Nutrition and disease in childhood

A window of opportunity?

Ilse I. M. Tromp

NUTRITION AND DISEASE IN CHILDHOOD: A WINDOW OF OPPORTUNITY?

Ilse I.M. Tromp

ACKNOWLEDGEMENTS

The general design of the Generation R Study was made possible by financial support from the Erasmus Medical Center Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), and an unrestricted grant from Europe Container terminals B.V and NutsOhra.

The work presented in this thesis was conducted in the Generation R study Group, in close collaboration with the Departments of Pediatrics and Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands.

ISBN: 978-94-6182-762-3

Cover design, layout & printing: Off Page, Amsterdam

NUTRITION AND DISEASE IN CHILDHOOD:
A WINDOW OF OPPORTUNITY?

Voeding en ziekte bij jonge kinderen:
kansen en mogelijkheden?

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 14 maart 2017 om 15.30 uur

Ilse Isabella Meswin Tromp
geboren te Gouda

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotor:	Prof.dr. H.A. Moll
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Voor mijn ouders

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* Denotes equal contribution within a manuscript

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General introduction

INFANT AND CHILDHOOD NUTRITION

Breastfeeding is the most desirable form of infant feeding [1, 2]. Breastmilk contains nutrients essential for normal growth and development but also immune-related components offering passive immunity in the gastrointestinal and respiratory tracts [1]. In addition, breastfeeding may provide maturation of the immune system in the long run [3]. Exclusive breastfeeding for 6 months is a global public health recommendation [4]. The primary aim of this global recommendation is to reduce infections in developing countries. However, problems of developing countries differ from those in industrialized countries and the evidence for the optimal duration of exclusive breastfeeding from industrialized countries is limited [5]. In industrialized countries, breastfeeding has been associated with a reduced risk of child infections, but also an reduced risk of overweight and obesity, and allergenic diseases [6].

Timely introduction of complementary feeding is of importance for both nutritional and developmental reasons such as for example neurodevelopmental, renal, and gastrointestinal maturation [7]. Complementary feeding comprises all solid and liquid foods other than breast milk or infants formula [7]. Recommendations regarding the introduction of solid foods has changed significantly over the past years [8]. Based on the optimal duration of exclusive breastfeeding, the WHO recommends complementary feeding to be introduced after the age of 6 months while breastfeeding continuous for up to two years of age or beyond [4]. However, recommendations vary between countries. Previous Dutch recommendations stated complementary feeding be introduced after the age of 6 months. Currently, Dutch youth health services recommends complementary feeding be introduced as of 4 months (17 weeks) of age [9]. The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition recommends the introduction of complementary feeding be delayed after 17 weeks of age, but no later than 26 weeks [7]. The American Academy of Pediatrics recommends the introduction of solid foods at 4-6 months of age [10].

After infancy the transition to ordinary foods takes place and dietary patterns emerge. A healthy diet by a moderate consumption of a varied diet including nutrient-dense foods is encouraged [11].

Diet and nutrition are increasingly recognized as modifiable risk factors for chronic disease development including obesity, cardiovascular disease and respiratory diseases [12, 13]. Nutrition is a critical determinant of health at every age but especially during critical stages of early development. However, there is currently still much debate on optimal feeding recommendations in early life for the prevention of disease. Also, not all parents follow early life feeding recommendations. The period of nutritional transition in infancy and diet in early childhood may influence later health and therefore provide a window of opportunity for disease prevention.

ALLERGIC DISEASE

The prevalence of allergic disease, including asthma and eczema, has increased substantially over the past decades and continuous to rise [14-16]. Although it appears that the prevalence of asthma among children has ceased to increase, asthma prevalence remains high [17]. Especially children are bearing the greatest burden of allergic disease [18]. Although genetic factors play an important role in individual susceptibility, it has been suggested that the rise in prevalence could only be explained by modern environmental factors [16]. The influence of environmental factors on the immune system is complex. Environmental factors including air pollution, dietary factors and behavioral changes associated with a Western lifestyle have been proposed as risk factor for allergic disease [19, 20].

The period of transition from breastmilk or formula feeding to the introduction of solid foods has been suggested to influence the development of allergic disease [21]. Regular and early exposure to food allergens during a critical period of development seems to promote the development of persistent oral tolerance [21, 22]. Recently, two randomized controlled trials in the United Kingdom examined the role of early introduction of allergenic food in the development of allergic disease [23, 24]. Both studies found early introduction of allergenic food to prevent food allergies in children. The timing of the window for the prevention of allergies is still not clear. Infant feeding recommendations for allergy prevention have changed significantly over the past decade [8]. In 2008, on the basis of available data, the ESPGHAN Committee on Nutrition recommended that complementary foods should not be introduced before 17 weeks and that foods should be added 1 at a time. There was no convincing evidence that delaying or avoiding the introduction of allergenic foods prevents the development of allergy [7]. However, there is still much debate on the timing of introduction of solid foods and allergy development [25].

After infancy a varied diet is recommended to insure sufficient intake of nutrients. However, dietary intakes of alpha-linolenic acid (ALA), iron and vitamin D are low in infants and young children in Europe [26]. Iron deficiency and vitamin D deficiency are the most common micronutrient deficiencies in young children, with a prevalence around 12% and 23% respectively in Western Europe [27]. Sun exposure is the main source of vitamin D [28, 29]. Lifestyle changes leading to decreased exposure to sunlight predispose to vitamin D deficiency. There are few dietary sources that naturally contain vitamin D including oily fish, egg yolk and nuts and some foods such as margarines and fruit juices are fortified with vitamin D [28, 29]. Vitamin D deficiency in childhood has been linked to adverse health consequences including rickets and growth failure [28, 29]. The American Academy of Pediatrics (AAP) recommends that all infants, children, and adolescents receive a minimum of 400 International Units (IU) of vitamin D daily through diet or supplementation to prevent rickets and vitamin D deficiency [30]. In the Netherlands it is recommended that all children till the age of 4 years receive 400 IU of vitamin D daily [31]. There is epidemiologic evidence that vitamin D deficiency may increase the risk of common chronic disease, including autoimmune diseases, infectious diseases, and cardiovascular disease [28, 29]. In children,

higher vitamin D concentrations have been linked to a reduced risk of allergic disease. However, a role of vitamin D in the prevention of allergic disease in children remains unclear.

Instead of studying the effect of specific nutrients the effect of overall diet might give more insight on the combination of foods. Children consume various foods with complex combinations of nutrients that may be interactive or synergistic. Dietary patterns in childhood have been linked to childhood obesity and the development of respiratory disease [12, 32]. It has been suggested that a more healthy diet in childhood, like a traditional Mediterranean diet, may reduce the risk of asthma in children [33] whereas a more unhealthy diet may increase the risk of respiratory symptoms [34, 35]. However, studies assessing the effect of overall diet, including a Western diet, in early childhood on respiratory health are few.

CELIAC DISEASE

Celiac disease is an autoimmune disorder resulting from both environmental and genetic factors. Permanent intolerance to gluten leads to chronic small enteropathy in genetically predisposed individuals. The prevalence of celiac disease has been estimated to approximate 1% [36, 37]. Celiac disease commonly appears in early childhood with classical symptoms including diarrhea, abdominal distension, and failure to thrive. However, individuals may also present with extra-intestinal symptoms or no symptoms. Most cases are undiagnosed [38]. Infant nutrition including breastfeeding and the timing of introduction of gluten have been suggested to play a role in triggering the onset of celiac disease. It has been suggested that a longer duration of breastfeeding and gradual introduction of gluten while breastfeeding may reduce the risk of developing celiac disease [2, 39]. Gradual introduction has been suggested to lead to the development of oral tolerance, also the response of the immune system to gluten may be modified by breastfeeding [40, 41]. In addition, delaying the introduction of gluten might increase the risk [42, 43]. However, results were inconsistent [40, 42, 44-48]. Since 2008 the ESPGHAN Committee on Nutrition recommended to avoid early (<4 months) and late (≥7 months) introduction of gluten and to introduce gluten gradually while the infant is still being breastfed to reduce the risk of celiac disease [7]. But, clear evidence was lacking. A more recent randomized controlled dietary intervention reported early (16-24 weeks) introduction of gluten not to reduce the risk of celiac disease. In addition, breastfeeding at the time of gluten introduction did not reduce the risk of celiac disease [49]. In the Netherlands youth health services recommends gluten to be introduced from the age of 4 months in small amounts for the prevention of celiac disease [50]. Studying whether infant feeding practices can reduce the risk of celiac disease might give new insight into the mechanisms underlying the development of this disease.

INFECTIOUS DISEASE

Infectious diseases are a leading cause of doctor visits and hospital admission in young children with most common reason being respiratory infection [51, 52]. Upper respiratory infections most often account for doctor consultations whereas lower respiratory infections

account for hospitalization [51, 52]. Most respiratory infections occur in infancy with a peak between 1 and 2 years of age [53]. Risk factors associated with respiratory infections in childhood are passive smoking [54], daycare attendance [55, 56], and vitamin D deficiency [57]. Positive health effects of breastfeeding during infancy have been reported, especially for infectious diseases [5]. Both the duration of breastfeeding as well as breastfeeding exclusivity have been found to be associated with the development of respiratory tract infections in infancy [2, 6, 58]. It has been suggested that breastfeeding may also have long-term health effects [3]. Breastfeeding may stimulate the immune system of the offspring with beneficial long-term effects [3]. Therefore, breastfeeding might protect against autoimmune diseases later in life, such as type 1 diabetes and inflammatory bowel disease, but may also have a positive effect on later cognitive function [2, 5]. Evidence for long-term effects of breastfeeding on the development of respiratory tract infections is limited [2, 5]. Studying the long-term health effects of breastfeeding might give more insight in the optimal duration of breast feeding for the long-term protection against infections after the termination of breastfeeding.

AIMS OF THIS STUDY

In this thesis we aimed to study the following aspects on early life nutrition and the development of childhood disease:

- Which maternal and infant factors are associated with the timing of introduction of complementary feeding?
- Is the timing of introduction of complementary feeding associated with diseases in childhood?
- Has breastfeeding a long-term health effect on respiratory tract infections in childhood?
- Are nutritional determinants in childhood of influence on the development of respiratory and allergic disease?

STUDY DESIGN

The Generation R Study

The studies presented in this thesis were conducted within the Generation R study. The Generation R study is a population based prospective cohort study from fetal life until young adulthood which is designed to identify early environmental and genetic determinants of growth, development and health during the life course. Participant eligible for this study were pregnant women with a delivery date from April 2002 through January 2006 living in Rotterdam, the Netherlands. The cohort included a total of 9778 mothers with their children enrolled throughout pregnancy. In each trimester during pregnancy assessments were conducted which included physical examinations, ultrasound measurements, and self-administered measurements. Information on health of the children was obtained by parent-reported questionnaires collected from birth until the child's age of 6 years and routine visits to the child health care centers. From 2003 onwards, data on nutritional intake of the child

was implemented in the study. At the age of 14 months a total of 5088 mothers received a food frequency questionnaire (FFQ) for their child. At the age of 6 years, all children were invited to the dedicated research facility in the Erasmus MC, Sophia children's hospital, to participate in different health outcome measurements. Venous serum samples were taken during the visit to the research center and serum 25-hydroxyvitamin D (25(OH) vitamin D) concentration and anti-tissue transglutaminase (anti-tTG) concentration was assessed in more than 4000 children.

OUTLINE OF THIS THESIS

Part I examines the timing of introduction of complementary feeding as possible risk factor for disease in childhood. Following the general introduction, **chapter 2** of this thesis describes the factors associated with the timing of introduction of complementary feeding. **Chapter 3** focuses on the timing of introduction of allergenic foods on the development of wheezing and eczema in childhood. In **chapter 4**, the influence of breastfeeding and the timing of introduction of gluten on the development of celiac disease autoimmunity is reported. **Part II** focuses on nutritional determinants of respiratory health. In **chapter 5,** the influence of breastfeeding on the development of respiratory tract infections after infancy is described. Dietary patterns and the development of respiratory symptoms are discussed in **chapter 6**. Further, in **chapter 7**, the influence of vitamin D status in children on the development of asthma-related outcomes and eczema is reported. Finally, **chapter 8** of this thesis provides an overall discussion on the main findings in the context of other studies. Also implications and directions for future research will be discussed. An overview of the studies described in this thesis is shown in Table 1.

Table 1: Overview of studies presented in this thesis

Chapter	Study group	n	Study design	Determinant	Outcome	Age
Part I The timing of introduction of complementary feeding						
2	Generation R	3561	Cross-sectional		Timing of introduction of complementary feeding	6 & 12 months
3	Generation R	6905	Longitudinal	Timing of introduction of allergenic foods	Wheezing and eczema	2-4 years
4	Generation R	1679	Cross-sectional	Duration of breastfeeding and timing of introduction of gluten	Anti-tissue transglutaminase antibody concentration	6 years
Part II Nutrition and respiratory and allergic disease						
5	Generation R	5322	Longitudinal	Breastfeeding	Upper and lower respiratory tract infections	2-4 years
6	Generation R (Dutch only)	2173	Longitudinal	Dietary patterns	Asthma-related symptoms and respiratory tract infections	2-4 years
7	Generation R	3815	Cross-sectional	25-Hydroxyvitamin D concentration	Asthma-related outcomes and eczema	6 years

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Part I

The timing of introduction of complementary feeding

2

Factors associated with the timing of introduction of complementary feeding

IIM Tromp, S Briede´, JC Kiefte-de Jong, CM Renders, VWV Jaddoe, OH Franco, A Hofman, H Raat and HA Moll.

Eur J Clin Nutr. 2013;67(6):625-30

ABSTRACT

Background: Many parents do not follow recommendations for the timing of introduction of complementary feeding. The aim of this study was to identify determinants associated with the timing of introduction of complementary feeding in a multiethnic birth cohort.

Methods: Subjects were 3561 mothers and infants participating in a prospective cohort study. The timing of introduction of complementary feeding and maternal and infant characteristics were obtained by parent-derived questionnaires. Regression analyses were performed to identify determinants for the timing of introduction of complementary feeding (<3, 3–6 and ≥6 months).

Results: In total, 62% of infants were introduced to complementary feeding before the age of 6 months. Determinants for very early (<3 months) introduction were being a single parent and infant day care attendance. Determinants for early (3–6 months) introduction were young maternal age, multiple parities, no infant family history of asthma, atopy and no infant history of allergy to cow's milk. Determinants for both very early and early introduction were low educational level and not fully breastfeeding for 4 months. Maternal educational level was only significantly associated with the timing of introduction in mothers of Western origin.

Conclusions: This study confirmed determinants for the timing of introduction of complementary feeding that have been identified by previous studies, which may be appropriate targets for education and guidance. Moreover, mothers whose infants attend day care and have a family history of asthma, atopy or allergy to cow's milk may need guidance to follow infant feeding recommendations.

INTRODUCTION

The first year of life includes many transitions in food consumption.1 Appropriate nutrition during infancy is essential for adequate growth, development and health [2]. The World Health Organization (WHO) recommends breastfeeding for the first 6 months of life with the introduction of complementary feeding thereafter [2–4]. The primary aim of the current WHO infant feeding recommendations is the reduction of infections in developing countries [4]. Advisory committees in developed countries, including the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the American Association of Pediatrics (APP) recommend delaying the introduction of complementary feeding, preferably until the age of 6 months and at least until 4 months of age [5–7]. The ESPGHAN also recommends the introduction of complementary feeding no later than 26 weeks [5, 6]. Many studies have focused on determining the optimal time to introduce complementary feeding. Very early introduction of complementary feeding has been suggested to be associated with adverse health outcomes like obesity and celiac disease later in life [8, 9]. However, late introduction of complementary feeding is also not recommended as exclusive breastfeeding will not be sufficient to meet the infant's nutritional needs after the age of 6 months, as well as for developmental reasons [5]. Many studies focused on determining factors associated with the initiation, duration and cessation of breastfeeding [10–12]. As both very early and late introduction of complementary feeding is not preferred and national studies indicate that many parents do not follow recommendations [7], identifying factors associated with the timing of introduction of complementary feeding is of importance. Several determinants associated with the timing of introduction of complementary feeding have been identified in previous studies in different countries [3, 4, 6, 13]. However, infant feeding practices have been found to differ between countries despite similar recommendations for the introduction of complementary feeding [6]. Differences in feeding practices have also been reported among subgroups of populations [7, 14]. Determining the factors associated with the timing of introduction of complementary feeding in a developed country with a multicultural population could give health care workers more insight and assist in identifying potential groups or individuals who are least likely to follow optimal feeding recommendations.

Therefore, the aim of this study is to determine maternal and infant characteristics associated with the timing of introduction of complementary feeding in infancy in a multiethnic birth cohort.

MATERIALS AND METHODS

Participants and study design

This study was embedded in the Generation R Study, a multiethnic population-based prospective birth cohort study from fetal life until young adulthood [15]. The Generation R Study included 9778 pregnant woman living in Rotterdam, the Netherlands, with a delivery date from April 2002 until January 2006. Informed consent for postnatal follow-up was provided by 7893 participants. At the child's age of 14 months, 5088 mothers received

a questionnaire about food consumption and the timing of introduction of complementary feeding (Supplementary Figure 2.1). Comprehensive data collection of food consumption was implemented in the study from 2003 onwards. The study was approved by the medical ethical review board of the Erasmus Medical Center, Rotterdam, the Netherlands.

Introduction of complementary feeding

At the child's age of 14 (±2) months, parents were asked to complete a questionnaire about food consumption and the timing of introduction of complementary feeding. The food products used in the questionnaire were selected based on food items frequently consumed among Dutch toddlers, as described in a previous survey [16]. Parents were asked at which age the following products were first introduced into the infant's diet: (full- or semi-skimmed) dairy products (with the exception of breastfeeding and formula-feeding), porridge, bread, biscuits, crackers, baby cookies, pasta, (breaded) meat products, vegetarian meat substitutes, fish, shellfish, vegetables, fruit, peanuts and nuts. The timing of introduction of complementary feeding was categorized as (I) before the age of 3 months, (II) at the age of 3–6 months and (III) at the age of 6 months and after. The categories are referred to as (I) very early, (II) early and (III) timely introduction of complementary feeding. The introduction of complementary feeding at the age of 6 months and after was defined as the reference group in accordance with the WHO recommendation which was currently followed during this study. Questionnaire response rate was 72% (n= 3643) and information on the timing of introduction of complementary feeding was available for 3603 infants.

Maternal and infants characteristics

Several maternal and infant characteristics were assessed as potential associated factors for the timing of introduction of complementary feeding. Data on maternal age, infant birth weight and gender were available from obstetric records assessed in midwife practices and hospital registries [15]. Prenatal questionnaires included information on maternal and infant ethnicity, maternal educational level, household income per month, marital status, maternal smoking and alcohol consumption during pregnancy, folic acid intake during pregnancy, multiple parities, family history of asthma or atopy and maternal body mass index at intake (questionnaire response rate: 91, 80 and 77% for early, mid and late pregnancy, respectively). Maternal educational level was defined according to the classification of Statistics Netherlands and categorized as follows: low (no education, primary school or <3 years of secondary school), mid (>3 years of secondary school, higher vocational training or bachelor's degree) and high (academic education) [17]. Ethnicity of the mother and infant was also categorized according to the classification of Statistics Netherlands into Western (Dutch, European, American-Western, Asian- Western, Oceanian and Indonesian) and non-Western (American non-Western, Asian non-Western, African, Turkish, Cape Verdean, Moroccan, Dutch Antillean and Surinamese) [18]. Net household income per month (≤€2200, >€2200), marital status (no partner, married/living together), family history of asthma or atopy (no, yes), maternal

smoking (never smoked during pregnancy, smoked during pregnancy), maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption during pregnancy), multiple parities (no, yes) and folic acid intake (no intake, started in first 10 weeks of pregnancy, started periconceptional) were defined into categories.

Data on breastfeeding were collected by a combination of delivery reports and postnatal questionnaires at the age of 2, 6 and 12 months (response rate: 82, 73 and 72%, respectively). Delivery reports included information on whether the infant was ever breastfed. Postnatal questionnaires included information on breastfeeding at the age of 2, 6 and 12 months and the age of breastfeeding cessation. Consequently, breastfeeding was categorized into the following groups: never breastfed, partial breastfeeding for at least 4 months and full breastfeeding for at least 4 months. Partial breastfeeding was defined as receiving both breast milk and infant formula and/or complementary feeding. Full breastfeeding was defined as receiving breastfeeding without any other infant formula, milk or complementary feeding. In addition, at the infant's age of 6 and 12 months, parents were asked by the questionnaire whether their child had attended a doctor for allergy to cow's milk in the first year of life (no, yes). At the age of 12 months, the questionnaire also included information on day care attendance. Day care attendance in the first year of life was defined as never, <16, 16–32 and ≥32 h per week.

Population for analyses

Only mothers and infants who returned the questionnaire were included in the analyses (n= 3643). Non-response analysis revealed that mothers who filled in the questionnaire, relative to non-responders, had a higher educational level (33 vs 21%), were of Western ethnicity (78 vs 41%), were more often married or had a partner (91 vs 81%), had a lower BMI (24.4 vs 26.0 kg/m2), more often drank alcohol during pregnancy (60 vs 43%) and took adequate folic acid supplementation during pregnancy (31 vs 12%) (Supplementary Table 2.1). To prevent clustering, twins were excluded from the analyses (n= 82). Therefore, data of 3561 mother and infants were available for statistical analyses (Supplementary Figure 2.1).

Statistical methods

To determine the factors associated with the timing of introduction of complementary feeding, two regression models were built. The first model included the factors associated with very early (<3 months) introduction of complementary feeding relative to timely (≥6 months) introduction of complementary feeding. The second model reflected the factors associated with early (3–6 months) introduction of complementary feeding relative to timely (≥6 months) introduction of complementary feeding.

Univariate logistic regression analyses were performed for the timing of introduction of complementary feeding and various potential associated factors as independent variables. All independent variables with a P-value <0.20 in the univariate analyses were selected

as candidate determinants for the multivariate analyses. Subsequently, multiple logistic regression analyses with a backward stepwise elimination procedure was performed with P <0.10 as the end-point, keeping only the strongest determinants. The Hosmer–Lemeshow test was applied to test the goodness of fit of the regression model.

To diminish potential bias associated with missing data, a multiple imputation procedure was used [19]. The multiple imputation procedure predicts values for missing data on the basis of the associations between missing data and other variables. Missing values of maternal and infant characteristics (~2.0–45.9%) were multiple imputed (n= 5 imputed datasets). The results of the five imputed analyses were pooled and reported as odds ratios (OR) and 95% confidence interval (CI) in this paper. A P-value <0.05 was considered as statistically significant. Statistical analyses were conducted using Statistical Package for Statistical Science Software version 17.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Study population

Maternal and infant characteristics of the study population are presented in Table 2.1 and Supplementary Table 2.2. Out of 3561 infants, 6% was introduced to complementary feeding very early (<3 months), 56% early (3–6 months) and 38% timely (≥6 months).

Factors associated with the timing of introduction of complementary feeding

Descriptive information on the determinants of interest and their associations with the timing of introduction of complementary feeding are presented in Table 2.2 and Supplementary Table 2.3.

Introduction before the age of 3 months. Relative to timely introduction, very early introduction of complementary feeding was inversely associated with higher maternal educational level and full breastfeeding for 4 months, and associated with a single-parent household and >32h of day care attendance per week.

Introduction at 3–6 months. Relative to timely introduction, early introduction of complementary feeding was inversely associated with maternal age, higher maternal educational level, family history of asthma or atopy, allergy to cow's milk and full breastfeeding for 4 months and associated with multiple parities.

Ethnicity and the timing of introduction of complementary feeding

A significant interaction was found between maternal educational level and ethnicity (P= 0.01 and P= 0.06 for mid- and high educational levels, respectively). Only in mothers of Western origin, higher maternal educational level was inversely associated with very early (<3 months) (OR: 0.36; 95% CI: 0.21–0.61 and OR: 0.18; 95% CI: 0.10–0.33) and early (3–6 months) introduction (OR: 0.52; 95% CI: 0.38–0.71 and OR: 0.41; 95% CI: 0.30–0.56) of complementary feeding (Table 2.3).

Table 2.1. Characteristics of the study population according to the timing of introduction of complementary feeding

	<3 months			3-6 months			≥6 months	
	Very early			Early			Timely	
n=3561	**n=217**	**%=6**	**P-value**	**n=2009**	**%=56**	**P-value**	**n=1335**	**%=38**
Maternal characteristics								
Age *Mean (SD)*	30.4	5.7	<0.01	31.2	5	<0.01	31.8	4.4
Ethnicity								
Western	136	5		1534	56		1088	39
Non-western	81	10	<0.01	475	59	<0.01	247	31
Educational level								
Low	55	10		346	63		152	27
Mid	121	7	<0.01	1060	57	<0.01	676	36
High	41	4	<0.01	603	52	<0.01	507	44
Household income per month								
≤2200 euro	79	8		557	58		332	34
>2200 euro	138	5	<0.01	1452	56	0.07	1003	39
Marital status								
Married/living together	173	5		1822	56		1250	39
No partner	44	14	<0.01	187	59	0.01	85	27
Maternal BMI *Mean (SD)*	24.6	4.5	0.30	24.6	4.3	0.04	24.2	4.1
Multiple parities								
No	125	6		1163	55		812	39
Yes	92	6	0.04	846	58	0.08	523	36
Smoking during pregnancy								
Never	151	5		1539	55		1100	40
Smoked during pregnancy	66	9	<0.01	470	61	<0.01	235	30
Alcohol use during pregnancy								
Never	108	8		794	56		510	36
Drank alcohol during pregnancy	109	5	0.05	1215	57	0.55	825	38
Folic acid intake								
Never	69	12		339	57		187	31
Start in first 10 weeks	62	5	0.03	651	58	0.51	412	37
Start periconceptional	86	5	<0.01	1019	55	0.10	736	40
Infant characteristics								
Gender								
Male	116	7		978	56		653	37
Female	101	5	0.21	1031	57	0.90	682	38
Ethnicity								
Western	133	5		1520	56		1081	39
Non-western	84	10	<0.01	489	59	<0.01	254	31
Birth weight *z*-score *Mean (SD)*	-0.25	1.00	<0.01	0.02	1.01	0.41	-0.01	1.06

Table 2.1. (continued)

	<3 months			3-6 months			≥6 months	
	Very early			Early			Timely	
n=3561	n=217	%=6	P-value	n=2009	%=56	P-value	n=1335	%=38
Breastfeeding								
Never	27	6		260	60		144	34
Non- fully for 4 months	154	7	0.60	1281	61	0.75	665	32
Fully for 4 months	36	4	0.02	468	45	<0.01	526	51
Family history of asthma or atopy								
Yes	99	6		940	53		724	41
No	118	7	0.03	1069	59	<0.01	611	34
Cow's milk allergy								
Yes	10	4		108	48		108	48
No	207	6	0.11	1901	57	<0.01	1227	37
Day care attendance								
Never	74	8		535	56		347	36
<16 hours	40	4	0.04	555	57	0.63	380	39
16-32 hours	81	5	0.07	845	57	0.75	566	38
≥32 hours	22	16	0.09	74	54	0.67	42	30

Abbreviations: BMI, body mass index; s.d, standard deviation. P-values were assessed by using univariate logistic regression analyses. % represents proportion by row.

DISCUSSION

Out of 3562 infants, 62% was introduced to complementary feeding before the age of 6 months. Infants with a single parent and who attended day care were more often introduced to complementary feeding very early (<3 months). Mothers who introduced complementary feeding early (3–6 months) were more often younger, had multiple parities and had an infant without a family history of asthma, atopy or a history of allergy to cow's milk. Mothers with a low educational level and those who did not fully breastfeed for 4 months were more likely to introduce complementary feeding very early and early. However, the socioeconomic gradient in complementary feeding practices was only present in mothers of Western origin.

Comparison with other studies is difficult as most studies defined early introduction of complementary feeding to be before the age of 4–6 months in accordance with the recommendations of the ESPGHAN and the APP. This study defined early introduction of complementary feeding to be before the age of 6 months according to the recommendation of the WHO which was currently followed in the Netherlands during the study period. The main determinants found in this study are confirmatory. Previous studies also found low maternal age, low maternal educational level, not fully breastfeeding and being a single parent to be determinants associated with early introduction of complementary feeding [3, 4, 6, 13]. In addition, this study found infants with a family history of asthma, atopy or a history of allergy to cow's milk to be more likely to be introduced to complementary

Table 2.2. Determinants for the timing of introduction of complementary feeding

	Univariate model (<3 vs. ≥6 months)	Multiple regression model (<3 vs. ≥6 months)	Univariate model (3-6 vs. ≥6 months)	Multiple regression model (3-6 vs. ≥6 months)
Maternal characteristics				
Age	0.94* (0.91-0.97)	-	0.97* (0.96-0.99)	0.98* (0.96-1.00)
Ethnicity				
Non-western vs. Western	2.60* (1.86-3.64)	-	1.36* (1.14-1.62)	-
Educational level				
Mid vs. Low	0.50* (0.33-0.74)	0.90 (0.56-1.44)	0.69* (0.55-0.86)	0.86 (0.68-1.09)
High vs. Low	0.23* (0.14-0.37)	0.49* (0.28-0.89)	0.52* (0.42-0.66)	0.74* (0.57-0.96)
Household income per month				
>2200 euro vs. ≤2200 euro	0.58* (0.42-0.80)	-	0.86 (0.74-1.01)	-
Marital status				
No partner vs. Married/living together	3.78* (2.49-5.73)	2.22* (1.39-3.56)	1.50* (1.13-2.00)	-
Maternal BMI	1.02 (0.98-1.06)	-	1.02* (1.00-1.04)	-
Multiple parities	1.19* (1.01-1.41)	-	1.08 (0.99-1.18)	1.23* (1.05-1.43)
Smoking during pregnancy				
Smoking during pregnancy vs. never	2.03* (1.32-3.11)	1.54 (0.98-2.42)	1.43* (1.19-2.71)	1.22 (1.00-1.50)
Alcohol use during pregnancy				
Drank alcohol during pregnancy vs. never	0.62* (0.40-0.96)	-	0.95 (0.79-1.13)	-
Folic acid intake				
Start in first 10 weeks vs. never	0.41* (0.21-0.80)	0.51 (0.25-1.02)	0.87 (0.59-1.29)	-
Start periconceptional vs. never	0.32* (0.18-0.55)	0.55 (0.29-1.04)	0.77 (0.57-1.03)	-
Infant characteristics				
Gender				
Female vs. Male	0.83 (0.61-1.12)	-	1.01 (0.88-1.16)	-
Ethnicity				
Non-western vs. Western	2.69* (1.87-3.87)	-	1.37* (1.15-1.63)	-
Birth weight (*z*-score)	0.80* (0.70-0.92)	-	1.03 (0.96-1.10)	-

Table 2.2. (continued)

	Univariate model (<3 vs. ≥6 months)	Multiple regression model (<3 vs. ≥6 months)	Univariate model (3-6 vs. ≥6 months)	Multiple regression model (3-6 vs. ≥6 months)
Breastfeeding				
Non-fully for 4 months vs. never	1.19 (0.63-2.25)	1.38 (0.81-2.35)	1.06 (0.77-1.45)	1.11 (0.78-1.58)
Fully for 4 months vs. never	0.35* (0.16-0.78)	0.48* (0.25-0.92)	0.49* (0.33-0.71)	0.53* (0.34-0.83)
Family history of asthma or atopy				
Yes vs. No	0.71* (0.52-0.96)	-	0.74* (0.64-0.85)	0.80* (0.69-0.93)
Cow's milk allergy				
Yes vs. No	0.57 (0.29-1.13)	-	0.65* (0.48-0.87)	0.63* (0.46-0.85)
Day care attendance				
<16 hours vs. never	0.49* (0.26-0.92)	0.63 (0.33-1.22)	0.95 (0.76-1.19)	-
16-32 hours vs. never	0.68 (0.45-1.02)	1.00 (0.65-1.53)	0.97 (0.79-1.18)	-
≥32 hours vs. never	2.43 (0.94-6.27)	2.77* (1.22-6.32)	1.12 (0.66-1.92)	-
Hosmer-Lemeshow test	-	χ^2 5.15 (*P*=0.72)	-	χ^2 11.34 (*P*=0.33)

Abbreviations: BMI, Body Mass Index; CI, confidence interval; OR, odds ratio for the introduction of complementary feeding relative to at 6 months and after per group or per unit of continuous predictor. X^2 for the goodness of fit of the model Values are represented as OR (95% CI) *signifies P-value <0.05.

Table 2.3. Ethnicity and the timing of introduction of complementary feeding

	Multiple regression model (<3 vs. ≥6 months)		Multiple regression model (3-6 vs. ≥6 months)	
	OR (95% CI)		OR (95% CI)	
	Western Ethnicity	Non-Western Ethnicity	Western Ethnicity	Non-Western Ethnicity
Maternal educational Level				
Mid vs. Low	0.36* (0.21 – 0.61)	1.01 (0.55 – 1.85)	0.52* (0.38 – 0.71)	0.98 (0.69 – 1.38)
High vs. Low	0.18* (0.10 – 0.33)	0.59 (0.21 – 1.64)	0.41* (0.30 – 0.56)	0.86 (0.51 – 1.45)

Abbreviations: CI, confidence interval; OR, odds ratio for the introduction of complementary feeding relative to at 6 months and after. Values are represented as OR (95% CI) *signifies P-value <0.05. $P_{interaction}$: 0.01 and 0.06 for mid- and high educational level respectively for comparison between <3 and ≥6 months and $P_{interaction}$: 0.01 and 0.06 for mid- and high educational level respectively for comparison 3–6 and ≥6 months.

feeding after the age of 6 months. The WHO also recommends delaying the introduction of complementary feeding after the age of 6 months for the prevention of asthma and allergic disease [20]. However, recent evidence lends little support for delaying the introduction of complementary feeding in order to prevent allergic disease.8 Mothers whose infants have an increased risk of allergic disease may be more conscious and aware of the introduction of complementary feeding and therefore less likely to introduce complementary feeding early or more likely to delay the introduction of complementary feeding for too long.

This study also found day care attendance for more than 32h a week to be a determinant for very early (<3 months) introduction of complementary feeding independent of socioeconomic status. However, in agreement with previous studies, this study did not find day care attendance to be a determinant for early introduction (3–6 months) of complementary feeding [3]. As confirmed by previous studies, this study did not find maternal ethnicity to be an independent determinant for the timing of introduction of complementary feeding [4, 6, 13]. However, it has been suggested that the socioeconomic gradient in complementary feeding practices may vary by maternal ethnic background [7, 14]. In addition, this study found that educational level was only associated with the timing of complementary feeding in mothers of Western origin.

The findings of this study may have several implications. The maternal and child determinants can assist health care workers and dieticians in identifying groups or individuals who are less likely to comply with infant feeding recommendations. Most determinants were confirmed in other studies as well and may be appropriate targets for education and guidance. Additionally, mothers whose infants attend day care and have a family history of asthma, atopy or a history of allergy to cow's milk may introduce complementary feeding differently. Mothers of these children may need additional guidance to follow infant feeding recommendations.

An important strength of this study was the large sample size and the multiethnic population. In addition, this study was able to examine a large number of potential factors

associated with the timing of introduction of complementary feeding. Another important strength of this study was that it identified determinants for both very early (<3 months) and early introduction (3–6 months) of complementary feeding.

A limitation of this study was that it could not specifically identify determinants for the introduction of complementary feeding using the recommendations of other advisory committees (that is, 16 weeks) [5–7]. However, this study identified determinants associated with the introduction of complementary feeding before the age of 6 months as recommended by the WHO and preferred by most advisory committees. Another limitation of this study was the retrospective character of the self-reported questionnaire on the timing of introduction of complementary feeding. Parents were asked to complete the questionnaire at the child's age of 14 (±2) months, about 8–11 months after the introduction of complementary feeding. Therefore, maternal recall bias cannot be excluded. However, this would have only influenced our results if, for example, mothers with a low socioeconomic background were more likely to misclassify having introduced complementary feeding after the age of 6 months instead of before the age of 6 months.

CONCLUSION

This study identified important maternal and infant determinants for the timing of introduction of complementary feeding. The determinants should assist health professionals in identifying groups and individuals who are least likely to follow optimal feeding recommendations. This study confirmed determinants that have been identified by previous studies, which may be appropriate targets for education and guidance. Moreover, mothers whose infants attend day care and who have a family history of asthma, atopy or a history of allergy to cow's milk may need guidance to follow infant feeding recommendations.

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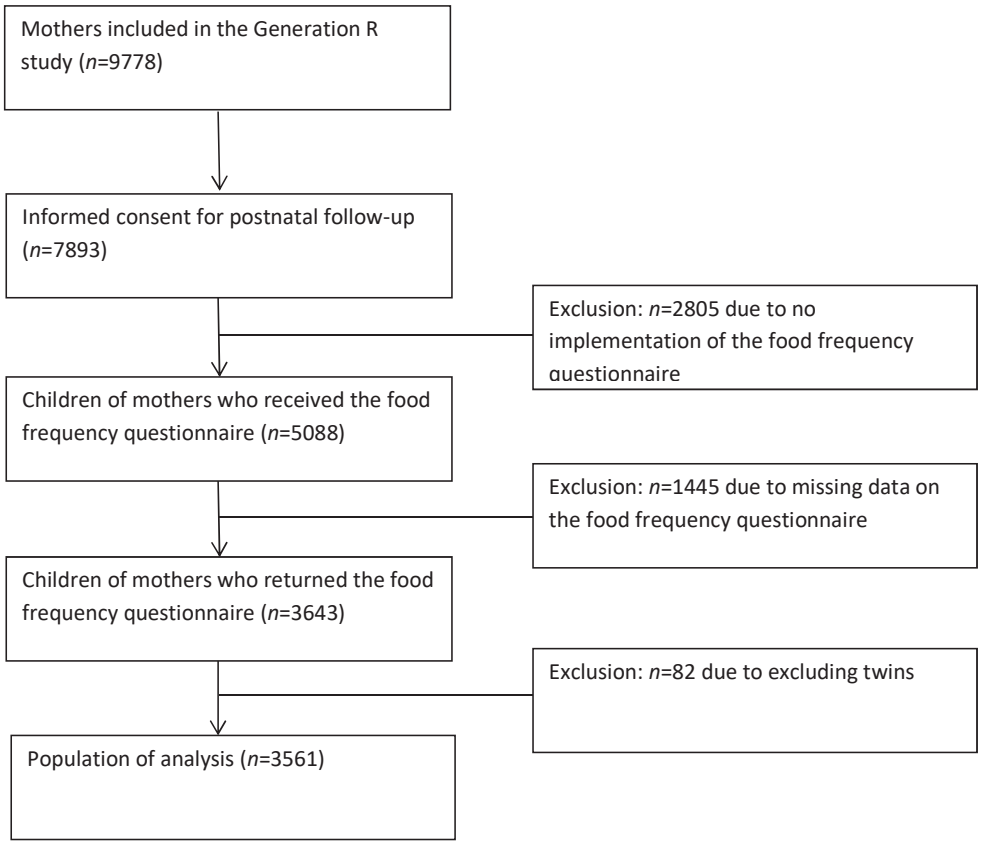
SUPPLEMENT CHAPTER 2



Supplementary Figure 2.1. Flow chart of the participants within the Generation R Study

Supplementary Table 2.1. Non-response analysis (n=3561)

	Responders	Non-responders	P-value
Maternal characteristics			
Age *Mean (SD)*	31.4 (4.6)	30.1 (6.1)	0.08
Ethnicity (%)			
Western	78	41	<0.01
Non-western	22	59	
Educational level (%)			
Low	15	37	<0.01
Mid	52	42	
High	33	21	
Household income per month (%)			
≤2200 euro	27	28	0.96
>2200 euro	73	72	
Marital status (%)			
Married/living together	91	81	0.02
No partner	9	19	
Maternal BMI *Mean (SD)*	24.4 (4.24)	26.0 (3.9)	0.03
Multiple parities (%)			
No	59	53	0.05
Yes	41	47	
Smoking during pregnancy (%)			
Never	79	80	0.86
Smoked during pregnancy	21	20	
Alcohol use during pregnancy (%)			
Never	40	57	0.04
Drank alcohol during pregnancy	60	43	
Folic acid intake (%)			
Never	15	36	<0.01
Start in first 10 weeks	31	12	
Start periconceptional	54	52	
Infant characteristics			
Gender (%)			
Male	49	48	0.84
Female	51	52	
Ethnicity (%)			
Western	77	43	<0.01
Non-western	23	57	
Birth weight (*z*-score) *Mean (SD)*	-0.01 (1.01)	-0.20 (1.11)	0.26
Breastfeeding (%)			
Never	9	0	0.09
Non- fully for 4 months	63	83	
Fully for 4 months	28	17	
Family history of asthma or atopy (%)			
Yes	49	56	0.46
No	51	44	

Supplementary Table 2.1. (continued)

	Responders	Non-responders	P-value
Allergy to cow's milk (%)			
Yes	6	0	0.15
No	94	100	
Day care attendance (%)			
Never	23	34	0.05
<16 hours	28	20	
16-32 hours	47	33	
≥32 hours	3	13	

Supplementary Table 2.2. Characteristics of the study population according to the timing of introduction of complementary feeding – Original data

	<3 months Very early			3-6 months Early			≥6 months Timely		Missing	
n=3521	**n=202**	**%=6**	**P-value**	**n=1999**	**%=57**	**P-value**	**n=1320**	**%=37**	**n**	**%**
Maternal characteristics										
Age *Mean (SD)*	30.3	5.7	<0.01	31.2	5	<0.01	31.8	4.4	0	0
Ethnicity									86	2
Western	122	5		1502	56		1062	39		
Non-western	65	9	<0.01	454	60	<0.01	230	31		
Educational level									129	4
Low	45	9		321	64		136	27		
Mid	106	6	<0.01	1022	57	<0.01	649	37		
High	36	3	<0.01	589	53	<0.01	488	44		
Household income per month									0	0
≤2200 euro	74	8		555	58		328	34		
>2200 euro	128	5	<0.01	1444	56	0.06	992	39		
Marital status									148	4
Married/living together	148	5		1743	56		1190	39		
No partner	38	13	<0.01	177	61	<0.01	77	26		
Maternal BMI *Mean (SD)*	24.5	4.6	0.37	24.5	4.3	0.05	24.2	4.1	291	8
Multiple parities									93	3
No	114	5		1134	56		788	39		
Yes	81	6	0.03	815	58	0.06	496	36		
Smoking during pregnancy									643	18
Never	117	5		1253	55		894	40		
Smoked during pregnancy	49	8	<0.01	381	62	<0.01	184	30		
Alcohol use during pregnancy									620	18
Never	82	7		657	57		416	36		
Drank alcohol during pregnancy	85	5	<0.01	991	57	0.41	670	38		
Folic acid intake									947	27
Never	38	10		233	59		124	31		
Start in first 10 weeks	45	6	<0.01	463	58	0.21	291	36		
Start periconceptional	62	4	<0.01	753	55	0.01	565	41		
Infant characteristics										
Gender									0	0
Male	108	6		974	56		647	38		
Female	94	5	0.24	1025	57	0.87	673	38		

Supplementary Table 2.2. (continued)

	<3 months Very early			3-6 months Early			≥6 months Timely		Missing	
	n=202	%=6	P-value	n=1999	%=57	P-value	n=1320	%=37	n	%
Ethnicity									70	2
Western	120	4		1490	56		1060	40		
Non-western	71	9	<0.01	473	61	<0.01	237	30		
Birth weight *z*-score *Mean (SD)*	-0.24	1.01	<0.01	0.01	0.99	0.77	-0.01	1.04	278	8
Breastfeeding									363	10
Never	16	6		175	63		86	31		
Non- fully for 4 months	134	7	0.62	1219	62	0.77	624	31		
Fully for 4 months	30	3	<0.01	407	45	<0.01	467	52		
Family history of asthma or atopy									153	4
Yes	83	5		894	54		691	41		
No	104	6	0.01	1013	60	<0.01	583	34		
Allergy to cow's milk									155	4
Yes	10	5		102	47		103	48		
No	179	6	0.18	1803	57	<0.01	1169	37		
Day care attendance									1218	35
Never	28	5		297	57		199	38		
<16 hours	20	3	0.06	369	58	0.85	253	39		
16-32 hours	47	4	0.40	614	57	0.96	414	39		
≥32 hours	7	11	0.06	35	57	0.59	20	32		

BMI: Body Mass Index; SD: standard deviation; *P*-values were assessed by using univariate logistic regression analyses. % represent proportion by row.

Supplementary Table 2.3. Determinants for the timing of introduction of complementary feeding - Original data

	Univariate model <3 vs. ≥6 months	Multiple regression model <3 vs. ≥6 months	Univariate model 3-6 vs. ≥6 months	Multiple regression model 3-6 vs. ≥6 months
Maternal characteristics				
Age	0.93 * (0.90-0.96)	-	0.97 * (0.96-0.99)	0.97 * (0.95-1.00)
Ethnicity				
Non-western vs. Western	2.46 * (1.76-3.43)	-	1.40 * (1.17-1.67)	-
Educational level				
Mid vs. Low	0.49 * (0.33-0.73)	-	0.67 * (0.53-0.83)	-
High vs. Low	0.22 * (0.14-0.36)	-	0.51 * (0.41-0.65)	-
Household income per month				
>2200 euro vs. ≤2200 euro	0.57 * (0.42-0.78)	-	0.86 (0.73-1.01)	-
Marital status				
No partner vs. Married/living together	3.97 * (2.60-6.07)	-	1.57 * (1.19-2.07)	-
Maternal BMI	1.02 (0.98-1.05)	-	1.02 (1.00-1.04)	-
Multiple parities	1.20 * (1.02-1.41)	-	1.09 (1.00-1.19)	1.39 * (1.14-1.69)
Smoking during pregnancy				
Smoking during pregnancy vs. never	2.04 * (1.41-2.94)	2.03 * (1.35-3.05)	1.48 * (1.21-1.80)	1.27 * (1.01-1.60)
Alcohol use during pregnancy				
Drank alcohol during pregnancy vs. never	0.64 * (0.46-0.89)	-	0.94 (0.80-1.10)	-
Folic acid intake				
Start in first 10 weeks vs. never	0.51 * (0.31-0.82)	0.64 (0.38-1.08)	0.85 (0.65-1.10)	-
Start periconceptional vs. never	0.36 * (0.23-0.56)	0.56 * (0.34-0.94)	0.71 * (0.56-0.91)	-
Infant characteristics				
Gender				
Female vs. Male	0.84 (0.62-1.13)	-	1.01 (0.88-1.16)	-

Supplementary Table 2.3. (continued)

	Univariate model <3 vs. ≥6 months	Multiple regression model <3 vs. ≥6 months	Univariate model 3-6 vs. ≥6 months	Multiple regression model 3-6 vs. ≥6 months
Ethnicity				
Non-western vs. Western	2.65 * (1.91-3.66)	2.14 * (1.40-3.29)	1.42 * (1.19-1.69)	-
Birth weight (*z*-score)	0.81 * (0.69-0.94)	-	1.01 (0.94-1.09)	-
Breastfeeding				
Non-fully for 4 months vs. never	1.15 (0.66-2.03)	-	0.96 (0.73-1.27)	1.11 (0.79-1.55)
Fully for 4 months vs. never	0.35* (0.18-0.66)	-	0.43* (0.32-0.57)	0.51* (0.36-0.72)
Family history of asthma or atopy				
Yes vs. No	0.67 * (0.49-0.92)	0.71 (0.49-1.02)	0.75 * (0.65-0.86)	0.74 * (0.62-0.89)
Allergy to cow's milk				
Yes vs. No	0.63 (0.33-1.24)	-	0.64 * (0.48-0.85)	0.61 * (0.42-0.89)
Daycare attendance				
<16 hours vs. never	0.56 (0.31-1.03)	-	0.98 (0.77-1.24)	-
16-32 hours vs. never	0.81 (0.49-1.33)	-	0.99 (0.80-1.24)	-
≥32 hours vs. never	2.49 (0.97-6.42)	-	1.17 (0.66-2.09)	-
Hosmer-Lemeshow test	-	χ^2 9.76	-	χ^2 11.89

Abbreviations: BMI, Body Mass Index; CI, confidence interval; OR, odds ratio for the introduction of complementary feeding relative to at 6 months and after per group or per unit of continuous predictor. χ^2 for the goodness of fit of the model Values are represented as OR (95% CI) *signifies P-value <0.05.

3

The introduction of allergenic foods and the development of reported wheezing and eczema in childhood

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Arch Pediatr Adolesc Med. 2011;165(10):933-8

ABSTRACT

Objective: To examine whether the timing of introduction of the allergenic foods cow's milk, hen's egg, peanuts, tree nuts, soy, and gluten is associated with eczema and wheezing in children 4 years of age or younger.

Design: Population-based prospective cohort study from fetal life until young adulthood.

Setting: Rotterdam, the Netherlands, from April 2002 through January 2006.

Participants: A total of 6905 preschool children participating in the Generation R study.

Main Exposure: Timing of introduction of cow's milk, hen's egg, peanuts, tree nuts, soy, and gluten collected by questionnaires at 6 and 12 months of age.

Main Outcome Measures: Information on the outcomes eczema and wheezing were obtained by questions from the age-adapted version of the "International Study of Asthma and Allergies in Childhood" core questionnaire and questionnaire data on parentally reported physician diagnosis for eczema.

Results: Of 6905 children, wheezing was reported in 31% at age 2 years and in 14% at ages 3 and 4 years. Eczema was reported in 38%, 20%, and 18% of children at the ages of 2, 3, and 4 years, respectively. The introduction of cow's milk, hen's egg, peanuts, tree nuts, soy, and gluten before the age of 6 months was not significantly associated with eczema or wheezing at any age after adjustment for potential confounders (P>.10 for all comparisons). The results did not alter after stratification according to the child's history of cow's milk allergy and parental history of atopy.

Conclusion: This study does not support the recommendation for delayed introduction of allergenic foods after age 6 months for the prevention of eczema and wheezing.

INTRODUCTION

The prevalence of atopic diseases in children has been increasing over the past few decades [1] and varies throughout the world [2]. Atopic diseases, including atopic eczema, asthma, allergic rhinitis, and food allergy, are common in childhood and cause a very significant burden [3, 4]. Atopic diseases are complex and multifactorial, involving genetic and environmental factors [5]. An important environmental factor that may influence the development of atopic diseases is early childhood nutrition. The first year of life includes many transitions in food consumption [6]. Introduction of complementary feeding is essential for both developmental and nutritional concerns. The timing of complementary feeding is particularly important given the maturation of the gastrointestinal and renal systems [7]. Health risks that have been suggested to be associated with early complementary feeding include excessive child weight gain [8], increased body mass index [9], respiratory illness during childhood [10], and autoimmune diseases (eg, type 1 diabetes mellitus [11] and celiac disease [12]).

Recommendations for the timing of complementary feeding vary. The European Academy of Allergology and Clinical Immunology [13], The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) [14], and the American Association of Pediatrics [15] recommend delaying introduction of solid foods until a child is 4 to 6 months of age. However, the ESPGHAN recommends that introduction of complementary feeding should not be delayed beyond the age of 6 ½ months [14]. There is no current convincing evidence that delayed complementary feeding beyond the age of 4 to 6 months is protective for the development of atopic diseases [13, 15-18]. In addition, few studies found early complementary feeding to be associated with an increased risk of atopic disorders such as eczema [16, 19, 20]. Some studies even found an increased risk for atopic disease in relation to delayed food introduction [21, 22].

It has been suggested that a family history of atopic disease is associated with a significantly increased risk for development of atopic disease in childhood [23]. However, avoidance or delayed introduction of potentially allergenic foods has not been convincingly shown to reduce allergies, either in children considered at risk for the development of allergy or in those not considered to be at risk [14]. Muraro et al [23] found that most children who develop atopic disease, particularly recurrent wheezing and asthma, during early childhood do not belong to high-risk groups for development of atopic disease.

Whether delayed introduction of allergenic foods could decrease the risk of atopic diseases is controversial. Therefore, our aim was to examine whether the timing of introduction of the following allergenic foods—cow's milk, hen's egg, peanuts, tree nuts, soy and gluten—is associated with eczema and wheezing in children up to 4 years of age. In addition, we aimed to assess whether the association differs between children with and those without a history of cow's milk allergy in the first year of life and those children with and those without a parental history of atopy.

METHODS

Participants and study design

This study was embedded in the Generation R study, a population based prospective cohort study from fetal life until young adulthood, and has been described in detail previously [24, 25]. Consent for postnatal follow-up was provided by a total of 7893 mothers with a delivery date between April 2002 and January 2006. The study was approved by the medical ethical review board of the Erasmus Medical Center, Rotterdam, the Netherlands.

Eczema and Wheezing

For children aged 2, 3, and 4 years, questions from the age adapted version of the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires on asthma and eczema were used. These questions were made suitable for younger children and have been used in several articles on this cohort [26]. Information was also available from questionnaire data on parentally reported physician diagnosis for eczema. Questionnaire response rates were 69%, 64%, and 63% at the ages of 2, 3, and 4 years, respectively. Eczema and wheezing were defined as present or absent in the child's second, third, and fourth years of life.

Introduction of allergenic foods and gluten in the first year of life

When the child was 6 and 12 months old, parents were asked by questionnaire about the child's age at first introduction of cow's milk (4855 children), hen's egg (4505 children), peanuts (4478 children), tree nuts (4431 children), soy (4658 children), and gluten (4734 children) in their infant's diet. Also at these times, parents were asked to complete a short food-frequency questionnaire about food products frequently consumed, according to a Dutch food consumption survey in children [27]. Subsequently, the reported food products introduced were crosschecked with the short food-frequency questionnaires. For example, if, when their child was 12 months old, the parents indicated that they had never introduced peanuts into their child's diet, yet when their child was 6 months old, the parents had indicated that their child had consumed peanut butter more than once, the introduction of this allergen was considered to be at or before 6 months of age. In addition, the first introductions of cow's milk and soy were also cross-checked with the type of bottle feeding (soy-based or based on fully hydrolyzed whey protein or not) used at the ages of 6 and 12 months.

Covariates

Variables possibly related to eczema and wheezing, such as sex, gestational age, and birth weight, were obtained from obstetric records assessed in midwife practices and hospital registries [25]. In addition, potential confounders and mediators were accessed through a combination of prenatal and postnatal questionnaires completed by both parents and included information on race/ethnicity; maternal socioeconomic status (SES); maternal smoking; parity; and family history of asthma, eczema, hay fever or allergic rhinitis, and

allergy to house dust. The race/ethnicity of the child was defined as follows: if both parents were born in the Netherlands, the race/ethnicity of the child was defined as Dutch; if one of the parents was born in a country other than the Netherlands, that country applied; if parents were born in countries other than the Netherlands, the country of the mother applied [28]. Maternal SES was defined according to educational level as follows: low level was defined as no education, primary school, or less than 3 years of secondary school; midlevel as more than 3 years of secondary school, higher vocational training, or a bachelor's degree; and high level as an academic education [29]. Postnatal questionnaires completed by the mothers at 6, 12, and 24 months included information on the general health of the child (ie, medication use, comorbidity), day care attendance, and the consumption of food products. Data on breastfeeding were collected by delivery reports and postnatal questionnaires at the ages of 2, 6, and 12 months. Breastfeeding was classified as (1) never; (2) exclusively for 6 months; (3) exclusively for 4 months and partially at 6 months; (4) exclusively for 4 months, with no breastfeeding at 6 months; (5) partially for 6 months; or (6) partially for 4 months, with no breastfeeding at 6 months. In addition, information on the presence of cow's milk allergy was obtained by questionnaire at the ages of 6 and 12 months by asking parents whether their child had been seen by a physician because of cow's milk allergy. When the child was 24 months old, parents were asked about the number of physician-attended respiratory tract infections acquired by the child and use of antibiotics during the past 12 months. Information on gastroenteritis was obtained by asking parents about their child's bowel movements and defined as any episode of diarrhea accompanied by fever. Body mass index (BMI) at 24 months was calculated from the child's weight and height available from child health centers. Being overweight was defined according to age- and sex-dependent BMI thresholds for young children from Cole et al [30].

Population for analyses

To avoid the influence of metabolic disorders and clustering, the following children were excluded in the analyses for this study: twinborn (238 children); siblings within the Generation R cohort (343 children); those with presence of a congenital heart condition (47 children); those with anemia between the ages of 12 and 24 months (58 children); and those with growth retardation, defined as height for age minus 2 standard deviations based on the Netherlands growth curves of children 12 to 24 months old (163 children) [31]. The presence of a congenital heart condition and anemia were defined according to parentally reported physician diagnosis obtained by questionnaire. Children whose parents did not provide informed consent for the use of questionnaire data were also excluded (135 children). To prevent bias associated with missing data, variables with missing values were multiple imputed (5 imputations) based on the correlation between the variable with missing values with other patient characteristics [32, 33] according to the Markov Chain Monte Carlo method. Consequently, data for 6905 children were available after multiple imputation for statistical analyses.

Statistical analysis

Logistic regression analyses were performed with eczema and wheezing at ages 2, 3, and 4 years separately as dependent variables. Introductions of allergenic foods in the first year of life were analyzed as independent variables and adjusted for potential confounders and mediators (ie, sex, maternal SES, race/ethnicity, maternal smoking, gestational age, birth weight, parity, breastfeeding, use of any antibiotics, day care attendance, gastroenteritis, number of respiratory tract infections, overweight, parental history of atopy). The selection of potential confounders and mediators was performed by the alteration in odds ratios (ORs). The potential confounder or mediator was kept in the multivariate model in case of an alteration of 10% or greater in OR. To assess whether the association between the timing of allergenic food introduction and wheezing and eczema was different among children with a history of cow's milk allergy and parental history of atopy vs those without, statistical interaction was evaluated by adding the product term of the independent variable and subgroup (independent variable×subgroup) as covariate to the univariate model. Stratified analyses by history of cow's milk allergy or parental history of atopy were performed when the statistical interaction was significant. The pooled results of the 5 imputed data sets are reported in this article as ORs and 95% confidence intervals (CIs). $P < .05$ was considered as statistically significant. The statistical analyses were performed using SPSS software (version 17.0 for Windows; SPSS Inc, Chicago, Illinois).

RESULTS

Study population

Maternal and child characteristics of the study population are presented in Table 3.1. Of 6905 children, 31% had reported wheezing at age 2 years and 14% at ages 3 and 4 years. Eczema was reported in 38%, 20%, and 18% of children at the ages of 2, 3, and 4 years, respectively.

Introduction of allergenic foods and gluten

The introduction of tree nuts before the age of 6 months was significantly associated with wheezing at 2 years of age (OR, 2.69; 95% CI, 1.25-5.73). However, this association was explained by sex, maternal SES, race/ethnicity, maternal smoking, gestational age, birth weight, parity, breastfeeding, use of any antibiotics, day care attendance, gastroenteritis, number of respiratory tract infections, overweight, and parental history of atopy (Table 3.2). No significant association was found between early introduction to tree nuts and wheezing at age 3 years (OR, 1.24; 95% CI, 0.70-2.20) or 4 years (OR, 1.30; 95% CI, 0.79-2.13) (Table 3.2). In addition, no significant association was found between early introduction to tree nuts and eczema up to age 4 years (Table 3.3). The introduction of cow's milk, hen's egg, peanuts, soy, and gluten to an infant's diet before the age of 6 months was not significantly associated with wheezing (Table 3.2) or eczema (Table 3.3) at any age. These results were independent of sex, maternal SES, race/ ethnicity, maternal smoking, gestational age, birth weight, parity, breastfeeding, use of any antibiotics, day care attendance, gastroenteritis, number of respiratory tract infections, overweight, and parental history of atopy. Additional

Table 3.1. Maternal and child characteristics in 6905 preschool children participating in the Generation R study

Characteristics	No.	%
Mother		
Socioeconomic status		
Low	766	11
Mid	4746	69
High	1393	20
Ever smoked during pregnancy		
No	5064	73
Yes	1841	27
Parents with a history of atopy	3274	47
Parity	2899	42
Child		
Male sex	3496	51
Race/ethnicity		
Dutch/Western	4380	63
Non- Western	2525	37
Gestational age at delivery, mean (SD), months	39.9	1.6
Birth weight, mean (SD),g	3431	540
History of cow's milk allergy in first year of life	846	12
Breast-feeding		
Never	781	11
6 months exclusive	345	5
4 months exclusive, partially at 6 months	1296	19
4 months exclusive, no breastfeeding at 6 months	357	5
6 months partially	795	12
4 months partially, no breastfeeding at 6 months	3331	48
Use of any antibiotics *	2839	41
Overweight *	1755	25
Respiratory tract infections, No.*		
0	3322	48
1-3 times	2369	34
≥4 times	1213	18
Day care attendance*	5137	74
Gastro-enteritis*	4599	67

* From ages 12 to 24 months.

adjustment for potential mediator history of cow's milk allergy did not alter the results for wheezing or eczema (data not shown).

Parental history of atopy and infants history of cow's milk allergy

A history of cow's milk allergy in the first year of life was more frequently found in children with reported wheezing and eczema than in children without reported wheezing and eczema ($P \leq .05$ for difference in history of cow's milk allergy at the ages of 2, 3, and

Table 3.2. Association between the introduction of allergenic foods and wheezing at ages 2, 3 and 4 years in 6905 preschool children participating in the Generation R study

		Age 2 y		Age 3 y		Age 4 y	
Allergenic food introduced at age ≤6 months	**Children, No. (%)**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR (95 % CI)[a]**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR (95 % CI)[a]**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR (95 % CI)[a]**
Cow's milk	4757 (69)	0.83 (0.43 – 1.57)	0.80 (0.44 – 1.43)	1.02 (0.84 – 1.23)	1.02 (0.85 – 1.22)	0.97 (0.77 – 1.21)	0.96 (0.77 – 1.19)
Hen's egg	1466 (21)	1.83 (0.95 – 3.51)	1.39 (0.84 – 2.28)	1.26 (0.83 – 1.91)	1.13 (0.84 – 1.51)	1.30 (0.99 – 1.70)	1.11 (0.91 – 1.34)
Peanut	1069 (15)	2.16 (0.75 – 6.13)	1.71 (0.60 – 4.83)	1.25 (0.81 – 1.93)	1.14 (0.77 – 1.66)	1.25 (0.79 – 1.97)	1.05 (0.69 – 1.61)
Tree nut	875 (13)	2.69 (1.25 – 5.73)[b]	2.41 (0.83 – 7.00)	1.24 (0.70 – 2.20)	1.11 (0.68 – 1.79)	1.30 (0.79 – 2.13)	1.12 (0.75 – 1.67)
Soy	2002 (29)	1.73 (0.99 – 3.01)	1.54 (0.96 – 2.46)	1.11 (0.80 – 1.53)	1.05 (0.80 – 1.38)	1.16 (0.87 – 1.55)	1.06 (0.84 – 1.35)
Gluten	3203 (46)	1.30 (0.94 – 1.79)	1.17 (0.86 – 1.60)	1.07 (0.86 – 1.31)	1.02 (0.83 – 1.26)	1.10 (0.94 – 1.28)	1.03 (0.87 – 1.20)

Abbreviations: CI, confidence interval; OR, odds ratio(compared with introduction at age older than 6 months).

[a] Adjusted for sex, socioeconomic status of the mother, race/ethnicity, smoking during pregnancy, gestational age at birth, birth weight, parity, breastfeeding, use of any antibiotics from ages 12 to 24 months, day care attendance from ages 12 to 24 months, gastroenteritis from ages 12 to 24 months, number of respiratory tract infections from ages 12 to 24 months, overweight from ages 12 to 24 months, and parental history of atopy.

[b] *P*=.06 after adjustment for unequal variances.

Table 3.3. Association between the introduction of allergenic foods and eczema at ages 2, 3 and 4 years in 6905 preschool children participating in the Generation R study

		Age 2 y		Age 3 y		Age 4 y	
Allergenic food introduced at age ≤6 months	**Children, No. (%)**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR (95 % CI)[a]**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR (95 % CI)[a]**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR (95 % CI)[a]**
Cow's milk	4757 (69)	0.92 (0.68 – 1.23)	0.91 (0.67 – 1.23)	0.88 (0.75 – 1.03)	0.88 (0.74 – 1.03)	0.95 (0.77 – 1.17)	0.95 (0.77 – 1.15)
Hen's egg	1466 (21)	1.27 (0.52 – 3.10)	1.10 (0.51 – 2.32)	0.84 (0.65 – 1.09)	0.87 (0.69 – 1.10)	1.11 (0.88 – 1.39)	1.05 (0.81 – 1.35)
Peanut	1069 (15)	1.36 (0.48 – 3.87)	1.11 (0.34 – 3.61)	0.95 (0.72 – 1.26)	0.99 (0.72 – 1.36)	0.94 (0.70 – 1.26)	0.87 (0.65 – 1.16)
Tree nut	875 (13)	1.64 (0.46 – 5.85)	1.54 (0.35 – 6.69)	1.09 (0.72 – 1.65)	1.16 (0.76 – 1.76)	1.12 (0.79 – 1.60)	1.06 (0.72 – 1.56)
Soy	2002 (29)	1.47 (0.74 – 2.92)	1.33 (0.72 – 2.44)	0.92 (0.75 – 1.14)	0.95 (0.75 – 1.19)	1.01 (0.82 – 1.23)	0.97 (0.80 – 1.17)
Gluten	3203 (46)	0.94 (0.69 – 1.28)	0.90 (0.71 – 1.14)	0.88 (0.76 – 1.02)	0.90 (0.76 – 1.06)	1.05 (0.85 – 1.29)	1.02 (0.81 – 1.27)

Abbreviations: CI, confidence interval; OR, odds ratio (compared with introduction at age older than 6 months).

[a] Adjusted for sex, socioeconomic status of the mother, race/ethnicity, smoking during pregnancy, gestational age at birth, birth weight, parity, breastfeeding, use of any antibiotics from ages 12 to 24 months, day care attendance from ages 12 to 24 months, gastroenteritis from ages 12 to 24 months, number of respiratory tract infections from ages 12 to 24 months, overweight from ages 12 to 24 months, and parental history of atopy.

4 years). A parental history of atopy was also more frequently found in children with reported wheezing (*P*=.24, *P* <.01, and *P*=.11 for difference in parental history at the ages of 2, 3, and 4 years, respectively) and eczema (*P* <.05 for difference in parental history at the ages of 2, 3, and 4 years) (Supplementary Table 3.1). Although a significant interaction was found with a history of cow's milk allergy for the introduction of peanuts and gluten (Supplementary Table 3.2), no significant association was found after stratification by history of cow's milk allergy (Supplementary Table 3.3). No interaction was found between the timing of introduction of food allergens and parental history of atopy (Supplementary Table 3.4).

COMMENT

This population-based prospective birth cohort study failed to demonstrate that the timing of introduction of allergenic foods (cow's milk, hen's egg, peanuts, tree nuts, soy, and gluten) was associated with eczema and wheezing in children 4 years or younger. The results did not alter after stratification for history of cow's milk allergy or parental history of atopy.

Various current feeding guidelines recommend complementary feeding to be introduced when a child is older than 4 to 6 months [13-15]. However, there is no current convincing evidence that delayed complementary feeding until a child is older than 4 to 6 months is protective for the development of atopic disease [13, 15-18]. Few previous studies found earlier complementary feeding, before a child is 4 months of age, to be positively associated with atopic diseases as eczema and wheezing [10, 19, 34]. In a birth cohort in the Christchurch Child Development Study in New Zealand, eczema rates were found to be significantly higher in infants who were introduced to solid foods before 4 months of age [19, 34]. A study of a cohort of children in Dundee, Scotland, found solid feeding before 15 weeks to be associated with an increased probability of wheezing during childhood [10]. However, these studies did not assess whether a longer delay of complementary feeding, until a child is older than 6 months, had a additional protective effect on eczema and wheezing.

The results of this study are in agreement with the findings of other birth cohort studies. The Influences of Lifestyle-related Factors on the Immune System and the Development of Allergies in Childhood (LISA) birth cohort study found no evidence supporting a delayed introduction of solid foods beyond age 6 months for the prevention of eczema at age 2 years [35] and no evidence supporting a delayed introduction beyond ages 4 or 6 months for the prevention of asthma at age 6 years [17]. Filipiak et al [18] also did not find evidence supporting delayed introduction of solid foods beyond 4 months of age or delayed introduction of the most potentially allergenic solids beyond 6 months for the prevention of eczema. In addition, a birth cohort study in the United Kingdom found no evidence for a protective effect of late introduction for the development of eczema or wheezing at ages 5 to 5 1⁄2 years [22]. Conversely, this last study found a significantly increased risk of eczema in relation to late introduction of the allergenic foods egg and milk. The KOALA birth cohort study [21]. found that a delayed introduction of cow's milk was associated with a higher risk

of eczema in the first 2 years of life. The latter association could possibly be explained by reverse causation since parents with a family history of atopy or infants with early symptoms of allergy may delay complementary feeding. Possible distortion by reverse causality has been suggested previously [35].

An important strength of this study is the large study population drawn from the general population. Several other studies selected children of atopic parents who are at higher risk of developing atopic diseases, which might have led to selection bias since atopic parents are more likely to introduce allergenic foods later in the infant's diet. An additional strength is the use of multiple imputation for missing data. Consequently, attrition bias was of minimum concern [32, 33].

Some limitations of the study have to be considered in the interpretation of the results. Information on the timing of allergenic food introduction was asked retrospectively when the child was 6 and 12 months old; therefore, minor misclassification because of recall bias cannot be excluded. However, this would have influenced our results only if parents of children with wheezing or eczema tended to misclassify having introduced allergenic foods after 6 months of age instead of before 6 months of age. Eczema and wheezing were diagnosed on the basis of parent-reported questionnaires. This could have led to misclassification of the outcome since physician diagnosis provides more accurate outcome diagnosis. Yet, we do not expect this misclassification to have influenced the effect of timing of food allergen introduction in particular, given that the outcome was measured after the introduction period. Another limitation of this study was that it could not examine the effect of allergenic food introduction before the age of 4 months in relation to eczema and wheezing. Thus, our study precludes conclusions on the effect of very early introduction of allergenic foods. However, Zutavern et al [17] found no evidence supporting a delayed introduction of solid foods beyond 4 or 6 months of age for the prevention of asthma at the age of 6 years. For eczema any effect of a delayed introduction of solids could not be excluded.

Asthma assessment among young children is based on asthmalike symptoms, such as wheezing, often reported by parents through self-administered written questionnaires. Early wheezing in infancy is, however, not a very strong and independent predictor of childhood asthma. Diagnosis of asthma is difficult in young children, owing to the nonspecificity of the symptoms and the fact that conventional lung function tests cannot be performed at such a young age [36]. Therefore, our results do not allow for conclusions regarding the introduction of allergenic foods and later development of asthma. However, previous studies found that an infant's diet has a greater effect on short-term outcomes of atopic diseases than on long term-outcomes [7]. Therefore, we do not expect the effect of the introduction of allergenic foods to influence the results for eczema and wheezing differently at ages older than 4 years.

We considered confounding and reverse causality in our analysis by adjusting for potential confounders and by evaluating statistical interaction for history of cow's milk allergy and parental history of atopy. However, residual confounding and residual reverse

causality cannot be fully excluded. Reverse causation may occur if a delayed introduction of allergenic foods is truly protective for wheezing and eczema and parents of high-risk infants were more likely to delay the introduction of allergenic foods after 6 months of age, which may cancel out the effect.

In conclusion, the results presented in this study do not support a delayed introduction of allergenic foods at an age older than 6months for the prevention of atopic diseases eczema and wheezing. Further studies in our cohort should focus on asthma and eczema at later ages in order to elucidate whether late introduction of food allergens delays the onset of atopic disease.

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SUPPLEMENT CHAPTER 3

Supplementary Table 3.1. History of cow's milk allergy and parental history of atopy in children with wheezing and eczema (n=6905)

	Children with wheezing	Children without wheezing	P value	Children with eczema	Children without eczema	P value
History of cow's milk allergy						
Year 2	16%	11%	<0.01	17%	10%	<0.01
Year 3	15%	12%	0.05	19%	11%	<0.01
Year 4	17%	12%	<0.01	18%	11%	<0.01
Parental history of atopy						
Year 2	49%	47%	0.24	49%	46%	<0.05
Year 3	52%	47%	<0.01	53%	46%	<0.01
Year 4	50%	47%	0.11	51%	47%	<0.05

Supplementary Table 3.2. Statistical interaction between the introduction of allergenic foods and history of cow's milk allergy

	Eczema			Wheezing		
	Year 2	Year 3	Year 4	Year 2	Year 3	Year 4
Allergenic food introduced at age ≤6 months	**P value**	**P value**	**P value**	**P value**	**P value**	**P value**
Cow's milk	0.60	0.33	0.28	0.70	0.73	0.34
Henn's egg	0.80	0.99	0.91	0.48	0.46	0.42
Peanut	0.24	0.63	0.53	0.03	0.49	0.38
Tree nut	0.27	0.43	0.13	0.99	0.61	0.50
Soy	0.09	0.31	0.21	0.54	0.33	0.39
Gluten	0.36	0.10	0.13	0.91	0.12	0.04

Supplementary Table 3.3. Association between the introduction of allergenic foods and wheezing stratified by history of cow's milk allergy

			No history of cow's milk allergy			History of cow's milk allergy	
	Allergenic food introduced at age ≤6 months	**No. (%)**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR 95 % CI)[a]**	**No. (%)**	**Univariate model OR (95 % CI)**	**Multivariate model 1 aOR (95 % CI)[a]**
Year 2	Peanut	826 (77)	2.17 (0.77-6.05)	1.65 (0.60-4.55)	244 (23)	1.62 (0.28-9.29)	1.52 (0.23-9.87)
Year 4	Gluten	2827 (88)	1.15 (0.96-1.38)	1.07 (0.88-1.30)	376 (12)	0.88 (0.57-1.35)	0.84 (0.53-1.35)

OR: odds ratio; 95% confidence interval. OR's are compared to introduction > 6 months of age.

[a] Adjusted for gender, SES mother, ethnicity, ever smoked during pregnancy, gestational age at birth, birth weight, parity, breast-feeding, use of any antibiotics between 12 and 24 months, day care attendance between 12 and 24 months, gastroenteritis between 12 and 24 months, number of respiratory tract infections between 12 and 24 months, overweight between 12 and 24 months, parental history of atopy.

Supplementary Table 3.4. Statistical interaction between the introduction of allergenic foods and parental history of atopy

Allergenic food introduced at age ≤6 months	Eczema			Wheezing		
	Year 2	Year 3	Year 4	Year 2	Year 3	Year 4
	P value	P value	P value	P value	P value	P value
Cow's milk	0.87	1.00	0.81	0.63	0.74	0.14
Henn's egg	0.79	0.85	0.85	0.72	0.81	0.98
Peanut	0.25	0.85	0.71	0.98	0.78	0.75
Tree nut	0.87	1.00	0.09	0.99	0.70	0.46
Soy	0.73	0.93	0.24	0.84	0.87	0.53
Gluten	0.29	0.64	0.70	0.72	0.25	0.30

4

Infant feeding and anti-tissue transglutaminase antibody concentrations

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Am J Clin Nutr. 2014;100:1095–101

ABSTRACT

Background: Celiac disease (CD) has emerged as a common, but largely undiagnosed health problem. Numerous studies examined the influence of infant nutrition on the development of diagnosed CD. However, results are still inconsistent. In addition, the effect of infant feeding practices on the development of potential forms of CD might be different.

Objective: The objective was to examine whether the timing of gluten introduction and breastfeeding duration are associated with CD autoimmunity (CDA) in children at the age of 6 y.

Design: This study was embedded in the Generation R Study, a population-based prospective cohort study. Participants included 1679 Dutch children who were positive for human leukocyte antigen (HLA) DQ2/DQ8. Data on the timing of gluten introduction (<6 mo compared with ≥6 mo) and duration of breastfeeding (<6 mo compared with ≥6 mo) were obtained by questionnaire. Serum samples were analyzed for anti-tissue transglutaminase (anti-tTG) concentrations at age 6 y. Anti-tTG concentrations were categorized into negative (<7 U/mL) and positive (≥7 U/mL) values. Positive anti-tTG concentrations were further categorized based on ≥10 times the upper limit of normal (ULN) values of the test kit (≥7–70 and ≥70 U/mL). Multivariable logistic regression analyses were performed.

Results: Positive anti-tTG concentrations were found in 43 children, 26 of whom had concentrations ≥10 times the ULN (≥70 IU/mL). The introduction of gluten from the age of 6 mo onward and breastfeeding for ≥6 mo were not significantly associated with positive anti-tTG concentrations. In addition, the timing of gluten introduction and duration of breastfeeding were not significantly associated with positive anti-tTG concentrations below or above 10 times the ULN.

Conclusions: Delayed introduction of gluten beyond the age of 6 mo does not increase the risk of CDA. In addition, breastfeeding for ≥6 mo does not decrease the risk of CDA in children at 6 y of age.

INTRODUCTION

Celiac disease [CD] has emerged as a common, but largely undiagnosed, health problem [1, 2]. Untreated CD is associated with excess morbidity in children and adults [3]. CD is characterized by an adaptive T cell–mediated response against gluten, classically resulting in chronic inflammation of the small intestinal mucosa and gastrointestinal complaints [4, 5]. However, the clinical presentation of CD has changed over the past few decades to include milder, nonclassic forms [6, 7]. Therefore, diagnosed CD only represents the more visible tip of the iceberg, ie, children having clinical symptoms, whereas asymptomatic or atypical cases are often missed [6].

Both human leukocyte antigen (HLA) DQ2/DQ8 carrier status and gluten exposure are prerequisites to develop CD. Breastfeeding and the timing of gluten exposure might influence the risk of CD. Underlying mechanisms remain uncertain but might involve a complex interplay between innate and adaptive immune responses, gut colonization, intestinal membrane permeability, genetic predisposition, and environmental factors such as infections and infant feeding habits [8–10].

Numerous studies examined the influence of infant nutrition, including breastfeeding and the timing of gluten introduction, on the development of diagnosed CD. On the basis of current literature, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition recommends to avoid both early (<4 mo) and late (>7 mo) introduction of gluten and to introduce gluten gradually while the child is still being breastfed [11]. However, results are still inconsistent in this respect [7, 9, 12–19]. The lack of consistency between studies may be due to the majority of these studies being retrospective (ie, comparing history of infant feeding practices in CD cases compared with controls) [13, 17, 18, 20] or ecological (ie, linking incidence rates of diagnosed CD to national infant feeding practices) [1]. These latter studies were mainly based on clinical CD. However, because subclinical, silent, or latent forms of CD might exist [4], it could be speculated that these forms have different etiologies, but these are not fully understood. Therefore, the effect of infant feeding practices on the development of subclinical, silent or latent forms of CD might be different [17, 21]. For example, a Swedish study found a decreased prevalence of symptomatic CD after new infant feeding recommendations were introduced but did not find any difference in the prevalence of undiagnosed (screening detected) CD [21]. However, a later replication within the same cohort study did not support these findings [19]. The aim of the current study was to examine whether breastfeeding duration and the timing of gluten introduction were associated with CD autoimmunity (CDA) in a population-based prospective cohort study in 6-y-old children.

SUBJECTS AND METHODS

Participants and study design

This study was embedded in the Generation R Study, a prospective population-based cohort study from fetal life onward, and has been described in detail previously [22]. A total of 8305

mothers with a delivery date from April 2002 through January 2006 provided consent for school age follow-up. Ethical approval for the study was obtained from the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam.

Duration of breastfeeding

Information with regard to breastfeeding was obtained by a combination of delivery reports and postnatal questionnaires at the ages of 2, 6, and 12 mo [22]. Mothers were asked by questionnaire whether they had ever breastfed their child and, if yes, at what age (in mo) they had stopped breastfeeding. Breastfeeding duration was categorized as reported by Størdal et al [14] but later dichotomized as <6 mo and ≥6 mo because only 1 infant still received breast milk at the age of 13 mo. Questionnaire response rates were 82%, 73%, and 72% at age 2, 6, and 12 mo, respectively.

Introduction of gluten in the first year of life

At the child's age of 6 and 12 mo, parents were asked by questionnaire the age of first-time introduction of gluten in their infant's diet. In addition, parents were asked to complete a short food-frequency questionnaire consisting of food products frequently consumed according to a Dutch food consumption survey in infants [23]. The timing of gluten introduction was cross-checked with the consumption of bread and biscuits and type of porridge (based on wheat or oats instead of rice) at the age of 6 and 12 mo, as described previously [24]. The timing of the introduction of gluten was categorized as <6 mo or ≥6 mo.

Anti-tissue transglutaminase concentrations

Anti-tissue transglutaminase (anti-tTG) concentrations were assessed in venous serum samples by using fluorescence enzyme immunoassay (ELiA Celikey IgA, PhadiaImmunocap 250; Phadia AB) at the Department of Immunology, Erasmus MC- University Medical Center Rotterdam, The Netherlands. The intra- and interassay CVs were below 10% and 15%, respectively. Of 8305 children participating at the age of 6 y, serum anti-tTG was available in 53% of children. We excluded 20 children in whom IgA concentrations were low, possibly indicating IgA deficiency. None of the participants were aware of tTG-IgA determination. The median anti-tTG concentration in the study population was 0.25 U/mL, varying from 0.10 to 125 U/mL. Anti-tTG concentrations were categorized into negative and positive by using the cutoff for clinical practice of 7 U/mL (Figure 4.1). Positive anti-tTG concentrations were further categorized into 2 categories based on ≥10 times the upper limit of normal (ULN) values of the test kit (cutoffs for positive anti-tTG: ≥7–70 and ≥70 U/mL) (Figure 4.1) [25].

Detection of HLA risk alleles

To capture whether the children carried the HLA-DQ risk type DQ2 or DQ8, a tag nucleotide polymorphism (single nucleotide polymorphism) approach was used as was described in detail previously [26, 27]. Children were genotyped for these single nucleotide polymorphisms

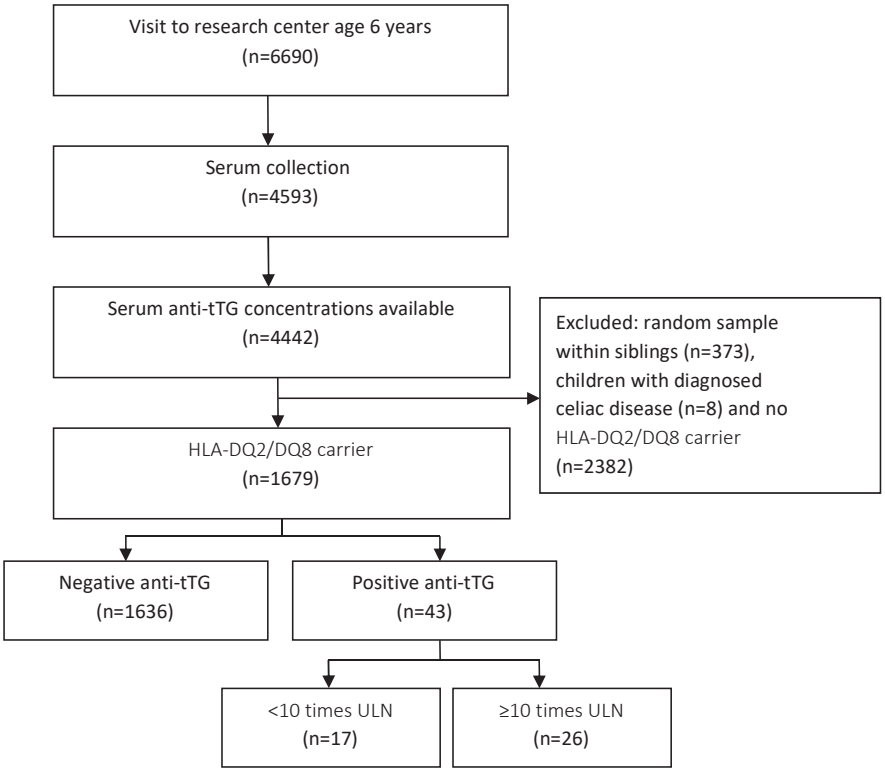

Figure 4.1. Flowchart of participants in the Generation R Study. anti-tTG, anti-tissue transglutaminase; HLA, human leukocyte antigen; ULN, upper limit of normal.

for HLA-DQ2 (rs2187668, rs2395182, rs4713586, and rs7775228) and DQ8 (rs7454108) [26] by using genome-wide Illumina 610 Quad Array. Genotype and allele frequencies were in Hardy-Weinberg equilibrium (rs2187668, P= 0.71; rs2395182, P= 0.85; rs4713586, P= 0.57; rs7775228, P= 0.92; and rs7454108, P= 0.76).

Covariates

Data on potential confounders such as fetal sex, gestational age, birth weight, and cesarean section (no differentiation was made between planned and unplanned) were obtained from obstetric records assessed in midwife practices and hospitals [22]. Maternal anti-tTG concentrations were measured during pregnancy. Data on other sociodemographic and lifestyle factors were obtained by a combination of pre- and postnatal questionnaires completed by both parents. This included information on ethnicity (Western or non-Western) [28], parity, maternal smoking during pregnancy, maternal educational level (low or high) [29], and household income per month (≤€2200 or ≥€2200) [30]. Postnatal questionnaires included information on vitamin D supplementation, gastrointestinal and respiratory tract infections, and day care attendance in the first year.

Population for analyses

Of all children with anti-tTG data available (n= 4442), we excluded from the analysis those with diagnosed CD (n= 8) at the age of 6 y and children who were HLA-DQ2/DQ8 negative (n= 2382). To prevent clustering, only one child per family within the Generation R cohort was included by random selection (n= 373). In total, 1679 children were available for statistical analyses (Figure 4.1).

Statistical analysis

First, independent Student's t test and chi-square tests were performed to test for differences in characteristics between groups of anti-tTG concentrations. Second, logistic regression analysis was performed with anti-tTG concentration at the age of 6 y as a dependent variable. Breastfeeding and the timing of gluten introduction were analyzed separately as independent variables and adjusted for major confounders. Because of the small numbers in the positive anti-tTG group, the selection of potential confounders in the multivariate model was restricted to those with an alteration of ≥10% in ORs [31]. To assess whether the association between breastfeeding or the timing of gluten introduction and anti-tTG concentrations differed by ethnicity and children with and without gastrointestinal infections during infancy, statistical interactions were evaluated by adding the product term of independent variable and subgroup (independent variable 3 subgroup) as covariates to the univariate model. To reduce attrition bias, multiple imputation of the exposures (ie, breastfeeding and timing of gluten introduction) and covariates was performed (n= 5 imputations). The multiple imputation procedure was based on the correlation between each variable with missing values with other subject characteristics [32]. The pooled results of the 5 imputed data sets are reported in this article as ORs and 95% CIs. A *P* value <0.05 was considered significant. The statistical analyses were performed in SPSS 20.0 for Windows (SPSS, Inc).

RESULTS

Maternal and child characteristics of the study population are shown in Table 4.1. In all 1679 HLA-DQ2/DQ8–positive children, 2.6% had positive anti-tTG concentrations and 97.4% had negative anti-tTG concentrations (Table 4.1 and Figure 4.1). Of children with positive anti-tTG concentrations (n= 43), 60% (n= 26) had values ≥10 times the ULN (≥70 IU/mL) (Table 4.1 and Figure 4.1). Breastfeeding for ≥6 mo was reported in 47% of children with positive and negative anti-tTG concentrations (Figure 4.2). The introduction of gluten from the age of 6 mo onward was reported in 56% and 64% of children with positive and negative anti-tTG concentrations, respectively (Figure 4.2).

Breastfeeding and anti-tTG concentrations

Relative to breastfeeding for <6 mo, breastfeeding for ≥6 mo was not significantly associated with positive anti-tTG concentrations (Table 4.2). In addition, breastfeeding

Table 4.1. Maternal and child characteristics according to anti-tTG concentration[1]

	Negative anti-tTG		Positive anti-tTG			
	n=1636 (97.4%)		≥7-70 IU/ml n=17 (1%)		≥70 IU/ml n=26 (1.6%)	
	n	%	n	%	n	%
Maternal characteristics						
Educational level						
Low	369	23	6	35	2	8
Mid	854	52	7	41	15	58
High	413	25	4	24	9	34
Household income per month						
≤2200 euro	732	45	9	53	5	19
>2200 euro	904	55	8	47	21	81[2]
Smoking during pregnancy						
Never	1203	74	13	76	20	77
Smoked during pregnancy	433	26	4	24	6	23
Caesarean section	213	13	2	12	2	8
Parity	711	43	7	41	10	38
Maternal anti-tTG during pregnancy[3]						
Negative	1242	99	12	100	20	100
Positive	6	1	0	0	0	0
Child characteristics						
Male	843	52	4	24[2]	8	31[2]
Ethnicity						
Western	1107	68	11	65	25	96[2]
Non-Western	529	32	6	35	1	4
Birth weight *z*-score[4]	-0.04	0.99	0.16	1.13	-0.28	1.14
Breastfeeding						
0 to <3 months	461	28	3	18	9	35
3 to <6 months	402	25	3	18	8	30
≥6 months	773	47	11	64	9	35
Introduction of gluten						
<6 months	586	36	5	29	14	54[2]
≥6 months	1050	64	12	71	12	46
Gastrointestinal tract infections first year	1072	66	11	65	18	69
Respiratory tract infections first year	893	55	10	59	18	69
Vitamin D supplementation age 6-12 months	874	53	10	59	12	46
Day care attendance first year	1099	67	12	71	24	92[2]

[1] *n*= 1679. Anti-tTG, anti-tissue transglutaminase.
[2] Significantly different from negative anti-tTG concentrations, P < 0.05.
[3] Was not multiple imputed.
[4] Values are means ± SDs.

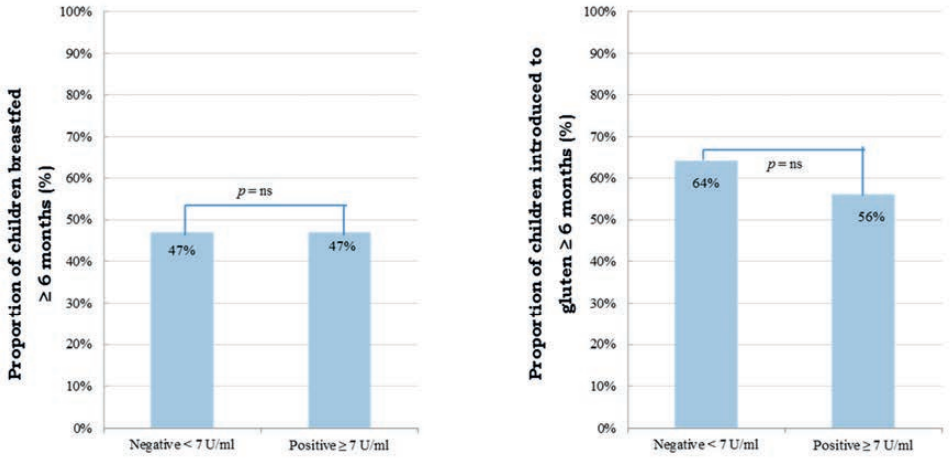

Figure 4.2. Duration of breastfeeding and timing of gluten introduction according to anti-tTG concentration. A: Proportion of children breastfed for ≥6 mo according to anti-tTG concentration [<7 U/mL (n= 1636) or ≥7 U/mL (n= 43)]. B: Proportion of children introduced to gluten from the age of 6 mo onward according to anti-tTG concentration [<7 U/mL (n= 1636) or ≥7 U/mL (n= 43)]. anti-tTG, anti-tissue transglutaminase.

for ≥6 mo was not significantly associated with positive anti-tTG concentrations below or above 10 times the ULN (≥70 IU/mL) (Table 4.3). In comparison with breastfeeding for <3 mo, breastfeeding between 3 and 6 mo and for ≥6 mo was not significantly associated with anti-tTG concentrations (data not shown). No significant interaction was found between breastfeeding and ethnicity or gastrointestinal infections during infancy (*P*-interaction > 0.50).

Timing of gluten introduction and anti-tTG concentrations

In comparison with the introduction of gluten before the age of 6 mo, the introduction of gluten from the age of 6 mo onward was not significantly associated with positive anti-tTG concentrations (Table 4.4). In addition, the introduction of gluten from the age of

Table 4.2. Association between breastfeeding duration and positive anti-tTG concentrations[1]

Breastfeeding	n (%)	Crude model	Multivariate model[2]
<6 months	885 (53)	Reference	Reference
≥6 months	794 (47)	1.07 (0.52-2.22)	1.20 (0.56-2.59)

[1] *n*= 1679. Values for the crude model are ORs (95% CIs), and values for the multivariate model are aORs.
[2] Adjusted for smoking during pregnancy, cesarean section, parity, day care attendance in first year, respiratory tract infections in first year, vitamin D supplementation at age 6–12 mo, household income per month, and maternal educational level. Additional adjustment for sex, ethnicity, birth weight z score, timing of gluten introduction, gastrointestinal tract infections in the first year, and maternal anti-tTG concentration did not provide an alteration of ≥10% in ORs.

6 mo onward was not significantly associated with positive anti-tTG concentrations below or above 10 times the ULN (≥70 IU/mL) (Table 4.5). No significant interaction was found between the timing of gluten introduction and ethnicity or gastrointestinal infections during infancy (*P*-interaction > 0.69).

Table 4.3. Association between breastfeeding duration and positive anti-tTG concentrations below and above 10 times ULN[1]

		≥7-70 IU/ml n=17		≥70 IU/ml n=26	
Breastfeeding	**n (%)**	**Crude model**	**Multivariate model[2]**	**Crude model**	**Multivariate model[2]**
<6 months	885 (53)	Reference	Reference	Reference	Reference
≥6 months	794 (47)	2.57 (0.56-11.75)	2.52 (0.59-10.84)	0.64 (0.24-1.73)	0.78 (0.27-2.27)

[1] *n*= 1679. Values for the crude model are ORs (95% CIs), and values for the multivariate model are aORs (95% CIs). anti-tTG, anti-tissue transglutaminase; aOR, adjusted OR; ULN, upper limit of normal.

[2] Adjusted for smoking during pregnancy, cesarean section, parity, day care attendance in first year, respiratory tract infections in first year, vitamin D supplementation at age 6–12 mo, household income per month, and maternal educational level. Additional adjustment for sex, ethnicity, birth weight z score, timing of gluten introduction, gastrointestinal tract infections in the first year, and maternal anti-tTG concentration did not provide an alteration of ≥10% in ORs.

Table 4.4. Association between the introduction of gluten and positive anti-tTG concentrations[1]

Gluten introduction	**n (%)**	**Crude model**	**Multivariate model[2]**
<6 months	605 (36)	Reference	Reference
≥6 months	1074 (64)	0.68 (0.34-1.35)	0.64 (0.31-1.31)

[1] *n*= 1679. Values for the crude model are ORs (95% CIs), and values for the multivariate model are aORs (95% CIs). anti-tTG, anti-tissue transglutaminase; aOR, adjusted OR.

[2] Adjusted for ethnicity and household income per month. Adjustment for sex, birth weight z score, smoking during pregnancy, breastfeeding, parity, cesarean section, day care attendance, vitamin D supplementation, gastrointestinal and respiratory tract infections in the first year, maternal educational level, and maternal anti-tTG concentration did not provide an alteration of ≥10% in ORs.

DISCUSSION

In this population-based prospective birth cohort, we did not find an association with breastfeeding duration and the timing of gluten introduction and positive anti-tTG concentrations in children with CDA at the age of 6 y.

The ESPGHAN Committee on Nutrition recommends that both early and late introduction of gluten should be avoided. In addition, it is recommended to introduce gluten in small amounts into the diet when the infant is still being breastfed [11]. However, evidence for avoiding very early and late introduction of gluten for the prevention of CD is inconsistent. We did not find a relation between the timing of gluten introduction and the risk of CD in childhood, which is in accordance with other studies [13, 15, 17]. In contrast, findings of 2

Table 4.5. Association between the introduction of gluten and positive anti-tTG concentrations below and above 10 times ULN[1]

Gluten introduction	n (%)	≥7-70 IU/ml n=17		≥70 IU/ml n=26	
		Crude model	Multivariate model[2]	Crude model	Multivariate model[2]
<6 months	605 (36)	Reference	Reference	Reference	Reference
≥6 months	1074 (64)	1.27 (0.37-4.37)	1.30 (0.38-4.41)	0.47 (0.19-1.13)	0.42 (0.17-1.02)

[1] *n*= 1679. Values for the crude model are ORs (95% CIs), and values for the multivariate model are aORs (95% CIs). anti-tTG, anti-tissue transglutaminase; aOR, adjusted OR; ULN, upper limit of normal.
[2] Adjusted for ethnicity and household income per month. Additional adjustment for sex, birth weight z score, smoking during pregnancy, breastfeeding, parity, cesarean section, day care attendance, vitamin D supplementation, gastrointestinal and respiratory tract infections in the first year, maternal educational level, and maternal anti-tTG concentration did not provide an alteration of ≥10% in ORs.

other prospective studies support a role for the timing of gluten introduction [9, 14]. Norris et al [9] found an increased risk of biopsy-confirmed CD in CDA-positive children for both early (≤3 mo) and late (≥7 mo) introduction of gluten [9]. Strikingly, before restricting their analyses to biopsy-confirmed CD, Norris et al did not find late introduction to increase the risk of CDA. This suggests that the timing of gluten introduction may be differentially associated with biopsy-confirmed and non–biopsy-confirmed CD. However, diagnosed CD is subject to bias, depending on the awareness of the pediatricians to make the diagnosis. In addition, Norris et al focused on high-risk children defined as having a firstdegree relative with type 1 diabetes or having HLA genotypes associated with CD and type 1 diabetes. Therefore, the results cannot be directly extrapolated to our study. Very recently, the Norwegian Mother and Child Cohort Study [14] found late (≥7 mo) but not early (≤4 mo) introduction to be borderline associated with an increased risk of CD (adjusted OR: 1.27; 95% CI: 1.01, 1.65). We could not confirm these results in our study, nor did we find the effect estimates in the same direction (adjusted OR: 0.64; 95% CI: 0.31, 1.3). However, the Norwegian Mother and Child Cohort Study included children with clinical CD instead of CDA, which may explain the different results.

The results of our study on breastfeeding and the development of CDA are in line with the findings of other prospective studies [9, 15, 33]. Størdal et al [14] also did not find breastfeeding for ≥6 mo (6–12 mo) to be associated with CD but found breastfeeding beyond 12 mo to be associated with an increased risk of CD. The majority of children in our cohort did not breastfeed after the age of 12 mo; therefore, we were unable to replicate this analysis [14]. Several studies did find breastfeeding to reduce the risk of CD [12, 17], especially breastfeeding at the time of [13, 34, 35] and beyond [12, 13, 19, 35] the introduction of gluten, as well as with gradual introduction of gluten while breastfeeding [12, 13, 19]. However, most of these studies were based on retrospectively collected data, which may lead to recall bias (ie, differential reporting on infant feeding practices in those with and without CD diagnosis) [13, 17, 18, 20, 35]. In addition, it remains unclear whether the protective

effect of breastfeeding is persistent or only delays the onset of symptoms and therefore CD diagnosis [12, 20, 34, 35]. For example, previous studies found longer breastfeeding [35] and breastfeeding exclusivity [18] only to delay the onset of CD in infancy. This may explain why we did not find any association between breastfeeding duration and CDA. Although we did not find breastfeeding duration and the timing of gluten introduction to be associated with CDA, we do not exclude the role of infant feeding in the development of CD. Breastfeeding and the timing of gluten introduction might not be protective for the development of subclinical, silent, or latent forms of CD but could protect against developing symptomatic CD. Also, gluten introduction while breastfeeding and the amount of gluten could be more important than the duration of breastfeeding and the timing of gluten introduction, as suggested by Ivarsson et al [13].

An important strength of this study is, first, the assessment of serum anti-tTG concentrations and HLA testing in the general pediatric population, which are good methods to detect clinically silent CD [6]. Previous studies selected children with diagnosed CD [17], or with symptomatic CD leading to CD diagnosis [13, 5], or focused on high-risk children [9]. Second, we subdivided positive anti-tTG concentrations (≥7 U/mL) into 2 groups based on 10 times the ULN value (≥70 IU/mL), because positive antitTG concentrations ≥10 times the ULN show high diagnostic accuracy [25]. Of all anti-tTG–positive children in our study population, >60% had anti-tTG concentrations ≥10 times the ULN, suggesting that these children likely have CD. Third, our study design provided information on a broad range of potential confounders, including ethnicity, socioeconomic status, smoking during pregnancy, and infections.

Some limitations should be taken into account in the interpretation of the results. Information on breastfeeding duration and the timing of gluten introduction was obtained by parental self-report. Nevertheless, only if misclassification of infant feeding practices were related to CD diagnosis would it have influenced our results, which is unlikely because questionnaires on feeding practices were completed before anti-tTG measurement at 6 y of age. In addition, participants in our study were unaware of tTG IgA determination, so a response bias is therefore highly unlikely. Also, it may be questioned whether our study had sufficient power to detect small differences in gluten introduction practices between children with and without CDA development. Our study had a power of 80% (at an α of 0.05) to detect at least a 22% difference in gluten at ≥6 mo to be significant between children with and without CDA. A recent study [19] (n= 13,279) found a difference of 2.5% in gluten at >6 mo to be significant, whereas Norris et al [9] found a difference of 12% to be significant (n= 1560). Although the sensitivity and specificity of anti-tTG are high [25, 36], only when anti-tTG concenetrations are ≥10 times the ULN in combination with symptoms and positive anti-endomysial antibody can (clinical) CD be diagnosed without duodenal biopsy [25]. According to the ESPGHAN, clinically diagnosed CD concerns children who visit the pediatrician because of symptoms, whereas subclinical CD is found by screening and must be verified by biopsy specimens. However, because our study did not have biopsy specimens, we examined the development of CDA. Therefore, final conclusions

concerning (subclinical) CD diagnosis should be made with caution. Furthermore, we adjusted for potential confounders in our analysis, but residual confounding cannot be fully excluded. Another limitation of this study is that our study cannot examine the effect of gluten introduction before the age of 4 mo, as well as the amount of gluten introduced in relation to anti-tTG concentrations. Although a previous study in Dutch infants showed that the majority of children receive gluten between 3 and 6 mo of age and not before that time [37], a window of opportunity in which gluten introduction might prevent CDA cannot be fully ruled out. In relation to breastfeeding, this study cannot examine the effect of breastfeeding at the time of and beyond gluten introduction. Hence, our study precludes conclusions on the effect of very early introduction of gluten, gradual introduction of gluten, and the introduction while breastfeeding.

In conclusion, the results suggest that a delayed introduction of gluten beyond the age of 6 mo does not increase the risk of CDA. In addition, breastfeeding for ≥6 mo does not decrease the risk of CDA in children at 6 y of age. Our study precludes conclusions on the timing of gluten introduction while breastfeeding.

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Part II

Nutrition and respiratory and allergic disease

5

Breastfeeding and the risk of respiratory tract infections after infancy

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In revision (PLoS One)

ABSTRACT

Background: The protection of breastfeeding against respiratory tract infections in the first year of life has often been suggested. Few studies examined the effect of breastfeeding on respiratory tract infections after infancy.

Objective: To examine the association between breastfeeding with lower respiratory tract infections (LRTI) and upper respiratory tract infections (URTI) after infancy up to 4 years of age (n= 5322).

Methods: This study was embedded in The Generation R study, a Dutch population-based prospective cohort study from fetal life until young adulthood. Information on breastfeeding duration (never; <3 months; 3-6 months; ≥6 months) and dose (never; partially until 4 months; predominantly until 4 months) were collected by questionnaire at 2, 6, and 12 months of age. Information on doctor attendance for LRTI and URTI were obtained by questionnaire at 2, 3, and 4 years of age.

Results: Breastfeeding for 6 months or longer, but not for less than 6 months, was significantly associated with a reduced risk of LRTI up to 4 years of age (aOR: 0.71; 95% CI: 0.51-0.98). Compared to never being breastfed, any breastfeeding duration was not associated with URTI after infancy. Partial and predominant breastfeeding until 4 months was not significantly associated with LRTI or URTI after infancy up to 4 years of age.

Conclusion: Breastfeeding duration for 6 months or longer is associated with a reduced risk of LRTI, but not URTI, in pre-school children whereas predominant breastfeeding until 4 months was not associated with respiratory tract infections. These findings are compatible with the hypothesis that the protective effect of breastfeeding for respiratory tract infections persist after infancy therefore supporting current recommendations for breastfeeding for at least 6 months.

INTRODUCTION

Infectious diseases, including respiratory tract infections, are a leading cause of morbidity and hospitalization in infants and children [1, 2]. There is much epidemiological evidence for the benefits of breastfeeding against a wide range of infections and illnesses [3, 4]. Breast milk contains various antimicrobial substances, anti-inflammatory components and factors that promote immune development [4, 5]. It enhances the immature immune system of the infant and strengthens defense mechanisms against infectious and other agents during the breastfeeding period [4-7]. Exclusive breastfeeding for the first 6 months of life with breastfeeding along with complementary feeding thereafter is recommended by the World Health Organization (WHO) [8]. The benefits have been found to be dose-dependent and related to the duration of breastfeeding [3, 4]. The protection of exclusive and prolonged breastfeeding against respiratory tract infections in the first year of life has often been suggested and also found in The Generation R Study [3, 4, 9, 10]. But, not all studies found breastfeeding exclusivity and duration to reduce the occurrence of respiratory tract infections [11, 12]. It is suggested that the influences of breast milk on the infant's immune system may persist beyond the breastfeeding period, as it not only provides passive immunity but also maturation of the immune system in the long run [13, 14]. Since breastfeeding might protect against diseases in adulthood such as type 1 diabetes and inflammatory bowel disease [3, 4, 15] a prolonged protection against respiratory tract infections after the first year of life seems plausible. However, only few studies have examined the effect of breastfeeding on respiratory tract infections after infancy and reported inconsistent results [16-21].

The aim of this study was to examine the association between breastfeeding and lower and upper respiratory tract infections after infancy up to 4 years of age.

SUBJECTS AND METHODS

Participants and study design

This study was embedded in the Generation R study, a population-based prospective cohort study from fetal life until young adulthood and has been described in detail previously [22]. In total, 9778 mothers with a delivery date from April 2002 through January 2006 enrolled in the study. Consent for postnatal follow-up was provided by 7893 participants (Supplementary Figure 5.1). The study was approved by the medical ethical review board of the Erasmus Medical Center, Rotterdam, the Netherlands.

Respiratory tract infections

Data on respiratory tract infections was obtained by postal parent-reported questionnaires at the age of 2, 3 and 4 years. Parents were asked whether their child had suffered from a respiratory tract infection in the previous year and had visited a doctor for the infectious disease. Upper respiratory tract infections were defined as having either a serious cold, ear infection or throat infection and lower respiratory tract infections by the presence

of pneumonia or bronchitis as described in our previous study [10]. Upper and lower respiratory tract infections were defined as present or absent in the second, third and fourth year of life. Questionnaire response rates were 76%, 69% and 73% at the age of 2, 3 and 4 years respectively.

Breastfeeding

Data on breastfeeding were collected by a combination of delivery reports and postnatal postal questionnaires at the age of 2, 6 and 12 months. By questionnaire the mothers were asked whether they had ever breastfed their child and at which age (in months) the child had stopped receiving breast milk. The duration of breastfeeding was categorized as (I) never breastfed, (II) breastfed for less than 3 months, (III) breastfed for 3-6 months, and (IV) breastfed for 6 months and longer. The majority of infants had stopped receiving breast milk before the age of 12 months, only 3 infants thereafter. An approximation of breastfeeding exclusivity was defined on the basis of parent reports of the child's age at which solid foods were first introduced, including the introduction of formula feeding. Predominant breastfeeding was defined as receiving breastfeeding without any other infant formula, milk or solid foods [23]. Breastfeeding dose was categorized as (I) never breastfed, (II) partially breastfed until 4 months, and (III) predominantly breastfed until 4 months. Partial breastfeeding was defined as receiving both breast milk and infant formula and/or solid foods. Questionnaire response rates were 82%, 73% and 72% at the age 2, 6 and 12 months respectively.

Covariates

Information on potential confounders, including mode of delivery, gender and gestational age, were obtained from obstetric records assessed in mid-wife practices and hospital registries [22]. Additional information was obtained by a combination of prenatal and postnatal questionnaires completed by both parents. The questionnaires included information on maternal age, maternal educational level, maternal marital status, maternal ethnicity, household income per month, maternal BMI before pregnancy, any maternal smoking during pregnancy, any maternal alcohol use during pregnancy, parity and parental history of atopy. Ethnicity of the mother was defined as follows: if both parents were born in The Netherlands, the ethnicity of the mother was defined as Dutch; if one of the parents was born in another country than The Netherlands, that country applied; if parents were born in different countries other than The Netherlands, the country of the mother applied [24]. Ethnicity of the mother was categorized into Western (Dutch, European, American-Western, Asian-Western) and non-Western (American non-Western, Asian non Western, African, Turkish, Cape Verdean, Moroccan, Dutch Antillean, Surinamese, Oceania, and Indonesian). Maternal educational level was defined as follows; low: no education, primary school or less than 3 years of secondary school, mid: more than 3 years of secondary school, higher vocational training or bachelor's degree, and high: academic education [25]. Household

income per month was categorized into two income-groups using the approximate monthly general labour income during the inclusion period of this study as cut off point (≤€2200 and >€2200) [26]. Postnatal questionnaire completed by the mother at the child's age of 6 months included information on smoke exposure of the child inside and outside the home. Postnatal questionnaires completed by the mother at age 12 months included information on vitamin D supplementation in the previous 6 months and questionnaires at age 12 and 24 months included information on day care attendance.

Population for analyses

Children whose parents did not provide informed consent for the use of postnatal questionnaire data (n= 1885) and children without information on respiratory tract infections at ages 2 to 4 years (n= 2015) were excluded from the analysis. To prevent clustering, only one child per family within the Generation R cohort was included by random selection (n=556 excluded). To reduce attrition bias, variables with missing values were multiple imputed (20 imputations) based on the correlation between the variable with missing values with other maternal and child characteristics (Supplementary Table 5.1) [27]. Consequently data of 5322 children were available after multiple imputation for statistical analyses (Supplementary Figure 5.1).

Statistical methods

First, independent Student's t test and chi-square test were performed to test for differences in characteristics between the 4 groups of breastfeeding duration. Second, logistic regression analyses by using generalized estimating equations (GEE) were performed. Regression analysis by GEE assesses the association between two variables by correction for the within subject's dependence as a result of the repeated observations on lower and upper respiratory tract infections (age 2, 3 and 4 years) since repeated measurements within one individual are frequently correlated [28]. An unstructured working correlation structure was used in the GEE analyses as adjustment for the dependency between the repeated measurements, since the within-subject correlation coefficient for lower and upper respiratory tract infections between the three time points were different (r= 0.13-0.32). Logistic regression analysis with GEE was performed with lower respiratory tract infections and upper respiratory tract infections as dependent variables and breastfeeding as independent variable. All analyses were adjusted for the age (time) at which observations of illness were assessed to account for potential confounding by age as well as clustering of repeated measurement. The selection of potential confounders was performed by the alteration in odds ratio (OR) and kept in the multivariable model in case of an alteration of ≥10% in OR [29]. The pooled results of the 20 imputed datasets were reported in this paper as odds ratio's (OR's) and 95% confidence intervals (95% CIs). A P-value <0 .05 was considered as statistically significant. The statistical analyses were carried out by using SPSS 22.0 for Windows (SPSS Inc, Chicago, IL).

STUDY POPULATION

Maternal and child characteristics are presented in Table 5.1 and Supplementary Table 5.1. Out of 5322 children, 14% had suffered from at least one episode of lower respiratory tract infection in the second year of life, 8% in the third year and 6% in the fourth year of life (Table 5.2). At least one episode of upper respiratory tract infection was reported for 44% of children in the second year of life, 36% in the third year and 31% in the fourth year of life (Table 5.2).

Table 5.1. Maternal and child characteristics (n=5322)

	Breastfeeding duration							
	Never n=893 (17%)		**<3 months n=1602 (30%)**		**3-6 months n=1093 (21%)**		**≥6 months n=1734 (32%)**	
	Number - % or mean ± SD		Number - % or mean ± SD		Number - % or mean ± SD		Number - % or mean ± SD	
Maternal characteristics								
Maternal age - Mean (SD)								
	30.7*	5.1	30.3*	4.9	31.5	4.6	31.8	4.8
Educational level – n (%)								
Low	230*	26	384*	24	129	12	262	15
Mid	496	55	881	55	566	52	856	49
High	167	19	337	21	398	36	616	36
Ethnicity – n (%)								
Western	623*	70	1057	66	786	72	1186	68
Non-Western	270	30	545	34	307	28	548	32
Household income per month – n (%)								
≤2200 euro	385	43	648	40	332*	30	710	41
>2200 euro	508	57	954	60	761	70	1024	59
Marital status – n (%)								
No partner	105	12	185*	12	88	8	157	9
Married/ Living together	788	88	1417	88	1005	92	1577	91
Maternal BMI before pregnancy (kg/m^2) - mean (SD)								
	23.9*	4.3	23.9*	4.4	23.1	3.6	23.1	3.6
Child exposure to smoke – n (%)								
Never	471*	53	929*	58	699*	64	1224	70
Prenatal smoking, no environmental smoking	51	6	115	7	77	7	113	7
Prenatal smoking and environmental smoking	198	22	340	21	154	14	160	9
Environmental smoking, no prenatal smoking	173	19	218	14	163	15	237	14
Alcohol use during pregnancy – n (%)								
Never	454*	51	724	45	356*	33	741	43
Drank alcohol during pregnancy	439	49	878	55	737	67	993	57

Table 5.1. (continued)

	Never n=893 (17%)		**<3 months n=1602 (30%)**		**-6 months n=1093 (21%)**		**≥6 months n=1734 (32%)**	
	Number - % or mean ± SD		Number - % or mean ± SD		Number - % or mean ± SD		Number - % or mean ± SD	
Parental history of atopy – n (%)								
No	462	52	833*	52	581*	53	840	48
Yes	432	48	769	48	512	47	894	52
Parity – n (%)								
0	468*	52	1037*	65	677*	62	947	55
≥1	426	48	565	35	416	38	787	45
Caesarean section – n (%)								
No	742*	83	1357*	85	943*	86	1547	89
Yes	152	17	245	15	150	14	186	11
Child characteristics								
Male – n (%)								
	464	52	818	51	554	51	832	48
Gestational age at birth – n (%)								
<37 weeks	52*	6	88*	5	61*	6	72	4
≥37 weeks	842	94	1514	95	1032	94	1662	96
Vitamin D supplementation age 6-12 months – n (%)								
No	450	50	941*	59	657*	60	786	45
Yes	444	50	661	41	436	40	948	55
Day care attendance first 2 years – n (%)								
No	248	28	369*	23	213*	19	423	24
Yes	646	72	1233	77	880	81	1311	76

* Significantly different from breastfeeding ≥6 months , $P< 0.05$

¶ Missing data for breastfeeding duration before multiple imputation n: 1083 (20%)

Table 5.2. Prevalence of lower and upper respiratory tract infections (n=5322)

	Age 2				**Age 3**				**Age 4**			
	Original data		**Multiple imputed**		**Original data**		**Multiple imputed**		**Original data**		**Multiple imputed**	
Outcome	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
LRTI												
No	4019	87	4591	86	3952	93	4884	92	4071	95	5011	94
Yes	594	13	732	14	316	7	438	8	201	5	311	6
Missing	709	13			1054	20			1050	20		
URTI												
No	2598	57	2954	56	2788	66	3412	64	3073	72	3666	69
Yes	1970	43	2368	44	1442	34	1910	36	1211	28	1656	31
Missing	754	14			1092	21			1038	20		

Duration of breastfeeding and respiratory tract infections

Compared to children who were never breastfed, breastfeeding for 6 months or longer was significantly associated with decreased risk of lower respiratory tract infections after infancy up to 4 years of age (aOR: 0.71; 95% CI: 0.51-0.98) (Table 5.3) (Supplementary Table 5.2). Breastfeeding for less than 3 months and breastfeeding for 3-6 months was not significantly associated with lower respiratory tract infections after infancy (Table 5.3) (Supplementary Table 5.2). Also, no significant association was found between breastfeeding for less than 3 months and 3-6 months, and upper respiratory tract infections (Table 5.3) (Supplementary Table 5.2). Compared to children who were never breastfed, breastfeeding for 6 months or longer was significantly associated with upper respiratory tract infections (OR: 0.78; 95% CI: 0.62-0.98). However, after correction for confounding variables the same trend was found, although non-significant (aOR:0.85; 95% CI: 0.69-1.05) (Table 5.3). The effects of the duration of breastfeeding on respiratory tract infections did not differ between the ages of 2, 3 and 4 years ($p_{interaction}$ >0.23 for lower and upper respiratory tract infections).

Dose of breastfeeding and respiratory tract infections

Partial breastfeeding until 4 months was significantly associated with a decreased risk of lower respiratory tract infections after infancy up to age 4 years (OR: 0.76; 95% CI: 0.59-0.99). However, the association did not remain significant after adjustment for confounders (aOR: 0.78; 95% CI: 0.59-1.02) (Table 5.4) (Supplementary Table 5.3). The same trend was found for predominant breastfeeding but not statistically significant (Table 5.4). Before multiple

Table 5.3. Association between breastfeeding duration and lower and upper respiratory tract infections up to age 4 years (n= 5322)

		Lower respiratory tract infections		**Upper respiratory tract infections**	
Breastfeeding	**n (%)**	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]
Never	893 (17)	Reference	Reference	Reference	Reference
<3 months	1602 (30)	0.75 (0.56-1.02)	0.75 (0.56-1.00)	0.87 (0.71-1.06)	0.86 (0.70-1.04)
3-6 months	1093 (21)	0.72 (0.48-1.06)	0.78 (0.53-1.13)	0.80 (0.64-1.00)	0.91 (0.73-1.12)
≥6 months	1734 (32)	**0.63 (0.46-0.87)**	**0.71 (0.51-0.98)**	**0.78 (0.62-0.98)**	0.85 (0.69-1.05)

OR: Odds Ratio; 95% confidence interval. OR's are compared to never-breastfed.

[a] Adjusted for caesarean section, maternal age, marital status, maternal ethnicity, maternal educational level, household income per month, maternal BMI before pregnancy, smoke exposure child, alcohol use during pregnancy, gender child, vitamin D supplementation age 6-12 months, day care attendance in the first two years of life, gestational age at birth, parity and parental history of atopy.

imputation, predominant breastfeeding was associated with lower respiratory tract infections (Supplementary Table 5.3). Partial breastfeeding until 4 months and predominant breastfeeding until 4 months was not significantly associated with upper respiratory tract infections after infancy (Table 5.4) (Supplementary Table 5.3). The effects of breastfeeding dose on respiratory tract infections did not differ between the ages of 2, 3 and 4 years ($p_{interaction}$ >0.59 for upper and lower respiratory tract infections).

Table 5.4. Association between exclusive breastfeeding and lower and upper respiratory tract infections up to age 4 years (n= 5322)

		Lower respiratory tract infections		**Upper respiratory tract infections**	
Breastfeeding	**n (%)**	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]
Never	862 (16)	Reference	Reference	Reference	Reference
Partially until 4 months	2870 (54)	**0.76 (0.59-0.99)**	0.78 (0.59-1.02)	0.87 (0.72-1.05)	0.89 (0.72-1.10)
Predominantly until 4 months	1590 (30)	0.66 (0.44-1.00)	0.72 (0.48-1.09)	0.84 (0.65-1.08)	0.93 (0.72-1.20)

OR: Odds Ratio; 95% confidence interval. OR's are compared to never-breastfed.
[a] Adjusted for caesarean section, maternal age, marital status, maternal ethnicity, maternal educational level, household income per month, maternal BMI before pregnancy, smoke exposure child, alcohol use during pregnancy, gender child, vitamin D supplementation age 6-12 months, day care attendance in the first two years of life, gestational age at birth, parity and parental history of atopy.

DISCUSSION

In this population-based prospective birth cohort study we found children who were breastfed for 6 months or longer to have a reduced risk of lower respiratory tract infections after infancy. Predominant breastfeeding until 4 months showed the same trend but this was not statistically significant. Also, similar direction of the effect estimates were found for the association between the duration and dose of breastfeeding and upper respiratory tract infections but not significant.

Various studies, including a previous study within our cohort, found exclusive breastfeeding for 6 months to be protective for the development of respiratory tract infections in infancy, thereby supporting the recommendation of the WHO [10, 19, 30]. Our study found breastfeeding for 6 months or longer to be associated with a reduced risk for lower respiratory tract infections after infancy till the age of 4 years. Contrary to our findings, a prospective longitudinal study found that breastfeeding duration, including breastfeeding longer than 6 months, was not associated with pneumonia or lung infection in 6 year old children [17]. However, the association was only examined in children who initiated breastfeeding whereas we also included children who were never breastfed. In

agreement with our findings on breastfeeding dose, Li et al [17] did not find breastfeeding exclusivity to be associated with lower respiratory tract infections. As for upper respiratory tract infections, we did not observe a significant association among children who were breastfed (duration and dose) compared to those who were never breastfed. Similarly, Chantry et al [19] found full breastfeeding for less than 6 months not to be associated with an increased risk of recurrent upper respiratory tract infections and recurrent otitis media in children 6-72 months of age. Li et al [17] also found no association between breastfeeding, including duration and exclusivity, and colds or upper respiratory tract infections among 6 year old children. The possibility that the protective effect of breastfeeding might wear off after breastfeeding cessation has previously been suggested [31-33]. Other studies that did find breastfeeding to be associated with a reduced risk of upper respiratory tract infections after infancy mainly focused on otitis media and mostly before the age of 3 years [20, 34, 35]. Some studies examined the effect of breastfeeding on respiratory tract infections in general. A Japanese study reported breastfeeding duration for 6-7 months to be borderline significantly associated with a reduced risk of hospitalization for respiratory tract infections between the age of 18-30 months [21]. Respiratory tract infections for which hospitalization is needed are often more serious and mainly infections of the lower respiratory tract [2, 36] which might explain the discrepancy between these latter results and those from our study. Conversely, another study did not find a protective effect of breastfeeding for all acute respiratory illness in children 1-6 years [20] which might be due to an overrepresentation of upper respiratory tract infections since these symptoms are more common in childhood [2].

The WHO definition of exclusive breastfeeding allows for ORS, drops and syrups but no other food or drink, not even water [23]. Therefore, this study examined the effect of predominant breastfeeding defined as no infant formula, milk or solid foods. This study cannot examine the effect of predominant breastfeeding per month neither for the duration of 6 months or longer due to small group size. The majority of mothers in the Netherlands do not continue breastfeeding after the age of 4 months [37]. Thus, our study precludes conclusions on the effect of exclusive breastfeeding for 6 months as defined by the WHO. In line with our findings, a birth cohort study from Hong Kong did not find exclusive and partial breastfeeding for 3 months to be associated with a reduced risk for hospital admissions for respiratory tract infections after the age of 6 months up to age 8 years [16]. However, Yamakawa et al.[21] did find exclusive breastfeeding at 6-7 months of age to be significantly associated with hospitalization for respiratory tract infections between the age of 18-42 months. Also, Li et al.[17] reported exclusive breastfeeding for 6 months and longer, compared to breastfeeding between 0 to 4 months, to be significantly associated with a reduced odds for ear, throat, and sinus infection at age 6 years. We performed multiple imputation to account for bias associated with missing data. Children with and without questionnaire data differed in socioeconomic background, ethnicity, and a selection towards a relative more healthy study population seems to be present [22]. However, this would only affect the interpretation of our results if the association between breastfeeding and respiratory

infections was different for children without questionnaire data compared with those with questionnaire data, which is unlikely. For the analyses on breastfeeding duration ≥ 6 months and lower respiratory tract infections, results were comparable in the original data (aOR: 0.56; 95% CI: 0.32-0.99) and after the multiple imputation procedure (aOR: 0.71; 95% CI: 0.51-0.98). However, for the analyses on predominant breastfeeding and lower respiratory tract infections the estimate in the original data analysis (aOR: 0.53; 95% CI: 0.30-0.93) slightly weakened after the imputation procedure (aOR: 0.72; 95% CI: 0.48-1.09). This would suggest that the missing data was not completely random and affected the uncertainty of the effect estimates for predominant breastfeeding.

An important strength of this study is the large study population drawn from the general population. On the basis of previous findings in our cohort, respiratory illnesses are socially patterned and related to several mother and child characteristics [38]. Our study design provided information on multiple potential confounders and allowed for follow-up into childhood. However, due to the observational nature of our study, residual confounding cannot be fully excluded. In addition, the prospective design made it possible to obtain information on breastfeeding at multiple time points during infancy therefore limiting recall bias. Whereas other studies examined the effect of respiratory tract infections in general, or focused on specific infections [20, 21, 34, 35], we examined the effect of breastfeeding on the development of lower and upper respiratory tract infections separately.

A weakness may be that the diagnosis of respiratory tract infection was obtained by parent-reported questionnaires at yearly intervals. The questions used to obtain information on respiratory infections were comparable to other studies. Parents were asked whether their child had suffered from a respiratory tract infection and whether they had visited a doctor for this infection since physician diagnosis is more accurate. However, this could have led to misclassification of the outcome as parents may not be able to distinguish between lower and upper respiratory tract infections and children who had not visited a doctor may even so have suffered from a respiratory tract infection. However, since the outcome was measured after the breastfeeding period we do not expect such misclassification to be differential and to have influenced the effect of the duration or dose of breastfeeding. Also, our study did not have information on the number of episodes of infection. Li et al.[17] found a relation between two or more visits to the physician and breastfeeding duration and exclusivity.

Several long-term effects of breastfeeding on the offspring have been reported [3-5, 15]. Different mechanisms for the stimulation of the immune response by breastfeeding have been suggested, among others transfer of anti-idiotypic antibodies and lymphocytes [6, 13]. However, the mechanism by which breastfeeding might add to a long-term protection remain unclear.

In conclusion, this study showed that breastfeeding duration for 6 months or longer is associated with a reduced risk of lower respiratory tract infections, but not upper respiratory tract infections, in pre-school children. Predominant breastfeeding until 4 months was not associated with a reduced risk for respiratory tract infections in childhood. These findings are

compatible with the hypothesis that the protective effect of the duration of breastfeeding for respiratory tract infections persist after infancy therefore supporting current WHO recommendations for breastfeeding for at least 6 months also in industrialized countries.

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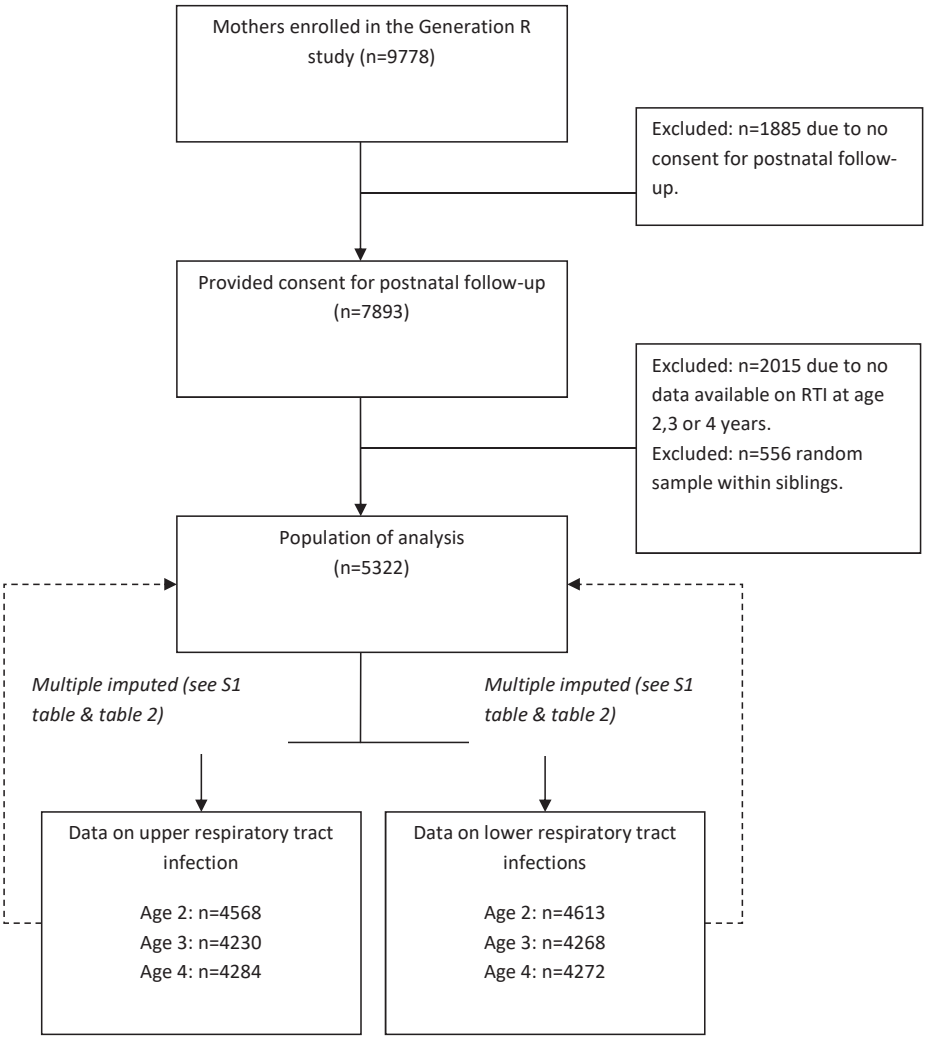
SUPPLEMENT CHAPTER 5



Supplementary Figure 5.1. Flowchart of the participants within the Generation R Study

Supplementary Table 5.1. Maternal and child characteristics (n=5322)

	Original data		Multiple imputed	
	n	%	n	%
Maternal characteristics				
Maternal age *Mean (SD)*	31.1	4.9	31.1	4.9
Missing	0	0		
Educational level				
Low	901	18	1004	19
Mid	2638	53	2799	52
High	1471	29	1519	29
Missing	312	6		
Ethnicity				
Western	3534	69	3652	69
Non-Western	1566	31	1670	31
Missing	222	4		
Household income per month				
≤2200 euro	1556	37	2075	39
>2200 euro	2704	64	3247	61
Missing	1062	20		
Marital status				
Married/ Living together	4538	90	4788	90
No partner	497	10	534	10
Missing	287	5		
Maternal BMI before pregnancy *Mean (SD)*	23.5	4.1	23.5	4.0
Missing	1325	25		
Smoking during pregnancy				
Never	3290	78	4114	77
Smoked during pregnancy	952	22	1208	23
Missing	1080	20		
Smoking in presence of child				
Smoking mother	543	16	912	17
Missing	1885	35		
Smoking in the home	174	5	392	7
Missing	1884	35		
Smoking in other places	448	13	802	15
Missing	1901	36		
Alcohol use during pregnancy				
Never	1803	42	2275	43
Drank alcohol during pregnancy	2466	58	3047	57
Missing	1053	20		
Parental history of atopy	2459	49	2606	49
Missing	322	6		
Multiparity	2111	41	2195	41
Missing	166	3		

Supplementary Table 5.1. (continued)

	Original data		Multiple imputed	
	n	%	n	%
Caesarean section	660	14	733	14
Missing	538	10		
Breastfeeding				
Never	398	9	893	17
<3 months	1466	35	1602	30
3-6 months	860	20	1093	21
≥6 months	1515	36	1734	32
Missing	1083	20		
Breastfeeding				
Never	406	10	862	16
Partially until 4 months	2647	64	2870	54
Predominantly until 4 months	1056	26	1590	30
Missing	1213	23		
Child characteristics				
Male	2667	50	2667	50
Missing	0	0		
Preterm birth[1]	268	5	272	5
Missing	25	1		
Vitamin D supplementation age 6-12 months	1873	45	2489	71
Missing	1112	21		
Day care attendance first 2 years	3140	95	4070	76
Missing	2013	38		

[1]Preterm birth is defined as <37 weeks.

Supplementary Table 5.2. Association between breastfeeding duration and lower and upper respiratory tract infections up to age 4 years (ORIGINAL DATA)

		Lower respiratory tract infections		Upper respiratory tract infections	
Breastfeeding	**n (%)**	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]
Never	398 (9)	Reference	Reference	Reference	Reference
<3 months	1466 (35)	**0.73 (0.55-0.96)**	0.71 (0.41-1.21)	0.97 (0.81-1.15)	0.79 (0.56-1.14)
3-6 months	860 (20)	**0.68 (0.50-0.92)**	0.59 (0.34-1.04)	0.87 (0.73-1.05)	0.95 (0.66-1.37)
≥6 months	1515 (36)	**0.59 (0.45-0.79)**	**0.56 (0.32-0.99)**	0.86 (0.72-1.02)	0.91 (0.63-1.31)

OR: Odds Ratio; 95% confidence interval. OR's are compared to never-breastfed.
[a] Adjusted for caesarean section, maternal age, marital status, maternal ethnicity, maternal educational level, household income per month, maternal BMI before pregnancy, smoke exposure child, alcohol use during pregnancy, gender child, vitamin D supplementation age 6-12 months, day-care attendance in the first two years of life, gestational age at birth, parity and parental history of atopy.

Supplementary Table 5.3. Association between breastfeeding dose and lower and upper respiratory tract infections up to age 4 years (ORIGINAL DATA)

		Lower respiratory tract infections		Upper respiratory tract infections	
Breastfeeding	**n (%)**	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]	**Univariate model OR (95 % CI)**	**Multivariable model 1 aOR (95 % CI)** [a]
Never	406 (10)	Reference	Reference	Reference	Reference
Partially until 4 months	2647 (64)	**0.71 (0.54-0.92)**	0.66 (0.39-1.11)	0.93 (0.79-1.10)	0.86 (0.61-1.21)
Predominantly until 4 months	1056 (26)	**0.52 (0.39-0.71)**	**0.53 (0.30-0.93)**	0.84 (0.70-1.01)	0.95 (0.66-1.37)

OR: Odds Ratio; 95% confidence interval. OR's are compared to never-breastfed.
[a] Adjusted for caesarean section, maternal age, marital status, maternal ethnicity, maternal educational level, household income per month, maternal BMI before pregnancy, smoke exposure child, alcohol use during pregnancy, gender child, vitamin D supplementation age 6-12 months, day-care attendance in the first two years of life, gestational age at birth, parity and parental history of atopy.

6

Dietary patterns and respiratory symptoms in pre-school children

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Eur Respir J. 2012; 40: 681–689

ABSTRACT

Background: Overall diet in early childhood may affect the development of respiratory symptoms. This study examined whether childhood dietary patterns are associated with respiratory symptoms in Dutch pre-school children, and whether this association could be explained by energy intake.

Methods: A prospective cohort study was performed in 2,173 children aged ≤4 yrs. Data on asthma-related symptoms were obtained by questions from the age-adapted version of the "International Study of Asthma and Allergies in Childhood" questionnaires. Data on respiratory tract infections, defined as episodes of physician attended fever with respiratory symptoms, was obtained by questionnaire. Principal components analysis was used to develop dietary patterns at 14 months of age.

Results: Compared with low adherence, high adherence to the "Western" dietary pattern was significantly associated with frequent wheeze at 3 yrs of age (relative risk (RR) 1.39, 95% CI 1.02–1.89) and frequent shortness of breath (RR 1.44, 95% CI 1.03–2.01) and respiratory tract infections (RR 1.54, 95% CI 1.08–2.19) at 4 yrs of age. However, this association was partially explained by energy intake.

Conclusion: A "Western" diet may increase the risk of frequent respiratory symptoms at 3 and 4 yrs of age. In some measure, this association was explained by energy intake.

INTRODUCTION

Atopic diseases are common in children and have been increasing in prevalence. Asthma is the most common chronic disease in childhood [1]. Infectious diseases, particularly of the respiratory tract are a leading cause of morbidity and hospitalisation in infants and children in industrialised countries [2]. Both asthma and infectious disease cause significant burden of disease in childhood [3], and identifying risk factors for the development of these diseases is of interest. The immune system undergoes substantial maturation from fetal life until childhood. The adequacy of this maturation process depends on environmental factors of which nutrition is suggested to play a role [4]. Diet during pregnancy has been suggested to be associated with asthma-related symptoms in the offspring [5, 6]. Duration and exclusiveness of breastfeeding has been found to be related to asthma and respiratory tract infections in infancy and childhood [2, 7, 8]. In addition, nutrition beyond the weaning period may also be of importance [4]. Relatively little attention has been paid to the influence of diet beyond the weaning period on respiratory symptoms. Traditional analyses in nutritional epidemiology often examine disease in relation to specific nutrients or foods [9]. Intake of specific nutrients and foods during childhood has a relationship with the development of asthma and respiratory tract infections [10, 11]. In addition, intake of calorie-rich foods has been associated with a higher prevalence of asthma [12]. However, children eat a variety of foods with complex combinations of nutrients that are likely to be interactive or synergistic [9]. Therefore, dietary pattern analysis examining the association of overall diet may be more predictive of disease risk. So far, studies examining the effect of overall diet in childhood mainly focused on a traditional Mediterranean diet. These studies found a Mediterranean diet in early life to be associated with the development of asthma-related symptoms in childhood [13, 14]. No studies have examined the effect of a Western diet in childhood on the development of respiratory symptoms in children. However, a Western diet has been found to be associated with an increased risk of frequent asthma attacks in adult females [15]. The aim of this study was to examine whether different childhood dietary patterns are associated with respiratory symptoms in Dutch children up to 4 yrs of age. A second aim was to examine whether this association could be explained by total energy intake.

SUBJECTS AND METHODS

Participants and study design

This study was embedded in the Generation R study, a population-based prospective cohort study from fetal life until young adulthood and has been described in detail previously [16]. In total, 9,778 mothers with a delivery date from April 2002 through to January 2006 enrolled in the study. Consent for post-natal follow-up was provided by 7,893 participants. Data collection on nutritional intake of the child was implemented in the study from 2003 onwards. In total, 5,088 mothers received a food-frequency questionnaire (FFQ) for their child at the age of 14 months (Figure 6.1). The study was approved by the medical ethical review board of the Erasmus Medical Center, Rotterdam, the Netherlands.

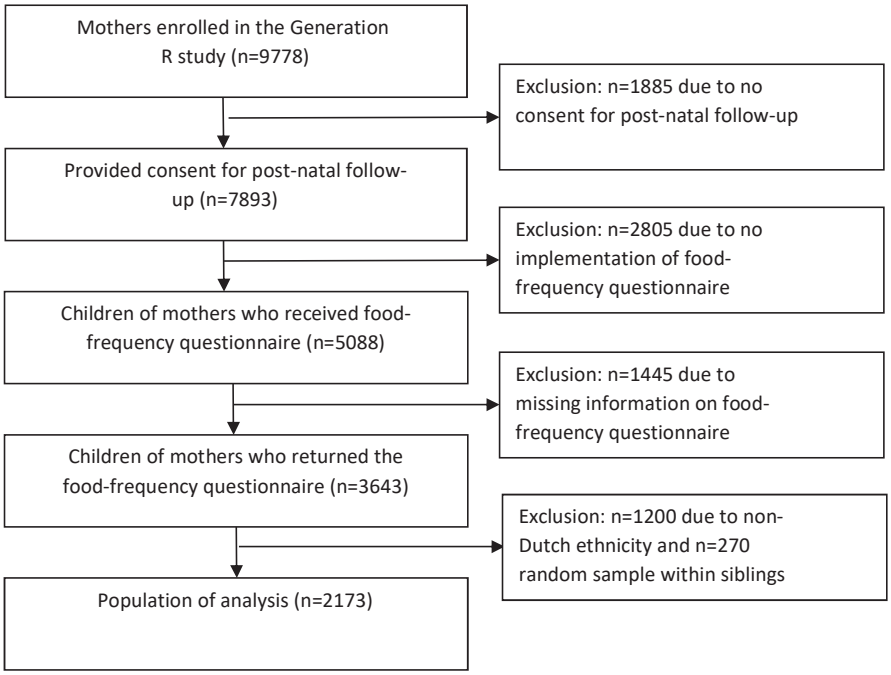

Figure 6.1. Flow chart summarising the participants of the Generation R study

Respiratory symptoms

Asthma-related symptoms

Data on asthma-related symptoms were obtained by questions adapted from the "International Study of Asthma and Allergies in Childhood" (ISAAC) core questionnaires on asthma at the age of 2, 3 and 4 yrs. These questions were made suitable for younger children and have been previously used in other studies [17]. Asthma symptoms, including wheezing and shortness of breath, were categorised according to frequency as follows: never, 1–3 times and ≥4 times [17]. Questionnaire response rates were 69%, 64% and 63% at the age of 2, 3 and 4 yrs, respectively.

Respiratory tract infections

Data on respiratory tract infections were obtained by parent-reported questionnaires at the age of 2, 3 and 4 yrs. Respiratory tract infections were defined by the number of parent-reported physician visit(s) for fever with respiratory symptoms; coughing, runny or blocked nose, or earache. Subsequently, respiratory tract infections were categorised according to frequency as follows: never, 1–2 times and ≥3 times [17].

Dietary patterns

At the child's age of 14 months (±2 months) parents were asked to complete a FFQ. The FFQ was developed in cooperation with the division of Human Nutrition of Wageningen University, Wageningen, the Netherlands, and based on an existing validated food questionnaire described in detail previously [18], and adapted with food products frequently consumed according to a Dutch food consumption survey in infants [19]. The FFQ was validated against 3-day, 24-h recalls in Dutch children at the age of 14 months. The intra-class correlation coefficients for macronutrients were as follows: total energy, 0.4; total protein, 0.7; total fat, 0.4; carbohydrates, 0.4; and dietary fibre, 0.7. The frequency of consumption of a food item was to be recorded per day, per week, or per month over the past 4 weeks. Subjects were asked to report their regular portion size relative to the standard portion size according to the Dutch table of regular portion sizes and household units [20]. Total nutrient content was calculated per item according to the Dutch Nutrient Composition table [21]. Response rate was 72% (n= 3,643) (Figure 6.1).

Covariates

Variables possibly related to the outcomes such as sex, gestational age and birthweight were obtained from obstetric records assessed in midwife practices and hospital registries [16]. Additional potential confounders were assessed by a combination of pre- and post-natal questionnaires completed by both parents. The questionnaires included information on maternal age, maternal socioeconomic status, maternal smoking during pregnancy, multiple parities and parental history of atopy. Maternal socioeconomic status was defined according to educational level as follows. Low: no education, primary school or <3 yrs of secondary school; mid: >3 yrs of secondary school, higher vocational training or bachelor's degree; and high: academic education. Data on breastfeeding were collected by a combination of delivery reports and post-natal questionnaires at the age of 2, 6 and 12 months. Breastfeeding was classified as (I) never, (II) exclusively for 6 months, (III) exclusively for 4 months and partially at 6 months, (IV) exclusively for 4 months, with no breastfeeding at 6 months, (V) partially for 6 months, (VI) partially for 4 months, with no breastfeeding at 6 months. Cow's milk allergy in the first year of life was assessed by questionnaire at the age of 6 and 12 months by asking parents whether their child had attended a doctor for cow's milk allergy. At the age of 12 months parents were asked about vitamin D supplementation during the past 6 months. Post-natal questionnaires completed by the mother at age 12 and 24 months included information on daycare attendance.

Population for analyses

To avoid the influence of culture differences in the definition of the dietary patterns, only children of Dutch origin were included in the analyses (n= 2,443). Siblings within the Generation R cohort were randomly selected and excluded (n= 270). To prevent bias associated with missing data, variables with missing values were multiple imputed (five imputations) based on the correlation between the variable with missing values with other

patient characteristics [22]. Consequently, n= 2,173 were available after multiple imputation for statistical analyses (Figure 6.1).

Statistical methods

The FFQ included 211 food items which were grouped into 21 different food groups. The 21 food groups were subjected to principal components analysis (PCA). The scree plot from the PCA showed a clear break in the curve after the second component revealing the presence of two dietary patterns with Eigen values of 3.4 and 1.7. The percentage of variance explained by the dietary patterns was 16.3% and 8.2%. In the PCA, varimax rotation was used to obtain uncorrelated factors. For reasons of interpretability, the population of analysis was categorised into tertiles according to their score for the dietary pattern as follows: low, moderate and high (using "low" score as reference category).

Log-binomial regression analyses were performed with the primary outcome variables; wheezing, shortness of breath and respiratory tract infections separately at the age of 2, 3 and 4 yrs. The defined dietary patterns at the age of 14 months were analysed as primary exposure and adjusted for potential confounders. The selection of potential confounders was performed by the alteration in relative risks and kept in the multivariate model in case of an alteration of ≥10% (multivariate model 1). To assess whether the association between the dietary patterns and respiratory symptoms was explained by total energy intake additional analysis were performed with adjustment for total energy intake resulting in a separate multivariate model (multivariate model 2). The pooled results of the five imputed datasets were reported in this paper as relative risks and 95% confidence intervals. A p-value <0.05 was considered as statistically significant. The statistical analyses were performed using Stata Statistical Software for Windows, release 11 (Stata Corp., College Station, TX, USA).

RESULTS

Study population

Maternal and child characteristics of the study population are presented in table 6.1 and the prevalence of the outcomes of interest in supplementary table 6.1.

Dietary patterns

The factor loadings of the food groups in the two dietary patterns present are shown in supplementary table 6.2. The individual factor loading scores for the food groups are correlation coefficients between the food products and the specific dietary pattern. Dietary pattern 1 was associated with starchy foods, fruit, vegetables, potatoes, vegetable oils, fish, legumes and meat. This pattern is referred to here as the "Health conscious" dietary pattern. Dietary pattern 2 was associated with refined grains, soups and sauces, savoury and snacks, other fats, sugar-containing beverages and meat. This pattern will be referred to as the "Western" dietary pattern.

Table 6.1. Maternal and child characteristics

Characteristics	Original data		After multiple imputation procedure	
Mother				
Maternal age yrs	31.8	±4.25	31.8	±4.25
Missing	0	0	-	-
Socioeconomic status %				
Low	230	10	232	11
Mid	1151	53	1157	53
High	777	36	784	36
Missing	15	1	-	-
Maternal smoking during pregnancy %	385	18	461	21
Missing	371	17	-	-
Multiple parities %	732	34	760	35
Missing	54	3	-	-
Parental history of atopy %	1121	52	1141	53
Missing	36	2	-	-
Child				
Male %	1082	50	1082	50
Missing	0	0	-	-
Standard deviation score birthweight	0.06	±1.01	0.06	±1.03
Missing	178	8	-	-
Breastfeeding %				
Never	213	10	229	11
6 months exclusive	33	2	49	2
4 months exclusive, partially at 6 months	371	17	387	18
4 months exclusive, no breastfeeding at 6 months	153	7	161	7
6 months partially	172	8	185	9
4 months partially, no breastfeeding at 6 months	1008	46	1161	53
Missing	223	10	-	-
History of cow's milk allergy first year %	124	6	237	11
Missing	444	20	-	-
Vitamin D supplement use 6-12 months %	750	35	801	37
Missing	127	6	-	-
Day care attendance first 2 yrs %	1493	69	1807	83
Missing	575	27	-	-
Total energy intake at 14 months	1276	±351	1276	±351
Missing	0	0	-	-

Data are presented as mean±SD or n (%). n= 2,173.

Dietary patterns and respiratory symptoms

High adherence to the "Western" dietary pattern was significantly associated with frequent shortness of breath (≥4) (RR 1.43, 95% CI 1.01–2.03) at age 2 yrs (table 6.2). High adherence to the "Western" dietary pattern was also significantly associated with frequent wheeze (≥4) (RR 1.39, 95% CI 1.02–1.89) and frequent shortness of breath (≥4) (RR 1.66, 95% CI 1.24–2.21) at age 3 yrs (tables 6.2 and 6.3). However, the association between the "Western" dietary pattern and frequent shortness of breath (≥4) at the age of 2 and 3 yrs was mainly explained by maternal age, maternal socioeconomic status, maternal smoking during pregnancy, parental history of atopy, multiple parities, standard deviation score (SDS) birthweight, sex, breastfeeding, vitamin D supplementation, daycare attendance and history of cow's milk allergy (table 6.2). High adherence to the "Western" dietary pattern was also significantly associated with frequent wheeze (≥4) (RR 1.70, 95% CI 1.22–2.36) and shortness of breath (≥4) (RR 1.44, 95% CI 1.03–2.01) at age 4 yrs (tables 6.2 and 6.3). However, the association between the "Western" dietary pattern and frequent wheeze (≥4) at age 4 yrs was mainly explained by the variables mentioned previously (table 6.3). Adherence to the "Western" dietary pattern was not significantly associated with frequent wheeze at age 2 yrs, or short-term wheeze (1–3 times) or shortness of breath (1–3 times) up to 4 yrs of age. High adherence to the "Western" diet was significantly associated with frequent respiratory tract infections (≥3) (RR 1.54, 95% CI 1.08–2.19) at 4 yrs of age (table 6.4). Adherence to the "Western" diet was not significantly associated with respiratory tract infections at 2 and 3 yrs of age or short-term respiratory tract infections (1–2 times) at 4 yrs of age. Adherence to the "Health conscious" diet was not significantly associated with respiratory symptoms up to 4 yrs of age (tables 6.2, 6.3 and 6.4).

Influence of total energy intake

After adjustment for total energy intake, high adherence to the "Western" dietary pattern remained significantly associated with frequent wheeze (≥4) (aRR 1.47, 95% CI 1.04–2.07) at 3 yrs of age and frequent respiratory tract infections (≥3) (aRR 1.46, 95% CI 1.00–2.13) at 4 yrs of age (tables 6.3 and 6.4). After additional adjustment for total energy intake adherence to the "Western" dietary pattern was no longer significantly associated with shortness of breath up to 4 yrs of age (table 6.2). Adherence to the "Western" dietary pattern remained not significantly associated with any respiratory symptom at age 2 yrs or short-term respiratory symptom up to 4 yrs of age. Adherence to the "Health conscious" dietary pattern remained not significantly associated with any respiratory symptom up to 4 yrs of age (tables 6.2, 6.3 and 6.4).

DISCUSSION

In this population-based prospective birth cohort study, we observed a higher risk of frequent respiratory symptoms among children at the age of 3 and 4 yrs who had a greater adherence to a "Western" diet in early childhood. However, this association was partially

Table 6.2. Association between the "Health conscious" versus "Western" dietary pattern and shortness of breath

	Shortness of breath					
	1-3 times			≥ 4 times		
Dietary pattern (n= 2173)	**Univariate model RR (95 % CI)**	**Multivariate model 1 aRR (95 % CI) [a]**	**Multivariate model 2 aRR (95 % CI) [b]**	**Univariate model RR (95 % CI)**	**Multivariate model 1 aRR (95 % CI) [a]**	**Multivariate model 2 aRR (95 % CI) [b]**
			Year 2			
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.96	0.93	0.95	0.46	0.84	0.85
	(0.71-1.30)	(0.69-1.27)	(0.70-1.28)	(0.55-1.31)	(0.56-1.27)	(0.58-1.26)
High adherence	1.07	1.05	1.09	0.83	0.93	0.94
	(0.81-1.43)	(0.78-1.41)	(0.79-1.51)	(0.47-1.91)	(0.46-1.91)	(0.55-1.60)
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.95	0.88	0.89	1.27	1.14	1.18
	(0.68-1.32)	(0.63-1.22)	(0.65-1.23)	(0.73-2.18)	(0.67-1.95)	(0.72-1.92)
High adherence	1.15	1.02	1.08	**1.43**	1.22	1.27
	(0.84-1.58)	(0.73-1.42)	(0.77-1.52)	**(1.01-2.03)**	(0.79-1.90)	(0.87-1.86)
			Year 3			
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.99	0.96	0.97	1.11	0.90	0.89
	(0.68-1.45)	(0.65-1.41)	(0.66-1.43)	(0.67-1.20)	(0.67-1.21)	(0.66-1.20)
High adherence	0.88	1.07	1.09	1.01	0.98	0.95
	(0.78-1.65)	(0.74-1.56)	(0.73-1.61)	(0.76-1.35)	(0.73-1.32)	(0.70-1.29)

Table 6.2. (continued)

	Shortness of breath					
	1-3 times			≥ 4 times		
Dietary pattern (n= 2173)	**Univariate model RR (95 % CI)**	**Multivariate model 1 aRR (95 % CI) [a]**	**Multivariate model 2 aRR (95 % CI) [b]**	**Univariate model RR (95 % CI)**	**Multivariate model 1 aRR (95 % CI) [a]**	**Multivariate model 2 aRR (95 % CI) [b]**
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.85	0.82	0.83	0.88	0.81	0.82
	(0.58-1.23)	(0.56-1.19)	(0.57-1.21)	(0.64-1.19)	(0.58-1.12)	(0.58-1.14)
High adherence	1.13	1.01	1.03	**1.66**	1.31	1.32
	(0.79-1.62)	(0.70-1.48)	(0.68-1.54)	**(1.24-2.21)**	(0.95-1.80)	(0.93-1.88)
	Year 4					
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.90	0.85	0.91	0.89	0.87	0.84
	(0.60-1.37)	(0.56-1.29)	(0.59-1.39)	(0.65-1.22)	(0.63-1.21)	(0.61-1.17)
High adherence	0.87	0.79	0.92	1.16	1.08	0.99
	(0.58-1.31)	(0.52-1.20)	(0.58-1.44)	(0.85-1.57)	(0.78-1.49)	(0.71-1.40)
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	1.12	1.08	1.15	1.09	1.03	1.01
	(0.76-1.67)	(0.73-1.62)	(0.76-1.73)	(0.79-1.51)	(0.74-1.44)	(0.72-1.41)
High adherence	0.85	0.68	0.81	**1.84**	**1.44**	1.36
	(0.56-1.30)	(0.44-1.07)	(0.50-1.32)	**(1.35-2.51)**	**(1.03-2.01)**	(0.95-1.96)

Data are presented as relative risk (RR) (95% CI) or adjusted (a)RR (95% CI). RRs are compared with low adherence to the dietary pattern. [a]: adjusted for maternal age, maternal socioeconomic status, smoking during pregnancy, parental history of atopy, multiple parities, standard deviation score birthweight, sex, breastfeeding, vitamin D supplementation at 6–12 months, daycare attendance in the first 2 yrs of life, and history of cow's milk allergy in the first year. [b]: adjusted for multivariate model 1 + total energy intake. Entries in bold are statistically significant.

Table 6.3. Association between the "Health conscious" versus "Western" dietary pattern and wheeze

	Wheeze					
	1-3 times			≥ 4 times		
Dietary pattern (n= 2173)	**Univariate model RR (95% CI)**	**Multivariate model 1 aRR (95% CI) [a]**	**Multivariate model 2 aRR (95% CI) [b]**	**Univariate model RR (95% CI)**	**Multivariate model 1 aRR (95% CI) [a]**	**Multivariate model 2 aRR (95% CI) [b]**
Year 2						
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	1.21	1.20	1.19	0.92	0.89	0.87
	(0.89-1.65)	(0.88-1.65)	(0.86-1.64)	(0.56-1.52)	(0.53-1.52)	(0.50-1.51)
High adherence	1.20	1.19	1.16	1.14	1.08	1.02
	(0.89-1.62)	(0.87-1.62)	(0.84-1.62)	(0.62-2.11)	(0.59-2.00)	(0.54-1.91)
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.88	0.86	0.84	0.87	0.82	0.80
	(0.65-1.18)	(0.64-1.16)	(0.62-1.14)	(0.56-1.36)	(0.53-1.27)	(0.51-1.23)
High adherence	0.97	0.94	0.89	1.14	0.98	0.90
	(0.73-1.30)	(0.68-1.29)	(0.63-1.25)	(0.74-1.74)	(0.59-1.63)	(0.50-1.63)
Year 3						
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.97	0.96	0.97	0.93	0.93	0.93
	(0.66-1.43)	(0.65-1.41)	(0.65-1.43)	(0.69-1.25)	(0.69-1.26)	(0.68-1.27)
High adherence	1.08	1.04	1.04	1.01	0.97	0.96
	(1.73-1.61)	(0.70-1.55)	(0.69-1.58)	(0.75-1.37)	(0.71-1.34)	(0.69-1.34)

Table 6.3. (continued)

	Wheeze					
	1-3 times			≥ 4 times		
Dietary pattern (n= 2173)	**Univariate model RR (95% CI)**	**Multivariate model 1 aRR (95% CI) [a]**	**Multivariate model 2 aRR (95% CI) [b]**	**Univariate model RR (95% CI)**	**Multivariate model 1 aRR (95% CI) [a]**	**Multivariate model 2 aRR (95% CI) [b]**
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.93	0.92	0.94	0.90	0.84	0.86
	(0.63-1.39)	(0.62-1.38)	(0.62-1.40)	(0.64-1.26)	(0.59-1.19)	(0.60-1.22)
High adherence	1.15	1.13	1.15	**1.75**	**1.39**	**1.47**
	(0.77-1.73)	(0.75-1.71)	(0.74-1.78)	**(1.31-2.34)**	**(1.02 -1.89)**	**(1.04-2.07)**
	Year 4					
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.81	0.78	0.79	0.98	0.96	0.93
	(0.56-1.19)	(0.53-1.16)	(0.53-1.17)	(0.69-1.39)	(0.67-1.37)	(0.65-1.32)
High adherence	0.98	0.94	0.94	1.25	1.19	1.09
	(0.67-1.44)	(0.64-1.39)	(0.63-1.42)	(0.92-1.70)	(0.87-1.64)	(0.78-1.52)
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.99	0.95	0.95	1.04	0.98	0.95
	(0.68-1.45)	(0.65-1.39)	(0.64-1.40)	(0.74-1.47)	(0.69-1.40)	(0.67-1.36)
High adherence	1.04	0.86	0.84	**1.70**	1.39	1.28
	(0.71-1.53)	(0.57-1.29)	(0.55-1.29)	**(1.22-2.36)**	(0.99-1.94)	(0.89-1.83)

Data are presented as relative risk (RR) (95% CI) or adjusted (a)RR (95% CI). RRs are compared with low adherence to the dietary pattern. [a]: adjusted for maternal age, maternal socioeconomic status, smoking during pregnancy, parental history of atopy, multiple parities, standard deviation score birthweight, sex, breastfeeding, vitamin D supplementation at 6–12 months, daycare attendance in the first 2 yrs of life, and history of cow's milk allergy in the first year. [b]: adjusted for multivariate model 1 + total energy intake. Entries in bold are statistically significant.

Table 6.4. Association between the "Health conscious" versus "Western" dietary pattern and respiratory tract infections

	Respiratory tract infections					
	1-3 times			≥ 4 times		
Dietary pattern (n= 2173)	Univariate model RR (95 % CI)	Multivariate model 1 aRR (95 % CI) [a]	Multivariate model 2 aRR (95 % CI) [b]	Univariate model RR (95 % CI)	Multivariate model 1 aRR (95 % CI) [a]	Multivariate model 2 aRR (95 % CI) [b]
			Year 2			
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.87	0.87	0.85	0.71	0.95	0.92
	(0.66-1.15)	(0.66-1.15)	(0.64-1.13)	(0.75-1.25)	(0.73-1.23)	(0.71-1.19)
High adherence	1.03	1.00	0.97	0.90	0.86	0.78
	(0.77-1.37)	(0.75-1.34)	(0.71-1.32)	(0.66-1.23)	(0.62-1.19)	(0.56-1.09)
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.98	0.99	0.97	0.92	0.89	0.87
	(0.74-1.29)	(0.75-1.30)	(0.73-1.28)	(0.71-1.19)	(0.69-1.16)	(0.67-1.13)
High adherence	1.04	1.04	0.99	1.11	1.01	0.93
	(0.79-1.37)	(0.78-1.38)	(0.73-1.36)	(0.86-1.43)	(0.78-1.32)	(0.70-1.24)
			Year 3			
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	1.17	1.16	1.17	1.07	1.08	1.08
	(0.87-1.58)	(0.86-1.57)	(0.87-1.58)	(0.79-1.46)	(0.79-1.48)	(0.79-1.48)
High adherence	1.22	1.18	1.22	1.14	1.13	1.12
	(0.89-1.66)	(0.85-1.65)	(0.87-1.69)	(0.81-1.61)	(0.80-1.60)	(0.78-1.61)

Table 6.4. (continued)

	Respiratory tract infections					
	1-3 times			≥ 4 times		
Dietary pattern (n= 2173)	**Univariate model RR (95 % CI)**	**Multivariate model 1 aRR (95 % CI) [a]**	**Multivariate model 2 aRR (95 % CI) [b]**	**Univariate model RR (95 % CI)**	**Multivariate model 1 aRR (95 % CI) [a]**	**Multivariate model 2 aRR (95 % CI) [b]**
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	1.03	1.02	1.02	0.94	0.91	0.91
	(0.78-1.37)	(0.77-1.35)	(0.77-1.36)	(0.69-1.29)	(0.67-1.25)	(0.66-1.26)
High adherence	1.05	1.01	1.03	1.29	1.12	1.12
	(0.66-1.67)	(0.69-1.49)	(0.66-1.60)	(0.92-1.79)	(0.79-1.59)	(0.79-1.59)
	Year 4					
Health conscious dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	0.91	0.90	0.88	1.08	1.07	1.03 (0.69-1.55)
	(0.68-1.21)	(0.67-1.21)	(0.65-1.17)	(0.75-1.55)	(0.72-1.59)	
High adherence	0.85	0.83	0.77	1.09	1.02	0.92 (0.61-1.39)
	(0.62-1.17)	(0.60-1.13)	(0.55-1.06)	(0.76-1.57)	(0.70-1.50)	
Western dietary pattern						
Low adherence	Reference	Reference	Reference	Reference	Reference	Reference
Moderate adherence	1.12	1.13	1.12	1.01	1.01	1.00
	(0.84-1.50)	(0.85-1.50)	(0.84-1.49)	(0.70-1.46)	(0.69-1.47)	(0.68-1.45)
High adherence	1.36	1.35	1.32	**1.83**	**1.54**	**1.46**
	(0.85-2.17)	(0.90-2.03)	(0.86-2.03)	**(1.28-2.61)**	**(1.08-2.19)**	**(1.00-2.13)**

Data are presented as relative risk (RR) (95% CI) or adjusted (a)RR (95% CI). RRs are compared with low adherence to the dietary pattern. [a]: adjusted for maternal age, maternal socioeconomic status, smoking during pregnancy, parental history of atopy, multiple parities, standard deviation score birthweight, sex, breastfeeding, vitamin D supplementation at 6–12 months, daycare attendance in the first 2 yrs of life, and history of cow's milk allergy in the first year. [b]: adjusted for multivariate model 1 + total energy intake. Entries in bold are statistically significant.

explained by total energy intake. No association was found between a "Health conscious" diet and respiratory symptoms up to 4 yrs of age.

Comparison with other studies of childhood dietary patterns and respiratory outcomes is difficult as most studies did not use PCA to obtain dietary patterns. However, our "Health conscious" dietary pattern (including starchy foods, fruit, vegetables, potatoes, vegetable oils, fish, legumes and meat) has some similarities with a Mediterranean diet. A study in Mexico found a Mediterranean dietary pattern to have a protective effect on asthma and asthma-related symptoms in children aged 6–7 yrs [23]. A Greek study found adherence to a Mediterranean diet to be modest protective for wheezing symptoms in children aged 7–18 yrs [24]. Two Spanish studies additionally found a Mediterranean diet in childhood to be protective for symptoms of asthma in children aged 4 and 6–7 yrs [25, 26]. However, these studies were of cross-sectional design and recall bias and reverse causality might be a serious concern. To our knowledge only one prospective cohort study examined the association between a dietary pattern in childhood and atopic disease, and also found childhood adherence to a Mediterranean diet not to be significantly associated with asthma-related symptoms [14].

It has been suggested that the increase in prevalence of asthma is related to adoption of a Western lifestyle including a Western diet [27]. The "Western" diet in this study was characterised by high intake of refined grains, soups and sauces, savoury and snacks, other fats, sugar-containing beverages and meat. Although no other studies examined the overall effect of a "Western-like" diet in childhood on the development of asthma-related symptoms in children, a French study did find a "Western" dietary pattern to be associated with an increased risk of frequent asthma attacks in adult females [15]. Individual food components of a "Western" diet in childhood have been found to be associated with asthma symptoms in children. Sugar consumption during the perinatal period was associated with severe asthma symptoms in 6- and 7-yr-old children [28]. An increased intake of saturated fatty acids was also found to be related to the risk of asthma in children [29].

Adjustment for energy intake is a standard procedure in nutritional epidemiology for standardising food and nutrient intake according to total food intake. However, most studies on dietary patterns and respiratory symptoms do not adjust for energy intake and found a Mediterranean diet to be protective for asthma-related symptoms [23, 25]. CHATZI et al. [14] did adjust for total energy intake and found childhood adherence to a Mediterranean diet not to be significantly associated with asthma-related symptoms. An association found between a dietary pattern consisting of high energy foods and a disease outcome may not be the effect of the foods themselves, but the effect of high calorie intake. It has been suggested that high calorie foods are associated with asthma [12]. Nevertheless, a Cochrane review showed only a small effect of calorie reduction on asthma [30]. Indeed, after adjustment for total energy intake the "Western" dietary pattern remained significantly associated with wheeze and respiratory tract infections. Therefore, the association between the "Western" dietary pattern and respiratory symptoms was only partially explained by total energy intake and needs further elucidation.

Our large study population drawn from the general population is an important strength of this study. An additional strength is the use of dietary patterns instead of single or few nutrients or food items. The effect of single nutrients may be too small to detect as the cumulative effects of multiple nutrients in a dietary pattern may be sufficiently large. Dietary patterns identified by PCA has the advantage of reducing a large number of correlated dietary measurements down to a small number of overall dimensions of diet that are uncorrelated [9].

The time window of exposure is becoming a key aspect in the study of diseases involving systems such as the immunological and respiratory systems [13]. Atopic disease often becomes manifest in early life, and it could be that the processes leading to atopic disease are initiated early in the immune development. This study assessed the effect of diet at the early age of 14 months, whereas most studies examined the effect of diet at school age. An additional strength of this study is that it examined the effect of dietary patters prior to the outcome of disease, contrary to other studies examining the effect of diet and outcome during the same time period, which may lead to recall bias and reverse causality in these studies.

Some limitations have to be considered in the interpretation of our results. Information on the outcomes was obtained by parent-reported questionnaires. This could have led to misclassification of the outcome, as physician diagnosis provides more accurate outcome diagnosis. However, we do not expect this misclassification to have influenced the effect of adherence to the dietary patterns, given that the outcome was measured after the FFQ was filled out and thus it will be unlikely that the misclassification was related to the child's diet. Several arbitrary decisions are involved in identifying dietary patterns by PCA. Decisions on combining food items into food groups, the number of factors to extract, the method of rotation, and the labelling of the components may influence the reproducibility of the findings [9]. Although we adjusted for potential confounders, residual confounding cannot be fully excluded, thereby precluding final conclusions regarding the causality of the study results.

Diagnosis of asthma is difficult in young children, due to the nonspecificity of the symptoms and the fact that conventional lung function tests cannot be carried out at pre-school age [17]. Therefore, asthma assessment among young children is still mainly based on asthma-related symptoms. Because the diagnosis of asthma is difficult in pre-school children, our results preclude us from making conclusions regarding the presence of asthma later in life. However, CAUDRI et al. [17] found wheezing and serious respiratory tract infections to be predictive for the development of asthma; in particular, frequent wheezing (≥4 times per year) and frequent respiratory tract infections (≥3 times per year) were predictive of asthma at the age of 7 to 8 yrs.

In conclusion, our findings suggest that a "Western" diet may increase the risk of respiratory symptoms at the age of 3 and 4 yrs. However, this association was moderately explained by total energy intake. This study does not support a protective effect of a "Health

conscious" diet on respiratory outcomes in children younger than 4 yrs of age. Further studies on respiratory outcomes to determine the association between diet and respiratory symptoms and the influence of total energy intake are worthwhile.

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SUPPLEMENT CHAPTER 6

Supplementary Table 6.1. Prevalence of outcomes

Outcome	Age 2		Age 3		Age 4	
	n	%	n	%	n	%
Wheeze						
Never	1654	76	1660	76	1689	78
1-3 times	346	16	184	9	199	9
≥4 times	174	8	329	15	286	13
Shortness of breath						
Never	1414	65	1618	75	1705	78
1-3 times	369	17	198	9	164	8
≥4 times	390	18	357	16	304	14
Respiratory tract infections						
Never	1097	50	1443	66	1409	65
1-2 times	491	23	388	18	396	18
≥3 times	586	27	342	16	367	17

Supplementary Table 6.2. Factor loadings of the food items in the "Health conscious" and "Western" dietary pattern in children aged 14 months (r >0.2)

		Factor loading	
Food group	**Mean intake g/d**	**Health conscious dietary pattern Pearson's correlation coefficient**	**Western dietary pattern Pearson's correlation coefficient**
Refined grains	14.9	-	0.57
Whole grains	61.9	-	-
Starchy foods	22.9	0.62	-
Dairy	626.0	-	-
Fruit	162.2	0.32	-
Soy substitutes	4.4	-	-
Vegetables	51.8	0.74	-
Potatoes	34.0	0.61	-
Soups and sauces	9.4	-	0.23
Savoury and snacks	3.9	-	0.59
Confectionary	27.8	-	0.72
Vegetable oils	0.56	0.50	-
Other fats	10.6	-	0.58
Fish	8.2	0.22	-
Shellfish	0.30	-	-
Meat	25.5	0.21	0.27
Eggs	1.9	-	-
Legumes	4.0	0.59	-
Sugar containing beverages	197.5	-	0.59
Non-sugar containing beverages	56.4	-	-
Composite dishes	102.3	-	-
Eigen value*		3.4	1.7
Variance explained (%)		16.3	8.2
Total energy intake		0.36	0.54

PCA was used as an extraction method in which the Pearson's correlation coefficients represent the relative contribution of that food group to the identified dietary pattern.
* The eigenvalue was used as indicator of the amount of variation explained by each dietary pattern.

7

25-Hydroxyvitamin D concentrations, asthma and eczema in childhood

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Clin Nutr. 2016; In Press

ABSTRACT

Background & Aims: A role of vitamin D in the development of respiratory and allergic disease in children remains unclear. It may be likely that vitamin D has an effect on airway inflammation, but only few studies examined the effect in children. We aimed to examine whether serum 25-hydroxyvitamin D (25(OH) vitamin D) concentrations are associated with the fraction of exhaled nitric oxide (FeNO), airway interrupter resistance (Rint), physician diagnosed asthma ever, wheezing and eczema in a population-based cohort study in 6 year old children.

Methods: Serum 25(OH) vitamin D concentration was assessed in 3815 children. 25(OH) vitamin D concentrations ≥75nmol/L were considered as sufficient, between 50-75nmol/L as insufficient, and <50nmol/L as deficient. FeNO and Rint were measured at the research center. Data on physician diagnosed asthma, wheezing, and eczema were obtained by parent-reported questionnaires.

Results: In comparison with sufficient 25(OH) vitamin D concentration, deficient concentrations were associated with elevated FeNO of ≥25ppb (OR: 2.54; 95% CI: 1.34-4.80). In addition, deficient and insufficient 25(OH) vitamin D concentrations were associated with a lower Rint (Z-score: -1.26; 95% CI: -1.66 to -0.85) (ß: -0.75; 95% CI: -1.08 to -0.42), and increased risks of eczema (OR: 1.65; 95% CI: 1.13-2.41) (OR: 1.44; 95% CI: 1.06-1.95). Insufficient 25(OH) vitamin D concentration were associated with a decreased risk of physician diagnosed asthma ever (OR: 0.59; 95% CI: 0.38-0.94).

Conclusions: Our results indicate that lower 25(OH) vitamin D levels are associated with elevated FeNO levels, but lower Rint values. Lower 25(OH) vitamin D levels are also associated with a decreased risk for asthma diagnoses but an increased risk for eczema.

INTRODUCTION

In the past decades the prevalence of allergic disease has increased and has therefore become a major public health problem [1]. The development of allergic disease might be influenced by exposures including diet and behavioral changes associated with a Western lifestyle [2]. Vitamin D may be another important environmental factor and the prevalence of vitamin D insufficiency has increased [3]. Potential extra-skeletal functions of vitamin D may play a role in the risk of asthma and allergic disease [4-9]. Sun exposure and dietary intake are the main source of vitamin D [7]. Changes in lifestyle, such as decreased exposure to sunlight, increased time spent indoors, and physical inactivity, are important determinants of vitamin D deficiency [6,10]. There is at present no universally accepted definition for optimal 25-hydroxyvitamin D (25(OH) vitamin D) concentrations [9]. The Institute of Medicine defined 25(OH) vitamin D concentration of <50 nmol/L as deficient [11]. However, the definition of vitamin D sufficiency by the Institute of Medicine is controversial since the recommendation was primarily based on the effects on bone health whereas it has been argued that extra-skeletal effects of vitamin D are as important [12]. Vitamin D has immunomodulatory effects [13]. The effect of vitamin D on immune function in relation to respiratory and allergic disease have been examined [13]. Epidemiological studies have shown inconsistent results. Different studies found low and high levels of vitamin D in children to be associated with and increased risk of asthma and allergy outcomes [14-18]. Other studies did not find an association [19, 20]. For the prevention of respiratory and allergic disease in children a role of vitamin D remains unclear [13, 21, 22]. Higher vitamin D levels have been associated with lower level of biomarkers of inflammation [23] and oxidative stress [24] in children. Therefore, it may be likely that vitamin D has an influence on airway inflammation. However, only few studies examined the effect in children as by measuring the fraction of exhaled nitric oxide (FeNO), which is a biomarker of airway inflammation [25, 26]. We aimed to examine the cross-sectional association between serum 25(OH) vitamin D concentrations and FeNO, Rint, physician diagnosed asthma ever, wheezing and eczema in a population-based cohort study in 6 year old children.

SUBJECTS AND METHODS

Participants and study design

These analyses were part of the Generation R study, which is a population-based prospective cohort study and has been described in detail previously [27]. All children were born from April 2002 through January 2006. The study was approved by the medical ethical review board of the Erasmus Medical Center, Rotterdam, the Netherlands. At the start of each phase of the study both parents were asked for written informed consent.

Asthma-related outcomes and eczema

Fractional exhaled nitric oxide (FeNO), which is a biomarker of eosinophilic airway inflammation, was measured at 6 years of age using the NIOX chemiluminescence analyzer

during a visit to the research center. Airway resistance (interrupter resistance (Rint), MicroRint, Micromedical, Rochester, Kent, UK) was measured during tidal breathing, with occlusion of the airway at tidal peak expiratory flow. To calculate Z-scores median values for no less than 5 acceptable measurements were calculated. The Z-scores were adjusted for the device that was used and for the child's height. Lung function measurements were obtained following the European Respiratory Society and American Thoracic Society guidelines [28]. Parents were asked to temporarily cease medication for respiratory conditions in children without symptoms 24 hours before the visit to the research center. Data on physician diagnosed asthma ever since birth was obtained by annual parent-reported questionnaires. Data on current wheezing was collected by parent-reported questionnaires at the age 6 years using questions from the International Study of Asthma and Allergies in Childhood (ISAAC) [29]. Also, data on current eczema was collected by parent-reported questionnaires at 6 years of age.

25-Hydroxyvitamin D concentration

25(OH) vitamin D concentration was assessed in thawed venous serum samples at the Endocrine Laboratory of the VU University Medical Center, Amsterdam. Serum 25(OH) vitamin D was measured using isotope dilution online solid phase extraction liquid chromatography-tandem mass spectrometry (ID-XLC-MS/MS) and has been described in detail previously [30]. Serum 25(OH) vitamin D was taken during a visit to the research center at the age of 6 years and available in 4167 children. Serum 25(OH) vitamin D was not available for children who expressed anxiety for blood sampling, whose parents did not provide informed consent for blood sampling, and when blood sample was not possible for logistic reasons (i.e. time constraints). The median 25(OH) vitamin D concentration in the study population was 64 nmol/L, varying from 4 to 211. On the basis of previous studies in the pediatric population, serum 25(OH) vitamin D concentrations ≥75nmol/L were considered as vitamin D sufficient, concentrations between 50-75 nmol/L were considered as vitamin D insufficient, and <50 nmol/L as deficient [15, 25, 30].

Covariates

Information on potential confounders including sex, gestational age, birth weight and mode of delivery were collected from obstetric records evaluated in mid-wife practices and hospital registries [27]. Further information on potential confounders was obtained using a combination of pre-and postnatal questionnaires completed by both parents. These questionnaires included information on ethnicity [31], maternal educational level, maternal marital status, household income per month (≤€2200 and >€2200), folic acid intake during pregnancy, maternal alcohol use during pregnancy, maternal smoking during pregnancy, multiparity and parental history of atopy. Ethnicity of the child was categorized into Western (Dutch, American, European, and Oceanian) and non-Western (Moroccan, Turkish, Dutch Antillean, Surinamese, Asian, Indonesian, African and Cape Verdean). Data on breastfeeding

were collected by questionnaires at 2, 6 and 12 months of age. Information on the presence of cow's milk allergy in the first year was obtained by questionnaire at the ages of 6 and 12 months. Questionnaires completed by the mother at age 12 months included information on vitamin D supplementation in the previous 6 months and questionnaires at the age of 12 and 24 months included information on day-care attendance in the first 2 years. Information on the time the child spent playing outside during daytime, walking or biking to school and the time spend watching television was assessed by parent-reported questionnaires at the age of 6 years. Also, data on respiratory tract infections in the previous year was obtained by questionnaire at the same time point. During a visit to the research center at the age of 6 years total body fat mass was measured using a Dual-energy X-ray absorptiometry (DXA) scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA), which analyzed fat, lean and bone mass of the total body using enCORE software v.13.6. We calculated age- and sex-specific z-scores for body fat percentage based on the total Generation R Study sample with body composition measurements available the age of 6 years.

Population for analyses

All children with 25(OH) vitamin D concentrations available (n= 4167) were included in the analyses. To prevent clustering, only one child per family within the Generation R cohort was included by random selection (n= 352). In total, 3815 children were available for statistical analyses (Supplementary Figure 7.1).

Statistical methods

Student's t test and chi-squared analyses were performed to examine differences between groups in 25(OH) vitamin D concentration. Logistic and linear regression analyses were performed for FeNO, Rint, physician diagnosed asthma ever, wheezing and eczema as dependent variables. FeNo was elog transformed to acquire normal distribution. Analyses were performed with FeNO as both continuous variable and as dichotomous variable using a threshold of 25 ppb, since it has been suggested that FeNO of ≥25 ppb indicates that eosinophilic inflammation is more likely [32]. 25(OH) vitamin D concentration at 6 years of age was analyzed categorically as independent variable (using sufficient 25(OH) vitamin D concentration as reference category) and adjusted for season of blood sampling and potential confounders. Additional analyses were performed with serum 25(OH) vitamin D concentration in quartiles based on the distribution of the cohort (using the first quartile as reference category). The selection of potential confounders was based on changes of 10% or greater on odds ratios (ORs) [33]. To assess whether the association between 25(OH) vitamin D concentration and asthma-related outcomes and eczema was different by gender, ethnicity, or children with and without a parental history of asthma or atopy, statistical interactions were assessed by adding the product term of independent variable and subgroup (independent variable × subgroup) as covariates to the univariate model. To account for potential reverse causality, sensitivity analyses were conducted in children without physician diagnosed

asthma ever. To prevent bias associated with missing data, missing values of the covariates were multiple imputed (n=20 imputations) based on the correlation between the variable with missing values with other patient characteristics [34]. Within the population for analyses the percentages of missing values were lower than 30%, except for folic acid intake (32%), breastfeeding (36%), vitamin D supplementation (39%), day care attendance (52%), history of cow's milk allergy (53%). Both the results of the original dataset (Supplementary Table 3) and the pooled results of the 20 imputed data sets were reported in this article as ORs and 95% confidence interval (95% CI) for the analyses on FeNO (dichotomous), physician diagnosed asthma ever, wheezing, and eczema. The results for the analyses on FeNO (continuous) and Rint were reported as ratio of geometric means (EXP(ß)) and change in standardized z-score (ß) and 95% confidence interval (95% CI). A p-value <0.05 was considered as statistically significant. IBM SPSS Statistics 22.0 for Windows (IBM Corp., Armonk, NY, USA) was used to carry out the statistical analyses.

RESULTS

Study population

Maternal and child characteristics of the study population are shown in Table 7.1 and Supplementary Table 7.1. In Supplementary Table 7.2 the prevalence of the outcomes of interest are shown. Of the 3815 children, 33%, were 25(OH) vitamin D sufficient, 37%, insufficient and 30% deficient. Mean 25(OH) vitamin D concentration for children with FeNO <25 ppb and FeNO ≥25 ppb, children with and without physician diagnosed asthma, wheezing, and eczema are shown in Figure 7.1.

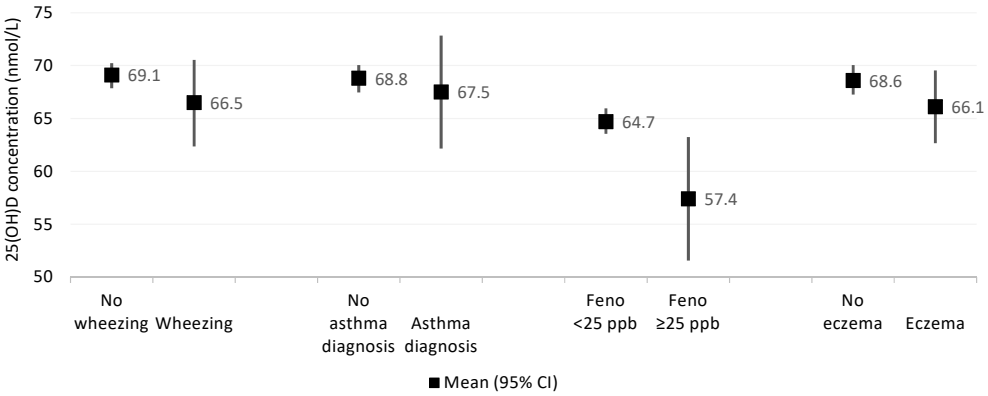

Figure 7.1. Mean 25(OH)D concentration in children with and without asthma-related outcomes and eczema

Table 7.1. Maternal and child characteristics (n=3815)

	25 Hydroxyvitamin D									
			Deficient <50 nmol/L			Insufficient 50-75 nmol/L			Sufficient ≥75 nmol/L	
	n= 3815		n=1156 (30%)			n=1393 (37%)			n=1266 (33%)	
Characteristics	**n**	**%**	**n**	**%**	**P-value**	**n**	**%**	**P-value**	**n**	**%**
Maternal characteristics										
Educational level					<0.01			0.11		
Low	919	24	431	37		271	19		217	17
Mid	1989	52	563	49		753	54		673	53
High	907	24	162	14		369	27		376	30
Household income per month					<0.01			<0.01		
≤2200 euro	1806	47	805	70		599	43		402	32
>2200 euro	2009	53	351	30		794	57		865	68
Marital status					<0.01			<0.01		
Married/ Living together	3291	86	896	78		1227	88		1168	92
No partner	525	14	260	22		166	12		98	8
Folic acid intake					<0.01			<0.01		
Never	1029	27	534	46		309	22		186	15
Start 1st 10 weeks	1203	31	338	29		460	33		404	32
Start periconceptional	1584	42	285	25		623	45		675	53
Smoking during pregnancy					<0.05			0.57		
Never	2840	74	826	71		1049	75		966	76
Smoked during pregnancy	975	26	331	29		344	25		300	24
Alcohol use during pregnancy					<0.01			<0.01		
Never	1736	46	670	58		608	44		458	36
Drank alcohol during pregnancy	2079	54	486	42		785	56		808	64
Parental history of atopy					<0.01			0.18		
	1820	48	499	43		674	48		647	51

Table 7.1. (continued)

	25 Hydroxyvitamin D									
			Deficient <50 nmol/L			Insufficient 50-75 nmol/L			Sufficient ≥75 nmol/L	
	n= 3815		n=1156 (30%)			n=1393 (37%)			n=1266 (33%)	
Characteristics	**n**	**%**	**n**	**%**	**P-value**	**n**	**%**	**P-value**	**n**	**%**
Multiparity					<0.01			<0.05		
	1681	44	590	51		599	43		492	39
Caesarean section					0.27			0.65		
	505	13	142	12		187	13		176	14
Child characteristics										
Season at blood sampling					<0.01			<0.01		
Summer	985	26	103	9		348	25		534	42
Fall	946	25	245	21		342	25		359	28
Winter	811	21	418	36		272	19		121	10
Spring	1073	28	390	34		431	31		252	20
Male					0.10			0.20		
	1966	52	612	53		726	52		628	50
Birth weight *z*-score *Mean (SD)*					<0.01			0.29		
	-0.078	1.00	-0.20	0.99		-0.01	1.00		-0.05	0.99
Ethnicity					<0.01			<0.01		
Dutch, American, other western	2508	65	443	39		997	71		1067	84
Maroccan, Turkish	530	14	293	25		168	12		69	6
Dutch Antilles, Surinamese	411	11	235	20		105	8		71	6
Asian, Indonesian	149	4	66	6		55	4		29	2
African, Cape Verdian	217	6	120	10		68	5		30	2
Breastfeeding					<0.05			0.18		
<6 months	2175	57	614	53		798	57		763	60
≥6 months	1640	43	542	47		595	43		503	40

Table 7.1. (continued)

	25 Hydroxyvitamin D									
			Deficient <50 nmol/L			Insufficient 50-75 nmol/L			Sufficient ≥75 nmol/L	
	n= 3815		n=1156 (30%)			n=1393 (37%)			n=1266 (33%)	
Characteristics	**n**	**%**	**n**	**%**	**P-value**	**n**	**%**	**P-value**	**n**	**%**
Vitamin D supplementation age 6-12 months					<0.01			<0.05		
	1895	50	672	58		670	48		553	44
History of cow's milk allergy first year					0.35			0.22		
	554	15	179	15		183	13		192	15
Day care attendance first two years					<0.01			0.06		
	2960	78	837	72		1087	78		1035	82
Respiratory tract infections *					0.06			0.44		
	1044	27	345	30		374	27		324	26
Playing outside *					<0.01			<0.01		
<1 hour	1560	41	631	55		567	41		362	29
≥1 hour	2255	59	525	45		826	59		904	71
Walking and biking to school * (h/day) *Mean (SD)*					0.85			0.30		
	0.14	0.14	0.14	0.15		0.13	0.14		0.14	0.14
TV watching * (h/day) *Mean (SD)*					<0.01			<0.01		
	1.50	1.11	1.90	1.33		1.40	1.02		1.24	0.85
Body fat percentage *z*-score * ¶ *Mean (SD)*	-0.01	0.99			<0.01			<0.01		
			0.14	1.34		-0.02	1.01		-0.14	0.86

* Age 6 years

¶ Body fat percentage z-score was not multiple imputed

25(OH) vitamin D concentration and asthma-related outcomes and eczema

Deficient 25(OH) vitamin D concentration, but not insufficient 25(OH) vitamin D concentration, was associated with elevated FeNO (OR: 2.54; 95% CI: 1.34-4.80) (Table 7.2; Supplementary Table 7.3). The association with continuous FeNO showed the same trend but became non-significant after adjustment for confounders (OR: 1.05; 95% CI: 0.98-1.12) (Table 7.2). In comparison with sufficient 25(OH) vitamin D concentration, deficient and insufficient 25(OH) vitamin D concentrations were associated with lower Rint (ß: -1.26; 95% CI: -1.66 to -0.85) (ß: -0.75; 95% CI: -1.08 to -0.42) (Table 7.2; Supplementary Table 7.3). Insufficient 25(OH) vitamin D concentration, but not deficient 25(OH) vitamin D concentration, was associated with a decreased risk of physician diagnosed asthma ever (OR: 0.59; 95% CI: 0.38-0.94) (Table 7.2; Supplementary Table 7.3). For wheezing, no association with 25(OH) vitamin D concentrations was observed (Table 7.2; Supplementary Table 7.3). In addition, deficient and insufficient 25(OH) vitamin D concentrations were associated with increased risks of eczema (OR: 1.65; 95% CI: 1.13-2.41) (OR: 1.44; 95% CI: 1.06-1.95) (Table 7.2; Supplementary Table 7.3). Analyses with 25(OH) vitamin D concentration as continuous variable showed comparable results (Table 7.3).

Subgroup and sensitivity analyses

A significant interaction was found between 25(OH) vitamin D categories and ethnicity for eczema (p=0.048 and p=0.14 for deficient and insufficient 25(OH) vitamin D concentrations, respectively). After stratification by ethnicity an association was found between deficient and insufficient 25(OH) vitamin D concentrations and increased risk of eczema in Western children (OR: 2.01; 95% CI: 1.24-3.24) (OR: 1.61; 95% CI: 1.12-2.32). No significant association was found for Non-Western children (Supplementary Table 7.4). No interaction was found between 25(OH) vitamin D concentration and gender or parental history of atopy (p-interaction >0.17). In the sensitivity analyses in children without physician diagnosed asthma ever, we observed similar results for the association between 25(OH) vitamin D deficiency and insufficiency and Rint (ß: -1.09; 95% CI: -1.61 to -0.56) (ß: -0.48; 95% CI: -0.89 to -0.07). Furthermore, in this subgroup the effect estimates for the association between 25(OH) vitamin D deficiency and FeNO ≥25 ppb remained in the same direction but lost statistical significance (OR: 1.73; 95% CI: 0.62-4.80) (Supplementary Table 7.5).

DISCUSSION

We observed that lower vitamin D levels were associated with elevated FeNO levels, suggestive of eosinophilic airway inflammation. Remarkably, lower vitamin D levels were also associated with lower Rint values, reflecting better airway patency, and lower risk of asthma diagnosis ever. We found no effect on the risk for wheezing but found lower vitamin D levels to be associated with increased risk for eczema.

At present, there is no agreement for optimal 25(OH) vitamin D concentrations [9]. The optimal vitamin D status for the prevention of allergic disease is unclear. Based on

Table 7.2. Association between 25 hydroxyvitamin D and asthma-related outcomes and eczema at age 6 years

n=3815		Crude model[a]	Multivariate model[b]
25(OH)D concentration	**n (%)**	**FeNO ≥25 ppb aOR (95% CI)**	
Deficient (<50 nmol/L)	1156 (30)	**2.90 (1.68-5.01)**	**2.54 (1.34-4.80)**
Insufficient (≥50-75 nmol/L)	1393 (37)	1.30 (0.76-2.21)	1.25 (0.72-2.18)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		FeNO EXP(ß) (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	**1.08 (1.02-1.15)**	1.05 (0.98-1.12)
Insufficient (≥50-75 nmol/L)	1393 (37)	1.04 (0.99-1.10)	1.03 (0.98-1.10)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Rint (ß) (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	**-1.09 (-1.44 to -0.75)**	**-1.26 (-1.66 to -0.85)**
Insufficient (≥50-75 nmol/L)	1393 (37)	**-0.73 (-1.05 to -0.41)**	**-0.75 (-1.08 to -0.42)**
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Physician diagnosed asthma ever aOR (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	0.96 (0.60-1.52)	0.85 (0.49-1.45)
Insufficient (≥50-75 nmol/L)	1393 (37)	**0.64 (0.42-0.98)**	**0.59 (0.38-0.94)**
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Wheezing aOR (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	1.19 (0.82-1.72)	1.15 (0.75-1.77)
Insufficient (≥50-75 nmol/L)	1393 (37)	1.03 (0.75-1.43)	0.99 (0.70-1.39)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Physician diagnosed eczema aOR (95% CI)[c]	
Deficient (<50 nmol/L)	1156 (30)	**1.64 (1.18-2.28)**	**1.65 (1.13-2.41)**
Insufficient (≥50-75 nmol/L)	1393 (37)	**1.34 (1.00-1.79)**	**1.44 (1.06-1.95)**
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference

OR: Odds ratio; 95% CI: 95% confidence interval. OR's are compared to sufficient 25OHD concentration. EXP(ß): Ratio of geometric means and (ß): Regression coefficient indicating the change in standardized z-score compared to the reference group; 95% CI: 95% confidence interval.

Entries in bold are statistically significant.

[a] Adjusted for season at blood sampling.

[b] Adjusted for season at blood sampling, smoking during pregnancy, alcohol use during pregnancy, caesarean section, day-care attendance first two years, ethnicity, household income per month, birth weight *z*-score, folic acid intake, marital status, breastfeeding, history of cow's milk allergy first year, vitamin D supplementation age 6-12 months, respiratory tract infections, playing outside, walking and biking to school, TV watching and body fat percentage z-score at the age of 6 years.

[c] Not additionally adjusted for respiratory tract infections age 6 years.

Additional adjustment for gender, parity, maternal educational level, parental history of atopy, and age mother did not provide an alteration of ≥ 10% in OR.

Table 7.3. Association between 25 hydroxyvitamin D and asthma-related outcomes and eczema at age 6 years

n=3815	Crude model[a]	Multivariate model[b]
FeNO ≥25 ppb		
OR (95% CI)	**0.98 (0.97-0.99)**	**0.98 (0.97-0.99)**
FeNO		
EXP(ß) (95% CI)	**0.99 (0.99-1.00)**	**0.99 (0.99-1.00)**
Rint		
(ß) (95% CI)	**0.02 (0.01-0.02)**	**0.02 (0.01-0.02)**
Physician diagnosed asthma ever		
OR (95% CI)	1.00 (0.99-1.01)	1.00 (0.99-1.01
Wheezing		
OR (95% CI)	1.00 (0.99-1.00)	1.00 (0.99-1.01)
Physician diagnosed eczema		
OR (95% CI)	0.99 (0.99-1.00)	0.99 (0.99-1.00)[c]

OR: Odds ratio; 95% CI: 95% confidence interval. OR's are compared to sufficient 25OHD concentration. EXP(ß): Ratio of geometric means and (ß): Regression coefficient indicating the change in standardized z-score compared to the reference group; 95% CI: 95% confidence interval.
Entries in bold are statistically significant.
[a] Adjusted for season at blood sampling.
[b] Adjusted for season at blood sampling, smoking during pregnancy, alcohol use during pregnancy, caesarean section, day-care attendance first two years, ethnicity, household income per month, birth weight *z*-score, folic acid intake, marital status, breastfeeding, history of cow's milk allergy first year, vitamin D supplementation age 6-12 months, respiratory tract infections, playing outside, walking and biking to school, TV watching and body fat percentage z-score at the age of 6 years.
[c] Not additionally adjusted for respiratory tract infections age 6 years.
Additional adjustment for gender, parity, maternal educational level, parental history of atopy**,** and age mother did not provide an alteration of ≥ 10% in OR.

skeletal health, the Institute of Medicine recommends 25(OH) vitamin D concentration of ≥50 nmol/L and more to be sufficient [11]. Since it has been argued that the cut-off of 50 nmol/L for vitamin D deficiency may be too low, we additionally classified vitamin D insufficiency using the cut-off used in previous studies [15, 25]. In agreement with a meta-analysis, we found both vitamin D deficiency and insufficiency to be associated with asthma-related outcomes and eczema [35]. Comparison of our findings to those of other studies on vitamin D and allergic disease is difficult due to differences in study design, age of participants, and outcomes. Furthermore, studies on vitamin D and allergic disease are inconsistent [22, 36]. For our findings on serum vitamin D concentrations and FeNO, only few studies were comparable. Similar to our results, a Taiwanese cohort study observed vitamin D deficiency (<50 nmol/L) and insufficiency (50-74.9 nmol/L) not to be associated with FeNO in children at the age of 6 years [25]. Checkley et al [26] also reported no association between 25(OH) vitamin D concentrations and FeNO. However, when using a cut-off of 25 ppb, we did find deficient 25(OH) vitamin D concentration to be associated with an increased odds of FeNO >25ppb. The threshold of 25 ppb has been suggested to indicate that eosinophilic

inflammation is likely [32]. To our knowledge no other study examined the association between vitamin D status and Rint. Therefore, we can only speculate about the association that was observed. Rint values reflecting airway resistance are generally increased in children with asthma [37, 38]. Therefore, our results that vitamin D insufficiency was associated with lower Rint values is surprising. Few studies report why Rint values may be reduced. First, Rint values may be lower when cheeks are not firmly supported, especially in younger children [38]. Nevertheless, we adjusted our Rint values for the technique during the measurement (eg. adequate breathing and support of cheeks). Another explanation may be potential treatment or interventions affecting airways resistance such as bronchodilators [37] since we also found a weak inverse association between insufficient, but not deficient, vitamin D concentrations and physician diagnosed asthma. Parents were asked to temporarily cease medication for respiratory conditions 24 hours before the visit to the research center in children without symptoms. Therefore, children who did experience symptoms prior to their visit might still have received medication. However, the results did not differ after exclusion of children with parent-reported physician diagnosed asthma. Last, Merkus et al [39] reviewed Rint values in different populations and found reduced values in children with recurrent rhinitis. Since previous studies have shown that low vitamin D levels are associated with a higher risk of rhinitis, this may have also been the case in our study [40]. This clearly deserves further elucidation. FeNO and Rint measurements both provide different information for the prediction of asthma. Caudri et al. showed that FeNO but not Rint is predictive for asthma [41]. In addition, after exclusion of children with parent-reported physician diagnosed asthma (Supplementary Table 7.5) the effect estimates for the association between 25(OH) vitamin D deficiency and FeNO ≥25 ppb remained in the same direction but lost statistical significance. Therefore, we speculate that the results on FeNO may be explained by airway inflammation due to uncontrolled asthma. Another Dutch birth cohort study reported higher serum vitamin D concentrations (median concentration 97nm and 68nm vs. 52nm) at the age of 4 years to be associated with a lower risk of parental reported asthma at age 4-8 years [17]. A meta-analysis of published studies, including European studies, on vitamin D and childhood asthma suggests that low vitamin D levels might increase the risk of asthma [35]. In contrast, we found insufficient vitamin D concentration to be associated with a lower risk of physician diagnosed asthma. The possibility of a U-shaped association (<50 nmol/L and ≥75 nmol/l vs. 50-75 nmol/L) between vitamin D levels and respiratory health has been previously suggested by Niruban et al [15]. However, similar direction of the effect estimates were observed for the association between vitamin D deficiency and diagnosed asthma ever but not significant. This might be explained by the small number of children with vitamin D deficiency and physician diagnosed asthma. Contrary to our study, two other studies did find 25(OH) vitamin D levels to be associated with wheezing in children [15, 16]. Although the latter studies did adjust for multiple confounders, we also considered prenatal factors and factors during infancy as potential confounders in our analyses. Therefore, the inconsistency with our findings might be explained by residual confounding. In addition, Keet et al [42] found the association between vitamin D and wheezing to vary by age, suggesting an

age-dependent relationship. Our results show that lower vitamin D levels are associated with an increased risk of eczema, but only in Western children and not in non-Western children. A randomized placebo-controlled trial observed oral vitamin D supplementation to lead to an improvement in atopic dermatitis severity in children, supporting a causal role [43]. It has previously been suggested that the association between vitamin D levels and eczema might vary by race [44]. Wegienka et al [44] found prenatal vitamin D levels to be inversely associated with eczema and found this association to be stronger in white children than in black children, however not statistically significant. In children with a white skin, the prevalence of eczema is lower and vitamin D levels higher than in children with a dark skin [45-47]. Skin color might vary within ethnic groups [48]. Our cohort consists of children from many different but also mixed races, making it difficult to interpret our findings. Hence, it may be valuable to further study the interaction between race i.e. skin color, vitamin D status and eczema.

An important strength of this study is the large study population drawn from the general population. Another strength is that we obtained data on several asthma-related outcomes including FeNO and Rint. We used FeNO and Rint measurements as an objective tool to support in the diagnosis of asthma in children [38, 49] in addition to self-administered questionnaire data. Also, our study design provided information on multiple potential confounders including sedentary activity and time spent outdoors. However, residual confounding cannot be fully excluded.

A limitation of this study is that the data on physician diagnosed asthma, wheezing, and eczema was collected by parent-reported questionnaires. This might have resulted in misclassification of the outcome as physician diagnosis is more precise. In a previous study in our cohort, we experienced that self-report mainly leads to overreporting of wheezing symptoms [50]. However, we believe it is unlikely that it would have influenced the directionality of our association with vitamin D since parents were not aware of their child's vitamin D status. Another limitations is that we only had one measurement of 25(OH) vitamin D concentration. However, a population based study in adults found 25(OH) levels to be highly correlated over time, providing support for the use of a single measurement [51]. The cross-sectional design of this study precludes us to make causal inference. Also, reverse causality may have influenced our results. Parents of children with early symptoms of allergy may be more inclined towards a healthy life style including supplementation of vitamin D, which might explain the adverse association we found between insufficient (but not deficient) vitamin D status and physician diagnosed asthma ever and Rint. In addition, it has been suggested that chronic inflammation may reduce circulating 25(OH) vitamin D [52]. Therefore, an inadequate vitamin D status may indirectly indicate disease rather than associated with disease pathogenesis.

In conclusion, we found that lower 25(OH) vitamin D levels are cross-sectional associated with elevated FeNO levels, suggestive of eosinophilic airway inflammation, but lower Rint values, meaning a better airway resistance. Lower 25(OH) vitamin D levels are also associated with a decreased risk for asthma diagnoses but an increased risk for eczema. Further studies are necessary to clarify the directionality of these observed associations.

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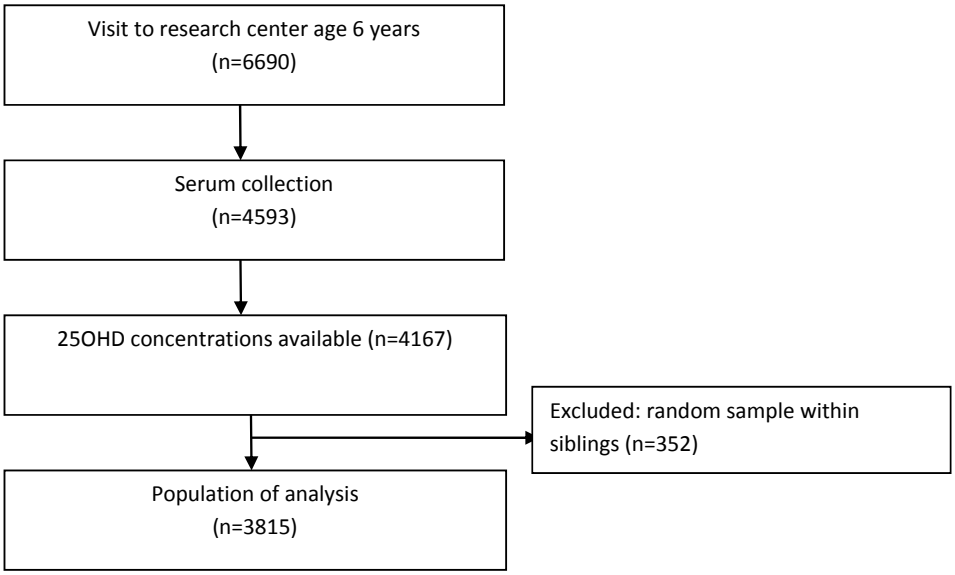
SUPPLEMENT CHAPTER 7



Supplementary Figure 7.1. Flowchart of the participants within the Generation R Study

Supplementary Table 7.1. Maternal and child characteristics (original data)

			25 Hydroxyvitamin D							
			Deficient <50 nmol/L			Insufficient 50-75 nmol/L			Sufficient ≥75 nmol/L	
	n= 3815		n=1156 (30%)			n=1393 (37%)			n=1266 (33%)	
	n	%	n	%	P-value	n	%	P-value	n	%
Maternal characteristics										
Educational level					<0.01			0.27		
Low	780	25	349	36		231	18		200	17
Mid	1807	52	475	49		694	55		638	53
High	856	23	147	15		348	27		361	30
Missing	372	10	185	16		120	9		67	5
Household income per month					<0.01			<0.01		
≤2200 euro	1212	43	505	65		412	39		295	30
>2200 euro	1611	57	270	35		636	61		705	70
Missing	992	26	381	33		345	25		266	21
Marital status					<0.01			<0.01		
Married/ Living together	3018	87	774	78		1136	88		1108	93
No partner	457	13	218	22		149	12		90	7
Missing	340	9	164	14		108	8		68	5
Folic acid intake					<0.01			<0.01		
Never	662	26	325	45		206	22		131	14
Start 1st 10 weeks	812	31	206	29		312	33		294	32
Start periconceptional	1127	43	189	26		435	45		503	54
Missing	1214	32	436	38		440	32		338	27
Smoking during pregnancy					<0.01			0.50		
Never	2458	75	671	71		910	75		877	76
Smoked during pregnancy	836	25	269	29		298	25		269	24
Missing	521	14	216	19		185	13		120	10

Supplementary Table 7.1. (continued)

			25 Hydroxyvitamin D							
			Deficient <50 nmol/L			Insufficient 50-75 nmol/L			Sufficient ≥75 nmol/L	
	n= 3815		n=1156 (30%)			n=1393 (37%)			n=1266 (33%)	
	n	%	n	%	P-value	n	%	P-value	n	%
Alcohol use during pregnancy					<0.01			<0.01		
Never	1365	46	506	58		476	44		383	37
Drank alcohol during pregnancy	1633	54	368	42		602	56		663	63
Missing	817	21	282	24		315	23		220	17
Parental history of atopy					<0.01			0.16		
	1660	48	419	44		622	48		619	51
Missing	354	9	192	17		106	8		56	4
Multiparity					<0.01			<0.05		
	1605	44	572	51		567	43		466	38
Missing	143	4	32	3		60	4		51	4
Caesarean section					0.26			0.57		
	439	13	125	12		161	13		153	14
Missing	482	13	141	12		170	12		171	14
Child characteristics										
Season at blood sampling					<0.01			<0.01		
Summer	985	26	390	34		431	31		252	20
Fall	946	25	103	9		348	25		534	42
Winter	811	21	245	21		342	25		359	28
Spring	1073	28	418	36		272	19		121	10
Missing	0	0	0	0		0	0		0	0
Male					0.10			0.20		
	1966	52	612	53		726	52		628	50
Missing	0	0	0	0		0	0		0	0

Supplementary Table 7.1. (continued)

			25 Hydroxyvitamin D							
			Deficient <50 nmol/L			Insufficient 50-75 nmol/L			Sufficient ≥75 nmol/L	
	n= 3815		n=1156 (30%)			n=1393 (37%)			n=1266 (33%)	
	n	%	n	%	P-value	n	%	P-value	n	%
Birth weight *z*-score *Mean (SD)*					<0.01			0.31		
	-0.08	1.00	-0.20	0.99		-0.01	1.00		-0.05	0.99
Missing	41	1	7	1		19	1		15	1
Ethnicity					<0.01			<0.01		
Dutch, American, other western	2477	67	429	40		989	72		1059	85
Maroccan, Turkish	510	14	278	26		165	12		67	5
Dutch Antilles, Surinamese	397	11	224	20		103	6		70	6
Asian, Indonesian	122	3	46	4		50	4		26	2
African, Cape Verdian	203	5	108	10		66	5		29	2
Missing	106	3	71	6		20	1		15	1
Breastfeeding					0.07			0.19		
<6 months	1591	65	377	63		613	64		601	67
≥6 months	857	35	224	37		340	36		293	33
Missing	1367	36	555	48		440	32		372	29
Vitamin D supplementation age 6-12 monthts					<0.01			0.19		
	1057	45	292	54		403	44		362	41
Missing	1470	39	610	53		478	34		382	30
History of cow's milk allergy first year					0.17			0.35		
	113	6	20	5		42	6		51	7
Missing	2017	53	764	66		692	50		561	44

Supplementary Table 7.1. (continued)

			25 Hydroxyvitamin D							
			Deficient <50 nmol/L			Insufficient 50-75 nmol/L			Sufficient ≥75 nmol/L	
	n= 3815		n=1156 (30%)			n=1393 (37%)			n=1266 (33%)	
	n	%	n	%	P-value	n	%	P-value	n	%
Day care attendance first two years					0.84			0.47		
	1734	95	347	95		690	95		697	95
Missing	1989	52	791	68		663	48		535	42
Respiratory tract infections *					0.14			0.62		
	809	26	220	28		306	26		283	25
Missing	722	19	372	32		214	15		136	11
Playing outside *					<0.01			<0.01		
<1 hour	1115	40	391	57		433	41		291	28
≥1 hour	1677	60	291	43		633	59		753	72
Missing	1023	27	474	41		327	24		222	18
Walking and biking to school * (h/day) *Mean (SD)*					0.49			0.17		
	0.13	0.14	0.13	0.15		0.13	0.13		0.14	0.14
Missing	798	21	379	33		247	18		172	14
TV watching * (h/day) *Mean (SD)*					<0.01			<0.01		
	1.41	1.04	1.81	1.29		1.34	0.96		1.21	0.82
Missing	843	22	407	35		264	19		172	14
Body fat percentage z-score * *Mean (SD)*					<0.01			<0.01		
	-0.01	0.99	0.14	1.12		-0.02	0.96		-0.14	0.87
Missing	59	2	19	2		19	1		21	2

* Age 6 years

Supplementary Table 7.2. Prevalence of outcomes

Outcome	n	%
FeNO *Median (Range)*		
	7.3	0.10-119
Missing	1051	28
FeNO		
<25	2660	96
≥25	104	4
Missing	1051	28
Rint *Mean (SD)*		
	-0.11	3.55
Missing	1159	30
Physician diagnosed asthma ever		
No	2191	94
Yes	136	6
Missing	1488	39
Wheezing		
No	2505	92
Yes	233	8
Missing	1077	28
Physician diagnosed eczema		
No	1859	85
Yes	326	15
Missing	1630	43

Supplementary Table 7.3. Association between 25 hydroxyvitamin D and asthma-related outcomes and eczema at age 6 years (original data)

n=3815		Crude model[a]	Multivariate model[b]
25(OH)D concentration	**n (%)**	**FeNO ≥25 ppb aOR (95% CI)**	
Deficient (<50 nmol/L)	1156 (30)	**2.90 (1.68-5.01)**	3.08 (0.31-30.47)
Insufficient (≥50-75 nmol/L)	1393 (37)	1.30 (0.76-2.21)	2.25 (0.51-10.05)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		FeNO EXP(ß) (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	**1.08 (1.02-1.15)**	0.95 (0.79-1.14)
Insufficient (≥50-75 nmol/L)	1393 (37)	1.04 (0.99-1.10)	0.98 (0.86-1.11)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Rint (ß) (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	**-1.09 (-1.44 to -0.75)**	-1.04 (-2.09 to 0.01)
Insufficient (≥50-75 nmol/L)	1393 (37)	**-0.73 (-1.05 to -0.41)**	-0.57 (-1.28 to 0.14)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Physician diagnosed asthma ever aOR (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	0.96 (0.60-1.52)	0.58 (0.13-2.50)
Insufficient (≥50-75 nmol/L)	1393 (37)	**0.64 (0.42-0.98)**	0.34 (0.10-1.09)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Wheezing aOR (95% CI)	
Deficient (<50 nmol/L)	1156 (30)	1.19 (0.82-1.72)	0.32 (0.08-1.24)
Insufficient (≥50-75 nmol/L)	1393 (37)	1.03 (0.75-1.43)	**0.12 (0.04-0.38)**
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference
		Physician diagnosed eczema aOR (95% CI)[c]	
Deficient (<50 nmol/L)	1156 (30)	**1.64 (1.18-2.28)**	1.76 (0.67-4.65)
Insufficient (≥50-75 nmol/L)	1393 (37)	**1.34 (1.00-1.79)**	1.61 (0.80-3.23)
Sufficient (≥75 nmol/L)	1266 (33)	Reference	Reference

OR: Odds ratio; 95% CI: 95% confidence interval. OR's are compared to sufficient 25OHD concentration. EXP(ß): Ratio of geometric means and (ß): Regression coefficient indicating the change in standardized z-score compared to the reference group; 95% CI: 95% confidence interval.

Entries in bold are statistically significant.

[a] Adjusted for season at blood sampling.

[b] Adjusted for season at blood sampling, smoking during pregnancy, alcohol use during pregnancy, caesarean section, day-care attendance first two years, ethnicity, household income per month, birth weight *z*-score, folic acid intake, marital status, breastfeeding, history of cow's milk allergy first year, vitamin D supplementation age 6-12 months, respiratory tract infections, playing outside, walking and biking to school, TV watching and body fat percentage z-score at the age of 6 years.

[c] Not additionally adjusted for respiratory tract infections age 6 years.

Additional adjustment for gender, parity, maternal educational level, parental history of atopy, and age mother did not provide an alteration of ≥ 10% in OR.

Supplementary Table 7.4. Association between 25(OH)D concentration and eczema stratified by ethnicity

	Western			Non-Western		
25(OH)D concentration	**n (%)**	**Crude model[a] OR (95% CI)**	**Multivariate model[b] OR (95% CI)**	**n (%)**	**Crude model[a] OR (95% CI)**	**Multivariate model[b] OR (95% CI)**
Deficient (<50 nmol/L)	1137 (37)	**1.76 (1.13-2.75)**	**2.01 (1.24-3.24)**	129 (17)	0.88 (0.48-1.60)	1.04 (0.54-1.99)
Insufficient (≥50-75 nmol/L)	740 (25)	**1.45 (1.03-2.04)**	**1.61 (1.12-2.32)**	416 (54)	0.83 (0.46-1.50)	0.93 (0.50-1.74)
Sufficient (≥75 nmol/L)	1164 (38)	Reference	Reference	229 (29)	Reference	Reference

OR: Odds ratio; 95% CI: 95% confidence interval. OR's are compared to sufficient 25OHD concentration.
Entries in bold are statistically significant.
[a] Adjusted for season at blood sampling.
[b] Adjusted for season at blood sampling, smoking during pregnancy, alcohol use during pregnancy, caesarean section, day-care attendance first two years, household income per month, birth weight *z*-score, folic acid intake, marital status, breastfeeding, history of cow's milk allergy first year, vitamin D supplementation age 6-12 months, playing outside, walking and biking to school, TV watching and body fat percentage z-score at the age of 6 years.

Supplementary table 7.5. Association between 25 hydroxyvitamin D and asthma-related outcomes at age 6 years in children without asthma diagnosis

n=2191		**Crude model[a]**	**Multivariate model[b]**
	n (%)	**FeNO ≥25 ppb aOR (95% CI)**	
Deficient (<50 nmol/L)	821 (37)	1.76 (0.72-4.28)	1.73 (0.62-4.80)
Insufficient (≥50-75 nmol/L)	521 (24)	1.14 (0.57-2.25)	1.14 (0.55-2.34)
Sufficient (≥75 nmol/L)	849 (39)	Reference	Reference
		FeNO EXP(ß) (95% CI)	
Deficient (<50 nmol/L)	821 (37)	1.06 (0.92-1.10)	0.98 (0.89-1.08)
Insufficient (≥50-75 nmol/L)	521 (24)	1.03 (0.96-1.10)	1.02 (0.95-1.10)
Sufficient (≥75 nmol/L)	849 (39)	Reference	Reference
		Rint (ß) (95% CI)	
Deficient (<50 nmol/L)	821 (37)	**-0.95 (-1.43 to -0.48)**	**-1.09 (-1.61 to -0.56)**
Insufficient (≥50-75 nmol/L)	521 (24)	**-0.48 (-0.88 to -0.09)**	**-0.48 (-0.89 to -0.07)**
Sufficient (≥75 nmol/L)	849 (39)	Reference	Reference

OR: Odds ratio; 95% CI: 95% confidence interval. OR's are compared to sufficient 25OHD concentration. EXP(ß): Ratio of geometric means and (ß): Regression coefficient indicating the change in standardized z-score compared to the reference group; 95% CI: 95% confidence interval.

Entries in bold are statistically significant.

[a] Adjusted for season at blood sampling.

[b] Adjusted for season at blood sampling, smoking during pregnancy, alcohol use during pregnancy, caesarean section, day-care attendance first two years, ethnicity, household income per month, birth weight *z*-score, folic acid intake, marital status, breastfeeding, history of cow's milk allergy first year, vitamin D supplementation age 6-12 months, respiratory tract infections, playing outside, walking and biking to school, TV watching and body fat percentage z-score at the age of 6 years.

8

General discussion

MAIN FINDINGS

The timing of introduction of complementary feeding

The transition from fluid-based nutrition to solid food is developmentally programmed but timing of developmental readiness differs by infant [1]. Infant feeding recommendations vary between countries but infants feeding practices have also been found to differ despite similar feeding recommendations [2, 3]. Infant feeding practices often do not follow infant feeding recommendations and early introduction of solid foods is common [1, 4]. Both early and late introduction of complementary feeding (solid foods and liquids other than breast milk or infant formula and follow-on formula) have been associated with health risks. Since the complementary feeding period accompanies a critical window of vulnerability, identifying maternal and infant factors associated with the timing of introduction of complementary feeding is of importance. Therefore, we aimed to identify determinants associated with the timing of introduction of complementary feeding (Chapter 2). Recommendations regarding the introduction of solid foods has changed significantly over the past years [1]. By the 1990s a delayed introduction of complementary feeding beyond the age of 6 months was recommended. This recommendation was current in the Netherlands during the study period. In our study population we found that 62% of infants were introduced to complementary feeding before the age of 6 months. Our findings are in accordance with other studies. In a Danish study [5], about 70% of infants were introduced to complementary feeding before the recommended age of 6 months and about 80% of infants in a Canadian study [6]. We found that being a single parent and infant day care attendance were determinants for very early (<3 months) introduction of complementary feeding. Young maternal age, multiple parities, no infant family history of asthma, atopy and no infant history of allergy to cow's milk were determinants for early (4-6 months) introduction of complementary feeding. Determinants for both very early and early introduction were low educational level and not fully breastfed for 4 months. Previous studies reported similar determinants for early introduction of complementary feeding including young maternal age, low maternal education, and short duration of breastfeeding [5, 7]. We found infants with a family history of asthma, atopy or a history of allergy to cow's milk more likely to be introduced to complementary foods beyond the age of 6 months. Mothers of infants with an increased risk of allergic disease may be more aware of the introduction of complementary foods and less likely to introduce complementary feeding early. It has previously been suggested that in families with a history of allergy, the perceived risk of infants feeding recommendations is an important factor for parental action [8].

The age of exposure to food allergens has been suggested to be important in the development of tolerance [9]. Formerly, a delayed introduction of solid foods beyond the age of 6 months was recommended and further delay in the introduction of allergenic foods was recommended for infants with a family history of allergy [1]. More recent guidelines recommend the introduction of solid foods >17 weeks of age for the prevention of allergies [9, 10]. Previous studies examining the association between the timing of introduction

of allergenic foods and the development of allergic diseases such as asthma and eczema have shown conflicting results. Therefore, we aimed to assess the effect of the timing of introduction of allergenic foods on the development of wheezing and eczema up to 4 years of age (Chapter 3). We found a delayed introduction of allergenic foods cow's milk, hen's egg, peanuts, tree nuts, soy, and gluten after the age of 6 months did not prevent wheezing and eczema in pre-school children. In addition, the association did not differ between children with and those without a history of cow's milk allergy in the first year of life and those children with and those without a parental history of atopy. A review examining the association between the introduction of solid foods and allergic disease, including asthma and eczema in children, also found no convincing evidence that avoidance or delayed introduction of allergenic foods reduced the risk for allergy development in high-risk and non-risk infants [9]. Recently, two randomized controlled trials in the United Kingdom examined the role of early introduction of allergenic food in the development of allergic disease. The Enquiring About Tolerance (EAT) study found that early introduction from the age of 3 months of allergenic food in sufficient quantity may be able to help prevent food allergies in children [11]. The Learning about Peanut Allergy (LEAP) study found the introduction of peanut between the age of 4 to 11 months to significantly decrease the frequency of the development of peanut allergy in high risk children [12]. The findings of both randomized controlled trials support current evidence that delayed introduction of allergenic foods may not reduce allergies [9, 10].

The rapid increase in celiac disease prevalence might suggest that besides genetics, environmental factors also play a role in the loss of immune tolerance to gluten. It has been hypothesized that the introduction of gluten prior to maturation of the gut barrier may lead to an immune response [13]. Also, there may be a period during infancy when the immune system is predisposed to tolerance development. Exposure to a food during this time could lead to persistent tolerance to that food [14]. The timing of introduction of gluten and breastfeeding has long been thought to play a role in the development of celiac disease. Both early and late introduction of gluten have been suggested to increase the risk of celiac disease development [15, 16] whereas breastfeeding duration and exclusivity, and gradual introduction of gluten while breastfeeding has been proposed to be protective for the development of celiac disease [17, 18]. Yet, results of mainly retrospective studies lacked consistency [15, 16, 19-23]. Therefore, we prospectively studied whether the timing of introduction of gluten and breastfeeding duration were associated with celiac disease autoimmunity in 6 year old children (Chapter 4). We found a delayed introduction of gluten beyond the age of 6 months not to increase the risk of celiac disease autoimmunity. Also, we found breastfeeding for 6 months or longer not to decrease the risk of celiac disease autoimmunity. The results of our study are in agreement with the findings of two randomized controlled trials. The Risk of Celiac Disease and Age at Gluten Introduction (CELIPREV) trial compared early and delayed introduction of gluten on the prevalence of celiac disease autoimmunity and overt celiac disease among children at risk. The study

reported the introduction of gluten at age 6 months compared with the introduction at 12 months to have no effect on the risk of celiac disease autoimmunity and overt celiac disease at 5 years of age. Also, breastfeeding had no effect on the development of celiac disease [24]. The PEVENTCD study, a randomized controlled trial in high-risk children, reported breastfeeding (exclusive or during gluten introduction) and early introduction (16-24 weeks) of small quantities of gluten not to influence the development of biopsy-confirmed celiac disease [25]. Therefore, the results of this latter study did not support the 2008 ESPGHAN Committee on Nutrition recommendation to avoid late (≥7 months) introduction of gluten and to introduce gluten gradually while the infant is still being breastfed to reduce the risk of celiac disease [10]. In addition, a recent updated systematic review reported breastfeeding and the age of gluten introduction to have no effect on the risk of celiac disease development in childhood [26]. Based on the new available evidence the ESPGHAN Committee on Nutrition recommendations now state that gluten may be introduced anytime between 4 (17 weeks) to 12 months of age. Breastfeeding should be promoted but neither any breastfeeding nor breastfeeding during gluten introduction reduces the risk of celiac disease [27]. Since gluten is central to the pathogenesis of celiac disease it remains the main environmental trigger.

Based on previous available evidence it has been suggested that the window of vulnerability in infancy for allergic disease most likely be allocated between 4 to 7 months of age [10]. Although the timing of the window to prevent allergies is still not clear, evidence for delaying the introduction of complementary foods in the infant's diet seems to be lacking.

Nutrition and respiratory and allergic disease

It is well known that breastfeeding is the most desirable nutrition for infants [28, 29]. Especially during infancy breastfeeding has been found to protect against infectious diseases [29, 30]. Human milk offers passive protection against infections in the infant via secretory IgA antibodies and most likely via other immune components such as lactoferrin, oligosaccharides, cytokines and others [28]. It has been suggested that immune factors such as anti-idiotypic antibodies as well as T and B lymphocytes and others factors may also actively stimulate the immune system of the infants with lasting effects [31, 32]. Whether the effect of breastfeeding on the development of respiratory tract infections proceeds after infancy is still not clear as evidence on long-term effects is lacking. For this reason, we assessed whether breastfeeding is associated with lower and upper respiratory tract infections after infancy up to 4 years of age (Chapter 5). We found breastfeeding for 6 months or longer to be associated with a reduced risk of lower respiratory tract infections in children. We did not find an association between breastfeeding dose and lower respiratory tract infections. In agreement with our findings, a systematic review and meta-analysis reported lack of breastfeeding as a risk factor for severe acute lower respiratory infections in children under 5 years of age [33]. When including studies in industrialized countries only, this latter study did not find an association between breastfeeding (lack of exclusive breastfeeding and no breastfeeding) and severe acute lower respiratory infections in children. However, only 2

case control studies from industrialized countries were included in this review [33]. We did not find the duration of breastfeeding and predominant breastfeeding to be associated with upper respiratory tract infections. Previous studies on breastfeeding and upper respiratory infections after infancy have shown conflicting results with some studies reporting an association [34, 35] and other studies a lack of [36, 37]. Comparison between studies examining the effect of breastfeeding on respiratory infections is difficult as the definition of breastfeeding varies. Also, studies focused on different outcomes with some studies examining the effect on respiratory tract infections in general and others specific respiratory infections. Whether the protective effect of breastfeeding on infectious diseases proceeds after infancy remains inconclusive.

A potential environmental factor related to the rise in asthma prevalence might be the changes in diet. Oxidative stress and inflammation are central in the clinical manifestation of asthma. Some foods such as vegetables and fruits have been proposed to have antioxidant, anti-allergic and anti-inflammatory properties which may have a protective effect on the development of asthma [38, 39]. Therefore, diet might modulate the risk of asthma and other respiratory diseases. Previous studies have focused on either individual nutrients or individual food groups. An overview of systematic reviews showed a beneficial effect of fresh fruits and antioxidant vitamins (vitamins C, E and D) on asthma or wheeze for which the protective effect was more clear in children [39]. More recently, some studies have examined the association between overall diet and asthma in children [39, 40]. Most studies focused on the effect of a traditional Mediterranean diet on the development of asthma symptoms. It has been suggested that adherence to a Mediterranean diet during childhood might have a protective effect on the development of asthma [40]. The Mediterranean diet refers to dietary patterns low in saturated fatty acids and rich in complex carbohydrates, fiber, antioxidants, monounsaturated fatty acids and n-3 polyunsaturated fatty acids (PUFA). A Mediterranean diet might influence asthma development through its ability to reduce the effect of oxidative stress and inflammation. Contrary, a more modern/Western dietary pattern low in antioxidants and high in saturated fat might increase the risk of asthma symptoms [41]. We studied whether a "Western" dietary pattern, characterized by high intake of refined grains, soups and sauces, savoury and snacks, other fats, sugar-containing beverages and meat and a "Health conscious" dietary pattern, characterized by starchy foods, fruit, vegetables, potatoes, vegetable oils, fish, legumes and meat are associated with respiratory symptoms in pre-school children (Chapter 6). We found a high adherence to a "Western" diet to be associated with an increased risk of respiratory symptoms including wheezing, shortness of breath, and respiratory tract infections. A review examining the association between consumption of a Western dietary pattern and asthma in adults did not find an association between a Western diet and asthma. However, a possible link between a Western diet and asthma morbidity might exist [41]. We found no association between a "Health conscious" dietary pattern and reduction of respiratory symptoms. Two systematic reviews and meta-analyses reported that adherence to a Mediterranean dietary pattern may prevent respiratory outcomes including wheezing and asthma in childhood [40, 42]. One of these latter studies did not observe this association in adults [42].

It has been suggested that vitamin D has immunomodulatory effects on allergen-induced inflammatory pathways [43, 44]. Therefore, vitamin D may play a role in the development of asthma and allergies. The role of vitamin D in the prevention of asthma and allergies in children remains controversial as studies found both negative and positive results [44]. We aimed to examine whether serum 25-hydroxyvitamin D (25(OH) vitamin D) concentrations are associated with asthma symptoms including the fraction of exhaled nitric oxide (FeNO), airway interrupter resistance (Rint), physician diagnosed asthma ever, wheezing and eczema in 6 year old children (Chapter 7). We found that 33% of children had sufficient 25(OH)D levels whereas 37% had insufficient and 30% deficient. A systematic review examining the role of 25 (OH) vitamin D in asthma found no association with asthma incidence in childhood (age 4-20 years) [45]. However, the studies varied greatly in the way that asthma was defined and measured [45]. For example, parental reports included questions ranging from symptoms, medication use to doctor diagnosis of asthma. In addition to self-administered questionnaire data, we used FeNO, a biomarker of airway inflammation, and Rint measurements, reflecting airway resistance, as an objective tool to support in the diagnosis of asthma in children [46, 47]. We found lower 25(OH) vitamin D levels to be associated with elevated FeNO levels, suggestive of eosinophilic airway inflammation. Few studies examining the association between vitamin D and FeNO found no association in children [48, 49]. In addition, a randomized controlled trial evaluating the effect of vitamin D treatment on fractional exhaled nitric oxide (FeNO) in children with mild asthma found no correlation between vitamin D and FeNO. However, the small sample size precludes conclusions [50]. We found lower vitamin D levels to be associated with lower Rint values. As lower Rint values reflect better airway patency an opposite effect was expected. To our knowledge no other study examined the association between vitamin D status and Rint and further studies are needed to elucidate our findings. Also, we found lower 25(OH) vitamin D levels to be associated with increased risk for eczema in Western children. A New Zealand cross-sectional study found no association between 25(OH) vitamin D concentration and eczema [51]. However, a randomized controlled trial found vitamin D supplementation to lead to an improvement in atopic dermatitis severity in children, supporting a causal role [52]. Despite the growing body of literature investigating the association between vitamin D and asthma and allergies in childhood, the role of vitamin D remains unclear as results are inconclusive.

METHODOLOGICAL CONSIDERATIONS

Study design

The studies described in this thesis were performed in the Generation R study, a prospective cohort study. Cohort studies provide an opportunity to observe populations prospectively, thereby identifying factors associated with a disease of interest. It allows to examine the relationship between different variables and multiple outcome measures and relevant covariates. However, to appreciate the results of this thesis, methodological considerations need to be taken into account.

Cohort studies can be prone to selection bias due to loss of study subjects. Initially, this may occur if a portion of the target study population does not participate and is systematically

different from the original study population with regard to exposure and outcomes status. In the Generation R study participating mothers more often had a higher socioeconomic status than non-participating mothers. This may have influenced the external validity. Moreover, selection bias may also occur when subjects are lost to follow up. However, selection bias could have influenced our findings only if the association between early life nutrition and disease was different for those who participated compared with those who did not participate.

Missing data are inevitable in epidemiological research and may also lead to biased results. The risk of bias depends on the reason for missing data. Missing data can be categorized as missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR) [53]. Missing data is MCAR when the missing observations are unrelated to any study variable (observed and unobserved). Missing data is MAR when systematic differences can be explained by other observed variables, but not by the variable with missing data itself whereas data MNAR is related to the missing variable itself [53]. We used multiple imputation to deal with missing data under the assumption that our data are MAR. However, in this thesis it was not possible to distinguish between data MAR and MNAR. When data was MNAR this could have affected the prediction of the missing values and therefore the uncertainty of the effect estimates observed.

Confounding and reverse causality

Epidemiological studies try to identify factors associated with disease outcome. However, other factors may also be associated with the exposure and affect the risk of developing the disease, thereby distorting the observed association between the exposure and disease. Sociodemographic and lifestyle factors are potential confounding variables in the association between early life nutrition and respiratory and allergic disease. Adjustment for these potential confounders is particularly important since lifestyle factors including diet have been found to cluster and also to be associated with health outcomes [54]. Although we adjusted our studies for multiple potentially confounding variables, residual confounding cannot be fully excluded. This may be due to the fact that some potential confounders were not (sufficiently) measured during the process of data gathering or because variables were measured with error.

We also considered the possibility of reverse causality. Reverse causality might influence the association between the timing of introduction of allergenic foods and wheezing and eczema, as parents with a family history of atopy or infants with early symptoms of allergy may delay the introduction of complementary feeding. Therefore, we evaluated whether the association was significantly different by history of cow's milk allergy and parental history of atopy (by assessing effect-modification). Reverse causality has been minimized in the association between dietary patterns and respiratory outcomes since we examined the effect of dietary patterns prior to the outcomes. In contrast, reverse causality may have influenced the observed association between vitamin D status and asthma and eczema.

Parents of children with early symptoms of allergy may be more inclined towards a healthy life style including supplementation of vitamin D. Also, children with symptoms of asthma may be less likely to spend time outdoors and therefore have lower vitamin D levels due to their illness. In addition, it has been suggested that an inadequate vitamin D status may indirectly indicate disease rather than associated with disease pathogenesis. Therefore, reverse causality cannot be fully excluded.

In observational research it is not possible to prove causality between early life nutrition and the reported outcomes. Only in a randomized controlled trial the risk of residual confounding and reverse causation can be excluded.

Nutritional assessment

In this thesis, a food frequency questionnaire (FFQ) was used to assess dietary intake in children. FFQs are commonly used in epidemiological studies. It is a widely used method for quantitative assessment of usual intake of groups or individuals. FFQs enables the assessment of long-term dietary intakes in a relatively simple, cost-effective, and less time consuming manner. However, FFQs may be less accurate than 24-hours diet recalls, dietary records, and nutritional biomarkers [55]. As diet may be influenced by factors including ethnicity and culture, FFQs should be developed for a specific study group. FFQs to assess dietary intake in children should be made suitable especially in terms of the types of foods that children consume. The FFQ used in the Generation R study, was specifically designed for the study population consisting of 1 year old children. It was tailored to foods frequently consumed by children 9-18 months of age according to a Dutch National Food Consumption Survey in 2002. The accuracy of information on dietary intake obtained by FFQ is highly dependent on the validity and reproducibility of the FFQ in the population it is intended for. The FFQ used in the Generation R study was validated against three day 24 hour recalls in Dutch children in the same age group. The intraclass-correlation coefficients were 0.4 for total energy, 0.7 for protein, 0.4 for total fat, 0.4 for carbohydrates, and 0.7 for dietary fiber. Although these correlations are not ideal, other validations studies of dietary measurements showed comparable coefficients [56].

Limitations of FFQs have to be considered in the interpretation of the results presented in this thesis. FFQs are self-reported measures of intake over an extended period of time. Because people may find it difficult to recall intake over a long period, FFQs may fail to truly reflect a person's long-term average intake. Thus, the estimated intake has an error that is often substantial which may lead to biased disease risk estimates. Dietary measurement error is mostly non-differential which is independent of the outcome of interest [57]. Misclassification of dietary intake may lead to attenuation of the effect estimate and wider confidence intervals, meaning an underestimation of the association and reduced statistical power to detect an associations. A second type of dietary measurement error that may occur is differential, which is related to the outcome of interest [57]. Because in the majority of studies described in this thesis, dietary intake was measured prior to the development of

the disease of interest, differential measurement error and thus recall bias may have been of minimum concern.

In the Generation R study we assessed dietary patterns in 1 year old children. Dietary patterns analysis capture the relationship between overall diet and its constituent foods, beverages and nutrients in relation to the outcome of interest. It thereby overcomes the collinearity among single foods and nutrients and considers the interactions between foods and nutrients in disease risk. Different dietary pattern assessment methods have been suggested [58]. In this thesis, dietary patterns were obtained through an *a posteriori* approach that was outcome-independent. The relationship between the dietary patterns and health outcomes were examined once the patterns had been defined. Principal component analysis (PCA) is a popular method for deriving dietary patterns. It makes use of the underlying relationships between food intakes to identify underlying patterns within a population [58]. However, several arbitrary decisions are involved in identifying dietary patterns by PCA including combining food items into food groups, the number of factors to retain, type of rotation, and the labelling of components [58]. These decisions are important for the interpretation of the dietary patterns as they may influence the reproducibility.

In this thesis, we adjusted the study on dietary patterns and disease outcome for total energy intake. Adjustment for total energy intake is a standard procedure in nutritional epidemiology. It addresses for the fact that total energy requirements are related to body size, metabolic efficiency, and physical activity. Failure to account for energy intake can obscure associations between diet and disease risk. An association found between a dietary pattern that represents a diet high in energy-dense foods and a disease outcome, may not be a real effect of the food themselves, but an association with actual energy intake. In addition to adjustment for the potential confounding effect of total energy intake, energy adjustment is used to adjust for measurement error in diet, which is based on the assumption that individuals tend to misreport to a similar degree and in the same direction. Different methods for energy adjustment have been described such as the residual method. However, the residual method for energy adjustment has not been applied in this thesis as it may be more difficult to pick up energy-dense dietary patterns when using PCA [59]. Therefore, we adjusted for total energy intake in the regression models after dietary patterns were defined. It has been suggested that energy adjustment in a later stage in the analysis is sufficient and that adjustment for energy intake before entry into PCA analysis is not necessary [59].

To assess the effect of dietary patterns on the development of respiratory outcomes over time, including changes in dietary intake, measurements at multiple time-points are needed. However, repeated measures of childhood diet was not available for the study presented in this thesis. We assessed the association between diet at the age of 1 year and respiratory symptoms in early childhood. With repeated measures the association could be adjusted for diet through early childhood and provide a more accurate assessment.

Specific biomarkers can be used to measure dietary intake of selected nutrients or dietary components. Nutritional biomarkers can provide an objective measure of dietary

intake or status that may provide more accurate measures than dietary intake estimates. We used vitamin D concentration as objective marker for nutrient status in children. However, measurement error may occur during processing, storage and laboratory assessment of the samples [60]. Since the measurement error is most likely to be random we do not expect this to have influenced our results.

Respiratory and allergic disease outcome assessment

The outcomes in this thesis were primary obtained from parent-reported questionnaires. Questions from the age-adapted version of the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires on asthma and atopic eczema were used [61]. The ISAAC questionnaire was developed for children 6-7 years and 13-14 years of age and used worldwide [61]. The questions used were made suitable for younger children between 1-4 years of age but was not validated for this age group. Diagnosing asthma can be difficult in young children [62]. Wheezing, coughing and other asthma-like symptoms can occur with conditions other than asthma such as viral respiratory infections. Objective tests to support the diagnosis of asthma include spirometry and bronchoprovocation. In children under the age of 5 years, lung function testing is not reliable due to the child's inability to perform reproducible expiratory maneuvers. Therefore, diagnoses are largely based on reported symptoms [62]. Since the outcomes of the studies in this thesis were mainly assessed by parent-reported questionnaires, misclassification could have occurred. However, we do not expect the misclassification to have influenced our results on the association between diet and the outcome of interest. Only when the misclassification was related to the exposure ie. breastfeeding, the timing of introduction of complementary feeding, and adherence to dietary patters would this have influenced the results.

In addition to parent-reported questionnaire data, we used the fraction of exhaled nitric oxide (FeNO) and the interrupter technique measuring airway resistance (Rint) during spontaneous breathing as objective measures to support asthma diagnosis. FeNO is a non-invasive marker of eosinophilic airway inflammation, a hallmark of asthma. Unlike other lung function tests, FeNO can be successfully obtained in young children [63]. Eosinophilic inflammation develops and increases in childhood. An association between FeNO in early childhood and asthma later in life has been suggested [64]. Therefore, FeNO as a biomarker might help to identify children at risk for school age asthma. Measuring Rint is another method that is feasible in young children [46]. Rint measurements have been found to be associated with asthma symptoms in children [65, 66]. However, whether Rint is a useful measure to diagnose asthma in preschool children is unclear. Few studies assessed the additional value of Rint [46]. One study in preschool children with asthma-like symptoms found Rint not to improve the prediction of asthma symptoms in childhood [67].

To assess celiac disease autoimmunity, we measured anti-tissue transglutaminase (anti-tTG) concentrations in venous serum. Clinical celiac disease can be diagnosed when anti-tTG concentrations ≥10 times the ULN in combination with positive anti-endomysial antibody

and symptoms are present [68]. Subclinical celiac disease is found by screening and must be verified by biopsy [68]. Since this is not the case in our study we examined the development of celiac disease autoimmunity and conclusions concerning celiac disease should be made with causation.

IMPLICATION FOR PUBLIC HEALTH AND FUTURE RESEARCH

In the Generation R study the association between nutrition in early life and the development of childhood respiratory and gastrointestinal disease has been investigated. Based on the current literature and the studies presented in this thesis some public health recommendations and direction for future research will be given.

Breastfeeding is suggested to protect against a wide range of diseases during infancy but also later in life. Especially in infancy, breastfeeding is known to protect against respiratory tract infections. Previous research, including our study, found this protective effect to proceed in early childhood. Therefore, we support current recommendations on breastfeeding for the duration of at least 6 months to reduce the risk for respiratory tract infections in children. Recent randomized controlled trials confirmed our results that breastfeeding does not reduce the risk for celiac disease.

Recommendations regarding the timing of introduction of complementary feeding has changed considerably over the past years. There is no evidence, including the results of our study, that avoiding or delaying the introduction of allergenic foods beyond the age of 6 months reduces the risk for allergic or celiac disease. In addition, recent randomized controlled trials reported that the introduction of allergenic food i.e. peanut, before 6 months of age might even protect for allergic disease. Therefore, on the basis of recent studies recommendations in The Netherlands that complementary feeding may be introduced into a child's diet as from 4 months of age may be valid.

Health professionals need to provide counseling to parents to follow infant feeding recommendations. In doing so, it is important that they are aware of groups and individuals who are less likely to follow these recommendations. Young mothers, mothers with a low educational level, and mothers of infants who attend day care may be appropriate targets for additional guidance and education. Also, mothers of children with a family history of asthma, atopy or a history of allergy to cow's milk may need guidance to follow infants feeding recommendations as they may be more likely to delay the introduction of complementary feeding due to previous recommendations.

The timing of introduction of gluten is not associated with celiac disease autoimmunity in childhood. Future research on early nutrition may focus on the introduction of other types of gluten (for example different wheat preparations), the optimal amount of gluten to be introduced, and duration of gradual amounts of gluten. Since gluten is central to the pathogenesis of celiac disease it remains the main environmental trigger. However, as the introduction of gluten in infancy does not appear to influence celiac disease development, further studies on other environmental factors that may contribute to the development of celiac disease may be valuable. An environmental factor that has been suggested to play

a protective role in the development of celiac disease are infections in early childhood [69]. Future research on childhood infections and celiac disease might give more insight into the mechanisms underlying the development of this disease.

We observed a unhealthy diet to be associated with respiratory symptoms. Different factors may influence food choices of mothers with young children. Most dietary interventions that have been implemented have focused on the prevention of childhood obesity as opposed to overall healthy diet behavior. Because dietary patterns are already present after the first year of life, and are likely to remain in childhood to adulthood, interventions improving dietary behavior may target mothers with young children [70]. The effect of additional dietary guidance for mothers of children from the age of 1 year on dietary behavior could be evaluated in a randomized controlled trial. Promoting healthy dietary practices could help to develop long-term positive eating behaviors and may contribute to the prevention of respiratory and other chronic diseases.

Previous studies and our study found vitamin D status to be associated with asthma symptoms and eczema in young children. However, we observed inconsistent associations. In the Netherlands, it is recommended that all children till the age of 4 years receive 400 IU of vitamin D daily [71]. Since vitamin D deficiency is still very common, health professionals should encourage parents to follow current recommendations on vitamin D supplementation in early childhood. Although vitamin D supplementation is advised for all children under the age of 4 years, this is especially important for children at risk of vitamin D deficiency [71]. Mothers of children with a darker skin type and children with limited sun exposure should be encouraged to continue the use of vitamin D supplementation after preschool age. Also, parents should be made aware that sun exposure is the main source of vitamin D and spending time outside could increase vitamin D levels. As the evidence is still inconclusive, well-designed clinical trials are warranted to examine the relation between vitamin D supplementation and the development of asthma and eczema in childhood.

Diagnosing asthma can be difficult in children under the age of 5 years. In older children, spirometry can support the diagnosis of asthma. This lung function test has been performed in 9 year old children in the Generation R study. Replication of the analyses on early life nutrition and asthma symptoms with spirometry, may give new insight in whether diet in early childhood is associated with the development of asthma in later childhood. In addition, allergen-specific immunoglobulin E (IgE) were assessed by skin-prick test at the age of 9 years. A positive skin test indicates sensitization which refers to the production of allergen-specific IgE. Most allergic responses are mediated by IgE antibodies specific for a particular allergen [72]. Atopic individuals who have a tendency to become sensitized and produce IgE antibodies in response to a particular allergen may develop typical symptoms of asthma or eczema. Allergen-specific IgE in combination with symptoms of asthma and eczema could support the diagnoses of an allergic disease.

Intestinal microbiome is an actively developing field. It has been suggested that the human intestinal microbiome is essential to the development of a healthy immune system. Early-life exposures, including infant feeding, have the capacity to influence the microbiome

composition. It has been suggested that in early childhood a window might exist when the effects of gut microbial dysbiosis are most influential in the immune development, with lasting effects on health and susceptibility to disease. An altered intestinal microbiome has been associated with immune-mediated diseases, including allergic diseases and asthma [73]. The intestinal microbiome might also play a role in the development of celiac disease. Similar environmental factors have been suggested to influence both the composition of the intestinal microbiome and celiac disease development, including mode of delivery and infant feeding. Prospective longitudinal studies from birth such as the Celiac Disease Genomic, Environmental, Microbiome, and Metabolomic Study (CDGEMM), might give more insight into how microbiome composition and metabolomic profiles may influence the development of disease [74, 75].

Nutrition in childhood has been linked to different health outcomes, but still much is unknown. In order to gain more insight in the influence of early nutrition on future respiratory and gastrointestinal health, further follow-up of the Generation R study cohort is valuable. Follow-up is needed to find out what the long term effects are of breastfeeding, the timing of introduction of complementary feeding, dietary patterns in young children, and nutrient deficiency in childhood.

CONCLUSION

Nutrition in early childhood may affect respiratory and allergic disease. We found breastfeeding for 6 months or longer to reduce the risk for respiratory tract infections in preschool children. Breastfeeding was not associated with a reduced risk for celiac disease autoimmunity. Delaying the introduction of allergenic foods beyond the age of 6 months does not prevent wheezing and eczema nor does it increase the risk of celiac disease autoimmunity. We found an unhealthy diet in early childhood to be associated with respiratory symptoms. Furthermore, we found lower vitamin D levels to be associated with asthma outcomes, but we observed inconsistent associations. We support current recommendations on breastfeeding, the timing of introduction of allergenic foods, and vitamin D supplementation. Early childhood may offer a window of opportunity to prevent disease or to identify those children who are at risk for severe disease. Improving early life nutrition may provide a possibility for prevention.

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9

Summary & Samenvatting

SUMMARY

The first year of life includes many transitions in food consumption. After a period of milk feeding including breast and formula feeding, gradual introduction of complementary feeding takes place, after which in early childhood dietary behaviors develop. Nutrition may be a critical determinant of health at every age but especially during critical stages of early development. Exclusive breastfeeding for 6 months is a global public health recommendation. However, as of 4 months of age complementary feeding may be introduced with dietary patterns emerging after infancy. The period of nutritional transition and diet in early childhood may provide a window of opportunity for disease prevention.

In this thesis we studied nutrition in early life and its association with allergic, respiratory and gastrointestinal health. We studied nutrition during different critical stages in infancy and early childhood and its effect on childhood health outcomes. Breastfeeding has been suggested to protect against respiratory tract infections in infancy for which its effect may proceed in early childhood. However, evidence for long-term effects of breastfeeding for the development of respiratory tract infections is limited. For the timing of introduction of complementary feeding a critical window has been suggested for the prevention of allergic and gastrointestinal disease. But, there is currently still much debate on optimal feeding recommendations in early life for the prevention of disease. Since children eat a variety of foods with complex combinations of nutrients, overall diet may be more predictive for disease risk. Yet, studies assessing the effect of dietary patterns in early childhood are few. Vitamin D deficiency is common in young children and has been linked to adverse health consequences, but a role of vitamin D in the prevention of disease is unclear.

The studies presented in this thesis were conducted within the Generation R study. Information on early life nutrition was obtained by FFQ and information on the different health outcomes by parent-reported questionnaires. At the age of 6 years, all children were invited to the research facility in the Erasmus MC, Sophia children's hospital, to participate in different health outcome measurements. Also, serum samples were taken during the visit to the research center and serum 25(OH) vitamin D concentration and anti-tissue transglutaminase (anti-tTG) concentration was assessed.

The timing of introduction of complementary feeding

The studies on the timing of introduction of complementary feeding are described in *chapter 2-4*. First, in *chapter 2* we identified determinants associated with the timing of introduction of complementary feeding. At the time the study was conducted delaying the introduction of complementary feeding beyond the age of 6 months was recommended. In our study population we found that 62% of infants were introduced to complementary feeding before the age of 6 months. We confirmed determinants for early introduction of complementary feeding that have been identified by previous studies such as young maternal age, low educational level, and not fully breastfeeding. In addition to these determinants, we found day care attendance to be a determinant for very early introduction

of complementary feeding. Health professionals may provide counseling to parents to follow infant feeding recommendations. Young mothers, mothers with a low educational level, and mothers of infants who attend day care may be appropriate targets for additional guidance and education. Also, we found infants with a family history of asthma, atopy or a history of allergy to cow's milk to be more likely to be introduced to complementary feeding after the recommended age of 6 months. Mothers of these children may need guidance to follow infants feeding recommendations as they may be more likely to delay the introduction of complementary feeding due to previous recommendations. Second, in *chapter 3* we assessed the association between the timing of introduction of allergenic foods cow's milk, hen's egg, peanuts, tree nuts, soy, and gluten and the development of wheezing and eczema in children 4 years of age and younger. In agreement with previous studies, we demonstrated that the timing of introduction of allergenic foods was not associated with eczema and wheezing. Therefore, our findings do not support previous recommendation for delayed introduction of allergenic foods after age 6 months for the prevention of eczema and wheezing. Also, the association did not differ between children with and those without a history of cow's milk allergy in the first year of life and those children with and those without a parental history of atopy. Third, in *chapter 4* we examined whether the timing of gluten introduction and breastfeeding duration are associated with celiac disease autoimmunity in children at the age of 6 years. We observed a delayed introduction of gluten not to increase the risk of celiac disease autoimmunity. In addition, breastfeeding does not decrease the risk of celiac disease autoimmunity. Future research on other environmental factors that may contribute to the development of celiac disease may be valuable.

Based on available evidence, current recommendations that complementary feeding may be introduced as of the age of 4 months may be valid since evidence for delaying the introduction of complementary foods in the infant's diet seems to be lacking.

Nutrition and respiratory and allergic disease

We assessed the association between breastfeeding with lower and upper respiratory tract infections after infancy up to 4 years of age in *chapter 5*. We found breastfeeding duration for 6 months or longer to be associated with a reduced risk of lower respiratory tract infections, but not upper respiratory tract infections, in pre-school children. Predominant breastfeeding until 4 months was not associated with respiratory tract infections. The results of this study suggest that the protection against respiratory tract infections may proceed after the first year of life. We support current recommendations on breastfeeding for the duration of at least 6 months to reduce the risk for respiratory tract infections in children.

In *chapter 6* we examined the association between dietary patterns in childhood and respiratory symptoms including wheezing, shortness of breath, and respiratory tract infections in pre-school children. In addition, we assessed whether this association could be explained by energy intake. We demonstrated that a Western diet, characterized by high intake of refined grains, soups and sauces, savoury and snacks, other fats, sugar-containing

beverages and meat, may increase the risk of frequent respiratory symptoms at 3 and 4 years of age. This association was in some measure explained by energy intake. We found no association between a "Health conscious" dietary pattern and reduction of respiratory symptoms. Because dietary patterns are already present after the first year of life, and are likely to remain in childhood to adulthood, interventions improving dietary behavior may target mothers with young children. Promoting healthy dietary practices could help to develop long-term positive eating behaviors and may contribute to the prevention of respiratory and other chronic diseases.

In *chapter 7* we aimed to examine the association between serum 25-hydroxyvitamin D (25(OH) vitamin D) concentrations and the fraction of exhaled nitric oxide (FeNO), airway interrupter resistance (Rint), physician diagnosed asthma ever, wheezing and eczema in 6 year old children. We found that lower 25(OH) vitamin D levels are associated with elevated FeNO levels, but lower Rint values. Lower 25(OH) vitamin D levels are also associated with a decreased risk for asthma diagnoses but an increased risk for eczema. Since vitamin D deficiency is very common in childhood, health professionals should encourage parents to follow current recommendations on vitamin D supplementation. Although vitamin D supplementation is advised for all children under the age of 4 years, this is especially important for children at risk of vitamin D deficiency. As the evidence is still inconclusive, well-designed clinical trials are warranted to examine the relation between vitamin D supplementation and the development of asthma and eczema in childhood.

Last, *chapter 8* provides a general discussion of the studies described in this thesis. Also, the methodological considerations of our studies, and the implication for public health and future research are addressed. The findings of this thesis suggest that nutrition in early childhood may affect respiratory and allergic disease. Early childhood may offer a window of opportunity to prevent disease or to identify those children who are at risk for severe disease. Improving early life nutrition may provide a possibility for prevention.

SAMENVATTING

In het eerste levensjaar vindt er veel verandering plaats in voedingsconsumptie. Na een periode van melkvoeding waaronder borst en flesvoeding vindt geleidelijke introductie plaats van bijvoeding, waarna al op de vroege kinderleeftijd voedingspatronen ontstaan. Voeding is mogelijk een belangrijke determinant van gezondheid in elke levensfase, maar met name in de kritische fases van de vroege ontwikkeling. Het geven van exclusief borstvoeding tot de leeftijd van 6 maanden wordt wereldwijd voor de volksgezondheid aanbevolen. Echter kan volgens de richtlijnen vanaf de leeftijd van 4 maanden gestart worden met het introduceren van bijvoeding, waarna zich na het eerste levensjaar geleidelijk voedingspatronen ontwikkelen. De vroege kinderleeftijd waarin veel verandering in voeding plaats vindt kan mogelijk een kans bieden voor ziekte preventie.

In dit proefschrift onderzochten we voeding in de eerste levensjaren in relatie tot allergische, respiratoire en gastro-intestinale gezondheid. We onderzochten het effect van voeding in de verschillende kritische fases- in het eerste levensjaar en vroege kinderleeftijd- op gezondheidsuitkomsten van deze kinderen. Het geven van borstvoeding zou mogelijk een beschermend effect hebben op het ontwikkelen van luchtweginfecties in het eerste levensjaar, waarna dit effect zou continueren op de vroege kinderleeftijd. Bewijs voor beschermende effecten van het geven van borstvoeding op het ontwikkelen van luchtweginfecties op de lange termijn is echter beperkt. Een periode waarin de introductie van bijvoeding mogelijk voor preventie van allergische en gastro-intestinale ziekte zou kunnen zorgen wordt gesuggereerd. Tot op heden is er echter nog veel discussie gaande over de meest optimale voedingsadviezen voor de preventie van ziekten. Sinds kinderen een verscheidenheid aan voedsel consumeren met complexe combinaties van nutriënten, zou het gehele dieet mogelijk meer voorspellend kunnen zijn voor het risico op ziekten. Echter zijn er weinig studies verricht, die het effect van voedingspatronen op de vroege kinderleeftijd hebben onderzocht. Vitamine D deficiëntie komt veel voor bij jonge kinderen en is gerelateerd aan slechte gezondheidsuitkomsten. Echter de rol van vitamine D voor de preventie van ziekte is onduidelijk.

De studies die in dit proefschrift worden gepresenteerd werden verricht in The Generation R Study. Informatie over voeding op de vroege kinderleeftijd werd verzameld door middel van voedselvragenlijsten, terwijl informatie over de verschillende gezondheidsuitkomsten door middel van de, door de ouders ingevulde vragenlijsten werden verkregen. Op zesjarige leeftijd werden alle kinderen uitgenodigd voor een bezoek aan het onderzoekscentrum in het Erasmus MC, Sophia kinderziekenhuis, voor deelname aan verschillende gezondheidsmetingen. Gedurende het bezoek aan het onderzoekscentrum werd er bloed afgenomen en werd er serum 25(OH) vitamine D concentratie en anti-tissue-transglutaminase (anti-tTG) concentratie bepaald.

Het tijdstip van introduceren van bijvoeding

De studies over het tijdstip van introduceren van bijvoeding worden beschreven in hoofdstuk 2-4. In hoofdstuk 2 identificeren we determinanten die zijn geassocieerd met het

tijdstip van introduceren van bijvoeding. Op het moment van de studie werd geadviseerd het introduceren van bijvoeding uit te stellen tot na de leeftijd van 6 maanden. In onze studie populatie constateerden we dat 62% van de zuigelingen alreeds vóór de leeftijd van 6 maanden aan bijvoeding waren geïntroduceerd. Onze studie vond dezelfde determinanten voor vroege introductie van bijvoeding die ook in eerdere studies zijn aangetoond, waaronder jonge leeftijd van moeder, laag educatie niveau van moeder, en het niet geven van volledige borstvoeding. Naast deze determinanten vonden we dat verblijf in een kinderopvang ook een determinant was voor vroege introductie van bijvoeding. Gezondheidsmedewerkers zouden ouders begeleiding kunnen bieden om zo voedingsadviezen voor zuigelingen op te volgen. Jonge moeders, moeders met een laag educatie niveau, en moeders van zuigelingen die naar een kinderopvang gaan, zouden een goede doelgroep kunnen zijn voor additionele begeleiding en educatie. Ook is gebleken dat het introduceren van bijvoeding bij zuigelingen met een familie geschiedenis van astma, atopie of geschiedenis van koemelk allergie, vaker wordt uitgesteld tot na de leeftijd van 6 maanden zoals voorheen werd geadviseerd. Moeders van deze kinderen hebben mogelijk begeleiding nodig om voedingsadviezen op te volgen, omdat zij meer geneigd zijn de bijvoeding uit te stellen vanwege voorgaande adviezen. In hoofdstuk 3 onderzochten we de associatie tussen het tijdstip van introduceren van allergene voedingsmiddelen zoals koemelk, kippen-ei, pinda's, noten, soja, en gluten, en het ontwikkelen van 'wheezing' en eczeem bij kinderen tot en met de leeftijd van 4 jaar. In overeenstemming met voorgaande studies laten we zien dat het tijdstip van introduceren van allergene voedingsmiddelen niet is geassocieerd met 'wheezing' en eczeem. De gevonden associatie verschilde niet tussen kinderen met en zonder een geschiedenis van koemelkallergie in het eerste levensjaar, en kinderen met en zonder familiegeschiedenis voor atopie. Onze resultaten ondersteunen hierin niet de voorgaande aanbeveling voor het uitstellen van het introduceren van allergene voedingsmiddelen tot na de leeftijd van 6 maanden, voor de preventie van 'wheezing' en eczeem. In hoofdstuk 4 onderzochten we of het tijdstip van introduceren van gluten en de duur van borstvoeding, geassocieerd zou zijn met coeliakie auto-immuniteit bij kinderen op de leeftijd van 6 jaar. Wij vonden dat met het uitstellen van het introduceren van gluten het risico op coeliakie auto-immuniteit niet wordt verhoogd. Ook vonden wij dat borstvoeding het risico op coeliakie auto-immuniteit niet verlaagd. Toekomstige studies naar andere omgevingsfactoren die kunnen bijdragen aan de ontwikkeling van coeliakie kunnen waardevol zijn.

Op basis van beschikbaar wetenschappelijke literatuur, en dat er geen bewijs is dat het uitstellen van bijvoeding bij zuigelingen gunstig is, is de huidige aanbeveling om de introductie van bijvoeding te starten vanaf de leeftijd van 4 maanden passend.

Voeding en respiratoire en allergische ziekte

In hoofdstuk 5 onderzochten we de associatie tussen het geven van borstvoeding met hoge en lage luchtweginfecties na het eerste levensjaar tot en met de leeftijd van 4 jaar. Wij vonden dat het geven van borstvoeding voor 6 maanden en langer, geassocieerd is met een

lager risico van lage luchtweginfecties. Dit in tegenstelling tot bovenste luchtweginfecties. Voornamelijk borstvoeding tot de leeftijd van 4 maanden was niet geassocieerd met luchtweginfecties. De resultaten van deze studie suggereren, dat het beschermende effect van borstvoeding voor luchtweginfecties continueert tot na het eerste levensjaar. Wij ondersteunen de huidige aanbeveling dat borstvoeding voor 6 maanden of langer het risico op luchtweginfecties vermindert.

In hoofdstuk 6 onderzochten we de associatie tussen voedingspatronen op de kinderleeftijd en respiratoire klachten zoals 'wheezing', kortademigheid en luchtweginfecties op jonge kinderleeftijd. Ook onderzochten we of deze associatie zou kunnen worden verklaard door energie inname. Wij vonden dat een 'Westers' dieet, gekarakteriseerd door hoge inname van geraffineerde granen, soep, sausen, snacks, andere vetten, suikerhoudende dranken en vlees het risico op frequente respiratoire klachten op de leeftijd van 3 en 4 jaar mogelijk doet toenemen. Deze associatie werd gedeeltelijk verklaard door energie inname. Wij vonden geen associatie tussen een "gezond" dieet patroon en respiratoire klachten. Omdat voedingspatronen na het eerste levensjaar al aanwezig, en mogelijk blijvend zijn van kinderleeftijd tot volwassenheid, zouden interventies gericht op het verbeteren van het eetgedrag zich moeten richten op moeders met jonge kinderen. Het promoten van een gezonde eetstijl kan bijdragen aan positief eetgedrag op de lange termijn en mogelijk bijdragen aan de preventie van respiratoire en andere chronische ziektes.

In hoofdstuk 7 onderzochten we de associatie tussen 25(OH) vitamine D concentraties en de fractie van stikstofmonooxide (FeNO) in uitgeademde lucht, luchtweg weerstand (Rint), dokter gediagnostiseerde astma, 'wheezing' en eczeem op de leeftijd van 6 jaar. Wij vonden dat lage 25(OH) vitamine D levels geassocieerd zijn met verhoogde FeNO levels, maar met lagere Rint waardes. Lage 25(OH) vitamine D levels zijn ook geassocieerd met een lager risico op astma diagnose maar een verhoogd risico op eczeem. Omdat vitamine D deficiëntie veel voorkomt bij kinderen zouden gezondheidsmedewerkers ouders kunnen motiveren de huidige aanbevelingen voor vitamine D suppletie op te volgen. Hoewel vitamine D suppletie wordt aanbevolen voor alle kinderen beneden de leeftijd van 4 jaar, is dit met name belangrijk voor kinderen met een verhoogd risico op vitamine D deficiëntie. Omdat het bewijs niet eenduidig is zijn clinical trials nodig om de relatie tussen vitamine D suppletie en de ontwikkeling van astma en eczeem op de kinderleeftijd te onderzoeken.

Tot slot geeft hoofdstuk 8 een algemene discussie van de studies beschreven in dit proefschrift. Ook de methodologische overwegingen van onze studies en de implicatie voor de volksgezondheid en vervolg onderzoek worden behandeld. De bevindingen van dit proefschrift suggereren dat voeding op jonge leeftijd mogelijk effecten heeft op respiratoire en allergische ziektes. De vroege kinderleeftijd zou daarom een kans bieden voor preventie van ziekte en het identificeren van kinderen met een verhoogd risico op ziekte. Het verbeteren van voeding op de kinderleeftijd biedt mogelijk een kans voor preventie.

10

PhD Portfolio

About the author

Dankwoord

PhD PORTFOLIO

Summary PhD training

Name PhD student:	Ilse Isabella Meswin Tromp
Erasmus MC Department:	Pediatrics – Generation R
PhD period:	2012 until 2016
Promotor:	Prof.dr. H.A. Moll
Co-promotor:	Dr. J.C. Kiefte-de Jong

TRAINING	Year	Workload
General courses		
Master's degree Health Sciences, specialization prevention and public health, VU University Amsterdam, the Netherlands	2008-2010	
Epidemiology and qualitative Methods		0.9
Methodology and applied biostatistics 1		1.9
Methodology and applied biostatistics 2		1.9
Dietetics and research		0.7
Policy and organisation of care		0.7
Prevention and public health		0.7
Public health in international context		0.7
Care and prevention research		0.9
Communication campaigns and research		0.7
Health and psychosocial factors of the elderly		0.7
Health promotion and disease prevention		0.7
Health psychology		0.7
Attended conferences and seminars		
Generation R research Meetings	2012-2015	0.5
Seminars at the department of Epidemiology	2012	1.0
Developmental Origins of Health and Disease (DOHaD), Rotterdam, The Netherlands - Poster presentation	2012	1.0
WEON 'Health and disease during the life course', Rotterdam, The Netherlands – Poster presentation	2012	1.0
European Academy Of Paediatrics Educational Congress & Mastercourse – EAP, Lyon, France - Oral presentation	2013	1.0
Congress of the European Academy of Paediatric Societies – EAPS, Barcelona, Spain - Oral presentation	2014	1.0
Symposium New insights in baby feeding and disease prevention, LUMC, Leiden	2015	0.3

ABOUT THE AUTHOR

Ilse Tromp was born on February 10th 1984 in Gouda, the Netherlands. In 2003, she graduated from secondary school on the island of Aruba. She obtained her Bachelor of Science degree in Medical Imaging and Radiation Therapy at Inholland University of Applied Sciences in 2008. In 2010, she obtained a Master of Science degree in Health Sciences, specialization Prevention and Public Health, at VU University Amsterdam. As part of the Master of Science program, she conducted her research project at the Generation R Study Group and the department of Pediatrics at Erasmus Medical Center in Rotterdam. Subsequently, she expended her research project in her PhD-project entitled 'Nutrition and disease in childhood: A window of opportunity?' under supervision of Prof.dr. H.A. Moll (Department of Pediatrics, Erasmus University, Rotterdam) and Dr. J.C. Kiefte-de Jong (Department of Epidemiology, Erasmus University, Rotterdam; Global Public Health, Leiden University College, The Hague). In 2011, Ilse started her medical education at the Erasmus University in Rotterdam. She is expected to graduate as a medical doctor in 2017.

DANKWOORD

Een proefschrift tot stand brengen doe je met veel mensen samen. Iedereen die op welke manier dan ook heeft bijgedragen aan het tot stand komen van dit proefschrift wil ik hartelijk bedanken. Een aantal personen wil ik hier kort noemen.

Allereerst wil ik mij promotor en copromotor bedanken, Prof.dr. Henriëtte Moll en Dr. Jessica Kiefte-de Jong. Beste Henriëtte, bedankt voor het vertrouwen en de vrijheid die je mij hebt gegeven in de afgelopen jaren. Toen ik na het afronden van de studie gezondheidswetenschappen aangaf verder te willen met een promotietraject, maar daarnaast ook te starten met de studie geneeskunde, was er enige twijfel. Door het vertrouwen, de vrijheid en steun die ik heb mogen ontvangen is dit toch mogelijk gebleken. Bedankt voor deze mooie kans! Lieve Jessica, bij jou begon het allemaal. Van stagebegeleider naar copromotor. Je bent er altijd voor mij geweest in de afgelopen jaren. Ook toen het allemaal wat minder ging. Ik bewonder je onuitputtelijke kennis maar heb nog meer waardering voor jou als persoon. Ik ben erg nieuwsgierig naar wat je allemaal nog gaat doen. Ik had mij geen betere copromotor kunnen wensen. Bedankt!

Prof.dr. J.C. de Jongste, hartelijk bedankt dat u plaats wilde nemen in de kleine commissie en voor het opnemen van de taak van secretaris. Prof.dr. H. Raat en Prof.dr. H.A. Smit, dank u voor het plaatsnemen in de kleine commissie en het beoordelen van het manuscript. Prof. dr. O.H. Franco, Prof.dr. J.P. Mackenbach, Prof.dr. S.G.M.A. Pasmans, Prof.dr. E.H.H.M. Rings, Dr. C.M. Renders, hartelijk dank voor uw bereidheid plaats te nemen in de grote commissie.

Prof.dr. Jaddoe, beste Vincent, bedankt voor het kritisch lezen van al mijn artikelen. Ook wil ik graag alle overige co-auteurs bedanken voor hun kritische en waardevolle feedback op mijn manuscripten en prettige samenwerking: Prof.dr. A. Hofman, Prof.dr. J.C. Escher, Prof. dr. H. Hooijkaas, Dr. L. Duijts, Dr. A. Lebon, Dr. J.H. de Vries, Dr. E.H. van den Hooven, Dr. A.C. Heijboer en Drs. M.A.E. Jansen.

Ook wil ik alle kinderen en hun ouders bedanken voor hun deelname aan het Generation R onderzoek. Zonder jullie was dit proefschrift niet tot stand gekomen. Bedankt voor jullie tijd en energie. Daarnaast wil ik alle focusmedewerkers, datamanagers, en bureaumedewerkers bedanken.

Tevens bedank ik mijn collega promovendi bij Generation R. Er is één persoon die ik in het bijzonder wil bedanken. Romy, bedankt voor je support op de momenten waarop ik het even niet meer zag zitten. We zijn nu bijna aan het einde van onze coschappen. Ik ben erg benieuwd naar wat gaat volgen.

Tot slot wil ik de belangrijkste personen in mijn leven bedanken. Al mijn familie, vriendinnen en vrienden die gedurende de jaren enthousiast bleven vragen naar mijn onderzoek.

Mijn paranimfen, Stephanie en Annika. Bedankt dat jullie op deze bijzondere dag aan mijn zijde willen staan.

Mijn lieve ouders (papa, mama & Ton), bedankt voor jullie onvoorwaardelijke liefde en steun. Zonder de door jullie gegeven kansen zou ik nooit zover gekomen zijn.

Jonathan, danki cu bo tey pami semper.

Danki!