Original Contribution

Antibiotic Use and Type 1 Diabetes in Childhood

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Indirect evidence is accumulating for an association between antibiotic use, especially in early childhood, and long-term immunologic health. The authors evaluated the association between antibiotic use in childhood and subsequent development of type 1 diabetes. A nationwide cohort study of all Danish singleton children born during 1995–2003 (n = 606,420) was conducted. Incidence rate ratios for type 1 diabetes comparing children according to antibiotic use were estimated. Antibiotic use was classified according to class, number of uses, and age at use. Use of any antibiotic was not associated with type 1 diabetes (rate ratio = 1.16, 95% confidence interval: 0.91, 1.50). Evaluation of type 1 diabetes risk according to number of courses of any antibiotic yielded no association between antibiotic use and type 1 diabetes, with an increase in rate ratio per course of 1.02 (95% confidence interval: 0.97, 1.07). No specific class of antibiotics was associated with type 1 diabetes, no specific age of use was associated with type 1 diabetes, and no specific age at onset of type 1 diabetes was associated with antibiotics. In a large nationwide prospective study, no association between antibiotic use and type 1 diabetes was found among Danish children.

anti-bacterial agents; diabetes mellitus, type 1; drug toxicity

Abbreviations: CI, confidence interval; RR, rate ratio.

Research hinting at a potential association between antibiotic use in childhood and immunologic health is accumulating (1–3). A commonly suggested mechanism by which antibiotic use in childhood could influence immunologic health is the well-known effect on the composition of the gut microflora (4). The gut microflora, in turn, interacts closely with the gut immune system and is likely to be an important factor in the development and maturation of the gut immune system (1). A dysfunctioning gut immune system has been proposed to play a role in the pathogenesis of type 1 diabetes (5). Antibiotics are some of the most common pharmaceuticals used in childhood, second perhaps only to vaccines, and type 1 diabetes is the most common autoimmune disease in childhood. A small number of controlled epidemiologic studies exist of the potential association between antibiotic use in childhood and type 1 diabetes (6–9), with some of them indicating an increased risk (6, 9). However, these studies have all been case-control studies, and most of them have focused on infections and vaccinations and have not analyzed the association in any detail. We conducted a population-based cohort study of the potential association between antibiotic use and type 1 diabetes in Danish children. We evaluated the association in detail, taking into account the type of antibiotic used, number of courses used, and age at use.

MATERIALS AND METHODS

The Danish Central Person Registry was introduced in 1968 and contains vital and demographic information on all people living in Denmark (10). This information is accessed via a unique personal identification number (the Central Person Registry number) that is also used in other nationwide registries. We constructed a cohort of all singleton children born in Denmark during 1995–2003. Using the Central Person Registry number, we were able to link information on antibiotics use, type 1 diabetes, and potential confounding variables to the cohort children.

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Type 1 diabetes

Cases of type 1 diabetes among cohort children in the period 1995–2004 were ascertained through the Danish National Hospital Registry (11). This registry contains diagnoses-specific information (coded by using the International Classification of Diseases, 10th Revision) on all hospitalizations, emergency room visits, and outpatient visits in Denmark. We used the code, E10, for insulin-dependent diabetes mellitus.

Antibiotic use

The nationwide Danish drug prescription registry was established in 1994 and includes detailed individual-level information on all prescriptions filled at pharmacies and aggregated data on hospital use—the only places where prescription medication is legally available in Denmark (12). Information on individual-level prescriptions includes the Central Person Registry number of the recipient, the date of filling the prescription, the Anatomical Therapeutic Chemical classification system code, and the number of daily defined doses in the prescription. The date of filling the prescription was considered the date of use for the purpose of this study. We obtained information on all systemic antibiotic use (Anatomical Therapeutic Chemical code J01) for the cohort children in the period 1995–2004. For some children (primarily in the 1995–1996 period), antibiotic use was not registered by using their own Central Person Registry number. In this case, a variable in the registry denoted the age of the receiving child, and all antibiotic use-ascribed Central Person Registry numbers of mothers and fathers of cohort children were ascribed to the cohort children. Antibiotics were classified accordingly: extended-spectrum penicillins, penicillin V, macrolides, and other systemic antibiotics. Use was quantified as courses, with all further use of the same class of antibiotics within 1 month of the first use ascribed to the same course. To be able to assess the effect of hospitalization use of antibiotics, we obtained information from the Danish National Hospital Registry on hospitalizations with serious bacterial infections, where antibiotics are highly likely to have been used for treatment, for all cohort children. The infections included were pyelonephritis, osteomyelitis, bacterial meningitis, bacterial pneumonia, and septicemia.

Potential confounding factors

Information on the following potential confounding factors was obtained from the Central Person Registry, the Danish Medical Birth Registry (13), and Statistics Denmark: child’s sex, birth order (1, 2, 3, ≥4), place of birth classified according to degree of urbanization (Copenhagen, Copenhagen suburbs, area with ≥100,000 population, area with 10,000–99,999 population, area with <10,000 population), ethnicity of the mother (Danish or not), mother’s age at birth (<20, 20–24, 25–29, 30–34, 35–39, ≥40 years), birth weight (<2,500, 2,500–2,999, 3,000–3,499, 3,500–3,999, ≥4,000 g), gestational age (<34, 34–36, 37–39, 40–41, ≥42 weeks), socioeconomic category of the father in the year preceding the year of birth (outside labor market, employment with unknown qualifications, employment with basic or no qualifications, employment with medium-level qualifications, top management or employment with high-level qualifications, self-employed or coworking spouse), and the educational level of the mother in the year preceding the year of birth (compulsory school, secondary school, vocational training or short tertiary education, medium or long tertiary education). The percentages of missing values for the variables, place of birth, birth weight, gestational age, ethnicity of the mother, socioeconomic category, and educational level, were 5.6%, 6.7%, 6.5%, 0.01%, 5.5%, and 9.3%, respectively.

Statistical analysis

Children in our cohort were followed for type 1 diabetes from 1 year of age until death, disappearance, emigration, type 1 diabetes, or January 1, 2005, whichever occurred first. The resulting person-years of follow-up were aggregated together with counts of type 1 diabetes according to antibiotic use and were analyzed by using Poisson regression (log-linear regression of the counts using the logarithm of the follow-up time as offset) (14). This produced incidence rate ratios according to antibiotic use. The number of courses of antibiotic use was considered a time-varying variable. The effect of antibiotic use on type 1 diabetes was evaluated through 2 effect measures: 1) rate ratios comparing type 1 diabetes incidence rates among antibiotic ever-users and never-users and 2) increases in rate ratios per course of antibiotics (i.e., a dose-response effect measure). Effect measures were always adjusted for age (in 1-year intervals) and calendar period (in 1-year intervals). The variables defined in the “Potential confounding factors” section above were included in the models if they were associated with type 1 diabetes. When adjusting for the potential confounding effect of variables with missing values, we used mode imputation. Multiple imputation using a Markov chain Monte Carlo approach (15) was also tested (data not shown). However, the change in estimate between this method and mode imputation proved negligible (<1%), and mode imputation was used for computational simplicity.

Ethics

Ethics approval was not required.

RESULTS

We included a total of 606,420 singleton children in our cohort. During 2,758,264 person-years of follow-up for type 1 diabetes, we identified 454 cases. The mean age of first type 1 diabetes hospital contact was 4.4 years (standard deviation = 2.3). Follow-up was terminated prematurely for 13,185 children because of death (n = 614), emigration (n = 12,169), or disappearance (n = 402).

A total of 538,298 children received at least 1 course of antibiotics, and the remaining 68,122 children accordingly received no courses. The average number of courses among antibiotics-exposed children was 4.8 courses (standard
deviation = 3.7). The average number of prescriptions was 5.6 (standard deviation = 4.7).

In Table 1, we present antibiotic use in the cohort children according to sex, age, and calendar period. Penicillin V was the most commonly used type of antibiotic (incidence rate = 292.6 courses per 1,000 person-years), followed closely by extended-spectrum penicillins (incidence rate = 288.3 per 1,000 person-years). Macrolides were the third most used type (incidence rate = 73.9 per 1,000 person-years). Other types of antibiotics saw little use in this cohort. Boys used more antibiotics than did girls (incidence rate ratio (RR) = 1.11, 95% confidence interval (CI): 1.11, 1.12). The age-specific incidence rates were lowest in the first period of life (incidence rate = 62.0 per 1,000 person-years) and type 1 diabetes risk; for example, the increase in rate ratio per course of extended-spectrum penicillin in the first 3 months of life was 0.99 (95% CI: 0.32, 2.25).

In Table 2, we present type 1 diabetes effect measures according to type of antibiotic used and number of courses. Antibiotic use in any specific age categories of the number of courses (1–2, 3–5, 6–10, and type of antibiotic. Antibiotic use in any specific age period, independent of type, was not associated with type 1 diabetes; for example, the increase in rate ratio per course of extended-spectrum penicillin in the first 3 months of life was 0.86 (95% CI: 0.32, 2.25).

In Table 3, we present increases in type 1 diabetes rate ratios per course according to the age-specific period of use and type of antibiotic. Antibiotic use in any specific age period, independent of type, was not associated with type 1 diabetes for example, the increase in rate ratio per course of extended-spectrum penicillin in the first 3 months of life was 0.86 (95% CI: 0.32, 2.25).

In Table 4, we present increases in type 1 diabetes rate ratios per course according to type of antibiotic and current age. The effect of antibiotic use, independent of type, was similar in all age groups (test of homogeneity for all types of antibiotics: \( P = 0.8124 \)). The use of macrolides was associated with type 1 diabetes in children 5–6 years of age, with an increase in rate ratio per course of macrolides of 1.15 (95% CI: 1.03, 1.38). However, the macrolide effect independent of current age was not associated with type 1 diabetes (increase in rate ratio per course = 1.15, 95% CI: 0.92, 1.43) and, in a test of homogeneity, the effect of macrolides was not different between age groups (\( P = 0.1237 \)). Given the direction of the results overall and the number of statistical tests performed in this study, we consider this 1 statistically significant association to be a chance finding. We estimated type 1 diabetes rate ratios according to categories of the number of courses (1–2, 3–5, 6–10, and type of antibiotic.
The increase in rate ratio per course, including further adjustment (other than age, calendar period, and maternal ethnicity) for child’s sex, birth order, place of birth, mother's age at birth, birth weight, gestational age, socioeconomic category of the father, and educational level of the mother, was 1.01 (95% CI: 0.97, 1.05)—almost identical to the estimate that included adjustment for only age, calendar period, and maternal ethnicity.

A number of further analyses were performed to evaluate the robustness of our results. Including only children with 2 or more type 1 diabetes hospitalizations as type 1 diabetes cases (n = 286) had little impact on the effect estimates (increase in RR per course = 1.04, 95% CI: 0.98, 1.11). The exclusion of children born during 1995–1996 when antibiotic prescriptions were likely to have been registered through parents had little impact on the effect measures (increase in RR per course = 1.02, 95% CI: 0.96, 1.09).

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Following the children for type 1 diabetes from birth instead of from 1 year of age had little impact on the results (increase in RR per course = 1.02, 95% CI: 0.98, 1.07). Changing the definition of 1 course of antibiotics to include only use within 14 days instead of 30 days had little impact on the effect measures (increase in RR per course = 1.02, 95% CI: 0.97, 1.07). Excluding never-users when estimating the increase in rate ratio per course had little impact on the effect estimates (increase in RR per course = 1.01, 95% CI: 0.95, 1.07).

**DISCUSSION**

We found no evidence of an association between antibiotic use and type 1 diabetes in childhood. This was true
Exposure to antibiotics was determined through a nationwide prescription drug registry. In Denmark, antibiotic exposure occurs legally through hospitalizations and through prescriptions. A limitation of our study is the failure to take hospitalization exposure into account directly. We indirectly evaluated this unmeasured exposure by including hospitalizations with serious bacterial infections, where antibiotics are highly likely to have been used, as an exposure proxy. We found no effect of this exposure category on type 1 diabetes risk. Exposure to antibiotics outside the hospital setting and the pharmacy setting is likely to be limited, especially among children, and exclusion of children classified as unexposed in this study when evaluating the dose-response between antibiotics and type 1 diabetes had little impact on our results. In a study by Muscat et al. (18) of self-medication with antibiotics in Denmark, only 1 of 164 antibiotic uses in children was from self-medication, specifically, leftover antibiotics.

A limitation in any study relying on prescription registries for drug use exposure is the potential discrepancy between filled and used prescriptions and the discrepancy between date of filling a prescription and date of use, resulting in the overestimation of antibiotic-exposed person-years of follow-up. However, because antibiotics are indicated for an acute event, an infection, such overestimation is likely to be limited.

We included a wide range of potential confounders (other than age and calendar period) in our study. However, no confounders could be identified, and fully adjusted results were almost identical to age- and calendar period-adjusted results, providing reassurance for the negligible impact of confounding in this study.

Other studies

It is well established that antibiotic use, especially use of extended-spectrum antibiotics, can modify the gut microbiota in humans, at least temporarily, and most pronounced in infancy and early childhood (4). Evidence for a subsequent effect of this on the gut immune system and systemic immunologic health is accumulating (1). Despite this, few controlled epidemiologic studies exist of the possible association between antibiotic use and long-term immunologic health. A number of studies have looked at antibiotic use and asthma, but results have been conflicting (19). A small number of studies have looked at antibiotic use and type 1 diabetes (6–9). Blom et al. (6) found that recent use of antibiotics was more common in cases than in controls. Use in infancy was similar in cases and controls, and the authors suggested that the observed association with recent use was due to confounding by indication. The EURODIAB Substudy 2 Study Group conducted a large case-control study of infections, vaccinations, and antibiotic use and type 1 diabetes (7). Antibiotic treatment evaluated through maternal recall or hospital notes was not significantly increased in cases compared with controls. Kilkkinen et al. (9) conducted a case-control study of both maternal use and use in childhood. Maternal use before pregnancy and a large number of courses during childhood (≥7 courses) were more common in cases than in controls. Cardwell et al. (8) conducted a case-control study of infections, vaccinations, and

Table 4. Increase in Type 1 Diabetes Rate Ratios per Antibiotic Use and Current Age Among Danish Children Born During 1995–2003 and Followed From 1 Year of Age Until January 1, 2005

<table>
<thead>
<tr>
<th>Type of Antibiotics Used and Current Age</th>
<th>Type 1 Diabetes</th>
<th>Rate Ratio</th>
<th>95% Confidence Interval</th>
<th>P_homogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td>0.8124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 years</td>
<td></td>
<td>0.98</td>
<td>0.89, 1.09</td>
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<tr>
<td>3–4 years</td>
<td></td>
<td>1.02</td>
<td>0.93, 1.12</td>
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<tr>
<td>5–6 years</td>
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<td>1.04</td>
<td>0.96, 1.14</td>
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<tr>
<td>≥7 years</td>
<td></td>
<td>1.03</td>
<td>0.93, 1.16</td>
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<tr>
<td>Penicillin V</td>
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<td></td>
<td></td>
<td>0.9036</td>
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<tr>
<td>1–2 years</td>
<td></td>
<td>0.95</td>
<td>0.80, 1.13</td>
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<tr>
<td>3–4 years</td>
<td></td>
<td>1.01</td>
<td>0.88, 1.15</td>
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<tr>
<td>5–6 years</td>
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<td>1.03</td>
<td>0.92, 1.15</td>
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<tr>
<td>≥7 years</td>
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<td>1.04</td>
<td>0.92, 1.17</td>
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<tr>
<td>Extended-spectrum penicillins</td>
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<td>0.7619</td>
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<tr>
<td>1–2 years</td>
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<td>1.05</td>
<td>0.93, 1.18</td>
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<tr>
<td>3–4 years</td>
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<td>1.01</td>
<td>0.91, 1.13</td>
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<td>1.05</td>
<td>0.96, 1.16</td>
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<tr>
<td>≥7 years</td>
<td></td>
<td>0.98</td>
<td>0.86, 1.10</td>
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<tr>
<td>Macrolides</td>
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<td>0.1237</td>
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<td>1–2 years</td>
<td></td>
<td>0.94</td>
<td>0.70, 1.26</td>
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<td>3–4 years</td>
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<td>0.84</td>
<td>0.63, 1.10</td>
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<td>1.20</td>
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<td>≥7 years</td>
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<td>1.09</td>
<td>0.87, 1.29</td>
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<tr>
<td>Other systemic antibiotics</td>
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<td>0.5477</td>
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<tr>
<td>1–2 years</td>
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<td>0.78</td>
<td>0.39, 1.55</td>
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<td>1.19</td>
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<td>5–6 years</td>
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<td></td>
<td>0.76</td>
<td>0.47, 1.23</td>
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</table>

a Adjusted for age, calendar period, and ethnicity of mother.

b Rate ratio not estimated for “hospitalization use” because of lack of cases in every age stratum.

Strengths and weaknesses

In any observational study, the potential impact of bias and confounding must be considered carefully. We identified cases through a nationwide hospitalization discharge registry. Nielsen et al. (16) evaluated the validity of type 1 diabetes diagnoses in this registry and found a specificity of 96% and a completeness of 91%. Similar validity results were obtained from a comparison of the hospitalization registry and a registry specific for type 1 diabetes in childhood (17). Increasing the specificity of case ascertainment in this study by including only cases with at least 2 type 1 diabetes hospitalizations had little impact on our results.

antibiotic use in early life and type 1 diabetes. Antibiotic use was similar in cases and controls. A number of animal studies of antibiotic use and type 1 diabetes have been conducted. Brugman et al. (20) found that the later development of type 1 diabetes in diabetes-prone rats was significantly associated with the composition of the intestinal flora. Furthermore, modulation of the intestinal flora through antibiotic treatment decreased the incidence and delayed the onset of type 1 diabetes in diabetes-prone rats. Schwartz et al. (21) observed similar results: Antibiotics significantly decreased the incidence of type 1 diabetes, this time in a mouse model. These results interestingly suggest that, under certain conditions, antigenic load in the gut can modify long-term immunologic health in animal models. Our results do not support the notion that these results have any clinical relevance in human children. However, our study was not designed to specifically test these hypotheses.

Public health importance

The current approach to drug safety has a number of limitations with respect to the long-term health effects of drug use in childhood. First, the duration of prelicensure clinical trials is limited. Second, little prelicensure testing has historically been conducted in children even though the growing and evolving nature of children presents more possibilities for adverse effects. Third, the spontaneous reporting systems of the postlicensure period can detect signals only when temporally related to drug use, that is, acute events. There is a clear need for large, analytical studies of the long-term health effects of drug use in susceptible groups, such as children (22), and especially so for drugs used as prevalently as antibiotics and for common chronic diseases, such as type 1 diabetes.

In conclusion, in a large, nationwide prospective study, we found no support for an association between antibiotic use and type 1 diabetes among Danish children.

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