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, GAD₆₅, 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), zygotic twins (1), a 10% progression rate yet low concordance rates among mono-
causal factors for many substances.

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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.

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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 linoleic 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, GAD65, 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 nontype 1 diabetes 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,
with positive results for any of the antitave on all subsequent visits. Children at the 9-month blood draw and negativity due to transplacental transmission at least one occasion. We excluded positivity for one or more of the three islet autoantibodies on more strict definition of IA, which in- cluded only the 11 children who were au- toantibody positive on two consecutive visits and 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 cal- culated tertile values for each variable, then modeled the variable as an ordered categorical variable and as separate cate- gories 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 con- tinuous 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 asso- ciated 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 his- tory 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 in- crease in dietary vitamin D intake during pregnancy (vitamin D SD 155.6 IU). The remaining exposure variables were non- significant univariately and remained so after adjustment for relation to a type 1 diabetic first-degree relative, HLA-DR ge- notype, and GDM (data not shown). Ex- ploratory analysis of persistent IA/ diabetes suggested a protective effect of vitamin D via food, although the effect was lower in magnitude and nonsignif-
In utero diet and islet autoimmunity

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

<table>
<thead>
<tr>
<th>Parameters (mean daily intake)</th>
<th>Affected</th>
<th>Unaffected</th>
<th>Unadjusted HR (95% CI)</th>
<th>Wald χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>206</td>
<td>0.49 (0.26–0.94)*</td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>Vitamin D intake via food (IU)</td>
<td>167.6</td>
<td>252.3</td>
<td>3.09 (0.88–10.83)</td>
<td>0.107</td>
<td></td>
</tr>
<tr>
<td>Vitamin D intake via supplements (IU) ≥400</td>
<td>13 (81.3)</td>
<td>116 (56.3)</td>
<td>0.94 (0.24–3.73)</td>
<td>0.830</td>
<td></td>
</tr>
<tr>
<td>EPA and DHA (ω-3 fatty acids) (g) ≥0.10</td>
<td>9 (56.3)</td>
<td>137 (66.5)</td>
<td>1.64 (0.24–1.71)</td>
<td>0.830</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (ω-3 fatty acid) (g)</td>
<td>1.3</td>
<td>1.3</td>
<td>1.16 (0.75–1.80)*</td>
<td>0.503</td>
<td></td>
</tr>
<tr>
<td>Arachidonic acid (ω-6 fatty acid) (g)</td>
<td>0.13</td>
<td>0.14</td>
<td>0.88 (0.5–1.56)*</td>
<td>0.672</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (ω-6 fatty acid) (g) &lt;7.92</td>
<td>4 (25.0)</td>
<td>71 (34.5)</td>
<td>1.0 (referent)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7.92–11.78</td>
<td>8 (50.0)</td>
<td>66 (32.0)</td>
<td>2.01 (0.55–7.37)</td>
<td>0.274</td>
<td></td>
</tr>
<tr>
<td>&gt;11.78</td>
<td>4 (25.0)</td>
<td>69 (33.5)</td>
<td>1.04 (0.26–4.16)</td>
<td>0.955</td>
<td></td>
</tr>
<tr>
<td>Ratio of ω-6 total/ω-3 total</td>
<td>7.9</td>
<td>7.8</td>
<td>1.13 (0.70–1.83)*</td>
<td>0.624</td>
<td></td>
</tr>
<tr>
<td>Caloric intake (kcal) &lt;1,718.88</td>
<td>5 (31.2)</td>
<td>68 (33.0)</td>
<td>1.0 (referent)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1,718.88–2,491.04</td>
<td>9 (56.2)</td>
<td>67 (32.5)</td>
<td>1.61 (0.54–4.82)</td>
<td>0.405</td>
<td></td>
</tr>
<tr>
<td>≥2,491.04</td>
<td>2 (12.5)</td>
<td>71 (34.5)</td>
<td>0.37 (0.07–1.93)</td>
<td>0.258</td>
<td></td>
</tr>
</tbody>
</table>

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.

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
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 levels in our cohort was that pregnant women might consume too little 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 studies, 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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