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Long-term use of antibiotics and risk of type 2 diabetes in women: a prospective cohort study

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Abstract

Background: Accumulating evidence suggested that long-term antibiotic use may alter the gut microbiome which has, in turn, been linked to type 2 diabetes. We undertook this study to investigate whether antibiotic use was associated with increased risk of type 2 diabetes.

Methods: This prospective cohort study included women free of diabetes, cardiovascular disease and cancer in the Nurses' Health Study (NHS 2008-2014) and NHS II (2009-2017). We evaluated the overall duration of antibiotics use in the past four years and subsequent diabetes risk with COX proportional hazards regression adjusting for demography, family history of diabetes, and lifestyle factors.

Results: Pooled analyses of NHS and NHS II (2837 cases, 703 934 person-years) revealed that a longer duration of antibiotic use in the past 4 years was associated with higher risk of diabetes (Trend $_{coefficient}$ = 0.09, 95%CI 0.04 to 0.13). Participants who received antibiotics treatment for a medium duration of 15 days to 2 months (HR 1.23, 95% CI 1.10 to 1.39) or long duration of over 2 months (HR 1.20, 95% CI 1.02 to 1.38) had higher risk of type 2 diabetes as compared with non-users. Subgroup analyses suggested that the associations were unlikely to be modified by age, family history of diabetes, obesity, smoking, alcohol drinking, physical activity, and overall diet quality.

Conclusions: A longer duration of antibiotic use in recent years was associated with increased risk of type 2 diabetes in women. Physicians should exercise caution when prescribing antibiotics particularly for long-term use.

Key words: Antibiotics, type 2 diabetes, prospective cohort study, gut microbiota, Nurses' Health Studies

Key Messages

- Accumulating evidence suggested that long-term antibiotic use may alter the gut microbiome which has, in turn, been linked to type 2 diabetes.

- This prospective cohort study showed that a longer duration of antibiotic use in recent years was associated with increased risk of type 2 diabetes in women.

- Physicians should exercise caution when prescribing antibiotics particularly for long-term use.

Introduction

Type 2 diabetes has become a global epidemic over the past few decades. The global

prevalence of diabetes has nearly doubled, rising from 4.7% in 1980 to 8.5% in 2014.[1] The

etiology of type 2 diabetes is complex, involving multiple genetic, behavioral and

environmental factors.[2] In recent years, researchers have turned their attention to the role of

human gut microbiota, which is essential for key metabolic processes such as energy

harvesting, production of neurotransmitters and hormones, and nutrient storage, in the

development of diabetes.[3,4] Accumulating studies support a causal role for the gut microbiota

in metabolic diseases.[5,6] The latest findings from the Integrative Human Microbiome Project

(iHMP) have revealed insights into pathways and responses that differ between

glucose-dysregulated and healthy individuals during health and disease.[7]

Antibiotics are one of the most widely prescribed medications, with a global consumption of

54 billion standard units in 2000 and 73 billion units in 2010.[8] Despite the irreplaceable role,

antibiotics may alter the gut microbiome and host health. A previous study showed that

broad-spectrum antibiotics could affect the abundances of 30% of the bacteria in the gut

community, causing rapid drops in taxonomic richness, diversity, and evenness.[7] The

alterations can persist for years; In some cases, the initial composition is never completely

recovered.[9,10] Considering the impact on gut microbiota, a number of studies investigated

antibiotics use and the development of metabolic disorders, and suggested that antibiotic use

could increase the risk of obesity [11,12] and diabetes.[13-15]

Although a number of observational studies have investigated antibiotics use and type 2

diabetes risk,[13-16] the relationship remains unclear. First, the results of these studies were

inconsistent showing either a positive[13-15] or a null association.[16] Second, since previous studies were carried out in general population which have a high rate of undiagnosed diabetes,[17] they cannot rule out the possibility that the positive association shown in these studies was because the participants seeking antibiotic treatment were more likely to have previously unrecognized with diabetes.[14,15] Finally, a large population-based, prospective cohort study is the most appropriate study design for investigating diabetes risk factors, but such a study investigating the effect of anbiotics is still lacking. In order to address these limitations, we evaluated antibiotic use and risk of type 2 diabetes among women enrolled in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS II).

Methods

Study population

The NHS is an ongoing prospective cohort study, beginning in 1976 and enrolling of 121 700 female nurses from 11 U.S. states aged 30 to 55 years. The NHS II, established in 1989, originally included 116 430 younger female registered nurses who were between the ages of 25 to 42 years from 14 states in the U.S.. The participants receive a biennial questionnaire since baseline to collect data on demographics, health-related behaviors, medical history, and newly diagnosed diseases, with a follow-up rate over 90% for each questionnaire cycle. The recruitment and data collection in NHS and NHS II are detailed previously.[18] The NHS and NHS II were approved by the Human Research Committee at the Brigham and Women's Hospital, Boston, MA. The study protocol was approved by the institutional review board (IRB) of the Brigham and Women's Hospital, and the IRB allowed participants' completion of

questionnaires to be considered as implied consent.

Previous studies have investigated antibiotic use and risk of colorectal adenoma,[19] all-cause and cause-specific mortality [20] using the NHS or NHS II dataset. For the current analysis, we restricted the participants to those without a diagnosis of diabetes (including type 1, type 2, and gestational diabetes). We also excluded the participants who had missing data on antibiotic use. (see Figure 1) The baseline was 2008 for NHS and 2009 for NHS II when data about antibiotic use in the past four years were collected. The end of follow-up was 2014 for NHS and 2017 for NHS II when the latest ascertainment of diabetes was carried out. Those who did not respond to any of the questionnaire cycles between 2008 and 2017 were considered as being lost to follow-up.

Assessment of antibiotic use

In 2008 and 2009, NHS and NHS II were asked about their total amount of time using antibiotics (excluding skin creams, mouthwash or isoniazid) during the past 4 years (none, 1–14 days, 15 days–2 months, 2–4 months, 4 months–2 years, 2–3 years, or over 3 years). The most common reason for using an antibiotic (respiratory infection, urinary tract infection, acne/rosacea, chronic bronchitis, dental and other reason) were collected in both cohorts.

Ascertainment of type 2 diabetes

On the subsequent biennial questionnaires, the participants were asked if they had ever been diagnosed with diabetes. Participants reporting physician-diagnosed type 2 diabetes were mailed a validated supplementary questionnaire regarding symptoms, diagnostic tests and

hypoglycaemic therapy to confirm self-reported diagnoses. Confirmed diabetes requires at least one of the following reported on the supplementary questionnaire in accordance with the American Diabetes Association criteria: 1) one or more classic symptoms (excessive thirst, polyuria, weight loss, hunger, pruritus, or coma) plus fasting plasma glucose (PG) ³126 mg/dl (7.0 mmol/L) or random PG ³200 mg/dl (11.1 mmol/L); 2) at least two elevated PG levels on different occasions (fasting PG³140 mg/dl and/or random PG³200 and/or PG³200 at ³2 hours on oral glucose tolerance testing) in the absence of symptoms; or 3) treatment with hypoglycemic medication (insulin or oral hypoglycemic agent). Two separate studies have shown high validity of self-reported type 2 diabetes diagnosis (>97%) through medical record reviews.[21,22]

Assessment of covariates

We selected covariates that may confound the association between antibiotics use and type 2 diabetes risk based on a literature review. In the baseline and follow-up questionnaires, we obtained updated information on age, ethnicity, marital status, living status, family history of diabetes, body mass index (BMI), smoking status, alcohol intake, menopausal status and postmenopausal hormone use, and history of hypertension and hypercholesterolemia. We calculated the 2010 Alternative Healthy Eating Index (AHEI-2010) to assess overall diet quality. Physical activity was measured by weekly expenditure of metabolic equivalents (METs) which has been validated in a previous study.[23]

Statistical analysis

This study calculated person-years from the date of return of the baseline questionnaire to the

date of diagnosis of diabetes, death, or the end of follow-up, whichever came first. We

evaluated the hazard ratios (HRs) and 95% confidence intervals (CIs) with multivariable

time-dependent proportional hazards models accounting for potential time-varying effects in

covariates. We stratified the analyses jointly by age (in months) and the year that the

questionnaire was returned. The participants were classified into non-use, short-term use

(1–14 days), medium-term use (15 days–2 months), and long-term use (2+ months) group,

taking the non-use group as the reference. We considered the antibiotic use duration as

categorical variables in the primary analysis and tested for trend by modeling the duration as

an ordinal variable (non-use:1, 1–14 days: 2, 15 days–2 months: 3, and 2+ months: 4). To

present the association in a clinically useful way, we calculated number needed to harm

(NNH) with the method described by Altman D.G.[24]

We generated missing covariate data (BMI, physical activity, Alternate Health Eating Index,

Menopausal status and postmenopausal hormone use, smoking, and days with alcohol

drinking per week) with multiple imputation. Five imputations were generated by the fully

conditional specification (FCS) method.[25] We evaluated potential non-linear associations

between continuous covariates and diabetes risk with cubic splines.[26] In the

multivariable-adjusted model 1, we controlled for ethnicity (*white, or other*), marital status

(*married, divorced, widowed, others*), living status (*alone, with families, or other*), history of

diabetes in a first-degree relative (*yes, or no*), BMI (*continuous*), menopausal status and

postmenopausal hormone use *(premenopausal, postmenopausal (never, past, or current*

menopausal hormone use). To control the potential confounding in lifestyle factors, we

further adjusted smoking status (*never, past, current*), days with alcohol drinking per week (*0,*

1-3, over 3days), physical activity (*continuous, MET-h/week*), and overall diet quality

(*continuous, AHEI score*) to the multivariable-adjusted model 2. We carried out regression in

NHS and NHS II separately and then pooled the effects with inverse variance weighted,

random effect meta-analyses. We tested the heterogeneity of associations between the two

cohorts using the Cochrane Q statistic and the I^2 statistic.

To verify potential interaction effects, we undertook subgroup analysis according to age,

family type 2 diabetes history, BMI, smoking, alcohol drinking, physical activity, and AHEI

score. We tested interactions by including interaction terms in the fully-adjusted Cox

regression model. To control potential confounding by the indication of antibiotics use, we

did sensitivity analyses by additionally adjusting for two surrogate indicators, including

rheumatoid arthritis and depression, which have been shown to be associated with increased

risk of infection.[27,28] To investigate the potential bias from healthcare utilization (i.e. the

participants with better healthcare utilization are likely to have a better access to antibiotics

and lower chance to be undiagnosed if they had diabetes), we adjusted physical examination

in the previous 2 years (*yes or no*) as a surrogate indicator. As hypertension and elevated

cholesterolemia may partially mediate or confound the effect of antibiotic on diabetes, we

adjusted for these covariates to evaluate the controlled direct effect or adjusted effect. We also

adjusted parity to investigate potential residual confounding effects. We performed the

analyses using SAS software, version 9.4 (SAS Institute, Cary, North Carolina, USA).

Results

This study included 114 210 participants, of which 50 810 were from NHS and 63 400 were from NHS II (Figure 1). Table 1 presents the baseline participant characteristics according to the duration of antibiotic use in the past four years. The participants with longer antibiotic use were more likely to have a higher BMI and higher rate of current postmenopausal hormone use.

We documented 1031 cases of type 2 diabetes during 260 765 person-years of follow-up in NHS and 1806 cases during 443 169 person-years in NHS II. The annual incidence of type 2 diabetes was 4.07/1000 person in NHS and 3.95/1000 person in NHS II. In the NHS, the adjusted HRs was 1.14(95%CI 0.96 to 1.37) for the medium-term use group (15 days–2 months) and 1.17 (95%CI 0.91 to 1.50) for the long-term use group (2+ months) as compared with non-users (Table 2). In the NHS II, we observed a dose-response relationship between antibiotic use duration and type 2 diabetes risk (Trend-coefficient = 0.10, 95%CI% 0.05 to 0.15). The HR in the fully adjusted multivariable models was 1.30 (95%CI 1.13 to 1.49) for the medium-term use group and 1.20 (95%CI 1.00 to 1.45) for the long-term antibiotic use group, as compared with the non-use group. In the meta-analysis of the two cohorts, the duration of antibiotic use was positively associated with diabetes risk (Trend-coefficient = 0.09, 95%CI% 0.04 to 0.13) with no major heterogeneity ($I^2 < 25\%$). Overall, the medium-term use group (HR 1.23, 95%CI 1.10 to 1.39) and long-term use group (HR 1.20, 95%CI 1.02 to 1.3) had higher risk of type 2 diabetes as compared with non-users. In the analyses limiting participants in those with complete covariates data, we observed no substantial changes in primary results.

To present the associations in a clinically useful way, we calculated NNHs based on the estimated HRs and incidence rate of type 2 diabetes in control group (*Supplementary Table S1*). Over 5 years, 102.6 (95%CI, 79.3 to 207.4) persons were needed to use antibiotics for 15 days - 2 months, and 114.7 (95%CI, 80.8 to 551.8) persons were needed to use antibiotics for 2+ months, to cause one case of type 2 diabetes.

To investigate the potential influence of the underlying infection for using antibiotics, we evaluated the HRs according to the primary reasons for using antibiotics (*Supplementary Table S2*). Taking respiratory infections as the reference, urinary tract infection (HR 1.01, 95% CI 0.70 to1.44), dental infection (HR 1.06, 95% CI 0.92, 1.23) and other infections (HR 1.00, 95% CI 0.90 to1.11) did not associate with different risk of type 2 diabetes risk. To confirm the independent effect of antibiotic use on type 2 diabetes risk, we additionally adjusted the primary reasons for using antibiotics in the regression model (*Supplementary Table S3*). Medium antibiotic use (HR 1.20, 95%CI 0.99 to 1.46) and long-term antibiotic use (HR 1.22, 95%CI 1.06 to 1.40) were associated with higher risk of diabetes as compared with short-term antibiotic users.

Table 3 shows the stratified analyses of antibiotic use in the past 4 years and type 2 diabetes risk. The associations between antibiotic use and risk of diabetes were unlikely modified by age, family diabetes history, BMI, smoking, alcohol drinking, physical activity, and AHEI score.

In the sensitivity analyses by additional adjustment for rheumatoid arthritis and depression, we observed no substantial changes in primary results (*Supplementary Table S4*). The results

were stable after additional adjustment for physical examination in the past 2 years and number of parity.

Discussion

In this prospective cohort of women, recent medium- and long-term antibiotic use was associated with an increased risk of type 2 diabetes, independent of traditional diabetes risk factors including demographic factors, family history of diabetes, obesity, and lifestyle factors. The association between antibiotic use and type 2 diabetes risk were unlikely to be modified by age, family history of diabetes, obesity, and lifestyle factors.

We did a systematic literature search and identified four observational studies that evaluated antibiotics and risk of diabetes (see the summary of these studies in th*e Supplementary Table S5*), of which three studies found a positive association.[13-15] A nested case-control study of 208 002 diabetic cases and 815 576 matched controls showed an association between past antibiotic exposure and diabetes risk.[14] In a nationwide register-based study of 170 504 cases and 1 364 007 matched controls from Denmark, the authors found an increased risk of type 2 diabetes with increasing intake of antibiotics. Increased use of antibiotics in type 2 diabetes patients was found up to 15 years before diagnosis of diabetes.[15] Despite the large sample size, the results of these two studies might be confounded by lifestyle factors and family history of diabetes. A retrospective cohort study of 14 361 veterans showed that one or more prescriptions of antibiotics, was associated with increased risk of diabetes (HR=1.13) when

compared to the non-prescription group. The individual class of antibiotics with significantly increased risk included cephalosporin, macrolide, and penicillin.[13] In a relatively small nested case-control study from the Alberta's Tomorrow Project (1 676 cases and 13 401 controls), the authors found no significant association after adjustment for clinical and lifestyle factors.[16] Important confounding factors in the Alberta's Tomorrow Project study have been controlled in our study and we still observed a positive association. Possible explanations for the inconsistency included different study design, participant characteristics, and study sample size.

One concern for the positive association is that the participants with undiagnosed type 2 diabetes have increased demand for antibiotics because they are more prone to develop infections.[29] Lacking of universal blood glucose screening may also influence the confirmation of study outcome. However, the influence would be minor because: 1) the study population are nurses who have rich medical knowledge and high awareness of diabetes symptoms for themselves; 2) US nurses have high frequency of blood glucose test since most of them have primary care provider; and 3) we adjusted physical examination in the previous 2 years and found no major change in the primary results.

The mechanism between antibiotic use and type 2 diabetes risk is still unclear. Accumulating evidence suggests that long-term use of antibiotics can lead a profound impact on gut microbiota[1,9,10,30] while gut microbiota play a causal role in metabolic disease development,[4-6,31] thus, it's reasonable to link antibiotics use with type 2 diabetes. Animal

models have shown that antibiotics may alter insulin sensitivity and glucose tolerance, as well as enhance lipid deposition by altering the gut microbiota composition.[32-34] In human, a randomized trial has shown that oral vancomycin significantly decreased intestinal microbiota diversity, bile acid dihydroxylation and peripheral insulin sensitivity in subjects with metabolic syndrome.[35] Furthermore, many antibiotics, such as fluoroquinolones and pentamidine may have increased risk of dysglycemia independently of the gut microbiota.[36-38]

One of our strengths is that this study was based on two well-established prospective cohorts with reasonably large sample sizes and high follow-up rates. The participants were nurses who were able to provide complete and accurate health information. Additionally, we comprehensively controlled for traditional diabetes risk factors, which minimized the potential confounding effect. Robust sensitivity analyses and the clear dose-response relationship also increased our confidence in the results.

This study has limitations. First, our results may be influenced by immortal time bias because at study entry, eligible participants should be free of diabetes during the past 4 years. However, the potential influence would be minor because 1) the rates of type 2 diabetes in the past four years were also increased with total duration of antibiotic use (NHS: None 2.33%, 1- 14 days 2.93%, 15 days - 2 months 3.28%, 2+ months 3.79%; NHS II: None 1.62%, 1- 14 days 1.98%, 15 days - 2 months 2.86%, 2+ months 3.82%), 2) the start of follow-up was after the end of immortal time period, which is a proposed approach to prevent immortal time bias.[39] Second, as an observational study, this study could not confirm a causal effect of

antibiotic use on type 2 diabetes. Third, the association between antibiotic use and diabetes risk may be confounded by the underlying infection. However, our analyses suggested that the risk of type 2 diabetes was similar among different types of infections, and the risk still increased with the duration of antibiotic use after additionally adjusting for the primary reason for using antibiotics. Forth, long-term antibiotic exposures may differ qualitatively from short term exposures. Though we have adjusted for the reasons and compared the risk of type 2 diabetes among different type of infection, we cannot rule out this concern because 1) the exact reason for using antibiotics was not collected, and 2) we cannot differentiate whether the participant used antibiotic one time over 2 months, or used antibiotic multiple times for difference reasons with a accumulating duration over 2 months. Fifth, our conclusion may be influence by recall bias. However, the influence would be minor as the participants are health professionals who are well-educated and familiar with personal antibiotic use. In addition, report of antibiotics use is expected to be non-differential to diabetes diagnosis, because the participants were unlikely to know there was association between antibiotics use and risk of diabetes (the first report of this association was published in 2015 while the time for collecting data about antibiotics use was much earlier (2008-2009)). Sixth, we were unable to evaluate the effect of specific class of antibiotics as such data were not collected. However, we did not find major differences according to the primary reasons for antibiotic use, which are closely associated with the type of prescription. Last, all included participants were women so the findings may be not applicable to men.

Conclusions

Overall, this prospective cohort study indicated that long-term antibiotic use in recent years was associated with increased risk of type 2 diabetes in women, independent of traditional diabetes risk factors. Long-term use of antibiotics has been linked with a series of health problems like obesity,[11,12] colorectal adenoma,[19] all-cause and cardiovascular mortality.[20] Our results add further evidence that physicians should exercise caution when prescribing antibiotics, particularly for long-term treatment. Further prospective studies are still required to confirm our findings.

Supplementary Data

Supplementary data are available online

and comments on data analysis.

Author Contributions

JY, YP, and JT had the initial concept and designed this study. JY, YP obtained the funding.

JY performed data analysis. JY and YH drafted the manuscript. All authors commented and

revised of the manuscript. Prof. Jinling Tang (jltang@cuhk.edu.hk) and Prof. Yihang Pan

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Conflict of interest: None declared.

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Figures

Figure 1. Flowchart of participant selection from the Nurses' Health Study and Nurses' Health Study II