Summer 8-8-2017

Length of Exclusive Breastfeeding and Obesity Risk in Children at Risk for Type 1 Diabetes

Krista Whitfield

Anita M. Nucci
Georgia State University

Barbara Hopkins
Georgia State University

LENGTH OF EXCLUSIVE BREASTFEEDING AND OBESITY RISK IN CHILDREN AT RISK FOR TYPE 1 DIABETES

By

KRISTA WHITFIELD

B.S., The University of Georgia, 2016

A Master's Project Submitted to the Graduate Committee

in the Department of Nutrition at Georgia State University in Partial Fulfillment

of the

Requirements for the Degree

MASTER OF SCIENCE

ATLANTA, GEORGIA

2017

Introduction

Type 1 diabetes (T1D) is an autoimmune disease that occurs when T lymphocyte cells attack and destroy beta cells in the pancreas.[1] The cause of T1D is considered to be a combination of genetic predisposition and environmental or lifestyle risk factors. Destruction of the pancreatic islet beta cells, which secrete insulin, leads to complete dependency on exogenous insulin to maintain glucose homeostasis. Insulin is a hormone that stimulates glucose uptake as well as lipid synthesis and is important for maintaining blood glucose levels.[1] In most cases of T1D, people have inherited risk factors from both parents. The most important genes implicated with susceptibility to T1D are the human leukocyte antigen (HLA) complex on chromosome 6.[2] Most young children with T1D carry either or both susceptibility haplotypes in the HLA class II region (90-95%).[2] In the United States, individuals with a first-degree relative with T1D have a 1 in 20 lifetime risk of developing T1D, compared to a 1 in 300 lifetime risk for the general population.[3] Caucasians have the highest rate of T1D, therefore, it is possible that these inherited risk factors are more common in Caucasians.[4] Maahs et al. (2010) conclude that the rates of T1D in non-Hispanic white youth are among the highest in the world (prevalence of T1D was 2.0/1,000 and the incidence was 23.6/100,000).[2] In 2002-2003, children with T1D (n=1905) were diagnosed in the SEARCH for Diabetes in Youth study (SEARCH) from a population of more than 10 million.[2] Rates were highest in non-Hispanic white youth as compared to other races/ethnicities and were slightly higher in females as compared to males (RR 1.028; 95% CI 1.025-1.030).[2] The EURODIAB ACE study group looked at the variation and trends in incidence of childhood diabetes in Europe between 1989 and 1994.[2] This study group found that the annual increase in the incidence rate of T1D was

3.4% (95% CI 2.5-4.4%).[2] The rates of increase were found to be highest in the youngest age group: ages 0-4 years 6.3% (95% CI 1.5-8.5%), 5-9 years 3.1% (95% CI 1.5-4.8%), and 10-14 years 2.4% (95% CI 1.0-3.8%).[2] However, since an environmental trigger is also involved in the development of the disease when an individual has already inherited a predisposition to diabetes, it may take years for T1D to develop in an individual with a predisposition.[4] In studies following relatives of people with T1D, researchers found that those who developed T1D later in life had certain autoantibodies in their blood for years prior to the development of the disease.[4] Four autoantibodies are markers of beta cell autoimmunity in type 1 diabetes: islet cell cytoplasmic autoantibodies (ICA), insulin autoantibodies (IAA), antibodies to the 65-kDa isoform of glutamic acid decarboxylase (GADA), and antibodies to the protein tyrosine phosphate-related IA-2 molecule (IA-2A).[5] Autoantibodies against GAD 65 are found in 80% of persons with type 1 diabetes.[6] Presence of ICA and IA-2A at diagnosis for type 1 diabetes range from about 69-90% and 54-75%.[6] Insulin autoantibodies are usually the first marker in young children at risk for diabetes and found in approximately 70% of young children at time of diagnosis.[6] The more antibodies present in an individual, the greater the risk of developing type 1 diabetes.[6]

Many people at risk for type 1 diabetes do not develop it. Researchers have examined associations between various environmental triggers and development of the disease. Cold climate, viruses, intestinal microbiota, infant diet, birth weight, and infant weight gain are environmental factors thought to play a role in the risk of developing T1D.[4] One trigger may be related to cold weather; more cases of T1D develop in the winter and more cases are seen in areas with cold climates.[7] According to Waernbaum

and Dahlquist (2016), there is an association with incidence of type 1 diabetes in children and low mean temperature independent of a possible effect of sunshine hours after adjustment for age, sex, and time trend.[8] Some researchers suggest viruses that have mild effects on some individuals could trigger T1D in others.[7] Enteroviral infection, in particular Coxsackie B4, showed an accelerated prediabetes progression in diabetes-prone NOD mice.[5] Early introduction of diet is thought to play a role in the development of T1D as it is less common in people who were breastfed and who were introduced to solid foods at later ages. The protection that breastfeeding can offer against the development of childhood obesity and T1D in children at risk for T1D is unknown and may be related to many different factors. The purpose of this project is to review the literature on the association between infant diet, including breastfeeding and complementary foods, and the development of obesity and T1D. This information will be used to prepare a secondary analysis proposal to examine the association between length of exclusive breastfeeding and obesity risk in children at risk for T1D for submission to the Presentations and Publications Committee of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study.

Specific Aim 1: To describe the association between length of exclusive breastfeeding and obesity in children at risk for T1D

Hypothesis 1: Shorter duration of exclusive breastfeeding predisposes children to obesity

Null Hypothesis 1: There will be no difference in obesity risk by length of exclusive breastfeeding

Specific Aim 2: To determine the association between length of exclusive breastfeeding and the development of obesity in children at risk for T1D is modified by maternal T1D status

Hypothesis 2: Risk of obesity will be higher in children who have a mother with T1D

Null Hypothesis 2: There will be no difference in obesity risk by maternal T1D status

Literature Review

Nutrition and Type 1 Diabetes

Breastfeeding and Infant Formula

Breastfeeding has several benefits for infants. There are three phases in breast milk production: colostrum (day 1 to 5 postpartum), transitional milk (day 6 to 15 postpartum), and mature milk (after day 15 postpartum).[9] Colostrum contains substances that offer general benefits, such as growth factors involved in the growth and development of cells in the digestive tract and transfer factors that may have general immune-activating properties.[10] In addition to immunoglobulins, colostrum contains neutrophils and macrophages, which secrete a range of immune-related components including cytokines and antimicrobial proteins and peptides, such as lysozyme, lactoferrin, and proline-rich polypeptides.[10] Phospholipids in human milk are an important source of energy for infants and are also providers of long-chain polyunsaturated fatty acids, which play an important role in the growth and brain development of neonates.[11] Fatty acids of high nutritional relevance such as arachidonic

acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) found in human milk are involved in child growth, visual acuity, and neurological development.[11] The World Health Organization (WHO) recommends exclusive breastfeeding up to six months of age.[12] Previous research has suggested that breast feeding for shorter than three months and early exposure to cow's milk proteins (such as bovine serum albumin) can trigger diabetes.[13] Some retrospective studies have shown a small reduction in the risk for T1D with breastfeeding; however, all but one of the prospective birth cohort studies failed to find a protective effect.[7]

These findings suggest that breastfeeding may play a protective role in the relationship between dietary factors and T1D.[7] Butalia et al. (2016) discusses a case-control study by Borch-Johnsen (1984) demonstrating that children with T1D were breastfed for shorter durations than their healthy siblings and general population.[14] Those who were exclusively breastfed for longer than 2 weeks were at decreased risk for developing T1D, but the protection was attenuated for those exclusively breastfed for more than 3 months.[14] It is possible that products with cow's milk-based protein may increase the risk for advanced beta-cell autoimmunity, whereas breastfeeding may be protective because breast milk has several antimicrobial and anti-inflammatory properties.[14] One possible mechanism is that early introduction of cow's milk protein may induce mucosal inflammation and increased gut permeability.[14] This increases the permeability of food antigens through the intestine, which leads to the stimulation of autoimmune processes, pancreatic islet inflammation, and destruction of beta cells.[14]

While breastfeeding may play a protective role in the risk for developing T1D, it is also important to look at other diet influences. In a double-blind, randomized trial

(TRIGR Pilot II) 230 infants genetically at risk for T1D were randomly assigned either the intervention formula (extensively hydrolyzed casein-based formula) or the control formula (80% intact milk protein and 20% hydrolyzed milk protein) whenever breast milk was not available.[7] At least one autoantibody developed in 17 of the children in the casein hydrolysate group (17%) and 33 in the control group (30%).[7] However, the larger phase three of the TRIGR study could not confirm this same effect on islet autoimmunity, and follow up of the study participants for T1D continues.[7] A higher cow's milk intake in children with islet autoimmunity might lead to T1D; however, the effect could be mediated by certain fatty acids present in cow's milk.[7] If this were confirmed, further dietary interventions to examine the preventive effect of diet on the development of On T1D could be conducted.

One recent paper published reported breastfeeding, other milk feeding, and complementary feeding patterns among infants in the TRIGR study. The large population (n=2159) consisting of participants from 15 different countries provides an assessment of infant feeding patterns in different regions of the world in mothers with and without T1D.[15] This paper documented that mothers with T1D breastfeed less than those unaffected by the disease.[15] During the first 3 days of life, the proportion of exclusively breastfed infants of mothers with T1D ranged from 81% in Northern Europe to 32% in Australia, but 94% of Australian mothers without T1D exclusively breastfed their infants during the first 3 days of life.[15] Sorkio et al. (2010) found that most (90%) of the infants of mothers with and without T1D were initially breastfed, but breastfeeding rates declined more among mothers with (50%) than without (72%) T1D at 6 months.[16] The feeding pattern data from the TRIGR study will allow for evaluation of how infant

diet is related to the development of autoimmunity and then progression to T1D by region at the completion of the study in 2017.[15]

Complementary Foods

The American Academy of Pediatrics recommends waiting until 6 months of age to start introducing solid foods, and to continue breastfeeding in combination with complementary foods until 12 months of age if possible.[17] Several studies have looked at the association between introduction of solid foods and risk for T1D. Children initially exposed to cereals between ages 0 and 3 months and those who were exposed at 7 months of age or older had increased hazard of islet autoimmunity (4.32; 95% CI 2.0-9.35 and 5.36; 95% CI 2.08-13.8; respectively) compared with those who were first exposed during the fourth through sixth month of life after adjustment for HLA genotype, family history of T1D, ethnicity, and maternal age.[18] The Finnish Diabetes Prediction and Prevention (DIPP) study reported that early introduction (by 4 months of age) of root vegetables increased the risk (1.75; 95% CI 1.11-2.75) of islet autoimmunity compared with a later introduction of root vegetables.[19] Researchers also reported that first exposure to egg before eight months of age was associated with an increased risk of islet autoimmunity.[14] All Babies in Southeast Sweden (ABIS) showed that less than daily consumption of vegetables (3-5 times per week) in the mother's diet was associated with increased risk (1.17; 95% CI 1.24-2.35) of islet autoimmunity.[14] It is important to interpret these study results with caution because there is a risk of false positive associations caused by multiple comparisons. There are some inconsistencies with the findings, but these studies support the idea that general antigenic stimulation is more

important than the actual antigen in this disease process.[14] In other words, the timing of introduction of certain solid foods could be more important than the type of food introduced. This could be due to immature immune response and the gut.

Introducing solid foods too soon may be a risk factor for developing islet autoimmunity due to immature immune response to foreign antigens and a more permeable gut in infants.[20] Abnormalities in gut permeability have been linked to the development of T1D.[20] In a multinational cohort study of children at increased genetic risk of T1D, a reduction in the risk of islet autoimmunity was observed in children that received probiotics via dietary supplements and/or via fortified infant formula before or at the age of 27 days compared with those who first received probiotics after 27 days or not at all.[20] Early probiotic exposure was associated with a 60% decrease in the risk of islet autoimmunity among children with the DR3/4 genotype but not among other genotypes.[20] While studies have shown that breastfeeding for at least six months may help reduce the risk of T1D, breastfeeding is not possible for some mothers for a variety of factors.[20] If mothers must use a formula, it appears that selecting a formula fortified with probiotics may be beneficial in helping reduce the risk of T1D in children genetically at risk.[20] More research should be conducted to determine the effect of supplemental probiotics on the development of autoimmunity and T1D in breastfed infants who are genetically at risk.

Micronutrients

Previous research has provided some evidence to support a potential role of vitamin D in the pathogenesis of T1D, and the factor believed to play a role is vitamin D

receptor (VDR).[14] The VDR gene is found in most tissues of the body, including the immune system.[14] The VDR gene is located on chromosome 12 and has a few allelic variants. Some of these variations of the gene have been associated with an increased risk for T1D.[14] Countries at northern latitudes where sunlight exposure is lower and vitamin D deficiency is more common have a higher incidence of T1D.[14] Several studies have reported lower levels of serum 25-OH vitamin D among patients with T1D compared with healthy controls.[14] The Endocrine Society Practice Guidelines on Vitamin D define vitamin D deficiency as 25(OH)D <20 nanograms/mL, insufficiency as 21-29 nanograms/mL, and sufficiency as $\geq$30 nanograms/mL.[21] On the contrary, a cohort study of maternal intake of vitamin D was not found to be protective in offspring developing T1D and beta-cell antibodies.[14] Virtanen and Knip (2003) reviewed the results of a few studies that evaluated vitamin D supplementation in infancy.[22] Vitamin D supplementation during infancy was inversely associated with the risk of T1D in a European case-control comparison, whereas vitamin D or cod liver oil use during infancy was not related to the risk of diabetes in a small Norwegian case-control series.[22] Due to contrary findings regarding vitamin D and the development of islet autoimmunity or T1D, further research is needed on this topic.

There is little research on other micronutrients associated with T1D. It is possible that vitamin E could play a role in preventing the development of T1D through its function as an important free radical scavenger as well as through its inhibition of N-nitroso compound formation in food and in the human organism.[22] In a Finnish case-control study within an adult cohort, an inverse relation was found between serum concentrations of alpha-tocopherol at baseline and the development of T1D 4-14 years

later; this association was independent of serum cholesterol levels and body mass index.[22] Serum selenium or retinol concentrations were not related to the risk of T1D in that same study.[22] An Australian case-control study reported that vitamin C supplementation was inversely related to the risk of T1D.[22] Zinc concentrations in drinking water were also observed to be inversely related to the risk of T1D in a Swedish case-control study.[22] Virtanen et al. (1994) found that maternal nitrite intake was positively associated with the risk of diabetes independent of the child's own intake and when adjusted for several sociodemographic factors.[23] Norwegian case-control findings of an inverse association between maternal cod liver oil supplementation during pregnancy and the risk of T1D in the offspring suggest that either vitamin D, vitamin A, or n-3 fatty acids (which are all abundant in cod liver oil) play a role in the development of T1D.[22] Some of the randomized placebo-controlled trials in subjects with recently diagnosed T1D suggest that nicotinamide delays the decay of β cell function, whereas other studies found no effect of nicotinamide.[22] Further research is needed on various micronutrients associated with the development of T1D.

Obesity and Risk of Type 1 Diabetes

An article by Nucci et al. (2012) aimed to evaluate the relationship between early growth and regional variations in T1D incidence in children with familial and genetic risk for T1D.[24] They obtained anthropometric indices between birth and 5 years of age in 2160 children participating in the TRIGR study among different regions.[24] They found that children in Northern Europe had the highest weight z-score between birth to 12 months of age, while those in Southern Europe and USA had the lowest weight and

length for height z-scores.[24] The study concluded that there are regional differences in early childhood growth that are consistent with the higher incidence of T1D in Northern Europe and Canada compared to Southern Europe.[24] This study allows for further evaluation of the association between growth (obesity) and progression to T1D.[24]

Kibirige et al. (2003) looked at the relationship between body mass index and age at diagnosis of T1D.[25] The relationship between fatness and age at diagnosis was examined in context of birth weight, weight change since birth, weight at diagnosis, BMI at diagnosis, and BMI 12 months later in 94 children aged 1–16 years (49 boys and 45 girls) presenting for management of acute-onset T1D.[25] The boys in this study were found to have a greater BMI than the girls, and they were presented with diabetes at a younger age.[25]

Birthweight and prevalence of overweight children have gradually increased in Sweden over recent decades, and this trend seems to parallel with increasing incidence of T1D occurring in childhood.[26] Dahlquist et al. (2005) observed in a population-based register study that the paralleling trend between increasing birthweight/overweight and increasing incidence of childhood T1D is seen in the younger age-at-onset groups, but not in the age groups older than 15 years at diagnosis.[26] One explanation could be that the overload of the beta cells due to increased insulin demand in the growing children may accelerate the process of beta-cell destruction and lead to an earlier onset on T1D.[26] This study concluded that high birthweight as a risk factor for T1D may be limited to young-onset cases.[26] The increase in T1D incidence in Sweden is seen in age groups younger than 10, but not in young adults, which could be explained by the increasing birthweight.[26]

Ljungkrantz et al. (2008) examined children's height and weight gain from birth to the time of diagnosis of T1D.[27] Growth charts from 316 children 0-16 years old up to the time of T1D diagnosis were compared with growth charts from age and gender matched controls.[27] Compared with controls in the year of diagnosis, children who developed T1D were taller (0.5 vs. 0.36 SDS) and heavier (0.7 vs. 0.45 SDS).[27] Children who developed diabetes at 5 years old or less gained more in weight than in height during the period between their third month and third year of life.[27] Children who were diagnosed between 6 and 10 years of age gained more in height before they were 5 years old.[27] The analysis from the study showed that a high weight or high BMI at 5 years old indicated, more than other measurements, a high risk (OR 1.6; 95% CI 1.02-2.38) for diabetes later during childhood, while height and weight at ages less than 5 years did not add any further information on diabetes risk.[27]

In conclusion, breastfeeding is thought to help reduce the risk of developing T1D in children genetically at risk. This could be due to a number of mechanisms including the hormones in breast milk, the delay in introduction of complementary foods containing foreign antigens that affect the islet autoimmunity with longer periods of breastfeeding, or the protective effect that breastfeeding has against overweight/obesity in children.

Breastfeeding and Obesity

Many studies have reported that children who are breastfed are more likely to maintain a healthy weight throughout childhood. The World Health Organization (WHO) and United States Department of Health and Human Services have concluded that breastfeeding for at least six months can help reduce the risk of obesity later in life.[28]

Human milk may be involved in growth and appetite control in the neonatal period and infancy, affecting the programming of energy balance regulation in both childhood and adulthood.[28]

A review article by Marseglia et al. (2015) provides a summary of what is known about the possible relationship between breastfeeding and risk of obesity in childhood.[28] Findings from different studies are discussed and possible mechanisms to explain the association between breastfeeding and obesity are mentioned. Human milk varies from day to day in composition, which influences metabolic state and diet of infant; it has been shown that a dose- and time-dependent association could correlate with a lower BMI in older children.[28] This paper also states prolonged duration and exclusivity of breastfeeding lead to lower growth rates during the first year of life and seem to lower risks of overweight and obesity in preschool aged children.[28] However, the data are controversial with regard to the effect that breastfeeding early in life has on short- and long-term obesity.[28] The results are from observational studies, which can be affected by many other confounding factors such as genetics, family structure, physical activity later in life, and future eating patterns.

A 2007 meta-analysis conducted for the WHO showed that breastfeeding was associated with a 22% reduced risk of obesity later in life.[28] In a 2013 study, researchers observed that exclusive breastfeeding for six to seven months of age was associated with decreased risk of overweight and obesity compared with formula feeding after adjusting for maternal factors (educational attainment, smoking status, and working status) and child factors (gender, television viewing time, and computer game playing time).[28] However, a cohort study of 8327 children from Hong Kong China did not find any

association between breastfeeding and BMI at seven years of age.[28] Not all authors agree on the relationship between breastfeeding and overweight, so there is still the need for further studies to clarify the association between the two.

The duration of breastfeeding can directly influence the infant's ability to self-regulate milk intake and the infant's growth. As a result, there are differences between growth parameters in infants who are breastfed for a short vs. long time period.[28] A meta-analysis about the duration of breastfeeding and obesity, using formula fed infants as the referent, noted that duration of breastfeeding and overweight were inversely correlated.[28] A shorter duration of breastfeeding is probably associated with an earlier introduction of solid food, which contains more protein than breast milk.[28] Shorter duration of breastfeeding was correlated with reduced appetite signaling which induces a greater number of feeding times. Recent studies have identified the role of the fat mass- and obesity-associated (FTO) gene in increasing BMI and adiposity. Abarin et al. (2012) hypothesized that the longer duration of breastfeeding, through its ability to interfere on the FTO gene, might reduce the risk of overweight later in life.[28] In 18 studies, duration of breastfeeding, if greater than 40 weeks, was positively related with a lower weight gain at one year.[28]

Several hormone molecules seem to be involved in the development of obesity in humans. Insulin, insulin-like growth factor I (IGF-1), leptin, adiponectin, ghrelin, obestatin, and resistin are hormone molecules involved in the development of obesity.[28] These hormones influence fat and lean body mass in healthy term infants and enhance appetite signaling, which promotes child satiety-responsiveness and decreases risk of over-eating.[28] Leptin promotes fetus growth, has a positive effect on satiety, increases

basal metabolism, and correlates with weight gain in newborns.[28] Leptin is synthesized and secreted in breast milk; it has been reported that higher serum leptin levels are found during the first month of life in breast-fed infants than in formula fed infants.[28] Adiponectin is released in breast milk. Adiponectin levels in newborns are directly associated with birth weight and length, insulin sensitivity, and levels of leptin, and inversely related with fat deposits and weight gain.[28] Ghrelin is present in human milk during lactation.[28] Ghrelin is directly associated with birth weight, birth length, and age; ghrelin is inversely related with weight gain in breastfed newborns of at least four months, but not in formula fed infants.[28] Obestatin is a hormone derived from the same gene that codes for ghrelin. Obestatin is synthesized by ductal epithelium of mammary gland or directly released from serum into breast milk and has been detected in colostrum and mature milk.[28] Obestatin is associated with less overfeeding, especially in the early stages of breastfeeding.[28] Although further studies are needed to clarify other factors associated with breastfeeding and weight gain, these findings do suggest that breast milk can play a critical role in metabolic development of newborns.

A study by Hunsberger (2014) aimed to show the association between breastfeeding and overweight children when considering family structure.[29] The researchers suggested that breastfeeding alone does not protect children from being overweight, but that other lifestyle and social factors play a role along with mothers who chose to breastfeed. The WHO recommends children be exclusively breastfed for at least six months because of other known benefits. The group that published this paper reviewed the exposure to exclusive breastfeeding and overweight in the "Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS

(IDEFICS)" study.[29] IDEFICS was a multi-center European study that involved eight different countries.[29] Only children who could be defined as being exclusively breastfed were included in the surveys for this study.[29] Exclusive breastfeeding and overweight/obesity combined were examined with adjustment for survey country, child age, sex and birth weight, household income, maternal education, maternal overweight/obesity, single or dual parent family structure, presence of one or more foreign born parents, and tobacco use during pregnancy.[29] In the fully adjusted model, breastfeeding exclusively for four to six months was protective for overweight/obesity when compared to children who were never exclusively breastfed.[29] Also, exclusive breastfeeding for six months showed more protection than four and five months combined.[29] When the role of family structure was investigated, being an only child was not protective for children becoming overweight/obese.[29] Although exclusive breastfeeding for four to six months can be protective for overweight/obesity, it is important to keep in mind that exclusive breastfeeding alone will not guarantee that children will not become overweight or obese due to other environmental factors such as family structure.[29]

A review by Spatz (2014) discussed the mechanism for how breastfeeding can influence future eating habits.[30] Infants learn about food and flavoring through both amniotic fluid and breastfeeding.[30] Human milk is influenced by maternal diet; when an infant breastfeeds, the palate is exposed to new tastes.[30] These early exposures to various tastes have an influence on flavor preferences of children that may later affect food choices.[30] Research conducted on Dutch children found that children at seven years of age who were breastfed for more than 16 weeks had a greater intake of fruits and

vegetables compared to those children who had never been breastfed.[30] The children who had been breastfed were also less likely to consume white bread, soft drinks, chocolate bars, and fried food.[30] These findings provide evidence that breastfeeding can be protective against children being overweight/obese due to the effects breastfeeding can have on future food choices in children.

Methodology

Study Population

The TRIGR study is an international T1D prevention trial designed to determine whether weaning to a hydrolyzed infant formula reduces the incidence of T1D in children with a first- degree relative with the disease and increased HLA-defined genetic risk.[5] Mothers with T1D diabetes were identified during pregnancy through endocrinologists or high-risk pregnancy services.[5] Fathers with T1D were identified by available history or data already in the medical record of the pregnant women, interviewing women at prenatal maternity clinic visits, and existing registries of T1D in some centers.[5] The newborn infants with a first-degree relative with T1D also had to fit the inclusion and exclusion criteria to be recruited. The inclusion criteria included: the biological parent and/or full (not half) sibling of the newborn infant had T1D as defined by the WHO; the infant's parents or legal guardians gave signed consent to participate; and the infant had one of four different genotypes listed in the study [HLA-DQB1*02/0302; HLA-DQB1*0302/x (x □DQB1*02, *0301, *0602); HLA-DQA1*05 -DQB1*02/y (y □DQA1*0201 -DQB1*02, DQB1*0302, *0301, *0602, *0603); HLA-DQA1*03 -DQB1*02/y (y □DQA1*0201 -DQB1*02, DQB1*0302, *0301, *0602, *0603)].[5] The

newborn infants could not be recruited if they met any of the exclusion criteria. Exclusion criteria for the study included: having an older sibling who had already been included in the TRIGR intervention; multiple gestation; parents were unwilling or unable to feed the infant cow milk (CM)-based products for any reason; the newborn infant had a recognizable severe illness; the gestational age of the newborn infant was less than 35 weeks; inability of the family to take part in the study (the family had no access to any of the study centers or the family had no telephone); the infant had received any infant formula other than Nutramigen prior to randomization; the infant was older than 7 days at randomization; and no HLA sample had been drawn before the age of 8 days.[5]

Recruitment for the study was carried out over the course of 4 years in 6 major centers in the USA, in 18 centers in Canada, in 51 centers in 12 European countries, and 3 centers in New South Wales, Australia. In order to facilitate recruitment and minimize any possible unintentional exposure to CM protein, attempts to identify and randomize eligible families were made before the child was born.[5]

Using data from the Childhood Diabetes in Finland (DiMe) Study Group, the German BABYDIAB study, and the DAISY study, a projected sample size of 2032 infants to be randomized for the trial was determined.[5] To achieve this number, the TRIGR study group determined that 4516 infants had to be screened assuming a frequency of 45% of the genotypes conferring increased risk.[5] Screening for TRIGR began on 1 May 2002 and the target enrollment was achieved by 1 September 2006.[5] A paper published on the recruitment and retention of the TRIGR study by Franciscus et al. (2013) stated that 5606 mothers registered worldwide, and 5000 of their infants were

randomized.[31] Of these, 2159 were HLA eligible and enrolled in the 8-month intervention and 10-year follow up phases of the study.[31]

Anthropometrics

The weight and length or height of subjects were recorded at the time of randomization (baseline). Anthropometrics were subsequently measured at the 3 month visit, 6 month visit, 9 month visit, 12 month visit, 18 month visit, 2 year visit, and at each annual visit thereafter until the maximum age of 14 years.[5]

Nutrition Assessment

In the TRIGR Study, the diet of the child and the compliance with avoiding intact CM proteins were assessed by interview at the delivery hospital.[5] The interview was conducted via telephone with parents/guardians when the infant was 2 weeks of age and 1, 2, 4 and 5 months old.[5] The interview was also conducted in person at the 3- and 6-month visits.[5] If the infant continued in the intervention after 6 months of age, telephone interviews were also done at 7 and 8 months old.[5] The dietary assessment was done via a structured form, including information on the duration of total and exclusive breastfeeding, the age at introduction, the duration of the study formula feeding, and the amount of study formula given per feeding.[5] The families were also asked about intake of allowed foods and non-recommended foods and food groups.[5] Measuring CM protein antibody levels from sera at 3 months and 6 months also assessed compliance with the avoidance of intact CM proteins.[5]

The dietary interview form given at the two-week follow-up call included two questions about what the baby had consumed at all during the first 3 days, and also what the baby consumed primarily in the first 3 days (Appendix A). Then, a few questions were asked about breastmilk consumption and study formula consumption; the subjects were asked if they needed more study formula and how much of the study formula they had at home at the time of 2 weeks. At 2 weeks, the families were asked about which types of foods and supplements were consumed and how frequently the baby had consumed these items since birth. The foods included: breast milk, study formula, strained potato/vegetables, strained fruit/fruit juices, foods containing oat, wheat, barley, or rye, foods containing corn, rice, buckwheat, or millet, foods containing pork, chicken, turkey, lamb, or game, and foods containing fish or egg or any other foods not previously mentioned. The supplements included vitamin D or cod liver oil, and other vitamins/minerals.

At months one through five, the dietary form included questions about a few additional foods not asked about at the two-week follow-up (Appendix B). These forms included questions about consumption of regular cow's milk/goat's milk based formula, Nutramigen or other hydrolyzed formula, soy-based formula, soured milk and sour milk products (buttermilk, cultured milk, yogurt), regular cow's milk/goat's milk, ice cream or cheese, foods containing beef, veal, or meat extract, sausage and other meat products containing beef, and any others not listed above that may contain lactic acid bacteria. An additional form was filled out at the six-month follow-up visit. This form included questions about the same foods asked about in the previous forms. Parents were also asked if the baby had already received the study formula and if the baby had received the

study formula daily for at least 2 months. Parents were asked to indicate how much study formula they still had at their home at the time of the 6-month visit, and how much unconsumed study formula the family returned after the intervention period.

Statistical Analysis

Frequency analysis will be used to describe the demographic and anthropometric characteristics of the total study population and stratified by maternal T1D status. Frequency analysis will also be conducted using the variables: region, maternal education, gender, HLA-genotype, method of delivery, birth weight and birth length, ponderal index (birth only), body mass index (BMI), length of exclusive breastfeeding (EBF), and maternal T1D status (MT1D). Normality statistics will be performed for the continuous variables. Mean and median values will be calculated for weight/age, length or height/age, and BMI/age (at birth and each subsequent time period) for the entire cohort and by MT1D status. Ponderal Index will be used at birth rather than BMI. Overweight and obesity status will be determined for each participant using International Obesity Task Force (IOTF) BMI cutoff points for overweight and obesity by age and gender. The rates (percentages) of overweight and obesity at birth and each subsequent time period will also be calculated.

In order to evaluate the association between length of exclusive breastfeeding and obesity by 10 years of age, a model will be created to assess the association between length of exclusive breastfeeding and obesity rate after adjusting for covariates. Another model will be created to evaluate the association between length of exclusive breastfeeding and growth rate. This model will assess the association between length of

exclusive breastfeeding and growth curves for weight, height/length, and BMI after adjusting for covariates (gender, method of delivery, HLA-genotype, birth weight, birth height/length, and MT1D). Weight/height data with suspected errors are corrected using statistical algorithm prior to analysis. Race was removed from the analysis because it was only documented in the U.S. Analysis will be divided by the following regions: Australia, Canada, Northern Europe, Southern Europe, Central Europe I, Central Europe II, and the United States. Type III SS model is the best because it controls for other variables. P-values will be viewed for significance.

Summary/Conclusion

The effect of exclusive breastfeeding on the development of overweight/obesity in children at risk for T1D is unknown. The purpose of this project was to review the literature for research that has evaluated the association between infant diet, including breastfeeding and complementary foods, and the development of obesity in children at risk for T1D. After completion of the literature review, a secondary analysis proposal was developed to investigate this relationship in a large population of children who participated in a large international T1D prevention trial. Previous research has reported that exclusive breastfeeding for greater than two weeks can be protective against developing T1D, whereas early exposure to cow's milk-based protein may increase beta-cell autoimmunity.[14] In addition, mothers with T1D breastfeed less frequently than those unaffected by the disease.[16] The timing of introduction to complementary foods seems to affect islet autoimmunity.[17] Studies have shown an association between weight status in children and development of T1D[25,27] while breastfeeding has been found to be protective

against overweight/obesity in children.[28] The proposal has been prepared to examine the association between length of exclusive breastfeeding and obesity risk in children at risk for T1D for submission to the Presentations and Publications Committee of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study. The TRIGR study data are ideally suited to answer this research question because there are controls for many important confounding factors pertaining to this research question. Furthermore, the study was conducted in an international population from birth to up to 14 years of age. A prospective study from birth will allow evaluation of relationships between exclusive breastfeeding and the development of overweight/obesity in children at risk for T1D during infancy, childhood and early adolescence.

References

1. Eringsmark RS, Lernmark A. The environment and the origins of islet autoimmunity and type 1 diabetes. *Diabet Med*. 2013;30(2):155-60.
2. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Chapter 1: Epidemiology of type 1 diabetes. *Endocrinol Metab Clin North Am*. 2010;39(3):481-497.
3. Redondo MJ, Fain PR, Eisenbarth GS. Genetics of type 1 diabetes. *Recent Prog Horm Res*. 2001;56:69–89.
4. Genetics of diabetes, 2013. American Diabetes Association Web Site. http://www.diabetes.org/diabetes-basics/genetics-of-diabetes.html?loc=db-slabnav. Updated January 27, 2017. Accessed April 27, 2017.
5. Study design of the trial to reduce IDDM in the genetically at risk (TRIGR). *Pediatr Diabetes.* June 2007;8(3):117-137.
6. Donner T, Champaneri S, Saudek C. Autoantibodies in type 1 diabetes. John's Hopkins Medicine Website. https://www.hopkinsguides.com/hopkins/view/Johns_Hopkins_Diabetes_Guide/547013/all/Autoantibodies_in_Type_1_Diabetes#7. Updated October 6 2015. Accessed April 14 2017.
7. Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. *The Lancet.* 2016;387(10035):2340-2348.
8. Waernbaum I, Dahlquist G. Low mean temperature rather than few sunshine hours are associated with an increased incidence of type 1 diabetes in children. *Eur J Epidemiol.* 2016;31(1):61-65.

9. Sala-Vila A, Castellote AI, Rodríguez-Palmero M, Campoy C, López-Sabater C. Lipid composition in human breast milk from Granada (Spain): changes during lactation. *J Nutr.* 2005;21:467–473.

10. Musumeci M, Musumeci S. A focus on colostrum: an overview. *Handbook of Dietary and Nutritional Aspects of Human Breast Milk.* 2013;5:133-143.

11. Antonio C, Késia DQ, Reyes B, Amparo A. Phospholipids in human milk and infant formulas: benefits and needs for correct infant nutrition. *Crit Rev Food Sci Nutr*. 2016;56(11):1880-1892

12. World Health Organization. *Breastfeeding.* Health Topics; 2017.

13. Perez-Bravo F, Oyarzu´n A, Carrasco E, Albala C, Dorman JS, Santos JL. Duration of breast feeding and bovine serum albumin antibody levels in type 1 diabetes: a case-control study. *Pediatr Diabetes.* 2003;4:157-161.

14. Butalia S, Kaplan GG, Khokhar B, Rabi DM. Environmental risk factors and type 1 diabetes: past, present, and future. *Can J Diabetes*. 2016;40(6):586-93.

15. Nucci AM, Virtanen SM, Sorkio S, et al. Regional differences in milk and complementary feeding patterns in infants participating in an international nutritional type 1 diabetes prevention trial. *Matern Child Nutr*. 2016.

16. Sorkio S, Cuthbertson D, Barlund S, et al. Breastfeeding patterns of mothers with type 1 diabetes: results from an infant feeding trial. *Diabetes Metab Res Rev.* 2010;26:206-211.

17. American Academy of Pediatrics. *Infant Food and Feeding.* 2017.

18. Norris JM, Barriga K, Klingensmith G, et al. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *JAMA.* 2003;290(13):1713-1720.

19. Virtanen SM, Takkinen H, Knip M, et al. Early introduction of root vegetables in infancy associated with advanced b-cell autoimmunity in young children with leukocyte antigen-conferred susceptibility to type 1 diabetes. *Diabet Med.* 2011;28(8):965-971.

20. Uusitalo U, Liu X, Yang J, et al. Association of early exposure of probiotics and islet autoimmunity in the teddy study. *JAMA Pediatr*. 2016;170(1):20-8.

21. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment, and prevention. *Rev Endocr Metab Disord.* 2017;18(2):153-165.

22. Virtanen SM, Knip M. Nutritional risk predictors of b-cell autoimmunity and type 1 diabetes at a younger age. *Am J Clin Nutr.* 2003;78(6);1053-67.

23. Virtanen SM, Jaakkola L, Räsänen L, et al. Nitrate and nitrite intake and the risk for type 1 diabetes in Finnish children. *Diabet Med* 1994;11:656–62.

24. Nucci A, Becker D, Virtanen SM, et al. Growth differences between North American and European children at risk for type 1 diabetes. *Pediatr Diabet.* 2012.

25. Kirbirige M, Metcalf, Renuka R, Wilkin TJ. Testing the accelerator hypothesis: the relationship between body mass and age at diagnosis of type 1 diabetes. *Am Diabet Assoc.* 2003; 26(10):2865-2870.

26. Dahlquist G, Pundziote-Lycka A, Nystrom L. Birthweight and risk of type 1 diabetes in children and young adults: a population-based register study. *Diabetologia.* 2005;48(6):1114-1117.

27. Ljungkrantz M, Ludvigsson J, Samuelsson J. Type 1 diabetes: increased height and weight gains in early childhood. *Pediatr Diabet.* 2008;9:50-56.

28. Marseglia L, Manti S, D'Angelo G, et al. Obesity and breastfeeding: the strength of association. *Women and Birth.* 2015;28:81-86.

29. Hunsberger M. Early feeding practices and family structure: associations with overweight in children. *Proc Nutr Soc.* 2014;73:132-136.

30. Spatz DL. Preventing obesity starts with breastfeeding. *J Perinat Neonatal Nurs.* 2014;28(1):41-50.

31. Franciscus M, Nucci A, Catteau J, et al. Recruitment and retention of participants for an international type 1 diabetes prevention trial: a coordinators' perspective. *Clinical Trials*. 2014;11(2):150-158.

Appendix A

Contact no. 2
Two Week Follow-up Call

DIETARY INTERVIEW 2 weeks

Study Center I__I__I__I__I__I Local Code I__I__I__I__I__I__I__I__I__I

Registration Code I__I__I__I__I__I__I Randomization Code I__I__I__I

Date I___I___I___I__I__I__I__I__I__I___I Form completed by _____ Code I__I__I__I__I
d d m m m y y y y Print name

DIET OF THE BABY DURING THE FIRST 3 DAYS (in the hospital or at home)

e. Has your baby received the following types of milk during the first 3 days? Mark (X) a response for each type of milk. Also, very tiny amounts should be recorded in the column "yes."

	Yes	No	Don't know
Own mother's breast milk			
Banked breast milk			
Study Formula			
Nutramigen			
Nutramigen LIPIL			
Other infant formula Brand name(s)? _____ National code(s) I__I__I__I , I__I__I__I			
Other type of milk Brand name(s)? _____ National code(s) I__I__I__I , I__I__I__I			

2. What type of milk did your baby primarily receive during the first 3 days? Circle the one most frequently used type of milk. If the baby has received two of them equally as often (e.g., its own mother's breast milk and the Study Formula), both numbers should be circled.

1. Own mother's breast milk
2. Banked breast milk
3. Study Formula
4. Nutramigen
5. Nutramigen LIPIL
6. Other infant formula, brand name? _____
   National code for the formula: I__I__I__I , I__I__I__I
7. Other type of milk, please give details_____
   National code for the type of milk: I__I__I__I , I__I__I__I
8. I don't know what type of milk my baby received primarily
9. None

Contact no. 2
Two Week Follow-up Call

DIETARY INTERVIEW 2 weeks

Study Center I__I__I__I__I__I Local Code I__I__I__I__I__I__I__I__I Registration Code I__I__I__I__I__I__I

DIET OF THE BABY DURING THE FIRST 2 WEEKS

3. Is your baby now receiving breast milk? Please give the mother the following options, and circle the one that is found most appropriate by her:
   1. Yes
   2. No, breast feeding was stopped at the age of _____ days
   3. No, my baby has not been breast fed at all

4. Has your baby started to receive anything other than breast milk or water (e.g., Study Formula, Nutramigen, juice, sugar water, strained potato/vegetables, baby cereals)?
   1. Yes
   2. No

   ¾ If yes: When did your baby receive it for the first time? At the age of ____ days

5. Has your baby already received the Study Formula?
   1. Yes
   2. No

   ¾ If yes: When did your baby receive the Study Formula for the first time? At the age of ____ days
   How long has the baby received the Study Formula daily so far? ____Days
   How much Study Formula has your baby received on average per feeding during the first two weeks?
   _____ Scoops Study Formula powder or _____ ml (milliliters) Study Formula liquid

   ¾ If no: Skip question 6 and go to question 7

6. Is your baby now receiving the Study Formula?
   1. Yes
   2. No

   ¾ If no: When did your baby receive the Study Formula last time? At the age of ____ days

7. How much unconsumed Study Formula do you have at home at the time of the 2-week call?
   Whole cases of formula _____ case(s)
   Individual cans of formula _____ can(s)

8. Do you need more Study Formula?
   1. Yes
   2. No

   ¾ If yes: Please complete the Study Formula Distribution Form (Form 302)

Contact no. 2
Two Week Follow-up Call

DIETARY INTERVIEW 2 weeks

Study Center |__|__|__|__|__| Local Code |__|__|__|__|__|__|__|__|__| Registration Code |__|__|__|__|__|__|

9. Which foods has your baby received since birth, and how often?

The purpose of this question is to assess how often your baby has consumed the foods listed below since birth. Mark (X) each row of the table appropriately to indicate how often the baby has received the food(s). If the baby has not consumed any food items in the row, mark the column "not at all". If the food was consumed more than 6 times per week, mark the appropriate "Times per day" box. Please do not use the last category "Other food item" unless it is absolutely necessary. Instead, try to use the specific food categories as much as possible for the foods consumed by the baby. There must be only one mark in each row.

Type of food	Average frequency since birth						
		Times per week			Times per day		
	Not at all	Less than 1	1-3	4-6	1-2	3-4	5 or more
Breast milk							
Study Formula							
Strained potato / vegetables							
Strained fruit / fruit juices							
Foods containing oat, wheat, barley or rye (e.g., baby cereals, bread, biscuits)							
Foods containing corn, rice, buckwheat or millet (e.g., baby cereals, bread, biscuits)							
Foods containing pork, chicken, turkey, lamb, or game (e.g., strained meat and vegetables)							
Foods containing fish (e.g., strained fish and vegetables)							
Egg							
Vitamin D supplementation or cod liver oil – please list:							
Other vitamins/minerals, please list:							
Other food item – please list:							

Contact no. 2
Two Week Follow-up Call

DIETARY INTERVIEW 2 weeks

Study Center |__|__|__|__|__| Local Code |__|__|__|__|__|__|__|__| Registration Code |__|__|__|__|__|__|

10. Has your baby received any foods that are <u>not allowed</u> during the dietary intervention period?

Foods not allowed during the dietary intervention period are mentioned in the table below. In case the baby has received any of those since birth, mark (X) each row of the table appropriately to indicate how often the baby has received the food(s). If the baby has not consumed any food items in the row, mark the column "not at all". If the food was consumed more than 6 times per week, mark the appropriate "Times per day" box. There must be only one mark in each row.

Type of food	Average frequency since birth						
		Times per week			Times per day		
	Not at all	Less than 1	1-3	4-6	1-2	3-4	5 or more
Regular cow's / goat's milk-based formula, as such or used in cooking Brand name(s)?_____							
Nutramigen* or other hydrolyzed formula** Brand name(s)?_____							
Soy-based formula** Brand name(s)?_____							
Soured milk and sour milk products (e.g., buttermilk, cultured milk, yogurt)							
Regular cow's / goat's milk, ice cream or cheese, as such, in commercial baby foods, or when used in cooking (e.g., baby foods)							
Foods containing beef, veal or meat extract (e.g., strained beef and vegetables)							
Sausage and other meat products containing beef							
Other – please list (e.g., milk containing lactic acid bacteria supplements)							

* Nutramigen can be given in the delivery hospital if the Study Formula is not available
** Other infant formulas than the Study Formula are not allowed during the dietary intervention period

Appendix B

Contact no. 3
One-Month Follow-up Call

DIETARY INTERVIEW 1 month

Study Center l__l__l__l__l__l Local Code l__l__l__l__l__l__l__l__l__l__l

Registration Code l__l__l__l__l__l__l Randomization Code l__l__l__l

Date l__l__l__l__l__l__l__l__l__l__l Form completed by _____ Code l__l__l__l__l
d d m m m y y y y Print name

1. Is your baby now receiving breast milk? Please give the mother the following options, and circle the one that is found most appropriate by her:
   1. Yes
   2. No, breast feeding was stopped at the age of _____ days
   3. No, my baby has not been breast fed at all

2. Has your baby started to receive anything other than breast milk or water (e.g., Study Formula, Nutramigen, juice, strained potato/vegetables, baby cereals)?
   1. Yes
   2. No

   ¾ If yes: When did your baby receive it for the first time? At the age of ____ days

3. Has your baby already received the Study Formula?
   1. Yes
   2. No

   ¾ If yes: When did your baby receive the Study Formula for the first time? At the age of ____ days
   How long has the baby received the Study Formula daily so far? ____days
   How much Study Formula has your baby received on average per feeding during the preceding two weeks?
   _____ scoops Study Formula powder or _____ ml (milliliters) Study Formula liquid

   ¾ If no: Skip question 4 and go to question 5

4. Is your baby now receiving the Study Formula?
   1. Yes
   2. No

   ¾ If no: When did your baby receive the Study Formula last time? At the age of ____ days

5. How much unconsumed Study Formula do you have at home at the time of the 1-month call?
   Whole cases of formula _____ case(s)
   Individual cans of formula _____ can(s)

6. Do you need more Study Formula?
   1. Yes
   2. No

   ¾ If yes: Please complete the Study Formula Distribution Form (Form 302)

Contact no. 3
One-Month Follow-up Call

DIETARY INTERVIEW 1 month

Study Center |__|__|__|__|__| Local Code |__|__|__|__|__|__|__|__|__| Registration Code |__|__|__|__|__|

7. Which foods has your baby received since the 2-week call, and how often?

The purpose of this question is to assess how often your baby has consumed the foods listed below since the 2-week call. Mark (X) each row of the table appropriately to indicate how often the baby has received the food(s). If the baby has not consumed any food items in the row, mark the column "not at all". If the food was consumed more than 6 times per week, mark the appropriate "Times per day" box. Please do not use the last category, "other food item", unless it is absolutely necessary. Instead, try to use the specific food categories as much as possible to describe the foods consumed by the baby. There must be only one mark in each row.

Type of food	Average frequency since the 2-week call						
		Times per week			Times per day		
	Not at all	Less than 1	1-3	4-6	1-2	3-4	5 or more
Breast milk							
Study Formula							
Strained potato / vegetables							
Strained fruit / fruit juices							
Foods containing oat, wheat, barley or rye (e.g., baby cereals, bread, biscuits)							
Foods containing corn, rice, buckwheat or millet (e.g., baby cereals, bread, biscuits)							
Foods containing pork, chicken, turkey, lamb, or game (e.g., strained meat and vegetables)							
Foods containing fish (e.g., strained fish and vegetables)							
Egg							
Vitamin D supplementation or cod liver oil – please list:							
Other vitamins/minerals, please list:							
Other food item – please list: _____							

TRIGR Form 305

Nutritional Prevention of Type 1 Diabetes North America Region

Contact no. 3

One-Month Follow-up Call

DIETARY INTERVIEW 1 month

Study Center I__I__I__I__I__I Local Code I__I__I__I__I__I__I__I__I__I Registration Code I__I__I__I__I__I__I

8. Has your baby received any foods since the 2-week call that are <u>not allowed</u> during the dietary intervention period?

Foods not allowed during the dietary intervention period are mentioned in the table below. In case the baby has received any of those since the 2-week call, mark (X) each row of the table appropriately to indicate how often the baby has received the food(s). If the baby has not consumed any food items in the row, mark the column "not at all". If the food was consumed more than 6 times per week, mark the appropriate "Times per day" box. There must be only one mark in each row.

Type of food	Average frequency since the 2-week call						
		Times per week			Times per day		
	Not at all	Less than 1	1-3	4-6	1-2	3-4	5 or more
Regular cow's / goat's milk-based formula, as such or used in cooking Brand name(s)?_____							
Nutramigen or other hydrolyzed formula* Brand name(s)?_____							
Soy-based formula* Brand name(s)?_____							
Soured milk and sour milk products (e.g., buttermilk, cultured milk, yogurt)							
Regular cow's / goat's milk, ice cream or cheese, as such, in commercial baby foods, or when used in cooking (e.g., baby foods)							
Foods containing beef, veal or meat extract (e.g., strained beef and vegetables)							
Sausage and other meat products containing beef							
Other – please list (e.g., milk containing lactic acid bacteria supplements)							

* Other infant formulas than the Study Formula are not allowed during the dietary intervention period