‘Mum, can I have Brussels sprouts again?’

Development of vegetable preferences during the first 2 years of life

Coraline Barends

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Abstract

Background and aim

Most children do not eat the recommended amount of vegetables. Their low vegetable intake may be attributed to their low preference for vegetables. Since the first years of life is a sensitive period in the development of taste preferences, and since taste preferences track over time, we started a longitudinal intervention study to investigate the effect of starting weaning with repeated exposure to vegetable purées on vegetable intake and liking during the first 2 years of life. The primary aim of this thesis was to investigate whether starting weaning with vegetables compared to weaning with fruit had an effect on the intake and liking of vegetables on the short and on the long term. The secondary aim was to investigate whether starting weaning with vegetables compared to weaning with fruit, influenced the preferences for sweet taste and daily intake of sugar.

Methods

First, we conducted an intervention study (n = 101) that investigated the effects of repeated exposure to either vegetable purées (vegetable groups) or fruit purées (fruit groups) on infants' acceptance of vegetable or fruit purées during the first 18 days of weaning. Intake of the purées and mothers' rated liking were measured in the lab. From a subsample (n = 60), we also measured liking by analysing the infants' facial expressions and behaviour after consuming green beans purée in the lab. In two follow-up studies, when the infants were 12 (n = 84) and 23 months of age (n = 81), the long-term effect of the intervention was measured on intake and mothers' rated liking of the purées in the lab. Additionally, infants' daily vegetable intake was assessed with 3-day food records at both follow-ups. At the second follow-up, also the influence of starting with vegetables or fruits on children's preferences for sweet and salty tastes (n = 81), were measured with sweetened and salted water solution and by calculating their daily mono- and disaccharides intake from the 3-day food records. Additionally, a systematic review investigated the current status of knowledge about effective strategies to increase vegetable intake in children younger than 3 y.

Results

The studies showed that the group of children who were repeatedly exposed to vegetables increased their vegetable intake from 24 ± 28 g to 45 ± 44g ($p < 0.001$), while the children who were repeatedly exposed to fruit increased their fruit intake from 46 ± 40 g to 66 ± 42 g ($p < 0.05$) . Interestingly, the first vegetable intake in the fruit group, which was directly after the 18 days of exposure to fruit purées, was as low as the first vegetable intake of the children in the vegetable group at day 1. This indicates that the repeated exposure to fruit did not influence the children's vegetable intake. These results were confirmed by the results of the facial expressions, showing a decrease in negative facial expressions after repeated exposure to green beans.

At the follow-ups, when the infants were 12 and 23 months of age, no differences between the vegetable and fruit groups in green beans or apple purée were found in the lab. Daily intake of vegetables at 12 months of age, was 38% higher ($p = 0.02$) in the vegetable group (75 ± 43 g) than in the fruit group (54 ± 29 g). At 23 months of age, no significant difference in daily vegetable intake was found between the groups. Also the 23 month olds' preference for sweet water solutions and their daily mono- and disaccharides intake did not differ between groups.

Finally, the systematic review of literature showed that counselling of the parents on healthy eating and nutrition did have a positive long-term effect on their children's' vegetable intake, although the effect was relatively small. The review further showed that repeated exposure was the most studied and also the most effective strategy, since all studies reported an increase in intake after repeated exposure to a vegetable. Also exposure to a variety of vegetables showed to have a positive effect on the intake of a new vegetable.

Conclusion

Weaning with repeated exposure to vegetables has a positive influence on vegetable intake until at least 12 months of age.

Contents

Chapter 1

General introduction

Despite health benefits of eating vegetables, most Dutch children do not meet the recommended vegetable intake (1, 2). The vegetable consumption of children is also low in other European countries and in the USA (3-6). Many strategies have been investigated to promote vegetable intake. A meta-analysis of different studies on the effect of school based-interventions to promote vegetable intake in 5-12 year old children showed that school-based interventions have no or minimal effect on children's vegetable consumption (7). This suggests that it is difficult to increase vegetable intake in school-aged children. On the other hand, most studies on repeated exposure to vegetables in weaning infants have shown immediate effects of increasing infants' vegetable intake (8-13), which encourages to familiarize children with vegetables at an early age. This thesis describes a longitudinal intervention study called 'Mum, can I have Brussels sprouts again?' that was aimed to promote vegetable intake and liking in young children starting from weaning. The studies in this thesis examine the short- and long-term effects of this intervention on vegetable intake and liking, and includes basic taste preferences for sweet and salt. In addition, the relevant literature is reviewed on studies that describe strategies to improve vegetable intake in children under the age of 3y. This general introduction starts with a brief overview of the development of food preferences and the factors that influence vegetable intake and liking. It ends by discussing the aim and presenting an outline of the thesis.

1.1 Development of food preferences

Although many factors influence the vegetable intake of children (e.g. availability, parent influence, texture, social economic status (SES), the most important factor for food intake is preference or liking of a food (14, 15). Studies that measured the preferences of new-born babies for basic tastes based on facial expressions and intake showed that they have an inborn preference for sweet tastes (16, 17). In addition, they have an inborn aversion to bitter and sour tastes (18). These preferences persists into preschool age (19, 20), and can be linked to foods containing these flavours (21). Although, taste preferences might persist over the course of childhood and even into adulthood, they are not set in plaster but are mouldable and influenced by experience. In fact, familiarity with a food might be the most important determinant of a child's liking for it (15, 22).

1.1.1 First flavour and food experiences

Exposure to flavours starts even before an infant is born. Infants have fully functioning taste buds already in the third trimester of gestation (23). When the infant is still in the mother's womb, he or she can taste flavours from the mother's diet by drinking from the amniotic fluid (24, 25). Studies have shown that new-born and weaning infants have a heightened preference for a flavour if their mother consumed food containing this flavour during the last trimester of pregnancy (26, 27). After birth, the mother's diet keeps influencing her infant's flavour experience through breastfeeding. While formula milk has a constant taste, the taste of mother's milk contains flavours from the mother's diet, which in turn can influence an infant's taste preferences (27). Since these early, and indirect sensory experiences influence long-term dietary outcomes, it is important mothers have a varied diet during pregnancy (28).

Cashdan (29) proposed that the first 2 or 3 years of life are the most sensitive period of taste-preference learning. Other studies suggest that this most sensitive period exists during the first year of life when children have their first taste experiences (30-32). For example, although infants have an inborn aversion to bitter tastes and probably to sour tastes (18), young infants accept bitter tasting formula quite easily after a few exposures (33, 34). Even if young infants might show negative facial expressions while drinking a bitter formula, they often continue drinking (21). On the other hand, it seems virtually impossible to introduce older children to bitter tasting vegetable drinks (35).

An important point in taste development is the process of weaning, when gradually complementary foods are integrated in an infant's diet, which until than only consisted of milk (36, 37). Most parents start weaning their infants between 4 to 6 months of age. During this period, infants remain open to new tastes. Sweet foods are usually accepted after 1 or 2 exposures (8), but more bitter tasting foods such as vegetables might require at least 8 exposures before an infant will accept them (8, 12, 38-40).

Between the ages of 1.5 and 2 years, children start showing reluctance to try new foods—food-neophobia (41)—which usually peaks at the age of 5 years and then declines (42). This reluctance to try new foods might serve as a safety mechanism to prevent children from eating toxic foods or non-foods. According to evolutionary theories, young infants accept food more easily than older infants because all the foods they receive are given by their parents and can therefore be considered safe (29). When infants become more independent, they are able to eat the things they pick up (plants, soil). Their bigger aversion to unknown tastes can be a defence mechanism that protects them from being poisoned. This also suggests the potential survival value of consuming only small

quantities of a potentially unsafe new food until experience shows that eating the food leads to no negative consequences. This reluctance to eat more than a small amount of a new food is usually referred to as food-neophobia, and it is frequently observed in humans (42) and omnivore animals (42). This may especially complicate the introduction of bitter vegetables at this age, as most children dislike bitter tastes (43-45). Furthermore, it underlines the importance of introducing vegetables in the first year of life, before food-neophobia emerges.

1.1.2 Food preferences track over time

Several studies showed that taste preferences developed at an early age track over time (46-48), i.e. they persist over a longer period (49). This also supports an early introduction of vegetables, since other studies also suggest that the food preferences of older children and adults have already been formed, and that, though taste development continues, most food preferences acquired at young age track into childhood and even into adulthood (46, 49-51).

1.1.3 Conclusion – development of food preferences

Although some basic taste preferences are present at birth, they are mouldable by experience. The first year of life when infants have their first experiences with food seems to be a particularly sensitive period in taste leaning. Furthermore, these taste preferences track over time. Therefore, the intervention to improve vegetable intake described in this thesis was carried out with young infants at the beginning of weaning. Since early taste preferences seem to track over time, increasing vegetable preferences during weaning might positively influence the preferences of infants for vegetables when they are older and so may contribute to a higher vegetable intake.

1.2 Exposure to vegetables

1.2.1 Repeated exposure

As noted before, familiarity with a food is an important predictor of food intake. A well-studied strategy used to familiarize infants with vegetables is repeated exposure to vegetables. Most studies on repeated exposure in infants found increases in intake directly after the exposure period, and some found increases in intake even over a longer period (8, 10, 12, 39, 40, 52). Repeated exposure in these studies consisted of exposure to

the same food for 8 to 10 consecutive days or in some studies every other day.

Early in weaning, intake of a particular food can increase after one or two exposures—though more than eight exposures may be needed to increase intake of bitter vegetables (8, 12, 38-40). Most parents stop offering a food if their infant has rejected it on fewer than five occasions, as they conclude from this experience that their child does not like the food (36). In fact, however, more exposures may be needed to get the child to accept the food.

The work of Maier et al. (12) showed that learned vegetable preferences were stable over a period of 9 months. Nine months after repeated exposure, 63% of infants still liked and ate a vegetable that was not liked initially, and an additional 12,5% still ate the vegetables but did not show signs of liking. However, at the start of the intervention described in this thesis, only limited research was done into the longer-term effects of repeated-exposure interventions.

While repeated exposure to a specific vegetable increases intake of that particular vegetable, exposure to a variety of vegetables increases the acceptance of a new vegetable (39, 52, 53). Exposure to variety seems to influence not only preferences for the exposed tastes; it seems also to influence preferences for foods with similar tastes. For instance, Mennella et al. (52) showed that daily consumption of a variety of fruits enhanced infants' initial intake of other fruit types but did not affect infants' initial intake of green beans. Exposure to a variety of vegetables, however, did enhance the acceptance of green beans. This also suggests that to start weaning only with vegetables, can have a positive effect on vegetable consumption in infants. Most studies on this subject, however, measured only short-term effects. It is not clear how these preferences develop over a longer period.

1.2.2 Starting with vegetables before fruits

In the period of weaning, an infant's diet gradually changes from a milk-only diet to one of greater variety that includes fruit, vegetables, cereals and meat. The Netherlands Nutrition Centre advises parents to start weaning their infants with soft-tasting fruits and vegetables that are not sour or bitter, because these are easily accepted by infants (54). Other health professionals have recommended that vegetables be introduced before fruit because starting with fruit is thought to interfere with bitter vegetable acceptance by reinforcing the inborn preference for sweet taste (39). However, no evidence has been found for this relationship.

1.2.3 Conclusion – exposure to vegetables

There is evidence that repeated exposure can increase preference for a particular food. In addition, research has shown a positive relationship between taste preferences and the intake of foods containing these tastes (21). Therefore, we hypothesized that when infants start weaning exclusively with vegetables their acceptance of vegetables will increase with repetition and consequently will remain later in life. Though at the start of weaning, infants might accept vegetables less easily than fruits, their acceptance of the vegetables may increase after a limited number of exposures. To investigate the longer-term effect, this thesis describes a study that combined repeated exposure with variety and investigates the effect until 18 months after the intervention.

1.3 Sweet and savoury preferences

As mentioned, the bitter taste of vegetables hinders liking and consumption of vegetables. On the other hand, infants are predisposed to like sweet and salty tastes (19, 55, 56). Studies showed that children ate foods and beverages containing fat and sugars, more than they ate vegetables (51, 57, 58). New-born infants already prefer sweetened water over plain water and the preference for salt emerges around the age of 4 months. Most children that are 2 years of age even have a greater preference for salty foods than adults (59). The preference for sweet and salty tastes can be explained with reference to their energy density. Unlike vegetables, most sweet and savoury foods have a high energy density (from carbohydrates and/or fat) (51, 60, 61), which causes a satisfied feeling after consumption. Though intake of energy is important for survival, a high consumption of energy-dense foods contributes to overweight and obesity (62).

1.3.1 Conclusion – sweet and savoury preferences

Children have an inborn preference for food containing sugar over bitter vegetables. It can therefore be hypothesized that weaning with fruits reinforces this inborn preference for sweetness whereas weaning with vegetables does not. Therefore, we expected that infants who started weaning exclusively with vegetables would accept vegetables more easily and have less preference for sweet foods containing sugar (e.g. candies) later in life than infants who started weaning with fruits.

1.4 Measuring food preferences

To investigate the eating behaviour of infants, studies usually assess the intake, the maternal ratings of infants' enjoyment, and duration of feeding (27, 32, 50, 53, 63, 64). Intake is most often used as the primary outcome measure for liking, though intake might be biased due to other influences like state of hunger, wanting or accessibility (65). Liking assessed by asking mothers to judge how much their infant likes a food, is subjective and open to bias. Since it is not always clear which criteria the mothers used to assess liking, it is difficult to make comparison across infants and across studies (66, 67). In addition, liking can also be rated by an external observer (53), which has the advantage that the observer is not biased by previous knowledge of the infant's food history. However, although the ratings from an external observer are more likely to be comparable between infants within one study, they are still subjective, which makes it difficult to use as a comparison across studies.

To avoid bias from subjective liking measurements, several studies have used video-recordings to measure children's facial expressions in reaction towards the food (10, 16, 64, 68-70). This measure is systematic and more objective, since the facial expressions are not interpreted by the observer, but the contraction of specific facial muscles are counted (71, 72). The studies that measured facial expressions, found that infants with higher food intakes, showed fewer negative facial expressions that are linked with distaste, which indicates good correspondence between these measures.

1.4.1 Conclusion – measuring food preferences

To have a complete view of food preferences in infants, multiple measures should be taken into account. Measures of intake and liking rated by the mother, could be complemented with the less biased measure of the infants' facial expressions after tasting the food.

1.5 Rationale and thesis outline

Most children do not eat enough vegetables. One reason for this is that vegetables are simply not very likable because of their bitter taste and low energy content. Since the first years of life seem to be a sensitive period in the development of taste preferences, and since taste preferences track over time, we started a longitudinal intervention study to investigate the effect of the first solid foods (vegetable or fruit purées) on vegetable

intake and liking during the first 2 years of life. The aim of this PhD project was to obtain knowledge on effective strategies to increase intake and liking of vegetables in young infants. We especially wanted to know if starting weaning with vegetables has a positive effect on longer-term vegetable intake.

Chapter 2 describes this intervention and its short-term effect. The intervention compared weaning with vegetables to weaning with fruits. We hypothesized that being exclusively weaned with vegetables at the age between 4 and 6 months results in a higher acceptance of vegetables than being exclusively weaned with fruits. We anticipated a symmetrical effect on fruit acceptance in infants weaned exclusively with fruits. We hypothesized that infants' acceptance of one particular type of vegetable or fruit would increase after repeated exposure to it. Infants' intake of vegetables and fruits and mothers' rated liking are taken as measures of acceptance.

In **chapter 3**, video recordings of the intervention described in **chapter** 2 were used to analyse the effect of the intervention on behaviours and facial expressions of infants as measures of liking and wanting.

In **chapter 4**, the long-term effect of the intervention is described. Follow-up measurements were taken when the infants were 12 and 23 months of age. We hypothesized that being exclusively weaned with vegetables results in a higher preference and intake of vegetables at 12 and 23 months than does weaning exclusively with fruits.

Chapter 5 describes whether children who started weaning with vegetables had a lower preference for sweet taste (measured with sweet solutions) and a lower daily mono- and disaccharide intake than infants who started weaning with fruits. Vegetables have, next to their bitter taste component, also a glutamate (umami) taste component. Since foods with an umami taste are often linked with a salty taste, we expected that children who started weaning with vegetables would have a higher preference for a salty taste.

The articles in this thesis concern a longitudinal study that investigated the efficacy of starting with vegetables, repeated exposure, and variety to improve vegetable intake on the short and long term. In **chapter 6**, a systematic review gives a detailed overview of articles investigating strategies to increase vegetable intake during the first 3 years of life.

Finally, **chapter 7** presents a general discussion of the studies in this this thesis and describes methodological considerations and implications of the findings.

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Chapter 2

Effects of repeated exposure to either vegetables or fruits on infants' vegetable and fruit acceptance at the beginning of weaning.

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2.1 Abstract

This study investigated the effects of repeated exposure to either vegetables or fruits on an infant's fruit and vegetable acceptance during the first 18 days of weaning. We hypothesized that repeated exposure to a type of vegetable or fruit, would increase its intake. Furthermore, we expected that being exclusively weaned with vegetables would result in a higher acceptance of vegetables than being exclusively weaned with fruits. To investigate this, a 19-day intervention study was conducted in 101 healthy infants, aged 4-6 months. Infants were randomly assigned to 1 of 4 treatment groups. Two groups received exclusively vegetable purées as targets every other day for 18 consecutive days; green beans was the target for one group and artichoke for the other group. The other two groups received exclusively fruit purées including either apple or plums as the target fruit. On day 19, the vegetable groups consumed their first fruit purée and the fruit groups their first vegetable purée. At the beginning of the study on days 1, 2 and at the end on days 17, 18 and 19, the infants were fed fruit or vegetable purée in our laboratory. On days 3 to 16, the parents fed their infants the fruit or vegetable purées at home.

Outcome variables were vegetable and fruit intake over time. Mean vegetable intake in the vegetable group increased significantly from 24 ± 28 g (mean ± SD) on days 1 and 2 to 45 ± 44 g on days 17 and 18. Fruit intake in the fruit group increased significantly from 46 ± 40 g to 66 ± 42 g. Fruit intake was significantly higher than vegetable intake from the start. Repeated exposure to fruit had no effect on the vegetable intake. The first intake of green beans in the fruit groups at day 19, was 24 ± 29 g and on average as low as the green beans intake in the vegetable groups at the 1st exposure on day 1 or 2. Similarly, the first apple intake in the fruit groups on day 1 or 2 of 47 ± 48 g did on average not differ from the first apple intake of 45 ± 49 g in the vegetable groups on day 19. The mean intake of green beans and plums increased significantly after repeated exposure. The intake of the target food artichoke stayed low and the intake of apple only increased slightly. These findings confirm that at the first exposure fruit acceptance is higher than vegetable acceptance. Weaning with vegetables, but not with fruits, may promote vegetable acceptance in infants.

2.2 Introduction

Most Dutch adults and children do not meet the recommended fruit and vegetable intake. The mean fruit and vegetable consumption of young adults is about half of the recommended intake (1). In Dutch children between 4 and 6 years old only 25% meets the recommendation of 50 -100 grams of fruits a day, and less than 1% meets the recommendation of 50-100 grams of vegetables a day. Two to 3 year olds do slightly better, but still only 17 to 21 % of them reaches the recommended intake of vegetable and fruit (1, 2).

Cashdan (3) proposed that the first 2 or 3 years of life are the most sensitive period in taste preference learning. Other researchers suggest that this most sensitive period is during the first year of life (4-6). For example, although infants have an inborn aversion to bitter and sour tastes (7), young infants accept bitter tasting formula quite easily after a few exposures (8). Early in weaning, intake of a particular food increases after one or two exposures (9), although more than 8 exposures may be needed to increase intake of more bitter vegetables (10-15). However, most parents already stop offering a food after they have given their infants a food less than 5 times and conclude, based on this experience, that their child does not like it (15). Furthermore, the work of Maier et al. (12) suggests that learned vegetable preferences are stable over a longer period. Nine months after repeated exposure, 63% of the infants still liked and ate a vegetable that was not liked initially. Other studies also suggest that in older children and adults, preferences have already been formed, and although taste development continues, most food preferences acquired at young age track into childhood and even adulthood (16-19). Hence it is important to know what foods and in which order these foods should be introduced in the first year of life (20).

In the period of weaning, the infants' diet gradually changes from exclusively milk to a diet with more variety including fruit, vegetables, cereals and meat. The Netherlands Nutrition Centre advises parents to start weaning their infants with soft tasting fruits and vegetables that are not sour or bitter because these are easily accepted by infants (21). Other health professionals have recommended to introduce vegetables before fruit, because starting with fruits is thought to reinforce the infants' inborn preference for sweet taste which interferes with the more bitter vegetable acceptance (14). However, no evidence has been found for this relationship. There is evidence that repeated exposure can increase preference for a particular food. In addition, research has shown a positive relation between taste preferences and the intake of foods containing these tastes (22). Therefore, we hypothesized that when infants start weaning exclusively with vegetables, their acceptance of vegetables will increase and consequently their acceptance of vegetables

later in life. Although at the start of weaning, infants might accept the vegetables less easily than the fruits, their acceptance of the vegetables may increase after a few exposures.

Weaning with particular vegetables could have a positive effect on the vegetable intake in general. Repeated exposure seems not only to influence the preferences for the exposed taste, but also appears to transfer to other similar foods. For instance, research of Birch et al. (9) suggests that daily consumption of a particular fruit enhanced the infants' initial intake of other fruit types, but did not affect the infants' initial intake of peas. In addition, Mennella et al. (23) showed that exposure to a variety of vegetables did enhance the acceptance of a new vegetable but exposure to a variety of fruits did not. This also indicates that starting weaning only with vegetables can have a positive effect on the vegetable acceptance in infants, and therefore also might positively influence the long-term vegetable intake.

The main objective of this study was to assess the effect of weaning exclusively with vegetables as compared to weaning exclusively with fruit, on the acceptance of fruit and vegetables after a 19 day intervention period. This is the first intervention that compares weaning with vegetables to weaning with fruits. We hypothesized that being exclusively weaned with vegetables, at the age between 4 and 6 months, would result in a higher acceptance of vegetables than being exclusively weaned with fruits. We anticipated a symmetrical effect on fruit acceptance in infants weaned exclusively with fruits. We hypothesized that the infant's intake of one particular type of vegetable or fruit would increase after repeated exposure to it.

2.3 Participants and methods

2.3.1 Participant characteristics

The participants were recruited from the area of Wageningen and Almere in the Netherlands where both the research locations were. They were recruited via local newspapers, maternity or infant welfare centers, postnatal care groups, and a mailing to subscribers of babyinfo.nl (a Dutch advertisement website that gives a box with free products for subscribers expecting a baby). Only healthy infants between 4 and 7 months old, who were not being weaned yet, were included in the study. Infants with known food allergies, swallowing or digestion problems, or other medical problems that could influence the ability to eat, were excluded. We included 101 parent-infant pairs in this 19

day intervention study. Two of them were not able to come the last 3 times to our lab and were excluded from further analysis. From six infants we do not have data of the 19th day, therefore they are only included in analysis about the first 18 days. In most cases the mother came all 5 times to our lab and therefore we provide characteristic information of the mothers, and refer to the mother instead of the parent. In 4 cases the father always accompanied the infants, and in six cases the father accompanied the infant on at least one of the 5 days.

Table 2.1 describes the characteristics of the infants and their mother for each of the four treatment groups. The mean age of the infants at the start of the intervention was 5.4 ± 0.8 (mean ± SD) months. At the start of the intervention, 53% of the infants were still breastfed, and 37% of them were still exclusively breastfed. From the formula fed infants, 19% had been breastfed for longer than 12 weeks. There were no significant differences in characteristics between the four treatment groups.

The study protocol was approved by the Medical Ethical Committee of Wageningen University, the Netherlands.

Table 2.1 Characteristics of the infants and their mothers categorized by the four treatment groups (infants that were repeatedly exposed to green beans, artichoke, apple or plums purée).

Characteristics	Green beans (n = 24)	Artichoke (n = 27)	Apple (n = 24)	Plums (n = 24)
Infants				
Age (days)	162 ± 24[a]	160 ± 23	165 ± 26	162 ± 23
Gender (% boys)	46%	59%	44%	56%
Weight (g)	7043 ± 799	6699 ± 846	6750 ± 779	7093 ± 929
Duration of breastfeeding (weeks)	14.1 ± 10.4	17.1 ± 10.3	14.6 ± 11,5	17.7 ± 10.0
Mother				
Age (y)	31 ± 3.7	30 ± 5.0	31 ± 6.0	32 ± 6.0
BMI (kg/m^2)	25.7 ± 4.8	24.8 ± 3.7	25.9 ± 4.7	25.2 ± 4.9
Education (n)				
Low	2	6	6	3
Middel	8	9	9	6
High	14	12	9	15

There were no significant differences ($p<0.05$) in percentages or means between the 4 conditions. [a] Mean ± SD for all such values. Weight was measured at the participants' latest visit to the infant welfare center. This was usually within a month before the measurement.

2.3.2 Study design

This 19 day intervention study was part of a longitudinal study on the development of vegetable and fruit acceptance in infants. The whole study design spans a 2 year period, and will be finished by the end of 2012.

Mother-infant pairs participating in this first part of the study were randomly assigned to 1 of 4 treatment groups. After being fed rice flour porridge for 5 days to get used to solid foods, two groups received exclusively vegetable purées consisting of either green beans or artichoke on every other day during 18 consecutive days. The other two groups received exclusively fruit purées consisting of either apple or plums every other day. On the other nine days, the vegetable groups received other types of vegetables and the fruit groups other types of fruits, selected by the researcher, to bring variety in the diet. On day 19, the vegetable groups received their first fruit and the fruit groups their first vegetable purée. On days 1, 2, 17, 18 and 19, the vegetables or fruits were given in one of the two laboratories. On days 3 to 16 the mothers fed their infant the vegetable or fruit purées at home (see Figure 2.1 for a detailed study design).

Vegetable groups:

	Lab		Home														Lab		
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Artichoke group	Art	GB	Art	Bro	Art	Cfl	Art	Bro	Art	Cfl	Art	Bro	Art	Cfl	Art	Bro	Art	GB	Apl
Green Bean gr.	GB	Art	GB	Bro	GB	Cfl	GB	Bro	GB	Cfl	GB	Bro	GB	Cfl	GB	Bro	GB	Art	Apl

Fruit groups:

	Lab		Home														Lab		
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Plum group	Pl	Apl	Pl	Ba	Pl	Pe	Pl	Ba	Pl	Pe	Pl	Ba	Pl	Pe	Pl	Ba	Pl	Apl	GB
Apple group	Apl	Pl	Apl	Ba	Apl	Pe	Apl	Ba	Apl	Pe	Apl	Ba	Apl	Pe	Apl	Ba	Apl	Pl	GB

Figure 2.1 Experimental design showing the purées given on each day of the intervention in the laboratory (lab) and during the home exposure period (Home) to the four different treatment groups of infants, consisting of the artichoke, green beans, plums and apple group. As target foods the infants received artichoke (Art), green beans (GB), plum (Pl), or apple (Apl) purée every other day. At home they received broccoli (Bro) and cauliflower (Cfl), or banana (Ba) and pear (Pe) purée on the other days.

We chose two targets foods that are commonly consumed in the Netherlands (green beans and apple purée), and two that are less commonly consumed (artichoke and plums).

The order at which the different type of vegetables and fruit were given on days 1 and 2 was counterbalanced. This order also affected the order on the rest of the days. Half of the infants in each group received the purées in the order as shown in Figure 2.1, with the target food on the uneven days, the other half received the target food on the even days.

The mothers were instructed not to introduce any other new foods during the 19 days of the study. If the mother thought the baby had more hunger for solid food, they were allowed to give the infant rice flour porridge. This porridge was also fed to the infants during the five days preceding the intervention, to accustom the infant to the act of eating a solid food.

2.3.3 Foods

The vegetable and fruit purées used were commercially available products from Nutricia (Danone). The jars with green beans, artichoke and apple purée contained 125 g purée, whereas the jars with plum purée contained 100g. Table 2.2 shows the ingredients and nutrient composition of all products used in the study. We chose green vegetables, and intentionally not vegetables like carrots or pumpkins, because we expected that the taste of the last two would resemble fruit more than green vegetables would. Other reasons for choosing these purées were more practical. The vegetables and fruits had to be regarded as safe (by the Netherlands nutrition centre) for infants between 4 and 6 months of age and had to be available in the assortment of Danone.

Sensory profiles of the foods (shown in Table 2.3) were provided to us together with the products. They had been obtained with an adult trained panel of 9 assessors to rate the sensory aspects of smell, taste and after taste. They used continuous, unstructured 100mm line scales from low (0/nil) to high (100/intensive). The vegetable products were evaluated at 40°C, and fruit products at room temperature. Vegetable and fruit products were evaluated separately. The profiles were used to examine whether or not the products characteristics could explain a potential difference in intake.

The sensory profiles of the foods used in this study showed that the artichoke purée had a much more bitter and sour taste than the green beans purée. Furthermore, the artichoke purée had a less sweet and a higher astringent mouth feel than green beans.

Table 2.2 Ingredients and nutrient composition per 100g of the four target foods

Type of purée	Ingredients	Energy		Protein	Carbo-hydrate	Fiber
		kJ	kcal	(g)	(g)	(g)
Green beans	Green beans (75%) Rice (17%), Water (8%)	130	31	1.8	5.4	2.4
Artichoke	Artichoke (25.2 %), Potato (29.1%), Modified corn starch Sunflower oil	183	43	0.9	9	1.2
Apple	Apple (100%)	196	46	0.3	10.8	1.5
Plums	Plums (62%), Sugar (5%) Rice flour, Water	356	84	0.5	20	n/a

n/a = this information was not available.

Table 2.3 Sensory profiles of the fruit and vegetable purées used during the study, compared between the fruit purées and between the vegetable purées separately

	Apple	Plum	Ba-nana	Pear	Green Beans	Arti-choke	Broc-coli	Cauli-flower
Taste								
Intensity	59[a]	71	79	64	71[b]	74	76	60
Sweet	49[a]	62	64	55	30[b]	11	28	20
Sour	44[a]	34	46	36	15[b]	47	12	9
Bitter	7	4	10	12	3[b]	22	22	20
After Taste								
Intensity	53[a]	58	69	54	58	63	62	52
Sweet	34[a]	47	54	42	-	-	-	-
Sour	38[a]	31	41	31	13[b]	41	13	12
Bitter	7	5	8	10	5[b]	18	17	18
Smell								
Intensity	53 [a]	56	83	62	74 [b]	82	79	70
Sweet	43 [a]	44	69	51	28 [b]	10	21	19
Sour	32 [a]	24	41	35	19 [b]	44	17	8
Mouth feel								
Thickness	35 [a]	34	52	24	51 [b]	34	49	65
Powdery	7 [a]	8	48	10	42 [b]	22	33	59
Astringency	34 [a]	33	43	35	14 [b]	41	19	16

Scoring on continuous, unstructured 100 mm line scales. from scale low (0/nil) to high (100/intensive).

[a] Significant difference between fruit purées

[b] Significant differences between vegetable purées

2.3.4 Procedure

Familiarisation to the procedures

To accustom the children to the testing procedures, the mothers fed their infants rice flour porridge with a supplied feeding spoon during 5 days prior to the intervention. By doing this the mothers also practiced the feeding guidelines that were used during the study. The feeding guidelines were provided in a letter that accompanied the porridge and they were also explained by telephone about two weeks prior to the intervention.

Measurements in the laboratory

To ensure that the infant was sufficiently hungry, the mother was asked not to feed the infant milk, other beverages, or solid foods within 1 hour before the lab feeding (24). Furthermore, the measurement in the lab was each day at the same time.

In the laboratory the mother fed the infant the purées in a quiet room, where the infant was placed in a baby chair. A bowl with 2 jars of vegetable purée was handed to the mother and the mother fed the infant at their usual rate until the end of the feeding was indicated by the infant (i.e. when it rejected the spoon more than 3 successive times). The infant had to taste at least 3 spoons of food. The mother was instructed to touch the infant's lip with the spoon with food. Usually the infants reacted to this by taking a bite or sucking the food from the spoon. The subsequent spoons were only presented in front of the infants mouth, without touching it. With a live camera connection we filmed the feeding session, to check whether the mothers followed the guidelines. When the mother kept trying after 3 rejected spoons, or stopped too soon, we entered the room and explained the mother to continue or to stop. After the first feeding session we gave them feedback on this, by explaining the guidelines again and giving some examples. At the end of each feeding session, the mother was asked to rate how much she thought the infant liked the food, using a 9-point scale, ranging from 1 = "dislikes very much" to 9 = "likes very much". The pre- and post-weight of the bowl including the spoon and bib was weighted to measure the actual intake.

Home-exposure period

At the end of the 2nd test-day at the lab, the mothers received the jars of puréed vegetables or fruits for the home exposure period.

Each jar was labelled with the date on which it had to be fed to the infant and numbered from 3 to 16 corresponding to the respective days of the intervention period. The feeding was carried out every day at about the same time and in the same way as during days 1

and 2 in the lab. After the feeding, the mothers rated on a 9-point scale how much the infant liked the purée, just like they did in the lab. In addition, the time of feeding and special circumstances were noted in a booklet, as a reminder for the mother to feed the child at the same time every day. The mother was instructed to empty both jars completely on a plate and to put all what was left over after the feeding, including the vegetable purée that was spilled on the table, floor, bib, child's face, etc., back in the jar and to seal the jar with the lid and put it in the refrigerator. Although some purée would be spilled in this manner, this percentage would be equal in all treatment groups. In order to have a standardized measure of home intake, the jars had been pre-weighted in the lab before the home exposure period, and after they were collected and were post-weighted again in the lab. If jars were missing or unopened we asked the parents about this, to check compliance.

2.3.5 Questionnaires/measures

Two weeks before the intervention each mother was asked to fill in two questionnaires, one with questions about her infant and the other with questions about herself. The infant questionnaire consisted of questions about the birthdate, weight, breastfeeding status (breastfed, formula fed, or combination of both), and breastfeeding history (length of exclusive breastfeeding and length of total breastfeeding).

The questionnaire for the mother consisted of questions about her own date of birth, weight, height, work status, and education level. Furthermore, it included a 10-item food neophobia questionnaire (25) translated in Dutch, to assess the mothers' neophobia. Neophobia was scored on a 7-point bipolar rating scale (1 = disagree strongly and 7 = agree strongly). After reversing the scores of 4 items that were stated in opposite direction, the responses to the ten items were summed per participant (possible ranges: 10–70).

The education level was classified into 4 categories: 1 = no education or primary school; 2 = lower or middle level secondary school; 3 = higher level secondary school or vocational education; 4 = college bachelor degree; university/ master degree. Since all mothers completed at least lower secondary school level, the categories were regrouped after data collection to: Low = lower or middle level secondary school; Middle = higher level secondary school or vocational education; high = college bachelor degree; university/ master degree. Work status was divided in the categories: 1 = 30 h or more; 2 = 8 to 29 h; 3 = less than 8 h/no work or student; and 4 = house wife.

2.3.6 Data analysis

All our data was analyzed using the statistical package IBM SPSS statistics 19. Differences in infants' and mothers' characteristics were compared between the green beans, artichoke, apple and plums group using one way analysis of variance for the continuous variables (infants' age, weight, breastfeeding duration, and mothers' age, BMI and neophobia). Chi-square tests were used to test for differences in the categorical variables (gender, milk feeding type, and mothers' education).

To measure the effect of weaning exclusively with vegetables as compared to weaning exclusively with fruits, both vegetable groups and both fruit groups were combined. The mean vegetable intake was compared with the mean fruit intake before and after the repeated exposure period using independent sample *t*-tests. To determine whether mean intake increased between the 1st and 9th exposure to the repeated exposed foods, paired *t*-test were conducted for the green beans, artichoke, apple and plums group separately. The same was done for the control foods (e.g. green beans in the artichoke group, see Figure 2.1) for the first and second exposure.

Since the infants just started weaning and their intake would increase also because they got more accustomed to eating practice, we checked whether vegetable intake was not influenced by repeated exposure to fruit. The first green beans intake of the fruit groups at day 19 was compared with the first green beans intake of the vegetable groups on day 1 or 2 using independent sample *t*-tests. Furthermore, home intake of the target vegetables and fruits was tested for a linear increase by regression analysis. Finally, Pearson's correlations between mothers' liking ratings and intake were calculated.

2.4 Results

2.4.1 Mean vegetable intake in the vegetable groups and fruit intake in the fruit groups before and after repeated exposure

Mean vegetable intake in the two vegetable groups increased significantly with 24 ± 28 g (mean ± SD) on days 1 and 2 to 45 ± 40 g on day 17 and 18 ($p < 0.001$) (see Figure 2.2). On the first two days, fruit intake in the fruit groups was 21 g more than vegetable intake in the vegetable groups ($t(99) = -2.91$, $p < 0.01$). Fruit intake increased significantly from 45 ± 44 g on days 1 and 2 to 66 ± 42 g on day 17 and 18 $p < 0.01$. On days 17 and 18 fruit intake in the fruit groups was still significantly higher than vegetable intake in the vegetable groups $t(97) = -2.43$; $p = 0.017$.

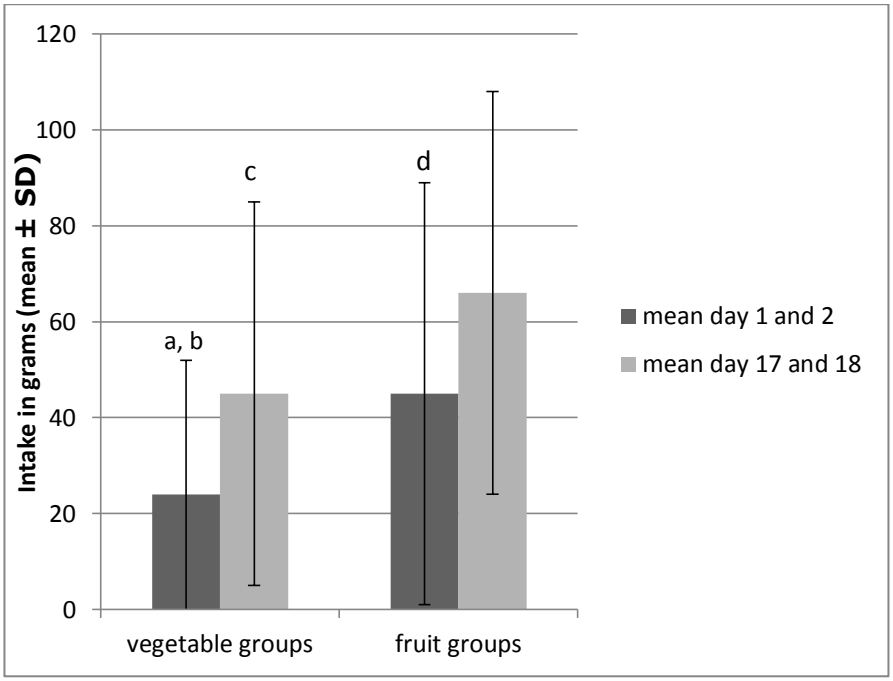

Figure 2.2 Intake of vegetable and fruit purées (mean ± SD) at days 1 and 2 and at days 17 and 18 of infants that are weaned with exclusively vegetable purées or fruit purées.
a = significantly different ($p < 0.01$) from the mean of days 1 and 2 in the fruit groups;
b = significantly different from the mean of days 17 and 18 in the vegetable groups ($p < 0.001$);
c = significantly different from the mean of days 17 and 18 in the fruit groups ($p < 0.01$);
d = significantly different from the mean of days 17 and 18 in the fruit groups ($p < 0.02$).

2.4.2 Effects of repeated exposure on intake in the four treatment groups

As shown in Table 2.4, the intake of the repeatedly exposed green beans and plums increased significantly between the 1st (day 1 or 2) and the 9th exposure (day 17 or 18). In the artichoke group, the artichoke intake at the ninth exposure was low and did not increase significantly from the 1st to the 9th exposure. At the first exposure, the mean apple intake was high 53 ± 53 g (mean ± SD) and increased to 66 ± 56 g but this was not statistically significant ($p = 0.103$).

2.4.3 Intake target food versus control food within treatment groups

In the green beans group, also the intake of the control food artichoke increased significantly (p = 0.042) (see Table 2.4). However, the increase of the green beans intake was 35 ± 66 g, while the increase of artichoke intake was only 18 ± 40 g (p = 0.125). On the contrary, in the artichoke group the intake of the control food green beans increased significantly while the artichoke intake did not.

In the apple and plums group the increase in intake of the target fruit (respectively apple and plum purée) was slightly higher than that of the control fruit that was given on days 2 and 18 (see Table 2.4).

Table 2.4 Intake of the target and control vegetables and fruits, on the 1st exposure (day 1 or 2) compared to the 9th exposure (day 17 or 18) to green beans, artichoke, apple and plums purée.

Group	Intake of:	Intake before home exposure period (day 1 or 2) in g	Intake after home exposure period (day 17 or 18) in g	*p*-value *t*-test
Green beans group (n= 24)	Green beans	24 ± 22[a]	59 ± 70	0.016
	Artichoke intake	*26 ± 23*	*43 ± 42*	*0.042*
Artichoke group (n = 27)	Artichoke intake	24 ± 40	27 ± 19	0.603
	Green beans	*21 ± 31*	*50 ± 53*	*<0.001*
Apple group (n = 24)	Apple intake	53 ± 53	66 ± 56	0.103
	Plums intake	*47 ± 43*	*51 ± 35*	*0.698*
Plums group (n = 24)	Plums intake	37 ± 35	75 ± 50	<0.001
	Apple intake	*43 ± 43*	*70 ± 43*	*0.033*

[a] Mean ± SD for all such values

2.4.4 Green beans intake after repeated exposure to fruits and apple intake after repeated exposure to vegetables

Repeated exposure to fruit had no effect on the vegetable intake and vice versa. The mean first green beans intake in the fruit groups at day 19 (24 ± 29 g) was just as low as the mean first green beans intake in the vegetable groups at the 1st exposure (23 ± 27 g)

(see Table 2.5). Also the mean first apple intake in the fruit groups on day 1 or 2 (47 ± 48 g) did not differ from the mean first apple intake in the vegetable groups on day 19 (45 ± 49 g).

Table 2.5 The first intakes of green beans in the vegetable groups on day 1 or 2 (n = 51) compared with that in the fruit groups on day 19 (n = 43) and the first intakes of apple in the vegetable groups on day 19 (n = 50) compared with that of the fruit groups on day 1 or 2 (n = 48).

	Vegetable groups	Fruit groups	*p*-value
1st green beans intake (g)	23 ± 27[a] (day 1 or 2)	24 ± 29 (day 19)	0.814
1st apple intake (g)	45 ± 49 (day 19)	47 ± 48 (day 1 or 2)	0.842

[a] Mean ± SD for all such values

2.4.5 Individual differences in intake

We found large differences in intake between the infants (see Figure 2.3). In the vegetable groups, a few infants ate considerably more than average on days 1 and 2, while in the fruit groups the intake was normally distributed. If in the artichoke group for example, the infant with the high intake of 175 g on day 1, was considered as an outlier and removed from the analysis, the intake of artichoke purée in the artichoke group would have increased from 18 to 27g ($p = 0.068$) instead of from 24 to 27 g. However, we chose to not remove the outliers since there were no abnormalities during the measurements or in the characteristics. Also, when the analysis were run without the outliers, the results did not change.

To explain the individual differences we assessed the correlation of intake with the characteristics breastfeeding duration and age at the start of weaning. The correlation of intake with age at the start of weaning was weak but not significant ($r = 0.18$, $p = 0.079$). Breastfeeding duration did not correlate significantly with intake ($r = 0.02$, $p = 0.787$).

2.4.6 Intake of target foods during home exposure period

In Figure 2.4 the intake of the four target foods during the home exposure period are shown. Similar to the intake in the lab, the artichoke intake did not increase at home and was low compared to the intake of the other target foods. Apple intake was, like in the lab, high from the start. Accordingly, both the artichoke and the apple groups did not show significant linear increase, whereas the green beans and the plums group did

(respectively: $r = 0.164$; $p < 0.05$ and $r = 0.167$; $p < 0.05$). When the total group was split in a younger and older group with the cut-off point at 5.4 months (mean age), we found a significant higher intake at the first exposure in the latter group (younger: 21 ± 24 g and older: 45 ± 48 g, $p = 0.002$).

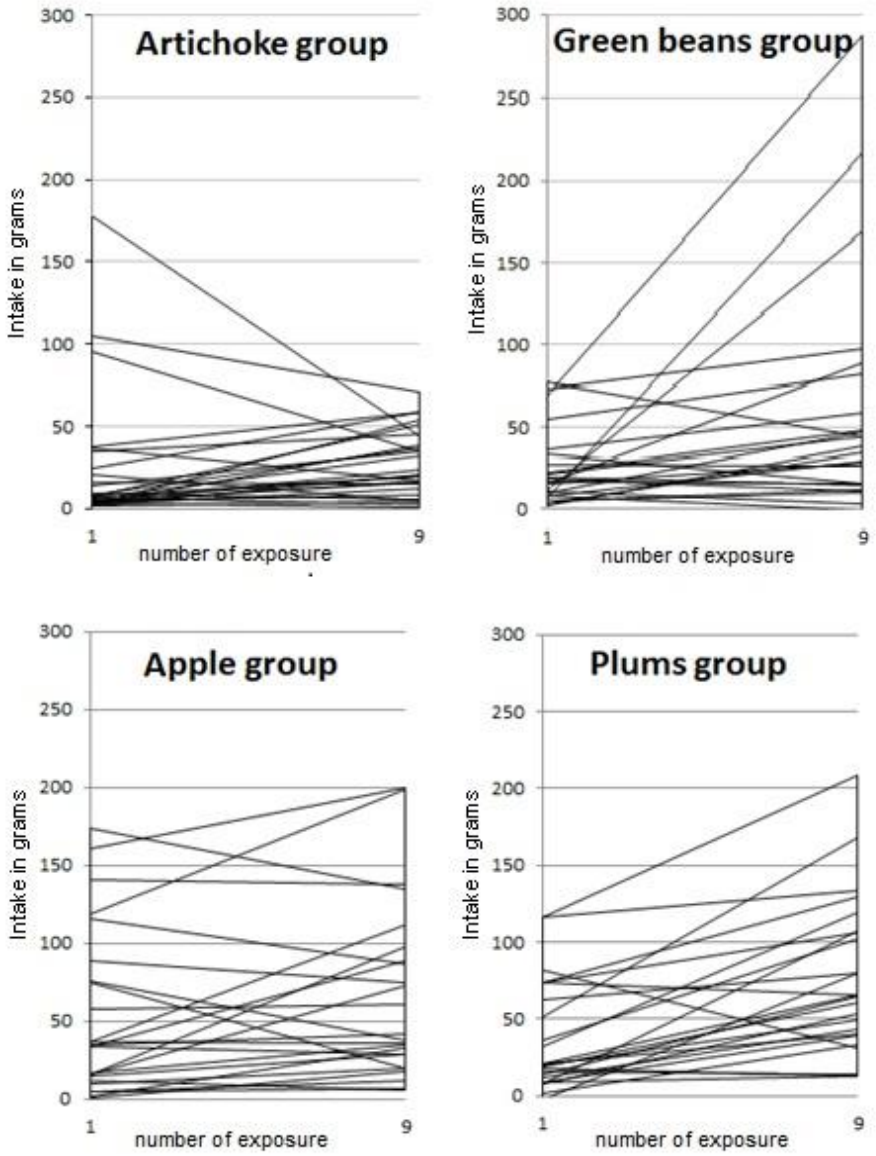

Figure 2.3 Intakes at the 1st and 9th exposure of the target foods of the individual infants in the Green Beans, Artichoke, Apple and Plums groups.

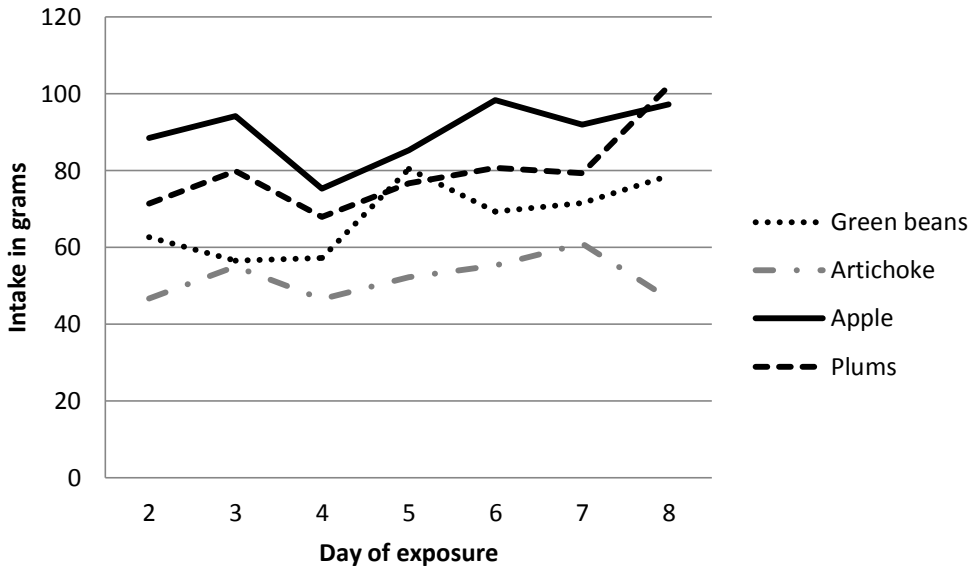

Figure 2.4 Mean home intake of green beans, artichoke, apple and plums purée of infants during weaning on exposure 2 -8.

2.4.7 Effect of repeated exposure on mothers' rated liking scores in the four treatment groups

Similar to intake, the mothers' liking ratings of green beans and plums increased significantly between the 1st and 9th exposure, with 0.8 and 1.6 points respectively (see Table 2.6). Apple and artichoke liking ratings did not increase significantly. Liking ratings correlated positively with intake. At the first exposure the correlation between intake and liking was $r = 0.53$, $p < 0.001$, at the ninth exposure this was $r = 0.54$, $p < 0.001$.

Table 2.6 Mothers' rating of infants' liking of the target and control vegetables and fruits, on the 1st exposure (day 1 or 2) compared to the 9th exposure (day 17 or 18) to green beans, artichoke, apple and plums purée.

Group	Liking of:	Liking before home exposure period (day 1 or 2)	Liking after home exposure period (day 17 or 18)	*p*-value *t*-test
Green beans group (*n* = 24)	Green beans liking	5.4 ± 1.9[a]	6.2 *± 1.8*	0.049
	Artichoke liking	*5.3 ± 2.0*	*5.4 ± 1.7*	*0.806*
Artichoke group (n = 27)	Artichoke liking	4.8 ± 2.2	5.2 ± 1.8	0.284
	Green beans liking	*4.6 ± 2.2*	*6.2 ± 1.6*	*0.003*
Apple group (n = 24)	Apple liking	5.8 ± 2.1	6.4 ± 1.2	0.110
	Plums liking	*5.9 ± 1.9*	*6.5 ± 1.3*	*0.200*
Plums group (n = 24)	Plums liking	5.7 ± 1.9	7.0 ± 0.6	0.001
	Apple liking	*6.1 ± 1.6*	*6.8 ± 1.0*	*0.074*

Scoring on a 9 points scale ranging from 1 = "dislikes very much" to 9 = "likes very much"
[a] Mean ± SD for all such values.

2.5 Discussion

The aim of our study was to investigate the effect of repeated exposure exclusively to vegetables or exclusively to fruits at the start of weaning on vegetable and fruit intake after weaning. Furthermore, we investigated the effect of repeated exposure to a particular vegetable or fruit type on its intake. The study showed that repeated exposure to green beans and plums significantly increased the intake of these foods. Because apple intake was already higher from the start, a smaller and not significant increase of apple intake was seen after repeated exposure to it. However, repeated exposure to artichoke had no effect on its intake. Vegetable intake was significantly higher after repeated exposure to vegetables than it was after repeated exposure to fruits. In addition, the vegetable intake after repeated exposure to fruit purées was the same as the vegetable intake on the first exposure in the vegetable group. Overall fruit intake was significantly higher than vegetable intake.

The intake results measured in our laboratory were supported by the results of the home exposure period and the liking ratings given by the mothers. Also at home, apple intake was highest at the start while artichoke intake was the lowest and stayed low during the study period. The liking ratings given by the mother for a specific food in the lab showed a

high and positive correlation with its measured intake in the lab, indicating that the amount of intake is one of the indicators for the mothers to evaluate whether their infant likes the food (12).

During this study the infants started weaning and learned how to eat. Although the infants had a 5-day practice period in which they were given a neutral tasting rice flour porridge to get used to eating solid food, they were still learning how to eat during the intervention. One could argue that the increase in intake could partly be caused by the learning effect of practicing how to eat and not of how to accept the taste. However, like Birch et al. (9) and Mennella et al. (23), we found that repeated exposure to a variety of fruit purées did not affect the vegetable intake. This finding supports the notion that this increase was not entirely due to learning how to eat solid food. The vegetable intake in the fruit group at day 19 was the same as the vegetable intake of the vegetable groups on days 1 and 2, while the fruit group ate purées for 18 more days. The fruit intake showed similar effects. The mean first encounter of fruit intake after 18 days of vegetable exposure was the same as the mean first encounter of fruit intake in the fruit group at days 1 and 2. These findings suggest that the increase of the target foods was due to the intervention and not just to learning how to eat from a spoon.

We expected that repeated exposure to all target foods would lead to an increase in intake and that nine exposures would be enough to increase also the intake of the vegetables that might be initially liked less. However, artichoke intake in the artichoke group did not increase. The sensory profile of artichoke shows that it is much more sour and less sweet than the green beans purée (Table 2.3). This can explain the low intake of artichoke, because infants have an innate dislike of sour tastes and a preference for sweet tastes (7). Schwartz et al. (26) found that the preference for sweet is still present at 6 months, a dislike for sour taste compared to water was found at 3 months, but not at six months. Although the green beans intake initially was also lower than the intake of the fruit purées, the repeated exposure did increase the intake. The artichoke purée was probably not palatable enough for most infants for the repeated exposure to have effect. Whether this is due to the combination of a low degree of sweetness and a relatively high degree in sourness, or that other factors played a role cannot be concluded based on our study design.

The artichoke intake did not increase in the artichoke group. Nevertheless, the mean intake of green beans in this group had increased at the end of the intervention. This could be due to the exposure to artichoke, but also the exposure to the broccoli and cauliflower at home could have played a role. The advice to wean infants with a variety of vegetables makes sense in this case. Not only it influences the intake of a new vegetable,

it also gives a change in tastes that might be less monotonous and is more appreciated by the infant. When a parent would give a type of food every day, which is not appreciated by the child during the first few times, this can be very discouraging and the parent may stop feeding this food. We do not think this means that parents should not feed their infants sour or bitter foods, like the Netherlands Nutrition Center recommends. Like our research showed, there are big differences in individual intake, and in some infants the intake of artichoke did increase. What the perfect balance is between variety and repeated exposure, may differ per child or per food. Giving a vegetable every other day might not have been optimal for artichoke, but seems to have worked well with green beans.

The finding that after repeated exposure to vegetables the mean intake of green beans was twice as high as after repeated exposure to fruits, suggests that repeated exposure to vegetables affected the vegetable intake but not the fruit intake. Vice versa we saw the same effect. The fruit intake after repeated exposure to fruits was significantly higher than after repeated exposure to vegetables. Furthermore, not only the intake of repeated exposed foods, but also that of the control vegetable in the vegetable groups and the control fruit in the fruit group increased. These findings are in line with the research of Birch et al. (9) and Mennella et al. (23), indicating that repeated exposure affects similar foods, but not foods from a different food category. Effects of different vegetables and of different fruits are similar because they belong respectively to the category vegetables and the category fruits. Vegetables and fruits belonging to different food categories are clearly experienced as different from each other. Within the categories a certain level of nutritional and sensory proximity should be present, but more research is necessary to define how these levels relate to each other (15).

Another factor that could have contributed to the increased intake of the control vegetable or fruit or was that the infants were exposed to a variety of different vegetables or fruits during the intervention, which increases the acceptance of a new taste (14, 23, 27). The Dutch Nutrition Center (21) advises to start weaning infants with a variety of different vegetables and fruits. Because of these recommendations, variety was integrated into the experimental design to prevent the infants from receiving a monotonous diet. However, we expected that the repeated exposure effect to a particular vegetable or fruit plus the variety of foods in the design would be stronger than the exposure to variation alone. This was not the case when comparing between foods within a category. However, as described before, it seems to be the case when comparing between vegetables and fruits. Infants that were repeatedly exposed to a variety of vegetables had a higher intake of green beans purée on day 19 than infants who were exposed to a variety of fruit purées.

A large variation in intake between the infants, as shown by the large standard deviations, was found. While the intake of most infants was just a few grams during the first exposure, some infants already ate more than 100 g at this occasion. It seemed that not all infants equally learned how to eat during the habituation period of 5 days prior to the intervention period. Although the rice flour porridge that was fed during the habituation period had no flavor and tasted the same as the milk it was made of, some mothers reported that infants did not like this porridge. Furthermore, the age difference may have played a role. Mothers decided when their child was ready to start with the study and thus to start weaning. The only restriction was that the infant had to be at least 4 months old and was not older than 6 months. We reminded the mothers that the infant welfare centers and the Dutch Nutrition Center (21) recommend to exclusively breastfeed infants until six months. Prior to the study, we recommended the mothers to issue with their child health practitioner the best time for their infant to start weaning. As a result some infants were only four, while others were already 6 months old. We found that infants younger than 5.4 months ate significantly less than older infants. Since children with different age were equally divided between the intervention groups this could not have influenced the different results between groups. Next to that, also some random factors may have led to the big variations in intake within the treatment groups. Sometimes infants were tired for unknown reasons, or stopped eating for a while because they pooped in their diaper. Nevertheless, we tried to keep the conditions as constant as possible by letting the mothers feed the purées to their infant every day at about the same time, with the same interval after the last feeding. Furthermore, all participants were randomly assigned to one of the four treatment groups, thus we can assume that the factors that led to the variations in intake were equally divided over the groups.

Mothers may also have influenced intake. Although the mothers had guidelines about how to feed the infant and to stop feeding only when the child rejected the spoon 3 consecutive times, they could have misinterpreted the infants behavior due to their own believes about the food. In our lab we corrected them if they did not follow the guidelines during the first session, but at home we had no control. Artichoke was the least known food by the mothers and some found it odd to give it to their infant, nevertheless, they fed it to their infant like the other purées. On the other hand, a lot of mothers asked questions about the plum purée, because they thought it was associated with diarrhea. However, the intake of plum purée was highest of all. This shows that, although the mothers may have had presumptions about the food, the infants still influenced how much they ate.

Finally, it is striking that intake of vegetable purées before and after the repeated exposure was significantly less than the intake of fruit purées. Already on day 1, when

infants had yet no experience with vegetable or fruit purées, the mean intake in the fruit groups was almost double than that in the vegetable groups. This is probably due to the innate preference for sweet taste and the experience with tasting sweet mother and formula milk. This difference did not disappear when infants were repeatedly exposed to a variation of vegetables. Although the vegetable intake in the vegetable group increased, so did the fruit intake in the fruit groups. By day 18 the fruit intake in the fruit group was still about 20 g more than the vegetable intake in the vegetable group. This difference is even more remarkable when the energy intake is considered (see Table 2.2), since the fruit purées had a higher energy density. Infants are able to compensate for energy intake and drink more milk when its energy content is lower (28). In this study, however, the fruit with the highest energy density was consumed the most. This can be explained both by the fact that foods with a higher energy density give a more satisfying feeling and that this fruit also contained more sugars and had a sweeter taste.

2.6 Conclusion

Vegetable intake was generally lower than fruit intake, however, repeated exposure to vegetables increased vegetable intake while repeated exposure to fruit increased fruit intake. Starting weaning exclusively with vegetables could be effective to stimulate vegetable intake. However, it is too early to recommend this to parents, because we do not know what would happen in the long term when the infants are further introduced to new fruit, vegetables and other foods. Nevertheless, our study clearly shows that weaning exclusively with vegetables has a positive influence on vegetable intake in the short term.

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Chapter 3

Infants' liking for green beans, assessed based on behavioural and facial expressions, increased after repeated exposure to vegetables at the start of weaning

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3.1 Abstract

Most children's diets do not meet the recommended amount of vegetable intake. In a previous study, we found that repeated exposure to vegetables increased children's intake of green beans. Since liking is an important predictor for intake, the aim of this study was to investigate the effect of repeated vegetable exposure on infants' liking for green beans, assessed with the help of behaviour and facial expressions.

The 19-day intervention was conducted on 101 four to seven month old infants, who were randomly assigned to a vegetable or a fruit group. The vegetable group received only vegetable purées for the first 18 days of weaning, with a specific vegetable every other day. The fruit group received only fruit purées during these 18 days, with a specific fruit every other day. On day 19, the fruit group received their first time vegetable purée. The feeding session on days 1, 2, 17, 18 and 19 were videotaped.

For present study, recordings of 60 mother-infant pairs were analysed regarding their acceptance and behaviour in response to the approaching spoon and the negative facial expressions in response to green bean purée. The recordings took place on days 1 or 2 and 17 or 18 for the vegetable group, and on day 19 for the fruit group.

After repeated exposure to vegetables, infants expressed significantly fewer negative facial expressions and their acceptance increased significantly. Comparing the vegetable group before and after repeated exposure to green beans with the fruit group's first exposure to green beans, it was found that there was only a significant difference between the negative facial expressions of the vegetable group after repeated exposure and the negative facial expressions of the fruit group after the first exposure. Additionally, a significant negative correlation was found between intake and facial expressions. These results support previous findings that liking of vegetables can be increased by repeated exposure to vegetables, but not by repeated exposure to fruits.

3.2 Introduction

Children's diets in the Netherlands are not meeting the recommended amount of vegetables (1-3), in part because liking of a food is the most important predictor of intake (4-6) and many children do not like vegetables due to the bitter taste and the low energy density (7, 8). During the first year of life, initial taste preferences are formed and infants are the most open to accepting new tastes. As taste preferences track over time (9), a higher preference for vegetables in infancy could also increase intake in later life. To investigate infants' eating behaviour, studies usually investigate the intake, the maternal ratings of infants' enjoyment, duration of feeding, infants' temperament, or liking (10-13). Intake, as the most common primary outcome measure, can be used as measurement for liking, but it might be biased due to other influences like wanting or accessibility (14). A more objective method for assessing liking is analysing the infants' facial expressions, which is supported by literature that indicates a relationship between the number of negative facial expressions and (dis)liking of food (15-17).

Since infants cannot verbally communicate, facial expressions give caregivers important non-verbal information (18). This idea is supported by Oster (19), who demonstrates that for infants with facial anomalies, the signal value of facial expressions is very robust. At birth, new-borns can already distinguish between the basic sweet, sour and bitter tastes, represented by stereotypical facial expressions (16, 20). Sweet tastes elicit positive facial expressions like sucking, while sour and bitter tastes cause negative facial expressions like nose wrinkling, lip pursing or gaping (16). Evolutionarily, bitterness indicates toxicity and defensive responses like gaping or nose wrinkling serve to protect the sense organs or prevent ingestion (16, 21). These facial expressions also warn the caregiver that the infant may have ingested poisonous food (16, 22). Currently, parents often stop offering food to infants after presenting the food less than 5 times, having the impression that the infant dislikes the offered food (11, 23-25). However, parents should continue feeding infants these foods in order to provide repeated opportunities for the infants to get used to the tastes, thereby increasing intake (18, 26-28). Although the dislike of strong bitter and sour tastes, as for most vegetables, is inborn, it is changeable through experience due to repeated exposure to vegetables (18, 26).

This study is part of the study of Barends et al. (29), which found that repeated exposure to vegetables at the start of weaning increased intake of vegetable purée in infants, but repeated exposure to fruit did not. Intake is influenced by factors such as availability and satiety, but most importantly by liking (4-6). Since liking of a food is an important predictor of future food intake (4), the aim of the present study was to investigate whether repeated exposure to vegetables increased the infants' liking of the vegetables. Liking was

assessed by the infants' nonverbal behaviour and negative facial expressions.

We hypothesised that the number of negative facial expressions would be reduced after repeated exposure to vegetables (vegetable group). By contrast, we expected that after starting weaning with fruit purées (fruit group), the number of negative facial expressions to green beans would not differ significantly from those of the vegetable group at the first exposure.

3.3 Material and methods

3.3.1 Participants

Participants were mother-infant pairs, recruited from the area of Wageningen and Almere in the Netherlands (29). The infants had to be healthy, between 4 to 7 months old and were not being weaned yet. Exclusion criteria were known food allergies, swallowing or digestion problems or other medical problems that could influence the ability to eat. In the end 101 mother-infant pairs were included, and randomly divided over 4 intervention groups (green beans, artichoke, apple and plum group, for more details see design below).

All feeding sessions were filmed, but since video analysis is very time consuming, videos of in total 60 infants were evaluated. From each of the four intervention groups 15 infants were randomly selected. Recordings of 2 infants of the green bean group, 2 of the artichoke group and 2 of the plum group had to be replaced. One of the videos had to be exchanged since the mother's head blocked the vision of the infant's face and could therefore not be coded. The other 5 recordings were replaced because of more than 4 refusals over 9 spoons. Replacing videos were randomly chosen from the same subgroup.

Because of the small sample sizes per subgroup for the present analysis, the infants of the green beans and artichoke group were considered as one group: the vegetable group (n = 30). The infants of the apple and plum group were considered as the fruit group (n = 30).

None of the characteristics of the infants differ significantly between the vegetable and the fruit group (Table 3.1), nor between infants from the initial artichoke, green beans, plums and apple group. Ethical approval for this study was given by the "Medical Ethical Committee of Wageningen University".

Table 3.1 Infants' characteristics categorised by the two subgroups expressed as mean (SD).

Infants' characteristics	vegetable group (n=30)	fruit group (n=30)
Age (days)	161 ± 24[1]	165 ± 24
Gender (% boys)	57	54
Weight (g)	6863 ± 851	6901 ± 753
Duration of breastfeeding (weeks)	16 ± 10	18 ± 11
Mothers age (years)	30 ± 4	31 ± 6
Mothers BMI (kg/m^2)	25.3 ± 4.2	25.2 ± 4.8

There were no significant differences ($p<0.05$) between the two subgroups.
[1] Mean ± SD for all such values.

3.3.2 Design of the study

The VaVo study was a longitudinal study that followed infants from weaning until the age of 2 y. It started with a 19-days intervention study to examine the development of fruit and vegetable acceptance by feeding the infants repeatedly fruit or vegetable purées. Further details are mentioned elsewhere (29).

In brief: for the intervention, the infants were randomly assigned to one of the four subgroups (schematic presentation of design see Figure 3.1). On the first day two subgroups were fed with vegetable purées (artichoke or green bean purée) and the other two subgroups with fruit purées (plum and apple purée). They received this purée every other day for the next 18 consecutive days exposing the infants 9 times to the target fruit/vegetable. On the other days other types of vegetables were offered to the vegetable groups and other types of fruits to the fruit groups to bring variety in their diet. On day 19 the vegetable groups received their first fruit and the fruit groups their first vegetable purée. On days 1, 2, 17, 18 and 19 the measurement session took place in the laboratory, where the mother-infant pairs were video recorded during the feeding. On days 3 to 16 the mothers weaned their babies at home. The mothers were asked not to feed their infant any other new food during the period of the study.

For the present study only the videos of the measurements in the lab on which the infants received green beans were analysed. To measure if repeated exposure to vegetables increased the liking for green beans, the facial expressions of the infants of the vegetable group were coded on the days 1 or 2 and 17 or 18 (depending on which day they received the green beans). Of the fruit group only the videos of day 19 were analysed, since this was the only day that the infants in this group received green beans purée (as their first vegetable).

Vegetable group:

		Lab		Home														Lab		
	day \ RE vegetable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Vegetable group	Artichoke	Art	(GB)	Art	Bro	Art	Cfl	Art	Bro	Art	Cfl	Art	Bro	Art	Cfl	Art	Bro	Art	(GB)	Apl
	Green beans	(GB)	Art	GB	Bro	GB	Cfl	GB	Bro	GB	Cfl	GB	Bro	GB	Cfl	GB	Bro	(GB)	Art	Apl

Fruit group:

		Lab		Home														Lab		
	day \ RE fruit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Fruit group	Plums	Pl	Apl	Pl	Ba	Pl	Pe	Pl	Ba	Pl	Pe	Pl	Ba	Pl	Pe	Pl	Ba	Pl	Apl	(GB)
	Apple	Apl	Pl	Apl	Ba	Apl	Pe	Apl	Ba	Apl	Pe	Apl	Ba	Apl	Pe	Apl	Ba	Apl	Pl	(GB)

Figure 3.1 Study design showing the purées given on each day of the intervention in the laboratory (Lab) and during the home exposure period (Home) to vegetable group and the fruit group. The infants were repeatedly exposed (RE) to artichoke (Art), green bean (GB), plum (Pl), or apple (Apl) purée every other day. At home they received broccoli (Bro) and cauliflower (Cfl), or banana (Ba) and pear (Pe) purée on the other days. The circles indicate the days analysed in present study.
Half of the participants received the purées on days 1 and 2 in a counterbalanced order. Depending on this order the sequence of the other days was adapted.

3.3.3 Procedure during feeding sessions

Feeding sessions took place in a quiet room in our lab. Sessions on day 1 or 2 and day 17 or 18 in the vegetable group took place at the same time of the day. The mother was asked not the feed the child within one hour before the session.

A bowl with 2 emptied jars of purée was handed to the mothers with one spoon and a bib. The green beans purée was served at a temperature of 42°C. The feeding guidelines were derived from methods of previous studies (18, 31, 32) and included that the mothers should fed their child at their usual pace until the spoon was rejected 3 consecutive times, and the child should have tasted at least 3 spoons.

With a live camera connection we checked whether the mothers followed the guidelines, and corrected them if necessary. These recordings were later used for coding the infants' facial expressions.

3.3.4 Coding procedure and measures

All 90 videos were coded by one coder, following the coding procedure of the Infant Behaviour, Facial Expression Coding System (IBFECS) developed by Madrelle et al. (33-35). Before coding the actual videos, the coder had to follow the training, described in the training manual accompanying the coding procedure, to be able to recognise the relevant behaviours and facial expressions. The description of the facial expressions in this manual are derived from the Facial Action Coding System (36) and the Babyfacs manual (37). After the training the coder completed a test consisting of 34 video clips. Cohen's kappa coefficient between coder and the by the manual provided answers was 0.65 (90% agreement), which is regarded as substantial agreement (38).

Of the 90 videos that were coded for this study, the first 9 spoons of each feeding were taken into account using The Observer (Noldus Information Technology). The coder was blinded for the group and day number. Video recordings of the feeding sessions to green beans were coded in 3 dimensions: rate of acceptance; behaviour towards the food; and negative facial expressions (Table 3.2). These 3 dimensions were separately coded and a score was calculated for each part.

Rate of acceptance was assessed by mouth movement which is defined as the early, late, enforced or refused opening of the mouth in reaction to the approaching spoon. The rate of acceptance was rated on a 4 point scale from 0 to 3 per spoon and the points of all 9 spoons were summed up per participant.

Table 3.2 Scoring scheme of acceptance, behaviours and facial expressions

Behaviours and facial expressions	Points per spoon
Acceptance of the food	
early acceptance (opens mouth when spoon approaches)	3
late acceptance (opens mouth when spoon is near)	2
enforced acceptance (opens mouth when spoon touches the lips, push	1
Refusal (infant does not open the mouth)	0
Negative behaviour	
looks away/ turns head/ looks down	1
arches back/ pulls body away	1
pushes spoon away	1
gets fussy/ cries	1
Positive behaviour	
leans forward/ reaches for food/ grasp spoon	1
Facial expressions	
inner brow raising *(AU 1*)*	1
brow lowering *(AU 4)*	1
squinting *(AU 7)*	1
nose wrinkling *(AU 9)*	1
upper lip raising *(AU 10)*	1
lip corners down *(AU 15)*	1
gaping *(AU 26/27)*	1

Scoring scheme derived from Madrelle et al. (33) and Hetherington et al. (35)
*Corresponding Action Units described in the Facial Action Code System by Ekman (36).

Positive and negative behaviours that were coded were the infant's response towards the approaching spoon (i.e. grabbing the spoon, lean forward, looking away, turning the head away, arching back, pushing the spoon away, getting fussy). To get the scores for the behaviours, the presence of the different behaviours per spoon were counted and summed up over the 9 spoons.

Since *negative facial* expressions are more discriminating than positive ones (12, 18), only negative expressions were taken into account (i.e. inner brow raising, brow lowering, squinting, nose wrinkling, upper lip raising, lip corners down and gaping). The score was calculated by counting the negative facial expressions in reaction to tasting the purée and summed up over the 9 spoons.

These scores were used for further statistical analysis. Intra-rater reliability was measured

by double coding 15 videos by the same coder and resulted in a Cohen's Kappa of 0.74 (89% agreement). Finally, to measure the inter-rater agreement index, these 15 videos were also coded by a coder who did the same training for a study at Leeds university (39) and this resulted in an Cohen's Kappa of 0.74 (89% agreement).

3.3.5 Other measures

The basic characteristics were collected before the 19-day intervention of the VaVo study (29). The mothers were asked to fill in questionnaires including questions about infant's age, weight, duration of breastfeeding and gender. Other outcome measures were infants' intake of green beans (in grams) during the filmed sessions in the laboratory. The pre- and post-weighting of the bowl including the spoons and bib was calculated to measure the actual intake. Mothers rated how much their children liked each feeding in the lab on a 9-point scale (mothers' rated liking).

3.3.6 Statistical analysis

The basic characteristics of the infants were compared between the vegetable and the fruit group by Wilcoxon signed-rank for the continuous variables (age, weight, duration of breastfeeding, intake) and by chi-square test for the categorical variable gender.

To examine the effect of repeated exposure to vegetables on liking for green beans, the behaviour, acceptance, total amount of negative facial expressions and number of every single negative facial expression on day 1/2 and day 17/18 in the vegetable group were compared using Wilcoxon signed-rank tests.

The negative and positive behaviour, mouth movement, total number of negative facial expressions and number of each single negative facial expression of the vegetable group on day 1/2 and day 17/18 were compared to those of the 1st exposure of green beans to the fruit group using Mann-Whitney tests.

Finally correlations between the number of facial expressions and intake, and facial expressions and mothers' rated liking were analysed by Spearman's rho. A multiple regression analysis was conducted to control for the possible confounders age, gender, weight, duration of breastfeeding and intake as independent variables and negative facial expressions as dependent variable.

3.4 Results

3.4.1 Effects of repeated exposure to green bean purée on negative facial expressions and behaviour in the vegetable group

The rate of acceptance of green bean purée, increased significantly in the vegetable group from a median score of 11 on day 1/2 to 13 on day 17/18 ($z = -2.69$, $p = 0.007$; see Table 3.3). In accordance, the number of negative facial expressions decreased significantly (23.3 vs. 15.9, $z = -2.63$, $p = 0.008$), with significant less expressions of brow lowering ($z = -2.10$, $p = 0.039$), squinting ($z = -2.13$, $p = 0.033$) and upper lip raising ($z = -3.07$, $p = 0.002$). Analysis of the infants' negative behaviour in response to green bean exposure showed no significant differences between day 1/2 and day 17/18.

3.4.2 Comparison between vegetable and fruit group

Exposure to green bean purée in the vegetable group on day 1/2 and day 17/18 was compared with the 1st exposure to green beans in the fruit group on day 19 (Table 3.3). At the 1st exposure to green beans behaviour, acceptance and total facial expressions in the vegetable group on day 1/2 did not differ from those in the fruit group on day 19. However, compared with the fruit group on day 19, significantly less total negative facial expressions ($z = -2.04$, $p = 0.041$), brow lowering ($z = -2.44$, $p = 0.015$), upper lip raising ($z = -2.36$, $p = 0.018$) and gaping ($z = -2.06$, $p = 0.039$) occurred in the vegetable group on day 17/18.

3.4.3 Correlations of acceptance, behaviour and facial expressions with intake and mothers' rated liking

The multiple regression showed no confounding of gender, age, weight and duration of breastfeeding on total facial expressions on day 1/2, day 17/18 as well as day 19.

In the vegetable group, intake and total negative facial expressions inversely correlated significantly on day 1/2 as well as on day 17/18 ($r = -0.45$, $p < 0.05$ and $r = -0.49$, $p < 0.01$). Intake and total negative facial expressions were also significantly inversely related in the fruit group at the 1st exposure ($r = -0.65$, $p < 0.001$) (see Table 3.4). Analyses done while excluding 4 outliers with a very high intake showed similar results.

When al 90 videos were analysed together, significant correlations were found between intake and total number of facial expressions as well as intake and behaviour. Also mothers' rated liking correlated with behaviour and facial expressions (see Table 3.4 for the extensive results).

Table 3.3 Comparison of behaviour, acceptance and facial expressions in vegetable group on day ½ and day 17/19 in response to green beans and fruit group at 1st exposure to green beans.

	Vegetable group		Fruit group	Sig *p* value		
Variable	**Day 1/2 (n=30)**	**Day 17/18 (n=30)**	**Day 19 (n=30)**	**Veg group day 1/2 vs day 17/18 [6]**	**Veg group day1/2 vs fruit group day 17/19 [7]**	**Veg group day17/18 vs fruit group day 19 [8]**
Intake	22 ± 21[1]	58 ± 66	27 ± 32	**0.004**	0.471	**0.025**
Mothers Rated liking	5.9 (3;7)	6.4 (6;7)	4.4 (3;6)	**0.002**	0.192	**>0.001**
Negative Behaviour [3]	1 (0;4) [2]	2 (0;4)	2 (2;5)	0.930	0.592	0.988
Positive Behaviour [3]	0 (0;0)	1 (0;0)	0 (0;0)	0.185	0.973	0.630
Rate of acceptance [4]	11 (9;14)	13 (9;19)	13 (8;17)	**0.007**	0.233	0.534
Total facial expressions [5]	23 (18;30)	16 (10;24)	20 (18;25)	**0.008**	0.234	**0.041**
Inner brow raising	2 (1;4)	1 (0;3)	2 (1;3)	0.236	0.677	0.284
Brow lowering	7 (5;9)	4 (3;7)	7 (4;9)	**0.039**	0.610	**0.015**
Squinting	1 (0;4)	0 (0;2)	1 (0;3)	**0.033**	0.504	0.149
Nose wrinkling	5 (2;7)	4 (2;7)	3 (2;6)	0.516	0.092	0.221
Upper lip raising	6 (4;8)	4 (2;5)	6 (4;7)	**0.002**	0.953	**0.018**
Gaping	0 (0;0)	0 (0;0)	0 (0;1)	0.169	0.593	**0.039**
Lip corner down	1 (0;2)	1 (0;2)	1 (0;2)	0.757	0.599	0.957

[1] Mean ± SD for all such values

[2] Median (25th;75th percentile) for all such values

[3] Behaviour means the infant's reaction towards the spoon. This can be negative or positive reactions.

[4] Rate of acceptance was measured by mouth movement which is defined as the early, late, enforced or refused opening of the mouth in reaction to the approaching spoon

[5] Total facial expressions is the sum of the single facial expressions.

[6] Comparison between Vegetable group days 1/2 and 17/18 (repeated exposure effect)

[7] Comparison between vegetable group on day 1/2 and fruit group on day 19. (first green beans intake of both groups)

[8] Comparison between vegetable group on day 17/18 and fruit group on day 19. (Green beans after repeated exposure to vegetables versus green beans after repeated exposure to fruit.

Table 3.4. Correlations (Spearman rho) between video coding and intake and mothers' rated liking ratings

		Intake	Mothers' rated liking
Beha-viours	Negative	-0.39***	-0.28**
	Positive	0.31*	0.13
	Rate of acceptance	0.60***	0.34***
Facial expressions	Total facial expressions	-0.59***	-0.41***
	Inner Brow raise	-0.27**	-0.17
	Brow Lowering	-0.51***	-0.44***
	Squinting	-0.41***	-0.30**
	Nose wrinkle	-0.29**	-0.17
	Upper lip raise	-0.40***	-0.30**
	Gaping	-0.12	-0.22**
	Lip corner down	-0.20	-0.09

* $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$

3.5 Discussion

The objective of this study was to assess the effect of repeated exposure to vegetables on infants' liking for green bean purée, assessed with regard to behavioural and facial expressions and in accordance with the manual of Hetherington et al. (33, 35). In a previous paper (29), we found that intake increased after repeated exposure to vegetables. The present study confirms that the increased intake is positively related to liking. After repeated exposure to vegetables, the infants expressed fewer negative expressions in response to green beans than before the repeated exposure; the acceptance score also increased. Furthermore, the fruit group showed significantly more negative facial expressions in response to their first green bean purée intake after repeated exposure to fruit purées than the vegetable group after repeated exposure to vegetable purées.

The rather strong correlations we found showed that the more the infants ate, the fewer negative facial expressions and behaviour and the more positive behaviour. Our data also supports the findings of other studies (34, 40, 41), which showed that negative facial expressions decreased the more the infant ate. This indicates that the increase in intake we found after repeated exposure to vegetables can indeed be explained by increased liking.

Rate of acceptance, behaviour and number of negative facial expressions did not differ

between the vegetable group and the fruit group at their first exposure to green beans, indicating similar (dis)liking of the food. However, there is a significant difference in negative facial expressions between the vegetable group on day 17/18, after repeated exposure to vegetables, and the fruit group on day 19, after repeated exposure to fruits. This finding is in line with the results of intake described in Barends et al. (29) and suggests that repeated exposure to fruits does not influence the liking for green beans. Mennella et al. (42) described similar results for intake. Repeated exposure to fruit did not increase the intake of green bean purée in infants, indicating that experience with one food category (fruits) does not generalise to a different food category (vegetables).

Early or late acceptance, measured by the opening of the mouth in response to the approaching spoon, is a new measure described by Hetherington et al. (35) and Nekitsing et al. (34). In the present study, the rate of acceptance in the vegetable group increased significantly after repeated exposure, indicated by an earlier opening of the mouth in response to the spoon. An earlier opening of the mouth in response to the spoon implies eagerness and it could therefore be a sign of increased liking for green beans. Hetherington et al. (35) describes that rate of acceptance is a sign of wanting the food. However, in our results, it is not clear if acceptance is increased by getting used to the taste of vegetables or by getting used to eating solid foods in general, since rate of acceptance in the vegetable group on day 17/18 did not differ from rate of acceptance in the fruit group, which was exposed to only fruit purées during the previous 18 days, on day 19. Furthermore, all infants practiced eating rice flour porridge only for 5 days preceding the intervention in order to get used to eating solid foods, so it is possible that infants were still not completely accustomed to the process of eating solids from a spoon at the start of the study. Therefore, rate of acceptance could be influenced by familiarisation with the procedure of eating solid foods from a spoon rather than by the taste of vegetables.

Mothers could have also influenced acceptance. We observed that some mothers sooner interpreted their infant's behaviour as refusal than others, although at the beginning of the study they received guidelines on how to feed their infants and were instructed to stop feeding after three consecutive refusals. Refusal was defined as not accepting the entry of the spoon into the infant's mouth. Mothers may have also had an impact on infants' facial expressions through their own facial expressions. They were instructed to keep a neutral face and open their mouth while the spoon was approaching the infant's mouth. Previous studies (12, 43) used masks on mothers; however, not masking mothers could lead to a stronger contact between the mother and the infant. The literature argued that masks ensure that the infants' facial expressions reflect their own liking for the food and are not an imitation of the mothers' facial expressions. However, infants could be

upset by the mask and mothers could be discouraged from taking part in the study if made to wear a mask(26). Additionally, not using masks simulates a more natural environment for the infant. Since mothers were instructed to keep a neutral face, the influence of mothers' facial expressions was limited. The strength of the mother's influence and whether scoring mouth movement could add further information about liking should be investigated more.

Due to time constraints, only a limited sample of 15 out of 25 infants per subgroup could be analysed. However, the sample was randomly selected and characteristics between the subgroups did not differ significantly. Random factors, like tiredness or fussiness, could be causes for variations in facial expressions, acceptance and behaviour, but this could not lead to a structural bias since the groups were randomly selected and these factors would have influenced results in all groups equally. Additionally, the coder was blinded for group and day number, which also diminishes biases.

Through measuring infants' facial expressions, this study supports the work of Barends et al. (29), which found that intake and liking (rated by the mothers) increased in response to repeated exposure to vegetable purées. Repeatedly feeding vegetables to infants gave them the chance to gain experience with vegetables, leading to an increase in liking. Therefore, these findings advocate the importance of early exposure to vegetables.

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Chapter 4

Effects of starting weaning exclusively with vegetables on vegetable intake at the age of 12 and 23 months

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4.1 Abstract

Background

The low vegetable intake in children may be attributed to their low preference for vegetables. During the first year of life, first taste preferences are formed, which may track over time. In a previous study to increase infants' vegetable intake and liking, we found that at the start of weaning, infants had a higher vegetable intake in the lab after repeated exposure to vegetable purées than to fruit purées. The current study is a follow-up of these infants at the age of 12 and 23 months, and examined whether the group that started weaning with vegetables continued eating more vegetables than the group that started weaning with fruits.

Methods

At 12 (n = 84) and 23 (n = 81) months of age the children's daily vegetable consumption was reported by their parents using a 3-day food diary. The intake of green beans and apple purée was measured in the laboratory.

Results

Reported daily intake of vegetables at 12 months of age was 38 % higher (P = 0.02) in the vegetable group (75 ± 43 g) than in the fruit group (54 ± 29 g), but was similar for both groups at 23 months of age (48 ± 43, 57 ± 35 g, respectively; NS). Both at 12 and 23 months of age, apple and green beans intake in the lab did not differ significantly between the groups.

Conclusion

These findings suggest that weaning exclusively with vegetables results in a higher daily vegetable consumption till at least 12 months of age. More research is needed to investigate how to maintain this effect.

4.2 Introduction

Most Dutch children do not meet the recommended vegetable intake (1, 2). According to the national food consumption survey only 17-21% of the 2 -3 year olds reach this recommendation of 50 to 100 g a day (1). The mean intake for 2-3 year old boys was 41 g (SD: 38 g) and for girls 36 g (SD: 31) per day. Also in other European countries as well as in the USA children's vegetable consumption is low (3-6). School-based interventions showed minimal effect on children's vegetable consumption (7). This raises the question what strategy should be used to improve vegetable consumption in children.

Since children's liking of a food strongly predicts its intake (8), increasing liking of vegetable can help increasing consumption. Several studies have shown that intake of specific vegetables increased after about 5 to 10 exposures to these vegetables during weaning (9-12). In these studies the repeated exposed vegetables were offered once a day on consecutive days. Increase in intake was usually measured between the first and the last exposure. The first year of life appears to be the most sensitive period in learning of taste preferences (13-16), which appear to be stable over a short time (17, 18). Furthermore, food patterns and preferences seem to track from early to late childhood (19) and even to early adulthood (20, 21). However, between the ages of 1.5 and 2 years, children start showing reluctance of trying new foods, caused by food-neophobia, which usually peaks at the age of 5 years and then declines (22). This may complicate especially introducing bitter vegetables at this age, since most children dislike bitter tastes (23, 24). This underlines the importance to introduce vegetables in the first year of life before food-neophobia emerges.

The Netherlands Nutrition Centre advises parents to start weaning their infants with soft sweet tasting fruits and vegetables that are not sour or bitter because these are easily accepted by infants (25). On the other hand, other health professionals recommended to introduce vegetables before fruit, because starting with fruits may intensify infants' inborn preference for sweet taste which interferes with the more bitter vegetable acceptance (10). In the study on repeated exposure during weaning of Gerrish and Mennella (10), some parents reported that they already had introduced fruits to their infants. The study showed that fruit had not negatively affected first carrot intake during the intervention, however, it is not clear whether it would affect the intake of green, less sweet, vegetables.

Weaning with specific vegetables could have a positive effect on vegetable intake in general. Repeated exposure seems not only to influence the preferences for the exposed

taste, but also to transfer to other similarly tasting foods (11, 12, 26). For instance, research of Birch et al. (11) showed that daily consumption of a particular fruit enhanced the infants' initial intake of other fruit types, but did not affect the infants' initial intake of green beans. Exposure to a variety of vegetables, however, did enhance the acceptance of green beans. This also indicates that starting weaning only with vegetables can have a positive effect on vegetable consumption in infants. These studies, however, only measured the short term effect. It is not clear how these preferences develop over a longer period.

We previously reported on a 19-day intervention study at the start of weaning (27) showing that repeated exposure to vegetables significantly increased the infants' vegetable intake from 24±28 g (mean ± SD) on days 1 and 2 to 45±44 g on days 17 and 18. Repeated exposure to fruit had no effect on vegetable intake. This paper concerns the follow-up at the ages of 12 and of 23 months, and assesses whether infants who started weaning exclusively with vegetables still have a higher vegetable intake than infants weaned exclusively with fruit. We hypothesized that being exclusively weaned with vegetables would result in a higher preference and intake of vegetables at 12 and 23 months than being exclusively weaned with fruits.

4.3 Subjects and methods

4.3.1 Subjects

The subjects had all participated in a 19-day intervention study between march 2010 and 2011 in Wageningen and Almere in the Netherlands (27), and were 101 parent-infant pairs randomly assigned to start weaning with either vegetable or fruit purées. Mean age at baseline (27) was 5.4±0.8 (mean ± SD) months (Table 4.1). Six and 18 months after baseline children were invited for the follow-up study. Eighty-four of them participated in the follow-up at the age of 12±1.4 months and 81 again at the age of 23±1 months. We have food record data of 71 of these children at 12 months and 69 children at 23 months. Of subjects that did not participate, the main reason was time constraints. Characteristics of participants of the follow-up and of subjects who did not participate were similar.

Informed consent was obtained from the parents for baseline and both follow-up studies. The study protocol was approved by the Medical Ethical Committee of Wageningen University, the Netherlands. The trial is registered at ClinicalTrials.gov, number ID: NCT01858337.

Table 4.1 Characteristics of the infants and their mothers in the vegetable and the fruit group.

	Vegetable group	Fruit group
Baseline (n) (27)	**51**	**50**
Infants' age (months)	5.3 (0.76)[a]	5.4 (0.79)
Gender (% boys)	53	50
12 months of age (n)	**42**	**42**
Infants:		
Gender (% boys)	52	50
Age (months)	12 (1.4)	12 (1.3)
Age at baseline (months)	5.3 (0.79)	5.4 (0.79)
Birth weight (g)	3528 (598)	3550 (615)
Weight (g)	9977 (985): n=33	9579 (954): n=34
Duration of breastfeeding (weeks)	15 (11)	17 (10)
Pickiness (4-point scale)[c]	1.9 (0.9)	1.8 (0.8)
Openness (4-point scale)[c]	3.5 (0.5)	3.6 (0.6)
Mothers[b]:		
Age (y)	31 (4)	33 (6)
BMI (kg/m^2)	26 (5)	25 (5)
Neophobia (0-100)[c]	29 (10)	28 (11)
Education Level (n)		
Low/middle/high	6 / 14 / 22	6 / 11 / 25
23 months of age (n)	**40**	**41**
Infants:		
Gender (% boys)	52	51
Age (months)	23 (1.1)	23 (0.9)
Age at baseline (months)	5.3 (0.79)	5.4 (0.78)
Birth weight (g)	3571 (612)	3520 (578)
Weight (g)	12814 (1251): n=29	12364 (1711): n=31
Duration of breastfeeding (weeks)	16 (11)	17 (11)
Pickiness (4-point scale)	2.3 (0.9)	2.1 (0.8)
Openness (4-point scale)	2.8 (0.7)	3.0 (0.5)
Mothers[b]:		
Age (y)	31 (4)	32 (6)
BMI (kg/m^2)	26 (4)	25 (5)
Neophobia (0-100)	27 (11)	29 (10)
Education Level (n)		
Low/middle/high	6/14/20	4/13/24

[a] Mean (SD) for all such values. [b] Measured at baseline

No significant differences in characteristics were found between the vegetable and fruit group. Characteristics were compared between the groups using t-tests for continuous variables, and chi-square tests for the categorical variables.

4.3.2 Experimental design

The baseline study at the start of weaning, that is described in more detail elsewhere (27), is briefly described below:

At the start of weaning, the infants had been randomly assigned to 1 of 4 groups. The infants had not been fed any solid food jet except a rice flour porridge that was given to them daily for at least five days preceding the intervention. During the first 18 days, half of the infants started weaning with only vegetable purées (vegetable group). They received green beans or artichoke purée every other day (9 times), and broccoli and cauliflower on the days in between. The other infants received only fruit purées (fruit group), which were apple or plum purée every other day (9 times) and banana and pear on the days in between. After the intervention the parents were free to feed their child the way they wanted.

This paper describes the follow up study when the infants were 12 and 23 months of age. The infants' daily consumption of vegetables and fruits at home was reported by their parents in a 3-day food diary. Also, intake of the same green beans and apple purées as during the baseline study was measured in our laboratory both at 12 and 23 months of age at two separate days in randomized order. We chose to measure apple and green beans intake, since all children had been exposed to these foods, at least once, during the intervention. We did not measure intake of other types of fruit and vegetables, because of the burden for the parent-infant pairs to come to our lab more often. The experimenter was not aware of a participant's treatment group. Parents knew whether their child was weaned with fruit or with vegetables during baseline (27), but were unaware of the study design and the fact that another group of children was weaned with another selection of purées.

4.3.3 Procedure

At 12 and 23 months of age mother/infant pairs visited our lab and were offered the purées in a quiet private room. The sessions took place both days at the same time. The mother was asked not the feed the child within one hour before the session. A bowl with 2 emptied jars of purée was handed to the mothers with one spoon for her to feed the child and one for the child. The green beans purée was served at a temperature of 42°C and apple purée at room temperature. The feeding guidelines were similar as during baseline (27) and were derived from methods of previous studies (10, 12, 28). The mothers fed their children at their usual pace until the spoon was rejected 3 consecutive times by the children. The children had to taste at least 3 spoons. When a child used his or her own spoon and finished, the mother continued feeding according to the guidelines.

With a live camera connection we checked whether the mothers followed the guidelines, and corrected them if necessary. Immediately after feeding, the mothers rated how much they thought their child liked the food. The pre- and post-weighting of the bowl including the spoons and bib was calculated to measure the actual intake.

After the measurement at day 1 or day 2, the mothers were asked to fill in a 3-day food record on three randomly assigned days.

4.3.4 Measurements

Outcome measures were infants' intake of green beans and apple purées in the laboratory at 12 and 23 months of age, and daily vegetable and fruit intake reported in a 3-day food record. On a 9-point scale the mothers' rated how much their children liked each feeding in the lab. They also rated how often their child ate the particular food at home and how much the child liked it.

Via questionnaires at the start of the 1st phase, information about the infants' and mothers' general characteristics were collected (27). The infant questionnaire consisted of questions about the birthdate, weight, breastfeeding status (breastfed, formula fed, or combination of both), and breastfeeding history (length of exclusive breastfeeding and length of total breastfeeding). The questionnaire for the mother consisted of questions about her own date of birth, weight, height, work status, and education level. Furthermore, it included a 10-item food neophobia questionnaire (29) translated in Dutch. Neophobia was scored on a 7-point bipolar rating scale (1 = disagree strongly and 7 = agree strongly). After reversing the scores of 4 items that were stated in opposite direction, the responses to the ten items were summed per participant (possible ranges: 10–70) (27).

At the follow-ups at 12 and 23 months of age, questionnaires about infants' characteristics were collected again. This time the questionnaire included a question about pickiness and one about openness to new foods, both rated on a 4-point scale.

4.3.5 Food records

The food records were collected according to standard procedures of the division of Human Nutrition from Wageningen University, which were previously validated (30). The parents recorded their children's food intake for 3 randomly assigned days. These days were only changed if the parents or day-care centre were not able to record that day. Parents weighed all separate foods consumed during dinner with a weighing scale provided, accurate to 1 gram. Quantities of other foods were reported using household

measures.

The parents received diaries for recording food intake and oral and written instructions on recording. The diaries were checked by students trained in dietary assessment. Information about poorly defined dishes and recipes and unclear items or amounts was obtained. Amounts of foods were calculated using standard portion sizes, or using the actual given weight in grams. Different types of fruits and vegetables were summed up to total fruit and vegetable intake respectively using the food grouping of the Dutch Food Composition Table (31). Energy intake of all foods was calculated using the same table. Finally, we calculated the usual daily fruit and vegetable intake by taking the average amount over 3 days.

4.3.6 Statistics

Characteristics of the children and mothers were compared between the vegetable and the fruit group using t-tests for continuous variables, and chi-square tests for the categorical variables.

To analyse differences in mean daily fruit and vegetable intake between the vegetable and the fruit group independent sample T-tests were used. Differences between groups in number of times that vegetables were offered were also analysed with independent sample T-tests. Additionally, to adjust for daily energy intake, analysis' of variance (ANCOVA) were conduct for daily vegetable intake, daily fruit intake, and daily macronutrient intake with (vegetable /fruit) groups as between-subject factors and daily energy intake as the covariate. Analyses were also run with other characteristics as covariates, but because this did not affect the outcomes, and these characteristics did not differ between the vegetable and fruit groups, only the outcomes with daily energy intakes as covariates are presented. Differences between the vegetable and fruit group in green beans and apple intake in the lab were analysed with independent t-test, and not adjusted for daily energy intake, because this data was not available from all infants that were tested in the lab.

We calculated Spearman's correlation coefficients between intake in the lab and the measures of: liking of the food in the lab and of the same food at home; daily intake of vegetables and of fruit at home to check for their coherence. Correlation between intake of the green beans and apple purée between 4-6, 12 and 23 months of age was calculated with Pearson's correlations.

Finally, correlation analyses were calculated between intake and other factors than weaning method. Pearson's correlations were used for continuous, and Spearman's

correlations for categorical variables. A repeated measures ANOVA, with (vegetable/fruit) group as between factor, was used to calculate the change in Pickiness and Openness between 12 and 23 months of age. Statistical analyses were performed using SPSS version 19 (IBM, Armonk, NY).

4.4 Results

No significant differences were found in the characteristics of the infants between the fruit and the vegetable groups (Table 4.1). Also the characteristics of the mothers measured at baseline did not differ between the groups.

4.4.1 Vegetable and fruit intake at home

At 12 months, children who had started weaning with vegetables had a significantly higher daily vegetable intake at home (75±43 g) than children started weaning with fruit (54±29 g; *P*=0.019; Figure 4.1). The amount of times a day that vegetables were offered did not differ significantly between groups. The children in the vegetable group received vegetables on average on 0.99±0.57 occasions a day and the children in the fruit group on 0.92±0.27 occasions (P=0.280). At 23 months the difference in vegetable intake between these groups had disappeared (Figure 4.2). The number of times the children were offered vegetables a day did also not differ between both groups (0.79 ±0.36 versus 0.84±0.32, *P*=0.613). The estimated daily vegetable intake of the vegetable group decreased significantly between 12 and 23 months of age (*P*=0.003) to a mean of 49±43g, whereas intake in the fruit group did not and was 57±35 g (*P*=0.36).

The estimated daily fruit intake at 12 and at 23 months did not differ significantly between the vegetable and the fruit group (figure 4.1), although it was 20 grams higher in the vegetable group at 12 months of age (*P*=0.16).

4.4.2 Daily energy intake

At 12 months of age the daily energy intake of the vegetable group (mean ± SD: 992±168 kcal) did not differ significantly from that of the fruit group (929±248 kcal; *p*=0.209; Table 4.2). With energy as covariate, the difference in vegetable intake between both groups was still significant (F(1,68)=4.41, *p*=0.039). In addition, no significant association was found (r=0.06, *p*=0.65) between vegetable and energy intake after removal of one outlier,

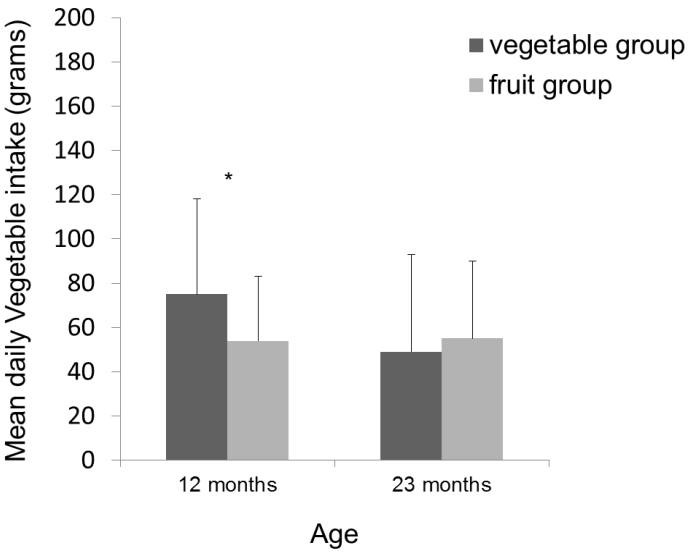

Figure 4.1 Mean daily vegetable intake (± SD) of the vegetable and fruit group, assessed with food records at 12 and 23 months of age.
* Significant difference between the groups assessed with t-test (*P* = .019)

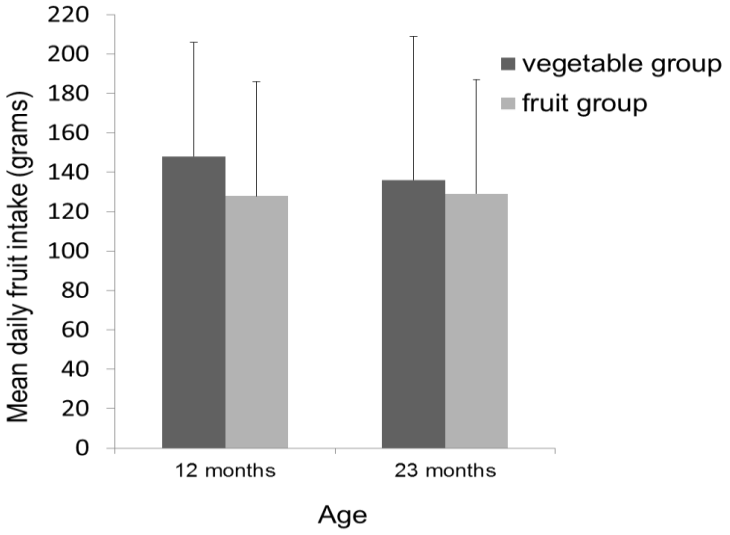

Figure 4.2 Mean daily fruit intake (± SD), of the vegetable and fruit group, assessed with food records at 12 and 23 months of age. No significant differences were found between the vegetable and the fruit group

consuming more than 1662 kcal/d. At 12 months of age, the groups only differed significantly in the intake of mono- and disaccharides, however, in energy percentages this difference was not significant (see Table 4.2).

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At 23 months of age the daily energy intake was higher in the vegetable group (1134±194 kcal) than in the fruit group (1025±194 kcal, *P*=0.022; table 4.2). The vegetable group also had significantly higher intakes of total fat and poly-saccharides, but in energy percentages these intakes did not differ between the vegetable and the fruit group (Table 4.2). In

addition, vegetable intake correlated significantly with energy intake (r =0.31, *P*=0.008). However, after controlling for energy intake the difference in vegetable intake between the vegetable and fruit group was still not significant (F(1,67)=3,0, *P*=0.09).

Fruit intake at both 12 and 23 months of age did not correlate significantly with energy intake (respectively: r =0.19, *P*=0.11; r =0.215, *P*=0.10). Also after controlling for energy intake, fruit intake in the vegetable and fruit groups was still similar at both 12 and 23 months of age.

4.4.3 Intake in the lab

Green beans intake in the lab at 12 months of age did not differ significantly between the vegetable group (106±109 g) and the fruit group (93±94 g; *P*=0.62). At 23 months the green beans intake for both groups had dropped significantly to a total mean of 44±75 g (*P*=<0.001). Intake did not differ between the vegetable group (47±75 g) and the fruit group (42±74 g; *P*=0.73).

Mean apple intake was relatively high and not significantly different between both the vegetable and the fruit group at 12 months (151±107 g vs 174±98; *P*=0.30) and 23 months (156±108 g vs 175±111 g, *P*=0.61).

Table 4.2 Mean daily energy and macronutrient intake, plus green beans and apple intake in the lab of the vegetable and the fruit group at 12 and 23 months of age.

		Vegetable group	Fruit group	P-value
12 months of age, daily intake:	n	33	38	
Energy	kcal	992 (168)[a]	929 (248)	0.209[b]
Fat	g	27 (7)	26 (11)	0.718
	en%[c]	24 (5)	25 (6)	0.521
Saturated fat	g	11 (3)	11 (6)	0.774
	en%	10 (2)	10 (3)	0.633
Protein	g	32 (9)	30 (8)	0.159
	en%	13 (3)	13 (2)	0.503
Carbohydrates	g	146 (31)	138 (41)	0.339
	en%	60 (5)	59 (5)	0.768
Mono- disaccharides	g	82 (22)	73 (17)	**0.049**
	en%	34 (6)	32 (6)	0.232
Polysaccharides	g	63 (16)	64 (32)	0.864
	en%	26 (5)	27 (7)	0.390
Dietary fiber (g)	g	13 (3)	12 (6)	0.450
	en%	3 (1)	3 (1)	0.576
23 months of age, daily intake (n):	n	35	34	
Energy (kcal)	kcal	1134 (194)	1025 (194)	**0.022**[d]
Fat (g)	g	35 (9)	30 (7)	**0.015**
	en%	28 (6)	27 (4)	0.352
Saturated fat	g	14 (4)	12 (3)	0.165
	en%	11 (3)	11 (3)	0.950
Protein (g)	g	38 (9)	35 (8)	0.066
	en%	14 (2)	14 (2)	0.914
Carbohydrates (g)	g	159 (38)	147 (34)	0.154
	en%	56 (7)	57 (4)	0.415
Mono- disaccharides	g	89 (31)	85 (27)	0.630
	en%	31 (9)	33 (6)	0.338
Polysaccharides	g	70 (20)	61 (14)	**0.036**
	en%	25 (5)	24 (4)	0.561
Dietary fiber (g)	g	12 (3)	11 (3)	0.261
	en%	2 (5)	2 (4)	0.534

[a] Mean (SD) for all such values. [b] ANCOVA comparing means between groups adjusted for daily energy intake. [c] en% = Energy percentages of the macronutrients. [d] Difference in energy intake and en% between groups are analyzed with independent T-test.

4.4.4 Correlations between lab intake, liking and daily intake

Both liking scores of the purées in the lab and the same food prepared at home correlated significantly with intake of that food in the lab (Table 4.3). However, green beans intake in the lab did not significantly correlate with liking of apple, and apple intake in the lab did not with liking of green beans.

4.4.5 Consistency of vegetable and fruit intake in the lab

Preferences for purées in the lab were relatively stable between the ages of 4-6 and 12 months, and between 12 and 23 months. This was shown by the correlations in intake of the purées between the different age periods. Green beans intake at 4-6 month of age correlated significantly with green beans intake at 12 months (r=0.36, *P*=0.001), and intake at 12 months with that at 23 months (r=0.30, *P*=0.01). For apple, only the intake between 12 and 23 months of age correlated significantly (r=0.32, *P*=0.005). For the intake of vegetables and fruits no significant correlations between 4-6 and 23 months of age were found.

Table 4.3 Spearman's correlations between intake of green beans and apple in the lab with the children's liking of the foods rated by the parents in the lab and at home.

	Liking green beans in the lab[a]	Liking green beans at home[b]	Liking apple in the lab	Liking apple at home
	r	*r*	*r*	*r*
intake in the lab:				
12 Months				
Green beans	0.85**	0.49**	0.13	0.19
Apple	0.15	0.14	0.64**	0.41**
23 months				
Green beans	0.76**	0.23*	0.18	0.08
Apple	0.04	-0.09	0.74**	0.34**

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

[a] Liking in the lab was rated by the mother when the child had finished eating the apple or green beans in the lab.

[b] Subsequently the mothers rated how much the child usually likes apple or green beans at home.

4.4.6 Influences of other factors on intake

Both pickiness and openness changed significantly between 12 and 23 months of age. There was a significant main effect of age in both pickiness and openness. Pickiness increased from 1.8±0.8 (mean ± SD) to 2.2±0.8 ($F(1,74) = 4,94$, $P = 0.001$) and openness decreased from 3.5±0.5 to 2.9±0.6 ($F(1,74) = 15,96$, $P < 0.0001$). No effect of condition on interaction between condition and pickiness or openness was found. Accordingly, pickiness correlated negatively with daily vegetable intake at 23 months of age ($r=-0.36$, $P<0.01$), while openness correlated positively with vegetable intake ($r=0.26$, $P<0.05$; Table 4.4).

Table 4.4 Correlations between intake and birth weight, age at the start of weaning, duration of breastfeeding, pickiness, and openness.

	Birth weight	Age start weaning	Total breast-feeding	Picky-ness	Open-ness
Apple intake					
12 months (n=83)	0.10	0.15	0.18	-0.30**	0.26*
23 months (n=79)	0.18	-0.05	-0.05	0.19	0.17
Green beans intake					
12 months (n=83)	0.11	-0.21	-0.05	-0.25*	0.30**
23 months (n=78)	0.01	0.08	0.02	-0.15	0.22#
Fruit home intake					
12 months (n=71)	0.38**	0.28*	0.27*	0.13	-0.18
23 months (n=68)	0.15	0.13	0.11	-0.21#	0.01
Vegetable home intake					
12 months (n=71)	0.05	-0.15	-0.20	-0.24*	0.07
23 months (n=67)	0.13	0.20#	0.02	-0.36**	0.26*

* p< 0.05; ** p< 0.01; # p<0.1

Pearson's correlations were used for continues variables, Spearman's' correlations for categorical variables.

NB. Subject number not always equals the total number of participants. In a few cases the children did not taste the apple or green beans purée in the lab, or we had no information about the children's characteristics given in this table.

4.5 Discussion

This longitudinal intervention study showed that weaning with vegetables led to a 38% higher daily vegetable consumption at home at the age of 12 months than weaning with fruits. The reported daily fruit intake at home however, did not differ between the

vegetable and fruit group, nor did the green beans and apple intake in the lab. At 23 months of age the effect in the vegetable group had disappeared. The green beans intake in the lab dropped dramatically in both groups, while the apple intake stayed the same.

Self-reports by food records are prone to misreporting (32). However, the procedures of our dietary assessments are highly standardized and validated (33). In the Netherlands vegetables are almost exclusively consumed during dinner, of which all the components were weighed on a scale. Finally, the energy and macro-nutrient intake of both groups were similar and normal. Thus, the errors in self-reported intakes by the diaries are probably small and comparable between the groups.

Daily vegetable intake is an important measure, because this is what actually had to be improved. We found that the daily vegetable intake of the vegetable and fruit group at 12 and 23 months of age was higher than that of Dutch children in the same age groups (34). Although daily vegetable intake in children might partly be influenced by the parents, who provide vegetables to the children, it does also correlate with children's own liking of vegetables children (35, 36). Because of the intervention parents may have been more motivated to feed their infants fruit and vegetables, which in turn increased their infants' intake of it. The finding that there is no significant difference between both groups in how often the children received vegetables suggests that the influence of the parents was equal in both groups. Furthermore, parent-infant pairs were randomly assigned to a group and were unaware of the design of the study. Moreover, the sample was diverse, since the mother-infant pairs were recruited in two different regions in the Netherlands. Therefore, a possible elevated vegetable intake still allows comparisons between groups showing that at 12 months the children weaned with vegetables had a 21 grams higher daily vegetable intake than those weaned with fruits.

The positive effect of weaning with vegetables on daily vegetable intake, was not found with green beans intake in the lab. Although the difference in green beans intake between the vegetable and the fruit group pointed in the same direction, this effect was not significant. The variance in intake in the lab (coefficient of variance = 100%) was much higher than in the reported vegetable intake at home (coefficient of variance =10%).

The lab intake of green beans may have been influenced by other factors than liking of vegetables, such as the unknown environment and age inappropriate texture of the purées. We used the same products as during weaning, while the 12 and 23 months old infants are used to eat more solid foods. Especially at 23 months of age most children are used to eat normal meals. This may also partly explain why the green beans intake at 23 months was so low.

The drop in vegetable intake we observed between 12 and 23 months of age is in line with an experimental study in which children older than 24 months of age consumed les vegetables than younger children (37). An explanation for this could be food-neophobia, which usually emerges and peaks at the age of 2 years (22). The significant increase in pickiness and decrease in openness to taste new foods at 23 months support this, since these characteristics are linked to food-neophobia (38, 39). Also, a higher score on pickiness and lower score on openness to try new foods was associated with a lower daily vegetable intake. However, we did not use an established scale to assess openness and pickiness, so no firm conclusions can be drawn from these results. Whether the effect of weaning with vegetables was totally disappeared at 23 months or was suppressed by food-neophobia is not clear.

Fruit intake both in the lab and at home were similar at 12 and 23 months of age. This is attributed to the sweeter taste of fruit, for which children have a hard wired inborn preference (40, 41). Furthermore, while most 23 months old children were not used to eat vegetable purées anymore, according to the mothers, many were used to eat apple purée (mousse). This could explain why only the intake of green beans purée dropped at 23 months and not the intake of apple purée. The liking for a product in the lab correlated with its intake, suggesting that the intake reflects liking. Liking ratings of green beans and apple purée in the lab also correlated with liking of these foods at home. Green beans intake did not correlate with apple liking and vice versa, indicating that the intake of purées in the lab reflected the liking of specific foods or food categories.

Our results confirm that repeated exposure to vegetables during weaning increases infants' vegetable intake across a 6 month period. However, the effect may not persist in the long-term. At twelve months of age, the effect on vegetable intake after the intervention was still present according to home intake but was vanished at 23 months. Therefore, an intervention during weaning might not be enough to stimulate longer-term vegetable preferences. Furthermore, this 6 months stability of taste preferences was supported by intakes in the lab. Although the difference in intake in the lab between the vegetable and fruit group was not maintained at 12 and 23 months of age, we found a relation between intakes at 6 and 12, and 12 and 23 months in the total group. Intake of the products at 6 months, however, did not correlate with intake at 23 months. These results indicate the importance to keep promoting vegetables during infancy. An intervention during weaning is valuable since it is easier to promote vegetables in 1 year olds that already have a higher preference for it.

We conclude that promoting vegetable intake during weaning, has a positive influence on vegetable intake until at least 6 months after weaning. To maintain a sufficient vegetable

intake, parents should keep trying to enhance vegetable intake also during the second year of life, since intake at 12 months predicts intake at 23 months. Whether the effect of weaning with vegetables totally disappeared at 23 months of age or food-neophobia erased the effect is not clear.

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Chapter 5

Does weaning with vegetables influence the preference for sweet and salty tastes?

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Submitted in revised form

5.1 Abstract

Low vegetable intake of children may be attributed to the bitter taste and low energy density. On the other hand, foods with high energy density, often having a sweet or salty taste, are in general more preferred because they give a satisfied and full feeling after consumption.

To investigate the effect of vegetables purées or fruit purées at the start of weaning on the development of preferences for sweet and salty taste, we compared the preference for sweetened and salted water solutions between infants who started the first 18 days of weaning with vegetables and infants starting with fruits. At the age of 23 months, 81 of the 101 infants who participated in the intervention during weaning, were tested in a follow-up study. Intake of sweetened and salted water expressed as ingestion rates (IR) was used as a measure of preference and compared between the vegetable and the fruit group. Ingestion rates (IR) were defined as the ratio between ingested taste solution and total water. Daily intake of vegetables, fruit and mono-and disaccharides were measured with 3 day- food records. No significant differences in IRsweet and IRsalt were found between the vegetable and the fruit group. IRsweet in both the vegetable and fruit group was not significantly different from 0.5, whereas IRsalt was lower than 0.5. Total intake of sweetened water, however, was significantly higher than total intake of plain water. Differences in IRsweet and total intake of sweetened water, may be caused by the not normally distributed sweetened water intake. Finally, groups did not differ in their daily mono- and disaccharides intake. In conclusion, the sweet and salty taste preferences in the 23 month olds, could not be explained by if they had started weaning with fruit or vegetables. Nor did children that started weaning with fruits have a higher daily intake of mono- and disaccharides at the age of 23 months.

5.2 Introduction

Most children do not meet the recommendations for vegetable intake. The Dutch national food survey of 2005/2006 showed that only 17 to 21% of the 2-3 year olds met the recommendations of 50-100 g of vegetables a day. Of the older children even less than 1% of the 4 to 6 years olds children met the recommendations of 100 – 150 g of vegetables a day (1). One of the factors causing a low intake could be the bitter taste of vegetables (2, 3). Already at birth infants show a rejection to bitter taste, and a preference for sweet taste (4-6). The preference for salt emerges around the age of 4 months (7).

Apart from the taste also the low energy density is believed to play a role in disliking of vegetables (3). Foods with a sweet (e.g. chocolate, cake) or salty taste (French fries, burgers) often are high in energy, because they contain a lot of sugar and/or fat. Although intake of energy is important for survival, a high consumption of energy dense foods contributes to overweight and obesity (8). Children consume a relatively large amount of sweet-tasting and fatty foods, including snacks and beverages, while their consumption of vegetables, which are low in energy density, is small (9, 10).

Although infants have inborn taste preferences, taste develops further throughout life. Especially, the first year of life is very important in developing taste preferences, since infants are very open to try new tastes at this age (11, 12), and food-neophobia has not yet emerged. Food neophobia usually emerges around the age of two years and lasts until the age of 6 (13). Furthermore, several studies have shown that intake of vegetables in young children increases after repeated exposure (14-19). Finally, early formed taste preferences are found to track further into childhood and even into adulthood (20, 21). Schwartz et al. (22) showed a relationship between infants' preferences for basic tastes, measured with intake of taste solutions, and acceptance of new foods bearing these tastes, rated on a 4 point scale by the infants' parents. Research on preferences for fruit and vegetables showed that repeated exposure to a variation of vegetables not only increased intake of the vegetable to which the infants were repeatedly exposed, but also of a vegetable to which the infants were not repeatedly exposed (17, 23, 24). Similarly, repeated exposure to a variation of fruits also increased intake of a fruit to which the infants were not repeatedly exposed (14, 24). Furthermore, Gerrish and Mennella (17), showed that daily consumption of fruit enhanced the infants' initial acceptance of carrots (which is a sweet tasting vegetable) but did not have an effect on the infants' initial acceptance of green beans. Exposure to a variation of vegetables, however, did enhance the acceptance of green beans. The positive effect of variation on acceptance of other/new vegetables was supported by a number of studies (17, 23-25).

To our knowledge, less research has been done yet on the relationship between vegetable preferences and the type of foods introduced at the start of weaning. The Dutch Nutrition Centre (26) advises parents to start weaning their infants with soft and sweet fruits and vegetables, because infants usually accept these foods easily. Other health professionals suggest that starting weaning with sweet tasting foods may facilitate the infants' inherent preference for sweet tastes and will interfere with vegetable acceptance. Therefore, it would be better to introduce vegetables before fruit (17). In the VaVo-study (14), we investigated this hypothesis. After an intervention with infants starting weaning for the first 18 days with a variation of fruit or vegetable purées (14), we found that first fruit intake was higher than first vegetable intake. The vegetable intake of children who were exposed to vegetables, however, was higher on day 17 and 18 of the exposure than vegetable intake on day 19 in children that were exposed to fruit during the previous 18 days.

In the present study, we investigated whether children who start weaning with vegetables have indeed a lower preference for sweet taste (measured with sweet solutions) and a lower daily mono- and disaccharide intake than infants who start weaning with fruits. Next to their bitter taste component, vegetables also have a glutamate, i.e. an umami taste component (27, 28). Since foods with umami taste are often linked with a salty taste(29), we expect that children who started weaning with vegetables will have a higher preference for a salty taste.

5.3 METHODS

5.3.1 Subjects

Subjects were 101 parent-infant pairs who had participated in a 19-day intervention study between March 2010 and 2011 in Wageningen and Almere in the Netherlands (14). In this study, they were randomly assigned to be weaned with either vegetable or fruit purées. Mean age at baseline (14) was 5.4±0.8 (mean ± SD) months. Eighteen months after baseline all children were invited for the follow-up study and eighty-one of them, with a mean age of 23±1 months, participated again. We have food record data of 69 of these children. The main reason of parents for not participating in this follow-up was time constraints. Characteristics of children and parents who did and did not participate were similar (Table 5.1).

The study protocol was approved by the Medical Ethical Committee of Wageningen University, the Netherlands.

Table 5.1 Characteristics of the infants and their mothers in the vegetable and the fruit group.

	Vegetable group	Fruit group
Baseline (n)(14)	51	50
Infants' age (months)	5.3 (0.76)[a]	5.4 (0.79)
Gender (% boys)	53	50
23 months of age (n)	40	41
Infants:		
Gender (% boys)	52	51
Age (months)	23 (1.1)	23 (0.9)
Age at baseline (months)	5.3 (0.79)	5.4 (0.78)
Birth weight (g)	3571 (612)	3520 (578)
Weight (g)	12814 (1251): n=29	12364 (1711): n=31
Duration of breastfeeding (weeks)	16 (11)	17 (11)
Pickiness (4-point scale)	2.3 (0.9)	2.1 (0.8)
Openness (4-point scale)	2.8 (0.7)	3.0 (0.5)
Mothers[b]:		
Age (y)	31 (4)	32 (6)
BMI (kg/m^2)	26 (4)	25 (5)
Neophobia (0-100)	27 (11)	29 (10)
Education Level (n)		
Low/middle/high	6/14/20	4/13/24

[a] Mean (SD) for all such values.
[b] Measured at baseline
No significant differences in characteristics were found between the vegetable and fruit group. Characteristics were compared between the groups using t-tests for continuous variables, and chi-square tests for the categorical variables.

5.3.2 Design

In the intervention study at the start of weaning, the infants had been randomly assigned to 1 of 4 groups. During the first 18 days, half of the infants started weaning with only vegetable purées (vegetable group). They received green beans or artichoke purée every other day, and broccoli and cauliflower on the days in between. The other infants received only fruit purées (fruit group), which were apple or plum purée every other day and

banana and pear on the days in between. The study is described in more detail in Barends et al. (14).

At 23 months of age, children's preferences for a sweet and a salty water solution were tested in the lab. The parents were asked to fill in a three-day food diary of their infant's intake. From this diary we assessed the children's daily intake of vegetables, fruit, total energy, and mono-, di- and polysaccharides.

The experimenter was not aware of a participant's treatment group. Parents knew whether their child was weaned with fruit or with vegetables during baseline (14) but were unaware of the purpose of the study and the fact that another group of children was weaned with another selection of purées.

5.3.3 Stimuli

For measuring the children's preferences for sweet and salty tastes, a sweetened and salted water solution was used. For the sweetened water we used a sucrose concentration of 0.4M (136.92 g/L). This is just above the mean preferred level in 5-10 year old children, as was found in a study of Pepino and Mennella (30). We chose this level to be able to distinguish between children who have a lower or a higher than average preference for sweet tastes. For the salted water we used a NaCl concentration of 0.1M (5.844 g/L). A study of Beauchamp et al. (31) showed that 7 to 23 months old infants preferred a 0.1 M solution over a 0.2M NaCl solution. Both solutions were made using bottles with Evian water with a pH of 7.2, containing 6.5 mg/L Sodium and 6.8 mg/L chloride.

The solutions were made beforehand using very precise calibrated scales (measuring in grams with 4 decimals). They were presented to the infants in commercially available infant feeding bottles, with nipples that were suitable for infants older than 6 months of age. The bottles each containing 50 ml of water or solution, were presented at room temperature and weighed beforehand and afterwards. This was done on a calibrated digital scale (2 decimals). The difference between these two measurements was used as the amount of total intake of the tastant and water presented.

5.3.4 Procedure

Parents were instructed not to let their child eat or drink for at least one hour before the test. The test took place in a quiet room, with no distractions. Each infant was accompanied by one parent, and was placed in a highchair, with the parent sitting next to it. To accustom the child to the situation, the child was allowed to play with a toy or a

book, while the parent was asked at what time the child's last meal was, in order to check compliance with the study instructions, and to sign the informed consent.

The procedure was adapted from previously described study methods (4, 32, 33). For each taste a sequence of four bottles was presented (Figure 5.1). During this test, 2 sessions with 4 feeding bottles were presented to the child; per session 2 bottles with water and 2 with water containing a tastant were presented. In one session the tastant was salty (salted water), and in the other session it was sweet (sweetened water). The order of the sessions was randomized over the participants and the method was executed double blind. Although the experimenter knew that a session always started and ended with a bottle of water, she was unaware of which tastant was presented in the second and third bottle.

The bottles were presented to the child by the experimenter during 45 seconds each, with a pause of 15 seconds between the bottles. The parent had a similar bottle with water, and also started drinking from it to show the child what to do. The 45 seconds started when the child started drinking from the bottle. When children stopped drinking, the researcher kept on presenting it till the end of the 45 seconds, while the parent continued to drink. The parent was allowed to help the infant to hold the bottle if the infant did not drink from it independently.

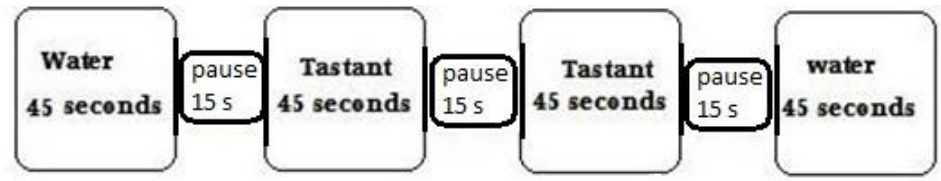

Figure 5.1 Taste sequence in which the different bottles were presented based on Schwartz et al. (4). Each bottle was presented for 45 seconds, followed by a 15 second pause. Tastant solutions were either 0.1 M salt (NaCl) or 0.40M sucrose. Intake was registrated as a measure for preference.

After the first session of 4 bottles the child was allowed to play for a while (at least 1 minute). When the child was fine with the situation, the child stayed in the baby-chair. Otherwise, the child could sit on the mother's lap or play in the room. The last session of 4 bottles were presented in the same way as in the first session. After the first session of 4 bottles the child was allowed to play for a while (at least 1 minute). When the child was

fine with the situation, the child stayed in the baby-chair. Otherwise, the child could sit on the mother's lap or play in the room. The second session of 4 bottles were presented in the same way as in the first session.

5.3.5 Outcome measures

In the sweet and salt preference test, intakes of the salted water, the sweetened water, and the plain water were measured. We described the test results using 'the Ingestion Rate' (IR). The IR is the ingestion volume of a tastant relative to the sum of the ingestion volumes of the particular tastant and of water (4). For example, IR for sweet tastant = total ingested volume of sweetened water / (total ingested volume of sweetened water + total ingested volume of plain water). When IR = 0.5 the children are indifferent to the taste compound. When IR > 0.5 they prefer the tastant over plain water, and when IR < 0.5 they prefer plain water over the tastant.

Children's liking of each water solution was rated by the children's mothers on a 9-point likert scale (1 = 'the child does not like it at all'; 9 = 'the child likes it very much'). Similar to the ingestion rate, also the liking rate (LR) was measured. LR for sweet tastant = sum of liking scores of total sweetened water solutions / (sum of liking scores of total sweetened water + sum of liking scores of total plain water).

5.3.6 Dietary assessment

Dietary intake of the children was assessed using food records that were collected according to standard procedures of the division of Human Nutrition from Wageningen University, and were previously validated (34). The parents recorded their children's food intake for 3 randomly assigned days. These days were only changed if the parents or daycare center were not able to record that day. Parents weighed all separate foods consumed during dinner with a weighing scale provided, accurate to 1 gram. Quantities of other foods were reported using household measures.

The parents received diaries and oral and written instructions for recording food intake. The diaries were checked by students in nutrition, who were trained in dietary assessment. If necessary, they asked the parents more detail about defined dishes and recipes, foods or amounts. Amounts of foods were converted into grams using standard portion sizes, or the actual given weight in grams was used (35). Different types of fruits and vegetables were summed up to total fruit and vegetable intake respectively using the food grouping of the Dutch Food Composition Table (36).

We calculated the usual daily fruit and vegetable intake by taking the average amount over 3 days. Daily energy intake and intake of mono and disaccharides were calculated using the Dutch Food Composition Table (36). We used the daily intake of the amount of mono- and disaccharides intake as a measure for the intake of 'sweet foods', since the sweet component in foods mainly consists of monosaccharaides (e.g. fructose and glucose) and disaccharides (e.g. sucrose, maltose, lactose).

5.3.7 Data analysis

Both the vegetable and the fruit group were analyzed to see whether preference for the sweetened and salted water differed from the preference for plain water. This was done by testing whether the Ingestion Rates (IR) were different than 0.5. For these analyses we used the non-parametric Wilcoxon signed-rank test, because IR was not normally distributed. The preference for sweetened and salted water of children who started weaning with fruits was compared to the preference in children who started weaning with vegetables, using Man-Whitney U tests. The same analysis was used for the liking rates (LR) of the sweetened and salted water. The relationship between IR and LR was analyzed using Spearman's correlations.

The 3-day food record data were processed with a nutrient computation program using the Dutch Food Composition table of 2011 (36). Daily intake of mono- and disaccharides were also compared between the vegetable and the fruit groups using ANCOVA with daily energy intake as a covariate.

The daily vegetable and fruit intake of the whole group was correlated with IRsalt and IRsweet, and daily intake of energy, mono- and disaccharides using partial correlations, corrected for daily energy intake (measured with the 3-day food records).

5.4 RESULTS

5.4.1 Intake of sweetened and salted water during taste preference test

In the taste preference test the mean of children's total intake of sweetened water (mean = 35 ml; SD = 31 ml) was higher than mean total intake of plain water (mean = 22 ml; SD = 16 ml; $p = < 0.0001$). Intake of salted water (mean = 9 ml, SD = 12 ml) was significantly lower than intake of plain water (mean = 18 ml; SD = 14 ml; $p < 0.001$).

5.4.2 Ingestion rates of sweetened and salted water in the vegetable and in the fruit group

The vegetable and the fruit group did not differ significantly in IRsweet ($p = 0.681$; Figure 5.2). In both groups the sweetened water was on average not liked more than plain water. IRsweet did not differ significantly from 0.5 in both the vegetable group (mean IR = 0.55; median = 0.63; $p = 0.088$) and the fruit group (mean IR = 0.52; median = 0.58; $p = 0.441$). IRsweet scores were not normally distributed (Figure 5.3), and the median scores showed that more than 50% of the children had an IRsweet > 0.6.

No significant difference in IRsalt between the vegetable and fruit group was found ($p = 0.191$) (Figure 5.2). Acceptance of salted water was significantly lower than acceptance of plain water in both the vegetable group (mean IR = 0.29; SD = 0.2; $p < 0.0001$) and the fruit group (mean IR = 0.39, SD = 0.2; $p = 0.001$) (Figure 5.2).

Sweetened water solution was preferred over salted water in both the vegetable and the fruit group as IRsweet was significantly higher than the IRsalt ($p < 0.001$ and $p < 0.01$ respectively for vegetable and fruit group).

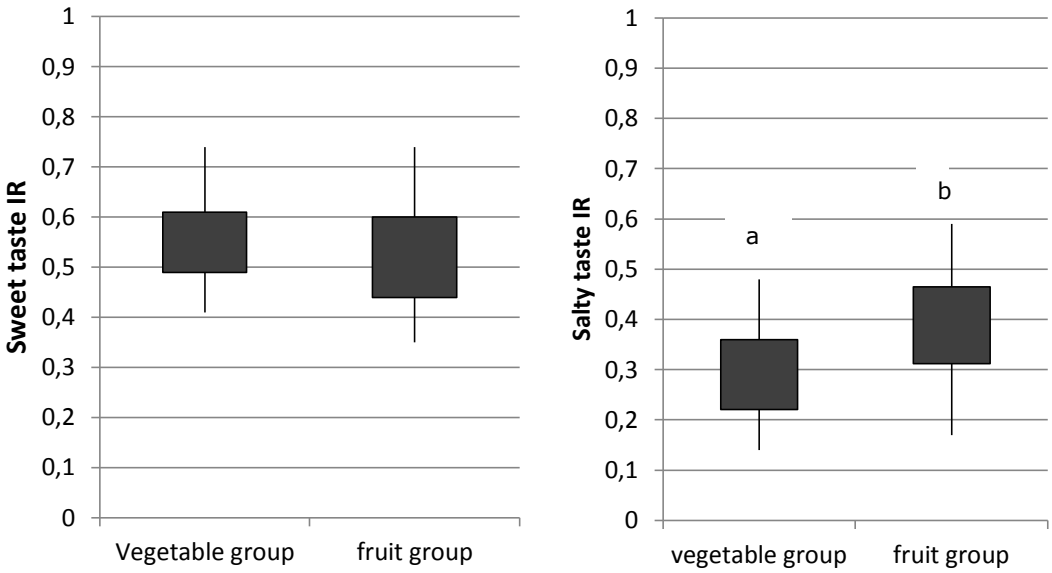

Figure 5.2. Boxplots of Ingestion Rates (IR), for sweet taste and salty taste. Each boxplot reflects the 95% Confidence interval. Mean values that were significantly different from 0.5 are marked with [a] ($P < 0.001$), or [b]($P<0.01$).

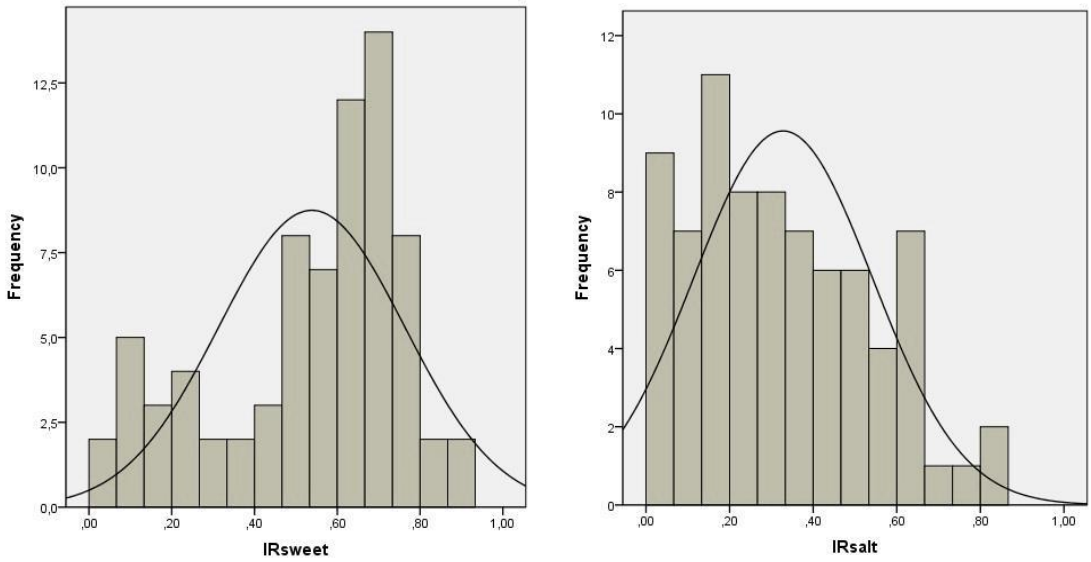

Figure 5.3 Distribution of IRsweet and IRsalt frequencies.

5.4.3 Liking rates of sweetened and salted water in the vegetable and in the fruit group

Mothers' rating of how much the children liked the sweetened (LRsweet) and the salted water (LRsalt) did not differ significantly between the vegetable and the fruit group.

Mothers' liking rates (LR) of sweetened water in both the vegetable group (LRsweet = 0.50; SD = 0.1; p = 0.98) and the fruit group (LRsweet = 0.48; SD = 0.12; p = 0.23) were not significantly different from 0.5. This means that, according to the mothers' ratings, the sweetened water was on average not liked more by the children than plain water. Liking of salted water was significantly lower than acceptance of plain water in both the vegetable group (LRsalt = 0.39; SD = 0.11; $p < 0.0001$) and the fruit group (LRsalt = 0.42, SD = 0.12; $p < 0.0001$).

Liking ratio of the sweetened and the salted water rated by their parent correlated highly with the intake ratio. The correlation between IRsweet and LRsweet was 0.76 ($p < 0.0001$). The correlation between IRsalt and LR salt was 0.79 ($p < 0.0001$).

5.4.4 Daily saccharides intake compared between the vegetable and fruit group

Daily mono- and disaccharide intake did not differ significantly between groups. The mean daily mono- and disaccharides in the vegetable group was 89 g (SD = 32g), and in the fruit group it was 85 g (SD = 27g; p = 0.124).

5.4.5 Relations between daily intake of vegetables, fruit, or daily intake of saccharides and IRsweet and IRsalt on the other hand

To see if preference for sweet and salt tastes is related to daily fruit and vegetable intake, IRsweet and IRsalt were correlated with daily fruit and daily vegetable intake independently from treatment group. Daily vegetable intake but not daily fruit intake was significantly correlated positively with IRsweet. IRsalt was not correlated with daily fruit or vegetable intake (Table 5.2).

We also analysed the relationship between daily mono-and disaccharide intake with IRsweet and IRsalt, however no significant correlations were found (Table 5.2).

Table 5.2 Correlations of IRsweet and IRsalt with daily intake of vegetable and fruit, and of mono-, and disaccharides.

	Daily vegetable intake *(r)*	Daily fruit intake *(r)*	Daily intake mono and di saccharides (g)
IRsweet	**0.30***	-0.01	-0.20
IRsalt	0.22#	0.14	0.05

*p<0.05; #p<0.1
Partial Correlation Sig. (2-tailed), corrected for daily energy intake

5.5 DISCUSSION

This study investigated the relationship between starting weaning with fruit or vegetables and later preferences for sweet and salty tastes at the age of 23 months. Also the relationship between the taste preferences and daily intake of fruit and vegetables was investigated. Against our expectations, the vegetable group did not have a lower preference for sweetened water compared with the fruit group and also their daily mono- and disaccharides intake was not lower than in the fruit group. Also no significant difference in salty taste preference was found between both groups. When daily fruit and

daily vegetable intake of the whole group were investigated, we did not find a correlation between IRsweet and daily fruit intake, but against expectations we did see a significant positive correlation with daily vegetable intake. No significant correlations were found between IRsalt and daily fruit or vegetable intake. However, a weak, although not significant, correlation was found between IRsalt and daily vegetable intake.

Like we expected, in the whole study group the intake of sweetened water was higher than the intake of plain water. However, this result was not supported by the ingestion rates. This was probably due to the fact that the IRsweet scores did not show a normal distribution. Although the biggest proportion of children had a IRsweet score above 0.5, also a relative big proportion had a very low IRscore under 0.3, which means that they preferred water over the sweetened water.

Moreover, our finding that the children rejected salted water over plain water, was contradictive to previous research that showed that children in the same age group prefer salted water over plain water (4, 33). Beauchamp et al. (31) showed that 4 to 24 month old infants preferred salted water over plain water, and that this preference had disappeared in children aged 31 to 60 months. This was attributed to the fact that older children and adults are not used to drink cold salty drinks, while younger infants are used to get a majority of their food intake through liquids. The precise age at which the preference for salty taste solution declines, however, is not clear. In the study of Beauchamp et al., a group of only 16 infants between 7 and 24 months was tested as one group, so the representation of infants around 23 months of age was probably small. Schwartz et al. (4) showed that 12 months olds still prefer salted water over plain water, but for children between 12 and 31 months there is no direct information from these two studies. Nevertheless, even though most children in our study disliked the salted water, most children did taste a few milliliters and therefore we think that the amount of intake still differentiated between children that had a lower or higher preference for salty taste. Most importantly, it is likely that the familiarity with salted water was the same in vegetables and fruit groups, so this could not have led to a group bias.

Children in the fruit group did not have higher sweet taste preferences than children in the vegetable group. When IRsweet was correlated with fruit and with vegetable intake of the whole sample, we did find a small positive correlation with daily vegetable intake, but not with daily fruit intake. However, there was also a trend for a weak correlation of IRsalt and vegetable intake ($p = 0.082$). So it might be that children who eat more vegetables have more preference for stronger tastes in general. This could be a result genetic predispositions influencing how strong the tastes are perceived by the children. Studies have shown that children who perceive the bitter taste component PROP as stronger have

lower vegetable intakes (2). Another study showed that adult PROP tasters also perceive sweet tastes stronger (37).

We used a validated procedure and our sample size was comparable to other studies using IR to measure basic taste preferences. It was not strictly double blind, since the experimenters knew the first and last bottle of a session were always water. However, they had strict guidelines on how to present all bottles in the same way, and they did not know which solution the 2nd and 3rd bottle contained. Both parents and children were blind to content of the bottles. Because it was a taste preference test the children could of course taste the difference between the solutions. Since all solutions and bottles looked exactly the same, we expected that the children would not differentiate between the bottles before tasting. However, after tasting expectations were formed about the other bottles that could have influenced the rest of the taste session. It was observed that in some cases children did not want to let go of a bottle they liked and sometimes refused to taste other bottles, because they wanted the liked bottle back. With these children it was more difficult to complete the procedure or keep the procedure completely strict. This, however, did not lead to a structural bias between groups, since missing data do not differ between the vegetable and the fruit group.

It is unlikely that environmental factors have influenced the results. The children were already used to the room from previous test sessions of vegetable and fruit purée intake, and liked to play with the familiar toy that was placed at the high chair. The experimenter enthusiastically presented the test as if it was a drinking game. Most children were familiar with drinking from a bottle. Children, who never drank from a bottle and did not manage to drink from it after trying, were allowed to drink with a straw. This could have led to less precise results. But again, this happened in both the vegetable and the fruit group, therefore it was not a source of bias for comparisons between groups.

Finally, our results from the Ingestion Rates are supported by how the mothers rated their children's liking of the solutions. Ingestion rate correlated strongly with mothers' rated liking and accordingly the liking ratings were similar to the ingestion ratings. Although parents partly rate their children's liking according how much they had consumed, they also took other factors into account such as children's behavior and facial expressions (38). These findings support that Ingestion Rates are a good way to measure liking and to compare liking of sweet and salt between groups.

5.6 CONCLUSION

In conclusion, we did not find evidence that starting weaning with vegetables or fruit influenced the preference for sweet and salty tastes. Nor did children that started weaning with fruits have a higher daily intake of mono- and disaccharides at the age of 23 months.

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Chapter 6

A systematic review of practices to promote vegetable acceptance in the first three years of life.

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6.1 Abstract

Most children do not meet the recommendations concerning vegetable intake. However, no clear universal guidelines exist on the best method of introducing and promoting intake of vegetables in infants and toddlers. In a systematic review we evaluated papers focussing on various strategies to introduce and promote consumption of vegetables in the first three years of life. Using a systematic search and selection approach, we selected 28 papers on this subject. Eighteen of these were experimental studies of which repeated-exposure was the most studied strategy. Other strategies were variety of exposure, flavour-flavour and flavour-nutrient learning, visual exposure, and nutritional counselling for the mothers. Factors studied in the remaining 10 observational studies included frequency of exposure, variety of exposure, age of introduction to solid foods, milk feeding method and maternal feeding practices or social learning/modelling.

The most effective strategy was repeated-exposure to vegetables. Flavour-flavour and flavour-nutrient learning did not produce additional benefit over repeated exposure in encouraging intake. Exposure to a variety of vegetables was effective in encouraging intake. Counselling was found to be minimally effective, since it only added a few grams of intake in comparison with the control groups. Effects of age of introduction of vegetables and milk feeding method in relation with vegetable consumption and acceptance differed between the studies. Based on the evidence of the papers reviewed the main recommendation to parents is to introduce vegetables at the beginning of complementary feeding by repeated exposure and exposure to vegetables varying day to day.

6.2 Background

Vegetables in the diet have a protective effect against obesity, diabetes type 2, cardiovascular disease and some types of cancer (1, 2) (3) (4). In a critical review of the literature on the role of vegetable and fruit intake in preventing chronic disease in adults, Boeing et al. (5) investigated the strength of evidence for supporting these effects. They found convincing evidence that increasing vegetable and fruit intake reduces the risk of hypertension, coronary heart disease, and stroke. For the association between consuming vegetables and fruit with weight gain, the authors concluded that there is possible evidence that an increased consumption of vegetables and fruit may prevent body weight gain (5). Since overweight is the most important risk factor for type 2 diabetes mellitus, an increased consumption of vegetables and fruit might also indirectly reduce the risk of type 2 diabetes mellitus.

The relationship between vegetable and fruit intake in children and a reduced risk of chronic diseases later in life is less clear. Some studies indicate that markers of chronic diseases are already present in childhood. For example, levels of blood pressure and serum lipid and lipoproteins are intermediate markers of disease that track from childhood into adulthood (6, 7) (8). However, whether a healthy diet in childhood can influence these markers of chronic disease is not clear. If a healthy diet during childhood protects against overweight then this will influence the expression of diabetes in adulthood (9), and reduce risk of coronary heart disease (10). Therefore, a healthy diet with a sufficient amount of vegetables in childhood may be helpful to prevent chronic diseases in adulthood, either directly or as a function of the effect on body weight.

Table 6.1 shows the vegetable consumption in children in several European countries and in the United States of America (USA). Despite the health benefits, most adults and children do not meet the recommendations concerning vegetable and fruit intake (11). In the Netherlands, the percentage of adults who consumed at least the recommended amount of vegetables increased with age but is low across age groups (between 3 and 14 %). Among children aged between 4 to 6 years old this percentage is less than 1%. Two to 3 year olds do slightly better, but still only 17 to 21 % of them reach the recommended level of vegetable intake (11). School-based interventions to increase fruit and vegetable intake showed some success in the increase of fruit intake, however they showed no or only minimal increases of vegetable intake (12).

Table 6.1 Mean vegetable intake in children per age group according to food surveys in five different countries across Europe plus the USA

	Age in years	Boys	Girls	Boys and girls
		Weight in grams ± SD		
Netherlands(1	<1	25 (n=164)	26 (n=169)	
	1	33 (n=314)	36 (n=294)	
	2 and 3	41 ± 38 (n=327)	36 ± 31 (n=313)	
	4 to 6	43 ± 36 (n=327)	44 ± 38 (n=312)	
Belgium(14)	1.5 to 3	70 ± 19 (n=102)	62 ± 20 (n=95)	
	4 to 6	75 ± 28 (n=236)	73 ± 32 (n=228)	
Italy(15)	0 to 2			61 ± 66 (n=52)
Poland(16)	0 to 2			112 ± 62 (n=400)
	4 to 6			69 ± 40 (n=182)
UK[8]	1.5 to 3			53 ± 29 (n=303)
USA(17)	2 to 5			~74 - 98 g (0.65 cups; n = 1202)

Low vegetable intakes in children can be a result of many factors (e.g. availability, parent influence, social economic status (SES), taste and texture of the vegetable), however, an important predictor of children's food intake is liking (18, 19). Liking for a food is mostly determined by a child's familiarity with it (19, 20). Thus to make children familiar with vegetables, they should be exposed to them. The first two to three years of life appear important for the development of healthy eating habits (21), as this is a period in which new foods are relatively easily accepted and first food preferences are formed. Between the ages of 1.5 to 2 years, children start showing more reluctance to try new foods –food neophobia– which usually peaks at the age of 5 years and then declines (22, 23).

Early food and taste experiences may have a long lasting influence on taste preferences. This was demonstrated by the work of Haller *et al.*(24) that indicated that the exposure to vanilla present in milk feeding in young babies was associated with a higher preference for vanilla flavoured foods in adult. Furthermore, a study by Mennella *et al.*(25) showed that infants who were fed with hydrolyzed formula milk in the first 3 months of life, four months later still had a higher preference for this milk than infants who were fed a regular formula milk. Also early established food habits tend to persist over time. Skinner *et al.*(26) found that patterns of food preferences in children at the age of two to three years were associated with those at the age of eight. Finally, a longitudinal study on the evolution of food preferences by Nicklaus *et al.*(27) demonstrated a relationship between

vegetable preferences in childhood, adolescence, and early adult life which was related to vegetable choices in early childhood. All these studies underline the persistence of early acquired taste preferences, which indicates that especially in this period a healthy diet is important.

Clear universal guidelines on the best method of introducing and promoting intake of vegetables in infants are lacking. Therefore, this systematic review will focus on various strategies to increase intake of vegetables, since vegetable intake in particular is reported to be too low in children. The objective of this paper is to give an overview of research on strategies to introduce and promote consumption of vegetables in the first 3 years of life.

6.3 Methods

6.3.1 Literature search

To identify relevant studies a literature search was carried out using the database Scopus (includes all the citations from PubMed and Embase from 1996 onwards). The search took place in March 2013 and was done in two steps. In the first step the list of search terms consisting of vegetables and/or fruit, different exposures to food, age of the children and a preliminary list of terms to be excluded from the search (exclude-list) were used. From the first 2000 titles found, the frequency of each different term in the abstracts was calculated in Excel. The most frequently used terms related to other, irrelevant research objectives were added to the 'not list'. For example, the words: pesticides, metal, and salmonella showed up frequently in the abstracts and were added to the exclude-list since they do not relate to our research question. Table 6.2 displays the final search string with the search terms and the complete 'exclude list' that was applied in the second search step.

The second step resulted in 2947 papers. From these 2947 papers, we excluded papers that were classified by Scopus in subject areas not relevant to our search, such as those listed below. This was done automatically by de-selecting the following subject fields (some articles were in more than one research field):
- Pharmacology, toxicology and pharmaceutics (120), Immunology and microbiology (81), Environmental science (195), Dentistry (48), Earth and planetary science (23), Physics and astronomy (19), Veterinary (14), Chemical engineering (11), Mathematics (6), This resulted in a remaining total of **2525** papers.

Table 6.2 Search terms and excluded terms of search string used to search the articles for this review.

Food	Exposure to food	Children (age)	Words that were excluded from the search strategy (exclude-list).
Vegetable Fruit	Accept* Learn/learning Consumption/ consume/ consuming Intake Program* Preference Liking/like Taste/tasting Introduc* Expose/exposure/ exposing Wean* Feed/feeding Complementary feeding	Toddler Early life Child* Infan* Baby*	acute OR allerg* OR asthma OR autism OR "birth weight" OR "blood concentration" OR bone OR carries OR caries OR cancer OR carcino* OR chronic OR diabet* OR disorder OR dentifrice OR diarrh* OR disable* OR disease* OR dysphagia OR deficiency OR hiv OR ill* OR infection OR malnutrition OR outbreak OR "physical activity" OR "plasma concentration" OR preterm OR prenatal OR pregnan* OR sick OR syndrom* OR vaccine OR advertis* OR cost* OR education OR "education program*" OR policy OR poverty OR sport OR "school program*" OR school OR television OR tv OR validat* OR animal* OR chimpanzee* OR colonization OR rat* OR adolesce* OR adult* OR elder OR elderly OR subject OR teen* OR women OR men OR gender OR acid OR alcohol OR adipos* OR arsenic* OR calcium OR caroten* OR fish* OR fluor* OR iron OR lead OR "low income" OR metal OR mercury OR nitrate OR pcb OR pesticides OR plasma OR protein OR salmonella OR serum OR supplement OR "vitamin D" OR zinc

6.3.2 Selection process

From this total of 2525 papers, a further selection was made using the criteria described in Table 3. Articles addressing healthy children of either gender, of any age from complementary feeding to 36 months old were included. Studies including children between of a broader age range, for example, 12 to 48 months, would only be included if separated results were given for the children under 36 months, or if the mean age was under 36 months. In longitudinal studies the children had to be under 36 months during the intervention, but results may have been gathered after this age. Similarly, in retrospective studies, effects of strategies applied before the age of 36 months, should be described. Articles with a more clinical or disease focus were excluded.

The inclusion and exclusion criteria were applied in three different runs. In the first run only the titles were manually screened and papers that clearly did not comply with the original search criteria were excluded (figure 6.1). From all papers that were not excluded

during the first run, the abstracts were taken into account in a second run. In this run the abstracts were scanned specifically for age of participants and whether vegetable and/or fruit intake were actually assessed. From the remaining articles the abstracts or, when the abstract was not informative enough, the whole articles were reviewed more thoroughly according to the criteria in Table 6.3. Through personal contacts, 3 additional papers which did not emerge from the original search were considered and were included in this review.

Inter-rater reliability was calculated with Cohen's Kappa from a set of 50 randomly selected papers from the 2nd run that were assessed by two reviewers. The Cohen's Kappa reliability score of the coding scheme was 0.92. The two reviewers discussed any disagreement and came to a consensus on the interpretation of selection rules. After adapting this to the selection rules, the remaining papers were assessed by one of the reviewers.

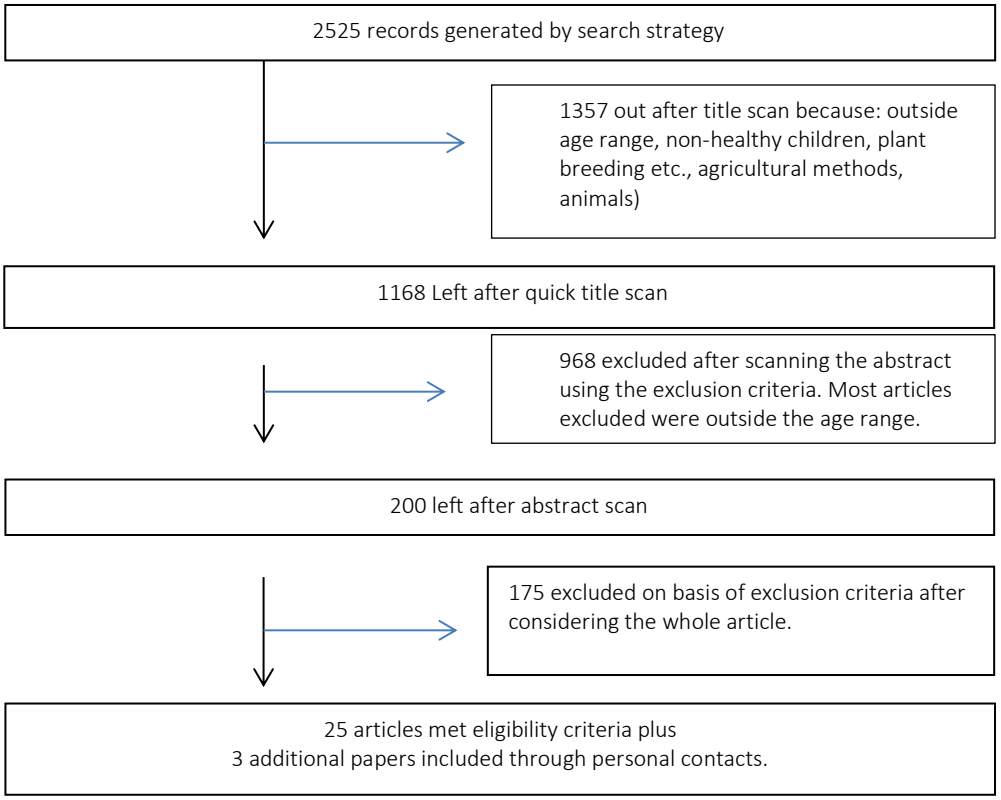

Figure 6.1. Flow diagram of the selection process of the papers

Table 6.3 Inclusion and exclusion criteria for the papers found with the search presented in table 6.2.

Inclusion	Exclusion
Study Population:	
Healthy children from 0 to 3 years old	Clinical or disease focus
Papers with longitudinal studies of which children were between 0 and 3 years old during at least a part of the intervention	Animal studies
Papers concerning pre-schoolers, toddlers, infants, baby's, without further age specification	
Outcome variables:	
Vegetable and fruit intake/acceptance/liking on short or long-term	
Papers on food refusal, picky eating etc.	
Method/strategy	
Introducing vegetables and/or fruit to children	
Feeding vegetables and/or fruit to children	
Taste learning strategies (e.g. repeated exposure, variety, responsive feeding, visual exposure, modelling) in relation to feeding vegetables to children	
Family food programs (if focused on vegetable intake of the child)	
Influence of breast/formula feeding	
Influence of age introduction to fruit/vegetables	
Type of papers	
International peer reviewed	Conference abstracts
Observational or intervention studies	Case studies with a number of subjects below 5
Clinical trial	Position papers not clearly based on literature
Meta-analysis	Papers which are focussed on developing a questionnaire methodology
Randomized controlled trial	Papers that show up and do not comply with the original search criteria.
Practice guidelines (for discussion)	Languages other than English
(systematic) reviews and position papers based on proper scientific literature (used for checking references)	Conference abstracts

6.4 Results

6.4.1 General characteristics of the studies

We reviewed 18 experimental and 10 observational studies. All data of vegetable intake and other measures of vegetable acceptance were extracted from the articles and summarised in tables 8 and 9. Some of the included studies, were not designed to specifically measure vegetable intake, but vegetable intake was a secondary outcome.

Quality was assessed by 2 authors independently, on a 12-point scale based on Hoyland et al. (28) and Jackson et al. (29), see Table 6.4. All studies had a good quality (between 10 and 12 points on a scale from 0 to 12). The general characteristics of the included studies are summarized in Table 6.5. Most studies were from the USA and UK and included both boys and girls.

Table 6.4 Rating criteria derived from Hoyland et al. (2009) (28) and Jackson et al. (2008) (29)

Review	No	Criterion	Score*
Introduction	1	Clear explanation of the scientific background and rationale for the investigation	0 or 1
	2	Clear hypothesis and/or objectives stated	0 or 1
Methodology	3	Appropriate design for the study objectives	0 or 1
	4	Clear description of sample, e.g. age (mean, SD, range), sex, n	0 or 1
	5	Clear description of setting/environment, e.g. location, laboratory/classroom/home	
	6	Clear description of the study procedure / data collection	0 or 1
	7	Clear description of outcome measures	0 or 1
	8	Provision of attrition and/or exclusion data and/ or appropriate handling of missing data	0 or 1
Result	9	Clear description of the data analysis	0 or 1
	10	Clear description of findings and in relation to the hypothesis/objectives	0 or 1
Discussion	11	Consideration of the methodological strengths of the study	0 or 1
	12	Consideration of the limitations of the study	0 or 1
Total Score *(QA)			N

* scoring 1 (yes) if the information is there in a useable form or 0 (no) if the information is not there in a useable form.

Table 6.5 General characteristics of the studies included in the studies

	Number of Intervention studies	Number of observational studies
Total number of studies (28), per country of origin		
Australia	-	3
Brazil	1	-
Canada	-	1
Denmark	1	-
Finland	1	-
France	1	1
France & Germany	1	-
Germany	1	-
The Netherlands	2	-
United Kingdom	4	3
United states of America	6	2
Number of participants (range)		
20 -50	7	
50-100	5	3
100-500	3	3
>500	3	4
*Focus study or intervention strategy**		
Repeated exposure	11	1
Frequency vegetable intake	-	1
Number of foods offered during first two months of complementary feeding	-	1
Repeated visual exposure	1	-
Variety	4	1
Flavour-flavour learning	3	-
Flavour nutrient learning	4	-
Age introduction to vegetables	-	4
Effects of milk feeding method	-	6
Participants of program/intervention on food/health info for mothers	5	-
Maternal feeding practices/behaviour/ modelling	-	2
Length of Follow up		
No follow up	8	2
< 1 month	3	-
1 month to <1 year	3	-
1 – 2 years	1	2
3 – 4 years	2	1
5 – 10 years	1	2
Retrospective questionnaires	-	3

* Studies can fall in multiple categories

Table 6.5 Continued

	Number of Intervention studies	Number of observational studies
*Vegetable intake/ liking assessment**		
Food pre- and post-weighed	12	1
Mothers' rated liking	8	1
Mothers' rated acceptance	-	1
Facial expressions	1	-
Interview (intake over last 3 months	2	-
Dietary range/ food frequency/intake questionnaire	-	4
Food preferences questionnaire	-	3
24-h recall	2	2
Food diary	1	1
Taste test	1	-

* Studies can fall in multiple categories

To facilitate logical reporting of the results, the articles were divided into intervention and observational studies and clustered according to method or strategy to introduce vegetables in the first three years of life. Most of these papers described intervention studies, with no follow up. In the majority of intervention studies intake was weighed and mother's rated liking was assessed, while in the observational studies food frequency and food preference questionnaires were mostly used.

6.4.2 Overview of applied practices

Table 6.6 shows the articles grouped by intervention-method or strategy. *Repeated exposure* was the subject of the largest number of papers. In this strategy the infant is offered the same vegetable across more than one occasion (up to 10 or more times). It is hypothesised that by doing this, the infants acquire familiarity to the taste and therefore increase the intake of the vegetable. Repeated exposure to vegetables can be achieved by feeding the same vegetable multiple days in a row, but also every two or three days, with other vegetables on the days in between. In studies testing *visual exposure* the children were only shown a vegetable in a picture book, but did not taste the food during the repeated exposures.

The studies on *flavour-flavour* and *flavour-nutrient* learning were characterised by a comparison with repeated exposure alone. In flavour-flavour learning, children are also offered a vegetable repeatedly but with a pleasant, usually sweet, taste added. The

Table 6.6 Papers about interventions to increase vegetable intake

Repeated exposure	Variety of exposed vegetables	Visual exposure	Flavour-flavour learning	Flavour – nutrient learning	Health and food information and counselling for mothers
Birch 1998 Gerrish 2001 Forestell 2007 Sullivan 1994 (l) Maier 2007 (l) Maier 2008 Mennella 2008 Caton 2012 Hausner 2012 Barends 2013 Remy 2013 (l)	Gerrish 2001 Forestell 2007 Maier 2008 Mennella 2008	Houston-Price, 2009	Caton 2012 Hausner 2012 Remy 2013 (l)	Caton 2012 Hausner 2012 De Wild 2013 (l) Remy 2013 (l)	Ponza 2004 (l) Talvia 2006 (l) Scheiwe 2010 (l) Vitolo 2010 (l) Whaley 2010 (l)

l = longitudinal

hypothesised effect of flavour-flavour learning is that after repeated exposure to a vegetable enriched with a pleasant taste, the infant associates the taste of the vegetable with that pleasant taste and consequently will increase intake of the plain version of the vegetable. Flavour-nutrient learning is a form of learning in which the vegetable is paired with additional energy rather than a distinctive, familiar and liked flavour. The hypothesised effect of flavour-nutrient learning is that after repeated exposure to a vegetable enriched with energy, the infant associates the taste of the vegetable with the satiating effect of the energy and consequently will increase intake of the vegetable. This is particularly relevant because it is thought that vegetables may be disliked by children since they are generally low in energy density, and children tend to prefer foods which are high in energy and/or taste sweet (30).

In observational studies (Table 6.7) measuring the effect of repeated exposure, the frequency of exposure to a vegetable was linked to later vegetable intake. Research on exposure to a *variety* of vegetables was always combined with at least three exposures. One study focussed on baby-led weaning. In this method of introducing solid foods to infants, the mother does not feed the infant with a spoon, but instead gives the infant soft finger foods that can be eaten with the hands.

Five studies investigated the effect of giving parents advice and counselling on healthy

Table 6.7 Papers about observational studies to increase vegetable intake

Frequency of exposure	Variety	Effect of age of introduction of solid foods / vegetables	Effect of milk feeding method/ duration	Maternal feeding practices/behavior /modeling
Coulthard 2010 (l) Townsend 2012 (r)	Lange 2013 (l)	Coulthard 2009 (l) Coulthard 2010 (l)	Mennella 2002 (r) Mennella 2006 (r)	Gregory 2011 (l) Townsend 2012 (r)
Howard 2012 (l)		Burnier 2011 (l) Lange 2012 (l)	Burnier 2011 (l) Grieger (r) Howart 2012 (l) Lange 2012 (l)	

r = retrospective; l = longitudinal

feeding behaviours. These were all longitudinal intervention studies but the methods and types of intervention differed. The counselling and dietary advice usually took place during multiple sessions during the first year of their child's life. In one study the counselling sessions continued yearly until the child was 10 years of age. Finally we found studies on the effect of breastfeeding compared with formula feeding on later vegetable intake. These studies are based on the hypothesis that the greater variety of flavours in breast milk influence taste preferences positively. All studies found on this subject were observational studies that used questionnaires to assess the milk feeding method.

6.4.3 Results of controlled randomized trials (Table 6.8, after references page 135)

Repeated exposure

All intervention studies on repeated exposure showed that this strategy increased vegetable intake directly during and after the exposure period (31-40). Most studies found increases between 25 and 40 grams (32-34, 39, 41). Three studies on repeated exposure to vegetables with older infants (between 7 month and three years of age) found an even greater increase in intake, but only with artichoke or a previously disliked vegetable and not for carrots where the change in intake was smaller (31, 35, 38). In all studies on repeated exposure, only a limited number of types of vegetables were tested: green beans, carrots, peas and artichoke and one study used spinach and endive soup. In most studies intake of carrots increased less than that of other vegetables or did not increase at all. A likely explanation is that carrots are generally well liked and intake of carrots was already relatively high pre-exposure.

Visual exposure

One study examined the effect of visual exposure on the acceptance of vegetables (42). This study showed that a storybook about specific unfamiliar and familiar vegetables increased the observed acceptance of unfamiliar vegetables, but decreased acceptance of familiar vegetables by 21 to 24 month old children. The main limitation of this study was that the observed acceptance was measured by willingness to try a new vegetable. Willingness to try does not necessarily predict greater intake nor does it necessarily predict greater liking. However, this could be viewed as a first step to developing liking.

Flavour-flavour learning and flavour-nutrient learning

The effects of flavour-flavour learning and flavour-nutrient learning were not more effective than repeated exposure (31, 38, 40). In the study of Caton *et al.* (31) vegetable intake increased significantly both in the flavour-flavour (FFL - sweet version) and in the flavour-nutrient learning (FNL – oil added) condition, but this increase was not significantly different from that of repeated exposure. Thus, adding flavour or energy to a novel vegetable eaten on at least 10 occasions did not have added value. The studies of Hausner *et al.* (38) and Remy *et al.* (40) had a similar design and used the same food products as that of Caton *et al.* They both found an increase in vegetable intake after repeated exposure and after FFL, but like in the study of Caton *et al.*, the FFL effect was not bigger than repeated exposure. In the study of Hausner *et al.*, the effect of repeated exposure was significantly higher than that of FFL, however, children assigned to the FFL condition consumed significantly more of the sweet puree than the plain puree. In both the study of Hausner and Remy no increase in intake of the novel vegetable was found after flavour-nutrient learning.

A strong point of these studies is that they compared the flavour-flavour and/or flavour-nutrient learning strategies with repeated exposure using exactly the same products across countries. However, the large positive effect on vegetable intake may be attributed to the novelty of the vegetable (artichoke); also intake of the target vegetable was especially high in the youngest children (weaning age) indicating that age of child may be more important than the strategy used in some circumstances (43).

Variety

The three intervention studies on exposure to a variety of different vegetables were effective in increasing vegetable intake of a new vegetable(32, 36, 37). In the study of Gerrish and Mennella(32), intake of carrots increased by 40 grams after 9 days of exposure to a variety of vegetables. This did not significantly differ from the increase in

intake of carrots of 46 grams after 9 days of exposure to carrots. Mennella *et al.* (37), found a higher increase in intake of green beans after 8 days for a variety of 2 different types of vegetables given each day, than with alternating each vegetable daily, or with repeated exposure to green beans alone. However, all three strategies increased the intake of green beans. On the other hand, exposure to a variety of fruits did not increase intake of green vegetables (37, 39).
The study of Maier *et al.*(36) compared 2 methods of exposure to variety, with a no variety group (Table 6.8). The combination of breastfeeding and the highest variety (a different vegetable daily) increased new food acceptance more than variety with a new vegetable every three days or repeated exposure to the same vegetable. This suggests that it is not simply variety but also how variety is applied, which produces the optimal outcome.

Starting complementary feeding with vegetables

The studies described above all address the question of how to introduce vegetables. The study of Barends *et al.*(39) also addressed the question of whether vegetables should be introduced before fruit, or vice versa. In an intervention study it was found that infants who started weaning with vegetable purees increased vegetable puree intake between day 1 and 18 from 24 ± 28 g to 45 ± 40 g (mean ± SD). Infants that started with fruit purees increased fruit puree intake from 45 ± 44 g to 66 ± 42 g. However, directly after 18 days of exposure to fruit purees, infants' intake of their first vegetable puree was still only 24 ± 29 g, which is as low as intake on day one of the infants that had started with vegetables. This indicates that to increase infants' vegetable intake, they should be exposed to vegetables, and exposing infants to a variation of fruit purees does not increase vegetable intake. The study suggests there is an advantage of starting complementary feeding with vegetables.

Counselling for mothers

Four of the five studies on the effects of counselling for mothers on healthy eating, demonstrated greater vegetable intake in (part of) the intervention groups than in the control groups. However, the effects were small. Also, not all studies corrected for differences in characteristics between intervention and control group. The study of Vitolio *et al.* (44) showed that after an intervention of 10 home visits with dietary counselling during the first year of life, more children consumed a higher amount of vegetables at 3-4 years of age than children in the control group. On a healthy eating index scale from 0 to 10, twice as many children in the intervention group scored above the 75th percentile of 1.67, compared to the control group. However, 1.67 out of 10 can be considered as a very

low score. Outcomes of the study were not adjusted for characteristics of the participants, since the characteristics did not differ significantly between the intervention and the control group. The study of Whaley et al. (45) also provided counselling during one year consisting of nutritional information and sessions about health goals and behaviour for parents. The only positive effect of the intervention was found among children younger than 2 years of age and not in those aged between 2-3 years. In the younger age group children in the intervention consumed vegetables on average 2.3 times a day versus 2.0 times a day in the control group (45). The intervention and control group were similar on individual characteristics, however the outcomes were adjusted for mother's education, language, and the baseline results. Ponza et al. (46) found no effect of a nutritional education intervention in a total sample of 4 to 24 months old children. When the results were analysed per age group, only significant differences in vegetable intake between intervention and control group were found among 4 to 6 months old children. In the intervention group the mothers received regular nutritional education that had started during pregnancy. However, the intervention was aimed at supplemental nutrition and supplemental foods were given to the participants. This also meant that a greater number of children who were formula fed were assigned to the intervention group, compared to the control group. Furthermore gender of the child and work status of the mother did not differ between the intervention and control group, but the mother's age, ethnicity, education and marital status did. Since the data were not adjusted for these characteristics, differences between groups could also be related to the differences in characteristics instead of the intervention (46). Another study followed children from 7 months of age until the age of 10 years (47). In the intervention group the parents, and their children from the age of 7 years, received regular dietary counselling based on a constructivist theory of learning. In the intervention group the mean vegetable intake, which was measured yearly, was only slightly higher (2.4 g a day) than in the control group. When analysis was done separately for both genders, the effect was only found in boys. Overall age and gender of the child were controlled as participants were randomised over the two groups.

The study of Scheiwe *et al.* (48) found no effect on children's vegetable intake 4 years after an intervention of monthly home visits during the 1st year of life to improve infant feeding practices. However, mothers from the intervention group did have better nutritional knowledge and more self-confidence (48). Since mother's education was significantly lower in the intervention group, there might have been more potential to gain nutritional knowledge, however, controlling for education did not change the outcomes. Of the four studies mentioned above, the study of Scheiwe *et al.*, had a relatively small sample size for the follow up, and therefore the authors suggest applying

caution to the conclusions. Although the other studies did find small advantages of vegetable intake in the intervention groups, the differences between groups were very small and therefore it is questionable if they are relevant.

6.4.4 Results of observational studies (Table 6.9 after references page 140)

Frequency of exposure

Three observational studies investigated repeated exposure to vegetables. They inquired about the times of repeated exposure via questionnaires. Two studies found a relationship between frequency of vegetable at or before the age of 1 and later vegetable intake(49, 50). Coulthard et al. (50) reported a correlation between the frequency of consumption of home cooked vegetables during complementary feeding, and the frequency of vegetable intake at the age of 7 years, but no such effect was found for manufactured, pre-cooked vegetables offered during complementary feeding. The study of Gregory et al. (49) followed children for 2 years and found that frequency of vegetable consumption at 1 year of age predicted vegetable intake at 2 years of age.

An observational study by Howard et al. (51) investigated the relationship between the number of repeated exposures to a new food and liking of fruits and vegetables in 23 month old children, but no significant association was found. However, the authors suggest that this result may reflect the question asked. Thus mothers were asked how often they had offered a new food before deciding whether they liked the food, rather than how often the child had tasted the food, and the question did not distinguish between categories of food such as vegetables. Therefore, this paper might not have measured actual frequency of exposure. Also it is not clear which foods were repeatedly offered to children.

Variety

One longitudinal observation study showed that new vegetable acceptance at the age of 15 months was significantly correlated with number of different types of foods offered during complementary feeding (52). This included all types of foods, thus when a high variety of foods was offered, particularly a large number of different types of vegetables, then this enhanced vegetable acceptance. The measure of acceptance of each food was done by the mothers at home since the beginning of complementary feeding, and was scored on a 4-point scale. Intake was not measured.

Milk feeding method

Two of the three papers on breastfeeding found a positive effect of breastfeeding on vegetable intake. One retrospective study in 2 to 8 year old children found a positive association between a healthy food pattern including vegetables and whether the child was ever breast-fed or not, after adjusting for confounders (i.e. age, gender, BMI and SES) (53). In addition, a longitudinal study found that, after adjusting for gender and SES, infants who were exclusively breastfed longer than 3 months were more likely to consume more than 2 servings of vegetables a day at the age of 4 years than infants who were exclusively breastfed shorter than 3 months (54). Lange et al. (52) examined the relation of exclusive breastfeeding duration and acceptance of new fruit and vegetables during the first 2 months of complementary feeding, but found no significant association.

The problem with all these studies is that they are difficult to compare, since they did not measure the effect of breastfeeding in the same way. One compared breastfeeding to not breastfeeding, while the others measured the effect of duration of (exclusive) breastfeeding. Also, the study that did not find an effect measured acceptance of a new vegetable, scored by the mother, whereas the other two measured actual consumption.

Two articles focused on the effect of hydrolysate based formula- milk, which has a bitter taste that is associated with brassica vegetables (55, 56). It was hypothesized that infants who were exposed to this type of milk would more readily accept vegetables, especially bitter tasting vegetables at 5 and 9 years of age. However, in both studies on this subject no differences were found in how often the children, who were or were not exposed to protein hydrolysate milk, consumed vegetables. The study of Mennella & Beauchamp (55) showed that in 4 year olds broccoli was more likely to be ranked as preferred by children that were fed soy-based or hydrolysate formula as a baby, than those that were fed a cow's milk-based formula. In contrast, the study of Mennella et al. (56) found that hydrolysate-milk fed infants consumed significantly less broccoli/cauliflower relative to carrots at the age of 6 to 11 months when compared to those who were currently fed milk based formula. The authors ascribe this lower broccoli/cauliflower consumption and liking to sensory specific satiety following repeated exposure to a particular flavour in mothers' milk or formula. However, the explanation is more likely to be related to monotony effects than sensory specific satiety.

Age of introduction to complementary foods

Studies that investigated the association between age of introduction to solid foods and vegetable intake, were all large scale, longitudinal observational studies.
Two studies investigated the effect of the age of introduction to vegetables on later intake. Lange *et al*. (52) found that the earlier vegetables were introduced, the higher the

acceptance was of new vegetables as rated by the mother. In contrast, Burnier *et al*. (54) found no relation between age of introduction to vegetables and vegetable consumption at 4 years of age. In this study vegetable consumption was associated with maternal education and the duration of exclusive breastfeeding. Another study found that when children were introduced to lumpy solid foods after the age of 9 months compared to the age between 6-9 months, they ate a smaller variety of vegetables at 7 years of age. Also a smaller proportion of these children consumed vegetables (57).
Results are difficult to compare because of the differences in age at which vegetable intake was measured. But there is no reason to doubt that age of measurement is an important factor in assessing the relationship. However, since studies were done in different countries, it is possible that cultural differences may play a role in both the ways in which solid foods are introduced and therefore the outcome.

Maternal feeding practices/behaviour/modelling/baby led weaning

One study investigated maternal feeding practices on later vegetable intake. Maternal modelling of healthy eating in the presence of their child at one year predicted higher child frequency of vegetable consumption at 2 years (49). Correlation between maternal use of pressure to eat at 1 year and lower vegetable consumption was close to statistical significance. Restriction did not significantly predict child frequency of consumption of fruits, vegetables or sweets over time.

Another study analysed the effect of baby-led weaning in comparison with spoon feeding (58). The results showed no effect of baby-led weaning on vegetable intake at 20 to 78 months of age. They found that exposure to vegetables was higher in the spoon-fed group, compared to the baby led group and that exposure was significantly associated with liking for vegetables. In this study the characteristics of the children in the baby-led weaning group were significantly different than those in the spoon-fed group. To control for this the results were measured with a matched sample of the initial group. This led to a relatively small sample and could have compromised power and representativeness of the sample.

6.5 Discussion

This paper reviewed the effect of different practices for vegetable introduction during the first three years of life on short or long-term vegetable intake. A systematic search was used to find all relevant papers on this subject. In order not to overlook relevant papers, we started with a search string that resulted in a large number of papers and then narrowed it down by manually scanning the titles and abstracts. Although we carefully selected the papers, we cannot rule out that we missed some papers in our review(59). The selection process was applied to 50 papers by two persons and we found a high inter-rater reliability for the selection process, which shows that our manual selection approach was reliable and consistent with the protocol.

Twenty-eight papers were reviewed on how to best introduce and promote vegetables during the first 3 years of life. One third of the papers involved repeated exposure, which was most often studied in the interventions. Two thirds of the papers described intervention studies, and the other papers were observational studies. We found papers describing interventions with six different strategies: repeated exposure, variety, visual exposure, flavour- flavour and flavour-nutrient learning, and counselling about healthy eating. We also found papers which reported on results from large scale, observational studies using self-report on the effect of frequency of exposure, variety, age of introduction of solid food, type of milk feeding, baby-led weaning and modelling of healthy eating. Studies were not necessarily designed to improve vegetable intake, nonetheless they all had vegetable consumption or liking as one of the outcome measures.

The most effective strategy to promote vegetable consumption appeared to be repeated exposure to vegetables. Also exposure to a variety of vegetables was effective in increasing intake. Counselling was found in most of the studies to give a significant increase in vegetable intake, but to a much lesser extent than repeated exposure (effect on vegetable intake was only a few grams). Flavour-flavour and flavour-nutrient learning did not produce any additional benefit over repeated exposure in encouraging intake, although preschool children seemed to prefer sweetened vegetable puree or high energy soups but this did not affect overall outcomes. For the effect of age of introducing vegetables and milk feeding method no consistent effects were found.

All 11 intervention studies on repeated exposure to a particular vegetable showed that this strategy positively affected intake of this vegetable. The size of the effect differed between the studies. Changes were biggest for novel or previously disliked vegetables, probably because of the potential for ceiling effects for already liked and familiar

vegetables. Exposure to a variety of vegetables was effective in increasing intake of a new vegetable and in the studies that investigated this, it was even more effective than repeated exposure to the specific vegetable itself(32).

Repeated exposure in weaning age infants consisted of exposures to the same food for 8 to 10 consecutive days. In two studies the repeatedly exposed vegetable was given every other day and another vegetable was given in the days in between. These two studies were actually a combination of repeated exposure and exposure to variation, but only looked at the increase in intake of the repeatedly exposed vegetable. In the other intervention studies on variation, the effect of variation on intake of a novel vegetable was measured. The variation was created by altering the vegetables each day for a sequence of 3 to 4 days, which was repeated 2 to 3 times. To translate these findings into practical advice to parents on how to apply a good combination of repeated exposure to vegetables with variation during complementary feeding, it would be interesting to compare different strategies of providing variation in an intervention. Also the type of vegetables should be taken into account. The results of our review suggest that alternating between orange and green vegetables is most effective. Variety of vegetables also leads to a higher intake of other new foods (e.g. chicken) (32). Variety of fruits did increase the intake of a new fruit, but did not increase the intake of a new vegetable. This suggests that especially for improving vegetable intake it is important that an infant is given a variety of vegetables, and that vegetables should be repeatedly offered.

Five of the repeated exposure studies had follow-ups that varied between one week and six months. Most of these studies found that the effect was stable or that vegetable intake in the intervention group increased more than in the control group. One of the two studies (40) that measured intake of the target vegetable until 6 months only found an effect of repeated exposure until 3 months after the intervention, but since this study involved weaning age babies, sustained effects may be weaker at follow ups when the diversity of foods eaten increases. All these results confirm that repeated exposure to a specific vegetable increases intake of this vegetable, while repeated exposure to a variety of vegetables increases intake of a new vegetable.

The effect of repeated exposure was also supported by the findings of two of the three observational studies investigating frequency of vegetable exposure. Results of these studies are however difficult to compare because they all measured different outcomes. Of the two studies that did find associations, one compared frequency of vegetable intake during complementary feeding with frequency of vegetable intake 6 years later (50), while the other associated it with liking in the short-term(58). Both these studies looked at vegetables as a group and not at individual vegetables, therefore it is plausible that these

results reflect exposure to a variety of vegetables rather than repeated exposure *per se*.

Flavour-flavour learning increased intake of the plain vegetable in all studies, but in two of the three the increase was significantly smaller than with repeated exposure (38, 40). Flavour-flavour learning has been shown to be effective in increasing vegetable liking in 4-6 year old children (60). However, these studies had small sample sizes and measured liking, not intake. In studies with consumption of sweet tasting drinks, flavour-nutrient learning has been shown to be effective in increasing intake of the neutral flavoured, i.e. unsweetened drink, in children 2-5 years of age (61, 62). In this review, however, flavour-nutrient learning was in two of the four studies as effective for vegetable intake as repeated exposure, while in the other two it did not increase intake significantly. These results point to the conclusion that both flavour-flavour learning and flavour-nutrient learning do not add additional benefit to simple repeated exposure to vegetables.

Exposure to the taste of vegetables was effective, however one study on visual exposure indicated that by showing children pictures of vegetables in a picture book, their willingness to try the unfamiliar vegetables could be increased. Since only one study investigating this strategy was included and it did not assess how much of the vegetables were eaten, therefore conclusions with regard to intake cannot be made. However, it would be an interesting strategy to investigate further, especially since neophobic toddlers are very reluctant to taste new foods and applying repeated exposure to the taste is than very difficult. But if showing a picture book about vegetables, is indeed effective tool to make children actually taste and eat the food, should be further investigated.

As is described in the previous paragraphs, a large number of studies investigated *how* to introduce vegetables to infants. Only few studies investigated *when* vegetables should be introduced, so no solid conclusions can be drawn on this subject yet. Vegetables seem to be more easily accepted when introduced at a younger age (52). One study found that starting complementary feeding with solid foods (including vegetables) after the age of 9 months, was related to a lower vegetable intake at the age of 7 years, than when solid foods were introduced between 6 and 9 months(57). Another study, that investigated the age of introduction to vegetables, did not find such a relation (54). Finally, although most studies on repeated exposure and exposure to a variety of vegetables took place at the beginning of commentary feeding, only one study addressed the question if vegetables should be introduced first, before introducing fruits. The results suggested that it is best to start complementary feeding with vegetables exclusively, since only exposure to vegetables, and not exposure to fruits, increased vegetable intake(39). Furthermore, it seems to be more important to focus on accustoming infants to vegetables than to fruit,

since the first intake of fruit puree was already twice as high as the first intake of vegetable puree. In a follow-up of this study (not included in this review), it was shown that children who started weaning with vegetables still had a higher vegetable intake at the age of 12 months than children who started weaning with fruits (63). This effect was no longer observed at the age of 23 months. Whether the effect of weaning with vegetables totally disappeared at 23 months of age or other factors, like the phase of food-neophobia, erased the effect is not clear. The authors suggest that only an intervention at the beginning of complementary feeding might not be enough to achieve longer term effects and parents should keep trying to enhance vegetable intake also during the second year of life. Children that started weaning with vegetables did have higher vegetable intakes at 12 months of age, therefore, this might make it easier to keep stimulating their vegetable intake.

Exposure to taste does not necessarily require eating the food itself. There is evidence that the mother's diet can influence their infants taste preferences through amniotic fluid during pregnancy and breast milk during breastfeeding (64). Also the higher variation of taste in mother milk compared to formula-milk is believed to increase acceptance of novel foods (65). In the studies presented in this review no relation between exclusive breastfeeding duration and acceptance or liking of a (new) vegetable was found on the short term (51, 52). Two other studies presented in this review do suggest a long-term effect of breastfeeding compared to formula feeding (53) and breastfeeding duration(54) on vegetable intake. Also the intervention studies about repeated exposure or variety, where breastfeeding was often taken as a control measure, imply that breastfeeding has a positive effect on vegetable intake. For example the study of Maier *et al.* (36) showed that breastfed infants had greater increases of vegetable intake during the intervention.

The age of introducing vegetables may interact with the effect of exclusive breastfeeding, because when mothers exclusively breast feed for longer, this postpones the introduction of solid foods including vegetables. This raises the question whether the impact of delayed introduction to solid foods and hence later introduction of vegetables even in breastfed infants could produce the unintended consequence of reducing vegetable acceptance.

Counselling of the parents on healthy eating was found to be effective on later vegetable intake of their children, in four of the five papers on this issue. In these studies the counselling ranged from multiple sessions during the 1st year of their child's life to regular counselling to the age of 10 years. Although counselling had some effect in four of the five studies, the effects on vegetable intake were relatively small. The difference in intake between the intervention group and the control group was in all cases not more than a few grams. Therefore it is questionable if these costly and time consuming intervention

programs add value to simply offering vegetables often within the family diet.

Especially during the first years of life parents have a substantial influence on children's eating habits. This suggests that it is important to provide parents with information and tools on how they can introduce vegetables to their children to promote acceptance. However, the implementation of effective methods must be paired with a high level of motivation by parents to enhance healthy eating in their children. Simply providing information, may not be enough. When it comes to their own eating habits, most adults know the importance of eating vegetables, however, only 3 to 14% eat a sufficient amount of vegetables (11). However, most mothers are motivated when it comes to healthy nutrition for their children (66). This review found that repeated exposure is an effective way to accustom infants to vegetables. However, the mean number of exposures before parents give up when they think the child does not like a vegetable is 5 times(67), while 5-10 times may be necessary. Also parents may be less aware of the importance of early taste learning and more aware of the importance of providing sufficient energy. Therefore, it is important to support parents with information and tools on the most effective way to promote healthy eating in their children.

In conclusion, based on the evidence of the papers reviewed the main recommendation to parents is to introduce vegetables at the beginning of complementary feeding by repeated exposure and feeding children a daily variety of different vegetables. This information should be included in complementary feeding advice given to parents by health care professionals and be included on child nutrition websites, i.e. different vegetables should be given every day for about 3-4 days and this should then be repeated.

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Table 6.8 Summaries of the included randomized controlled trials, with results on vegetable (V) and fruit (F) consumption and/ or liking

Authors (year) (ref #)	Sample	Data collection	Focus paper	Design of study	Reported findings on vegetable and fruit intake, acceptance or liking
Sullivan and Birch (1994) **(34)**	N = 36 Mean age: 22 weeks (range: 17-27 Country: USA	Intake was weighed. Mothers rated liking (ML).	Repeated exposure (RE); Adding salt	*Intervention:*(home) 4 groups (random): 10 x RE once a day on consecutive days to salted peas, unsalted peas, salted green beans or unsalted green beans *Measures* Intake salted and unsalted food on 2 separate days: (1) before the 10-days RE, (2) immediately after RE; and (3) one week after RE. Intake control food before and after RE	After RE intake significantly increased, regardless of which vegetable was consumed and whether or not the V contained added salt ($F(2,64) = 16.54$, $P < .001$). Increase was 28 to 63 g. for salted vegetables and 36 to 58 g for unsalted V. One week after RE intake of V did not change significantly. Breastfed infants showed larger increases in V consumption (39 to 72 g) than formula fed infants (25 to 46 g; $F(1,32) = 9,74$, $P < 0.01$). Rated liking showed same effect as intake.
Birch, Gunder, Grimm-Thomas et al. (1998)**(41)**	N = 39 Mean age: 24 weeks (range: 16–31) Country: USA	Intake was weighed.	Repeated Exposure (RE)	*Intervention* 4 groups: 2 were RE to peas purée. Two groups were RE to banana purée. RE to target food once a day for 10 days. *Measures* Pre- and post-exposure intake of target, same (different brand), similar (F-F, V – V) and different food (V – F).	RE increased mean intake of the target food from 35 to 72 g ($p < 0.01$). Same food increased from 59 to 65 g (ns). Similar food increased from 60 to 77 g ($P < 0.01$). No effect on a different food.
Gerrish and Mennella (2001)**(32)**	N = 48 Mean age 4.6 months (SD: 0.2) Country: USA	Intake was weighed. Mothers rated liking.	Repeated exposure (RE); Variety	*Intervention:* Acceptance evaluated of a novel V (puréed carrot) and meat (puréed chicken) after a 9-days RE at home in 3 groups of infants. (1) was fed only carrots, the target V; (2) was fed only potatoes, a V that differed in flavor from carrots; (3) was fed a variety of vegetables that did not include carrots. *Measure* Intake and ML of carrot purée pre- and post RE in the lab. Intake of meat last day in the Lab.	Infants carrots intake increased significantly when exposed to either carrots (50 ± 6 g to 90 ± 11 g) or a variety of V (62 ± 12 to 108 ±11; $P < 0.05$), but not those exposed to potatoes. RE to a variety of V also facilitated the acceptance of the novel food, puréed chicken, and daily experience with fruit enhanced the infants' initial acceptance of carrots.

Authors (year)	Sample	Data collection	Focus paper	Design of study	Reported findings on vegetable and fruit intake, acceptance and liking
Ponza, Devaney, Ziegler et al. (2004) **(46)**	N = 3017. Age: 4-24 months. 821 WIC* participants Country: USA	24-hour dietary recall.	Comparison between WIC and non-WIC participants.	Sample was drawn from the new parents database and divided in 2 groups: (1) WIC participants and (2) non-WIC participants. *Measure* Two 24-hour dietary recalls were collected.	No differences in percentages of infants that consumed V between the WIC and the non-WIC group. In the 4-6 months old group alone total % of children consuming V between WIC and non-WIC participants was the same. A significant higher proportion of children consumed cooked and raw V than the non-WIC group. But baby food V and deep yellow V were (not significantly) more consumed in the non-WIC group.
Talvia, Räsänen, Lagström et al. (2006) **(47)**	N = 1047 Age: 7 months old at start of the study, follow until ten years of age. Country: Finland	Intake was measured in grams and energy % with 4-day food records.	Parents dietary counseling.	*Prospective intervention:* 2 groups: (1) intervention; (2) control Families in the intervention received counselling based on a constructivist theory of learning. The children's diets were discussed and parents were encouraged to include a.o. more vegetables in the diet. A nutritionist met the intervention families at 1- to 3-months intervals until the child was 2 years old, and biannually thereafter. Children aged >7 counselling also received separately counselling from their parents *Measurement:* Intake was measured yearly with 4-day food records from 1 to 10 years of age.	In girls the energy % of F and V intake was slightly higher in intervention group than in control. In boys this was only the case at 6 of the 10 time points. (*not more than 2% difference)* V consumption increased with age. No differences between gender. Intervention children consumed more V than control (mean difference: 2.4 g/d, p <0.001) (only significant in boys (3.2 g/d) not in girls (1,5 g/d).
Forestell and Mennella (2007)	N = 45 Mean age 5.8 months (SEM: 0.2,	Intake was weighed. Mothers rated	Repeated exposure (RE); variety	*Intervention:* 2 groups: (1) RE to green beans, (2) RE to green beans and after 1 hour peaches. 8 consecutive days.	Initially infants ate more calories from peaches than from green beans. RE to green beans, with or without peaches, increased consumption of green beans (from

(33)	range: 4-8m) months. Country: USA	liking. Facial expressions.		*Measure*: Acceptance of both foods, was assessed before and after the home-exposure.	56.8 to 93.6 g; p <0.05). Only infants who experienced green beans with peaches displayed fewer facial expressions of distaste during feeding.
Maier, Chabanet, Schaal et al. (2007) **(35)**	N = 49 Mean age: 6.9 months (SD: 0.9). Country: Germany	Intake was weighed. Mothers rated liking.	Repeated exposure (RE)	*Intervention study:* From a larger study infants who disliked at least one vegetable were selected. Mothers were asked to offer a disliked vegetable on alternate days for 16 days, and to offer a well-liked one (carrot purée) on the other days. *Measure:* Amount eaten and acceptance were measured at each meal.	Intake of disliked vegetable increased from 39 ± 29 g at first exposure to 174 ± 54 g at the 8th exposure. Similar to that of the liked vegetable (186 ± 68 g). Nine months later, 63% of the infants were still eating and liking the initially disliked vegetable. A similar pattern of results was found for mother-reported liking ratings.
Maier, Chabanet, Schaal et al. (2008) **(36)**	N = 147 Mean age: 5.2 months (SEM: 0.1, range 4-7 m) months. Country: Germany and France	Intake was weighed. Mothers rated liking.	Repeated exposure (RE) and variety during weaning	*Intervention:* 3 groups received their first vegetable (carrot purée) in the lab and, 9 days at home, either (1) carrots every day; (2) 3 vegetables changed every 3 days; or (3) 3 vegetables changed daily. On the 12th and 23rd days they received new vegetable purées, zucchini tomato then peas. *Measure:* Acceptance of new foods was measured by intake and by liking ratings of mothers and observer.	Intake day 1 same in breast and formula fed and in 3 conditions. Breastfeeding (p < 0.001) and variety (p <0.001) increased new food acceptance. Frequency of change was more effective than number of vegetables fed. The combination of breastfeeding and high variety produced greatest new food intake. Liking day one. Intake scores were supported by liking scores.

Authors (year)	Sample	Data collection	Focus paper	Design of study	Reported findings on vegetable and fruit intake, acceptance and liking
Mennella, Nicklaus, Jagolino et al. (2008) **(37)**	N = 74 Mean age: 6.6 months (SEM: 0.3, range: 4-9) Country: USA	Intake was weighed. Mothers rated liking.	Repeated exposure (RE) and variety during weaning.	Study 1: *Intervention:* 2 groups. During meal at 8X home exposure. (1) RE to only pears (Pear Group). (2) variety to: fed a different fruit than the one of the previous 2 days (not pears). *Measurement:* Evaluated infants' intake, liking, length and rate of feeding of pears on days 1 and 11 and green beans on days 2 and 12. Study 2: *Intervention:* 3 groups. During meal at 8X home exposure. (1) only green beans. (2) Between-Meal (BM) variety: green and orange vegetables alternated daily. (3) BM–Within meal Variety: two vegetables each day (one green, one orange). Pair of vegetables varied from day-to-day, 1 of the pair was experienced the prior day. *Measures:* Infants' Intake, liking, length and rate of feeding of green beans on days 1 and 11 and alternating spoonfuls of carrots and spinach on Days 2 and 12 in lab.	Study 1: In both groups pear intake increased, but not green beans intake. Study 2: Increase Green beans intake group (1) from 15 to 24 cal ($p < 0.08$). Group (2) 20 to 26 cal ($p < 0.08$). Group (3) 12 to 27 cal ($p < 0.05$). Increase carrot-spinach intake: only in group (3): 9 to 16 cal ($p < 0.05$)
Houston-Price, Butler, & Shiba (2009) **(42)**	N = 20 infants Mean age: 23.2 months (range 21-24 m) Country: UK	Tasting test. Number and order of foods tasted were counted.	Visual exposure	*Intervention:* 2 groups. In each group parents read a different picture book with their child every day for two weeks. About a familiar and an unfamiliar fruit and vegetable. *Measurement:* In a 'taste test' following the exposure period they were offered all eight foods shown in the two books: the four vegetables followed by the four fruits. Number of foods the children tasted and the order in which they did were video recorded.	Children tasted more familiar than unfamiliar foods ($p = 0.046$). Overall no exposure effect. However, exposure served to decrease children's willingness to taste familiar V, it increased their willingness to taste unfamiliar V. Order of tasting only sign. effect in F. Exposure F were tasted before non-exposed F. Children displayed neophobic behaviour towards foods to which they had not been exposed, but not towards exposed foods.

Scheiwe, Hardy, & Watt et al. (2010) **(48)**	N = 101 Mean age at follow-up: 4 years 7 months. (range: 4 - 5 years and 2 m) Country: UK	interviews (most questions from 'National diet and nutrition survey' NDNS).	long-term effects of peer-led infant feeding intervention during first year of life.	*4-year follow-up of intervention:* *2 groups: (1) intervention, (2) control.* Monthly home visits (during the 1st year of life) from trained volunteers to improve infant feeding practices. *Follow-up measurement:* children's eating (a.o. F and V) and drinking habits, general and dental health, and BMI. Data collected via structured face-to-face interviews and postal questionnaires.	No evidence that the intervention had an important positive effect on children's F and V consumption. Mothers from the intervention group had better nutritional knowledge and confidence.
Vitolo, Rauber, Campagnolo et al. (2010) **(44)**	N = 345 Age range: 3-4 years. Intervention during first year of children's life. Country: Brazil	24-h recall.	Nutritional intervention. Dietary advice to parents. During first year of infants life.	*Follow up of intervention:* 2 groups (random): (1) intervention, (2) control. (1) 10 counseling home visits during 1st year of their child's life. Dietary advise and introduction of complementary foods. (2): Measurement: Dietary intake was assessed using two 24-h food recalls. Overall diet quality was determined by the Healthy Eating Index on a 10-pont scale. 10 => recommended amount. One of the ten components of the scale concerned V intake.	The prevalence of poor diet in the intervention group was lower compared with the control group. Intervention group had higher scores on healthy eating index for V intake (1.53 ± 2.10) than control (1.00 ± 1.84, p = 0.005). For the vegetable component 31.7% of the children in the intervention group scored above the 75 percentile of 1.67, compared to 16.7% of the children in the control group ($p < 0.05$).
Whaley, McGregor, Jiang et al. (2010) **(45)**	N = 821 Mean (SD) age at baseline: 23 (9.1) months. Country: UK	Phone survey with questions about food intake.	Dietary advice to parents.	*Intervention:* 2 groups: (1)Intervention: WIC with individual nutrition education. (2) Control: regular WIC participants and control participants. At baseline, 6 months, and 12 months past baseline parents of children in the intervention group got nutrition information and a session of motivational interviewing about health goals and behaviour. Parents filled in questionnaires about food intake, physical activity and television watching.	In children aged ≤2 year the amount of V intake a day was higher in the intervention group ($F_{1,298} = 3.7$, $P = 0.05$).

Authors (year)	Sample	Data collection	Focus paper	Design of study	Reported findings on vegetable and fruit intake, acceptance, and liking
Caton, Ahern, Remy et al. (2012) **(31)**	N = 72 Mean age: 23.6 months (SEM: 0.9, range:9-38 m) Country: UK	Intake was weighed.	Repeated exposure (RE); Flavour-flavour learning (FFL); Flavour-nutrient learning (FNL)	*Intervention:* 3 conditions (RE, FFL and FNL). 10 exposures to a novel vegetable. RE: plain artichoke purée. FFL: sweet (sucrose) added. FNL: added energy using oil *Measures:* Pre- and post-intervention intake measures of plain artichoke and carrot purée (control vegetable). Durability assessed 2 weeks post RE. Intake of Artichoke purée once a week for 3 weeks. Last week also carrot.	During 10 exposures intake of both vegetables increased (P=0.001); Artichoke sign. more than carrot (P=0.001) and to the same extent in all three conditions. This effect was persistent up to 5 weeks post-intervention. No sign. condition effect immediately after intervention. Elevated intake in RE condition at post-test (p=0.02). Sign vegetable by condition effect between RE and FFL. Artichoke intake post-test higher in RE group than in FFL group (p=0.024), not between RE and FNL and FFL and FNl.
Hausner, Olsen, & Møller (2012) **(38)**	N = 106. Mean age: 28 months (range 22-38) At nurseries. Country: Denmark	Intake weighed.	Repeated exposure (RE); Flavor-flavor (FF) learning; Flavor-nutrient (FN) learning	*Intervention: (at nurseries)* Nurseries were randomly assigned to one of three learning strategies. 3 groups: Children were exposed 10 times to unmodified purée (RE), a sweetened purée (FF) or an energy dense purée with added fat (FN). *Measurement:* Pre-testing with an unmodified artichoke purée. Post-test with unmodified purée, 3 and 6 months after last exposure to monitor long-term effects of learning.	Intake of purée increased in the RE and FF condition, and was unchanged in the FN condition. RE changed children's intake by the 5^{th} exposure, FF learning by the 10^{th}. RE led to the largest increase in intake of unmodified purée at post-test and over 6 months. Children following FF learning consumed more of the sweet purée than of unmodified purée. About 30–40% of the children were resistant to acceptance changes.
De Wild, de Graaf & Jager (2013) **(68)**	N = 28 Mean age: 36 months (SD:7.3, rage: 21 – 46) Country: the Netherlands	Intake weighted. Paired preference test.	Repeated exposure (RE); Flavour-nutrient (FN) learning	*Intervention:* *2 groups were fed soups for 7 weeks 2x a week at nursery. (1)High- energy (HE) spinach soup and low-energy (LE) endive soup. (2)HE endive soup and LE spinach soup* *Measures*: Preference and ad libitum intake (with a maximum of 200 g) of both vegetable products	After completion of intervention, 28 children met criteria for FNL, and were included in further data analysis. Sign.increase (58 g) in intake for both vegetable soups (stable over time), irrespective of the energy content. This indicates a robust effect of mere exposure on intake, but no FNL.

				(LE), measured before, shortly after the intervention period, and 2 and 6 months following conditioning to assess longer-term effects.	Preference results showed a sign. shift in liking for the vegetable soup consistently paired with high energy, supporting FNL
Remy, Issanchou, Chabanet et al. (2013) (40)	N = 95 Age: 4-8 months. Country: France	Intake, liking rated by mothers	Repeated exposure (RE); Flavour-flavour (FF) learning; Flavour-nutrient (FN) learning	*Intervention:* 3 conditions (RE, FFL and FNL). 10 exposures to a novel vegetable. RE: plain artichoke purée. FFL: sweet (sucrose) added. FNL: added energy using oil *Measures:* Pre- and post-intervention intake measures of plain artichoke and carrot purée (control vegetable). Durability assessed 2 weeks post RE. Intake of Artichoke purée once a week for 3 weeks. Last week also carrot.	No effect of breastfeeding duration on initial intake. Amount of V eaten before starting the study, influenced artichoke intake at pre-exposure (+8 ± 2 g/vegetable previously eaten; P = 0.0001). Post exposure: Both intake of RE (+63%, p = 0.0001) and FFL (+39%, p = 0.007) group increased sign. Not in FNL group. Intake of RE and FFL similar. Learning was stable up to 3 months post exposure. No effect of number of V eaten before starting the study on liking at pre-exposure. Liking increased only in RE group (+39%; P = 0.005)
Barends, de Vries, Mojet et al. (2013) (39)	N = 101 mean age 5.4 months (SD, range 4-6) Country: the Netherlands	Intake was weighed. Mothers rated liking.	Repeated exposure (RE)	*Intervention: Home and Lab* 4 groups: 18 consecutive days exposure to variation of V with 9x RE to: (1) green beans, (2) Artichoke. Or variation of F with RE to (3) Apple, (4) Plums. Measurements: Day 1 and 2 intake measurements and liking in the lab of exposed vegetable and other vegetable in V groups. Exposed fruit and other fruit in fruit groups. Day 19 1st F in vegetable group and 1st V in fruit group.	Mean V intake in vegetable group increased sign. from 24 ± 28 g (mean ± SD) on days 1 and 2 to 45 ± 44 g on days 17 and 18. 1st intake of green beans in the fruit groups at day 19, was 24 ± 29 g and as low as the green beans intake in the vegetable groups at the 1st exposure on day 1 or 2. 1st apple intake in the fruit groups on day 1 or 2 of 47 ± 48 g did on average not differ from the first apple intake of 45 ± 49 g in the vegetable groups on day 19. Mean intake of green beans increased sign. both in groups 1 (p=0.016) and 2 (p<0.001). Intake of artichoke only increased sign. in group 1 (p=0.042) but not in group 2 (p=0.603). Liking correlated with intake.

[1] QA = quality assessment score. Other abbreviations used in this table: F = fruit; V= vegetables; RE = repeated exposure; FFL = flavour-flavour learning; FNL = flavour-nutrient learning; WIC = a Special Supplemental Nutrition Program for Women, Infants and Children

Table 6.9 Summaries of the included observational studies, with results on vegetable (V) and fruit (F) consumption and/ or liking

Authors (year)	Sample	Data collection	Focus paper	Design of study	Reported findings on vegetable and fruit intake
Mennella and Beachamp (2002) **(55)**	N = 102 Mean age: 4.9 years (range 4 – 5 years) Country: USA	Food frequency questionnaire. List most preferred vegetables and fruits.	Effect of milk feeding method on later V and F preferences.	*Retrospective.* Children were divided into three groups based on their early feeding experiences Milk-based formula only Soy-based formula (were usually fed milk-based the first 1 or 2 months and then switched to soy. Hydrolysate-based formula (were usually fed milk-or soy-based for the first 3 months). *Measurement*: Age appropriate taste test to examine preferences for foods and the formulas Mothers completed a food frequency questionnaire and listed the children's most preferred V and F.	No differences were found in how often the children ate F and V. Broccoli was more likely to be ranked as preferred by children that were fed soy-based ($p < 0.005$) or hydrolysate ($p > 0.03$)formula, than those that were fed a milk-based formula.
Mennella, Kennedy, & Beauchamp (2006) **(56)**	N = 74 Mean age: 8.6 (SD: 0.2, range 6-11 months) Country: USA	Intake was weighed. Mothers rated liking.	Effect of milk feeding method on V and F preferences.	*Observational experiment:* 2 groups on type of formula feeding: (1) milk-based formula. (2) hydrolysate formula. Intake and mothers rated liking of puréed broccoli/cauliflower was determined during one test session and puréed carrots on the other.	No group differences in amount of carrots consumed. Hydrolysate fed infants consumed sign. less broccoli/cauliflower relative to carrots than milk based fed infants (65 vs 101 g, $p=0.04$). Hydrolysate fed infants were rated to enjoy broccoli/ cauliflower less than milk-based fed infants (45,8% vs 78%, $p=0.03$). No difference in enjoyment of carrot.
Coulthard, Harris, and Emmett (2009)	N = 7821 Age all: 7 year olds who participated in ALSPAC study at	Questionnaire about introduction of vegetables (at	Effect of age of introduction of solid foods on V and F intake.	*Longitudinal study:* Groups: Children were divided into three groups based on the age at which they were first introduced to 'lumpy' solids: (1) <6	Fewer children in group (3) ate vegetables ($p = 0.001$) than children introduced to lumpy foods between 6-9 months. *(about 7% les children).*

(57)	6 and 15 months of age. Country: UK	age of 15 months). Dietary range questionnaire (at age 7 years).		months 12.1%. (2) 6-9 months, 69.8% (3) ≥10 months, 18.1% *Measurement:* Self-report questionnaires completed by the mother about her child at 6 months, 15 months, and 7 years postpartum about food foods eaten and feeding difficulties.	They also ate fewer portions and fewer types of V. In addition they were reported as having significantly more feeding problems at seven years Of group (1)<6 months more children ate green V and tomatoes ($p = 0.001$).
Coulthard, Harris, & Emmett (2010) **(50)**	N = 7821 Age all: 7 years. Data collection started at 6 months of age. Country: UK	Questionnaire about introduction of vegetables (at age of 6 months). Dietary range questionnaire (at age 7 years).	Longitudinal effects of frequency, type, and age of introduction of fruit and vegetable intake during weaning.	*Longitudinal observational study:* Mothers completed self-reported questionnaires about the introduction of vegetables during weaning at 6 months and FFQ at 7 years postpartum. Relation between frequency of consumption of types of F and V at or before 6 months of age and fruit and V consumption at 7 years of age were calculated.	Frequency of consumption of home-cooked V (and not ready prepared) at 6 months correlated ($\beta = 0.14$; $P < 0.001$) with frequency of V consumption at 7 years. Age of introduction moderates frequency effect. But does not affect intake at 7 years directly.
Burnier, Dubois, & Girard (2011) **(54)**	N = 1549 Age all: 4 years. Data collection started at 5 months of age. Country: Canada	Interviews and questionnaires about infants feeding at 5 months of age. 24-h dietary recall of food consumption at 4 years of age.	Breastfeeding and age of introduction to vegetables	*Longitudinal observational study*: Collection of information on children's food consumption patterns and behaviors of the infants from the age of 5 months and once a year thereafter. At 4 years of age a 24-h dietary recall. *Analysis*: Relation between exclusive breastfeeding duration and V intake.	Age of introduction to V was not related to V consumption at 4 years of age. Infants that were breastfed longer than 3 months and infants with a mother that had a university degree had a higher change to get more than 2 servings of V a day ($p < 0.05$)

Authors (year)	Sample	Data collection	Focus paper	Design of study	Reported findings on vegetable and fruit intake, liking and acceptance
Gregory et al. (2011) **(49)**	78 mothers completed questionnaires when infants were 1 and 60 of them also when they were 2 years of age. Country: Australia	Questionnaire maternal feeding practices. Child food frequency questionnaire.	Longitudinal effects of maternal feeding practices on vegetable and fruit intake. Frequency vegetable consumption.	*Longitudinal observational study*: A self-report questionnaire was used for mothers to record demographic and anthropometric information, as well as measures of maternal feeding practices, child food consumption, and food availability. Prospective relationship between maternal feeding practices and young children's frequency of consumption of F, V and sweets were calculated.	Frequency of vegetable consumption at 1 year of age predicted V intake at 2 years of age. Maternal use of pressure to eat at 1 year predicted lower child frequency of F consumption at 2 years and approached significance for lower vegetable consumption. Maternal modeling of healthy eating at 1 year predicted higher child frequency of V consumption at 2 years. Restriction did not significantly predict child frequency of consumption of F, V or sweets over time.
Grieger et al. (2011) **(53)**	N = 1071 Mean age: 2.5 years (SEM: 0.0, range 2-3) N=1216 Mean age: 6 years (range 4-8) Country: Australia	24- hour food recall by interview.	Influence breastfeeding on later food intake	*Cross-sectional study:* Sample divided in breastfed and non-breastfed. *Measurement*: Food and nutrient intake data were collected on two occasions using a 24 h food recall. A computer-assisted personal interview (CAPI) was conducted in the child's home including questions about milk and breastfeeding history.	A positive association was found between breast-feeding and the healthy, meat and vegetable food pattern (r = 0.267) (β = 0.23; p=0.03). No data on vegetable intake alone.
Howard, Mallan, Byrne et al. (2012) **(51)**	N = 245. Mean age 24 months (SD:1) Country: Australia	Questionnaire of early feeding taken at 13 months of age. Food preferences questionnaire at 23 months. Question about	Number of novel food exposure	*Cross-sectional observational study:* Sample drawn from control group of NOURISH RCT study. Breastfeeding duration measured with characteristics questionnaire at 4, 13 and 23 months of age. Child food preferences were collected at 23 months of age with food preferences	Breastfeeding duration and number of repeated exposures to new food were not significantly associated with vegetable or other food liking at this age.

		novel food exposure.		questionnaire. Number of repeated food exposures was assessed in a questionnaire item. The effects of repeated exposure to new foods and child food neophobia on toddlers' liking of V, F and non-core foods and the proportion never tried were examined via hierarchical regression models.	
Townsend & Pitchford (2012) **(58)**	155 children aged 20-78 months. Country: UK	Food preference questionnaire.	Weaning style Exposure	*Observational:* 2 groups based on information questionnaire. (1) baby-led weaning. (2) spoon-fed weaning. *Measurement:* Questionnaires on child's preference for 151 foods and exposure (frequency of consumption). Food preference and exposure data were analyzed using a case controlled matched sample to account for the effect of age on food preference. Weaning style was investigated with the whole sample.	Exposure to V, F, carbohydrates, protein, meals and sweets was higher in the spoon-fed group, compared to the baby led group. Exposure was associated with liking vegetables ($r_s = 0.47$, $p<0.0001$). No association was found for carbohydrates, sweet foods, fruits, and meals. This suggests that only for carbohydrates, weaning style was more influential than exposure on preference ratings
Lange, Visalli, Jacob et al. (2013) **(52)**	N = 203 Followed from 0 to 15 months of age. Country: France	Weekly food diaries Acceptance score.	Breastfeeding duration Age of food introduction and food variety	*Longitudinal study:* Infants' milk diets were recorded in food diaries one week per month during 1st year of life. Mothers recorded each food offered to the infant from the beginning of weaning to the age of 15 months. The acceptance of these foods was scored by the mother on a 4 point scale form very negative (e.g. spitting out foo) to very positive (e.g. infant ate spoon immediately with relaxed face or smile)	F and V were the least well-accepted categories at the beginning of weaning. The earlier V were introduced, the higher the acceptance of new V was ($p=0.04$). New food acceptance was correlated with the number of different foods offered in the first two months of weaning, particularly for fruits ($p = 0.01$) and vegetables ($p < 0.0001$). Exclusive breastfeeding duration did not influence new V and F acceptance.

QA = quality assessment score. Other abbreviations used in this table: F = fruit; V= vegetables; RE = repeated exposure; FFL = flavour-flavour learning; FNL = flavour-nutrient learning; WIC = a Special Supplemental Nutrition Program for Women, Infants and Children

Chapter 7

General Discussion

It is hard to increase children's intake of vegetables. A vast amount of research has shown that vegetable intake in children does increase after repeated exposure to vegetables. However, if children do not want to eat vegetables, they do not get exposed to them and as a consequence do not get used to their taste.

In an attempt to tackle this problem, we directed our research on young infants who are still open to new tastes. The aim of this PhD thesis was to investigate whether first exposures to solid food (fruit or vegetables) could influence preferences for vegetables.

This was investigated on a short term **(chapter2)** and on a longer term **(chapter 4)**. Both the increase of intake **(chapters 2, 4)** and liking **(chapter 3)** were investigated. Intake of vegetables and fruit was measured in the lab under controlled conditions, and intake of vegetables during daily life was measured at 2 different time points **(chapter 4)**. The effect of weaning with vegetables or with fruit on children's preferences for sweet and salty tastes, was also investigated (**chapter 5**, sweet and salt solutions test, sugar intake). In addition, a review was carried out to identify the most effective strategy for increasing vegetable intake in children younger than 3 years of age **(chapter 6).**

In this chapter the main results of the studies will be discussed.

7.1 MAIN FINDINGS

The main findings of this research are displayed in **Table 7.1.**

Chapter 2 reported that the group of children who were repeatedly exposed to vegetables increased their vegetable intake while children who were repeatedly exposed to fruit increased their fruit intake. Although the fruit intake of the children in the fruit group was already as high as the vegetable intake of the children in the vegetable group after repeated exposure to the vegetables, their fruit intake after repeated exposure to fruit increased significantly. Interestingly, the first vegetable intake of the children in the fruit group at day 19 was only 24 ± 29 g, which is similar to the vegetable intake of children in the vegetable group at day 1. This indicates that the repeated exposure to fruit did not influence the children's vegetable intake.

Chapter 3 reported that analysis of facial expressions showed that while the intake increased, the number of negative facial expressions decreased and the rate of acceptance (measured by infants opening their mouth in response to an approaching spoon) increased. Intake can be influenced by multiple factors. The increase in rate of acceptance showed that the children were more willing to eat after repeated exposure

and opened their mouths sooner for an approaching spoon. On the other hand, facial expressions are (involuntary) responses to the food, which are likely to reflect liking or disliking of the food. Therefore, the results in chapter 3 show that willingness to eat and preference for the repeatedly exposed food both increased.

Chapter 4 investigated whether the intervention at weaning had a longer-lasting effect on the fruit and vegetable intake of the infants. When the infants reached 12 and 23 months of age, their intake of apples and green beans purée was measured in the lab. No significant differences between the groups were found, however. We found that apple purée intake was higher than green beans purée intake in both groups. When fruit and vegetable intake were measured at home (with 3-day food records), we found that, at 12 months of age, children who started weaning with vegetables had a significantly higher daily vegetable intake than children who had started weaning with fruits. At 23 months of age, however, this effect was gone.

We hypothesised that starting weaning with fruit would facilitate the inborn preference for sweet taste. We expected that children who started weaning with vegetables, which are not sweet, would develop a lower preference for sweet taste. In **chapter 5,** this hypothesis was tested by measuring the preference 23-month-old children exhibited for sweet water solutions and their daily mono- and disaccharides intake. No differences between the vegetable and the fruit group were found, which indicates that the intervention had no lasting effect on the preference for sweet.

Finally, the review in **chapter 6** gives an overview of different strategies used to increase vegetable intake that have been studied in children under 3 years of age. The review showed that counselling parents on healthy eating and nutrition has some positive long-term effects on children's vegetable intake, although these effects were small. The review further showed that repeated exposure was both the most studied and the most effective strategy, since all studies reported an increase in intake after repeated exposure to a vegetable. Exposure to a variety of vegetables is also found to have a positive effect on the intake of a new vegetable.

Table 7.1. Overview of the main research findings

Chapter	Topic	Results
2	Effect of repeated exposure to either vegetables (*vegetable group*) or fruit (*fruit group*) during weaning on intake	- Intake of vegetable purées increased from 24 ± 28[1] g to 45 ± 44g ($p < 0.001$) in the vegetable group. - Intake of fruit purées increased from 46 ± 40 g to 66 ± 42 g ($p < 0.01$) in the fruit group. - The first intake of green beans purée in the fruit group on day 19, after 18 days of exposure to fruit purées, was 24 ± 29 g and on average as low as the first green beans intake of the vegetable group on day one.
3	Effect of repeated exposure to either vegetables or fruit during weaning on Facial expressions	- Total of negative facial expressions over the first nine spoons of green beans purée per session, decreased from 23 (18;30)[2] to 16 (10;24); $p < 0.01$ in the vegetable group. - Total of negative facial expressions over the first nine spoons of green beans purée in the fruit group on day 19, was 20 (18;25) and was on average as high as that in the vegetable group on day one.
4	Long-term effect on vegetable intake by starting weaning with exclusively vegetables or fruit	- Reported daily intake of vegetables at 12 months of age was 38 % higher ($p = 0.02$) in the vegetable group (75 ± 43 g) than in the fruit group (54 ± 29 g), but was similar for both groups at 23 months of age (48 ± 43, 57 ± 35 g, respectively; NS). - Both at 12 and 23 months of age, intake of apple and green beans purée in the lab did not differ significantly between the groups.
5	Effect of weaning exclusively with vegetables or fruit on sweet and salt preferences at the age of 23 months.	- Preferences for sweetened solution did not differ between the vegetable group (Mean IR = 0.55)[3] and the fruit group (mean IR = 0.52) ($p = 0.441$). - Daily mono- and disaccharides intake did not differ significantly between the vegetable group (89 ± 32 g) and the fruit group (85 ± 27 g) ($p = 0.124$).
6	Review of strategies to increase vegetable intake in young children under the age of 3 years.	The most effective strategy was repeated exposure to vegetables. Exposure to a variety of vegetables was also effective in encouraging intake. Counselling was found to be minimally effective, since it only added a few grams of intake in comparison with the control groups. Flavour-flavour and flavour-nutrient learning did not produce additional benefit over repeated exposure in encouraging intake. Inconsistent effects were found for age at introduction of vegetables and milk feeding method in relation with vegetable consumption and acceptance.

[1] mean ± SD for all such values

[2] Median (25thl 75th percentile) for all such values.

[3] IR = ingestion rates measured as intake of taste solution compared to intake of plain water. A rate of 0.5 means that preference for the taste solution is equal as preference for water.

7.2 METHODOLOGICAL CONSIDERATIONS

Some methodological and practical choices were made with respect to the design and execution of the study. In this chapter, the implications of these decisions will be described.

7.2.1 Study population

The mother-infant pairs that participated in this study were recruited through a number of different sources. In all cases the parents had to volunteer to participate. We recruited during a period of 1 year until we found enough participants. This means that the participants were not picked randomly from the population, and that we therefore cannot rule out that the possibility that the mothers, who were interested to participate, already were more interested than averagely in healthy eating. However, the study was not designed to test a sample that was representative for the whole Dutch population, but rather to compare infants who were weaned with vegetables with infants who were weaned with fruit. Since the mother-infants pairs were randomly allocated to a treatment group, there is no reason to think that the mothers' characteristics would differ between the vegetable and fruit group. To be sure the test groups were similar, we compared characteristics of the mothers that could influence the intake of their infants (e.g., age, education, BMI, food neophobia and weeks of breastfeeding) (1-4). No significant differences were found. This means that, with our data, we can say that weaning with vegetables does increase vegetable intake compared to weaning with fruit. However, the magnitude of the effect in both groups could be different in other populations. To minimize this possibility we recruited mother-infant pairs in two different regions in the Netherlands (Wageningen and Almere) to get a more diverse sample of participants.

The age at which the infants started with the intervention (**chapter 2**) varied between 4 and 6 months. This age difference could have influenced the initial intake. Mothers decided when their child was ready to start weaning, and thus decided to start with the intervention. The only restriction was that the infant had to be at least 4 months old and not older than 6 months. Prior to the study, we recommended that the mothers discuss with their child health practitioners the best time for their infant to start weaning. As a result some infants were only four months old, while others were already six months old. Although some children are ready to start weaning sooner than others, we found that infants younger than 5.4 months ate significantly less than older infants. Since children with different ages were equally divided between the intervention groups this could not have influenced the results between the groups.

A strong point of the study was that we followed infants until they were 23 months of age, which was on average 18 months after the intervention during weaning. Also we had good compliance rates. We started with 101 mother-infant pairs. At 12 months of age, 83% of the initial group participated again, of which 85% filled in the food records. At 23 months of age, 80% of the initial group participated again, which was 96% of the children that participated at 12 months of age. Of these participants 85% filled in the food records. To rule out the possibility of structural differences between participants of the follow-ups and subjects that did not participate, we compared their characteristics, but we did not find significant differences. In addition, taking into account the equal amount of participants and drop-outs in both the vegetable and the fruit groups, it is unlikely that this would have influenced the results.

7.2.2 Test food

We had a few considerations and limitations regarding the choice for the vegetable and fruit purées that were used during the intervention.

The vegetable and fruit purées used for the intervention during weaning **(chapter 2)** were also used in the follow-up measurements **(chapter 4)** when the children were 12 and 23 months of age. This was done to keep the taste and structure constant, so that differences in taste an structure could not affect the results. During the course of the study, the children experienced a large development in oral-motor skills as is normal for infants between 4 and 24 months of age (5). At the start, most were able only to suckle puréed food from the spoon, but at the end they were able to bring the spoon to their mouths by themselves and eat as adults do (though in a slightly clumsy manner). This made it impossible to choose a test food with a structure that is suitable for both 4-month and 23-month-old infants. Because the ability to chew on lumpy foods develops during the first year of life (6, 7), and the World Health Organization (WHO) recommends to introduce lumpy solids between the ages of 6 and 9 months (8), we used smooth puréed vegetables and fruits suitable for infants as young as 4 months of age. Most children of 12 and 23 months, however, were not used to eating vegetable purées anymore, which could explain the low green beans purée intake in the lab that was found in both the vegetable and the fruit group.

For the intervention **(chapter 2),** green vegetables were chosen over vegetables like carrots or pumpkins to distinguish clearly between fruit and vegetables, as we expected that the taste of orange vegetables would resemble the taste of fruit more than green vegetables. A study of Gerrish and Mennella (9) indicated that infants who were already daily exposed to fruit purées had higher intakes of carrots than infants with no prior fruit

exposure, while Mennella et al. (10) showed that exposure to a variety of fruit did not influence green beans intake. Therefore we chose Green beans, Artichoke, Broccoli and Cauliflower as our four target vegetables. We wanted one target vegetable that was well known by the parents and another target vegetable that was unknown by most parents. We included an unknown vegetable to minimize both the effect of the parents own liking of the food and the chance that the infants already were exposed to it through amniotic fluid and breastfeeding (1, 11-13). Green beans (well-known vegetable) and artichoke ('uncommon' vegetable) met these requirements.

Taste of artichoke

The review (**chapter 6**) describes 10 other studies of the effectiveness of repeated exposure with a number of different vegetables (9, 10, 14-20). These studies suggest that repeated exposure to all types of vegetables would lead to an increase in intake, and that 9 exposures would be enough to increase also the intake of vegetables that might initially be liked less. However, unlike 2 recent studies (19, 21, 22), artichoke intake in the artichoke group did not increase in our study. This might be caused by the difference in sensory profile between the artichoke purée used in those studies and the one that we used. The sensory profile of the artichoke purée that we used, shows that it is sourer, and bitterer and less sweet than the green beans purée **(Table 2.3, chapter 2**). This can explain the low intake of artichoke, because infants have an innate dislike of sour tastes and a preference for sweet tastes (23). Although the intake of green beans was also lower than the intake of the fruit purées, repeated exposure did increase the intake. The artichoke purée was probably not palatable enough to most infants for the repeated exposure to have an effect. Our study design does not allow us to conclude whether this is due to the combination of a low degree of sweetness and a relatively high degree in sourness, or that other factors played a role.

7.2.3 Sweet- and salt-water solutions

When the children in our study were 23 months of age we tested their sweet and salt preferences with sweetened- and salted-water solutions **(chapter 5).** This method of testing taste preferences in infants was used in studies by Beauchamp et al. (24) and Schwartz et al. (25). The method, however, was developed for younger infants and used drinking bottles from which the children tasted the solutions. Not all of the children in our study were still accustomed to drinking from bottles, however, which could have affected. Furthermore, while younger infants are used to getting a majority of their food intake through liquids, older children and adults are not used to drinking cold, salty drinks. This may explain why the 23-month-old children rejected salted water over plain water,

thereby contradicting previous findings which showed that infants prefer salt water over plain water (24, 25). Nevertheless, though most children in our study disliked the salted water, most children did taste a few millilitres of it, and we therefore think that the test still differentiated between children with had a lower and children with higher preference for salty tastes. Most importantly, it is not likely that the unfamiliarity with salt water differed between the vegetable and fruit groups, so this could not have led to a group bias.

7.2.4 Internal and environmental factors

Our studies used intake as the main measure for how much the children accepted or liked fruit and vegetables. We also used the mothers' rated liking and the infants facial expressions and behaviour **(chapter 3).** Internal factors (such as hunger or sleep) and environmental factors may have influenced the intake of the purées **(chapter 2 and 4)** and the sweet and salty solutions **(chapter 5)**. To minimize the influence of these factors, we asked parents not to feed their infants 1 hour prior to the measurements in the lab and to come to the lab for all the measurements at the same time or day. With the young infants, it was not always possible to test at the same time each day, since some infants lacked a strict time schedule and were fed on demand. In these instances, we were more flexible with time of day so long as the time since the last feeding was about the same.

Setting

Measurements were carried out in the infants natural environment (by food records kept by the parents) and in the lab (by measuring intake). Both methods have their advantages and disadvantages. Although direct measurements of intake are more precise than measurements of intake using food dairies recorded by the parents (26), the lab differs from the infants' natural environment and could therefore have influenced intake. To minimize the unfamiliarity of the environment, the lab was quiet and tranquilly decorated. At the start of each session, the children could become accustomed to the room and the baby chair while playing with a toy. Throughout the study, the sessions in the lab always took place in the same room, so the infants got used to the environment.

During the intervention in **chapter 2**, the setting in the lab resembled the setting at home, where the child often would sit in a similar chair and would be fed similar fruit and vegetable purées. For the 12- and 23-month olds, the setting in the lab probably differed from the way they would eat fruit and vegetables at home. As mentioned before, toddlers are not used to eating purées anymore. Furthermore, vegetables usually are consumed as part of a dinner consisting of multiple ingredients. Therefore, the intake of purées in the

lab cannot be compared with daily fruit and vegetable intake. Rather it serves as a comparison between both intervention groups of how much they like or accept the food.

Mothers influence on intake and liking measurements

During the measurements of vegetable and fruit intake in the lab with the intervention during weaning **(chapter 2 and 3),** and also with the follow-up measurements **(chapter 4),** it was mostly the mother who fed the infants the purées. To minimize the mothers' influence we provided standardized guidelines about how to feed their infants and instructed them to stop feeding only when the child rejected the spoon 3 consecutive times. Although they could have misinterpreted the behaviour of their infants due to their own beliefs about the food, we were able to follow the sessions via a live video connection and hence were able to correct the decisions of the mothers if they did not follow the guidelines during the first session.

Mothers may also have influenced the facial expressions of their children **(chapter 3)** in response to their own facial expressions (27, 28). They were instructed to keep a neutral face and to open their mouths as the spoon was approaching the infant's mouth. The instruction for mothers to open their mouths was not only to show the infant what to do, but also because this is what mothers automatically/unconsciously do when they see their child eating. In previous studies, mothers wore a mask to prevent them from influencing their infants' facial expressions with their own facial expressions (29, 30). However, in our study we chose to keep the feeding as natural as possible, in order not to upset the infants.

Food-records

To measure the daily vegetable and fruit intake when the infants were 12 and 23 months of age, we asked the parents to fill in 3-day food records **(chapter 4)**. Daily vegetable and fruit intake **(chapter 4)** was possibly influenced by the parents, since there were no feeding guidelines and the parents provided vegetables and fruit to their children according to their own choice.

Self-reports by food records are prone to misreporting (26, 31, 32), which can lead to biases such as over- or underestimation or social desirability. Since the parents knew that the study was about fruit and vegetable intake, they could have (unconsciously) overestimated fruit and vegetable intake due to social desirability. The fact that their child had received only vegetable or only fruit purées during the first 18 days of the intervention could have influenced the parents. Therefore, we cannot rule out the

possibility that the parents in the vegetable group were more vegetable orientated than the parents in the fruit group. To minimalize this possibility, both groups were provided with the same information about the importance of fruit and vegetables in children's diets, and were advised to continue complementary feeding with both fruit and vegetables. Furthermore, they were unaware of the study design, and hypotheses. Nevertheless, although the parents provide availability of the foods by deciding what the children eat, the children decide how much they eat. Studies on children's vegetable and fruit intake, show that intake correlates with liking (33-35). Many children refuse to eat the vegetables that their parents offer them. On the other hand, when parents do not give vegetables to their children, the children have no chance to eat it. Studies have shown that availability of fruit and vegetables, had a positive influence on children's intake of fruit and vegetables (36, 37). Because of the intervention in **chapter 2**, parents may have been more motivated to feed their infants fruit and vegetables, which in turn may have increased their infants' intake of it. Compared to the children involved in the Dutch national food survey (38) the children in our study had a higher fruit and vegetable intake both at 12 and 23 months of age. The finding that there is no significant difference between children that started weaning with vegetables and children that started weaning with fruits in how often the children received vegetables suggests that the influence of the parents was equal in both groups **(chapter 4).** Therefore, a possible elevated vegetable and fruit intake caused by the intervention, still allows comparisons between groups.

7.3 Discussion and interpretation of results - recommendations for further research

The next section, considers the research questions regarding the effect of repeated exposure and weaning with exclusively vegetables on increase in vegetable acceptance in the short and long term. Suggestions for future research will be given. In addition, the findings from the chapters 2 through 6 will be compared and interpreted.

7.3.1 Repeated exposure versus variety

The studies in this thesis confirm previous research which found that repeated exposure to vegetables increases vegetable acceptance (9, 14-16, 18, 39-41). In addition, the review in **chapter 6** concludes that repeated exposure and exposure to a variety of vegetable are 2 effective methods to stimulate vegetable intake. The study at weaning in **chapter 2** combined both repeated exposure and variety. To differentiate between the effect of

repeated exposure and variety, both the intake of the repeatedly exposed vegetable or fruit and a novel vegetable (in the vegetable groups) or fruit (in the fruit groups) were measured. In the group that was repeatedly exposed to green beans, the increase in green beans intake was higher than that of the novel vegetable – artichoke. Also in the groups that were repeatedly exposed to respectively apple or plums, were the increases in intakes of the exposed fruit slightly higher than of the novel fruit, but this was not statistically significant.

In the artichoke group however, no increase in artichoke intake was found while the intake of the novel vegetable green beans did increase significantly. The low palatability of artichoke probably undermined the repeated exposure effect, since both the green beans and the artichoke group had lower artichoke intake than green beans intake. The fact that the infants in the artichoke group, who were repeatedly exposed to artichokes alternating with broccoli and cauliflower, did increase their intake of green beans, indicates that this increase was caused by exposure to vegetables varying day to day. This result is supported by previous studies, showing that between meal variety of vegetables, increased the acceptance of a new vegetable (9, 10, 17). Furthermore, our results indicate that it was not due to variation only, since the combination of repeated exposure to apples or plums alternated with exposure to banana and pear seemed to have no effect on green beans intake in the fruit group, indicating that, to increase an infant's vegetable intake, the infant should be exposed to vegetables.

For a better understanding of the most effective use of repeated exposure and variety, different designs of intervals of exposure and variety should be compared. The study of Maier at al. (17) showed that when infants were fed a variety of 3 vegetables alternating daily for 9 days in a row, the intake of a new food was higher than when 3 vegetables were each fed 3 days in a row. This indicates that a higher variety is more effective. An overview of previous studies on repeated exposure and variety is given by Mennella and Trabulsi (42). These studies show that repeated exposure increases the intake of the exposed food (9, 10, 15, 18), while variety of vegetables increases the intake of a novel vegetable (9, 10). Our results confirm these studies, since in our groups the acceptance of the novel (or unexposed) vegetable did increase in both vegetable groups. Considering also the health benefits of a diet with a wide variety of fruit and vegetables (43), this emphasises the importance of combining repeated exposure with a day to day variety of vegetables.

7.3.2 Starting with vegetables or with fruit

The intervention study described in this thesis is one of the first to investigate the effect of starting weaning with vegetables versus starting with fruit. Our results show that starting weaning with vegetables increases intake and liking of vegetables (**chapters 2 and 3**). The results also show that fruit intake was higher than vegetable intake already at the first exposure. This might trigger mothers into feeding their infants (sweet) fruits since this is what babies seem to enjoy most and because at the end of the day all mothers want is a content baby. However, since vegetables are less popular than fruit, they have to learn to pay particular attention to the possible consequences of such behaviour. Our results showed that the group that started weaning with vegetables doubled its vegetable intake (from 24 ± 28 g to 45 ± 44 g), while weaning with fruit did not influence vegetable intake. When infants of the vegetable group finally were exposed to their first fruit purée, the intake of this purée was comparable to their vegetable intake (both about 46 g). In contrast, for the fruit group, which was first repeatedly exposed to fruit purées, the intake of the infants' first vegetable – green bean purée- was much lower (24 ±29 g) than the intake of the first fruit purées (66 ± 42 g). This indicates that vegetable acceptance needs more attention.

Our study design in **chapters 2 to 4** did not show how infants of the fruit group, who started weaning with fruit purées, were introduced to vegetable purées after day 19 of the intervention period when they received their first vegetable. All we know is that the fruit group had a lower daily vegetable intake when they were 12 months of age. Although we provided parents from both groups with the same information about complementary feeding, which advised them to continue weaning with both vegetable and fruit purées, we have no exact data on how they subsequently introduced fruit and vegetables. An interesting future study would be to investigate the effect of repeated exposure to vegetables after infants have been already repeatedly exposed to fruit. Will intake of the vegetable increase as much as in the group that starts with vegetables? What if infants who started weaning with vegetables **(chapter 2)** are repeatedly exposed to fruit after day 19 and the infants that start with fruits now are repeatedly exposed to vegetables? Will the original vegetable group at the end still eat more vegetables, or do the effects of the first and the second intervention level each other out? In other words, will future research corroborate the important finding/proposition that repeated exposure to vegetables needs to occur at the very start of weaning?

7.3.3 Short and long term effect

The positive effect of starting weaning with vegetables on vegetable intake and liking that was found in **chapter 2 and 3** on the short term was investigated on the longer term in **chapter 4**. This study confirms that repeated exposure to vegetables during weaning increases infants' vegetable intake across a 6 month period. However, the effect may not persist in the long run. At twelve months of age, the effect on vegetable intake after the intervention was still present according to home intake but had vanished at 23 months. Therefore, an intervention during weaning might not be enough to stimulate longer-term vegetable preferences. Alternatively, food neophobia usually emerges between the ages of 1.5 and 2 years (44), which could complicate the introduction of vegetables at this age (22). **Chapter 4** further shows that pickiness increased between 12 and 23 months of age, which indeed points to the emergence of food neophobia (45, 46).

The difference in green beans intake between the vegetable and the fruit group found after the intervention at weaning, was not maintained at the follow-ups at 12 and 23 months of age. Nevertheless, we did find a positive correlation in intakes of green beans purée in the total group between 4-6 and 12 months of age and between 12 and 23 months of age. However, no correlation was found between 4-6 and 23 months of age. This means that intake at 4-6 months of age is related to intake at 12 months of age. Since the results of the food-records showed that the vegetable group had a higher daily vegetable intake at the age of 12 months than the fruit group, a new intervention to stimulate vegetable intake at this age could to prolong heightened vegetable intake to the age of 23 months and further. This could be investigated, for example in a study with 3 research conditions: 1) intervention only during weaning, 2) intervention only around 12 months of age, 3) interventions both during weaning and at 12 months of age, and a control group without intervention. This way, it can be determined if an interventions to increase vegetable during weaning and at 12 months, adds value to one another.

Although, the intervention during weaning might not have been effective enough to stimulate vegetable intake until the age of 23 months, we conclude that it was also valuable for the long term, as it is easier to promote vegetables in 1 year olds that already have a higher preference for them.

7.3.4 Integrating all findings

To determine the effect of the repeated exposure intervention, we used intake as the main measure. In **chapter 2** the effect was measured in the short term. Intake of the purées was measured all 19 days of the intervention. It was measured in the lab on days 1, 2, 17, 18 and 19, and measured at home on the other days. Although measured intakes of

the purées at home were higher than in the lab, they showed the same effects of increase in intake. In addition, liking as rated by the mothers correlated strongly with intake. Furthermore, in **chapter 3,** the results were confirmed with the measure of facial expressions. Since liking is an important predictor for future intake (33-35), it is important to know if liking increased also. Although intake partly reflects liking, it is an indirect measure that is also influenced by other factors. As in other studies, we measured how much the mothers thought the infants liked the food (1, 15, 16, 30). Of course this is also an indirect measurement. Mothers base their score of liking on the infants facial expressions and intake (30) but they might also be influenced by their own preferences with respect to the food (47). **In chapter 3** liking was measured by coding the infants' behaviours and negative facial expressions. This method is more objective, since specific facial expressions and behaviours were coded according to structured rules by an independent and trained rater (48). The results confirm findings elsewhere that together with intake also liking, measured with facial expressions, increased (15, 49).

In the study described in **chapter 3** we measured only facial expressions and behaviour from the sessions in which infants were exposed to green beans, as this was the only vegetable that both the vegetable and the fruit group had been exposed to (although the fruit group was exposed to it only on day 19). Since the results from facial expressions are similar to those of intake alone, one could argue that the data from the facial expressions did not add additional value, especially since coding facial expressions is a very time-consuming activity. However, data from facial expressions might give more information when no differences are found in intake. Several intervention studies on liking of food and beverages report effects on facial expressions, while no effects on intake were found (1, 15, 49). Furthermore, the scale we used gave more information than just facial expressions (48, 50); it also measured the acceptance score, which measures wanting of the food (51). These wanting scores gave information that differed from the liking scores. Like intake and liking, the rate of acceptance for green bean purée increases after exposure to vegetables, but it is not significantly different from wanting scores of green beans purée in the fruit group. This may indicate that liking of vegetables was influenced only by exposure to vegetables and not by exposure to fruit, while the willingness to take a bite (wanting) was the same in both groups. Unfortunately for the children in the fruit group, they got an unpleasant surprise after tasting the spoons with green beans purée, which they showed by their negative facial expressions.

To see if the intervention had an effect in the longer-term, we measured intake of green beans purée and apple purée again in the lab when the children were 12 and 23 months of age (**chapter 4**). To get a better idea of their normal daily intake, we also measured their daily food intake with a 3-day food record. However, results from measurements in the

lab did not support the results of the 3-day food records. Measurements of intake of green beans and apple purée in the lab showed no significant differences between the vegetable and fruit group at 12 and 23 months of age (intakes of the vegetable group were only slightly higher than intakes of the fruit group). On the other hand, the 3-day food records revealed that at 12 months of age, the children in the vegetable group had a considerably higher daily vegetable intake than children in the fruit group. Since the 3-day food records are a good reflection of the actual daily intake of the children, and since this daily intake is what needs to be improved, we consider these results to better represent liking and intake of vegetables than the measured intakes of green beans in the lab. Although intake in the lab is probably less influenced by the parents, the puréed green beans may not have been suitable for the 12 and 23 month olds anymore. The results in the lab did point in the same direction, but the differences between groups were small and the effect was far from significant.

One of the hypotheses of this thesis was that when infants start weaning with vegetables, they develop lower preferences for sweet tastes or for foods containing high levels of sugar. In the preference test with sugar-water solutions described in **chapter 5,** no differences were found between groups. This indicates that the intervention during weaning has not influenced sweet taste preference of the children at 23 months of age. Because at this age the difference in vegetable intake between the vegetable and fruit group was also gone, this result is not surprising. We cannot say whether the intervention had a short-term effect on sweet taste preference, as it was not measured during or right after the intervention and when the children were 12 months of age. However, it is safe to say that the intervention did not influence sweet taste preference on the longer term, since neither the preference for the sweetened water solution nor the daily mono-and disaccharides intake, differed between the groups.

Finally, the results from the review in **chapter 6** support the results from **chapters 2 and 4**. The review concluded that the most effective strategy for promoting vegetable consumption appeared to be repeated exposure to vegetables (9, 10, 16, 18-21, 52). The studies on repeated exposure found effects up to 9 months after the intervention, but no longer follow-ups are described. Exposure to a variety of vegetables is also effective in increasing intake (9, 10, 17). Counselling was found in most of the studies to have some positive effect on vegetable intake, but to a much lesser extent than repeated exposure (the effect on vegetable intake was only a few grams) (53-56). finally, Flavour-flavour and flavour-nutrient learning did not produce additional benefit over repeated exposure in encouraging intake of a plain vegetable (19-21, 57).

This chapter ends with a final note for parents:

What can parents with young infants learn from all of this? “Keep feeding vegetables to your child” might be easier said than done. And what to do when food-neophobia emerges?

The results of this thesis speak for themselves. Repeated exposure to a variety of vegetables at the start of weaning does help. But preaching and practicing are two different things. Future research should focus on how to motivate mothers and how to help them to feed their children in an effective way. Furthermore, not all children are the same. We saw in our study that some children ate everything without even a blink, while others kept refusing everything. In an article based on three studies on learning to eat vegetable performed in the UK, Denmark and France, Caton et al. (22) described that infants could be divided into 4 categories: learners, plate cleaners, non-eaters and others /variable. More research should be done to determine how best to encourage these non-eaters to eat vegetables.

However, parents should keep in mind, that even while infants might show expressions of distaste, it often takes 5 to 10 exposures before the infant accepts the vegetable. Finally, not all vegetables have to be liked. If your child keeps refusing a vegetable even after 10 times, you might focus more on vegetables that your child does eat, as long as there is variety and it includes green vegetables.

7.4 Conclusion

The main conclusion of this research is that weaning with a combination of repeated exposure and a day to day variety of vegetable purées has a larger positive influence on vegetable intake until at least the age of 12 months than does weaning with a variety of fruit purées. To maintain this effect, parents should keep trying to enhance vegetable intake also during the second year of life, since intake at 12 months predicts intake at 23 months. Furthermore, the fruit intake was always higher than the vegetable intake, which shows out that learning to like vegetables needs more attention than learning to like fruits. Since parents may be not aware of the importance of early taste learning, it is important to support parents with information and tools on the most effective way to promote healthy eating in their children.

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Summary

Background

Most children in the Netherlands do not eat the recommended amount of fruit and vegetables. Especially vegetable consumption is far below the recommendations. Low-energy density and the bitter taste of vegetables contribute to this low intake. School-based interventions to promote vegetable intake in children have been shown to have no or minimal effect on children's vegetable intake. On the other hand, most studies on repeated exposure to vegetables in infants, have shown immediate effects of increasing infants' vegetable intake. Although, infants have an inborn aversion to bitter and sour tastes, they accept new foods relatively easily during their first year of life and repeated exposure could help to increase this acceptation.

Aim of this thesis

This thesis describes a longitudinal intervention study, called: 'Mum, can I have Brussels' sprouts again?' It's not something that many children would ask. This study aimed to investigate whether children's vegetable intake and preferences could be increased by starting weaning with only vegetable purées. The chapters in this thesis describe short- and long-term results of this study.

Chapters

Chapter 2 describes an intervention study (n = 101) that investigated the effect of repeated exposure to either vegetable purées or fruit purées on infants' acceptance of vegetable effects of repeated-exposure to vegetables (vegetable group) or fruit (fruit group) during the first 18 days of weaning. This was tested in an intervention study with 101 infants sub-divided in 2 vegetable and 2 fruit groups. One vegetable group received green beans every other day, and the other group artichoke. On the other days, the infants received broccoli or cauliflower purée. The infants in the fruit groups received apple or plums every other day and pear or banana on the days in-between. This study showed that during these 18 days, infants from the vegetable group had significantly increased their vegetable intakes from 24 ± 28 g to 45 ± 44 g after 18 days of fruit exposure. Infants from the fruit groups increased their fruit intake from 46 ± 40 g to 66 ± 42 g. Interestingly, the first green beans intake in the fruit group on day 19, which was directly after the 18 days of exposure to fruit purées, was as low as the first green beans intake in the vegetable groups on day 1. This indicates that repeated exposure to fruit did not influence infants' vegetable intake. On the other hand, the infants who had been repeatedly exposed to green beans as well as those who had been repeatedly exposed to artichoke, showed similar increases in green beans intake.

Chapter 3 describes the analysis of the infants' behavior and facial expressions during the intervention described in chapter 2. Results showed that after repeated exposure to vegetables, the amount of negative facial expressions in reaction to green beans puree had decreased. This indicates that their liking for green beans had increased. In addition they accepted the spoons with purée faster after repeated exposure. Finally, infants from the fruit group, who had been exposed to fruit purées for 18 days, showed more negative facial expressions in response to their first green beans purée than infants from the vegetable groups, who had been repeatedly-exposed to vegetable purées. This indicated that the infants from the vegetable group had developed a higher liking for green beans than the infants from the fruit group.

Chapter 4 describes the long-term effect of the intervention. In two follow-up studies, when the infants were 12 and 23 months of age, vegetable and fruit intake were measured again. In the time between the first intervention and the follow-ups we did not control the children's diets, and parents were free to feed their children whatever they thought was best. At both 12 and 23 months of age, no differences in green beans intake were found between the initial vegetable and fruit group. Both groups had on average low intakes. Daily vegetable intake was also measured using 3-day food diaries. Results showed that at 12 months of age vegetable intake in the vegetable groups was on average 40% higher than in the fruit group. At 23 months of age, no significant difference in daily vegetable intake was found between the groups.

In **chapter 5**, the long-term effect of starting weaning with vegetables or fruits (during the intervention from chapter 2) on the infants' preferences for sweet taste was further investigated. This was measured with intake of sweetened solutions at the follow-up when infants were 23 months of age. The results showed no significant differences between the vegetable and the fruit group. Also the infants daily mono- and disaccharide intake (calculated from the 3-day food diaries) did not differ between the groups, which indicates the intervention had no lasting effect on the preference for sweet.

Chapter 6 gives a review of different strategies and practices used to increase vegetable acceptance that have been studied in children under 3 years of age. The review showed that counselling parents on healthy eating and nutrition has some positive long-term effects on children's vegetable intake, although these effects were small. The review further showed that repeated exposure to vegetables is most studied and also most effective strategy to increase vegetable intake. Exposure to a variation of vegetables was shown to have a positive effect on intake of a new vegetable.

In **chapter 7** the main findings and conclusions are discussed. The main conclusion of this thesis is that weaning with a combination of repeated exposure and a day to day variety of

vegetable purées increases vegetable intake in weaning infants. Starting weaning with vegetable purées leads to higher vegetable intake up until 12 months of age, compared to starting weaning with fruit purées. For longer lasting effects parents might need to keep trying to enhance their children's vegetable intake, also during the 2nd year of life. The results of this thesis indicate that learning to like vegetables needs more attention than learning to like fruits. Since parents may be not aware of the importance of early taste learning, it is important to support parents with information and tools on effective ways to promote vegetable intake in their children.

Samenvatting

(Summary in Dutch)

Achtergrond

De meeste kinderen in Nederland eten onvoldoende groente en fruit. Vooral de groente consumptie is ver onder de aanbevolen hoeveelheid. De lage groente inname is on andere te wijten aan de lage energie dichtheid van groente en de overwegend bittere smaak. Uit onderzoek blijkt dat het moeilijk is om de groente inname bij kinderen te verhogen. Interventies op lagere scholen gericht op de verhoging van groente inname, hebben in het algemeen weinig tot geen effect. Onderzoek naar herhaald aanbieden van groente bij baby's laten echter wel een toename in groente inname zien. Alhoewel kinderen geboren worden met een afkeer voor bittere smaken, staan ze vooral tijdens hun eerste levensjaar nog erg open staan voor nieuwe smaken en helpt herhaaldelijk aanbieden de acceptatie te verhogen. Omdat kinderen fruit makkelijker accepteren, beginnen ouders meestal met fruit als bijvoeding, terwijl starten met groente juist beter zou kunnen zijn voor de acceptatie van groenten op latere leeftijd.

Doel van dit proefschrift

Dit proefschrift beschrijft een longitudinaal interventie onderzoek genaamd: 'Mum, can I have Brussels sprouts again?' ('Mama, mag ik weer spruitjes?'). Het zou mooi zijn als kinderen deze vraag aan hun ouders zouden stellen. Het doel van de studie was om the onderzoeken of bijvoeding met in het begin alleen groente een positieve invloed zou hebben op de acceptatie in inname van groente. De hoofdstukken in dit proefschrift beschrijven de korte en lange termijn resultaten van dit onderzoek.

Hoofdstukken

Hoofdstuk 2 beschrijft de effecten zijn van het herhaaldelijk geven van groente (groente groep) of fruit (fruit groep) tijdens de eerste 18 dagen dat een kind bijvoeding krijgt. Dit is onderzocht in een interventie bij 101 baby's die onderverdeeld waren in 2 groente groepen en 2 fruitgroepen. De ene groente groep kreeg om de dag sperzieboontjes puree en de andere groep artisjok puree. In de tussenliggende dagen kregen ze broccoli of bloemkool puree. De kinderen in de fruit groepen kregen om de dag appel puree of pruimpuree en op de tussenliggende dagen banaan en peer. Hieruit bleek dat de gemiddelde groente inname bij baby's uit de groente groep na 18 dagen was toegenomen van 24 ± 28 g naar 45 ± 44 g. Bij de baby's uit de fruit groep, die dagelijks een fruithapje hadden gehad, was de fruit inname toegenomen van 46 ± 40 g naar 66 ± 42 g. Opvallend was dat vanaf dag 1 de fruit inname in de fruit groep significant hoger was dan de groente inname in de groente groep. Toen de kinderen in de fruit groep op dag 19 voor het eerst een groente hapje kregen, aten zij hier echter maar gemiddeld 24 gram van, wat gelijk was

aan de groente inname van de groente groep op dag 1. Dit wijst erop dat herhaald aanbieden van fruithapjes geen invloed heeft op de groente inname en dat de kinderen dus aan groente moeten worden blootgesteld om de groente inname te verhogen. De soort groente waaraan de kinderen waren blootgesteld lijkt niet uit te maken. Zowel de baby's die om de dag artisjok hadden gehad, als de baby's die om de dag sperzieboontjes hadden gehad, hadden aan het eind van de interventie een hogere inname van sperzieboontjes dan de baby's die alleen fruithapjes hadden gehad.

In **hoofdstuk 3** word beschreven dat analyse van het gedrag en de gezichtsuitdrukkingen van de kinderen, liet zien dat na blootstelling aan groente puree, niet alleen de inname van sperzieboontjes was toegenomen, maar dat de dit ook lekkerder waren gaan vinden. Het aantal negatieve gezichtsuitdrukkingen was afgenomen. Verder was na herhaaldelijk blootstelling aan groente de acceptatie ervan toegenomen. Dit was te zien aan hoe snel ze hun mond openden in reactie op een naderende lepel met puree. Verder werd aangetoond dat de baby's uit de groente groep de sperzieboontjes lekkerder vonden dan de baby's uit de fruit groep, aangezien de laatste meer negatieve gezichtsuitdrukkingen lieten zien.

In **hoofdstuk 4** word het lange termijn effect van de interventie beschreven. In 2 follow-up studies toen de kinderen gemiddeld 12 en 23 maanden oud waren, hebben we de groente inname thuis en in het lab nog eens gemeten. In het lab werd er geen verschil meer gevonden in de inname van sperzieboontjes puree. In beide groepen werd er gemiddeld erg weinig van gegeten. De dagelijkse groente inname, gemeten doormiddel van voedingsdagboekjes die de ouders op 3 dagen in een week bijhielden, was bij 12 maanden in de groentegroep gemiddeld 40% hoger dan in de fruit groep. Bij 23 maanden zagen we geen significant verschil meer.

In **hoofdstuk 5** word het lange-termijn effect van de eerste hapjes op de voorkeur voor zoete smaken beschreven. We verwachtten dat kinderen die de eerste 18 dagen alleen groentehapjes hadden gehad, een lage voorkeur voor zoet zouden ontwikkeling dan kinderen die met fruithapjes waren begonnen. Aangezien groente hapjes minder zoet en juist bitterder zijn dan fruithapjes, verwachtten we dat de groentehapjes de aanboren voorkeur voor zoete smaken minder zou versterken dan fruithapjes. Dit was gemeten bij de kinderen toen ze gemiddeld 23 maanden oud waren, doormiddel van hun inname van suikerwater tijdens een smaaktest. Verder werd aan de hand van de 3-daagse voedingsdagboekjes hun dagelijkse inname van mono- en disacharide (suikers) berekend. Hierin werden geen verschillen tussen de kinderen uit de groente- en fruitgroep gevonden. Dit laat zien dat de interventie toen de kinderen voor het eerst bijvoeding kregen, geen effect had op de latere voorkeur voor zoet.

In **hoofdstuk 6** wordt een overzicht gegeven van verschillende onderzoeken die tot nu toe zijn gedaan naar strategieën die kunnen worden toegepast bij kinderen onder de 3 jaar, om hun groente inname op korte of lange termijn te verhogen. Dit review laat zien dat het herhaald aanbieden van groente de meest effectieve strategie is om groente inname te verhogen. Verder heeft blootstelling aan variatie van verschillende groenten een positief effect op de acceptatie van een nieuwe (onbekende) groente.

In **hoofdstuk 7** worden de belangrijkste bevindingen en conclusies van dit proefschrift besproken. Hieruit word geconcludeerd dat bij het herhaald aanbieden van groente aan baby's, de inname en de waardering hiervoor wordt verhoogd. Verder heeft beginnen met bijvoeding met alleen groente hapjes, een positieve invloed heeft op de voorkeur en inname van groente, in vergelijking met beginnen met fruithapjes. Dit effect houdt stand tot de leeftijd van 12 maanden. Dit effect zou mogelijk verder in stand gehouden kunnen worden als ouders hun kind ook tijdens het 2^{e} levensjaar blijven stimuleren om groente te eten. Verder wijzen de resultaten uit dit proefschrift erop dat het 'leren waarderen' van groente meer aandacht nodig heeft dan het leren waarderen van fruit. Aangezien niet alle ouders op de hoogte zijn van het belang van het vroeg aanleren van smaken, is het belangrijk om ouders op een goede manier te informeren over effectieve manieren om hun kinderen aan groente te laten wennen.

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About the author

Curriculum Vitae
List of publications
Overview of completed training activities

Curriculum Vitae

Coraline Barends was born in Lelystad, April 27th 1981. After completing secondary school at `ISG Arcus' in Lelystad (2000), she started her study in Psychology at Utrecht University and finalised the study with a Masters program in Developmental Psychology in 2005. In 2004 she did an internship at a play-therapy practice, where she learned to conduct psychological assessment of, and play therapy with 4 to 11 year old children. In 2005 she finalised the study with a literature thesis on 'Emerging Adulthood' and a Masters research thesis on: 'Assessing the influence of age on the Big-Five personality traits'.

After traveling through south east Asia for 3 months she returned to work as a human resources planner/coordinator and at a Home Care organization, where she also worked during the last year of her Masters.

In 2007 she enrolled in another Masters program 'Applied Cognitive Psychology' at Utrecht University, and did her internship at TNO Defence and Safety in Soesterberg, at the division of Human Factors. There she wrote a literature review on how to build social intelligence in a robotic interface. For her Masters research thesis she implemented this in a robot (iCat) and investigated if this robot was more capable in motivating children than a neutral version of the robot. During this internship Coraline realised how much she liked to do (practical) research.

In November 2008, Coraline was appointed as a PhD candidate at the Division of Human Nutrition at the Wageningen University. Her research focussed on the development of infants' preferences for vegetables and to increase vegetable intake in young children. During her PhD project, Coraline attended several (international) conferences and courses, was involved in teaching and supervised students. She was also involved in developing a method and training manual to analyse facial expressions in infants.

List of publications

Publications in peer-reviewed journals

Barends C, de Vries J.H., Mojet J, de Graaf C. (2013). Effects of repeated exposure to either vegetables or fruits on infants' vegetable and fruit acceptance at the beginning of weaning. Food quality and preference, 29(2):157 - 65.

Barends, C., de Vries, J. H., Mojet, J., & de Graaf, C. (2014). Effects of starting weaning exclusively with vegetables on vegetable intake at the age of 12 and 23 months. Appetite, 81, 193-199.

Submitted publications

Barends, C., Gatzemeier, Graaf, C., & de Vries, J. H. Infants' liking for green beans, assessed based on facial expression, increased after repeated exposure to vegetables at the start of weaning. *Submitted*

Barends, C., de Vries, J. H., Mojet, J., & de Graaf, C. Does weaning with only vegetables influence the preference for sweet and salty tastes. *Submitted in revised form*

Barends, C., de Vries, J. H., Weenen, H., Warren, J., Hetherington, M.M. & de Graaf, C. Systematic review on strategies to promote vegetable consumption in the first three years of life. *Submitted in revised form*

Nekitsing, C., Madrelle, J., **Barends, C.**, de Graaf, C., Morgan, S., Parrot, H., Weenen, H. & Hetherington, M. M. Application and validation of the Infant Behaviour, Facial Expression Coding System (IBFACS) to assess liking and wanting in infants at thee time of complementary feeding. *Submitted in revised form* .

Hetherington, M. M., Nekitsing, C., Madrelle, J., **Barends, C**., de Graaf, C., Morgan, S., Parrot, H. & Weenen, H. Developing a novel tool to assess liking and wanting in infants at the time of complementary feeding: the Infant Behaviour, Facial Expression Coding System (IBFECS). *Submitted*

Published abstracts

Barends, C., Mojet, J., De Vries, J., & De Graaf, K. (2011). Effects of repeated exposure to either fruits or vegetables during the first 18 days of weaning on infant's fruit and vegetable acceptance. *Appetite*, *57*(2), 553.

Barends, C., De Vries, J., & De Graaf, C. (2012). Effect of repeated exposure to either fruits or vegetables during the first 18 days of weaning on fruit and vegetable intake of infants at 12 months. *Appetite*, *59*(2), 620. *The 36th annual meeting of the British Feeding and Drinking Group, March 29th and 30th 2012, Brighton, UK*

Barends, C., De Vries, J., & De Graaf, C. (2013). The effects of weaning with either fruits or vegetables at the age of 4–6 months on vegetable and fruit acceptance at 12 and 23 months. Appetite, 71, 471. *Special Section: The 37th Annual Meeting of the British Feeding and Drinking Group, April 4th-5th 2013, Loughborough, UK*

Overview of completed training activities

Discipline specific courses and activities

- 6th European conference on sensory and consumer research, 2014 (Copenhagen, Denmark, oral presentation)
- 36th Annual meeting The British Feeding and Drinking Group, 2013 (Loughborough, UK, poster presentation)
- Habeat Workshop, 2013 (Wageningen, The Netherlands, oral presentation)
- ViVa International congress, 2013 (St. Andrews, UK, poster presentation)
- 35th Annual meeting The British Feeding and Drinking Group, 2012 (Brighton, UK, oral presentation)
- 34th Annual meeting The British Feeding and Drinking Group, 2012 (Belfast, UK, oral presentation)
- Opaline meeting 'Understanding the early development of food preference and eating behaviour in children', 2012 (Dijon, France, oral presentation)
- 'Eet Compleet' workshop, 2012 and 2013 (the Netherlands, oral presentations)
- Course 'Food Perception and Food Preference', 2011 (Wageningen, The Netherlands)
- 9th Pangborn Sensory Science Symposium, 2011 (Toronto, Canada, oral presentation)
- Symposium on consciousness, 2011 (Stichting koninklijk paleis Amsterdam, Amsterdam, The Netherlands)
- European conference on facial expression, 2010 (Duisburg, Germany)
- Training 'How to study parent-child interaction using The Observer' 2009 (Wageningen, The Netherlands)
- Course 'Facial Action Unit Coding system workshop', 2009 (Berkeley, USA)
- 8th Pangborn Sensory Science Symposium, 2009 (Florance, Italy)
- Wageningen Nutritional Science Forum, 2009 (Arnhem, The Netherlands)

General courses and activities

- PhD introduction week, 2009 (Bergeijk, The Netherlands)
- PhD Competence assessment, 2009 (Wageningen, The Netherlands)
- Scientific writing, 2009 (Wageningen, The Netherlands)
- Effective behaviour in your professional surroundings, 2009 (Wageningen, The Netherlands)

- Teaching and supervising MSc thesis students, 2010 (Wageningen, The Netherlands)

Optional activities

- PhD study tour to Denmark, Sweden, and Finland, 2009
- Staff seminars, 2008-2013
- Literature group ‘Oldsmobiles’, 2008-2012

Colophon

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