The Effect of Childhood Cow's Milk Intake and HLA-DR Genotype on Risk of Islet Autoimmunity and Type 1 Diabetes: The Diabetes Autoimmunity Study in the Young (DAISY)

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Abstract

Background—Cow's milk intake has been inconsistently associated with islet autoimmunity (IA) and type 1 diabetes (T1D) development. Genetic and environmental factors may modify the effect of cow's milk on IA and T1D risk.

Methods—The Diabetes Autoimmunity Study in the Young (DAISY) follows children at increased T1D risk for IA (presence of autoantibodies to insulin, GAD65 or IA-2 twice in succession) and T1D development. We examined 1,835 DAISY children with data on cow's milk intake: 143 developed IA, 40 subsequently developed T1D. Cow's milk protein and lactose intake were calculated from prospectively collected parent- and self-reported food frequency questionnaires (FFQ). High risk HLA-DR genotype: HLA-DR3/4,DQB1*0302; low/moderate risk: all other genotypes. We examined interactions between cow's milk intake, age at cow's milk introduction, and HLA-DR genotype in IA and T1D development. Interaction models contained the base terms (e.g., cow's milk protein and HLA-DR genotype) and an interaction term (cow's milk protein*HLA-DR genotype).

Results—In survival models adjusted for total calories, FFQ type, T1D family history, and ethnicity, greater cow's milk protein intake was associated with increased IA risk in children with low/moderate risk HLA-DR genotypes (Hazard Ratio (HR): 1.41, 95% Confidence Interval (CI): 1.08–1.84), but not in children with high risk HLA-DR genotypes. Cow's milk protein intake was associated with progression to T1D (HR: 1.59, CI: 1.13–2.25) in children with IA.

Conclusions—Greater cow's milk intake may increase risk of IA and progression to T1D. Early in the T1D disease process, cow's milk intake may be more influential in children with low/ moderate genetic T1D risk.

Keywords

cow's milk protein; childhood diet; HLA-DR genotype; Islet Autoimmunity

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Introduction

Both genetic and environmental factors play a role in the autoimmune destruction of pancreatic beta cells, leading to the development of islet autoimmunity (IA) (1) and subsequent type 1 diabetes (T1D). Many dietary factors have been investigated for their role in the T1D disease process. In particular, cow's milk has been researched intensively since animal studies first suggested it may be diabetogenic (2). However, human studies have produced contradictory results: cow's milk intake in childhood has been associated with both an increased risk of IA (3–5) and T1D (6, 7), as well as decreased T1D risk (8). These inconsistent findings may be due to the modifying effects of infant exposures or the underlying genetic profile.

The age at which cow's milk is introduced in infancy may produce a priming effect (9), whereby exposure to bovine insulin in cow's milk formula may lead to loss of tolerance to insulin and spur the initiation of insulin-specific T-cells. Once the body is `primed' in infancy, childhood cow's milk intake may continue to trigger the formation of insulin-specific T-cells, thus increasing IA and T1D risk in susceptible individuals. Not accounting for both infant exposure and current cow's milk intake may explain the aforementioned inconsistent results regarding current cow's milk intake, as well as the conflicting reports suggesting that children introduced to cow's milk earlier in infancy have either an increased risk of IA and T1D (8, 10–14), no difference in risk (6, 15, 16), or even a decreased risk of T1D (17).

The underlying genetic profile may also modify the effect of cow's milk on the T1D disease process. The recent rise in T1D incidence (18–20), coupled with data suggesting an increasing penetrance of moderate-risk HLA-DR genotypes (21, 22), suggests that environmental factors may be becoming more influential in T1D disease etiology (23). Moreover, the pressures of an increasingly permissive environment may be more easily observed in children with moderate or low risk HLA-DR genotypes, whereby dietary exposures might have a stronger association with disease development in this group. However, explorations of an interaction between childhood cow's milk intake and the HLA-DR genotypes have not yet produced significant results (6, 24).

Using prospective data from a population of children with increased risk for T1D, we tested for associations between childhood cow's milk intake and risk of IA and progression to T1D. We also tested for interactions between childhood cow's milk intake and either age of cow's milk introduction or HLA-DR genotype for association with IA and progression to T1D. We hypothesized that earlier introduction to cow's milk in infancy would interact with increased cow's milk intake in childhood to increase IA and T1D risk. We also hypothesized that the increased IA and T1D risk associated with cow's milk intake would be more evident in children with low/moderate risk HLA-DR genotypes.

Methods

The Diabetes Autoimmunity Study in the Young (DAISY) is a prospective study of two groups of young children at increased risk for developing T1D. One group consists of

unaffected siblings and offspring of patients with T1D, identified and recruited between birth and age 8 years. The second group consists of babies born at St. Joseph's Hospital in Denver, Colorado, and screened by umbilical cord blood samples for diabetes-susceptibility alleles in the HLA class II region. This group is representative of the general population of the Denver Metropolitan Area. Cord blood is sent to Roche Molecular Systems, Inc., Alameda, CA for PCR-based HLA class II typing. The details of the newborn screening (25) and follow up (16) have been published elsewhere. The DAISY study has enrolled approximately 2,500 children at increased risk for T1D from 1993 to 2006.

Prospective follow-up of DAISY children enrolled at birth included clinic visits at 9, 15 and 24 months. For children not enrolled at birth, follow-up began at the time of enrolment and continued prospectively for both cohorts annually from the ages of 2 to 15 years (25), with the option to continue follow-up until age 25 years. At every visit, blood was drawn and tested for insulin (IAA), protein tyrosine phosphatase (IA2), and glutamic acid decarboxylase (GAD) autoantibodies using radio-immunoassays, as described previously (26–28). The cut-off for positivity was established as the 99th percentile of healthy controls. If a child was autoantibody positive, they were put on an accelerated visit schedule of every 3–6 months. IA was defined as testing positive for ≥1 autoantibody on two consecutive visits, or autoantibody positive on one visit and diabetic on the next consecutive visit. Details of intensive monitoring and T1D diagnosis protocol have been described previously (29). The Colorado Multiple Institutional Review Board approved all study protocols. Informed consent was obtained from the parents/legal guardians of all children. Assent was obtained from children age 7 years and older.

Data for infant diet were collected during telephone or face-to-face interviews at 3, 6, 9, 12, and 15 months of age for children followed from birth. At each interview, mothers were asked to report the date of introduction of all formulas, milks and foods containing cow's milk that the infants consumed during the previous 3 months. For children enrolled in DAISY later in childhood, these data were collected via questionnaire at the time of enrollment.

A semi-quantitative food frequency questionnaire (FFQ) (developed by the Nutrition Questionnaire Service Center, Boston, MA) was used to collect the child's diet after age 1 year via parental report. This FFQ has been validated using multiple 24 hour recalls and biomarkers in the DAISY population (30, 31). Parents completed the questionnaire regarding their child's diet in the previous year, starting when the child was 2 years old and annually thereafter. Food intake was classified into 9 response categories ranging from "never or less than once per month" to "6+ times per day." Average daily intake was calculated by multiplying each item's intake frequency by its nutrient content and summing the nutrient contributions of all foods. Starting in 2003, DAISY began using the Youth Adolescent Questionnaire (YAQ) (also developed by the Nutrition Questionnaire Service Center, Boston, MA) so that children age 10+ years could report their own diets (32). The 152-question YAQ is based on the 145-question FFQ. The YAQ was designed to be user-friendly for adolescents, and has been both validated against 24-hour diet recalls and shown to be reproducible in this age group (33, 34). A comparison of the FFQ and the YAQ in the DAISY population showed that diet data from the FFQ and the YAQ can be used in the

same analysis if the analysis includes an indicator variable defining which survey method with which a nutrient was collected (32). FFQs and YAQs were checked for feasibility of reported caloric intake and completeness prior to analysis.

We examined cow's milk protein and lactose as indicators of childhood cow's milk intake. Previous studies have used servings of cow's milk (6) and cow's milk products (3) as the exposure variable. We calculated cow's milk protein and lactose intake (daily averages) from the reported servings of: skim milk; 1 or 2% milk; whole milk; cream; frozen yogurt, sherbet or low-fat ice cream; regular ice cream; yogurt; butter; cottage or ricotta cheese; cream cheese; other cheese; dairy coffee drink; and milk-based soups such as chowder. The USDA Nutrient Database for Standard Reference (SR) Release 10 was used to calculate nutrient values for the diet surveys collected from January 1996 – May 1999, SR Release 11 from June 1999 – February 2003, SR Release 14 from March 2003 – February 2007, SR Release 19 from March 2007 – March 2011, and SR Release 21 from April 2011 – present. Dietary data were linked to an autoantibody measurement if the one-year time period of the questionnaire encompassed the time directly preceding the visit at which the autoantibody was measured.

Prior to analysis, we excluded 762 DAISY subjects due to: missing childhood dietary data (n = 702), missing data for introduction to cow's milk in infancy (n = 51), or missing ethnicity data (n = 9). The analysis cohort had a significantly higher proportion of children with the high risk HLA genotype, a family history of T1D, or a non-Hispanic white ethnicity, compared to the DAISY subjects not included in the analysis. The analysis cohort did not differ from the DAISY subjects not included in the analysis in terms of IA incidence or sex. For the analysis with IA development as the outcome of interest (`IA Analysis Cohort'), we analyzed multiple diet records per subject up until the age of first autoantibody positive visit (in those that developed IA) or age at last follow-up. We analyzed 12,688 records for 1,835 DAISY children with infant and childhood diet data for the development of IA; 143 children in this cohort developed IA. Ten of these children were removed from the analysis due to left-censoring. For the analysis of diet and the progression to T1D in children with IA (`T1D Analysis Cohort'), we examined a single dietary measure corresponding to the age at first IA positive visit for the 143 children that had developed IA. Forty children from this IA positive cohort developed T1D. We did not analyze longitudinal data for the progression from IA to T1D, as it may be differentially incomplete for the following reason. When a child was diagnosed with T1D outside of a scheduled DAISY visit, we did not contact the family after diagnosis to collect an FFQ (regarding the diet over the previous year), because their recollection of the child's pre-diagnosis diet may be altered by the intense dietary advice they received at diagnosis.

The data analysis for this paper was generated using SAS software, Version 9.3 of the SAS System for Windows. [Copyright © 2002–2010] SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA. For all analyses, hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox regression, to account for right-censored data. While this method does not account for interval censoring, this should have minimal impact on our analyses given that our intervals are short (~1 year). A clustered time to event

analysis was performed treating siblings from the same family as clusters, and robust sandwich variance estimates (35) were used for statistical inference. For the analyses of time to development of IA, the childhood diet variables (cow's milk protein and lactose) were treated as time-varying covariates, and we adjusted for the HLA-DR genotype (HLA-DR3/4,DQB1*0302 vs. all other genotypes), T1D family history, ethnicity (non-Hispanic white vs. other), diet survey type (FFQ or YAQ), and total calories. For the analysis of progression to T1D in IA positive children, the childhood dietary intake variables corresponding to the time at first IA positivity were treated as fixed covariates, and we adjusted for age at first IA positivity in addition to the above listed covariates. Based on our *a priori* hypotheses, we explored the following two interactions: HLA-DR genotype and childhood cow's milk intake, and timing of cow's milk introduction in infancy and childhood cow's milk intake. Interaction models contained the base terms (e.g., childhood cow's milk protein intake and HLA-DR genotype) and an interaction term (childhood cow's milk protein intake*HLA-DR genotype). Age at introduction to cow's milk in infancy and childhood cow's milk intake were treated as continuous variables in the interaction terms. The hazard ratios and 95% confidence intervals for cow's milk introduction presented in Table 2 were calculated from the interaction model for specific ages in infancy where age at introduction to cow's milk was modeled as a continuous variable.

We tested non-supplemental dietary vitamin D as a potential confounder in our analyses of childhood cow's milk protein intake because vitamin D has been hypothesized to protect against T1D (36–38), and liquid milk is fortified with vitamin D in the United States. Non-supplemental vitamin D was not associated with either the IA or progression to T1D outcomes in our cohort, so we did not include it as a confounder in our analyses.

Results

Cow's Milk Intake and Development of IA

The IA Analysis Cohort was 48% female, 76% non-Hispanic white, and has been followed for an average of 9.9 years. DAISY children who developed IA were significantly more likely to have the high risk HLA-DR genotype than those who did not develop IA (36% vs. 22%) (Table 1). As expected, childhood lactose intake was strongly correlated with childhood cow's milk protein intake (Spearman's rho = 0.92, p < 0.0001). Therefore, in the interest of space, we present herein only the results from the cow's milk protein intake models; the results from the lactose intake models are similar.

Childhood cow's milk protein intake was not associated with IA development (Table 2). However, we did find a significant interaction between childhood cow's milk protein intake and HLA-DR genotype (interaction p-value = 0.01) (Table 2). Childhood cow's milk protein intake was associated with IA development in children with low/moderate genetic risk for T1D (HR: 1.41 95% CI: 1.08–2.25), but not in children with high genetic risk for T1D. The interaction between age at cow's milk introduction and childhood cow's milk protein intake was marginally significant (interaction p-value = 0.07) (Table 2). Increased childhood cow's milk protein intake was associated with increased IA risk in children who were exposed to cow's milk very early in infancy (for example: HR for childhood cow's milk protein intake in children introduced to cow's milk at one month: 1.36 95% CI: 1.07–1.72, or three months:

1.25 95% CI: 1.01–1.55) but not in those who were exposed to cow's milk later in infancy (ie, at six months or nine months of age) (Table 2).

In order to explore a more stringent definition of IA, we redefined the IA outcome to be the age at first autoantibody positivity for children who were positive for two or more autoantibodies at some point in their follow-up, or autoantibody positive on one visit and diabetic on the next consecutive visit. There were 68 children in the IA Analysis Cohort that met this stricter definition of IA. We found that increased childhood cow's milk protein intake was marginally associated with this stricter IA outcome (HR: 1.30, 95% CI: 0.98 – 1.72). We found no significant interactions between cow's milk introduction in infancy and childhood cow's milk protein intake (interaction p-value = 0.97), nor between childhood cow's milk protein intake and HLA genotype (interaction p-value = 0.20), in the association with this stricter definition of IA.

Cow's Milk Intake and Progression to T1D in Children with IA

The T1D analysis cohort was 49% female and 80% non-Hispanic white. Children with IA that developed T1D were younger at IA development, and were significantly more likely to have the high risk HLA genotype, a first degree relative with T1D, or be non-Hispanic white compared to children with IA that did not develop T1D. Children that developed T1D were also younger at T1D development than their non-T1D counterparts at their most recent visit (Table 3).

Childhood cow's milk protein intake was associated with progression to T1D in children with IA (HR: 1.59, 95% CI: 1.13 – 2.25) (Table 3). There was no significant interaction between childhood cow's milk protein intake and age at introduction to cow's milk (interaction p-value = 0.88). Additionally, the interaction between childhood cow's milk protein intake and HLA genotype was not significant (interaction p-value = 0.16).

Discussion

This prospective analysis of children at increased genetic risk for T1D revealed that cow's milk may influence the entire T1D disease process. However, the risk of IA conveyed by cow's milk intake was dependent on the child's HLA-DR genotype, while the risk of progressing to T1D conveyed by cow's milk intake was not dependent on HLA-DR genotype. The complexity of the association between cow's milk intake and the T1D disease process should be further studied as it may shed light on the biologic mechanisms underlying the diet's role in disease development.

Prior to the development of IA, a child's HLA-DR genotype modified the effect of cow's milk intake on IA risk. Children with low/moderate risk HLA genotype had increased IA risk with greater cow's milk intake, while children with the high risk HLADR genotype were not affected by cow's milk intake. Other studies have also shown cow's milk intake to be associated with increased IA risk (4, 5). Notably, a recent nested case-control study within a Finnish prospective cohort study of children at increased risk for T1D, similar to DAISY, found that cow's milk intake was marginally associated with increased IA risk (3). However,

our study is the first to identify a significant interaction between childhood cow's milk intake and HLA-DR genotype on IA risk.

The biologic mechanism underlying this interaction may be related to the gut's response to cow's milk protein. Gut epithelial cells do not normally express HLA-DR antigens, but will do so if the epithelium becomes inflamed (39). Evidence suggests that cow's milk protein induces increased HLA-DR expression and activates cell-mediated immunity when the epithelial cells have been irritated by injury, inflammation, or infection (40). An aberrant cell-mediated immune response can lead to the development of autoimmunity. It is possible that children with high risk HLA-DR genotypes are prone to an abnormal cell-mediated immune response, thus they may develop IA in the presence of epithelial inflammation regardless of dietary factors. In children with low/moderate risk HLA genotypes, however, the combination of gut inflammation and greater cow's milk protein exposure may be necessary to trigger IA development.

Interestingly, we did not find a significant interaction between HLA-DR genotype and childhood cow's milk protein intake associated with T1D development, as we saw with IA development. Similarly, this interaction was not significant in either of the two Finnish case-control studies examining T1D risk factors that tested the interaction between cow's milk intake and HLA-DR genotype (6, 24).

Instead, we found a marginal association between cow's milk protein intake and IA development in children that had two or more autoantibodies. We also identified a significant association between childhood cow's milk intake and T1D development in children with IA, suggesting that, once the body has begun to attack its own beta cells, the continued exposure to cow's milk may exacerbate the autoimmune process. These findings highlight the increasing influence of cow's milk protein as a child progresses towards T1D development. In agreement with our results, two case-control studies conducted in children and adolescents (age 0–16 years) have shown childhood cow's milk intake increases risk of T1D (6, 7). These studies had predominantly non-Hispanic white populations that were similar in age to our study population. In contrast, one case-control study found increased cow's milk intake resulted in a *decreased* risk of T1D in children under five years of age (8). This discrepancy in findings may be due to the different ages of the study populations. A dietary factor that is protective against T1D development in the early years may increase disease risk later in childhood (or vice versa), due to the changing demands of a growing and developing body.

Finally, our analysis showed only marginal evidence of an interaction between current cow's milk intake and introduction of cow's milk in IA risk. Thus, our study lends modest support to the `priming' hypothesis, which posits that exposure to cow's milk formula in infancy may lead to loss of tolerance to insulin and spur the initiation of insulin-specific T-cells. Childhood cow's milk intake would then continue to trigger this immune reaction, thus increasing IA and T1D risk in susceptible individuals. Many studies have observed that early introduction of cow's milk formula increases IA and T1D risk (8, 10–14). However, few studies have examined the interaction between cow's milk introduction in infancy and childhood cow's milk intake in the pathogenesis of T1D. Virtanen *et al*., examining a T1D

outcome, also did not observe a significant interaction between age at introduction of supplementary milk and childhood milk intake (6). Our intriguing (albeit non-significant) observation should be followed up in other, larger cohorts.

Strengths of this study include the availability of HLA-DR genotype information and the prospective, longitudinal nature of the data, with frequent follow-up intervals and dietary data collection prior to IA development. While our sample size is relatively small, the DAISY children are older and have longer follow-up time than the children in most prospective studies that track diet and detect IA development. Our ability to detect significant gene/environment interactions suggests that our sample size was sufficient. However, the interaction we describe between age at introduction to cow's milk in infancy and cow's milk intake in childhood in our analysis of an IA outcome did not reach statistical significance, suggesting that a larger cohort may be necessary to fully answer this research question. Finally, the DAISY cohort was selected for having increased genetic risk of developing T1D, therefore our results are not generalizable to the US pediatric population.

In summary, in children with low/moderate HLA-DR genotypes, greater childhood cow's milk protein intake may increase the child's risk of developing IA. Moreover, greater childhood cow's milk protein intake predicts progression from IA to T1D. These findings suggest that cow's milk may be diabetogenic when consumed throughout childhood, and may impact both early and later stages of T1D development. The results also suggest that the effect of dietary exposures may only be detected in children with low/moderate risk genotypes in the initial development of islet autoantibodies, but once a child develops IA, progression to T1D may be more dependent on the child's dietary exposures than on HLA-DR genotype. Stratification by HLA-DR genotype should be considered when analyzing IA and T1D risk in dietary studies, especially in studies focusing on the earliest stages of the T1D disease process.

Acknowledgements

References

1. Verge CF, Gianani R, Kawasaki E, et al. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes. 1996; 45:926–933. [PubMed: 8666144]

2. Elliott RB, Martin JM. Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? Diabetologia. 1984; 26:297–299. [PubMed: 6376238]

3. Virtanen SM, Nevalainen J, Kronberg-Kippila C, et al. Food consumption and advanced á cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes: a nested case-control design. American Journal of Clinical Nutrition. 2012; 95:471–478. [PubMed: 22237062]

4. Wahlberg J, Vaarala O, Ludvigsson J, et al. Dietary risk factors for the emergence of type 1 diabetes-related autoantibodies in 21/2 year-old Swedish children. The British Journal of Nutrition. 2006; 95:603–608. [PubMed: 16578935]

5. Virtanen SM, Hypponen E, Laara E, et al. Cow's milk consumption, disease-associated autoantibodies and type 1 diabetes mellitus: a follow-up study in siblings of diabetic children.

Childhood Diabetes in Finland Study Group. Diabetic Medicine. 1998; 15:730–738. [PubMed: 9737801]

6. Virtanen SM, Laara E, Hypponen E, et al. Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: a nested case-control study of siblings of children with diabetes. Childhood diabetes in Finland study group. Diabetes. 2000; 49:912–917. [PubMed: 10866042]

7. Verge CF, Howard NJ, Irwig L, Simpson JM, Mackerras D, Silink M. Environmental factors in childhood IDDM. A population-based, case-control study. Diabetes Care. 1994; 17:1381–1389. [PubMed: 7882806]

8. Rosenbauer J, Herzig P, Giani G. Early infant feeding and risk of type 1 diabetes mellitus-a nationwide population-based case-control study in pre-school children. Diabetes/metabolism research and reviews. 2008; 24:211–222. [PubMed: 17968982]

9. Paronen J, Knip M, Savilahti E, et al. Effect of cow's milk exposure and maternal type 1 diabetes on cellular and humoral immunization to dietary insulin in infants at genetic risk for type 1 diabetes. Finnish Trial to Reduce IDDM in the Genetically at Risk Study Group. Diabetes. 2000; 49:1657–1665. [PubMed: 11016449]

10. Kimpimaki T, Erkkola M, Korhonen S, et al. Short-term exclusive breastfeeding predisposes young children with increased genetic risk of Type I diabetes to progressive beta-cell autoimmunity. Diabetologia. 2001; 44:63–69. [PubMed: 11206413]

11. Holmberg H, Wahlberg J, Vaarala O, et al. Short duration of breast-feeding as a risk-factor for beta-cell autoantibodies in 5-year-old children from the general population. The British Journal of Nutrition. 2007; 97:111–116. [PubMed: 17217566]

12. Alves JG, Figueiroa JN, Meneses J, Alves GV. Breastfeeding protects against type 1 diabetes mellitus: a case-sibling study. Breastfeeding Medicine. 2012; 7:25–28. [PubMed: 21819204]

13. Virtanen SM, Rasanen L, Ylonen K, et al. Early introduction of dairy products associated with increased risk of IDDM in Finnish children. The Childhood in Diabetes in Finland Study Group. Diabetes. 1993; 42:1786–1790. [PubMed: 8243824]

14. Svensson J, Carstensen B, Mortensen HB, Borch-Johnsen K. The Danish Study Group of Childhood Diabetes. Early childhood risk factors associated with type 1 diabetes--is gender important? European Journal of Epidemiology. 2005; 20:429–434. [PubMed: 16080591]

15. Couper JJ, Steele C, Beresford S, et al. Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. Diabetes. 1999; 48:2145–2149. [PubMed: 10535447]

16. Norris JM, Barriga K, Klingensmith G, et al. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. JAMA. 2003; 290:1713–1720. [PubMed: 14519705]

17. Savilahti E, Saarinen KM. Early infant feeding and type 1 diabetes. European Journal of Nutrition. 2009; 48:243–249. [PubMed: 19263185]

18. Vehik K, Hamman RF, Lezotte D, et al. Increasing incidence of type 1 diabetes in 0- to 17-year-old Colorado youth. Diabetes Care. 2007; 30:503–509. [PubMed: 17327312]

19. DIAMOND Project Group. Incidence and trends of childhood Type 1 diabetes worldwide 1990–1999. Diabetic Medicine. 2006; 23:857–866. [PubMed: 16911623]

20. Patterson CC, Gyurus E, Rosenbauer J, et al. Trends in childhood type 1 diabetes incidence in Europe during 1989–2008: evidence of non-uniformity over time in rates of increase. Diabetologia. 2012; 55:2142–2147. [PubMed: 22638547]

21. Steck AK, Armstrong TK, Babu SR, Eisenbarth GS. Type 1 Diabetes Genetics Consortium. Stepwise or linear decrease in penetrance of type 1 diabetes with lower-risk HLA genotypes over the past 40 years. Diabetes. 2011; 60:1045–1049. [PubMed: 21307077]

22. Vehik K, Hamman RF, Lezotte D, et al. Trends in High-Risk HLA Susceptibility Genes Among Colorado Youth with Type 1 Diabetes. Diabetes Care. 2008; 31:1392–1396. [PubMed: 18356404]

23. Vehik K, Dabelea D. The changing epidemiology of type 1 diabetes: why is it going through the roof? Diabetes/metabolism research and reviews. 2011; 27:3–13. [PubMed: 21218503]

24. Saukkonen T, Virtanen SM, Karppinen M, et al. Significance of cow's milk protein antibodies as risk factor for childhood IDDM: interactions with dietary cow's milk intake and HLA-DQB1 genotype. Childhood Diabetes in Finland Study Group. Diabetologia. 1998; 41:72–78. [PubMed: 9498633]

25. Rewers M, Bugawan TL, Norris JM, et al. Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). Diabetologia. 1996; 39:807–812. [PubMed: 8817105]

26. Yu L, Rewers M, Gianani R, et al. Antiislet autoantibodies usually develop sequentially rather than simultaneously. J Clin Endocrinol Metab. 1996; 81:4264–4267. [PubMed: 8954025]

27. Barker JM, Barriga KJ, Yu L, et al. Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). J Clin Endocrinol Metab. 2004; 89:3896–3902. [PubMed: 15292324]

28. Yu L, Robles DT, Abiru N, et al. Early expression of antiinsulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. Proc Natl Acad Sci,USA. 2000; 97:1701–1706. [PubMed: 10677521]

29. Stene LC, Barriga K, Hoffman M, et al. Normal but increasing hemoglobin A1c levels predict progression from islet autoimmunity to overt type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). Pediatric Diabetes. 2006; 7:247–253. [PubMed: 17054445]

30. Parrish LA, Marshall JA, Krebs NF, Rewers M, Norris JM. Validation of a food frequency questionnaire in preschool children. Epidemiology. 2003; 14:213–217. [PubMed: 12606888]

31. Brady HL, Lamb MM, Sokol RJ, et al. Plasma micronutrients are associated with dietary intake and environmental tobacco smoke exposure in a paediatric population. Public Health Nutrition. 2007; 10:712–718. [PubMed: 17381951]

32. Lamb MM, Ross CA, Brady HL, Norris JM. Comparison of children's diets as reported by the child via the Youth/Adolescent Questionnaire and the parent via the Willett food-frequency questionnaire. Public Health Nutrition. 2007; 10:663–670. [PubMed: 17381935]

33. Rockett HR, Breitenbach M, Frazier AL, et al. Validation of a youth/adolescent food frequency questionnaire. Prev Med. 1997; 26:808–816. [PubMed: 9388792]

34. Rockett HR, Wolf AM, Colditz GA. Development and reproducibility of a food frequency questionnaire to assess diets of older children and adolescents. J Am Diet Assoc. 1995; 95:336–340. [PubMed: 7860946]

35. Wei LJ, Lin DY, Weissfeld L. Regression Analysis of Multivariate Incomplete Failure Time Data by Modeling Marginal Distributions. Journal of the American Statistical Association. 1989; 84:1065–1073.

36. Stene LC, Joner G, Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. American Journal of Clinical Nutrition. 2003; 78:1128–1134. [PubMed: 14668274]

37. EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. Diabetologia. 1999; 42:51–54. [PubMed: 10027578]

38. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001; 358:1500–1503. [PubMed: 11705562]

39. Fais S, Pallone F, Squarcia O, et al. HLA-DR antigens on colonic epithelial cells in inflammatory bowel disease: I. Relation to the state of activation of lamina propria lymphocytes and to the epithelial expression of other surface markers. Clinical and experimental immunology. 1987; 68:605–612. [PubMed: 3308219]

40. Chung HL, Lee JJ, Kim SG. Cow's milk protein induced changes in the expression of HLA-DR antigens on colonic epithelial cells. Annals of Asthma, Allergy and Immunology. 2003; 90:348–350.

Table 1

Description of the IA Analysis Cohort: The Diabetes Autoimmunity Study in the Young

Variable	Did not develop IA (N = 1,702 children)	Developed IA (N = 133 children)
High risk HLA-DR3/4,DQ8 genotype	21.6%	36.1%[†]
Family history of T1D	49.2%	57.1%
Female	47.7%	50.4%
Non-Hispanic white ethnicity	75.7%	78.2%
Mean (SD) age at introduction of cow's milk (mos)	4.2 (3.8)	4.7 (4.0)
Mean (SD) age at last visit or development of IA (yrs)	10.2 (5.0)	7.0 (3.9)
	N = 12,043 visits	**N = 645 visits**
Mean (SD) childhood total calories[*]	2068.2 (730.9)	2052.88 (592.6)
Mean (SD) childhood cow's milk protein intake (g)[*]	28.17 (13.93)	30.84 (14.17)
Mean (SD) childhood lactose intake (g)[*]	29.4 (17.5)	31.7 (17.9)

[*] As reported by the parent on the FFQ, or by the child on the YAQ

[†] t-test for continuous variables and chi-square test for categorical variables p-value < 0.05

Table 2

Interactions between Childhood Cow's Milk Intake, Age at Introduction to Cow's Milk in Infancy, HLA-DR Genotype and Islet Autoimmunity Development: The Diabetes Autoimmunity Study in the Young

Dietary Exposure		Hazard Ratio[*]	95% Confidence Interval	p-value
Childhood cow's milk protein intake[‡,§]		1.18	0.94 – 1.48	0.15

	Interaction Variable	Hazard Ratio [*]	95% Confidence Interval	p-value for interaction
Childhood cow's milk protein intake[‡]	Low/moderate HLA-DR genotype[†]	1.41	1.08 – 1.84	**0.01**
		0.85	0.61 – 1.18	
	High HLA-DR genotype[†]			
Childhood cow's milk protein intake[‡§]	Cow's milk introduced at one month	1.36[¶]	1.07 – 1.72	0.07
	Cow's milk introduced at three months	1.25[¶]	1.01 – 1.55	
	Cow's milk introduced at six months	1.11[¶]	0.87 – 1.41	
	Cow's milk introduced at nine months	0.99[¶]	0.72 – 1.35	

[*] Hazard ratio represents a one standard deviation change in the diet variable

[†] Low/moderate ≠ HLA-DR3/4,DQ8 genotype, High = HLA-DR3/4,DQ8 genotype

[‡] Adjusted for total caloric intake, food frequency questionnaire type, family history of T1D, and ethnicity

[§] Also adjusted for HLA-DR genotype

[¶] The Hazard Ratios and 95% Confidence Intervals presented for cow's milk introduction at specific months in infancy are calculated from a survival model in which age at introduction to cow's milk was a continuous variable.

Table 3

Associations between Cow's Milk Intake and Progression to Type 1 Diabetes in Children with Islet Autoimmunity: The Diabetes Autoimmunity Study in the Young

Variable	Did not develop T1D (n = 103)	Developed T1D (n = 40)
High risk HLA-DR3/4,DQ8 genotype	29%	50% [†]
Family history of T1D	51%	73% [†]
Female	50%	53%
Non-Hispanic white ethnicity	75%	93% [†]
Mean (SD) age at introduction of cow's milk (mos)	4.83 (4.08)	4.89 (4.12)
Mean (SD) age at development of IA (yrs)	7.61 (3.89)	4.58 (2.94) [†]
Mean (SD) age at last visit or T1D development (yrs)	14.09 (3.75)	9.58 (3.52) [†]
Mean (SD) childhood total calories [*]	2045.00 (738.83)	2115.52 (648.69)
Mean (SD) childhood cow's milk protein intake (g) [*]	28.65 (16.13)	33.07 (15.73)
Mean (SD) childhood lactose intake (g) [*]	29.84 (20.52)	32.59 (18.62)

Dietary Exposure	Hazard Ratio [*]	95% Confidence Interval	p-value
Cow's milk protein intake[‡]	1.59	1.13 – 2.25	**0.009**

[*] As reported by the parent on the FFQ, or by the child on the YAQ

[†] test for continuous variables and chi-square test for categorical variables p-value < 0.05

[‡] Adjusted for total caloric intake, food frequency questionnaire type, HLA-DR status, family history ofTID, age at first IA positivity and ethnicity