Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

Associations of breastfeeding with childhood autoimmunity, allergies, and overweight: The Environmental Determinants of Diabetes in the Young (TEDDY) study

Sandra Hummel,[1] Andreas Weiß,[1] Ezio Bonifacio,[2] Daniel Agardh,[3] Beena Akolkar,[4] Carin A Aronsson,[3] William A Hagopian,[5] Sibylle Koletzko,[6,7] Jeffrey P Krischer,[8] Åke Lernmark,[3] Kristian Lynch,[8] Jill M Norris,[9] Marian J Rewers,[10] Jin-Xiong She,[11] Jorma Toppari,[12,13] Ulla Uusitalo,[8] Kendra Vehik,[8] Suvi M Virtanen,[14,15,16,17] Andreas Beyerlein,[1] and Anette-G Ziegler,[1] the TEDDY Study Group

[1]Institute of Diabetes Research, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg, Germany; and Forschergruppe Diabetes, Technical University Munich, at Klinikum rechts der Isar, Munich, and Forschergruppe Diabetes eV, Neuherberg, Germany; [2]DFG Center for Regenerative Therapies Dresden, Faculty of Medicine, TU Dresden, Dresden, Germany; [3]Department of Clinical Sciences, Lund University, Skåne University Hospital, Malmö, Sweden; [4]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA; [5]Pacific Northwest Diabetes Research Institute, Seattle, WA, USA; [6]Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Munich, Germany; [7]Department of Pediatrics, Gastroenterology and Nutrition, School of Medicine, Collegium Medicum University of Warmia and Mazury, Olsztyn, Poland; [8]Health Informatics Institute, Morsani College of Medicine, University of South Florida, Tampa, FL, USA; [9]Department of Epidemiology, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; [10]Barbara Davis Center for Childhood Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; [11]Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, Augusta, GA, USA; [12]Department of Pediatrics, Turku University Hospital, Turku, Finland; [13]Institute of Biomedicine, Research Centre for Integrative Physiology and Pharmacology, and Centre for Population Health Research, University of Turku, Turku, Finland; [14]Health and Well-Being Promotion Unit, Finnish Institute for Health and Welfare , Helsinki, Finland; [15]Unit of Health Sciences, Faculty of Social Sciences, Tampere University, Tampere, Finland; [16]Center for Child Health Research, Tampere University and Tampere University Hospital, Tampere, Finland; and [17]The Science Center of Pirkanmaa Hospital District, Tampere, Finland

ABSTRACT

Background: Breastfeeding has beneficial effects on numerous health outcomes.

Objectives: We investigated whether breastfeeding duration is associated with the development of early childhood autoimmunity, allergies, or obesity in a multinational prospective birth cohort.

Methods: Infants with genetic susceptibility for type 1 diabetes ($n = 8676$) were followed for the development of autoantibodies to islet autoantigens or transglutaminase, allergies, and for anthropometric measurements to a median age of 8.3 y (IQR: 2.8–10.2 y). Information on breastfeeding was collected at 3 mo of age and prospectively thereafter. A propensity score for longer breastfeeding was calculated from the variables that were likely to influence any or exclusive breastfeeding. The risks of developing autoimmunity or allergy were assessed using Cox proportional hazards models, and the risk of obesity at 5.5 y of age was assessed using logistic regression with adjustment by the propensity score.

Results: Breastfeeding duration was not associated with a lower risk of either islet or transglutaminase autoimmunity (any breastfeeding >6 mo, adjusted HR: 1.07; 95% CI: 0.96, 1.19; exclusive breastfeeding >3 mo, adjusted HR: 1.03; 95% CI: 0.92, 1.15). Exclusive breastfeeding >3 mo was associated with a decreased risk of seasonal allergic rhinitis (adjusted HR: 0.70; 95% CI: 0.53, 0.92; $P < 0.01$). Any breastfeeding >6 mo and exclusive breastfeeding >3 mo were associated with decreased risk of obesity (adjusted OR: 0.62; 95% CI: 0.47, 0.81; $P < 0.001$; and adjusted OR: 0.68; 95% CI: 0.47, 0.95; $P < 0.05$, respectively).

Conclusions: Longer breastfeeding was not associated with a lower risk of childhood (islet or transglutaminase) autoimmunity in genetically at-risk children but was associated with decreased risk of seasonal allergic rhinitis and obesity at 5.5 y of age. *Am J Clin Nutr* 2021;00:1–9.

Keywords: breastfeeding, islet autoimmunity, celiac disease, obesity, allergic disease

Introduction

Autoimmunity, allergy, and obesity are genetically and environmentally determined (1–4) and their frequencies are increasing in children (5–8). Changes in lifestyle and environmental factors in recent decades may contribute to the increased frequencies of these disorders. Among modifiable lifestyle factors in early childhood, breastfeeding is thought to have a protective effect against numerous health outcomes, including autoimmunity, allergic disease, and obesity (9).

The findings of beneficial effects of breast milk on autoimmunity are largely based on retrospective studies, which reported reduced risks of type 1 diabetes and celiac disease with prolonged breastfeeding (10, 11). However, prospective birth cohort studies

Am J Clin Nutr 2021;00:1–9. Printed in USA. © The Author(s) 2021. Published by Oxford University Press on behalf of the American Society for Nutrition. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

and meta-analyses failed to identify a protective association conclusively (10–13), and 1 prospective study indicated that breastfeeding for >12 mo may actually increase the risk of celiac disease (14). Also, previous results from the TEDDY (The Environmental Determinants of Diabetes in the Young) study indicated that continued breastfeeding at the time of gluten introduction may increase the risk of celiac disease–associated autoimmunity (15).

Studies on the effects of breastfeeding on allergic disease have also yielded inconsistent results (16, 17). Although there is more convincing evidence for a protective association of breastfeeding with overweight or obesity risk in later life, there is substantial heterogeneity between studies (18). The heterogeneous effects of breastfeeding are likely due to the many confounders that affect both breastfeeding behavior and the outcomes of multifactorial diseases, and to the retrospective design of most of these studies.

The TEDDY study is a large birth cohort study prospectively following children with increased genetic susceptibility for type 1 diabetes and celiac disease in the United States, Finland, Sweden, and Germany for the development of islet and transglutaminase autoantibodies (19), which represent the more frequent manifestations of autoimmunity in childhood. It collects detailed prospective information on environmental exposures and childhood conditions, such as allergy, as well as demographic and anthropometric data, such as growth. The study monitors children for the earliest signs of autoimmunity and reports of allergy, including reactions to food, pollen, plants, drugs, and animals. Therefore, it is ideally suited to look for associations between breastfeeding and disease in early childhood accounting for variables affecting breastfeeding behavior. Here, we used information from this unique cohort to investigate whether breastfeeding is associated with the development of 3 different childhood outcomes: autoimmunity, allergy, and obesity.

The TEDDY Study was supported by grants U01 DK63829, U01 DK63861, U01 DK63821, U01 DK63865, U01 DK63863, U01 DK63836, U01 DK63790, UC4 DK63829, UC4 DK63861, UC4 DK63821, UC4 DK63865, UC4 DK63863, UC4 DK63836, UC4 DK95300, UC4 DK100238, UC4 DK106955, UC4 DK112243, UC4 DK117483, and contract no. HHSN267200700014C from the National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Allergy and Infectious Diseases, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institute of Environmental Health Sciences, CDC, and Juvenile Diabetes Research Foundation. Supported in part by NIH/National Center for Advancing Translational Sciences Clinical and Translational Science awards UL1 TR000064 (to the University of Florida) and UL1 TR002535 (to the University of Colorado), by German Federal Ministry of Education and Research grants FKZ 01KX1818 (to A-GZ) and FKZ 01EA1905 (to SH), and by the German Federal Ministry of Education and Research (to the German Center for Diabetes Research—DZD eV).

The funders had no impact on the design, implementation, analysis, and interpretation of the data.

Supplemental Tables 1–7, Supplemental Figure 1, and Supplemental Acknowledgments are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to SH (e-mail: sandra.hummel@helmholtz-muenchen.de).

Abbreviations used: GAD, glutamic acid decarboxylase; HLA, human leukocyte antigen; SDS, SD score; TEDDY, The Environmental Determinants of Diabetes in the Young; tTG, tissue transglutaminase.

Received September 22, 2020. Accepted for publication February 19, 2021.

First published online 0, 2021; doi: https://doi.org/10.1093/ajcn/nqab065.

Methods

Study population

The TEDDY study screened 424,788 newborns for type 1 diabetes–associated human leukocyte antigen (HLA) genotypes between 2004 and 2010, of which 8676 children were enrolled and followed up in 6 centers located in the United States, Finland, Germany, and Sweden. The study design, eligibility, and methods have been published in more detail elsewhere (6, 19). We used data up to 31 December, 2017. Written informed consent was obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and participation in the prospective follow-up. The study was approved by local institutional review boards and is monitored by an external advisory board formed by the NIH.

Dietary assessment

Information on the infant's diet during the first 3 mo of life was collected by questionnaires at the first clinical visit at 3–4 mo of age. The primary caretakers were asked whether the infant was still receiving breast milk. If they responded "no," they were asked when breastfeeding was stopped or whether the child had never been breastfed. If they responded "yes," the caretakers were asked to prospectively record the date when breastfeeding was stopped in a booklet which was reviewed at each clinical visit (every 3 mo ≤4 y of age). The definition of exclusive breastfeeding included small amounts (<5 mL/d) of nonnutritious drinks such as tea, water, and dietary supplements (including vitamins and minerals, such as vitamin D). The duration of any breastfeeding was the age at the last time the child was breastfed. Breastfeeding included feeding breast milk either at the breast and/or by bottle and feeding with the child's mother's breast milk or donated breast milk (which was the case in 45 children). The TEDDY study did not provide any specific recommendations or advice on infant feeding to the families.

Assessment of outcomes

Autoimmunity.

Blood samples were obtained every 3 mo until 4 y of age and biannually thereafter for the analysis of islet autoantibodies [insulin-, glutamic acid decarboxylase- (GAD), and insulinoma antigen-2 autoantibodies]. From 2 y of age and every year thereafter, samples were tested for autoantibodies to tissue transglutaminase (tTG). If a child tested positive at 2 y, earlier blood samples that had been collected from birth onward were also analyzed to determine the age at which tTG antibodies first became detectable. Autoantibodies were measured by radiobinding assays as previously described (20–22).

In this study, autoimmunity was defined as positivity for islet autoantibodies and/or tTG autoantibodies. Positive for islet autoantibodies was defined as positive for ≥1 islet autoantibody in 2 reference laboratories on ≥2 consecutive visits. Positive for tTG autoantibodies was defined as positive on ≥2 consecutive visits. The date of seroconversion (time to first autoantibody) was defined as the date on which the first of the 2 consecutive positive samples was collected. For children who developed both islet and

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

tTG autoantibodies, the date of seroconversion was defined as the date of the first of the 2 autoantibody entities.

Allergy.

Information on the development of allergies during the first 3 mo was obtained at the first clinical visit at 3–4 mo of age. The primary caretakers were asked whether the child had developed any allergy. If they responded "yes," the start date, allergy type, and whether the allergy was confirmed by a health care professional were recorded. The caretakers were asked to prospectively record this information in the TEDDY booklet. The booklets were reviewed at all future clinical visits. A positive allergy outcome was defined as confirmation of the allergy by a health care professional, and included the development of any allergy, including food allergies, seasonal allergic rhinitis, drug, animal, or other allergies. In addition, the outcomes food allergies and seasonal allergic rhinitis were assessed separately. The start date of the first allergy, the first food allergy, or seasonal allergic rhinitis was used to indicate the time from birth to the event, regardless of the development of further allergies.

Obesity.

Anthropometric outcomes were assessed at 5.5 y of age at the 66-mo visit or at the next closest visit between the ages of 54 and 72 mo, a range which corresponds to preschool age for the children in all 4 countries. The height and weight of the child were measured by trained personnel at the TEDDY clinics, as previously described (23). Height and weight were used to calculate BMI, which was transformed to SD score (SDS) values using WHO reference values (24). SDS values < -5 or >5 were deemed implausible and therefore excluded. BMI-SDS values were used to define obesity (BMI-SDS >2).

Statistical analysis

Children were classified into different groups according to any breastfeeding duration ($\leq$6 mo and $>$6 mo) and exclusive breastfeeding duration ($\leq$3 mo and $>$3 mo), respectively. In a sensitivity analysis, children were classified according to any breastfeeding duration $\leq$12 mo and $>$12 mo.

The influence of several confounders which may affect either the exposure (breastfeeding duration) or the outcomes (autoimmunity, allergy, obesity) was controlled for by calculating propensity scores, which were included in the final models instead of using conventional covariate adjustment. The propensity score was defined as the probability of a child being breastfed for a longer duration (i.e., any breastfeeding $>$6 mo, $>$12 mo in the sensitivity analysis, or exclusive breastfeeding $>$3 mo), given a set of pregnancy- and birth-related covariables known from previous studies to affect breastfeeding duration. The propensity scores were estimated as previously described (25), using a logistic regression model in which these pregnancy- and birth-related variables were included as covariates and any breastfeeding $>$6 mo ($>$12 mo in the sensitivity analysis) or exclusive breastfeeding $>$3 mo as dependent variables. The following covariates were included in the calculation of the propensity score: country of origin, maternal diabetes during pregnancy (preexisting or gestational diabetes, yes/no), maternal prepregnancy BMI, gestational weight gain [classified as inadequate, adequate, or excessive according to Institute of Medicine guidelines (26)], maternal smoking during pregnancy (yes/no), maternal age at delivery, maternal education (high compared with middle/low), parity (firstborn child yes/no), gestational age, birth weight, and cesarean delivery (yes/no). The score resulting from the ORs for each of the included variables in the model originally ranged between 0 and 1; for interpretational purposes the score was multiplied by the factor 10. To test how breastfeeding groups were distributed across the propensity score ranges, quartiles were calculated. The risks of developing islet- and/or celiac disease–associated autoimmunity and allergy in each breastfeeding category (i.e., any breastfeeding $\leq$6 mo compared with $>$6 mo and exclusive breastfeeding duration $\leq$3 mo compared with $>$3 mo, respectively, in the main analysis and any breastfeeding $\leq$12 mo compared with $>$12 mo in the sensitivity analysis) were computed using multivariable Cox proportional hazards models for the any and exclusive breastfeeding categories and expressed as HRs with 95% CIs adjusted for the propensity score. For the separate autoimmunity outcomes, islet autoantibodies and tTG autoantibodies, competing risk was accounted for by censoring children at the time of their first autoimmune (either islet or tTG autoantibodies) event. In the sensitivity analysis, Cox proportional hazards models were computed for the outcomes insulin autoantibodies or GAD autoantibodies as first appearing islet autoantibodies and Cox proportional hazards models for the autoimmunity outcomes were further adjusted for HLA genotype and having a first-degree relative with type 1 diabetes or celiac disease. The risks of being obese at 5.5 y of age by breastfeeding category were determined by logistic regression, including the respective propensity score as a covariate.

Several children developed the outcome islet autoimmunity or allergy before 6 mo of age. To avoid bias by the fact that children, who developed the outcome early, were breastfed longer and were therefore in the longer breastfeeding category, sensitivity analyses were performed excluding children who developed the outcome during the first 3 mo (for the exclusive breastfeeding analysis) or the first 6 mo (for the any breastfeeding analysis).

All analyses were carried out with SAS version 9.4 (SAS Institute) and R version 3.6.3 (R Foundation for Statistical Computing).

Results

Risks of developing childhood autoimmunity, allergy, and obesity

At the time of the analysis, the children had been followed to a median age of 8.3 y (IQR: 2.8–10.2 y). Information on breastfeeding was available for 8615 of 8676 children (4255 girls) (**Table 1**), including 6810 children with complete information for the calculation of the propensity score. All children were assessed for islet autoantibodies and allergies, 5968 for tTG autoantibodies, and 4927 had anthropometric measurements at 5.5 y of age (**Figure 1**). Among children with breastfeeding data, 1677 developed autoimmunity (680 islet autoantibodies, 1132 tTG autoantibodies), 1266 developed allergy (577 food allergy,

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

TABLE 1 Description of the study population[1]

Variable	*n*	*n* (%)/median [IQR]
Duration of any breastfeeding, mo	8576	
≤6		3982 (46.4)
>6		4594 (53.6)
Duration of exclusive breastfeeding, mo	8611	
≤3		6699 (77.8)
>3		1912 (22.2)
Females	8615	4255 (49.4)
Country	8615	
USA		3694 (42.9)
Finland		1825 (21.2)
Germany		580 (6.7)
Sweden		2516 (29.2)
Maternal/birth-related variables		
Maternal age at delivery, y	8615	30 [27–34]
Maternal prepregnancy BMI, kg/m^2	8372	23.6 [21.3–27.0]
Excessive weight gain during pregnancy (yes)	8300	3985 (48.0)
Maternal diabetes during pregnancy (yes)	8252	836 (10.1)
Maternal smoking during pregnancy (yes)	8373	1068 (12.8)
High maternal education (yes)	7501	4110 (54.8)
Preterm delivery (gestational age <37 wk)	8601	477 (5.5)
Birth weight, g	8381	3500 [3156–3840]
Cesarean delivery (yes)	8610	2235 (26.0)
Firstborn child (yes)	7455	3335 (44.7)

[1]Total $n = 8615$.

378 seasonal allergic rhinitis), and 262 were obese at 5.5 y of age. The cumulative risk of autoimmunity was 30.0% by 10 y of age, with cumulative risks of 12.1% for islet autoantibodies and 20.6% for tTG autoantibodies. The cumulative risk of any allergy (including food allergies, seasonal allergic rhinitis, or other allergies) by 10 y of age was 20.4%, with cumulative risks of 9.1% for food allergy and 6.7% for seasonal allergic rhinitis. The prevalence rate of being obese at 5.5 y was 5.3%. A total of 18 children developed all 3 entities (**Supplemental Figure 1**).

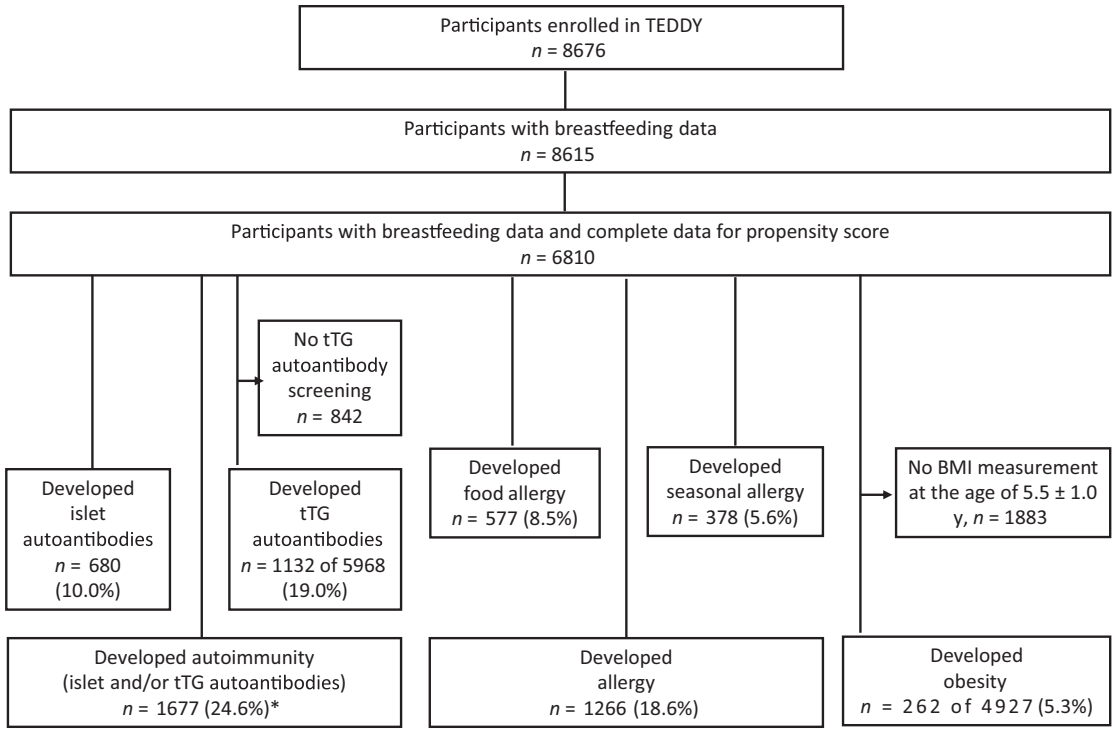


FIGURE 1 Flowchart of the study population. ∗For the outcome autoimmunity, information on tTG autoantibodies was not available for 842 of 6810 children. TEDDY, The Environmental Determinants of Diabetes in the Young; tTG, tissue transglutaminase.

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

TABLE 2 Propensity score model: logistic regression model for the probability of any breastfeeding >6 mo or exclusive breastfeeding >3 mo

	Any breastfeeding >6 mo		Exclusive breastfeeding >3 mo	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Country				
USA	Reference		Reference	
Finland	1.62 (1.40, 1.87)	<0.0001	1.26 (1.07, 1.48)	<0.01
Germany	1.35 (1.08, 1.69)	<0.01	2.92 (2.29, 3.71)	<0.0001
Sweden	1.17 (1.03, 1.33)	0.02	1.68 (1.46, 1.95)	<0.0001
Pregnancy-related variables				
Maternal diabetes during pregnancy (yes)	0.87 (0.72, 1.04)	0.13	0.61 (0.48, 0.77)	<0.0001
Maternal age at delivery, y	1.04 (1.03, 1.05)	<0.0001	1.01 (1.00, 1.03)	0.08
Maternal smoking during pregnancy (yes)	0.46 (0.39, 0.55)	<0.0001	0.54 (0.43, 0.68)	<0.0001
Maternal prepregnancy BMI, kg/m^2	0.95 (0.94, 0.96)	<0.0001	0.96 (0.95, 0.98)	<0.0001
Inadequate gestational weight gain	0.97 (0.83, 1.13)	0.70	0.86 (0.73, 1.02)	0.09
Excessive gestational weight gain	0.78 (0.69, 0.88)	<0.0001	0.75 (0.66, 0.86)	<0.0001
High maternal education	2.18 (1.94, 2.44)	<0.0001	1.77 (1.55, 2.02)	<0.0001
Gestational age, wk	1.01 (0.97, 1.05)	0.65	1.07 (1.02, 1.13)	<0.01
Birth-related variables				
Cesarean delivery (yes)	0.69 (0.60, 0.78)	<0.0001	0.56 (0.48, 0.66)	<0.0001
Birth weight, g	1.00 (1.00, 1.00)	<0.0001	1.00 (1.00, 1.00)	<0.001
Firstborn child (yes)	0.92 (0.83, 1.03)	0.17	0.65 (0.58, 0.74)	<0.0001

Propensity for prolonged breastfeeding duration

Table 2 shows propensity score models for any breastfeeding >6 mo and for exclusive breastfeeding >3 mo. The propensity for any breastfeeding >6 mo or exclusive breastfeeding >3 mo was increased in children from European countries, in children of mothers with higher maternal age and high education, and in children with increased birth weight. Propensity for longer breastfeeding was decreased in children of mothers who reported smoking during pregnancy, who had a higher prepregnancy BMI, and who had excessive gestational weight gain, and in children delivered by cesarean delivery. In addition, the propensity for exclusive breastfeeding >3 mo was increased in children with increasing gestational age and decreased in children of mothers with diabetes during pregnancy and in firstborn children. The proportion of children with longer breastfeeding duration increased with every quartile of the propensity score (**Supplemental Table 1**).

Breastfeeding and childhood autoimmunity

The duration of any and exclusive breastfeeding was not associated with the risk of autoimmunity (adjusted HR for any breastfeeding >6 mo: 1.07; 95% CI: 0.96, 1.19; $P = 0.20$ compared with ≤6 mo; adjusted HR for exclusive breastfeeding >3 mo: 1.03; 95% CI: 0.92, 1.15; $P = 0.63$ compared with ≤3 mo) (**Table 3**). Similarly, no significant associations were observed when islet autoantibodies (adjusted HR for any breastfeeding >6 mo: 1.03; 95% CI: 0.88, 1.22; $P = 0.69$ compared with ≤6 mo; adjusted HR for exclusive breastfeeding >3 mo: 0.95; 95% CI: 0.79, 1.14; $P = 0.57$ compared with ≤3 mo) (Table 3) and tTG autoantibodies (adjusted HR for any breastfeeding >6 mo: 1.08; 95% CI: 0.95, 1.23; $P = 0.24$ compared with ≤6 mo; adjusted HR for exclusive breastfeeding >3 mo: 1.08; 95% CI: 0.94, 1.24; $P = 0.27$ compared with ≤3 mo) (Table 3) were analyzed separately and when insulin autoantibodies or GAD autoantibodies as the first appearing autoantibodies were analyzed (**Supplemental Table 2**). Results were similar when adjusting for additional covariables that have been associated with islet autoimmunity previously, such as HLA genotype or having a first-degree relative with type 1 diabetes or celiac disease (**Supplemental Table 3**), and when using 12 mo as a cutoff for the any breastfeeding categories (**Supplemental Table 4**). No child developed autoimmunity during the first 3 mo and 9 children developed islet autoantibodies between 3 and 6 mo of age. Results were similar after excluding these children from the analysis (**Supplemental Table 5**).

Results were also similar when analyzing whether breastfeeding duration was associated with the risk of developing autoimmunity during the first 24 mo of age (**Supplemental Table 6**).

Breastfeeding and allergy

Exclusive breastfeeding >3 mo was significantly associated with a decreased risk of seasonal allergic rhinitis compared with exclusive breastfeeding ≤3 mo (adjusted HR: 0.70; 95% CI: 0.53, 0.92; $P = 0.01$) (**Table 4**). Results were similar when excluding 50 children who developed any allergy during the first 3 mo (adjusted HR: 0.70; 95% CI: 0.52, 0.91; $P < 0.05$) (**Supplemental Table 7**). Any breastfeeding duration was not significantly associated with the risk of seasonal allergic rhinitis [adjusted HR: 0.87; 95% CI: 0.70, 1.08; $P = 0.19$, for any breastfeeding >6 mo (Table 4); and adjusted HR: 0.91; 95% CI: 0.70, 1.19; $P = 0.50$, for any breastfeeding >12 mo (Supplemental Table 4)], also after excluding children who developed allergies during the first 6 mo (Supplemental Table 7). Breastfeeding duration was not associated with the risk of food allergy (adjusted HR for any breastfeeding >6 mo: 0.96; 95% CI: 0.80, 1.15; $P = 0.64$ compared with ≤6 mo; adjusted HR for exclusive breastfeeding >3 mo: 0.99; 95% CI: 0.81, 1.21; $P = 0.90$ compared with ≤3 mo) (Table 4, Supplemental Tables 4, 7). Similarly, breastfeeding duration was not associated with the risk of any first allergy (Table 4, Supplemental Tables 4, 7).

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

TABLE 3 Multivariate Cox proportional hazards model for autoimmunity (including islet- and/or celiac disease–associated autoimmunity), ≥1 islet autoantibodies, and tissue-transglutaminase autoantibodies according to the duration of any and exclusive breastfeeding

	Autoimmunity			Islet autoantibodies			t-Transglutaminase autoantibodies		
Variables	*n*	HR (95% CI)	*P* value	*n*	HR (95% CI)	*P* value	*n*	HR (95% CI)	*P* value
Any breastfeeding									
≤6 mo	2751	Reference		2751	Reference		2298	Reference	
>6 mo	4030	1.07 (0.96, 1.19)	0.20	4030	1.03 (0.88, 1.22)	0.69	3653	1.08 (0.95, 1.23)	0.24
Propensity score[1]	6781	1.06 (1.03, 1.09)	<0.0001	6781	1.07 (1.02, 1.12)	<0.01	5951	1.05 (1.01, 1.09)	<0.05
Exclusive breastfeeding									
≤3 mo	5169	Reference		5169	Reference		4484	Reference	
>3 mo	1641	1.03 (0.92, 1.15)	0.63	1641	0.95 (0.79, 1.14)	0.57	1484	1.08 (0.94, 1.24)	0.27
Propensity score[1]	6810	1.10 (1.06, 1.14)	<0.0001	6810	1.12 (1.05, 1.19)	<0.001	5968	1.08 (1.03, 1.14)	<0.01

[1]Propensity score on the 10-fold probability for each individuum to be breastfed for >6 mo (any breastfeeding) or >3 mo (exclusive breastfeeding), respectively, based on logistic regression–derived estimates of the following variables: country, maternal diabetes, maternal age, firstborn child, maternal education, maternal smoking during pregnancy, maternal prepregnancy BMI, gestational weight gain, birth weight, cesarean delivery, and gestational age.

Breastfeeding and obesity

The risk of obesity at 5.5 y of age was associated with breastfeeding duration. Any breastfeeding for >6 mo and exclusive breastfeeding for >3 mo were associated with a decreased risk of obesity (any breastfeeding >6 mo, adjusted OR: 0.62; 95% CI: 0.47, 0.81; $P < 0.001$ compared with ≤6 mo; exclusive breastfeeding >3 mo, adjusted OR: 0.68; 95% CI: 0.47, 0.95; $P < 0.05$ compared with ≤3 mo) (**Table 5**). Any breastfeeding >12 mo was not associated with obesity risk (Supplemental Table 4).

Discussion

Using data from the TEDDY study, we found that breastfeeding duration beyond 6 mo and exclusive breastfeeding longer than 3 mo were not associated with protection from developing autoimmunity associated with type 1 diabetes and celiac disease. In contrast to autoimmunity, seasonal allergic rhinitis was less frequent in children with longer exclusive breastfeeding and obesity at 5.5 y was less frequent in children with longer any or exclusive breastfeeding.

The finding that a longer duration of any or exclusive breastfeeding was not associated with a lower risk of autoimmunity in genetically at-risk children supports results from smaller national prospective studies (10, 27–30), whereas beneficial effects of breast milk were reported from several retrospective studies. Inconsistent results on the effect of breastfeeding on islet- and celiac disease–associated autoimmunity are likely due to differences in study design and differences in the selection of the many confounders that affect breastfeeding duration. In contrast to previous studies, we included a propensity score for the probability of longer breastfeeding duration in the multivariable models instead of conventional covariate adjustment. This approach has been increasingly applied in observational studies to avoid bias due to baseline characteristics of the study participants (31). The propensity score was defined as the probability of a child being breastfed for a longer duration, given a set of pregnancy- and birth-related covariables known from previous studies to affect breastfeeding duration. By the inclusion of the propensity score in the multivariable model we attenuated the risk of systematic differences in pregnancy- or birth-related baseline characteristics between children in the shorter and longer breastfeeding categories when estimating the effect of breastfeeding on autoimmunity, allergy, and obesity.

Because breast milk is among the earliest environmental factors to which children are exposed, it could be hypothesized that its protective effect is limited to the early development of autoimmunity. However, we did not observe any differences in the association when analyzing the risk of developing autoimmunity during the first 24 mo of age. Therefore, an age-dependent association between breastfeeding and autoimmunity risk is unlikely.

There is evidence that the association between breastfeeding and allergy risk may depend on allergy type (32). Indeed, when performing separate analyses for food allergies and seasonal allergic rhinitis, which were the most commonly reported allergies in our cohort, we observed that shorter exclusive breastfeeding was associated with an increased risk of seasonal allergic rhinitis but not with the risk of food allergies. The lack

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

TABLE 4 Multivariate Cox proportional hazards model for the risk of any allergy, any food allergy, and seasonal allergic rhinitis according to the duration of any and exclusive breastfeeding

	Any allergy			Food allergy			Seasonal allergic rhinitis		
Variables	*n*	HR (95% CI)	*P* value	*n*	HR (95% CI)	*P* value	*n*	HR (95% CI)	*P* value
Any breastfeeding									
≤6 mo	2739	Reference		2751	Reference		2751	Reference	
>6 mo	4021	0.94 (0.83, 1.06)	0.28	4030	0.96 (0.80, 1.15)	0.64	4030	0.87 (0.70, 1.08)	0.19
Propensity score[1]	6760	0.96 (0.93, 1.00)	<0.05	6781	1.02 (0.97, 1.08)	0.38	6781	0.93 (0.88, 0.99)	<0.05
Exclusive breastfeeding									
≤3 mo	5152	Reference		5169	Reference		5169	Reference	
>3 mo	1637	0.94 (0.81, 1.08)	0.35	1641	0.99 (0.81, 1.21)	0.90	1641	0.70 (0.53, 0.92)	0.01
Propensity score[1]	6789	0.87 (0.82, 0.91)	<0.0001	6810	0.96 (0.89, 1.03)	0.20	6810	0.83 (0.76, 0.91)	<0.0001

[1]Propensity score on the 10-fold probability for each individuum to be breastfed for >6 mo (any breastfeeding) or >3 mo (exclusive breastfeeding), respectively, based on logistic regression–derived estimates of the following variables: country, maternal diabetes, maternal age, firstborn child, maternal education, maternal smoking during pregnancy, maternal prepregnancy BMI, gestational weight gain, birth weight, cesarean delivery, and gestational age.

TABLE 5 Multivariate logistic regression analysis of the risk of obesity at 5.5 y of age according to the duration of any and exclusive breastfeeding

	Obesity		
Variables	*n*	OR (95% CI)	*P* value
Any breastfeeding			
≤6 mo	1793	Reference	
>6 mo	3122	0.62 (0.47, 0.81)	<0.001
Propensity score[1]	4915	0.78 (0.73, 0.84)	<0.0001
Exclusive breastfeeding			
≤3 mo	3650	Reference	
>3 mo	1277	0.68 (0.47, 0.95)	<0.05
Propensity score[1]	4927	0.65 (0.58, 0.74)	<0.0001

[1]Propensity score on the 10-fold probability for each individuum to be breastfed for >6 mo (any breastfeeding) or >3 mo (exclusive breastfeeding), respectively, based on logistic regression–derived estimates of the following variables: country, maternal diabetes, maternal age, firstborn child, maternal education, maternal smoking during pregnancy, maternal prepregnancy BMI, gestational weight gain, birth weight, cesarean delivery, and gestational age.

of a protective effect of breastfeeding on food allergy risk is consistent with previous reports (32). In contrast, meta-analyses on the effect of breastfeeding on seasonal allergic rhinitis were inconclusive, probably owing to varying methods and outcome definitions between studies (32, 33). Unlike meta-analyses, we can exclude the possibility that differences in study design between countries may have affected the associations. There are several possible mechanisms that have been proposed on how breastfeeding could protect from seasonal allergic rhinitis, such as the modulation of gut microbiota, the maturation of the immune system (32, 34), and beneficial effects of breastfeeding on lung development (34).

We observed that longer breastfeeding duration was associated with a reduced risk of obesity at 5.5 y of age after adjusting for propensity score. This study further complements the available evidence by showing a protective association of breastfeeding on overweight risk in a multinational setting accounting for regional differences in growth patterns (35) and breastfeeding behaviors (36), in addition to childhood dietary intake, which has been associated with overweight risk in the same cohort previously (23).

This study has several strengths. It was performed in a large number of children who were prospectively followed for the development of islet and tTG autoantibodies using the same protocol with documentation of breastfeeding duration, anthropometric measurements, and allergies. This unique cohort allowed us to assess the role of breastfeeding on childhood health outcomes prospectively, including autoimmunity, allergies, and becoming overweight, and to avoid some of the challenges often encountered in meta-analysis of smaller studies, particularly inconsistencies in study design and definitions of breastfeeding and outcomes. In addition, the prospective and longitudinal assessment of breastfeeding practices and outcomes enabled us to evaluate reverse causation, which may be caused by changes in breastfeeding practices as an effort to reduce disease risk after notification of a positive autoantibody result or the appearance of the first symptoms of food intolerance. Exclusion of children with an outcome in the first months did not change

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

the findings, suggesting little influence through the modification of breastfeeding by a positive autoantibody result or an allergy.

A limitation of our study is that, although we only included family-reported allergies that were confirmed by a health care professional, we cannot ascertain that the diagnosis was confirmed according to current diagnostic guidelines, which may have led to a higher prevalence of allergies (37). Furthermore, the definition of allergy was not limited to IgE-mediated allergies but included all types of allergies. To refine the allergy definition, we performed a subanalysis on the outcome seasonal allergic rhinitis, an IgE-mediated allergy with a prevalence in our study that was comparable with previous reports from the same countries (7). We were not able to investigate the outcome of asthma, because this information was not available from the allergy questionnaire, and we had no information on family history of allergies, which might affect breastfeeding behavior. A family history would, however, be expected to increase allergy risk, and, therefore, would unlikely be a factor in the protection from allergy afforded by breastfeeding. Second, owing to the observational study design, we were unable to prove causality for significant associations and we cannot exclude the possibility that other factors not included in the study may have acted as confounders in this analysis. Because we observed a strong collinearity between the propensity score and the continuous breastfeeding variables, our analysis was limited to categorized breastfeeding duration. Relatively few of the TEDDY participants were exclusively breastfed for 6 mo and any breastfed for 24 mo, which is the WHO recommendation (38), and therefore 3 mo was chosen to categorize the groups for exclusive breastfeeding duration and 6 mo for any breastfeeding duration. To assess if longer breastfeeding duration may be beneficial, we in addition used 12 mo to categorize any breastfeeding duration. However, no significant associations of longer breastfeeding duration with any of the outcomes were observed, suggesting that in particular breastfeeding during the first months affects the risk of seasonal allergic rhinitis and obesity. Moreover, exclusive breastfeeding definition was not based on the strictest definition as proposed by the WHO, because it included feeding of small amounts of nonnutritious drinks and dietary supplements. Finally, the study population was selected based on an HLA genotype conferring risk of type 1 diabetes and the results may not be generalizable to children with other HLA genotypes.

In conclusion, our study showed that longer breastfeeding duration was not associated with protection from autoimmunity but was associated with reduced risk of seasonal allergic rhinitis and with reduced risk of becoming obese at 5.5 y in children with genetic susceptibility for type 1 diabetes.

For a list of members of the TEDDY Study Group, please see the Supplemental Acknowledgments.

The authors' responsibilities were as follows—SH, KL, KV, ÅL, MJR, WAH, J-XS, JT, BA, JPK, EB, and A-GZ: contributed to study design; SH, DA, CAA, SK, JMN, UU, SMV, ÅL, MJR, WAH, J-XS, JT, BA, JPK, EB, and A-GZ: contributed to acquisition of the data; SH, AW, AB, DA, CAA, SK, JMN, UU, SMV, ÅL, MJR, WAH, J-XS, JT, BA, JPK, EB, and A-GZ: contributed to interpretation of the data; SH, EB, and A-GZ: contributed to analysis of the data; SH: drafted the manuscript; AW and AB: performed statistical analysis and contributed to drafting of the manuscript; KL and KV: contributed to statistical analysis; KL, KV, DA, CAA, SK, JMN, UU, SMV, ÅL, MJR, WAH, J-XS, JT, BA, JPK, EB, and A-GZ: critically reviewed the manuscript; ÅL, MJR, WAH, J-XS, JT, BA, JPK, EB, and A-GZ: contributed to study concept; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data availability

Data described in the article and code book will be made available upon request from the National Institute of Diabetes and Digestive and Kidney Diseases Central Repository at https://repository.niddk.nih.gov/studies/teddy/.

References

1. Gurnani M, Birken C, Hamilton J. Childhood obesity: causes, consequences, and management. Pediatr Clin North Am 2015;62:821–40.
2. Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of allergy and asthma in early life. J Allergy Clin Immunol 2017;140:1–12.
3. Marson A, Housley WJ, Hafler DA. Genetic basis of autoimmunity. J Clin Invest 2015;125:2234–41.
4. Krischer JP, Lynch KF, Lernmark Å, Hagopian WA, Rewers MJ, She J-X, Toppari J, Ziegler AG, Akolkar B. Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by 6 years of age: the TEDDY Study. Diabetes Care 2017;40:1194–202.
5. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, Absah I. Increasing incidence and altered presentation in a population-based study of pediatric celiac disease in North America. J Pediatr Gastroenterol Nutr 2017;65:432–7.
6. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study. Ann N Y Acad Sci 2008;1150:1–13.
7. Asher MI, Montefort S, Björkstén B, Lai CKW, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 2006;368:733–43.
8. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, Adams RJ, Aekplakorn W, Afsana K, Aguilar-Salinas CA, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 2017;390:2627–42.
9. Agostoni C, Przyrembel H. The timing of introduction of complementary foods and later health. World Rev Nutr Diet 2013;108:63–70.
10. Szajewska H, Shamir R, Chmielewska A, Pieścik-Lech M, Auricchio R, Ivarsson A, Kolacek S, Koletzko S, Korponay-Szabo I, Mearin ML, et al. Systematic review with meta-analysis: early infant feeding and coeliac disease – update 2015. Aliment Pharmacol Ther 2015;41:1038–54.
11. Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. Lancet 2016;387:2340–8.
12. Cardwell CR, Stene LC, Ludvigsson J, Rosenbauer J, Cinek O, Svensson J, Perez-Bravo F, Memon A, Gimeno SG, Wadsworth EJK, et al. Breast-feeding and childhood-onset type 1 diabetes: a pooled analysis of individual participant data from 43 observational studies. Diabetes Care 2012;35:2215–25.
13. Silano M, Agostoni C, Sanz Y, Guandalini S. Infant feeding and risk of developing celiac disease: a systematic review. BMJ Open 2016;6:e009163.
14. Størdal K, White RA, Eggesbø M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics 2013;132:e1202–9.
15. Aronsson CA, Lee H-S, Liu E, Uusitalo U, Hummel S, Yang J, Hummel M, Rewers M, She J-X, Simell O, et al. Age at gluten introduction and risk of celiac disease. Pediatrics 2015;135:239–45.
16. Oddy WH. Breastfeeding, childhood asthma, and allergic disease. Ann Nutr Metab 2017;70(Suppl 2):26–36.
17. Miliku K, Azad MB. Breastfeeding and the developmental origins of asthma: current evidence, possible mechanisms, and future research priorities. Nutrients 2018;10:995.
18. Patro-Gołąb B, Zalewski BM, Kołodziej M, Kouwenhoven S, Poston L, Godfrey KM, Koletzko B, van Goudoever JB, Szajewska H. Nutritional

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

interventions or exposures in infants and children aged up to 3 years and their effects on subsequent risk of overweight, obesity and body fat: a systematic review of systematic reviews. Obes Rev 2016;17: 1245–57.
19. The TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. Pediatr Diabetes 2007;8:286–98.
20. Bonifacio E, Yu L, Williams AK, Eisenbarth GS, Bingley PJ, Marcovina SM, Adler K, Ziegler AG, Mueller PW, Schatz DA, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for National Institute of Diabetes and Digestive and Kidney Diseases consortia. J Clin Endocrinol Metab 2010;95:3360–7.
21. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, Rewers MJ, She J-X, Simell OG, Toppari J, et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015;58:980–7.
22. Liu E, Lee H-S, Aronsson CA, Hagopian WA, Koletzko S, Rewers MJ, Eisenbarth GS, Bingley PJ, Bonifacio E, Simell V, et al. Risk of pediatric celiac disease according to HLA haplotype and country. N Engl J Med 2014;371:42–9.
23. Beyerlein A, Uusitalo UM, Virtanen SM, Vehik K, Yang J, Winkler C, Kersting M, Koletzko S, Schatz D, Aronsson CA, et al. Intake of energy and protein is associated with overweight risk at age 5.5 years: results from the prospective TEDDY study. Obesity 2017;25: 1435–41.
24. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:76–85.
25. Vehik K, Lynch KF, Wong MC, Tian X, Ross MC, Gibbs RA, Ajami NJ, Petrosino JF, Rewers M, Toppari J, et al. Prospective virome analyses in young children at increased genetic risk for type 1 diabetes. Nat Med 2019;25:1865–72
26. Rasmussen KM, Yaktine AL,editors. Weight gain during pregnancy: reexamining the guidelines. Washington (DC): Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines; 2009.
27. Norris JM, Beaty B, Klingensmith G, Yu L, Hoffman M, Chase HP, Erlich HA, Hamman RF, Eisenbarth GS, Rewers M. Lack of association between early exposure to cow's milk protein and β-cell autoimmunity: Diabetes Autoimmunity Study in the Young (DAISY). JAMA 1996;276:609–14.
28. Chmiel R, Beyerlein A, Knopff A, Hummel S, Ziegler A-G, Winkler C. Early infant feeding and risk of developing islet autoimmunity and type 1 diabetes. Acta Diabetol 2015;52:621–4.
29. Hakola L, Takkinen H-M, Niinistö S, Ahonen S, Nevalainen J, Veijola R, Ilonen J, Toppari J, Knip M, Virtanen SM. Infant feeding in relation to the risk of advanced islet autoimmunity and type 1 diabetes in children with increased genetic susceptibility: a cohort study. Am J Epidemiol 2018;187:34–44.
30. Couper JJ, Steele C, Beresford S, Powell T, McCaul K, Pollard A, Gellert S, Tait B, Harrison LC, Colman PG. Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. Diabetes 1999;48:2145–9.
31. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. Multivariate Behav Res 2011;46:399–424.
32. Lodge CJ, Tan DJ, Lau MXZ, Dai X, Tham R, Lowe AJ, Bowatte G, Allen KJ, Dharmage SC. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. Acta Paediatr 2015;104:38–53.
33. Güngör D, Nadaud P, LaPergola CC, Dreibelbis C, Wong YP, Terry N, Abrams SA, Beker L, Jacobovits T, Järvinen KM. Infant milk-feeding practices and food allergies, allergic rhinitis, atopic dermatitis, and asthma throughout the life span: a systematic review. Am J Clin Nutr 2019;109:772S–99S.
34. Ogbuanu IU, Karmaus W, Arshad SH, Kurukulaaratchy RJ, Ewart S. Effect of breastfeeding duration on lung function at age 10 years: a prospective birth cohort study. Thorax 2008;64:62–6.
35. Nucci AM, Becker DJ, Virtanen SM, Cuthbertson D, Softness B, Huot C, Wasikowa R, Dosch HM, Akerblom HK, Knip M, et al. Growth differences between North American and European children at risk for type 1 diabetes. Pediatr Diabetes 2012;13:425–31.
36. Hummel S, Vehik K, Uusitalo U, McLeod W, Aronsson CA, Frank N, Gesualdo P, Yang J, Norris JM, Virtanen SM, et al. Infant feeding patterns in families with a diabetes history – observations from The Environmental Determinants of Diabetes in the Young (TEDDY) birth cohort study. Public Health Nutr 2014;17:2853–62.
37. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy 2014;69:992–1007.
38. WHO. Complementary feeding: report of the global consultation and summary of guiding principles for complementary feeding of the breastfed child. Geneva: World Health Organization; 2002.

Downloaded from https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqab065/6218071 by University of Liverpool user on 11 April 2021

On-line Supplementary Material

Online Supplementary Table 1: Proportion of TEDDY children with any breastfeeding ≤6 months compared to > 6 months and exclusive breastfeeding ≤ 3 months compared to > 3 months according to their propensity score - quartile.

Propensity score any breastfeeding >6 months

			Any breastfeeding	
			≤6 months	**>6 months**
Quartiles	Range of Propensity score	N	N (%)	N (%)
1	0.8-4.7	1,695	1,133 (66.8)	562 (32.2)
2	4.7-6.3	1,695	715 (42.2)	980 (57.8)
3	6.3-7.4	1,695	566 (33.4)	1,129 (66.6)
4	7.4-9.2	1,696	337 (19.9)	1,359 (80.1)

Propensity score exclusive breastfeeding >3 months

			Exclusive breastfeeding	
			≤3 months	**>3 months**
Quartiles	Range of Propensity score	N	N (%)	N (%)
1	0.2-1.5	1,703	1,528 (89.7)	175 (10.3)
2	1.5-2.3	1,702	1,413 (83.0)	289 (17.0)
3	2.3-3.2	1,702	1,223 (71.9)	479 (28.1)
4	3.2-7.0	1,703	1,005 (59.0)	698 (41.0)

Online Supplementary Table 2: Cox proportional hazards model for insulin autoantibodies (IAA) and glutamic acid decarboxylase autoantibodies (GADA) as first appearing islet autoantibodies according to the duration of any and exclusive breastfeeding.

	IAA first			**GADA first**		
Variables	**n**	**HR (95% CI)**	**p-value**	**N**	**HR (95% CI)**	**p-value**
Any breastfeeding						
≤6 months	2,751	Reference		2,751	Reference	
>6 months	4,030	1.16 (0.88, 1.52)	0.30	4,030	1.01 (0.78, 1.30)	0.96
Propensity score[a]	6,781	1.06 (0.98, 1.15)	0.15	6,781	1.07 (0.99, 1.15)	0.08
Exclusive breastfeeding						
≤3 months	5,169	Reference		5,169	Reference	
>3 months	1,641	1.05 (0.79, 1.40)	0.75	1,641	0.96 (0.73, 1.26)	0.78
Propensity score[a]	6,810	1.08 (0.98, 1.20)	0.12	6,810	1.12 (1.02, 1.24)	<0.05

Abbreviations: HR, hazard ratio; CI, confidence interval;

[a] Propensity Score on the probability for each individuum to be breastfed for more than 6 months (any breastfeeding) or more than 3 months (exclusive breastfeeding), respectively, based on logistic regression derived estimates of the following variables: country, maternal diabetes, maternal age, first-born child, maternal education, maternal smoking during pregnancy, maternal pre-pregnancy BMI, gestational weight gain, birthweight, caesarean section, and gestational age

Online Supplementary Table 3: Multivariate Cox proportional hazards model for autoimmunity (including islet and/or celiac disease associated autoimmunity), one or more islet autoantibodies and for tissue-transglutaminase autoantibodies according to the duration of any and exclusive breastfeeding, adjusted for propensity score for prolonged breastfeeding duration, HLA genotype and having a first-degree relative with type 1 diabetes.

	Autoimmunity		**Islet autoantibodies**		**t-Transglutaminase autoantibodies**	
Variables	**HR [95% CI]**	**p-value**	**HR [95% CI]**	**p-value**	**HR [95% CI]**	**p-value**
Any breastfeeding						
≤6 months	Reference		Reference		Reference	
>6 months	1.08 (0.97, 1.20)	0.17	1.04 (0.88, 1.23)	0.67	1.09 (0.96, 1.24)	0.20
Propensity score[a]	1.07 (1.03, 1.10)	<0.0001	1.08 (1.02, 1.13)	<0.01	1.06 (1.02, 1.10)	<0.01
Sex						
Female	Reference		Reference		Reference	
Male	0.76 (0.69, 0.84)	<0.0001	1.14 (0.98, 1.33)	0.09	0.62 (0.55, 0.70)	<0.0001
HLA Genotype						
DR4-DQ8/DR4-DQ8	Reference		Reference		Reference	
DR3-DQ2/DR4-DQ8	1.41 (1.22, 1.62)	<0.0001	1.30 (1.06, 1.60)	<0.05	1.39 (1.16, 1.66)	<0.001
DR3-DQ2/DR3-DQ2	2.24 (1.93, 2.61)	<0.0001	0.68 (0.52, 0.89)	<0.01	3.32 (2.77, 3.97)	<0.0001
other	0.63 (0.52, 0.76)	<0.0001	0.83 (0.65, 1.06)	0.14	0.37 (0.28, 0.50)	<0.0001
Family history of autoimmunity						
None	Reference		Reference			
Family history of T1D	1.42 (1.23, 1.63)	<0.0001	2.11 (1.74, 2.56)	<0.0001	-	

Family history of CD	1.96 (1.65, 2.34)	<0.0001	-		2.42 (2.00, 2.92)	<0.0001
Exclusive breastfeeding						
≤3 months	Reference		Reference		Reference	
>3 months	1.00 (0.89, 1.12)	0.98	0.94 (0.79, 1.13)	0.53	1.04 (0.90, 1.19)	0.62
Propensity score[a]	1.10 (1.06, 1.14)	<0.0001	1.12 (1.06, 1.20)	<0.001	1.08 (1.03, 1.13)	<0.01
Sex						
Female	Reference		Reference		Reference	
Male	0.78 (0.70, 0.85)	<0.0001	1.15 (0.99, 1.34)	0.07	0.63 (0.56, 0.70)	<0.0001
HLA Genotype						
DR4-DQ8/DR4-DQ8	Reference		Reference		Reference	
DR3-DQ2/DR4-DQ8	1.41 (1.22, 1.62)	<0.0001	1.30 (1.06, 1.60)	<0.05	1.39 (1.16, 1.66)	<0.001
DR3-DQ2/DR3-DQ2	2.24 (1.93, 2.60)	<0.0001	0.69 (0.53, 0.90)	<0.01	3.31 (2.77, 3.96)	<0.0001
other	0.64 (0.53, 0.77)	<0.0001	0.84 (0.66, 1.08)	0.17	0.38 (0.28, 0.50)	<0.0001
Family history of autoimmunity						
None	Reference					
Family history of T1D	1.40 (1.22, 1.62)	<0.0001	2.10 (1.74, 2.55)	<0.0001	-	
Family history of CD	1.95 (1.64, 2.32)	<0.0001	-		2.39 (1.98, 2.89)	<0.0001

Abbreviations: HR, hazard ratio; CI, confidence interval

[a] Propensity score on the 10-fold probability for each individuum to be breastfed for more than 6 months (any breastfeeding) or more than 3 months (exclusive breastfeeding), respectively, based on logistic regression derived estimates of the following variables: country, maternal diabetes, maternal age, first-born

child, maternal education, maternal smoking during pregnancy, maternal pre-pregnancy BMI, gestational weight gain, birthweight, caesarean section, and gestational age

Online Supplementary Table 4: Multivariate Cox proportional hazards model for autoimmunity and allergy outcomes and multivariate logistic regression analysis for obesity at 5.5 years of age according to **any breastfeeding >12 months** (Children, who developed the outcome during the first 12 months were excluded).

	Autoimmunity			**Islet autoantibodies**			**t-Transglutaminase autoantibodies**		
Any breastfeeding	**n**	**HR (95% CI)**	**p-value**	**n**	**HR [95% CI]**	**p-value**	**N**	**HR (95% CI)**	**p-value**
≤12 months	5,247	Reference		5,248	Reference		4,577	Reference	
>12 months	1,436	1.06 (0.95, 1.19)	0.31	1,436	0.99 (0.82, 1.21)	0.94	1,306	1.05 (0.92, 1.21)	0.46
Propensity score[a]	6,683	1.06 (1.03, 1.09)	<0.001	6,684	1.05 (1.00, 1.11)	<0.05	5,943	1.05 (1.02, 1.09)	<0.01
	Any Allergy			**Food allergy**			**Seasonal allergic rhinitis**		
Any breastfeeding	**n**	**HR (95% CI)**	**p-value**	**n**	**HR [95% CI]**	**p-value**	**N**	**HR (95% CI)**	**p-value**
≤12 months	5,009	Reference		5,139	Reference		5,290	Reference	
>12 months	1,371	0.99 (0.84, 1.17)	0.95	1,405	1.01 (0.78, 1.31)	0.92	1,455	0.91 (0.70, 1.19)	0.50
Propensity score[a]	6,380	0.95 (0.91, 0.99)	<0.01	6,544	1.00 (0.94, 1.06)	0.93	6,745	0.94 (0.89, 1.00)	0.05
	Obesity								
Any breastfeeding	**N**	**OR (95% CI)**	**p-value**						
≤12 months	3,741	Reference							
>12 months	1,174	1.00 (0.72, 1.37)	1.0						
Propensity score[a]	4,915	0.75 (0.70, 0.80)	<0.0001						

Abbreviations: HR, hazard ratio; OR, Odds ratio; CI, confidence interval

[a] Propensity score on the 10-fold probability for each individuum to be breastfed for more than 6 months (any breastfeeding) or more than 3 months (exclusive breastfeeding), respectively, based on logistic regression derived estimates of the following variables: country, maternal diabetes, maternal age, first-born child, maternal education, maternal smoking during pregnancy, maternal pre-pregnancy BMI, gestational weight gain, birthweight, caesarean section, and gestational age

Online Supplementary Table 5: Multivariate Cox proportional hazards model for the risk of autoimmunity (including islet and/or celiac disease associated autoimmunity), one or more islet autoantibodies and for tissue-transglutaminase autoantibodies according any breastfeeding duration after excluding nine children who developed one or more islet autoantibodies during the first 6 months.

	Autoimmunity			**Islet autoantibodies**			**t-Transglutaminase autoantibodies**		
Variables	**n**	**HR (95% CI)**	**p-value**	**n**	**HR (95% CI)**	**p-value**	**N**	**HR (95% CI)**	**p-value**
Any breastfeeding									
≤6 months	2,747	Reference		2,874	Reference		2,294	Reference	
>6 months	4,025	1.07 (0.96, 1.19)	0.20	4,025	1.04 (0.88, 1.23)	0.68	3,650	1.08 (0.95, 1.23)	0.25
Propensity score[a]	6,772	1.06 (1.03, 1.10)	<0.001	6,772	1.07 (1.02, 1.12)	<0.01	5,944	1.05 (1.01, 1.09)	<0.05

Abbreviations: HR, hazard ratio; CI, confidence interval

[a] Propensity score on the 10-fold probability for each individuum to be breastfed for more than 6 months (any breastfeeding) based on logistic regression derived estimates of the following variables: country, maternal diabetes, maternal age, first-born child, maternal education, maternal smoking during pregnancy, maternal pre-pregnancy BMI, gestational weight gain, birthweight, caesarean section, and gestational age

Online Supplementary Table 6: Multivariate Cox proportional hazards model for the risk of autoimmunity (including islet and/or celiac disease associated autoimmunity), one or more islet autoantibodies and for tissue-transglutaminase autoantibodies **during the first 24 months of age** according to any and exclusive breastfeeding duration.

	Autoimmunity			**Islet autoantibodies**			**t-Transglutaminase autoantibodies**		
Variables	**n**	**HR (95% CI)**	**p-value**	**n**	**HR [95% CI]**	**p-value**	**n**	**HR (95% CI)**	**p-value**
Any breastfeeding									
≤6 months	2,751	Reference		2,751	Reference		2,298	Reference	
>6 months	4,030	0.96 (0.78, 1.17)	0.67	4,030	0.94 (0.72, 1.24)	0.67	3,653	0.93 (0.69, 1.25)	0.63
Propensity score[a]	6,781	1.05 (0.99, 1.12)	0.08	6,781	1.11 (1.02, 1.20)	<0.05	5,951	0.98 (0.90, 1.07)	0.67
Exclusive breastfeeding									
≤3 months	5,169	Reference		5,169	Reference		4,484	Reference	
>3 months	1,641	0.98 (0.78, 1.22)	0.84	1,641	1.08 (0.81, 1.45)	0.61	1,484	0.87 (0.62, 1.21)	0.41
Propensity score[a]	6,810	1.15 (1.07, 1.24)	<0.001	6,774	1.14 (1.03, 1.27)	<0.05	5,968	1.13 (1.01, 1.27)	<0.05

Abbreviations: HR, hazard ratio; CI, confidence interval

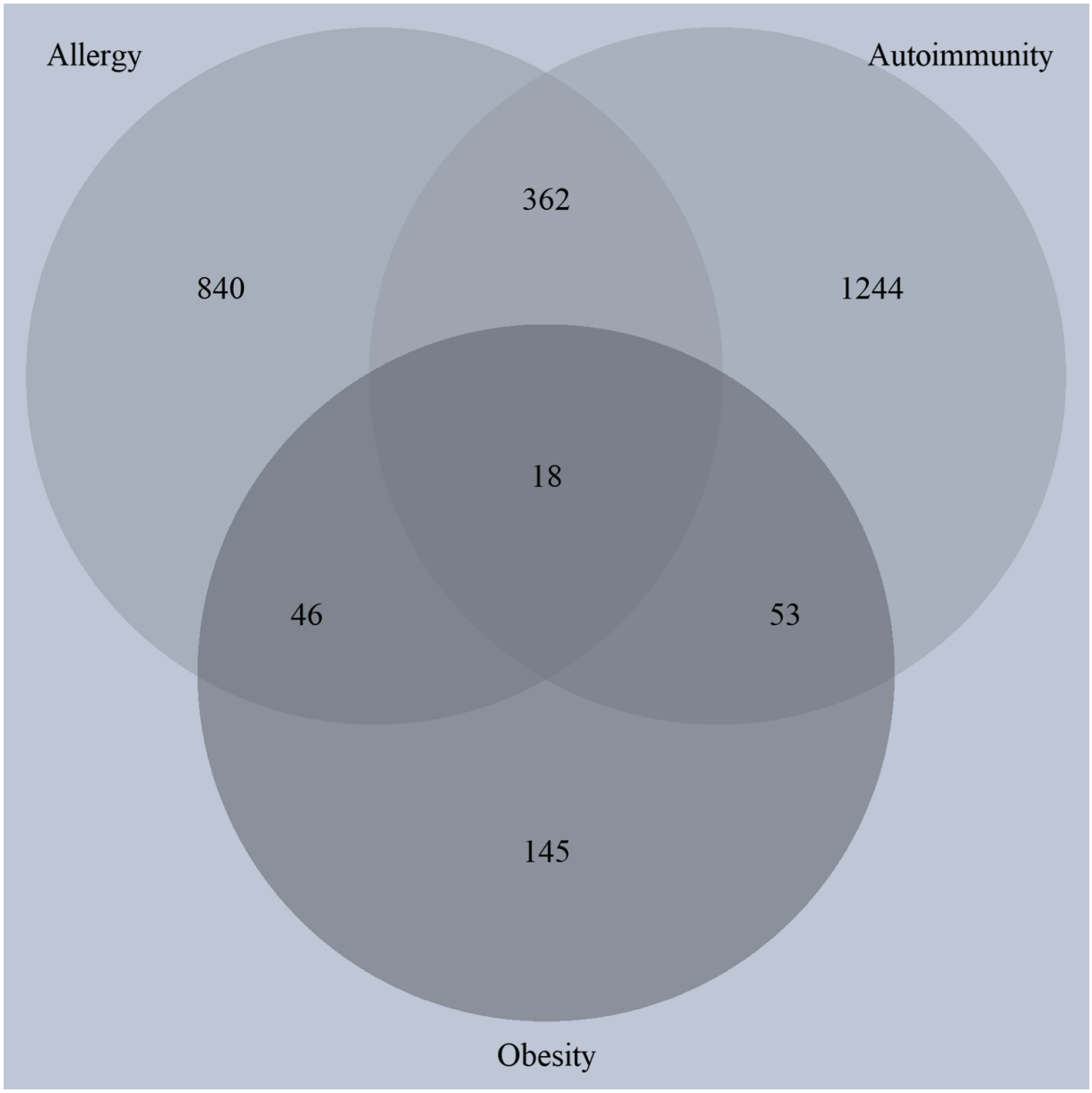
[a] Propensity score on the 10-fold probability for each individuum to be breastfed for more than 6 months (any breastfeeding) based on logistic regression derived estimates of the following variables: country, maternal diabetes, maternal age, first-born child, maternal education, maternal smoking during pregnancy, maternal pre-pregnancy BMI, gestational weight gain, birthweight, caesarean section, and gestational age

Online Supplementary Table 7: Multivariate Cox proportional hazards model for the risk of any allergy, any food allergy and seasonal allergic rhinitis according to any and exclusive breastfeeding duration after excluding children who developed any allergy during the first 3 months or 6 months respectively.

	Any Allergy			**Food allergy**			**Seasonal allergic rhinitis**		
Variables	**n**	**HR (95% CI)**	**p-value**	**n**	**HR [95% CI]**	**p-value**	**n**	**HR (95% CI)**	**p-value**
Any breastfeeding									
≤6 months	2,681	Reference		2,681	Reference		2,666	Reference	
>6 months	3,962	0.98 (0.86, 1.11)	0.72	3,962	1.00 (0.83, 1.22)	0.97	3,951	0.88 (0.70, 1.10)	0.25
Propensity score[a]	6,643	0.95 (0.92, 0.99)	<0.01	6,643	1.00 (0.95, 1.06)	0.98	6,643	0.93 (0.88, 1.00)	<0.05
Exclusive breastfeeding									
≤3 months	5,125	Reference		5,125	Reference		5,125	Reference	
>3 months	1,635	0.95 (0.83, 1.10)	0.52	1,635	1.03 (0.84, 1.26)	0.79	1,635	0.70 (0.53, 0.92)	<0.05
Propensity score[a]	6,760	0.87 (0.82, 0.91)	<0.0001	6,760	0.96 (0.89, 1.03)	0.22	6,760	0.84 (0.76, 0.92)	<0.001

Abbreviations: HR, hazard ratio; CI, confidence interval;

[a] Propensity score on the 10-fold probability for each individuum to be breastfed for more than 6 months (any breastfeeding) or more than 3 months (exclusive breastfeeding), respectively, based on logistic regression derived estimates of the following variables: country, maternal diabetes, maternal age, first-born child, maternal education, maternal smoking during pregnancy, maternal pre-pregnancy BMI, gestational weight gain, birthweight, caesarean section, and gestational age



Online Supplementary Figure 1. Numbers of children with autoimmunity, allergy, and/or obesity.

+

Online Supplementary Acknowledgement

The TEDDY Study Group

Colorado Clinical Center: Marian Rewers, M.D., Ph.D., PI[1,4,5,6,9,10], Aaron Barbour, Kimberly Bautista[11], Judith Baxter[8,911], Daniel Felipe-Morales, Kimberly Driscoll, Ph.D.[8], Brigitte I. Frohnert, M.D.[2,13], Marisa Stahl, M.D.[12], Patricia Gesualdo[2,6,11,13], Michelle Hoffman[11,12,13], Rachel Karban[11], Edwin Liu, M.D.[12], Jill Norris, Ph.D.[2,3,11], Stesha Peacock, Hanan Shorrosh, Andrea Steck, M.D.[3,13], Megan Stern, Erica Villegas[2], Kathleen Waugh[6,7,11]. University of Colorado, Anschutz Medical Campus, Barbara Davis Center for Childhood Diabetes.

Finland Clinical Center: Jorma Toppari, M.D., Ph.D., PI[¥^1,4,10,13], Olli G. Simell, M.D., Ph.D., Annika Adamsson, Ph.D.[^11], Sanna-Mari Aaltonen[^], Suvi Ahonen[*±§], Mari Åkerlund[*±§], Leena Hakola[*±], Anne Hekkala, M.D.[µ¤], Henna Holappa[µ¤], Heikki Hyöty, M.D., Ph.D.[*±6], Anni Ikonen[µ¤], Jorma Ilonen, M.D., Ph.D.[¥¶3], Sinikka Jäminki[*±], Sanna Jokipuu[^], Leena Karlsson[^], Jukka Kero M.D., Ph.D.[¥^3, 13], Miia Kähönen[µ¤11,13], Mikael Knip, M.D., Ph.D.[*±5], Minna-Liisa Koivikko[µ¤], Merja Koskinen[*±], Mirva Koreasalo[*±§2], Kalle Kurppa, M.D., Ph.D.[*±12], Jarita Kytölä[*±], Jutta Laiho, Ph.D.[*6], Tiina Latva-aho[µ¤], Laura Leppänen[^], Katri Lindfors, Ph.D.[*12], Maria Lönnrot, M.D., Ph.D.[*±6], Elina Mäntymäki[^], Markus Mattila[*±], Maija Miettinen[§2], Katja Multasuo[µ¤], Teija Mykkänen[µ¤], Tiina Niininen[±*11], Sari Niinistö[§2], Mia Nyblom[*±], Sami Oikarinen, Ph.D.[*±6], Paula Ollikainen[µ¤] , Zhian Othmani[¥], Sirpa Pohjola [µ¤], Jenna Rautanen[±§], Anne Riikonen[*±§2], Miia Pekkola[*±], Minna Romo[^], Satu Simell, M.D., Ph.D.[¥12], Aino Stenius[µ¤11], Päivi Tossavainen, M.D.[µ¤], Mari Vähä-Mäkilä[¥], Eeva Varjonen[^11], Riitta Veijola, M.D., Ph.D.[µ¤13], Irene Viinikangas[µ¤], Suvi M. Virtanen, M.D., Ph.D.[*±§2]. [¥]University of Turku, *Tampere University, [µ]University of Oulu, [^]Turku University Hospital, Hospital District of Southwest Finland, [±]Tampere University Hospital, [¤]Oulu University Hospital, §Finnish Institute for Health and Welfare, Finland, [¶]University of Kuopio.

Georgia/Florida Clinical Center: Jin-Xiong She, Ph.D., PI[1,3,4,10], Desmond Schatz, M.D.[*4,5,7,8], Diane Hopkins[11], Leigh Steed[11,12,13], Jennifer Bryant[11], Katherine Silvis[2], Michael Haller, M.D.[*13], Melissa Gardiner[11], Richard McIndoe, Ph.D., Ashok Sharma, Stephen W. Anderson, M.D.[^], Laura Jacobsen, M.D.[*13], John Marks, DHSc.[*11,13], P.D. Towe*. Center for Biotechnology and Genomic Medicine, Augusta University. *University of Florida, Pediatric Endocrinology. [^]Pediatric Endocrine Associates, Atlanta.

Germany Clinical Center: Anette G. Ziegler, M.D., PI[1,3,4,10], Ezio Bonifacio Ph.D.[*5], Cigdem Gezginci, Anja Heublein, Eva Hohoff[¥2], Sandra Hummel, Ph.D.[2], Annette Knopff[7], Charlotte Koch, Sibylle Koletzko, M.D.[¶12], Claudia Ramminger[11], Roswith Roth, Ph.D.[8], Jennifer Schmidt, Marlon Scholz, Joanna Stock[8,11,13], Katharina Warncke, M.D.[13], Lorena Wendel, Christiane Winkler, Ph.D.[2,11]. Forschergruppe Diabetes e.V. and Institute of Diabetes Research, Helmholtz Zentrum München, Forschergruppe Diabetes, and Klinikum

rechts der Isar, Technische Universität München. *Center for Regenerative Therapies, TU Dresden, ¶Dr. von Hauner Children's Hospital, Department of Gastroenterology, Ludwig Maximillians University Munich, ¥University of Bonn, Department of Nutritional Epidemiology.

Sweden Clinical Center: Åke Lernmark, Ph.D., PI[1,3,4,5,6,8,9,10], Daniel Agardh, M.D., Ph.D.[6,12], Carin Andrén Aronsson, Ph.D.[2,11,12], Maria Ask, Rasmus Bennet, Corrado Cilio, Ph.D., M.D.[5,6], Susanne Dahlberg, Emelie Ericson-Hallström, Annika Björne Fors, Lina Fransson, Thomas Gard, Monika Hansen, Susanne Hyberg, Fredrik Johansen, Berglind Jonsdottir, M.D., Ph.D.[11], Helena Elding Larsson, M.D., Ph.D.[6,13], Marielle Lindström, Markus Lundgren, M.D., Ph.D.[13], Marlena Maziarz, Ph.D., Maria Månsson Martinez, Jessica Melin[11], Zeliha Mestan, Caroline Nilsson, Kobra Rahmati, Anita Ramelius, Falastin Salami, Anette Sjöberg, Birgitta Sjöberg, Carina Törn, Ph.D.[3], Ulrika Ulvenhag, Terese Wiktorsson, Åsa Wimar[13]. Lund University.

Washington Clinical Center: William A. Hagopian, M.D., Ph.D., PI[1,3,4,5,6,7,10,12,13], Michael Killian[6,7,11,12], Claire Cowen Crouch[11,13], Jennifer Skidmore[2], Christian Chamberlain, Brelon Fairman, Arlene Meyer, Jocelyn Meyer, Denise Mulenga[11], Nole Powell, Jared Radtke, Shreya Roy, Davey Schmitt, Sarah Zink. Pacific Northwest Research Institute.

Pennsylvania Satellite Center: Dorothy Becker, M.D., Margaret Franciscus, MaryEllen Dalmagro-Elias Smith[2], Ashi Daftary, M.D., Mary Beth Klein, Chrystal Yates. Children's Hospital of Pittsburgh of UPMC.

Data Coordinating Center: Jeffrey P. Krischer, Ph.D.,PI[1,4,5,9,10], Rajesh Adusumali, Sarah Austin-Gonzalez, Maryouri Avendano, Sandra Baethke, Brant Burkhardt, Ph.D.[5,6], Martha Butterworth[2], Nicholas Cadigan, Joanna Clasen, Kevin Counts, Christopher Eberhard, Steven Fiske[8], Laura Gandolfo, Jennifer Garmeson, Veena Gowda, Belinda Hsiao, Christina Karges, Qian Li, Ph.D.[2,3], Shu Liu, Xiang Liu, Ph.D.[2,3,8,13], Kristian Lynch, Ph.D. [5,6,8], Jamie Malloy, Cristina McCarthy[11], Jose Moreno, Hemang Parikh, Ph.D.[3], Cassandra Remedios, Chris Shaffer, Susan Smith[11], Noah Sulman, Ph.D., Roy Tamura, Ph.D.[1,2,11,12,13], Dena Tewey, Michael Toth, Ulla Uusitalo, Ph.D.[2], Kendra Vehik, Ph.D.[4,5,6,8,13], Ponni Vijayakandipan, Melissa Wroble, Jimin Yang, Ph.D., R.D.[2], Kenneth Young, Ph.D. *Past staff: Michael Abbondondolo, Lori Ballard, Rasheedah Brown, David Cuthbertson, Stephen Dankyi, David Hadley, Ph.D., Kathleen Heyman, Francisco Perez Laras, Hye-Seung Lee, Ph.D., Colleen Maguire, Wendy McLeod, Aubrie Merrell, Steven Meulemans, Ryan Quigley, Laura Smith, Ph.D.[8,11].* University of South Florida.

Project scientist: Beena Akolkar, Ph.D.[1,3,4,5,6,7,9,10]. National Institutes of Diabetes and Digestive and Kidney Diseases.

Autoantibody Reference Laboratories: Liping Yu, M.D.^[5], Dongmei Miao, M.D.^, Polly Bingley, M.D., FRCP*[5], Alistair Williams*, Kyla Chandler*, Ilana Kelland*, Yassin Ben Khoud*, Huma Zahid*, Matthew Randell *. ^Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, *Bristol Medical School, University of Bristol, UK.

HLA Reference Laboratory: William Hagopian[3], MD, PhD, Christian Chamberlain, Jared Radtke, Sarah Zink. Pacific Northwest Research Institute, Seattle WA. (Previously Henry Erlich, Ph.D.[3], Steven J. Mack, Ph.D., Anna Lisa Fear. Center for Genetics, Children's Hospital Oakland Research Institute.)

Repository: Sandra Ke, Niveen Mulholland, Ph.D. NIDDK Biosample Repository at Fisher BioServices.

Other contributors: Thomas Briese, Ph.D.[6], Columbia University. Suzanne Bennett Johnson, Ph.D.[8,11], Florida State University. Eric Triplett, Ph.D.[6], University of Florida.

Committees:

[1]Ancillary Studies, [2]Diet, [3]Genetics, [4]Human Subjects/Publicity/Publications, [5]Immune Markers, [6]Infectious Agents, [7]Laboratory Implementation, [8]Psychosocial, [9]Quality Assurance, [10]Steering, [11]Study Coordinators, [12]Celiac Disease, [13]Clinical I