Association between maternal gluten intake and type 1 diabetes in offspring: national prospective cohort study in Denmark

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ABSTRACT

OBJECTIVE

To examine the association between prenatal gluten exposure and offspring risk of type 1 diabetes in humans.

DESIGN

National prospective cohort study.

SETTING

National health information registries in Denmark.

PARTICIPANTS

Pregnant Danish women enrolled into the Danish National Birth Cohort, between January 1996 and October 2002.

MAIN OUTCOME MEASURES

Maternal gluten intake, based on maternal consumption of gluten containing foods, was reported in a 360 item food frequency questionnaire at week 25 of pregnancy. Information on type 1 diabetes occurrence in the participants' children, from 1 January 1996 to 31 May 2016, were obtained through registry linkage to the Danish Registry of Childhood and Adolescent Diabetes.

RESULTS

The study comprised 101 042 pregnancies in 91 745 women, of whom 70 188 filled out the food frequency questionnaire. After correcting for multiple pregnancies, pregnancies ending in abortions, stillbirths, lack of information regarding the pregnancy, and pregnancies with implausibly high or low energy intake, 67 565 pregnancies (63 529 women) were included. The average gluten intake was 13.0 g/day, ranging from less than 7 g/day to more than 20 g/day. The incidence of type 1 diabetes among children in the cohort was 0.37% (n=247) with a mean follow-up period of 15.6 years (standard deviation 1.4). Risk of type 1 diabetes in offspring increased proportionally with maternal gluten intake during pregnancy (adjusted hazard ratio 1.31 (95% confidence interval 1.001 to 1.72) per 10 g/day increase of gluten). Women with the highest gluten intake versus those with the lowest gluten intake (≥20 *v* <7 g/day) had double the risk of type 1 diabetes development in their offspring (adjusted hazard ratio 2.00 (95% confidence interval 1.02 to 4.00)).

CONCLUSIONS

High gluten intake by mothers during pregnancy could increase the risk of their children developing type 1 diabetes. However, confirmation of these findings are warranted, preferably in an intervention setting.

WHAT IS ALREADY KNOWN ON THIS TOPIC

In an animal model of type 1 diabetes, a gluten free maternal diet during pregnancy almost completely prevented type 1 diabetes in offspring

However, human studies have not shown an association between maternal gluten intake during pregnancy and the risk of type 1 diabetes in offspring

WHAT THIS STUDY ADDS

In a study population of 67 565 pregnancies (63 529 women), the incidence of type 1 diabetes among children in the cohort was 0.37% (n=247) with a mean follow-up period of 15.6 years (standard deviation 1.4)

The risk of type 1 diabetes in offspring increased proportionally with maternal gluten intake during pregnancy (adjusted hazard ratio 1.31 (95% confidence interval 1.001 to 1.72) per 10 g/day increase of gluten intake)

Mothers with the highest gluten intake versus those with the lowest gluten intake (≥20 *v* <7 g/day) had double the risk of type 1 diabetes development in their offspring (adjusted hazard ratio 2.00 (95% confidence interval 1.02 to 4.00))

Introduction

The incidence of type 1 diabetes is highest in countries following a western lifestyle,[1] and, until recently,[2] it has been increasing at a rate of 3-4% per year, especially in children below 5 years of age in Europe.[3] This increase is faster than can be accounted for by genetic drift, pointing to the importance of environmental factors. Gluten proteins found in wheat, rye, and barley are believed to be important in diabetes development[4]; they are rich in proline and glutamine,[5] which makes them highly hydrophobic and partly resistant to intestinal degradation. These properties make them more immunogenic than other dietary proteins, which are efficiently hydrolysed into single amino acids or dipeptides or tripeptides.

In an animal model of colitis, wheat gliadin and related peptic fragments were shown to activate mouse macrophages and human monocytes to produce proinflammatory cytokines. This action differed from any other food proteins.[6] The amino acid sequence of wheat gliadin further facilitates the presentation on human leucocyte antigen (HLA)-DQ2 and HLA-DQ8.[4]

The effect of diet on the development of diabetes has been consistently shown in animal models of type 1 diabetes, the BioBreeding rat,[7] and the non-obese diabetic (NOD) mouse. In NOD mice fed a lifelong gluten free diet, we found that incidence of diabetes fell from 64% to 15%.[8] We then found that dietary gluten fed to healthy BALB/c mice changed the cytokine pattern in T cells towards an inflammatory cytokine profile and increased the proportion of T helper 17 cells (associated with development of autoimmunity, specifically in the pancreatic lymph nodes).[9 10] A gluten free diet also was shown to induce an anti-inflammatory profile in NOD mice.[9] The incidence

of diabetes in the mice offspring was reduced even further from 64% to 8% if mothers were fed a gluten free diet only during pregnancy.[11] This reduction was accompanied by an increase in islet number as well as decreased insulitis[12] in the offspring.

Coeliac disease and type 1 diabetes share the same genetic background, with HLA being the predominant factor.[13] Other common non-HLA loci also confer risk to both diseases.[14] Coeliac disease is more prevalent among children diagnosed with having type 1 diabetes,[15] and patients with diabetes plus unrecognised coeliac disease have an earlier onset of type 1 diabetes. If coeliac disease is diagnosed first, the risk of an individual developing type 1 diabetes decreases.[16] In addition, the introduction of a gluten free diet in patients with a new diagnosis of type 1 diabetes diminishes the need for exogenous insulin, thereby prolonging the patients' period of remission.[17 18]

Prenatal exposure to gluten could be relevant to type 1 diabetes development, because the process leading to islet autoimmunity may begin in fetal life. Seroconversion has a peak incidence or median at around 9-12 months of age,[19–21] and thymic deletion of potentially self reactive T cells occurs mainly during the prenatal and neonatal periods, suggesting that these early stages could be a window for disease prevention.[22] In two previous studies investigating maternal intake of gluten and the development of islet autoimmunity in HLA conferred individuals at risk, researchers found no association.[23 24] The aim of the present study was to investigate, in a large prospective birth cohort, whether maternal gluten intake is associated with the risk of type 1 diabetes in offspring.

Methods

Population and study design

The study was based on data from the Danish National Birth Cohort,[25] in which pregnant Danish women were enrolled from January 1996 to October 2002. Eligible individuals were all pregnant women in Demark who were fluent in Danish. During the recruitment period, 91 745 mothers were enrolled, but because women were allowed to enter the study more than once, the total number of pregnancies was 101 042. Women were recruited during their first antenatal visit to the general practitioner at 6-10 weeks of pregnancy. The study covered about 35% of all births in Denmark during the recruitment period. Enrolled women participated in two telephone interviews at 12 and 30 weeks of gestation that collected extensive data on maternal lifestyle as well as past and present health characteristics. From these interviews, information on potential confounders were extracted. In addition, a food frequency questionnaire was sent to women at about 25 weeks of pregnancy. Familial follow-ups were conducted at six and 18 months postpartum, when information on breastfeeding was collected. Additional follow-ups were conducted when the children were 7, 11, and 14 years old. Selection bias for the present cohort was evaluated in a previous study, which found no difference in associations between maternal exposures (such as maternal smoking and prepregnancy weight) and preterm delivery and fetal growth, among those women recruited and those not recruited into the study.[26]

Outcome measures

Data about the development of type 1 diabetes in the children was obtained by unique linkage of person specific identifiers (from the Danish Civil Registration System (CPR)) to the Danish Registry of Childhood and Adolescent Diabetes (DanDiabKids), which covers children aged 0-18 years with a diagnosis of type 1 diabetes. For this study, the register covered diagnoses occurring from 1 January 1996 to 31 May 2016. The registry is validated annually.[3 27] The time of clinical diagnosis of type 1 diabetes was set as the first day of insulin treatment in accordance with the EURODIAB criteria.[28] The diagnosis in the DanDiabKids register was based on clinical presentation. Any doubt about the diagnosis was resolved by autoantibodies, C peptide, and genetic testing.

Quantification of gluten intake

Maternal diet was assessed at around week 25 of pregnancy by a validated food frequency questionnaire of 360 items that covered food intake during the previous four weeks.[29] Food and nutrient intakes were then quantified on the basis of assumptions of standard portion sizes and by use of Danish food composition tables[30 31] containing nutrient information of about 1030 foods available on the Danish market. If items in the questionnaire were not directly covered by records in the food composition tables, recipes were made with raw ingredients. For foods containing gluten, the amount of wheat, rye, and barley could be directly calculated for items that were based on recipes (bread, crispbread, pasta, lasagne, fast foods, cakes, and desserts); these amounts were estimated by expert judgments for other items such as beer, meatballs, and dumplings. The amount of gluten was then calculated on the basis of the protein content of wheat, rye, and barley with the conversion factors of 0.80, 0.65, and 0.50, respectively.[32–35] As a quality check, we compared our estimated amount of gluten for individual food items to comparable work conducted in the Nurses' Health Study.[36] Quantified gluten intake based on the Harvard data[36] strongly correlated with our own intake estimates (Spearman's r~0.95).

Cohort attrition

Of 101 042 pregnant women recruited from 1996 to 2002, 70 188 filled out and returned the food frequency questionnaire. Multiple pregnancies (n=1559), pregnancies ending in abortions (n=79), stillbirths (n=214), or lack of information on these pregnancy outcomes (n=96) left 68 240 women available for analysis. In our analysis, we also excluded 343 women with implausibly high (<25 000 kJ/day) or low (<2500 kJ/day) energy intake. In addition, we decided a priori

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to exclude women who had been diagnosed with having type 1 diabetes before pregnancy (n=332) to prevent potential genetic confounding. These exclusions left us with 67 565 pregnancies, corresponding to 67% of the full cohort and 96% of those women who completed the food frequency questionnaire. The 67 565 pregnancies represented 63 529 individuals, because 3992 and 44 women entered the study two and three times, respectively. The authors had access to the necessary data.

Statistical analysis

By use of summary statistics and thorough visual inspection, all variables were checked for coding errors values that were clearly implausible. Maternal gluten intake was categorised by percentiles (<10, 10-20, 20-50, 50-80, 80-90, ≥90). We then examined maternal offspring characteristics across these categories of intake using the mean and standard deviation for continuous variables and percentages for dichotomous variables. With these same categories of exposure, the association between maternal gluten intake during pregnancy and offspring risk of type 1 diabetes was examined by Cox regression. We used offspring age from birth up to May 2016 as the underlying timescale censoring if death or emigration from Denmark occurred (1217 events). Because women could enter the study repeatedly through different pregnancies, we used a robust sandwich covariance matrix estimate to account for interdependent observations. To test for linear trend, we used the median intake for each of the six gluten categories, entering these values as continuous variables in our regression models.

In our analysis, associations were first examined by an unadjusted model and then by two adjusted models (referred to as models 1 and 2). Characteristics that might influence the risk of type 1 diabetes were identified a priori and included as potential confounders in our adjusted analysis. In model 1, we adjusted for:

- Mother's age at childbirth (<25, 25-35, ≥35 years; 0.9% (n=58) missing)
- Body mass index before pregnancy (<18.5, 18.5-25, 25-30, ≥30; 5.8% (n=3924) missing)
- Parity (0, 1, <2; 4.4% (n=2946) missing)
- Smoking during pregnancy (never, occasional, <15 cigarettes/day, daily smokers ≥15 cigarettes/day; 0.7% (n=481) missing)
- Parental socioeconomic status (high/intermediate level proficiency, skilled worker, unskilled worker/unemployed, or student; 4.6% (n=3103) missing)
- Breastfeeding duration (0, 1, 2, 3, 4, 5, <6 months; 27.8% (n=18 760) missing)
- Caesarean section (yes/no; no missing data)
- Offspring sex (male/female; no missing data)
- Total energy intake (by quintiles; no missing data) was also included to account for potential confounding by total energy intake.[37]

Information on breastfeeding was extracted from the telephone interviews conducted at six and 18 months postpartum, which had much lower participation (72.4%) than the two prenatal interviews where information on most covariates was extracted. In model 2, additional adjustments were made for pre-existing maternal type 2 diabetes (no missing) and suspected gestational diabetes mellitus cases (no missing).

In our sensitivity analysis, we examined the association between maternal gluten intake during pregnancy and offspring risk of type 1 diabetes, stratifying by maternal age (<30 *v* ≥30 years), offspring sex (boys *v* girls), maternal body mass index before pregnancy (<25 *v* ≥25), parity (nulliparous *v* parous), and pre-existing diabetes (no diabetes *v* type 2 diabetes or gestational diabetes mellitus). Together with the stratified results for women with gestational diabetes mellitus or type 2 diabetes, the association between maternal gluten intake and offspring risk of type 1 diabetes was also reported from women with underlying type 1 diabetes, although these women were a priori excluded from our data. The influence of time (offspring age at type 1 diabetes diagnosis) was examined in groups, with the end of follow-up occurring at 10 years of age on one hand and with follow-up starting at 10 years on the other. Computer codes are available on request.

Patient and public involvement

The study was conducted on historical data, so we were unable to involve patients. We have invited patients to help us develop our dissemination strategy.

Results

Mean maternal gluten intake was 13.0 g/day (standard deviation 5.3) and a total of 247 (0.37%) offspring with type 1 diabetes were identified over a mean follow-up period of 15.6 (standard deviation 1.4) years. Women in the highest versus lowest category of gluten intake (≥20 *v* <7 g/day, table 1) were more likely to be of normal weight (that is, with a body mass index of 18.5-25; 66.3% *v* 59.3%), to be non-smoking (75.0% *v* 70.0%), to have breastfed their offspring for more than one month (65.8% *v* 59.7%), and to be parous (51.7% *v* 41.9%). In absolute terms, only minor differences were observed in familial socio-occupational status and other characteristics across categories of increased maternal gluten intake. Pre-existing diabetes (type 2 or gestational diabetes mellitus) was not related to gluten intake.

With respect to maternal diet (table 2), intake of both whole and refined grains increased, as expected, substantially with higher gluten intake. Women in the highest intake category were eating excessive amount of grains per day compared with among those in the lowest intake category (about 477 g v about 112 g). In addition, energy intake increased considerably with higher absolute gluten intake, with women in the lowest intake category (<7 g/day) having a mean energy intake of 7.2 mJ/day compared with 13.6 mJ/day among women in the highest intake category (≥20g/day). However, in terms of nutrient density, the

Table 1 | Characteristics of study participants (n=67 565) according to gluten intake. Data are mean (standard deviation) for continuous variables and number (%) for categorical variables. Values presented in table are before imputation of missing values; the number (%) of missing values are given in the first column

		Gluten intake by percentile (g/day; median (range))						
	All participants n=67 565	<10% (5 (0-7); n=6756)	10-20% (8 (7-9); n=6756)	20-50% (11 (9-13); n=20 269)	50-80% (14 (13-17); n=20 270)	50-80% (18 (17-20); n=6756)	≥90% (22 (20-66); n=6756)	P*
Maternal characteristics								
Maternal age (years)								
<25	5985 (8.9)	932 (13.8)	657 (9.7)	1641 (8.1)	1551 (7.7)	531 (7.9)	673 (10.0)	<0.01
25-35	26 452 (39.2)	2775 (41.1)	2801 (41.5)	8081 (39.9)	7750 (38.2)	2524 (37.4)	2521 (37.3)	
≥35	35 070 (51.9)	3046 (45.1)	3294 (48.8)	10 528 (51.9)	10 950 (54.0)	3967 (54.7)	3555 (52.6)	
Missing	58 (0.9)	4 (0.0)	5 (0.1)	19 (0.1)	19 (0.1)	5 (0.1)	7 (0.10)	
Body mass index before pregnancy								
<18.5	2913 (4.3)	266 (3.9)	249 (3.7)	757 (3.7)	924 (4.6)	325 (4.8)	392 (5.8)	<0.01
18.5-25	43 203 (63.9)	4009 (59.3)	4199 (62.1)	12 810 (63.2)	13 230 (65.3)	4476 (66.2)	4479 (66.3)	
25-30	12 435 (18.4)	1427 (21.1)	1350 (20.0)	3883 (19.2)	3623 (17.9)	1082 (16.1)	1070 (15.8)	
≥30	5090 (7.5)	589 (8.7)	563 (8.3)	1644 (8.1)	1378 (6.8)	486 (7.2)	430 (6.4)	
Missing	3924 (5.8)	465 (6.9)	396 (5.9)	1175 (5.8)	1115 (5.5)	388 (5.7)	385 (5.7)	
Gestational age (days)	280.3 (12.4)	280.0 (12.7)	280.2 (12.6)	280.4 (12.5)	280.5 (12.0)	280.5 (12.0)	280.1 (12.4)	0.10
Preterm pregnancy	2989 (4.4)	323 (4.8)	321 (4.8)	921 (4.5)	830 (4.1)	283 (4.2)	311 (4.6)	0.05
Parity								
0	31 596 (48.9)	3575 (52.9)	3501 (54.0)	9546 (47.1)	10 349 (46.8)	3588 (44.9)	2987 (46.1)	<0.01
≥1	33 023 (48.9)	2828 (41.9)	2978 (44.1)	9819 (48.4)	9086 (44.8)	2901 (52.7)	3491 (51.7)	
Missing	2946 (4.4)	353 (5.2)	278 (4.1)	904 (4.5)	835 (4.1)	298 (4.4)	278 (4.1)	
Smoking during pregnancy								
Non-smoker	50 747 (75.1)	4729 (70.0)	5017 (74.3)	15 336 (75.7)	15 475 (76.3)	5126 (75.9)	5064 (75.0)	<0.01
Occasional smoker	8253 (12.2)	926 (13.7)	871 (12.9)	2463 (12.2)	2392 (11.8)	795 (11.8)	806 (11.9)	
Daily smoker	8084 (12.0)	1043 (15.4)	818 (12.1)	2311 (11.4)	2284 (11.3)	792 (11.7)	836 (12.5)	
Missing	481 (0.7)	58 (0.9)	51 (0.8)	159 (0.8)	119 (0.6)	44 (0.7)	50 (0.7)	
Parental socioeconomic position								
High level proficiency	35 759 (52.9)	3141 (46.5)	3528 (52.2)	10 822 (53.4)	11 075 (54.6)	3665 (54.2)	3528 (52.2)	<0.01
Medium level proficiency	17 547 (26.0)	1900 (28.1)	1844 (27.3)	5338 (26.3)	5083 (25.1)	1702 (25.2)	1680 (24.9)	
Low level proficiency	7752 (11.5)	1003 (14.9)	774 (11.5)	2233 (11.0)	1043 (5.2)	738 (17.0)	431(6.4)	
Students	3404 (5.0)	347 (5.1)	320 (4.7)	926 (4.6)	2185 (10.8)	337 (5.0)	819 (12.1)	
Missing	3103 (4.6)	365 (5.4)	291 (4.3)	950 (4.7)	884 (4.4)	315 (4.7)	298 (4.4)	
Months of breastfeeding								
0-1	5362 (7.9)	670 (9.9)	606 (9.0)	1611 (8.0)	1534 (7.6)	478 (7.1)	463 (6.9)	<0.01
1-6	29 704 (44.0)	1573 (23.3)	2766 (40.9)	8775 (43.3)	9335 (46.1)	3203 (47.4)	3164 (46.8)	
≥6	13 829 (20.5)	2461 (36.4)	1512 (22.4)	4207 (20.8)	3697 (19.6)	1282 (19.0)	1285 (19.0)	
Missing	18 760 (27.8)	2052 (30.4)	1873 (27.7)	5676 (28.0)	5431 (26.8)	3202 (47.4)	1844 (27.3)	
Caesarean section	5362 (15.1)	1071 (15.9)	1115 (16.5)	3141 (15.5)	2899 (14.3)	988 (14.6)	1002 (14.8)	<0.01
Suspected gestational diabetes mellitus	625 (0.9)	51 (0.8)	53 (0.8)	198 (1.0)	210 (1.0)	56 (0.8)	57 (0.8)	0.15
Type 2 diabetes diagnosed before pregnancy	522 (0.8)	50 (0.7)	52 (0.8)	163 (0.8)	153 (0.8)	52 (0.8)	52 (0.8)	0.99
Offspring characteristics								
Type 1 diabetes	247 (0.37)	20 (0.30)	20 (0.30)	69 (0.34)	73 (0.36)	30 (0.44)	35 (0.52)	0.18
Age at diagnosis (years)	9.5 (3.9)	10.4 (3.3)	7.5 (3.9)	8.9 (4.0)	10.1 (3.8)	9.8 (4.0)	9.3 (4.1)	0.43
Male	34 658 (51.3)	3470 (51.4)	3532 (52.3)	10 432 (51.5)	10 366 (51.1)	3477 (51.5)	3381 (50.0)	0.20
Birth weight (kg)	3.59 (0.57)	3.54 (0.58)	3.59 (0.57)	3.59 (0.57)	3.60 (0.57)	3.61 (0.57)	3.58 (0.58)	<0.01

*P values evaluated by *t* test for linear trend for continuous variables and x^2 test for categorical variables.

energy coming from protein, carbohydrate, and fat was relatively similar across categories of gluten intake.

Maternal gluten intake was significantly associated with increased risk of type 1 diabetes in offspring in both unadjusted and covariate adjusted analyses (table 3). Compared with offspring born to mothers with the lowest gluten intake (<7 g/day), offspring of those with the highest intake (≥20 g/day) had double the risk of being diagnosed with having type 1 diabetes during follow-up (hazard ratio 2.00 (95% confidence interval 1.02 to 4.00)). Risk of type 1 diabetes in offspring was positively associated with maternal gluten exposure during pregnancy: the association was significant (Ptrend=0.016) and increased monotonically. Only minor differences were observed between the unadjusted and covariate adjusted analyses.

In the stratified analyses (table 4), the association between maternal gluten intake during pregnancy and offspring risk of type 1 diabetes was more pronounced among older (age ≥30 years), parous, and overweight or obese (body mass index before pregnancy ≥25) mothers, with a non-significant but positive increase in risk observed in the other groups. A non-significant trend was observed between maternal gluten intake and offspring risk of type 1 diabetes among the few (n=1147, seven cases of type 1 diabetes in offspring) women with pre-existing type 2 or gestational diabetes mellitus (hazard ratio 2.08 (95% confidence interval

Table 2 | Maternal diet of study participants (n=67 565) according to gluten intake. Data are mean (standard deviation)

	All participants (n=67 565)	Gluten intake by percentile (g/day; median (range))*					
		<10% (5 (0-7))	10-20% (8 (7-9))	20-50% (11 (9-13))	50-80% (14 (13-17))	50-80% (18 (17-20))	≥90% (22 (20-66))
Total energy intake (mJ/day)	10.1 (0.3)	7.2 (0.2)	8.2 (0.2)	9.2 (0.2)	10.7 (0.2)	11.9 (0.2)	13.6 (0.3)
Protein (E%)	15.7 (2.4)	16.7 (3.0)	16.4 (2.6)	16.0 (2.3)	15.5 (2.3)	15.0 (2.2)	14.6 (2.2)
Carbohydrate (E%)	51.4 (5.8)	50.9 (7.1)	51.5 (5.9)	51.4 (5.6)	51.4 (5.5)	51.4 (5.4)	51.5 (5.7)
Sugar (E%)	8.2 (4.7)	10.2 (6.3)	8.8 (5.2)	8.4 (4.7)	7.8 (4.3)	7.4 (4.0)	7.1 (3.8)
Fibre (E%)	2.2 (0.6)	1.6 (0.5)	2.0 (0.5)	2.1 (0.5)	2.3 (0.5)	2.4 (0.5)	2.4 (0.5)
Fat (E%)	31.4 (6.3)	31.1 (7.1)	30.5 (6.2)	31.0 (6.0)	31.5 (6.2)	31.9 (6.4)	32.3 (6.8)
Monounsaturated fatty acids (E%)	9.9 (2.2)	10.0 (2.5)	9.7 (2.1)	9.8 (2.1)	9.9 (2.2)	10.0 (2.2)	10.0 (2.4)
Polyunsaturated fatty acids (E%)	4.6 (0.9)	4.2 (1.1)	4.4 (0.9)	4.6 (0.9)	4.7 (0.8)	4.9 (0.9)	4.9 (0.9)
Saturated fatty acids (E%)	12.8 (3.5)	13.1 (3.7)	12.6 (3.4)	12.7 (3.3)	12.8 (3.4)	12.9 (3.5)	13.1 (3.7)
Whole grain (g/day)	156 (108)	41 (29)	126 (70)	146 (40)	226 (106)	246 (101)	326 (155)
Refined grain (g/day)	67 (69)	46 (26)	49 (31)	63 (35)	78 (76)	102 (91)	138 (109)
Breakfast cereals (g/day)	25 (45)	25 (42)	30 (46)	26 (45)	24 (43)	16 (43)	13 (39)

E%=percentage of total energy intake.
*All dietary variables presented in the table were significantly correlated (all P<0.001), as measured by Spearman's *r*.

0.92 to 4.69) per 10 g/day increase in gluten intake). Women with no underlying type 2 or gestational diabetes mellitus (n=66 418, 240 cases of type 1 diabetes in offspring) showed a close to significant positive trend with gluten intake (hazard ratio 1.27 (95% confidence interval 0.96 to 1.68) per 10 g/day increase in maternal gluten intake). The association between maternal gluten intake and type 1 diabetes in offspring was more pronounced among male offspring. No substantial differences were observed when stratifying by offspring age at diagnosis of type 1 diabetes.

In stability analyses, we firstly examined the association between total energy intake in mothers and risk of type 1 diabetes in offspring, because absolute intake of gluten and total energy intake were strongly related (table 2). We saw no association with total energy intake and offspring risk of type 1 diabetes (P_{trend}=0.44, data not shown). When expressing gluten intake as energy adjusted residuals[37] instead of absolute intake as presented in table 2, we also observed a significant positive association with risk of type 1 diabetes in offspring (P_{trend}=0.028, table S1). Secondly, because we used relatively extreme categories for the highest and lowest gluten intake in mothers (<10% and ≥90% intake groups), the association with risk of type 1 diabetes in offspring using groups of 20% as an explanatory variable was also explored (that is, <20%, 20-40%, 40-60%, 60-80%, ≥80% intake groups; table S2). Again, a significant association was observed (P_{trend}=0.035), although the effect estimates were more modest, after collapsing the extreme categories.

Finally, unadjusted associations were explored between women who had one or more covariates were missing (n=21 305, 75 cases of type 1 diabetes in offspring) and those with complete covariate information (n=46 505, 175 cases of type 1 diabetes in offspring). When modelling exposure as linear per 10 g/day increase in gluten intake, without adjusting for covariates (in order to obtain comparable estimates), we found that the effect estimates for the missing (β=1.22 (95% confidence interval 0.82 to 1.81)) and complete case groups (β=1.30 (1.01 to 1.69)) were similar with overlapping confidence intervals.

Discussion

Principal findings

In this prospective cohort study of pregnant women, we found that maternal gluten intake during pregnancy was strongly associated with the subsequent risk of their offspring developing type 1 diabetes, with risk increasing proportionally (hazard ratio 1.31 (95% confidence interval 1.001 to 1.72) per 10 g/day increase of gluten intake). A twofold risk of offspring developing type 1 diabetes was found when comparing mothers

Table 3 | Association between maternal gluten intake and offspring type 1 diabetes (n=67 565)

Maternal gluten intake, by percentile*	No (%) of cases of type 1 diabetes in offspring/total	Hazard ratio (95% CI) of type 1 diabetes diagnosis in offspring		
		Unadjusted model	Adjusted model 1	Adjusted model 2
Continuous intake, per 10 g/day increse	—	1.28 (1.03 to 1.58)	1.31 (1.002 to 1.72)	1.31 (1.001 to 1.72)
10%	20 (0.30)/6761	1.00 (reference)	1.00 (reference)	1.00 (reference)
10-20%	20 (0.30)/6769	0.99 (0.53 to 1.84)	1.07 (0.57 to 1.99)	1.06 (0.57 to 1.99)
20-50%	69 (0.34)/20 289	1.13 (0.9 to 1.86)	1.32 (0.75 to 2.30)	1.31 (0.75 to 2.30)
50-80%	73 (0.36)/20 268	1.19 (0.73 to 1.96)	1.46 (0.82 to 2.61)	1.46 (0.82 to 2.60)
80-90%	30 (0.44)/6750	1.47 (0.84 to 2.59)	1.82 (0.93 to 3.53)	1.81 (0.93 to 3.53)
≥90%	35 (0.52)/6728	1.72 (0.99 to 2.97)	2.03 (1.02 to 4.01)	2.00 (1.02 to 4.00)
P_{trend}	—	0.013	0.016	0.016

Adjusted model 1=adjusted for maternal body mass index before pregnancy, age, parity, smoking status, parental socioeconomic status, total energy intake, breastfeeding duration, caesarean section, and offspring sex; adjusted model 2=same as model 1 but also adjusted for pre-existing maternal type 2 diabetes and suspected gestational diabetes mellitus.
*Median intake of gluten in the <10%, 10-20%, 20-50%, 50-80%, and ≥90% percentile categories were 5, 8, 11, 15, 18, and 22 g/day, respectively.

Table 4 | Stratified analyses of risk of type 1 diabetes in offspring versus maternal gluten intake during pregnancy, by maternal characteristics

Maternal characteristics	No (%) of cases of type 1 diabetes in offspring/ total No	Hazard ratio (95% CI) of type 1 diabetes diagnosis in offspring per 10 g/day increase of maternal gluten intake	
		Unadjusted model	Adjusted model
Maternal age			
<30 years	134 (0.41)/32 461	1.29 (0.97 to 1.72)	1.13 (0.78 to 1.63)
≥30 years	113 (0.32)/35 104	1.28 (0.93 to 1.77)	1.57 (1.09 to 2.29)
Parity			
Not parous	120 (0.37)/32 738	1.32 (0.96 to 1.82)	1.15 (0.75 to 1.76)
Parous	127 (0.36)/34 827	1.24 (0.93 to 1.65)	1.49 (1.06 to 2.09)
Body mass index before pregnancy			
<25	164 (0.34)/45 506	1.34 (1.02 to 1.75)	1.17 (0.82 to 1.65)
≥25	83 (0.44)/19 059	1.20 (0.85 to 1.69)	1.68 (1.08 to 2.60)
Maternal diabetes			
No pre-existing diabetes or gestational diabetes mellitus	240 (0.36)/66 418	1.24 (1.00 to 1.55)	1.27 (0.96 to 1.68)
Pre-existing type 2 diabetes or suspected gestational diabetes mellitus	7 (0.61)/1147	2.32 (1.18 to 4.57)	2.08 (0.92 to 4.69)
Type 1 diabetes*	9 (2.71)/332	0.90 (0.31 to 2.67)	0.63 (0.16 to 2.40)
Offspring sex			
Male	132 (0.38)/34 658	1.63 (1.27 to 2.10)	1.41 (1.02 to 1.96)
Female	115 (0.35)/32 907	0.93 (0.65 to 1.32)	1.19 (0.76 to 1.85)
Offspring age at diagnosis			
≤10 years	130 (0.44) /67 565	1.25 (0.93 to 1.67)	1.27 (0.88 to 1.82)
<10 years	117 (0.18) /66 576	1.31 (0.95 to 1.79)	1.36 (0.91 to 2.03)

Adjusted model=adjusted for maternal body mass index before pregnancy, age, parity, smoking status, parental socioeconomic status, total energy, breastfeeding duration, pre-existing maternal type 2 diabetes, and suspected gestational diabetes mellitus. When stratifying by age and body mass index, these variables were included as covariates in the adjusted model.
*It was decided a priori to exclude these women from our analyses, but the association is reported here under "maternal diabetes" for consistency.

who had the highest versus lowest gluten intakes (≥20 *v* <7 g/day; hazard ratio 2.00 (95% confidence interval 1.02 to 4.00)). Our stratified analyses suggest that parous, older, or overweight/obese women might be more sensitive to gluten intake than those who are younger or of normal weight. However, these results are at best suggestive, owing to the loss of statistical power when stratifying by individual maternal characteristics, and more evidence is needed to confirm these suggestive trends. Likewise, the mechanism that might be responsible for this effect is not known, but could include increased inflammation[38–41] or increased intestinal permeability.[42 43]

Gluten intake and total calorie intake were correlated in our dataset, and high maternal body mass index or gestational diabetes is associated with both type 1 and 2 diabetes in children.[44–46] As a result, it could be hypothesised that the association observed in our present study could have been caused by high body mass index rather than by gluten. The involvement of body mass index is probably not the case, because we noted that high gluten intake inversely correlated with maternal body mass index. Thus, if diabetes development was triggered by high maternal body mass index, we would not observe a dose dependent association of gluten intake in the mothers, but rather an inverse effect.

Strengths and limitations of study

A major strength of our study was the study design. The data were gathered from one of the largest prospective birth cohorts, increasing the power in the presented associations, and the prospective design eliminated recall bias because food frequency questionnaires were collected before knowledge of the disease outcome. The use of a food frequency questionnaire is considered to be a reproducible and valid method to assess diet during pregnancy,[47 48] and the register used to identify children with type 1 diabetes (DanDiabKids) is annually validated.[27] Furthermore, comprehensive registry and interview data collected during and after pregnancy made it possible to adjust for a large number of confounders and perform sensitivity analyses, and our results were robust to adjustment for well established predictors of type 1 diabetes in offspring. Our use in Denmark of unique ID numbers (from the Danish Civil Registration System (CPR)) for every citizen enabled a follow-up of our cohort that was near 100% for identifying incident cases of type 1 diabetes in the children through linkage to the DanDiabKids register, which has full national coverage.

Despite the large study size, the statistical power of our study was still modest because of the low number of cases of type 1 diabetes (n=247) in our study population, which was reflected by some associations that were only just below the threshold set for formal significance (α=0.05). In addition, the role of unmeasured or unidentified confounders can never be fully excluded in observational studies. Confirmation of our findings in another comparable but independent dataset is therefore warranted. Dietary assessment methods that rely on the participants' ability to report their habitual diet are inevitably subject to uncertainties, owing to the inherent difficulty in recalling diet accurately. However, food frequency questionnaires have proved useful for ranking individuals according to quantified dietary intake of foods and food constituents in large scale epidemiological studies, where the use of precise methods such as dietary records are not feasible.[49]

Although gluten containing foods are easy to identify, and the protein fractions of gluten in wheat, rye, and barley are known, gluten is also intentionally added during production to certain types of flour, bread, and other foods, which we were unable to account for. This unaccountable addition of gluten would lead to a systematic underestimation of intake. Also relevant to our intake estimation was that statistical associations including essential nutrients are commonly examined by transforming crude intake to nutrient densities or energy adjusted residuals.[37] The underlying assumption is that the energy intake is roughly proportional to the physiological needs of each individual, meaning that it is the energy density of the nutrient under consideration that in most cases is the determinant of health. However, this assumption does not hold for non-essential nutrients or other substances that might be detrimental in absolute terms. Gluten is not an essential nutrient, and studies on gluten and type 1 diabetes in laboratory animals suggest that it is the absolute amount of gluten that influences the risk of type 1 diabetes.[4] As a result, we based our analyses on the absolute gluten intake. Justifying this approach, and despite a strong correlation between absolute gluten and total energy intake (table 2), we found no indication in our sensitivity analyses to suggest that maternal energy intake was related to offspring type 1 diabetes risk. The same conclusions were reached when we used a more traditional way of accounting for energy intake, using energy adjusted residuals[37] (table S1).

Another limitation was that we do not know whether mothers with a low intake of gluten during pregnancy also serve a low gluten diet to their infants. This factor could be important for type 1 diabetes development because the amount, timing,[50–52] and mode of gluten introduction all seem to affect disease development.[4] However, in our animal experiments, a gluten free diet fed to mothers during pregnancy was far more effective in preventing diabetes in the offspring than a gluten free diet fed to the offspring,[11] suggesting that the intrauterine environment could be the decisive factor for the development of type 1 diabetes.

Comparison with other studies

To our knowledge, only two other studies have looked at the association of gluten intake in pregnancy and risk of type 1 diabetes in children. In both studies, researchers investigated at-risk children (HLA conferred susceptibility) and the development of β cell autoantibodies. Lamb and colleagues[23] investigated a cohort of 642 children whose mothers filled out a food frequency questionnaire covering the third trimester. Of these children, 27 developed autoimmunity. Virtanen and colleagues[24] studied a cohort of 3723 infants, of whom 138 developed autoimmunity and whose mothers filled out a food frequency questionnaire postnatally, covering the eighth month of pregnancy. These two studies did not find an association between maternal gluten intake and development of autoantibodies in the children. Their findings differ from to our study results, and could be explained by multiple differences in the design of the two studies: inclusion of high risk study populations, use of a postnatally completed food frequency questionnaire, different outcomes and exposure times, insufficient statistical power, shorter follow-up time, or a setup that allowed for adjustment for fewer confounders. Thus, statistically well powered studies with information on potential confounders and evaluating childhood diabetes have currently been lacking to firmly establish whether prenatal gluten exposure is a risk factor for childhood diabetes.

Conclusions and policy implications

The possible effect of maternal gluten intake on risk of type 1 diabetes in offspring might be related to the complex interplay between diet, immune development, microbiota, and intestinal permeability, which could all affect the pathogenesis of type 1 diabetes. There are regional differences in the effect of probiotics and type 1 diabetes development as well as in the microbiome and structure of gluten and in the gluten content of crops. The effect of gluten intake during pregnancy could therefore vary geographically. In humans, the establishment of the microflora early in life might affect children's risk of chronic immune disorders; babies delivered by caesarean section have a 23% increased risk of onset of type 1 diabetes in childhood.[53]

It was previously assumed that fetuses were sterile, but evidence suggests that the establishment of the fetus microflora could already start in utero, transferred from maternal intestinal microbiota.[54] The colonisation of the child's intestine continues to develop after vaginal birth through contact with vaginal bacteria and maternal faeces, and is subsequently affected by breastfeeding and early infant feeding. Maternal intestinal and vaginal microbiota and the composition of breast milk are influenced by maternal diet (eg, gluten intake).[55] Thus, high gluten intake, as reported during pregnancy, could reflect habitual diets that shape the maternal, and thus the neonatal, microbiome.

Our study suggests that high gluten intake in pregnancy might be a risk factor for type 1 diabetes in offspring. The association is moderate, suggesting a 50% reduction in type 1 diabetes incidence among the children of mothers with the highest versus lowest gluten intake. This magnitude is comparable to results from other studies looking at other potential protective factors (that is, breastfeeding and D3 vitamin supplementation) in early childhood.[56 57] However, more evidence is needed before changes to dietary recommendations could be justified. Confirmation is warranted, preferably in an intervention setting, or in other cohort studies. In this context, the safety of substituting gluten containing foods for other foods and nutrients should be investigated as well as the possibility of obtaining a larger effect by adherence to a completely gluten free diet. Whether a diet with a low gluten content during pregnancy changes the incidence of coeliac disease in children should be investigated, although a recent study did not support this concern.[58]

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Contributors: JCA initiated the study. SFO was responsible for the data collection. TIH, CG, THO, LH, and SFO were responsible for the dietary database within the Danish National Birth Cohort, including quantification of gluten intake, and JS for type 1 diabetes case validation. TIH and LH carried out the statistical analyses. JCA and TIH drafted the work. JCA, TIH, and KJ wrote the final version of the manuscript. All authors participated in the interpretation of the results as well as revision of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. TIH, CG, THO, and SFO had full access to the server where data were kept. Access by other authors was granted under supervision of those with who had direct access to data. JCA is the guarantor.

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Data sharing: Computer codes for the statistical analyses are available on request. The data underlying the presented results in this paper can be shared by sending a request via the regular mechanism for obtaining access to data from the Danish National Birth Cohort (https://www.ssi.dk/English/RandD/Research%20areas/Epidemiology/DNBC/For%20researchers/How%20to%20apply%20for%20data.aspx).

The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Web appendix: Supplementary tables