Food consumption and advanced β cell autoimmunity in young children with *HLA*-conferred susceptibility to type 1 diabetes: a nested case-control design[1–4]

Suvi M Virtanen, Jaakko Nevalainen, Carina Kronberg-Kippilä, Suvi Ahonen, Heli Tapanainen, Liisa Uusitalo, Hanna-Mari Takkinen, Sari Niinistö, Marja-Leena Ovaskainen, Michael G Kenward, Riitta Veijola, Jorma Ilonen, Olli Simell, and Mikael Knip

ABSTRACT

Background: Evidence for the role of food consumption during childhood in the development of β cell autoimmunity is scarce and fragmentary.

Objective: We set out to study the associations of longitudinal food consumption in children with the development of advanced β cell autoimmunity.

Design: Children with advanced β cell autoimmunity (n = 232) (ie, with repeated positivity for antibodies against islet cells) together with positivity for at least one of the other 3 antibodies analyzed or clinical type 1 diabetes were identified from a prospective birth cohort of 6069 infants with *HLA-DQB1*–conferred susceptibility to type 1 diabetes who were born in 1996–2004, with the longest follow-up to the age of 11 y. Repeated 3-d food records were completed by the families and daycare personnel. Diabetes-associated autoantibodies and diets were measured at 3–12-mo intervals. Four control subjects, who were matched for birth date, sex, area, and genetic risk, were randomly selected for each case.

Results: In the main food groups, only intakes of cow-milk products (OR: 1.05; 95% CI: 1.00, 1.10) and fruit and berry juices (OR: 1.09; 95% CI: 1.02, 1.12) were significantly, although marginally, associated with advanced β cell autoimmunity. The consumption of fresh milk products and cow milk–based infant formulas was related to the endpoint, whereas no evidence was shown for consumption of sour milk products and cheese. The intake of fat from all milk products and protein from fresh milk products was associated with risk of advanced β cell autoimmunity.

Conclusion: Intakes of cow milk and fruit and berry juices could be related to the development of advanced β cell autoimmunity. This trial was registered at clinicaltrials.gov as number NCT00223613. *Am J Clin Nutr* 2012;95:471–8.

INTRODUCTION

The incidence of type 1 diabetes continues to increase, especially in young children in high- and middle-income countries (1, 2). For example, in Finland, greater than a linear increase in the incidence rate in children $\leq$4 y old has been observed during the past decade (1). The environmental factors responsible for this continuous increase in the disease incidence remain to be elucidated. For example, one may ask whether the ways the gut immune system reacts to infections, foods, or toxins have changed or whether novel or altered exposures or their combinations play a role.

Evidence for a putative role of food consumption during childhood in the development of β cell autoimmunity and clinical type 1 diabetes is scarce and comes mainly from hypothesis-generating studies. The mean cow-milk consumption and mean intake of energy from animal products of the populations were directly, and energy intakes from vegetables were inversely, associated with their incidences of type 1 diabetes in ecologic comparisons (3, 4). In adolescent populations, the mean consumption of dietary fats, fruit, and vegetables was directly

[1] From the Unit of Nutrition, Department of Lifestyle and Participation, National Institute for Health and Welfare, Helsinki, Finland (SMV, CK-K, HT, LU, SN, and M-LO); the School of Health Sciences, University of Tampere, Tampere, Finland (SMV, SA, LU, H-MT); the Science Center of Pirkanmaa Hospital District, Tampere, Finland (SMV, LU, and MK); Statistics, Department of Social Research (JN) and the Departments of Virology (JI) and Pediatrics (JI and OS), University of Turku, Turku, Finland; the Department of Epidemiology and Population Health, Medical Statistics Unit, London School of Hygiene and Tropical Medicine, London, United Kingdom (MGK); the Department of Pediatrics, University of Oulu, Oulu, Finland (RV); the Department of Virology, University of Eastern Finland, Kuopio, Finland (JI); the Department of Pediatrics, Tampere University Hospital, Tampere, Finland (MK); the Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland (MK); and the Folkhälsan Research Center, Helsinki, Finland (MK).

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[4] Address correspondence to SM Virtanen, Unit of Nutrition, Department of Lifestyle and Participation, National Institute for Health and Welfare, Helsinki, Finland, Mannerheimintie 166, 00300 Helsinki, Finland. E-mail: suvi.virtanen@thl.fi.

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associated with the incidence of type 1 diabetes in the respective populations (5). Cow milk, wheat, and soy have inconsistently increased, and hydrolyzed proteins have decreased, the rate of diabetes in diabetes-prone animals used as models for immune-mediated diabetes (6). Roots, potatoes, or milk contaminated by microbes that produce toxins destructive to β cells could increase risk of autoimmune diabetes (7, 8).

Most of the studies in humans have focused on infant feeding, especially on the duration of breastfeeding and the age at introduction of certain foods (9, 10). Findings as to whether breastfeeding would protect from, or the early introduction of cow milk, cereals, fruit, or roots would contribute to, pre– or clinical type 1 diabetes are inconclusive (10).

In case-control settings, cow milk (11), sugar and bread (12), and soft drink and egg (13) consumption were linked to increased risk of type 1 diabetes as were intakes of foods that are important sources of *N*-nitroso compounds and nitrite (mainly meat products) (14, 15). In a prospective cohort of initially healthy siblings of children with type 1 diabetes, a high cow-milk intake was related to the development of clinical and preclinical type 1 diabetes (16, 17). In a case-control study nested within a prospective cohort of individuals with increased genetic susceptibility to type 1 diabetes, several fatty acids that are biomarkers of milk intake were directly but weakly linked to advanced β cell autoimmunity at or close to the time of seroconversion (18). In another cohort of high-risk individuals, n−3 fatty acid intake and status were inversely related to early β cell autoimmunity (19). Pilot findings of a randomized, double-blind trial suggest that it may be possible to manipulate spontaneous β cell autoimmunity by substituting conventional cow milk–based infant formula with a hydrolyzed one during the first 6–8 mo of life (20).

We set out to assess prospectively whether longitudinal food intakes during childhood, especially the intake of cow milk–based infant formulas, other cow-milk products, cereals, meat products, fish products, dietary fats, vegetables, roots and potatoes, fruit, and sweets, are associated with risk of emergence of diabetes-associated autoantibodies in a population-based cohort of young children with *HLA-DQB1*–conferred susceptibility to type 1 diabetes.

SUBJECTS AND METHODS

Subjects

In the DIPP[5] study, which is a prospective population-based cohort study (21), newborn infants from the areas of 3 university hospitals in Finland are screened for *HLA-DQB1*–conferred susceptibility to type 1 diabetes by using cord blood samples. Infants who carry increased genetic susceptibility [*HLA-DQB1*02/0302* heterozygous and *DQB1*0302*/x-positive subjects (x stands for homozygosity or a neutral allele)] are being monitored for diabetes-associated autoantibodies, growth, and viral infections at 3–12-mo intervals. Families were offered the opportunity to take part in a randomized, double-blinded intervention trial with intranasal insulin when their child tested repeatedly positive for $\geq$2 autoantibodies (22). The intervention with intranasal insulin had no effect on the progression rate to clinical type 1 diabetes. Procedures were approved by the local ethics committees.

The DIPP Nutrition Study falls within the framework of the DIPP study (23). The present analysis comprised a case-control series selected from at-risk children born between 2 September 1996 and 31 August 2004 at Oulu University Hospital and between 20 October 1997 and 5 September 2004 at Tampere University Hospital (n = 6069; 78% of the children who were invited), with the longest follow-up to the age of 11 y. Cases were defined as children who were repeatedly positive for ICAs and at least one other diabetes-associated autoantibody out of IAAs), antibodies to the 65-kD isoform of GADAs, and IA-2As or children with type 1 diabetes. Cases came from the entire DIPP Nutrition cohort, including twins and other multiple births as well as additional index siblings (ie, younger eligible siblings who were born during the recruitment period). First, all possible control subjects who fulfilled the matching criteria were selected from the entire DIPP Nutrition cohort, including twins and other multiple births as well as index siblings. The matching criteria included a birth date within 3 mo, the same sex, the same research area (Oulu or Tampere district), and the same genotype (high or moderate risk). In addition, control subjects could not come from the same family as cases. Control children must have been observed at the visit when the case turned out to be a case, and control subjects had to be seronegative and without diabetes up to that point. From the series of all possible control children who fulfilled the matching criteria, 4 control children were randomly selected for each case.

Genetic methods

HLA-DQB1 alleles were analyzed as described previously (24). In brief, a part of the second exon of the *HLA-DQB1* gene was amplified by using a primer pair with a biotinylated 3′ primer. Biotinylated PCR products were transferred to streptavidin-coated microtitration plates, denatured, and hybridized with sequence-specific probes labeled with lanthanide chelates: europium, terbium, or samarium. Two hybridization mixtures were used; one mixture contained probes that hybridized with *DQB1*0602* and **0603*, *DQB1*0603* and **0604*, and a consensus sequence, and the other mixture contained probes that were specific to the *DQB1*02*, **0301*, and **0302* alleles. After appropriate incubations and washings, the specific hybridization products were detected by using 3-color time-resolved fluorescence of the lanthanide chelates.

Immunological methods

Of the 4 type 1 diabetes–associated autoantibodies analyzed, ICAs were used as the primary screening tool for β cell autoimmunity. When a child seroconverted to positivity for ICA for the first time, all of the child's preceding (starting from birth) and subsequent samples were analyzed for IAA, GADA, and IA-2A. ICAs were quantified by a standard indirect immunofluorescence method, IAAs were quantified with a microassay, and GADA and IA-2A were quantified with specific radiobinding assays as described previously (23). ZnT8A were analyzed with

[5]Abbreviations used: DIPP, Type 1 Diabetes Prediction and Prevention; GADA, antibodies to the 65-kD isoform of glutamic acid decarboxylase; IAA, insulin autoantibody; IA-2A, antibodies to islet antigen 2; ICA, islet cell antibody; ZnT8A, zinc transporter 8 autoantibodies.

a radiobinding assay modified from that described by Wenzlau et al (25) by using the 4.1 chimeric recombinant plasmid that encodes the COOH-terminal part (amino acids 268–369) of both the ZnT8 amino acid 325 arginine allele and the ZnT8 amino acid 325 tryptophan allele (a gift from J Hutton, University of Colorado Denver). The disease sensitivity of this assay was 60%, and the disease specificity was 100%, in accordance with the 2010 Diabetes Autoantibody Standardization Program. The cutoff for positivity was set at 0.61 relative units, which corresponded to the 99th percentile in 250 nondiabetic control subjects. Transplacentally transferred autoantibodies were excluded from the analyses.

In the 6069 children with genetic risk of type 1 diabetes, 601 children (9.9%) were at least twice positive for ICA, and 222 children (3.7%) were repeatedly positive for ICA plus at least one other antibody. In the latter group, 175 children tested at least twice positive for IAA, 159 children tested positive for GADA, and 146 children tested positive for IA-2A. Of the children, 135 children (2.2%) had progressed to clinical type 1 diabetes at a median age of 4.4 y (range: 1.0–10.5 y of age). In these children, 92 children had been repeatedly positive for ICA plus at least one other autoantibody before developing diabetes. However, 17 of the remaining 43 children had or had had one or more autoantibodies in one single sample before or at the time of diagnosis, and no blood samples were available for 14 children. For the 12 persistently seronegative children, the median time since the last blood sample drawn was 4.7 y (range: 0.3–10.2 y) before diagnosis. Therefore, we decided to include clinical type 1 diabetes in the autoantibody endpoint. This inclusion resulted in 265 children (4.4% of all children) who were positive for the ICA plus at least one other antibody (including type 1 diabetes) endpoint (ie, with advanced β cell autoimmunity; this term is used throughout the text for the endpoint). None of the children were randomly assigned for the intranasal insulin trial before testing repeatedly positive for ICA plus at least one other autoantibody. Likewise, the 43 children who were not repeatedly positive for ICA plus at least one other autoantibody, but who developed diabetes, were not randomly assigned for the intranasal insulin trial. Of the 265 children with advanced β cell autoimmunity, 33 children did not have any food records available, which resulted in 232 cases with food-record data. Of these cases, 10 cases seroconverted to positivity for the advanced β cell autoimmunity endpoint by 1 y, 75 cases seroconverted to positivity for the advanced β cell autoimmunity endpoint by 2 y, 123 cases seroconverted to positivity for the advanced β cell autoimmunity endpoint by 3 y, 190 cases seroconverted to positivity for the advanced β cell autoimmunity endpoint by 5 y, and all 232 cases seroconverted to positivity for the advanced β cell autoimmunity endpoint by 10 y of age.

Dietary methods

Information on diet was collected by means of a 3-d food record at the age of 3 and 6 mo and at 1, 2, 3, 4, and 6 y. In the current analysis data from 232 case and 926 control children were available. The dietary (ie, food-record) data of both cases and control subjects were used up to the time when the endpoint of advanced β cell autoimmunity was reached for the first time. Three-day food records were used for 214 case and 799 control children at 3 mo, 193 case and 792 control children at 6 mo, 171 case and 761 control children at 1 y, 94 and 429 control children at 2 y, 58 case and 285 control children at 3 y, 37 case and 161 control children at 4 y, and 7 case and 46 control children at 6 y of age, respectively.

Research nutritionists visited the research clinics 4 times annually for training and motivation of the research staff. In addition, each new research nurse or doctor received individual training. In the standardized training, the following examples of issues were emphasized: aims of the study and importance of food-data collection; knowledge of food items and new foods on the market; the type of foods eaten, whether homemade, commercial, or from a restaurant; knowledge of the composition of foods and dishes (brand names, recipes, and preparation and processing methods); knowledge of dietary supplements and foods enriched with nutrients (brand names); ability to check amounts of foods and drinks; what information was or was not necessary in the recording; practice in checking of food records (eg, by real examples); and why it was important to settle recording dates beforehand. The research personnel were informed about the progress of the study and about childhood nutrition overall. Each research nurse had a manual of operations for the nutrition study that contained detailed information on the conduct of the study at each visit, methods used, and all study material. The research nurses gave advice to the families before recording how to complete food records and checked the food records during the respective visits. Separate food records were given for day care personnel. The type of day care, source of food, and contact information was inquired, and the menus and recipes were asked in detail. Families were asked to check the food records completed at the day care with the day care personnel. When necessary, research nurses or nutritionists called the day care personnel or the kitchen of the day care. Families and day care personnel received written advice, including an example of a 1-d food record. Families and day care personnel were also asked to record eating times and locations. In the advice for families, emphasis was put on the frequency and completeness of meals and snacks, amounts of foods and drinks, types and amounts of dietary fats used in food preparation, in salads, and on bread, types of milks and milk products used, and types of breads used. Families were encouraged not to change the children's eating because of the food recording. Probing was used to get information on between-meal snacks, juices, sweets, dietary supplements, and other easily forgettable foods. Brand names were asked for all commercial infant foods, infant formulas, and dietary supplements. Continuously updated food-product brochures (eg, of baby foods, infant formulas, dietary fats, milks and milk products, breads, cereals, and functional foods) and a list of dietary supplements available helped in the identification of foods and dietary supplements at study centers. A booklet of food portion sizes, household measures, and food product brochures were used for the estimation of amounts of foods used.

An annually updated national food database was also used (26). The food database and connected software enabled the summarization of the intake of each food from different food items (eg, that of milk from different milk products and foods that contained milk). An infant-food database for commercial baby foods and infant formulas was developed and regularly updated for the current study. The database included all the commercial baby foods and infant formulas available in Finland, which meant that all the intakes of these foods were coded by using specific codes.

In the current analyses, the absolute daily consumption of the following main food groups were used: cow-milk products (which included cow milk–based infant formulas, fresh milk products, sour milk products, and cheese); gluten-containing cereals (rye, wheat, and barley); other cereals (oats, rice, buckwheat, corn flour, and millets); meat and meat products (unprocessed and processed meat); fish and fish products; eggs; dietary fats (vegetable oils, margarines, butter, and butter spreads); vegetables; roots and potatoes; fruit and berries; fruit and berry juices; and sweets and sugar. Among milk products, the variables cow milk–based infant formulas, all fresh milk and all sour milk products, cheese and protein from fresh milk products, and fat from all milk products were used. Protein amounts were calculated from fresh milk products because the protein composition in, eg, cheese is different from that in fresh milk products. Protein in cheese is more in the form of amino acids and small peptides. The composition of fatty acids does not change during food processing, and therefore, fat from all milk products was calculated. We also calculated the sum of all liquid foods (beverages and liquid milk products).

Sociodemographic and perinatal characteristics

Information on child sex, maternal education, and diabetes status of the first-degree relatives was collected by a structured questionnaire completed by parents after the delivery (**Table 1**). In the current study cohort, vocational education was more closely associated with dietary factors than was general education, and we chose to use maternal vocational education as the adjusting factor. Vocational education leads to an occupation and is received mainly after general education (comprehensive school and upper secondary general education). Information on diabetes status was updated from medical registries. Of case children, 29 children (12.5%) had a first-degree relative with diabetes at the time of the study (ie, 17 children had a father, 10 children had a mother, and 5 children had a sibling with diabetes). Of case children. 3 children had several first-degree family members with diabetes. Of control children, 44 children (5.5%) had a first-degree relative with diabetes (ie, 20 children had a father, 23 children had a mother, and 2 children had a sibling with diabetes). In control children, one child had several first-degree family members with diabetes. Information on perinatal characteristics was received from the Medical Birth Registries of the Oulu and Tampere University Hospitals. Absence of maternal vocational education, high-risk *HLA* genotype, familial diabetes, and low gestational age were associated with advanced β cell autoimmunity in the total cohort (27).

TABLE 1
Baseline characteristics of participating case and control children with food-record data

Characteristic	Cases (n = 232)	Control subjects (n = 803)[1]
Sex of the child [n (%)]		
Boys	143 (61.6)	497 (61.9)
Girls	89 (38.4)	306 (38.1)
HLA-DQB1–conferred risk [n (%)]		
High (*DQB1*02/*0302*)	162 (69.8)	569 (70.9)
Moderate (*DQB1*0302/x*)[2]	70 (30.2)	234 (29.1)
Familial diabetes [n (%)]		
Yes	29 (12.5)	44 (5.5)
No	198 (85.3)	732 (91.2)
Missing information	5 (2.2)	27 (3.4)
Maternal vocational education [n (%)][3]		
Academic	57 (24.6)	178 (22.2)
Upper secondary vocational education	84 (36.2)	361 (45.0)
Vocational school or course	59 (25.4)	210 (26.2)
None	25 (10.8)	35 (4.4)
Missing information	7 (3.0)	19 (2.4)

[1] A total of 102 children served as control subjects for more than one case but were included only once in the total number of control subjects. Of control subjects, 11 became a case at a later date and were counted only as cases.

[2] *x* not equal to *02, *0301, or *0602.

[3] Education that leads to an occupation and mainly received after general education (comprehensive school and upper secondary general education).

Statistical methods

The estimation of risk was based on a nested case-control design set up within the cohort. In a nested case-control design, each case is associated with k randomly selected matched control subjects (in the current study, k = 4). Control subjects were randomly sampled without replacement from the set of individuals who fulfill the matching criteria and were disease-free at the time when the case was diagnosed. One way to view this design is to regard time as one of the matching criteria. To eliminate the potential effects of confounding factors, matched control subjects were used.

The standard procedure for estimation in nested case-control designs is to use conditional likelihood logistic regression analysis (28, 29). However, in our setting, exposure variables were measured repeatedly at different ages before the onset of disease. Measurements of the same individual tend to be correlated, and this needed to be accounted for. To make full and proper use of this design, we used a generalized estimating equation framework to estimate regression coefficients (30). With the sandwich estimator of variance, this approach results in consistent estimates of regression coefficients, valid SEs, and improved precision in terms of narrower CIs than does an approach that uses only one measurement per individual. A complete description of the method and a simulation study used to validate it in the current framework is available on request. Possibilities of age-dependent effects according to age were investigated by appropriate interaction tests.

Analyses were conducted on all observed data and, to reduce potential bias associated with missing explanatory variables and to allow individuals with incomplete sets of explanatory variables to be included, on multiply imputed data (31). In the use of multiple imputation, we assumed that the missingness mechanism was missing at random, although small departures from this were unlikely to be critical. The process of generating plausible values was based on the sequential imputation regression method and software (32). As many sources of variation were included in the imputation models as computationally possible. These sources were within-individual dietary data, which included breastfeeding (no or yes); the outcome (for case or control subject) and its possible interaction with event time; the matching criteria; birth date as a continuous variable as well as the birth month as a categorical variable to account for temporal variation over the period of recruitment and seasonal differences; diabetes

of the mother and father; and maternal age and vocational education of the mother (no or yes). Details of the method for the current study design were provided previously (33). Ten sets of imputed missing values were generated after repeating 50 within-time iterations twice over the follow-up time. Variable estimates and their SEs were calculated according to Rubin's rules (34). All dietary variables were log(x + 1) transformed before the analysis. SAS software (version 9.2; SAS Institute) was used in the analyses.

RESULTS

The consumption of fresh milk products increased by age, whereas cow milk–based infant formulas were mainly consumed to the age of ≤1 y (**Figure 1**). Amounts of sour milk products and cheeses consumed by children at any age were relatively small (Figure 1). (*See* supplemental Figure 1 under "Supplemental data" in the online issue for a presentation of the consumption of different cow-milk products of cases and control subjects by age and supplemental Figures 2A and 2B for those of other main food groups.)

In the main food groups, only the intake of cow-milk products and that of fruit and berry juices was significantly associated with advanced β cell autoimmunity (**Table 2**). A more detailed analysis of cow-milk products revealed that both the consumption of fresh milk products and the consumption of cow milk–based infant formulas were related to the endpoint (**Table 3**). Intakes of fat from cow-milk products and protein from fresh milk products were both associated with risk of advanced β cell autoimmunity, whereas intakes of sour milk products and cheese were not associated with risk of advanced β cell autoimmunity (Table 3). Estimates for total milk products or those of any of the subgroups did not change when adjusted for the total amount of beverages and liquid milk products, and adjustment for vitamin D or n−3 fatty acid intake did not change the results [eg, for total milk products, ORs (95% CIs) adjusted for vitamin D and n−3 fatty acid intakes were 1.05 (1.00, 1.11; $P = 0.049$) and 1.06 (1.01, 1.12; $P = 0.026$), respectively. Mean (±SD) vitamin D and n−3 fatty acid intakes from foods (others than breast milk) and supplements together were 10.6 ±4.6 μg and 236 ± 354 mg at 3 mo, 12.0 ± 4.5 μg and 546 ± 401 mg at 6 mo, 11.1± 4.5 μg and 853 ± 439 mg at 1 y, 7.3 ± 4.8 μg and 1032 ± 504 mg at 2 y, 5.2 ± 4.3 μg and 1203 ± 518 mg at 3 y,

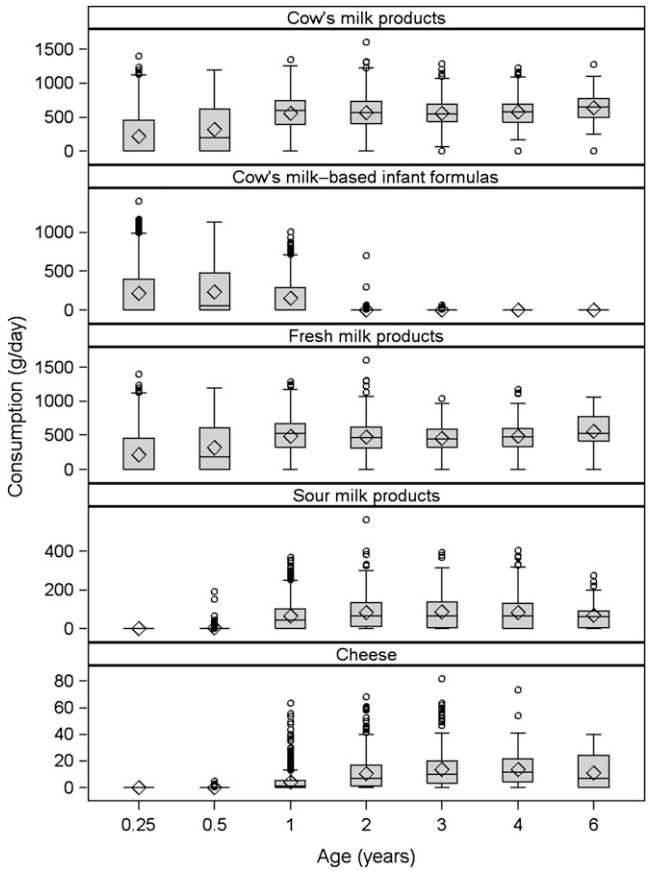

FIGURE 1. Consumption of total cow-milk products, cow milk–based infant formulas, fresh milk products, sour milk products, and cheese by age at 3 ($n = 1013$) and 6 ($n = 985$) mo and 1 ($n = 932$), 2 ($n = 523$), 3 ($n = 401$), 4 ($n = 198$), and 6 y ($n = 53$) y. Diamonds represent mean values, central bars in box plots represent medians, boxes represent IQRs, whiskers represent smallest and largest nonoutlier values, and circles represent outliers (ie, observations that lie >1.5× the IQR lower than the first quartile or 1.5× the IQR higher than the third quartile.

TABLE 2
Risk of advanced β cell autoimmunity associated with the amount of foods consumed longitudinally until the development of advanced β cell autoimmunity[1]

Food group (g)	Values	*P*
Cow-milk products	1.05 (1.00, 1.10)	0.032
Rye, wheat, and barley products	1.07 (0.94, 1.20)	0.305
Other cereals[2]	0.98 (0.88, 1.09)	0.436
Meat and meat products	0.97 (0.87, 1.09)	0.629
Fish and fish products	0.96 (0.87, 1.06)	0.381
Eggs	1.03 (0.90, 1.20)	0.650
Dietary fats	1.08 (0.91, 1.29)	0.361
Vegetables	1.00 (0.90, 1.11)	0.977
Roots and potatoes	1.02 (0.93, 1.12)	0.631
Fruit and berries	1.04 (0.94, 1.14)	0.503
Fruit and berry juices	1.09 (1.02, 1.17)	0.015
Sweets and sugar	1.00 (0.87, 1.14)	0.959
Beverages and liquid milk products	1.05 (0.99, 1.12)	0.078

[1] All values are ORs; 95% CIs in parentheses. Data from 232 case and 926 control children were used in the analysis. A generalized estimating equation framework with the sandwich estimator of variance was used to estimate the regression coefficients.

[2] Other cereals included oats, rice, buckwheat, corn flour, and millets.

TABLE 3
Risk of advanced β cell autoimmunity associated with the amount of cow-milk products consumed longitudinally until the development of advanced β cell autoimmunity[1]

Food (g)	Values	*P*
Cow-milk products	1.05 (1.00, 1.10)	0.032
Cow milk–based infant formulas	1.05 (1.01, 1.09)	0.017
Fresh milk products	1.05 (1.00, 1.10)	0.037
Sour milk products	1.03 (0.97, 1.09)	0.340
Cheese	0.99 (0.87, 1.12)	0.839
Protein from fresh milk products	1.13 (1.00, 1.26)	0.042
Fat from all milk products	1.11 (1.01, 1.22)	0.028

[1] All values are ORs; 95% CIs in parentheses. Data from 232 case and 926 control children were used in the analysis. A generalized estimating equation framework with the sandwich estimator of variance was used to estimate regression coefficients.

4.5 ± 3.6 μg and 1336 ± 572 mg at 4 y, and 5.7 ± 3.7 μg and 1545 ± 650 mg at 6 y, respectively. Likewise, adjustment for gestational age (first quarter compared with other quarters), familial diabetes, or maternal vocational education did not change the results (data not shown). When the intake of fruit and berry juices was adjusted for the total amount of beverages and liquid milk products, it was no longer significantly associated with the endpoint ($P = 0.100$). Log ORs for cow-milk products, fat from all milk products, and protein from fresh milk products at different ages and the overall estimate are presented in **Figure 2**.

In addition, we assessed how the estimates for the amount of fresh milk products from observed data and multiple imputation were related to advanced β cell autoimmunity. Because both assessment methods gave very similar estimates of ORs, we believed that missing observations did not cause substantial bias or loss of precision to the analysis. Thus, only results for complete cases are presented. Also, we performed 2 types of additional analyses (*see* supplemental tables under "Supplemental data" in the online issue) by *1*) excluding children with ICA and GADA repeatedly positive as the endpoint and *2*) adding ZnT8A results that were not yet available from all children and having repeated positivity to 2 biochemical autoantibodies (out of IAA, GADA, IA-2, and ZnT8A) in addition to ICA as the endpoint. In both of these analyses, the number of endpoints decreased somewhat (from 232 to 210 and 184, respectively), which decreased the statistical power. The results in terms of ORs remained practically the same, although the findings were less significant.

DISCUSSION

Our findings from the current nested case-control analysis of the largest prospective cohort series thus far reported suggested that the intake of cow-milk products is weakly associated with signs of advanced β cell autoimmunity in children with increased *HLA*-conferred genetic susceptibility to type 1 diabetes. Also the intake of fruit and berry juices was directly but marginally associated with the autoimmunity endpoint.

The major virtues of the current study were a well-defined study population, a high participation rate, and the use of an endpoint that reflects advanced β cell autoimmunity. In most cases, positivity for a single autoantibody specificity represents harmless nonprogressive β cell autoimmunity, whereas the presence of ≥2 autoantibodies usually reflects a progressive process that only rarely reverts (35). In our study, the collection of dietary data before the development of the autoantibody endpoint excluded the possibility of a differential bias in the selection of subjects and in the reporting of dietary habits.

The major challenge of the current study was the measurement of the diets of children as accurately as possible and to exclude biases in the measurement of food consumption as much as possible. To minimize reporting errors because of the recording process, we gave the recording dates for the families and day cares in advance and stressed that keeping the record should not influence what the child eats or drinks. Research nurses and doctors who advised the families and checked food records in study centers as well as nutritionists who entered the dietary data and called families, day cares, and study centers received continuous training and motivation. Also, all of the training material was continuously updated. The day-to-day variation in intakes of foods and nutrients increases by age (36), which makes mean intakes are less representative at the individual level as the child gets older.

The current observation of a direct association between the child's longitudinal cow-milk intake and development of advanced β cell autoimmunity is in accordance with several previous case-control and cohort findings with endpoints that varied from early pre–type 1 diabetes to clinical disease (11, 16, 17,

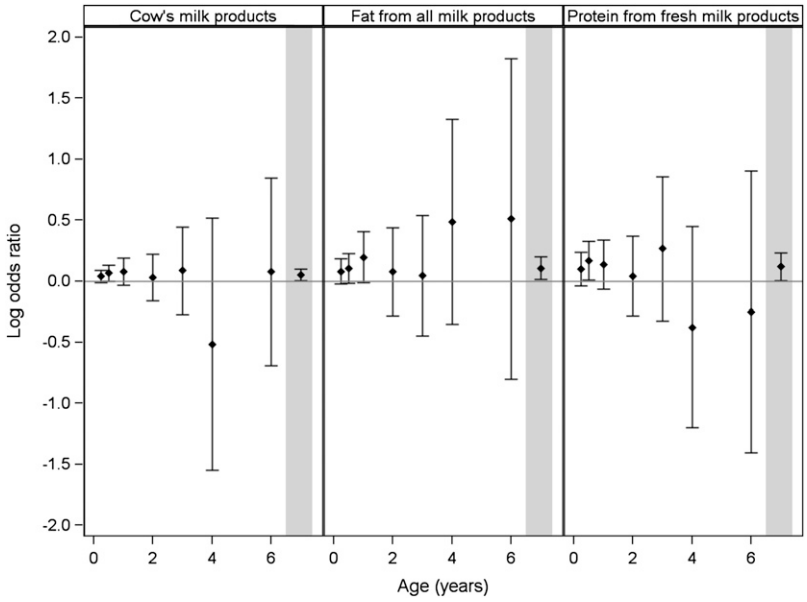

FIGURE 2. Log ORs and CIs for cow-milk products, fat from all milk products, and protein from fresh milk products at different ages and the overall estimate (highlighted with the gray background). Conditional likelihood logistic regression was used to estimate regression coefficients at each time, and the overall estimate was based on analysis of generalized estimating equations. The respective numbers of case and control children were 214 and 799 children at 3 mo, 193 and 792 children at 6 mo, 171 and 761 children at 1 y, 94 and 429 children at 2 y, 58 and 285 children at 3 y, 37 and 161 children at 4 y, and 7 and 46 children at 6 y.

37). In 2 case-control studies, cow-milk intake was inversely related to risk of type 1 diabetes (14, 38). Observations from the pilot study of the first nutritional primary prevention trial for type 1 diabetes showed that the development of signs of β cell autoimmunity can be delayed by giving, whenever breast milk was not available, hydrolyzed formula instead of a regular cow milk–based one during the first 6–8 mo of life in children with at least one family member affected by type 1 diabetes in addition to a risk-conferring *HLA* genotype (20). Furthermore, we recently observed that such serum fatty acids, which act as biomarkers of consumption of milk and ruminant meat fat were directly, although marginally, associated with advanced pre–type 1 diabetes at or before the time of seroconversion (18). There are conflicting findings as to whether a very early introduction of cow milk would increase risk of preclinical or clinical type 1 disease and whether breast milk would be protective (10). In a population-based cohort with small a number of disease endpoints, Savilahti and Saarinen (39) reported that very early exposure to cow milk–based infant formula was inversely associated with risk of clinical type 1 diabetes during the first 7 y of life .

It is possible that the observed association between cow-milk intake and advanced β cell autoimmunity reflected a causal relation. The current findings did not clarify whether protein or fat or some other component in cow milk could explain this association. Sour milk products and cheese, which were not associated with advanced β cell autoimmunity in the current analyses, do differ in their composition from fresh milk products. Proteins in sour milk products are partly split to smaller peptides. Sour milk products may contain probiotic microbes. In hard cheese, which was the main cheese type in the diet of children in the current study, the proteins are, to a large extent, split to small peptides and amino acids. However, the amounts of sour milk products and cheese consumed were very small in the current study, which also may have hampered our ability to find associations.

There is increasing evidence that the gut-associated lymphoid tissue is involved in the development of type 1 diabetes (40, 41). According to a hypothetical model suggested by Vaarala et al (41), the abnormal activation of intestinal epithelium increases the permeability of food antigens through the intestine, which leads to the stimulation of autoimmune processes and to the release of cytokines and, furthermore, to pancreatic islet inflammation and β cell destruction (41). The early introduction of cow-milk proteins may induce mucosal inflammation and increased gut permeability. Enhanced humoral immune responses to cow-milk proteins have been observed in children with newly diagnosed type 1 diabetes and in infants who progressed to overt type 1 diabetes later in childhood (42). This effect may be due to increased consumption of cow milk, enhanced immunological reactivity, or increased intestinal permeability in children who develop type 1 diabetes (10).

Saturated fatty acids have received less attention than milk proteins as a potential risk factor for type 1 diabetes. In in vitro and in vivo studies, it has been observed that saturated fatty acids are generally cytotoxic to β cells in type 2 diabetes (43–45). Lipid intermediates and increased activation of lipid-mediated signals may play a role in fatty-acid induced lipotoxicity (46). Metabolic dysregulation of lipids has been detected to precede early islet autoimmunity in children who later progressed to type 1 diabetes (47). In normoglycemic BB rats with preexisting insulitis, lipids decreased β cell function, which suggested that lipotoxity might also contribute to the pathogenesis of type 1 diabetes (45). In vitro studies and an in vivo model in rats suggested that saturated fatty acids may be more detrimental to β cells in type 2 diabetes when the glucose concentration is already increased (glucolipotoxicity) (48–50), although glucolipotoxicity has not been shown in humans (45). Formula-fed infants have higher glucose concentrations than do breast fed infants (51).

In conclusion, our findings from this case-control analysis nested in a prospective population-based cohort of individuals with increased genetic risk of type 1 diabetes suggest that intakes of cow milk and fruit and berry juices are weakly related to the development of advanced β cell autoimmunity in Finnish children.

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