and 3.1 +/- 0.8; both $p < 0.001$). Finally, Danish children were less neophobic (2.7 +/- 0.8) than Dutch and Greek children (both 3.1; $p < 0.001$). No significant differences were found for food responsiveness. Overall, we noticed significant differences between the countries for most variables, indicating heterogeneous sample populations in the different countries. Consequently, the model to predict vegetable intake was run per country.

### Modelling intake by breastfeeding duration

Table 6.3 shows the significant predictors of initial vegetable intake per country. Breastfeeding duration was a significant predictor in Denmark (B $_{BF}$ = 0.35; $p < 0.01$) and the Netherlands (B $_{BF}$ = 0.20; $p = 0.02$), where a longer breastfeeding duration

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predicted a higher initial vegetable intake, but not in Greece ($p = 0.20$). Age did not prove to be a significant predictor of vegetable intake in any of the three countries. Gender was a marginal predictor (B gender = 9.13; $p = 0.06$) for children's vegetable intake in Denmark only. Danish boys, who had been breastfed longer, tend to consume more vegetables.

**Table 6.3** Relationship between breastfeeding duration and children's vegetable intake using a General Linear Model with age as covariate and gender as fixed factor per country. Bold means $p < 0.05$ and italics means $p < 0.10$.

| Model | DK | GR | NL |
|---|---|---|---|
| n | *143* | *213* | *391* |
| **Intercept** | | | |
| B | 10.46 | *29.28* | 34.16 |
| $p$ | 0.46 | 0.15 | **< 0.001** |
| **Breastfeeding** | | | |
| B (SE) | 0.35 (0.12) | 0.19 (0.15) | 0.20 (0.85) |
| $p$ | **< 0.01** | 0.20 | **0.02** |
| **Age** | | | |
| B (SE) | -0.07 | 0.05 (0.39) | -0.02 (0.17) |
| $p$ | 0.78 | 0.90 | 0.90 |
| **Gender**[1] | | | |
| B (SE) | 9.13 (4.89) | 5.03(4.60) | 4.96 (3.61) |
| $p$ | *0.06* | 0.28 | 0.17 |

DK = Denmark; GR = Greece; NL = the Netherlands
[1] Reference is girls
B = regression coefficient; SE = standard error; $p$ = $p$ value.

The outcome of the Danish model implies that each additional week of breastfeeding duration resulted in an increase in vegetable consumption of 0.35g (see beta coefficients Table 6.3). Hence, a child that had been breastfed for 1 year (52 weeks) on average ate 18g more vegetables than a child that had not been breastfed. The model showed an increase of 0.20g of vegetable intake in The Netherlands for each additional week of breastfeeding, indicating that Dutch children who had been breastfed for 1 year ate 10g more vegetables than Dutch children who had not been breastfed.

## Implications of duration of breastfeeding on vegetable intake

The outcome after performing the GLM per country using different cut-off values for breastfeeding duration (i.e. 6 months; 1 year; 2.5 years) is shown in Table 6.4. The results show that breastfeeding duration remained a significant predictor of initial vegetable intake in The Netherlands for all cut-off values, with approx. 0.45g increase in

vegetable intake per additional week of breastfeeding using a cut-off value of 6 months and 1 year. With 2.5 years as cut-off value, the benefit of breastfeeding levelled off to approximately 0.20g per week. In Greece, using 6 months and 1 year as cut-off values yielded breastfeeding duration as a significant predictor for initial vegetable intake with a 0.84g and 0.62g increase in vegetable intake per additional week of breastfeeding, respectively. This effect disappeared with the highest cut-off value of 2.5 years. In Denmark, however, only a long cut-off value of 2.5 years yielded breastfeeding as a significant predictor (0.35g increase in vegetable intake per additional week of breastfeeding), whereas shorter cut-off values for breastfeeding duration resulted in a non-significant relationship between breastfeeding and children's initial vegetable intake. This implies that the relatively high percentage of Danish mothers who breastfed for a long period are responsible for the positive relationship seen in our overall model for Denmark.

**Table 6.4.** Implications: GLM performed per country using different cut-off values for breastfeeding duration. Bold indicates $p < 0.05$.

| Time (cut-off value) | DK | GR | NL |
|---|---|---|---|
| **6 months bf[1]** (26 weeks) | | | |
| n | 24 | 167 | 287 |
| B ($p$) | 0.16 (0.82) | 0.84 (**0.02**) | 0.46 (**0.03**) |
| Grams[2] | n.a. | n.a. | n.a. |
| **1 year bf** (52 weeks) | | | |
| n | 100 | 198 | 364 |
| B ($p$) | 0.14 (0.47) | 0.62 (**< 0.01**) | 0.43 (**< 0.001**) |
| Grams[2] | 7.2 | 32.1 | 22.2 |
| **2.5 years bf** (130 weeks) | | | |
| n | 143 | 213 | 389 |
| B ($p$) | 0.35 (**< 0.01**) | 0.19 (0.20) | 0.21 (**0.02**) |
| Grams[2] | 18.2 | 10.0 | 10.6 |

DK = Denmark; GR = Greece; NL = the Netherlands
[1] According to the WHO recommendation
[2] Predicted additional g of vegetables eaten by children after one year of breastfeeding compared to children who were not breastfed.
n.a. = not applicable, could not be calculated for 1 year since GLM was based on cut-off value of 6 months breastfeeding duration.
n = number subjects; B = regression coefficient; $p$ = $p$ value.

Table 6.4 shows, per country, the predicted additional amount of vegetables (in grams) eaten by children who have had 1 year of breastfeeding compared to a child who was not breastfed. To compare between the countries, we describe here the output of the GLM using a cut-off value of 1 year. An additional week of breastfeeding in Denmark, Greece, and The Netherlands results in approximately 0.14g, 0.62g, and 0.43g extra vegetable

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intake, respectively. This indicates that, after 1 year of breastfeeding, children's initial vegetable intake was approximately 7g, 32g, and 22g higher in Denmark, Greece, and The Netherlands, respectively, than that of children who had not been breastfed.

**Table 6.5** Spearman's rho coefficients between measured child characteristics and initial vegetable intake and vegetable liking per country ($p < 0.10$, italic; $p < 0.05$, bold; $p < 0.01$, grey highlight).

| | DK | | GR | | NL | |
|---|---|---|---|---|---|---|
| **Vegetable** | **Intake** | **Liking** | **Intake** | **Liking** | **Intake** | **Liking** |
| **Age (months)** | -.- | -.- | -.- | -.- | -.- | -.- |
| **BMI z-score** | -.- | -.- | -.- | -.- | -.- | -.- |
| **Bf duration** | -.- | -.- | 0.14 | **0.23** | **0.14** | *0.11* |
| **Food neophobia** | **-0.33** | *-0.17* | **-0.26** | **-0.20** | **-0.28** | **-0.25** |
| **Food enjoyment** | -.- | -.- | -.- | **0.15** | **0.19** | **0.19** |
| **Food fussiness** | -.- | -.- | -0.17 | **-0.26** | **-0.24** | **-0.29** |
| **Satiety responsiveness** | -.- | -.- | -.- | -.- | -.- | *-0.10* |
| **Food responsiveness** | -.- | -.- | -.- | -.- | *0.09* | 0.14 |
| **Maternal education** | -.- | -.- | -.- | -.- | *0.08* | -.- |
| **Vegetable liking** | **0.32** | - | **0.36** | - | **0.49** | - |

-.- = non-significant
DK = Denmark; GR = Greece; NL = the Netherlands

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## Other relevant relationships

Table 6.5 shows the Spearman correlations indicating the relationship between initial vegetable intake and child characteristics and maternal education. For none of the three countries was a significant relationship found between intake and age, BMI z-score, or maternal education. Neither were satiety responsiveness and food responsiveness correlated with vegetable intake in any of the three countries. Food fussiness was negatively correlated to children's vegetable intake in Greece ($\varrho$ = -0.17; $p < 0.05$) and in The Netherlands ($\varrho$ = -0.24; $p < 0.01$). Enjoyment of food was positively correlated to children's vegetable intake only in The Netherlands ($\varrho$ **= 0.19;** $p < 0.01$). All countries showed a negative relation between food neophobia and children's vegetable intake ($\varrho$ = -0.26 to -0.33; all $p < 0.01$) and a moderately strong correlation between vegetable

liking and intake (all rhos > 0.3; $p$ < 0.01). So, these relations indicate that children with a higher liking ate –as expected– more vegetables, and more neophobic children had a lower initial vegetable intake, as did children with higher scores on food fussiness (Greece and The Netherlands). Furthermore, Dutch children who enjoyed food more had a higher initial vegetable intake. The relation between child's characteristics and vegetable liking are also shown in Table 6.5. Almost all child characteristics that were related to initial vegetable intake were also related to vegetable liking (i.e. the same pattern was observed).

## Discussion

The aim of the current study was to investigate the relationship between breastfeeding duration and vegetable intake at a later age (2–6 years) and whether child eating characteristics, and age and gender, influence this relationship. Breastfeeding duration contributes positively to later vegetable intake in all three countries. As expected, children who were breastfed for a longer period consumed more vegetables. Age and gender did not predict vegetable intake in our sample. We noticed differences in the outcome of this relation across and between the countries using different cut-off times for breastfeeding duration. Overall, this indicates that the relationship between breastfeeding duration and later vegetable intake exists, but is not straightforward. The observed differences between the countries are mediated by breastfeeding practices; Greek mothers breastfed for a short period (15 weeks); a relatively higher percentage of Danish mothers breastfed for a long period (41 weeks), and Dutch mothers breastfed for a moderate period (21 weeks). Differences and variations in breastfeeding duration between the countries can be explained by cultural and social aspects. Women in Denmark typically take 9–12 months maternity leave, whereas Greek and Dutch policy prescribes shorter maternity leave, respectively, 17 and 16 weeks. Our results further show that, as expected, children who were more neophobic and food fussy had a lower initial vegetable intake, whereas children who enjoyed food more and liked vegetables more had a higher vegetable intake. No relation between intake and satiety responsiveness or food responsiveness was found in our population.

Our results are in line with studies by Möller et al. [7] and Burnier et al. [2], who found that longer exclusive breastfeeding was associated with a higher vegetable intake at the age of 4 and 5 years in The Netherlands and Canada. The mixed findings found in the current study across European countries are in line with other HabEat research that reported mixed findings on the relation between breastfeeding and vegetable intake across four European cohorts [27]. For vegetables, a positive relation was found for children breastfed for 3–6 months (versus never breastfed) in France and the United Kingdom, but the association was not significant in Greece and Portugal. One possible explanation for

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these intra-country differences is the way vegetable intake was measured. Each country used its own FFQ to assess the children's diet, and these FFQs differed in number of questions asked to assess fruit and vegetable consumption. They used as main outcome whether a child did or did not reach a certain eating frequency of fruit or vegetables per day. In our study, vegetable intake was measured in the same way across the studies and reflected actual vegetable intake in grams. Consequently, this could not have biased our outcome, and therefore it is less likely that this has affected the differences that we found across the countries.

In contrast to our study, Cooke [9] did not find a significant relation between breastfeeding and vegetable consumption among 2–6-year-olds in the UK; breastfeeding ('breastfed only' or 'breast- and bottle-fed') was a significant predictor of children's fruit intake compared to bottle-fed children, but this was not the case for vegetable intake. Other factors such as gender, enjoyment of food, food neophobia, and adults' intake played a role in children's vegetable intake. A very similar study to our study whitin the Habeat programme was Caton's study [8] where vegetable intake was actually measured during experiments. They also reported that child characteristics could play a role in vegetable intake. In Caton's study [8], child characteristics such as age, enjoyment of food, and satiety responsiveness were significant predictors of initial vegetable intake: younger children who enjoyed food more and scored lower on satiety responsiveness consumed more vegetables. Additionally, their results showed that younger children were less fussy, enjoyed food more, and had lower satiety responsiveness –characteristics which contributed to increased acceptance of a novel food (i.e. artichoke puree). Contrary to our results, breastfeeding duration did not influence initial vegetable intake in their study. Differences in the definition of total breastfeeding duration between the studies could have mediated or confounded the effect. Caton et al. defined breastfeeding duration as the period until formula milk was introduced to the breastfed child; whereas in our study breastfeeding duration was defined as the total period of breastfeeding. Our study showed that eating characteristics such as food neophobia, food fussiness, enjoyment of food, and vegetable liking were associated with vegetable intake. So, in line with previous studies, factors such as food neophobia, food fussiness, enjoyment of food, and vegetable liking contribute to vegetable intake [9, 23, 28]. All these studies point to the fact that, besides breastfeeding, other factors such as child (eating) characteristics seem to play a role in predicting later vegetable intake.

There are several explanations for the observed differences in the effect of breastfeeding duration on children's vegetable intake across the three European countries. First, the length of the breastfeeding period appears to be an important factor that may affect the outcome. As mentioned before, average breastfeeding duration was longest in Denmark, followed by The Netherlands, and then Greece. Furthermore, Greece used a category scale for assessing breastfeeding duration with the maximum category referring to

12 months or longer instead of an open question filling out the number of weeks of breastfeeding. This could explain why no effect was found in Greece when the longer breastfeeding duration was used. Recent work by Perrine et al. confirms that the length of the breastfeeding period plays a crucial role. In this Australian cohort, only children breastfed for 12 months or longer had a higher odds ratio for vegetable consumption frequency at 6 years. Children breastfed for 6 months or longer had a higher odds ratio for consuming more fruit at 6 years of age, but not vegetables [29].

Second, another explanation is that the relation between breastfeeding and vegetable consumption is complex and not straightforward. Also, the mechanism by which breastfeeding contributes to later vegetable intake remains unclear. Research has shown that flavours like carrot, garlic, vanilla, ethanol, and nicotine transfer via breast milk to the infant [4, 30-35]. However, whether other flavours are also transferred via breast milk is less clear. The mechanism may also be related to the continuous variation of in mothers' milk concerning flavour diversity and intensity, because of mothers' dietary variety. Early flavour exposure through the maternal diet can be mediated via mothers' milk influencing offsprings' response to flavours [6]. Correspondingly, variety in a child's diet early in life seems to improve acceptance of new foods. Maier et al. [3] emphasized that the combination of breastfeeding and a high variety experience was most effective in increasing intake of new foods, including vegetables, directly after weaning and two months later. Although Lange et al. [36] did not find an effect of exclusive breastfeeding on higher acceptance of new food, an effect was found for higher acceptance of vegetables. Both studies stress the importance of offering variety early in life in combination with breastfeeding as the most powerful mechanism for later vegetable acceptance. This suggests that, besides breastfeeding, other strategies such as offering variety are important in vegetable liking and intake in young children.

Third, another possibility is that, as children get older, other factors become more important for vegetable intake and start to overrule an early effect of breastfeeding. A factor such as food neophobia can interrupt vegetable intake. Food neophobia is low in infants and very young children, but emerges around 2 years of age, and peaks between the age of 2 and 6 years [37, 38]. Enjoyment of food, availability, and parental vegetable intake are also regarded as important factors regarding children's vegetable intake [9, 39, 40]. Also, differences in timing and type of complementary feeding practices and intra-country differences in practices regarding mothers returning to work may have contributed to the intra-country differences found.

One strength of the current study is that the data used in the analyses were based on actual vegetable intake data measured in real life settings. To our knowledge, Caton et al.'s [8] recent study is the only other in which actual vegetable intake in relation to breastfeeding was investigated based on different experiments. Most studies are based

on self-reported and/or retrospective data on vegetable consumption in which food frequency questionnaires or recalls are used, often only asking for frequency or servings per day, but these are not the most precise instrument to assess vegetable consumption [2, 7, 9, 27, 29]. The present study's actual measurement of vegetable intake gives a more accurate measure that validates our outcome.

Limitations of the present study include the self-reported information about breastfeeding history, which may not be as accurate as prospective report and may explain some differences between the countries. Despite the use of similar questionnaires for child and parent characteristics and the use of a joint template for comparing the 10 studies, we did not harmonize all answer categories. Thus, it is recommended that future research employ more consistent data collection. This implies using questionnaires with exactly the same response categories.

Another limitation is that maternal education in our sample was relatively high. It remains unclear whether the positive relationship in our study can be extrapolated to less educated populations. Future research among more diverse samples with regard to education level is recommended.

To conclude, breastfeeding duration contributes to vegetable intake in 2–6-year-old children, but the mechanism of this relation and the required duration of breastfeeding remain unclear. It appears that a complex mix of factors such as vegetable liking and some child characteristics (like food neophobia and enjoyment of food) play a role in vegetable intake. On the basis of the current study's findings, total breastfeeding duration and other child characteristics may be more important than exclusive breastfeeding alone. Therefore, the WHO recommendation should be complemented with other strategies (especially in countries where mothers tend to breastfeed for shorter periods). Because it remains a challenge to encourage children to eat sufficient vegetables, further research should continue in this area in order to develop effective interventions that start at a young age.

## Acknowledgements

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# Chapter 7

General discussion

The overall aim of this research was to investigate the underlying learning mechanisms and modifying factors which play a role in developing vegetable preferences in 2–5-year-old children. This chapter summarizes the main findings of the research and discusses outstanding methodological issues and key findings in a broader context, followed by a discussion of their implications and recommendations for further research.

## Main findings

Table 7.1 presents the main findings of the studies performed in this thesis. We observed a clear effect of repeated exposure on vegetable acceptance: ad libitum *intake* increased from 50% up to 300% after the interventions and this increased intake remained stable in time, two and six months later (chapters 2, 3, and 4). In general, it was shown that children increased their vegetable intake independently of flavouring the vegetable or adding more energy to it. Overall, the effect of flavour–flavour learning (FFL), flavour–nutrient learning (FNL), or taste modification on vegetable intake was not more effective than repeated exposure (RE) (chapters 2, 3, and 4). The strategy of offering a choice caused only a small positive change in vegetable consumption (chapter 5). Other modifying factors like breastfeeding history, vegetable liking, and age seem to play a role in this process. Older children who liked vegetables more tend to eat more vegetables when offered a choice. Food neophobic children ate fewer vegetables. Further, breastfeeding duration was positively associated with a higher vegetable intake in 2–6-year-old children across three European countries (chapter 6). Gender and age had no influence.

For *preference*, flavour–nutrient learning was effective, shortly after the intervention, regarding the vegetable previously paired with energy, but the effect was not robust over time (chapter 2). Flavour–flavour learning had no effect on preference (Chapter 3). However, preference changed towards the vegetable flavour to which children were exposed during the intervention, but irrespective of the strategy used (chapter 4). This indicated that there was no strong evidence found in this thesis that strategies such as FFL, FNL, or diluting/hiding a vegetable are more effective in changing vegetable preference than RE alone.

Repeatedly offering a vegetable is seen as the most powerful strategy to enhance young children's vegetable intake (chapters 2, 3, and 4), whereas FNL and FFL are not more effective than RE. These findings indicate that RE is most important in the development of food preferences in toddlers and pre-schoolers.

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**Table 7.1** Overview of findings in the thesis: effect of different learning mechanisms and several modifying factors on children's vegetable preference and intake.

| Outcome: *preference* | | | |
|---|---|---|---|
| *Mechanism* | *Findings* | *Effect* | *Chapter* |
| **Repeated exposure** | *Inconsistent effect of RE on preference* | -<br>+/- | 2, 3<br>4 |
| **Flavour–nutrient learning** | *Effect of FNL on preference immediately after the conditioning period (p = 0.04) but disappeared (p = 0.09) in the long term (i.e. 2 and 6 months after the conditioning period).* | + | 2, 4 |
| **Flavour–flavour learning** | *No effect of FFL on preference in the short term (immediately after the conditioning period and in the long term (2 and 6 months after the conditioning period).* | - | 3, 4 |
| **Taste modification (pure, dilute, hide)** | *Overall, preference for spinach over French beans improved after the intervention (p < 0.001); however, not in total number and no differences were discerned between the groups.* | +/- | 4 |
| Outcome: *intake* | | | |
| *Mechanism/factor* | *Findings* | *Effect* | *Chapter* |
| **Repeated exposure** | *Main effect of exposure on intake of vegetable soup (p < 0.0001) directly after the conditioning period and over time (2 and 6 months after the conditioning period: p < 0.0001).* | ++ | 2, 3, 4 |
| | *Main effect of exposure on intake of freeze-dried vegetable crisps (p < 0.0001) directly after the conditioning period and over time (2 and 6 months after the conditioning period: p < 0.0001).* | ++ | |
| | *Main effect of exposure on spinach intake (p < 0.001).* | ++ | |
| **Flavour–nutrient learning** | *No effect of energy on vegetable soup intake.* | - | 2, 4 |
| | *Children ate fewer sandwiches (p < 0.0001) after consumption of the high energy soup compared to the low energy soup.* | | |
| **Flavour–flavour learning** | *No effect of flavouring on intake of freeze-dried vegetable crisps.* | - | 3, 4 |
| | *Children ate more vegetable crisps in combination with tomato ketchup (conditioned) compared to children who were unconditioned during the conditioning period.* | | |
| **Choice-offering** | *Offering a choice had a small effect (p = 0.09) on increasing vegetable intake in children.* | +/- | 5 |
| **Vegetable liking, Age** | *Vegetable liking (p < 0.001) and age (p = 0.06) predicted vegetable intake and mediated the effect of choice-offering.* | +<br>+/- | |
| **Taste modification (pure, dilute, hide)** | *No effect of different taste experiences on spinach intake.* | - | 4 |
| **Food neophobia** | *Food neophobia score had a negative effect on intake, with neophobic children being less responsive to the intervention (p = 0.008).* | + | |
| **Breastfeeding duration** | *Breastfeeding duration contributes to higher vegetable intake in 2–6-year-old children across three European countries.* | + | 6 |
| **Gender, Age** | *Age and gender had no effect on vegetable intake* | -<br>- | |

However, strategies like FNL and FFL can be used to encourage initial tasting of vegetables since one of the conditions for learning to eat/like vegetables is that actually tasting is required [1]. Flavouring or adding extra energy can stimulate children to taste the initially disliked vegetables. Factors like breastfeeding duration, vegetable liking, and food neophobia status are important for children's vegetable intake (chapters 4, 5, and 6). Children who are more reluctant to try novel food proved to have a lower vegetable intake during and after the interventions. Our results indicate that neophobic children are not responsive to the different strategies (chapters 4 and 6). Children who generally liked vegetables more prior the interventions consumed, as expected, more vegetables during and after the interventions (chapters 4 and 5). Breastfeeding duration contributes positively to later vegetable intake in 2–6-year-old children across three European countries (chapter 6). Children who were breastfed for a longer period consumed more vegetables. Age and gender did not predict later vegetable intake. Additionally, context factors like environment/positive ambience and peer pressure seem to stimulate children's vegetable intake (chapters 2, 3, 4, and 5).

## Methodological considerations

The methodological issues relating to the research described in this thesis have been discussed in the various chapters. However, before the general discussion and interpretation of the results, it is important to consider a number of methodological issues, such as the selected test foods, the study designs, the test environment, the participants, and the outcome measures.

### Study foods

Foods used in the studies had to meet certain criteria, for example the target vegetables had to be neutral or disliked and relatively novel to successfully perform the experiments which investigated the learning mechanisms. Since vegetables in general are disliked by children because of their appearance and bitter taste [2-4], we wanted to ensure that the products used in the experiments were of interest to the children, thereby enhancing their willingness to taste and eat, so that we would be able to test the efficacy of the different learning mechanisms on children's vegetable preference and intake. Therefore, some of the used (novel) products were developed especially for the studies after consultation with a Dutch chef (Pierre Wind) specialized in working with school-aged children. One of his main pieces of advice was to make food more attractive/appealing for children by making the food more exciting and by presenting food more as an experience. Therefore, we aimed in our studies at optimizing both the product and the serving context in order to encourage children to consume their vegetables. For

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example, initially we planned to test the learning mechanisms with vegetable purees. However, Pierre Wind convinced us that children of this age (2–4 years old) tend to think of purees as 'childish', i.e. not what adults eat. Hence, we decided to change to soups (chapter 2) and crisps (chapter 3), a more exciting product for toddlers. Dutch children are not used to having vegetable soups or vegetable crisps at lunchtime, but this proved to be a successful approach. Apart from a small subgroup, almost all the children enjoyed the experience and tried/consumed our novel products.

In order to test the effect of differences in the energy content of the foods (chapter 2; FNL), large contrasts in energy density are required (100 kcal/100 g) [5, 6]. Another criterion for successful FNL to be able to occur is that the target vegetables had to be neutral/disliked and relatively novel [7]. We developed two green vegetable soups (spinach and endive) in two energy variants (low and high energy) with an energy difference of 119 kcal between the high and the low vegetable soup. Dutch toddlers are not used to consuming a pure green vegetable soup. To add energy we used both sunflower oil and malto-dextrine in our products; therefore, differences in sensory characteristics between the low and high variant were minimized. Other studies used only sunflower oil or only malto-dextrine to add extra energy [3, 8-10], and this influenced the taste (oily; sweetened) of the products. We believe that these two soup options were sufficiently varied to optimize the criteria for successful flavour–nutrient learning and that we were able to investigate energy learning properly.

Vegetables crisps (parsnip and red beet) were used to test flavour–flavour learning (chapter 3), a relatively unknown vegetable and a novel product for toddlers. Tomato ketchup (TK) was selected as a liked and familiar dip and was preferred over the neutral dip. The preference for TK remained stable over time, with rated preferences for TK varying from 73% to 79%. As a neutral dip was offered in the unflavoured condition, a similar procedure of dipping occurred in both conditions and therefore could not affect the outcome.

In these studies, products like vegetable soups and vegetable crisps proved not to be more effective than repeatedly offering a vegetable without flavouring or adding extra energy. However, the attractive appearance and way of presentation of the soups and crisps may have contributed to children's willingness to at least try to taste the novel products. What remains unclear, however, is the extent to which exposure to vegetable tastes via, for example, crisps/soups will transfer to a better acceptance and intake of vegetables cooked normally.

We wanted to test whether masking or hiding a vegetable is an effective way to increase children's vegetable acceptance. Spinach ravioli was used to cover spinach. Unfortunately, parents reported that the children liked the spinach ravioli less than the other products (i.e. pure spinach, creamed spinach, beans). Although the children did not equally like

the products served during the intervention period, intake during the intervention did not differ among the groups. Liking scores could possibly have influenced our outcome; however, we assumed that children were similarly exposed to the spinach taste, because there were no differences in intake among the different groups (i.e. products) during the intervention.

For the other experiments, we chose to work with more regularly eaten vegetable products (chapters 4 and 5) [6, 11]. For practical reasons – since part of the intervention was not performed at the day-care centres but in the home situation – the target vegetables had to fit into a typical Dutch meal of potatoes/rice/pasta, vegetables, and meat/fish/ vegetarian product. The six target vegetables used in the choice study were generally liked and familiar to the children and it is therefore assumed that the possible effect of liking on intake was constant and therefore did not influence the outcome.

## Study designs

We used within-subject designs to test the efficacy of FNL, FFL, and RE (chapters 2 and 3). One of the advantages of using a within-subject design is that inter-individual variability of the children plays a minor role because the subjects are their own control. Alternatively, we could have chosen between-subject designs. With a between-subject design, two or more groups of subjects are tested by different conditions; in this way, carryover effects are minimized. Another advantage is that it requires fewer measurements, thus reducing the risk of boredom. However, to generate useful and reliable data, a larger number of participants are required for a between-subject design.

We chose a within-subject design over a between-subject design when testing the efficacy of FNL and FFL compared to RE for several reasons. First, there is large variation in vegetable intake between children [6, 11]: the standard deviation is often higher than the average vegetable intake (chapters 2–6). Second, it is hard to recruit a large number of children in this age group to participate in scientific research. Our number of participants was based on a significance level of $p = 0.05$ and a power of 80%. We allowed for a dropout rate and children's unwillingness to eat of 10–15%, and our number of children is in line with earlier similar studies [12-14]. Therefore, we assume that the numbers used in the studies were correct to detect possible effects of the learning mechanism.

To minimize carryover effects from one condition to another condition, between-subject designs were used to detect possible influences of different taste experiences and offering a choice (chapters 4 and 5). Here, we chose a between-subjects design because we wanted to compare two or more conditions that could have influenced one another. Consequently, we needed more children in total to test the effect of choice-offering and taste modification. In the choice-offering study and the taste modification study

(chapters 4 and 5), we used a random intercept model to take into account the individual variation in intercepts between participants and the correlation between observations in the same participant. In this way, we tried to take account of the individual differences in intake between and within children, which could have influenced our outcome.

In our choice study (chapter 5), we offered a variety of six vegetables to test whether choice matters. Offering a variety of vegetables has been observed to increase vegetable intake in other studies [15-17]. The distinction between variety and choice as an intervention is sometimes not straightforward, because choice-offering implies that more than one vegetable at a time is provided, hence a bigger variety. In our study, two of the six target vegetables were offered an equal number of times (i.e. twice) in both conditions. As a consequence of the design, the remaining four vegetables were offered more often (i.e. five times) in the choice group than in the no-choice group (i.e. twice). One could argue that if an effect was detected it was due to more frequent exposure (variety) or choice-offering. We did find that some vegetables were eaten more than others, but this was regardless of the choice status or the frequency with which the vegetables were offered during the intervention. Although we acknowledge that offering variety can positively affect intake, it did not explain the outcomes in our study, indicating that choice-offering itself had a moderate effect.

## Test environment

To minimize interference with the children's daily routine, researchers were not present during the consumption tests. To stimulate intake, products were served at the same time during the experiments: 10–15 minutes before lunchtime at the day-care centres so that children would be reasonably hungry. We assumed that possible differences in hunger/satiety status between children would be randomly distributed across the different groups. Children were seated at tables in small groups (5–10 children) together with a day-care staff member. Researchers and day-care leaders were instructed to behave as normally as possible to facilitate a relaxed atmosphere for the children. This test environment may have had a positive effect on children's vegetable intake, due to the presence of other children (peer pressure), the positive ambience of the day-care centres, or modelling by observing day-care leaders consuming the vegetable products as well. Consequently, these social context effects could have modified our outcome. However, we expected these social context effects to be equally distributed across our conditions, and we consider it unlikely that this caused a systematic effect on vegetable intake in one of the conditions selectively.

Some of the studies took place in an in-home situation, with as little interference in the normal dinner routine as possible (chapters 4 and 5). We believe this strengthens the face validity of the studies. A potential drawback of this approach is that there is

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less experimental control in a naturalistic/ecological setting. Parents participating in the in-home studies (chapters 4 and 5) proved to be committed to the intervention and motivated to comply with the procedures; 95% returned completed food diaries. These numbers indicate that parents and children who participated in the studies showed high involvement and compliance with the procedures. Therefore, we consider it unlikely that factors like experimental control or deviations from the procedures caused a systematic effect on our results. It cannot, however, be excluded that factors like parental style (e.g. positive reinforcement), family composition (e.g. one or more siblings present), meal composition (e.g. rice/pasta and/or meat/fish/vegetarian product) may have influenced the outcome. However, we assume that variations in these factors would be randomly distributed across the different conditions.

## Study participants

Participants were recruited via the day-care centres in Wageningen, the Netherlands. Recruitment via day-care centres could be a source of selection bias, since only the working population is reached. Children of relatively highly educated parents participated in our studies since the population of Wageningen is relatively highly educated ('university town'). Another potential source of selection bias could be that parents who have an interest in healthy behaviour such as eating vegetables are more willing to enrol in our studies. Although these factors could have influenced our outcome, we emphasize that we investigated the efficacy of different underlying learning mechanisms and not the effect of parental involvement or education. To test the effect of parental education level we would need a more diverse sample, but this was beyond our scope. Since our sample consists of a relatively highly educated population, we cannot extrapolate our results to other education groups.

## Outcome measure preference

As described in the general introduction, liking was measured indirectly as relative preference and seen as reliable to measure liking in such young children (2–5 years of age). Although we assumed that this was the best possible option for measuring preference, it is probably difficult – especially for children as young as 2 years of age – to perform such a test, because of their limited verbal skills, limited concentration span, and limited cognitive capabilities [18, 19]. Therefore, it is questionable whether the outcomes for preference are truly reliable in our studies conducted with pre-schoolers. This could explain the differences in our findings between the robust effect on intake found in all our studies compared to inconsistent or hardly any effect on preference, whereas we expected that changes in preference would be in line with increased intake as preference is seen as an important predictor of food intake [20-23].

Ideally, children should be tested alone when performing a preference test to minimize the influence of other children's performance. However, because of the participants' very young age, children were not tested alone but were allowed to conduct the preference test together with one other child to make it more comfortable for them. Children were seated in a quiet room together with a researcher and if necessary attended by a day-care leader. Despite day-care leaders and researchers being instructed to behave in a neutral way so as not to influence the children, we cannot exclude the possibility that children behaved differently in response to the presence of the test leader or the day-care staff member. Some children, especially the youngest ones, can be shy or unwilling to go to an unknown person. Also, we cannot exclude the possibility that some of the 2–3-year-old children made a random choice just to please the test leader. We noticed that children just before the age of 4 years old (in the Netherlands just before attending primary school) were more mature and more confident in their behaviour and ability to express themselves during the preference tests. All in all, these differences between the youngest (2 years old) and the oldest (4 years old) children may have confounded our findings on preference to a certain extent, and so the findings should be regarded with caution.

## Confounding factors

In the choice-offering study (chapter 5), adjustments were made in the model for gender, age, and vegetable liking prior the intervention. Vegetable liking is related to vegetable intake, and children eat more as they grow older, as do boys [5, 6, 23-27]. These confounders were taken into account to investigate the relation between choice-offering and vegetable intake. We also made adjustments in the model for gender and age, examining the relation between breastfeeding duration and vegetable intake (chapter 6). Vegetable liking was not taken into account here since it may also be connected to vegetable intake indirectly, via breastfeeding duration. Hence, it was impossible to disentangle the effects of breastfeeding duration and of vegetable liking on initial vegetable intake. Because our primary objective was to explore the relation between breastfeeding duration and later vegetable intake in children, we decided not to include vegetable liking in our model, although we acknowledge the potential moderating effect of liking on intake.

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## Key findings in a broader context

### Children's vegetable preference and intake

Repeatedly offering vegetables affected *intake*, with ad libitum intake of vegetable products being substantially higher post intervention and with longer-term potential. These findings were consistent across the interventions (chapters 2, 3, and 4) and in line with other research conducted within the HabEat programme in other European countries, where repeated exposure was also shown to be a strong mechanism to increase young children's intake of a novel vegetable taste (i.e. artichoke puree, salsify puree) [9, 10, 28-30]. The HabEat studies also reported an effect of RE over time varying from five weeks up to six months after the interventions across the different studies. In our experiments, we used novel products as well (chapters 2 and 3). However, in the taste modification study (chapter 4), we used a more common but disliked vegetable as target vegetable and observed an effect of repeated exposure. This result is in line with previous findings that observed an effect of repeated exposure on more common – but in general disliked – vegetables [31-33]. Therefore, these results indicate that repeated exposure is a powerful strategy to enhance young children's intake of both novel and familiar but disliked vegetables in the short term and in the long term.

We observed mixed results regarding changes in *preferences* across our studies. We noticed an effect on preference, directly after the intervention, towards the vegetable previously paired with energy. However, this effect was not robust over time (chapter 2). Other strategies to shape food preferences such as flavour–flavour learning, taste modification, and repeated exposure did not result in a positive change towards the target vegetables used in the interventions (chapters 2, 3, and 4). Although the majority of comparable studies conducted within the HabEat project looked solely at intake and not at preferences as outcome measure [9, 28, 29], one of these studies did look at liking as an outcome measure [10]. This study reported that, after exposure, the target vegetable was as much consumed and as much liked as the control vegetable in the RE group. This indicates that learning mechanisms such as FFL and FNL were not effective in changing the liking score. Our findings contrast with a number of studies performed earlier in the field that observed an effect on liking [13, 14, 33-35]. These inconsistencies in findings may be due to the different products used in those studies (not always related to vegetables) and due to the different age of the children investigated (older children: 4–8 years of age).

### Underlying learning mechanisms in relation to food preferences

We studied the potential learning mechanisms flavour–nutrient learning and flavour–flavour learning to understand the underling mechanisms involved in the shaping of

food preferences. Adding energy or flavouring did not produce any robust effect over and above that yielded by repeated exposure across our studies on preference or intake, despite conditions meeting many of the proposed requirements to detect an FNL or an FFL effect (chapters 2 and 3). These requirements include, among others: 1) target vegetables have to be neutral/disliked and relatively novel; 2) there should be sufficient difference (i.e. 100kcal/100 g) in energy content between the low and the high energy variant of the vegetable product (FNL); 3) the added flavour has to be familiar and already liked (FFL); and 4) children have to be sufficiently hungry.

### Flavour–nutrient learning

The requirement of ingestion of a sufficient amount of energy was met and other conditions/requirements such as novel stimuli favoured the occurrence of flavour–nutrient learning. Participants were served at the same time, 10–15 minutes before lunchtime; therefore as much as possible controlled for their hunger state. We found that children ate fewer sandwiches after being exposed to a higher density pre-load, supporting the idea that children can adjust their energy after consuming a high energy load [12, 36-39]. In sum, it appears that the requirements for FNL to occur were met. Our (negative) findings contrast with work in animals that has consistently shown a positive effect of FNL [40-42]. To explain these discrepancies, two options seem plausible. First, FNL works well in animals but not in humans. Second – and we consider this more likely – FNL possibly works in human beings as well but may be more difficult to demonstrate or detect due to less experimental control and the inability to create 'extreme' conditions. For example, studies in rats apply food deprivation to animals by applying food restriction schedules, making them very hungry, before commencing the experiment. In addition, foods can be administered to animals using sham-feeding paradigms with intragastric infusions. It is obvious that for young children, for ethical reasons, these conditions cannot be applied or created. It would be unethical, for example, to severely restrict food intake in toddlers on the day of testing to make them extremely hungry before offering them the vegetable products used in our studies.

Hence, whether FNL can also increase consumption in humans as reported in animal studies remains to be determined. We acknowledge that the present findings on FNL are not very robust, although one can wonder whether more than one learning strategy is active at a time. FNL may have had additive or modifying effects that strengthen the effect of mere exposure. So, a learning mechanism such as flavour–nutrient learning can support *initial* exposure to vegetables as it may promote children's willingness to taste/eat the vegetable products, thereby ensuring repeated exposure, but FNL is not more effective than *repeated* exposure in increasing vegetable intake in toddlers.

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### Flavour–flavour learning

Similar to our findings for FNL, we failed to observe an additional effect of adding a flavour compared to repeatedly offering vegetables without flavouring (chapter 3). Previous studies reported an effect of flavour–flavour learning, although this was not more effective than repeated exposure [9, 10, 28, 31, 43] and therefore is in line with our results. We offered small portions of vegetable crisps with a dip low in kcal per total serving (23 to 33 kcal) to prevent a learning mechanism such as FNL occurring at the same time. Our results showed that participants did not differ in adjusting their energy intake when consuming flavoured vegetables compared with unflavoured vegetables; this suggests that it is indeed unlikely that FNL occurred, as we did not observe the compensatory effect on sandwich eating during the regular lunch. Although we failed to find an additional effect of FFL, we did notice a higher intake of vegetables during the intervention in the flavoured as compared to the unflavoured condition. Yet, this potential effect of flavouring disappeared post intervention when vegetables were presented without the dip. This has also been observed in other studies [31, 43, 44] and suggests that flavouring can have added value in that it promotes initial tasting of vegetables in young children. Here, we showed that using a dip was effective as a strategy to encourage children to taste vegetables, and this might be an easy/practical way to enhance young children's vegetable consumption. Other studies confirm the use of a dip as an effective strategy to increase young children's vegetable liking and/or intake [31, 43, 44].

### Long term

We observed an effect of repeated exposure on vegetable intake not only directly after the interventions but also persistent over time (up to 6 months after the interventions; chapters 2 and 3). It seems that, if children learn to like a specific taste, they will recognize this taste over time. One might speculate whether the positive environment could have contributed to the effect we observed, since these findings suggest that, besides the effect of the intervention itself, the environment/context can support the effect.

## Other strategies to improve vegetable liking and intake

### Taste modification

Hiding or masking vegetables did not prove to be a more effective strategy than repeatedly offering a vegetable to change preference or increase vegetable intake (chapter 4); even children who were not exposed to the target vegetable increased their intake post intervention. We expected that masking the pure, often disliked, vegetable taste would be an effective way to stimulate children's vegetable intake as reported in two studies [38, 45]. It can be reasoned that in our study the target vegetable was more visible and therefore the vegetable was less hidden while being eaten; this in turn could have influenced our outcome. Since masking/covering vegetables is seen as a popular strategy

often applied in real life, it is of great interest to investigate whether strategies such as masking/blending/mixing vegetables have an additional effect and contribute to taste preferences and intake.

### Choice-offering

We expected that offering a choice would stimulate and increase children's vegetable intake. This hypothesis was not entirely confirmed in this thesis. We observed that choice, after correcting for age, gender, and vegetable liking, has a tendency to predict vegetable intake, but our findings are not robust (chapter 5). Children as young as toddlers like to make their own choices in order to develop a sense of autonomy and individuality [46], but one could wonder whether vegetables are of interest at this age. Choice can be effective if the topic reflects the participants' needs [47], and generally vegetables are often not liked in the first place. Children showed that they appreciated having a choice [48], and vegetable intake improved after a choice of unfamiliar vegetables was offered [49], although older children were involved. Our results indicate that choice-offering has potential as a strategy to promote vegetable intake in older children. It seems that younger children benefit from repeatedly being offered vegetables to make them familiar with the taste; and as they get older a strategy like choice-offering can be appropriate to stimulate vegetable consumption.

### Context factors

Besides the influence of different learning mechanisms and strategies to improve children's vegetable preference and liking, social context factors can play a role in this.

Our results show that 2–5-year-old children met the lower limit of the recommended vegetable intake of 50 grams per day when consuming vegetables in an in-home situation. However, when they consumed vegetables at day-care centres, they increased their intake up to 150–200 grams post intervention, thereby meeting the upper limit of the recommended vegetable intake of 150 grams per day [6]. These findings indicate that, besides the effect of the intervention itself, the environment/context can modify the effect. Previous studies have reported that social context effects such as peer pressure and role modelling can contribute to encouraging food acceptance in children [50-54]. Children are responsive to adapting to peers' food choices and intake by eating the same amounts of food [52, 54]. Peer modelling also proved to be effective in interventions aimed at improving fruit and vegetable intake [53-56]. In our studies, children were seated together in small groups (5–10 children) during the pre- and post-tests at the day-care centres, and this could have facilitated intake via peer pressure. Addessi et al. [57] also indicated that children are more likely to eat new food if others are eating the same food than when others are merely present or eating another kind of food. In addition, the day-care staff can have an important role in children's dietary intake at the day-care

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centre by being a positive role model eating healthy foods along with the children [50, 58].

Additionally, the time of offering the vegetable products (i.e. just before lunchtime) could have contributed to the effect. In the Netherlands, dinner is the main meal where children eat vegetables, whereas lunch commonly consists of a sandwich meal without vegetable dishes. So, at the traditional hot meal in the evening, most parents have to ensure that their children eat a sufficient amount of vegetables, but most parents are both employed outside the home and young children are often tired at that time of the day.

Our results could be used to plead for the introduction of a hot lunch including vegetables at the day-care centre, although this is not common practice in the Netherlands, or to make use of different meal or snack times to serve children more/sufficient vegetables to stimulate their intake. In that way, parents will have less worry about their children' daily vegetable intake, and in the meantime maintain a pleasant meal situation at home.

## Individual differences in child characteristics

Our studies have shown that differences in individual characteristic traits such as food neophobia, breastfeeding duration, and age seem to influence food preferences and therefore play a role in vegetable intake in toddlers/pre-schoolers (chapters 4, 5, and 6). Gender did not modify the effects on vegetable intake in this thesis (chapters 5 and 6). We expected girls to eat more vegetables than boys [25]; however, this was not confirmed in this thesis. One possible explanation can be that the effect of gender is not present in children as young as 2–3 years of age. Toddlerhood is the timespan to explore food by experiences (trial and error), and probably as children get older other factors in the environment become more prominent in influencing eating behaviour.

Longer breastfeeding duration contributes to higher vegetable intake (chapter 6). The relation between breastfeeding duration and vegetable intake is not straightforward, and it appears that a complex mix of other factors such as vegetable liking and food neophobia play a role in vegetable intake as well. Children's levels of food neophobia were measured in almost all the studies in this thesis and, as expected, higher scores on food neophobia corresponded with lower vegetable intake in children [23, 59-61]. Neophobic children proved to be less responsive to the interventions. This indicates that difficult eaters may need other approaches to stimulate vegetable intake. Our findings are confirmed in other research which indicates that it remains difficult to get this specific group to eat (more) vegetables [8, 25, 59].

Similar work within the HabEat group reported that child characteristics such as age, enjoyment of food, and satiety responsiveness were significant predictors of initial vegetable intake [8]. The results showed that younger children were less fussy, enjoyed

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food more, and had lower satiety responsiveness – characteristics which contribute to increased acceptance of a novel food. Younger children seem to be more responsive to novel food, whereas applying a strategy like choice-offering seems to work better with older children. So, in line with previous studies, factors such as food neophobia, food fussiness, enjoyment of food, and vegetable liking contribute to vegetable intake [59, 61, 62]. All these studies point to the fact that, besides breastfeeding, other factors such as child (eating) characteristics seem to play a role in predicting later vegetable intake.

## Implications and suggestions for further research

From the perspective of children's reduced vegetable intake causing an unbalanced diet and possible health problems, it is important to understand how children's food preferences are shaped and which factors play a role in this. With better understanding, we can gain more insight into the development of vegetable preferences and this could help us to promote healthy eating habits in children. In this thesis, we studied the working of different learning mechanisms and strategies involved in shaping food preferences and consumption.

We noticed inconsistent, or hardly any, effect on preference in our studies, whereas we expected that changes in preference would be in line with increased intake as preference is seen as an important predictor of food intake [20-23]. This suggests that we need more reliable methods to measure preferences in toddlers. However, it remains challenging to test toddlers, and methods available for preference testing that generate reliable and replicable results from young children are limited [18]. Toddlers can make decisive 'yes/no' distinctions, although they have difficulty making complex decisions and have a limited attention span. Since food preferences are an important predictor of food intake, it is of great interest to develop a more valid tool for measuring preference/liking in toddlers (2–4-year-old children).

We learned that strategies such as flavour–nutrient learning and flavour–flavour learning had no additional effect over repeated exposure in stimulating vegetable intake. However, they might help to stimulate initial intake since one of the conditions for learning to like vegetables is to actually taste them. Therefore, strategies like flavour–nutrient learning and flavour–flavour learning can assist in overcoming the initial resistance to tasting disliked vegetables. In this thesis, we worked mainly with novel or disliked vegetables, and it remains questionable whether children will taste vegetables in their plain, unadulterated form. Future research should explore whether the positive effects of repeatedly offering novel vegetables in an appealing form (e.g. red beet vegetable crisps with a dip) will generalize to their acceptance when they are offered and prepared in the common way (e.g. cooked red beets).

In this thesis, we found some indication for generalization (chapter 4); however, it remains unclear on which factors/reasons this is based. We noticed that children increased their intake of a more familiar, initially disliked, vegetable after repeated exposure; even children in the control condition increased their intake. This suggests that repeatedly offering (any) vegetable seems to stimulate vegetable intake. It may be of interest to investigate the conditions necessary for learning from one vegetable to another vegetable (i.e. generalization). One can wonder whether certain sensory properties such as colour and texture play a role, and this remains to be determined by future studies.

Our findings imply that repeatedly offering a vegetable in a trusted positive environment is a powerful strategy to increase young children's vegetable intake. For that reason, it is of interest to further investigate the possibilities of serving vegetables in a setting like a collective lunch or making use of different meal or snack times at the day-care centre to stimulate and increase children's vegetable consumption.

We have assumed that a strategy such as choice-offering is less effective in younger children. It might be worthwhile to explore whether there is minimum age at which to apply a strategy like choice-offering, and future studies should therefore focus on different age groups. This is of interest because a strategy like repeated exposure is effective in young children's acceptance and consumption of a novel or unfamiliar vegetable. Future research is needed to investigate whether strategies like choice-offering are effective in increasing intake of the already accepted taste to further increase consumption of a relatively familiar vegetable to comply with the recommendations.

We have shown in this thesis that an individual characteristic trait such as food neophobia and a factor such as breastfeeding duration seem to influence food preferences and therefore play a role in vegetable intake in toddlers/pre-schoolers. To further understand why a specific group of children (food neophobic children) is not responsive to repeated exposure or even masking/diluting a vegetable taste, we need to explore different approaches towards this group to stimulate vegetable intake. A strategy known to be effective in stimulating young children's food intake at the age of the onset of food neophobia is to start early with exposure to a variety of vegetables [8, 63-67]. Still, based on current findings, it remains difficult to apply a strategy such as repeated exposure as an effective tool for food neophobic children.

We also observed that length of breastfeeding seems to play a role in children's later vegetable acceptance. However, the optimum breastfeeding duration and the mechanism behind it remain unclear. Our findings suggest that the length of breastfeeding, some child characteristics, and other strategies like variety-offering may be more important than solely exclusive breastfeeding. Recent work has shown that adding vegetable purees to milk during complementary feeding contributes to taste acceptance [68]. Further research should investigate the efficacy of vegetable flavouring in milk or other relevant

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products as a possible way to shape vegetable preferences early in life before the onset of food neophobia. Other recent work showed that even starting as young as weaning age with a combination of RE and a day to day variety of vegetables is an effective way to stimulate vegetable intake in infants until at least the age of 12 months [69]. It is of interest to further investigate to start weaning with a variety of vegetables for a longer period and to follow these children over time.

## Main/general conclusion(s)

This thesis demonstrates that repeatedly offering a novel or disliked vegetable is highly effective in promoting vegetable intake in toddlers and pre-school children. Repetition seems to be the way to teach young children to accept, and shape their acceptance of, novel/disliked foods. Other strategies such as flavouring, adding energy, or taste modification can help to promote initial tasting of vegetables in young children. More strategies are needed such as choice-offering to boost already liked/familiar vegetable intake when children get older.

Individual differences such as age, vegetable liking, breastfeeding duration, and food neophobia status play a role in determining children's vegetable consumption. It is important to keep this in mind for the development of more specific strategies for difficult eaters/non-eaters. Context factors such as a positive atmosphere and environment are essential for children to learn to like vegetables and can contribute to vegetable consumption.

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# Summary

Children's vegetable consumption is far below recommendations. Dutch children's diets, like the diets of many children in Europe and the US do not contain enough fruit and vegetables and have been associated with a high prevalence of childhood obesity. Despite the increased awareness of the importance of vegetable consumption for health, it remains challenging to improve children's vegetable intake. Liking of vegetables does not come naturally or easy to most children. Since food preferences are central to food intake, it is important to understand how they are shaped and which factors play a role in this. So far, research on the formation of vegetable preferences has focused mainly on infants and school age children but is not elaborately investigated in toddlers/pre-schoolers. This thesis describes the research conducted to investigate whether underlying mechanisms (e.g. repeated exposure, flavour-nutrient learning, flavour-flavour learning) and modifying factors such as age, gender, breastfeeding history, food neophobia play a role in developing 2–5-year-old children's acceptance of vegetables.

The **first chapter** provides background information on children's vegetable intake, food preferences, learning mechanisms which play a role in establishing food preferences and intake, and describes other relevant factors involved in vegetable preference and intake. Additionally, the research questions and the outline of the thesis are presented.

**Chapter 2** describes the effect of flavour–nutrient learning and repeated exposure as mechanisms to increase toddlers' preference for, and intake of, green vegetables. Flavour nutrient learning (FNL; temporarily adding energy) could be an effective mechanism to change preferences. Forty healthy toddlers were included in a randomized intervention study. During an intervention period of 7 weeks, the children consumed vegetable soups (endive and spinach) twice per week before lunch time at the day care. Half of the group received a high-energy variant of one soup and a low energy variant of the other flavour, whereas for the other half the order was reversed. The intake for both vegetable soups increased (~47% increase compared to baseline) after the conditioning period, but this was independent from the energy manipulation. The positive effects on intake remained stable for at least 6 months after conditioning. This indicates a robust effect of mere exposure on intake, but no additional effect of FNL. For preference, however, results showed a difference in liking for the vegetable soup consistently paired with high energy, supporting FNL.

In **chapter 3,** we wanted to test the efficacy of the learning mechanisms flavour-flavour learning (FFL) and repeated exposure (RE). Temporally pairing or adding an already liked, known flavour to an unknown or disliked flavour can contribute to accepting the novel flavour. During an intervention period of seven weeks, 39 toddlers consumed red beet and parsnip crisps at day-care centres. Half of the group received red beet crisps with a dip of tomato ketchup (conditioned), and parsnip with a neutral white sauce (unconditioned), whereas for the other half the order was reversed. Vegetable intake

increased by approximately 300% (from baseline) after the intervention, but regardless of the condition. The increase in intake persisted over time and was still present two and six months afterwards. We observed no shifts in preference for one vegetable over the other as a consequence of FFL. These findings reflect that RE but not FFL proved to be the primary mechanism explaining increased vegetable consumption in young children and, notably, one with longer-term potential.

Besides above mentioned learning mechanisms, different preparation techniques like blending, mixing, mashing, pureeing or seasoning are often used by parents in practice to improve the vegetable intake in children**. Chapter 4** describes whether strategies like masking or covering the original often disliked vegetable taste is an effective technique in stimulating children's vegetable intake. Cooked spinach was our test product. One hundred and three children were randomly assigned to one of the four groups: pure (pure spinach), diluted (spinach a la crème), hidden (spinach ravioli) and control (green beans). Children consumed their vegetable products six times at home during the main meal. Pre and post-tests -in which ad libitum intake and preferences were measured- were conducted at the day cares. All groups increased their spinach intake from pre- (53.4 g +/- 56.7) to post-exposure (90.6 g +/- 75.0) by an average increase of 70%. These results indicate that toddlers increased their ad libitum intake, irrespective from whether they were exposed to the pure spinach taste, or to a diluted taste, or whether the vegetable was 'hidden'.  However, the effect on intake did depend on the child's neophobia status, with neophobic children being less responsive to the intervention.

**Chapter 5** investigates the influence of the strategy of offering a choice of vegetables. Seventy children were randomly assigned to a choice or a no-choice condition, and exposed 12 times to six familiar target vegetables at home during dinner. In the choice group, two selected vegetables were offered each time, whereas the no-choice group only received one vegetable a time. Average vegetable intake in the choice group was higher compared to the intake in the no-choice group, respectively 58 and 49 gram. These results showed a small, but hardly robust effect of choice-offering on vegetable intake in the expected direction (i.e. choice-offering increases vegetable intake). In addition, it seems that individual characteristics such as baseline vegetable liking and age predicted vegetable intake to be higher when the child liked vegetables better and with older age.

**Chapter 6** describes the relation between breastfeeding and later vegetable intake in 2–6-year-old children. Here, the results of 10 intervention studies performed under the EU research programme HabEat across Europe are investigated. This enabled us to study the relationship between breastfeeding history and other relevant factors, and the child's actual vegetable consumption as measured prior to the intervention in these studies. We used a conceptual model to predict later vegetable intake. Breastfeeding duration contributes positively to later vegetable intake in all countries. As expected,

children who were breastfed for a longer period consumed more vegetables. Age and gender did not predict vegetable intake in our sample.

Finally, **chapter 7** discusses the main outcomes and conclusions of this thesis. We showed that repeatedly offering a novel or disliked vegetable in a trusted positive environment is highly effective in promoting vegetable intake in toddlers and pre-schoolchildren. Repetition seems to be the way to teach/learn young children to accept, and shape their acceptance of novel/disliked foods. Other strategies such as flavouring, adding energy or taste modification may still be helpful in promoting the willingness to try and taste vegetables in young children. Additional strategies are needed such as choice-offering to boost already liked/familiar vegetable intake when children get older.

Individual differences in child characteristics such as food neophobia, breastfeeding duration and age play a role in shaping food preferences and therefore they need more attention when applying strategies to promote children's vegetable acceptance. These results can be used by parents, caregivers and public health to stimulate children's vegetable consumption to maintain a more balanced diet in children.

# Acknowledgements

"Je zult je diamanten niet in ver weg gelegen bergen of in gindse zeeën vinden,
je diamanten liggen in je eigen achtertuin als je ernaar graaft".

*Rusell Conwell* (1843-1925)

Bovenstaande is ook van toepassing op het onderzoek wat ik de afgelopen vijf jaar heb gedaan. Dit werk had ik nooit kunnen verrichten zonder de hulp van vele mensen die dicht bij me hebben gestaan tijdens deze periode. Ik ben heel blij dat ik zoveel lieve mensen om me heen heb gehad in deze tijd: samen bereik je meer dan alleen.

Een van mijn belangrijkste drijfveren voor het uitvoeren van dit onderzoek zijn de kinderen geweest. Ik zie de kindertijd als een heel bijzondere en belangrijke fase die van invloed is op de rest van je leven, en gezonde voeding ligt aan de basis hiervan. Ik vind hun gedrag fascinerend en hun originele, eerlijke reacties op alles wat ik aanbood, gaven mij nieuwe inzichten. Kinderen zijn daarom mijn grootste inspiratie bron geweest bij het werk. Zonder de kinderen (en hun ouders) die deel hebben genomen aan de onderzoeken had dit boekje nooit tot stand kunnen komen. Ook de gastvrijheid die ik heb mogen beleven op de kinderdagverblijven van de overkoepelende organisatie Kinderopvang Wageningen heb ik als bijzonder prettig ervaren. Speciaal wil ik graag Marion van Leeuwen bedanken voor het altijd bereid zijn om mee te denken en klaar te staan voor ons. Ik vond het heel bijzonder om met jou te mogen samenwerken. We konden lezen en schrijven met elkaar.

Een andere inspiratie bron voor dit boekje is mijn liefde voor groente. Deze liefde is bij mijzelf met de paplepel ingegoten. Mijn herinneringen gaan terug naar vroeger, waar mijn vader in zijn vrije tijd een (grote) moestuin bestierde, mijn moeder heerlijke gerechten met veel groente bereidde en wij als gezin aan tafel alles opaten. Ook stonden mijn ouders samen vaak in de keuken om alle groente te blancheren en in te maken want er was vaak veel meer van het land afgekomen dan wij konden consumeren. Mijn zus en ik zijn op die manier blootgesteld aan vele verschillende groente en zijn jullie daar nog steeds dankbaar voor.

Dit boekje had niet tot stand kunnen komen als Kees niet het lef had gehad om mij aan te nemen. Heel fijn dat je mij een kans gaf als toch niet standaard en geen piep jonge PhD student meer. Bedankt voor je vertrouwen in mij. Het was altijd fijn om met jou van gedachten te wisselen over het onderwerp. Jouw ervaring is van ontelbare/onnoembare/onschatbare waarde.

Gerry, je bent echt een topcoach en daarnaast ook een ijzersterke supervisor, een echte powervrouw. Je wist mij te helpen, te stimuleren en mij uit te dagen om telkens weer een niveau hoger te komen. Daarnaast, was jij er altijd voor mij en kon ik altijd mijn verhaal bij jou kwijt. Ik zal nooit vergeten hoe rustig jij bleef en mij bijstond toen bleek

dat de usb stick waar de presentatie op stond alleen maar lege resultaten weergaf tijdens mijn allereerste congres.

Dear HabEat colleagues, too many to be mentioned here, it was really a great honour to work together with so many talented people. I was so lucky to be part of an international project when I just started as a freshman. Thanks for all the fruitful discussions, feedback and nice gala diners we shared together on our annual meetings, workshops and conferences. Special thanks to Sylvie Issanchou. You are very supportive and always interested in my research, I really appreciated it.  I want to give a special wink to Annemarie Olsen, Per Møller, Sophie Nicklaus, Lucy Cooke, Sam Caton, Marion Hetherington and Gertrude Zeinstra.

Vincenzo Fogliano, Marion Hetherington, Jaap Seidell and Hugo Weenen, I would like to thank you for the critical reading of my thesis and for the willingness to participate in my committee.

Onderzoek doen, betekent ook data verzamelen en dit werk was nooit tot stand gekomen zonder de hulp van alle studenten die mij hierbij geholpen hebben. Annelies van Dijk, Eline Houben, Daniela Briceno, Monique Zwinkels, Jacelien Balkema-van Berkum, Renske van Asten en Roosmarijn Kralemann bedankt voor de gezellige uurtjes die we voornamelijk op de kinderdagverblijven en in de keukens samen hebben doorgebracht. We hebben honderden kilo's groentes samen verwerkt, verpakt, uitgedeeld en geserveerd!

All members of the Sensory science 'eetclub'/'tasty talk' and old colleagues (especially Sanne Griffioen-Roose, Pleunie Hogenkamp, and Anne Wanders), thanks for sharing so many interesting research topics with me and all the feedback I received from you.

Collega's van het Biotechnion en Agrotechnion, teveel om iedereen persoonlijk op te noemen, er was altijd wel een 'slachtoffer' te vinden in de gang, trappengat, of bij het koffie automaat om even van gedachten te wisselen zowel persoonlijk als zakelijk. Er wordt zoveel mooi onderzoek gedaan allemaal met mensen in relatie tot gezondheid. Op deze manier dragen ook wij ons steentje bij aan de maatschappij, daar kunnen we trots op zijn.

Jacqueline, Karen, Gea, Jasmijn en Didi waar zouden we zijn zonder jullie? Heel bijzonder hoe jullie altijd klaar staan voor een ander en voor mij.

Voor bijna al mijn studies zijn er 'nieuwe' producten ontwikkeld. Oh, wat was ik blij met je Els als jij weer heerlijk praktisch en realistisch naar al mijn ideeën keek. Ook Monica en haar dochter Amber bleken geïnteresseerd te zijn in mijn onderzoek. Hoewel Amber niet altijd blij was als ze weer eens proefkonijn voor de te testen producten mocht zijn. Monica, super dat ook jij altijd meedacht en mij een beetje in de gaten hield vanaf de zijlijn.

Gertrude en Coraline, wij deelden de passie voor het onderzoek doen naar kinderen en hun eetgedrag. Hoe heerlijk was het om met jullie hierover te kunnen sparren en van gedachten te wisselen. Gertrude, daarnaast ben je voor mij altijd een grote steun geweest en kon ik altijd terecht met mijn vragen bij jou.

De Wageningense clan Karin, Sanna, Simone en Wati, altijd dicht in de buurt en altijd bereid om met mij te wandelen, lunchen, theetje of wijntje te drinken en mee te denken over het leven! Bedankt voor alle heerlijke en eerlijke uren.

Beautyclub (beauty & brains!) heerlijk hoe wij de sociale/gezellige dingen van het leven delen en dit ook hebben weten te gieten in een avondprogramma waarbij we tegelijkertijd iets nuttigs doen. Dankbaar ben ik voor het feit dat we altijd van die heerlijke pittige discussies hebben en het heel verhelderend is om andere visies/meningen te horen. Laten we zo scherp blijven!

Heidi, mijn soulmate, ik kan je altijd bellen en dan begrijp je direct wat ik bedoel. We hebben niet veel woorden nodig omdat we zelfs van een afstand (Haarlem-Wageningen) elkaar aanvoelen. Echter als we elkaar dan zien lijkt het wel een soort waterval van woorden. Ik waardeer het zeer dat jij altijd kritisch blijft en de juiste vragen weet te stellen. Ook jouw humor is zeer ontspannend.

Elise mijn roomie en maatje, wat was het heerlijk verfrissend en verhelderend om met jou een kamer te mogen delen. Helaas Colombia is niet naast de deur en ben je niet bij mijn verdediging, maar we keep in touch for life.

Mijn paranimfen, Valeri en Annemarie. Valeri, trouwe vriendin vanuit een eerdere Wageningse periode. Tijdens onze studententijd deelden we al lief en leed en ik hoop dat we dit nog jaren voorzetten. Annemarie, ik heb jou pas echt leren kennen tijdens 'our last stretch of the PhD'. We hebben heerlijke lunch wandelingen samen gemaakt en hadden altijd genoeg zaken om over te praten. Ik vind het heel fijn dat jullie aan mijn zijde staan en zitten tijdens mijn verdediging.

Lieve zus, Dymph wat ontzettend jammer dat je er niet bij kan zijn maar je bent altijd aanwezig in mijn hart. Bedankt voor je inspiratie voor het ontwerp van het boekje en voor de vele gezellige keren als je weer eens langs kwam vanuit de States. Jacques, je bent echt een trouwe supporter die niet alleen mijn 'papers' las maar ook de informatie brochures die naar de ouders van de deelnemers werden verstuurd, werden gelezen en gekeurd door je.

Lieve pap en mam, ja ik zie jullie nog zitten op één van de woensdagen als jullie naar Wageningen kwamen, samen brieven vouwen aan de keukentafel om deelnemers te werven voor één van mijn studies. Jullie leven altijd met mij mee en zijn zeer geïnteresseerd, hoe fijn is dat. Wat heftig dat je op deze speciale dag er niet bij bent lieve pap, maar geestelijk ben je zeker van de partij. Mijn gedachten gaan terug naar

samen eten aan tafel met verse, eerlijke producten en gezelligheid. Ik ben jullie hier heel dankbaar voor en ik hoop dat weer door te kunnen geven aan de volgende generatie(s).

Oenie ook wil ik graag jou bedanken voor het altijd klaar staan als achterwacht thuis als ik naar één van mijn congressen ging en Christiaan ook op reis was.

En dan last but not least, mijn kleine eigen familietje : Christiaan, Chris, Floris en Eva, mijn rots in de  branding, mijn steun en toeverlaat, mijn alles. Ik geloof dat ik nergens zo goed ontspan en mezelf kan zijn als ik maar bij jullie samen ben en hoe heerlijk is dat. Jullie waren dus de beste afleiding ever tijdens dit proces. Bovendien had ik 3 gratis proefpersonen erbij. Er is menig groente pureetje, chipje of soepje geproefd thuis! Christiaan, jij bent de beste personal coach die je maar kan wensen. Je bent er altijd voor mij als ik je nodig heb, je kunt goed luisteren en stelt kritische vragen. Hoe bijzonder is het, als je samen op een rots aan de Noorse fjorden de wetenschap bedrijft. Wat fijn dat we zoveel dingen in het leven samen delen. Ik hou van je en ik hoop dat we nog vele avonturen samen mogen beleven!

"It always seems impossible until it's done".

*Nelson Mandela* (1918-2013)

# About the author

## Curriculum vitae

Victoire Wilhelmina Theresia de wild was born on 19 June 1966 in Tilburg, The Netherlands. After completing secondary school (Athenaeum-B) at 'Mill Hill College' in Goirle, she started to study Food Technology at the Wageningen University in 1985 with specialisation in process technology, product quality and logistics. As part of her study she conducted two master projects, the first one at the division of food processing about the separation of fatty acids with membrane technology and the second one at the division of management about a critical analyse of quality systems in the animal feeding industry. She performed an intern ship at the University of Guelph, school of engineering, Ontario, Canada, about the effect of using different process conditions on the quality of wieners.

After obtaining her master's degree in 1991, Victoire worked as a food expert at the Dutch Board for margarines, fats and oils on producing an European overview of the legalised additives in margarine. In 1992 she joined Cargill BV and started as a technical management trainee. Here she performed different jobs in the Netherlands, Brazil and the United Stated of America. She coordinated and implemented an integral quality system for several fruit juice factories, set up a package line for the retail industry, was a production supervisor and member of the management team. In 2000 she moved to France together with her family. In 2002 they returned and after a couple of years taking care of her family, she started to study Psychology at the University of Leiden. In 2009 she completed cum laude a BSc degree and was interested to work in the research field.

In 2010 she moved from Haarlem to Wageningen and was appointed as a PhD candidate at the Division of Human Nutrition at the Wageningen University. The PhD project was carried out within an European project 'HabEat', on understanding how infants and children develop and form food habits and on exploring strategies to change these habits within early childhood. During her PhD project, Victoire joined the educational programme of the Graduate school VLAG. She attended several (international) conferences and courses, and was involved in teaching and supervising students at BSc and MSc level. She was a member of the research committee of the Division of Human Nutrition. Currently, Victoire is working on education and research at the sensory science and eating behaviour group of Wageningen University.

## List of publications

### Publications in peer reviewed journals

**Victoire W.T. de Wild**, Cees de Graaf, Gerry Jager. Effectiveness of flavour nutrient learning and mere exposure as mechanisms to increase toddler's intake and preference for green vegetables. Appetite. 2013; 64: 89-96.

**Victoire W.T. de Wild**, Cees de Graaf, Gerry Jager. Efficacy of repeated exposure and flavour-flavour learning as mechanisms to increase preschooler's vegetable intake and acceptance. Pediatric Obesity. 2015; 10(3): 205-212.

**Victoire W.T. de Wild,** Cees de Graaf, Hendriek C. Boshuizen, Gerry Jager. Influence of choice on vegetable intake in children: an in-home study. Appetite. 2015; 91: 1-6.

### Submitted papers

**Victoire W.T. de Wild,** Cees de Graaf, Gerry Jager. Efficacy of offering vegetables in different taste experiences on its acceptance in toddlers.

**Victoire W.T. de Wild**, Gerry Jager, Annemarie Olsen, Vassiliki Costarelli, Eric Boer, Gertrude G. Zeinstra. Relation between breastfeeding duration and later vegetable intake in 2–6-year-old children in 10 studies across Europe.

### Abstracts

**Victoire de Wild,** Gerry Jager, Kees de Graaf. Effect of flavour nutrient learning as a mechanism to increase children's preference for vegetables. Appetite. 2012; 59(2): 637. (Abstract for the British Feeding and Drinking group, annual meeting 2012, Brighton, UK, oral presentation).

**Victoire de Wild,** Kees de Graaf, Gerry Jager. Repeated exposure more effective than flavour flavour learning as mechanism to increase vegetable consumption in pre-school children. Appetite. 2013; 71: 473 (Abstract for the British Feeding and Drinking group, annual meeting 2013, Loughborough, UK, poster presentation).

**Victoire de Wild,** Kees de Graaf, Gerry Jager. Repeated exposure more effective than flavour flavour learning as mechanism to increase vegetable consumption in pre-school children. 2013 (Abstract for end symposium of VIVA project, EU FP7 Marie Curie, St Andrews's, Ireland, poster presentation).

**Victoire de Wild,** Kees de Graaf, Gerry Jager. Repeated exposure more effective than flavor flavor learning as mechanism to increase vegetable consumption in pre-school children. 2013 (Abstract for the 10th Pangborn Sensory Science Symposium 2013, Rio de Janeiro, Brazil, oral presentation).

Zeinstra G., Kooijman V., Costarelli V., de Graaf C., Hausner H., Jager G., Kremer S., Moller P., Moschonis G., Olsen, A., **Wild, V**. Strategies to increase vegetable consumption in 3-to 6-year-olds: theory and practice. Annals of Nutrition and Metabolism. 2013; 63:40. (Abstract for the International Union of Nutritional Sciences, International congress of Nutrition, Granada Spain, 2013).

**Victoire de Wild,** Kees de Graaf, Gerry Jager. Freedom of choice: Effect of choice-offering on vegetable intake in toddlers in an at home situation. 2013 (Abstract for the Dutch NOW retraite days Voeding, Deurne, The Netherlands, oral presentation).

**de Wild V.W.T.**, Ahern S.; Bouhlal S.; Caton S.; de Graaf C.; Hetherington M.; Issanchou S.; Jager G.; Möller P.; Nicklaus S.; Olsen A.; Remy E.; Zeinstra G.G. Strategies for learning to eat and like new vegetables: Effects of different learning strategies on increasing vegetable intake and liking. Results from different studies completed in Europe. 2014 (speaker at end symposium of HabEat project, EU FP7, Dijon, France, oral presentation).

**Victoire W. T. de Wild,** C. de Graaf, H. C. Boshuizen, G. Jager. Choice-offering as a strategy to stimulate vegetable intake in young children: an in-home study. Appetite. 2015; 89: 304. (Abstract for the European Childhood Obesity Group, annual congress 2014, Salzburg, Austria, oral presentation).

**Victoire de Wild,** Kees de Graaf, Gerry Jager. Effect of offering vegetables in different taste gradients on its acceptance in toddlers. (Abstract for the British Feeding and Drinking group, annual meeting 2015, Wageningen, the Netherlands, poster presentation).

## Overview of training activities

### Discipline specific courses

Course 'Sensory perception & food preferences', 2013 (Graduate School VLAG Wageningen, The Netherlands).

Course 'Regulation of energy intake; the role of product properties', 2012 (Graduate School VLAG Wageningen, The Netherlands).

Course 'Public health interventions in real-life settings', 2010 (Graduate School VLAG Wageningen, The Netherlands).

### Conferences and meetings

39th Annual Meeting of the British Feeding & Drinking Group, 2015 (Wageningen, The Netherlands; poster presentation).

15e 'Food for Thought, Healthy Start' work conference with Alliance the Gelderse Vallei, 2015 (Ede Hospital, The Netherlands, oral presentation).

HabEat Annual meetings & stakeholder workshops, 2010-2014 (Athena, Greece; Porto, Portugal; Warsaw, Poland; Dijon France: oral and poster presentations).

Meetings WEVO (Werkgroep Voedingsgewoonten/nutritional habits), 2012-2015 (The Netherlands).

24th Annual meeting of the European Childhood Obesity Group Congress, 2014 (Salzburg, Austria; oral presentation).

3th National Congress Childhood Obesity, 2014 (Ede, The Netherlands).

Nutritional Science Days, 2013 (Deurne, The Netherlands; oral presentation).

Symposium 'Sugar, Light or Water?', 2013 (VU University, Amsterdam, The Netherlands).

Symposium 'Children eating vegetables & fruit', 2013 (HabEat workshop, Wageningen, The Netherlands).

VIVA, International congress on Vegetables & eating habits in young children, 2013 (St Andrew's, Scotland; poster presentation).

36th Annual Meeting of the British Feeding & Drinking Group, 2012 (Brighton, UK; oral presentation).

## General courses

Course 'Career perspectives', 2015 (Wageningen Graduate Schools, Wageningen, The Netherlands).

Statistical course 'Longitudinal data analysis, Mixed models', 2013, (Graduate School VLAG, Wageningen, The Netherlands).

Course 'Scientific Writing', 2012 (Wageningen Graduate Schools, Language Services, Wageningen, The Netherlands).

Course 'Techniques for Writing and Presenting Scientific Papers', 2011 (Wageningen Graduate Schools, Wageningen, The Netherlands).

Educational course 'Teaching and supervision thesis students', 2011 (Wageningen University, Wageningen, The Netherlands).

Ethical course 'Philosophy and Ethics of Food Science and Technology', 2011 (Wageningen Graduate Schools, Wageningen, The Netherlands).

Course 'Science, the press & general public: communication & intentions', 2010 (Wageningen Graduate Schools, Wageningen, The Netherlands).

Course 'Didactic skills', 2010-2011 (Wageningen University competence studies, Wageningen, The Netherlands).

## Optional courses and activities

Staff seminars and research presentations, 2010-2015, Division of Human Nutrition, Wageningen, The Netherlands.

Preparation PhD research proposal, 2010.