In Utero Dietary Exposures and Risk of Islet Autoimmunity in Children

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OBJECTIVE — The goal of this study was to examine whether maternal dietary intake of vitamin D, ω-3 fatty acids, and ω-6 fatty acids during pregnancy is associated with the appearance of islet autoimmunity (IA) in offspring.

RESEARCH DESIGN AND METHODS — The Diabetes Autoimmunity Study in the Young (DAISY) is recruiting at birth and following children at increased risk for type 1 diabetes, as determined by HLA-DR genotype or by family history of type 1 diabetes. A total of 233 mothers of newly recruited DAISY subjects were asked to recall their intake of food and nutritional supplements during the third trimester of pregnancy using the Willett food frequency questionnaire. Children were followed for an average of 4 years (range 0.8–7.3 years) for the appearance of insulin, GAD_{65}, and IA-2 autoantibodies. Sixteen children developed at least one autoantibody during this period. Unadjusted and adjusted hazard ratios (HRs) for the development of IA were estimated with survival analysis using a Weibull distribution.

RESULTS — Maternal intake of vitamin D via food was significantly associated with a decreased risk of IA appearance in offspring, independent of HLA genotype, family history of type 1 diabetes, presence of gestational diabetes mellitus, and ethnicity (adjusted HR = 0.37; 95% CI 0.17–0.78). Vitamin D intake via supplements, ω-3 fatty acids, and ω-6 fatty acids intake during pregnancy were not associated with appearance of IA in offspring.

CONCLUSIONS — Our findings suggest that maternal intake of vitamin D through food during pregnancy may have a protective effect on the appearance of IA in offspring.

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Type 1 diabetes is a T-cell–mediated autoimmune disease characterized by the destruction of insulin-producing β-cells of the pancreas. The causes of type 1 diabetes are unknown, yet low concordance rates among monozygotic twins (1), a 10% progression rate among those genetically susceptible (2), migratory studies (3), and increasing worldwide incidence rates (4) suggest that genetic factors interact with environmental factors in the development of type 1 diabetes. These environmental factors have not been clearly identified.

Type 1 diabetes is preceded by a preclinical stage termed islet autoimmunity (IA) that signals the beginning of β-cell destruction with the presence of autoantibodies against islet autoantigens. IA appears typically by 2 years of age and sometimes as early as 3 months of age in individuals with type 1 diabetic first-degree relatives (5–7). Autoantibodies can be persistent for months or years, often predicting clinical diagnosis, or can be transient. Transient autoantibody positivity has not been associated with genetic risk factors (7,8), suggesting that environmental factors may be involved in suppressing persistent β-cell destruction.

Increased expression of proinflammatory cytokines may be associated with the appearance and persistence of IA (9). Vitamin D and the ω-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to modify the immune response by suppressing proinflammatory cytokines and promoting anti-inflammatory cytokines (10–13). The ω-6 fatty acids, specifically arachidonic acid, have been shown to promote the proinflammatory cytokine prostaglandin E_2, which may increase progression of IA toward type 1 diabetes in individuals with high genetic risk for type 1 diabetes (14). Animal studies (15–18) and epidemiological studies (19–21) have observed the protective effect of vitamin D or ω-3 fatty acids on type 1 diabetes risk.

In utero exposures may influence the child's risk of IA and type 1 diabetes. Evidence for this stems from observations concerning prenatal exposure to congenital rubella (22) and intake of cod liver oil during pregnancy (21). Recent evidence suggests the possibility of autoimmune reactivity against β-cells in utero (23,24), underscoring the importance of studying in utero exposures when investigating potential type 1 diabetes prevention strategies.

Vitamin D, ω-3 fatty acid, and ω-6 fatty acid status in the fetus and newborn child is dependent on maternal intake during pregnancy (25–27). Considering the rapid increase in type 1 diabetes incidence among 0–5 year olds (28) and early appearance of IA, maternal intake of certain dietary nutrients during pregnancy may provide sufficient in utero exposure to these nutrients, offering early protec-

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Abbreviations: DAISY, Diabetes Autoimmunity Study in the Young; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFQ, food frequency questionnaire; GDM, gestational diabetes mellitus; IA, islet autoimmunity; IAA, insulin autoantibody.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

tion from or promotion of IA in infancy or early childhood.

Previous studies have been limited because they assessed exposures after the appearance of disease, which could lead to bias, and/or they have only examined supplemental exposures as opposed to exposures via foods, which may result in misclassification. Therefore, we examined whether maternal dietary and/or supplemental intakes during pregnancy of vitamin D, ω-3 fatty acids, and ω-6 fatty acids are associated with the appearance of IA in children using dietary intake data that were measured before the appearance of the outcome.

RESEARCH DESIGN AND METHODS

Study population

Informed consent was obtained from the parents of each study subject at enrollment, and the Colorado Multiple Institutional Review Board approved all study protocols. Subjects were identified through the Diabetes Autoimmunity Study in the Young (DAISY), a birth-cohort recruited from the Denver metropolitan area that investigates the natural history of IA in infants and children at moderate to high risk for developing type 1 diabetes. DAISY children were recruited in two ways. The first was through a screening program of all children born at St. Joseph Hospital in Denver, Colorado, via testing of umbilical cord blood samples for diabetes-susceptibility alleles in the HLA region. Details on screening are available elsewhere (29). Results of HLA typing placed subjects into three risk groups that are determined by the odds of developing type 1 diabetes by the age of 20 years: high 1:16; moderate 1:75 (in non-Hispanic Caucasians) or 1:230 (in Hispanics); or low <1:300. The second method of DAISY recruitment was through identifying children with first-degree type 1 diabetic relatives through the Colorado type 1 diabetes registry, the Barbara Davis Center (Denver, CO), the Children's Hospital (Denver, CO), or media publicity.

Collection of maternal diet during pregnancy

Maternal third trimester exposures were assessed via a self-administered Willett Food Frequency Questionnaire (FFQ) sent to mothers at DAISY enrollment ~2–3 months after delivery. Assessments began in January 1996. Our cohort comprised 233 children whose mother completed an FFQ. The FFQ inquired about the usual intake of food and vitamins over a defined period of time. It listed foods with serving sizes and nine options for frequency of intake ranging from never or less than once per month to six or more serving sizes per day. For vitamin supplements, the FFQ inquired about brand name, constituents, dose, and frequency. The FFQs were sent to the Channing Laboratory at Harvard University for scanning and nutritional data analysis. Nutrient scores were computed by multiplying the frequency of intake by the nutrient content of the item using data from the U.S. Department of Agriculture, food manufacturers, and other published sources. Data on serum concentrations of vitamin D and erythrocyte membrane fatty acids during pregnancy were not available.

Intakes of vitamin D via food and vitamin D via supplements were analyzed separately. Vitamin D intake via food was analyzed as a continuous variable in international units (IU). Vitamin D intake via supplements was dichotomized using a cutoff of 400 IU to distinguish between those above and below the recommended daily consumption of vitamin D. Indicators of ω-3 fatty acid intake were measured through the intake of linolenic acid and a combined intake of EPA and DHA. A dichotomization value of 0.1 g for EPA and DHA intake was chosen based upon recent research that reported daily intake of 0.1 g EPA and DHA reflects an average frequency of fish intake of one to three servings per month (30). In our cohort, we assumed that women acquired EPA and DHA through fish consumption. The ω-6 fatty acid intake was captured through intake of linoleic acid and intake of arachidonic acid. We also calculated the ratio of total ω-6 fatty acid intake to total ω-3 fatty acid intake.

Descriptive data collection

We examined sociodemographic and pregnancy indicators to determine their association with IA, which were collected with structured questionnaires. Sociodemographic indicators included child's sex, mother's age at time of delivery, mother's educational attainment at time of delivery (≤high school versus >12 years), mother's income at time of delivery (<$30,000 vs. ≥$30,000), and ethnicity (non-Hispanic Caucasian versus "Other"). The "Other" ethnicity category included Hispanic (18.92%), American Indian (0.90%), Asian (0.90%), African American (2.70%), and biracial (1.35%). Variables relating to pregnancy included child's birth weight, a preterm or post-term delivery date (yes/no), cesarean delivery (yes/no), breast-feeding duration (<3 months vs. ≥3 months), and development of gestational diabetes mellitus (GDM) (yes/no). We also examined genetic risk factors as indicated by subject's relation to a type 1 diabetic first-degree relative (yes/no) and subject's diabetes-susceptibility HLA-DR genotype (HLA-DR3/4, DQ8 versus other genotypes).

Measurement of autoantibodies and definition of outcome

To determine the presence of IA, we collected serum from blood draws at ages 9 months, 15 months, 2 years, and annually for the remainder of the study. We used radioimmunoassays for insulin, GAD_{65}, and IA-2 autoantibodies. Insulin autoantibodies (IAAs) are measured by a micro-insulin autoantibody assay with sensitivity of 58%, specificity of 99%, and interassay coefficient of variation 11% as described previously (31). The combined anti-GAD and IA-2 radioassay is performed in duplicate on a 96-well filtration plate, and radioactivity is counted on a TopCount 96-well plate β-counter as described previously (32). The levels of both antibodies are expressed as an index (sample cpm − negative control cpm)/(positive control cpm − negative control cpm). In the 1995 Immunology of Diabetes Society Workshop, the GAD antibody assay had 82% sensitivity and 99% specificity using sera from new-onset diabetic patients aged <30 years. The interassay coefficient of variation was 6%. The IA-2 assay had 73% sensitivity and 100% specificity, and the interassay coefficient of variation was 10% (32). All samples with IAA, GAD antibody, or IA-2 levels exceeding the 99th percentile and a random 10% of the remaining samples were retested in a blinded manner for quality assurance. For GAD antibody and IAA, we used the 99th percentile based on testing 198 nondiabetic control subjects aged 0.4–67 years (0.01 for IAA, and 0.032 for GAD antibody) as the cutoff for positivity. The single highest value (100th percentile) for IA-2 among the control subjects,

Table 1—*Demographic and pregnancy characteristics and risk of IA in offspring*

Characteristic	Affected	Unaffected	Unadjusted HR (95% CI)	Wald χ^2 *P*
n	16	206		
Age at last follow-up (years)*	4.3 ± 1.9	4.0 ± 1.4	NA†	NA†
Child's sex (% male)	7 (43.7)	101 (49.0)	0.87 (0.32–2.33)	0.779
Child's ethnicity (% non-Hispanic Caucasian)	15 (93.7)	152 (73.8)	5.29 (0.7–40.06)	0.133
Maternal age at delivery (years)	31.3 ± 6.0	29.7 ± 5.8	1.26 (0.8–1.96)‡	0.331
Maternal education at delivery (% ≤12 years)	4 (25.0)	48 (23.3)	1.09 (0.35–3.39)	0.878
Income at delivery (% <$30,000/year)§	4 (26.7)	48 (23.8)	1.04 (0.33–3.28)	0.942
Birth weight (g)§	3,122.9 ± 564.9	3,286.4 ± 527.7	0.74 (0.47–1.18)‡	0.238
Pre- or post-term delivery§	2 (13.3)	69 (33.7)	0.31 (0.07–1.37)	0.148
Cesarean delivery	1 (6.2)	43 (20.9)	0.27 (0.04–2.04)	0.226
Breast-fed <3 months§	6 (37.5)	66 (32.3)	1.3 (0.47–3.56)	0.619
GDM	3 (18.8)	3 (1.5)	9.64 (2.74–33.97)	0.006
First-degree type 1 diabetic relative	6 (37.5)	62 (30.1)	1.71 (0.62–4.75)	0.302
HLA-DR3/4, DQ8 genotype	14 (87.5)	129 (62.6)	3.17 (0.72–14.01)	0.166

Data are means ± SD or *n* (%) unless otherwise indicated. *For affected, average age at first positive test for IA is 2.5 ± 1.7 years; †outcome determined by difference in age of affected subject at first positive visit and age at last negative visit; ‡HR associated with incremental values: maternal age = 5 years, birth weight = 500 g; §missing observations include income: one in the affected group (6.2%) and four (1.9%) in the unaffected group, birth weight: one from the unaffected group (0.5%), pre- or post-term delivery: one in each affected (6.2%) and unaffected group (0.5%), and breast-feeding duration: two missing observations from unaffected group (1.0%).

0.07, was used as the cutoff for positivity for this assay.

For the purposes of this study, the definition of IA was positivity for one or more of the three islet autoantibodies on at least one occasion. We excluded positivity due to transplacental transmission of autoantibodies, as indicated by positivity at the 9-month blood draw and negative on all subsequent visits. Children with positive results for any of the antibodies tested were placed on an accelerated blood draw schedule of every 3–6 months for the duration of the study. Of the 16 children who met this definition of IA, 5 have since been diagnosed with type 1 diabetes, 6 were autoantibody positive at last follow-up, and 5 have subsequently tested negative for autoantibodies. We also did some exploratory analysis with a more strict definition of IA, which included only the 11 children who were autoantibody positive on two consecutive visits and still positive (or diabetic) at the last visit.

Statistical analysis

We used parametric survival analysis (PROC LIFEREG) and specified the Weibull distribution to estimate all univariate and adjusted hazard ratios (HRs) and calculated corresponding approximate 95% CI (33,34). We started accrual of follow-up time from birth. Due to fixed-interval blood draws, the exact timing of IA development was unknown and varied for affected subjects. Thus, the outcome for all affected subjects was interval censored, and the interval was determined by the difference in the age of the affected subject at the first positive visit and their age at the last negative visit. Two subjects were positive at their first blood draw (~9 months of age) and were left censored. Subjects not considered affected had variable lengths of follow-up and were right censored. There were 11 pairs of siblings (9.4%) in the cohort. One sibling from each of the 11 families was randomly deleted to avoid violating the assumption of independence.

Variables for which we did not have a priori reasons for choosing a categorization value were tested for log linearity of outcome. To test log linearity, we first calculated tertile values for each variable, then modeled the variable as an ordered categorical variable and as separate categories based upon tertile values, and evaluated linearity using the likelihood ratio test ($P < 0.20$ indicated nonlinearity). For all models, variables meeting the assumption of log linearity were entered as continuous variables, whereas variables violating the assumption of log linearity were entered as tertiles.

Tests for confounding began with a saturated survival model and proceeded in a manual backward stepwise fashion. Variables were retained in the final model if their exclusion would have resulted in a >10% change in the magnitude of the HR for the dietary variable of interest. The SAS V8e statistical software package was used for all analyses.

RESULTS — The average age at last follow-up for the affected and unaffected groups is 4.3 ± 1.9 and 4.0 ± 1.4 years, respectively. Age at first positive IA test was 2.5 ± 1.7 years and ranged from 0.76 to 6.0 years. Univariate analysis revealed a significantly increased hazard of IA associated with mothers having GDM (HR 9.64; 95% CI 2.74–33.97) (Table 1).

Maternal intake of vitamin D through food was associated with a decreased risk of IA in offspring univariately (HR 0.49; 95% CI 0.26–0.94) (Table 2), and after adjustment for HLA genotype, family history of type 1 diabetes, presence of GDM, and ethnicity (adjusted HR 0.37; 0.17–0.78) (Table 3). This HR represents a 63% decrease in risk associated with an SD increase in dietary vitamin D intake during pregnancy (vitamin D SD 155.6 IU). The remaining exposure variables were nonsignificant univariately and remained so after adjustment for relation to a type 1 diabetic first-degree relative, HLA-DR genotype, and GDM (data not shown). Exploratory analysis of persistent IA/diabetes suggested a protective effect of vitamin D via food, although the effect was lower in magnitude and nonsignifi-

Table 2—*Maternal dietary exposures during pregnancy and risk of IA in offspring*

Parameters (mean daily intake)	Affected	Unaffected	Unadjusted HR (95% CI)	Wald χ^2 *P*
n	16	206		
Vitamin D intake via food (IU)	167.6	252.3	0.49 (0.26–0.94)*	0.059
Vitamin D intake via supplements (IU)				
≥400	13 (81.3)	116 (56.3)	3.09 (0.88–10.83)	0.107
EPA and DHA (ω-3 fatty acids) (g)				
≥0.10	9 (56.3)	137 (66.5)	0.64 (0.24–1.71)	0.380
Linolenic acid (ω-3 fatty acid) (g)	1.4	1.3	1.16 (0.75–1.80)*	0.503
Arachidonic acid (ω-6 fatty acid) (g)	0.13	0.14	0.88 (0.5–1.56)*	0.672
Linoleic acid (ω-6 fatty acid) (g)†				
<7.92	4 (25.0)	71 (34.5)	1.0 (referent)	
7.92–11.78	8 (50.0)	66 (32.0)	2.01 (0.55–7.37)	0.274
>11.78	4 (25.0)	69 (33.5)	1.04 (0.26–4.16)	0.955
Ratio of ω-6 total:ω-3 total	7.9	7.8	1.13 (0.70–1.83)*	0.624
Caloric intake (kcal)†				
<1,718.88	5 (31.2)	68 (33.0)	1.0 (referent)	
1,718.88–2,491.04	9 (56.2)	67 (32.5)	1.61 (0.54–4.82)	0.405
>2,491.04	2 (12.5)	71 (34.5)	0.37 (0.07–1.93)	0.258

Data are *n* (%) unless otherwise indicated. *HR represent risk for an SD difference in intake: SD vitamin D = 155.6 IU; SD linolenic acid = 0.69 g; SD arachidonic acid = 0.08 g; SD ratio ω-6 total to ω-3 total = 2.00; †categorized as tertiles because variable did not meet the assumption of log linearity.

cant compared with the less restrictive IA model (Table 3).

CONCLUSIONS — Our findings suggest that maternal intake of vitamin D through food during pregnancy may have a protective effect on the appearance of IA in offspring. While other studies have examined related questions (21), our study is the first to examine in utero dietary exposures using a cohort design in which exposure is assessed and then the cohort is followed for the outcome. This design is free of recall bias, a weakness commonly attached to study designs in which exposures are assessed after the appearance of disease. Also, the DAISY cohort was created by selectively screening for subjects at increased genetic risk of type 1 diabetes. Although this results in a cohort that is not representative of the general population, the cohort is otherwise free of exposure-dependent selection bias. In addition, our study design accounted for the level of exposure through dose and frequency assessments, aspects not accounted for in past epidemiological reports (19,21).

FFQs are appropriate for assessing diet in pregnant women (35–38), although there is a tendency for overestimation of intake (39). While the period of recall was relatively short, we recognize that nondifferential misclassification of dietary exposure was also possible because each mother assessed her third trimester intake at ~2–3 months after delivery. However, overestimation and nondifferential misclassification are more likely to bias the measure of association toward the null rather than away from the null and therefore would not explain our results regarding dietary vitamin D. In fact, given the potential for nondifferential misclassification, our analyses may actually be underestimating the effect of maternal intake. Only a biomarker of vitamin D could better confirm vitamin D exposure, as it would account for vitamin D acquired through diet as well as sunlight, the latter of which we were unable to account for in this study. However, exposure to sunlight is a readily available source of vitamin D in Colorado and would likely not differ among affected and unaffected groups. In the DAISY children, plasma 25, hydroxyvitamin D levels were significantly associated with vitamin D intake as measured by the Willett FFQ, suggesting that the questionnaire was producing a valid measure of vitamin D intake (40).

The previous epidemiological studies that observed the protective effect of vitamin D (19–21) used type 1 diabetes as the outcome. However, considering that IA is the initial signal of β-cell destruction, that it often appears early in life, and that it can last for years before clinical diagnosis of type 1 diabetes, identifying exposures associated with the appearance of IA, regardless of its persistence, offers an opportunity for type 1 diabetes prevention at its most primitive stage. Our outcome definition includes transient IA subjects. While transient IA more weakly predicts progression toward type 1 diabetes compared with persistent IA, it is our observation that 13% of the DAISY subjects who lost their antibodies gained them back at a later date (M.R., J.M.N., unpublished data). Interestingly, 11.7% ($n = 2$) of the 17 DAISY subjects followed from birth who have developed type 1 diabetes were classified with transient IA at some time before type 1 diabetes diagnosis (M.R., J.M.N., unpublished data). The number of subjects who developed at least one autoantibody was relatively small, and CIs for significant results under this outcome were wide, implying that further studies are needed to verify this association.

Because high-risk genetic factors and

Table 3—*Multivariate survival analysis examining maternal vitamin D intake via food during pregnancy and risk of IA and persistent IA*

Risk factors*	IA adjusted HR (95% CI)	Wald χ^2 *P*	Persistent IA adjusted HR (95% CI)	Wald χ^2 *P*
n	16		11	
Vitamin D intake via food (IU)	0.37 (0.17–0.78)	0.020	0.58 (0.27–1.28)†	0.205
First-degree type 1 diabetic relative	5.15 (1.45–18.28)	0.021	6.74 (1.62–28.03)	0.031
HLA-DR 3/4, DQ8 genotype	9.79 (1.69–56.83)	0.029	9.14 (1.50–55.85)	0.054
GDM	7.78 (1.83–33.03)	0.011	2.05 (0.22–19.02)	0.528
Child's ethnicity (% non-Hispanic Caucasian)	6.97 (0.88–55.46)	0.088	Not calculable‡	NA

*All variables entered into model simultaneously; †HR represents risk for a 155.6 IU SD difference in intake; ‡all affected were non-Hispanic Caucasian.

multiple appearances of islet autoantibodies on more than one occasion significantly influence progression toward type 1 diabetes (8), we explored an outcome defined with persistent IA (i.e., positive for an autoantibody on two consecutive occasions and still positive or type 1 diabetes at last visit). However, restricting our outcome reduced our already small affected group by >30%. The trend of protection with vitamin D via food remained, but statistical significance was not observed with this or any other exposure. This is likely due to the small number of persistent IA subjects, resulting in weak power to detect a significant difference between affected and unaffected groups.

We did not find a significant association between combined intake of EPA and DHA and risk of IA in offspring. The power to detect a significant difference between the affected and unaffected groups with respect to this exposure was limited. We had only 12% power to detect a significant result with respect to a combined intake of EPA and DHA given that 91% of the unaffected group reported intake of <0.10 g EPA and DHA daily. One concern regarding the EPA and DHA levels in our cohort was that pregnant women might consume too little fish to provide a valid estimate of EPA and DHA, considering women are advised against fish consumption during pregnancy due to increased risk of fetal mercury exposure. Indeed, the levels of EPA and DHA intake are slightly lower in our cohort than levels reported by other cohorts (30) for women aged 34–59 years using the same FFQ (median daily intake, 0.14 and 0.17 g, respectively). Therefore, additional studies in populations consuming more fish may be necessary to further address fish intake during pregnancy and IA in offspring.

Interestingly, we did not find an association between vitamin D intake via supplements and IA, which is similar to observations in another epidemiological study (21). The reasoning is not clear as to why we observed an association with vitamin D intake via food and not via supplements. The inconsistency could be due to residual confounding unaccounted for in the analysis in that women who take less than the recommended amount of vitamin D may be different from those who take the recommended amount or more. Alternatively, the inconsistency might be explained by differences in power due to differences in the distribution of the two variables, in that we had 48% power to obtain a significant result given that 90% of the unaffected group received at least 400 IU of vitamin D supplementation. However, these explanations are likely too simplistic considering the nonsignificant HR for the vitamin D supplement variable was in the opposite direction of the vitamin D intake via food variable. Perhaps in utero, the bioavailability of vitamin D may be greater when acquired through food, even if the food is simply fortified with vitamin D, as opposed to manufactured multivitamin supplements taken alone. Alternatively, an unidentified nutrient that is available in foods containing vitamin D or a combination of vitamin D and this nutrient could be responsible for the association between vitamin D via food and IA in offspring. To design effective prevention strategies, studies are needed to determine whether the protective factor is vitamin D itself, a nutrient that accompanies vitamin D in foods, or an interaction between vitamin D and another nutrient.

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