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Maternal nitrate and nitrite intakes during pregnancy and risk of islet autoimmunity and type 1 diabetes – the DIPP cohort study

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Abbreviations: DIPP; The Type 1 Diabetes Prediction and Prevention Study, ICA; Islet cell antibodies, IAA; insulin autoantibodies, GADA; glutamic acid decarboxylase autoantibodies, IA-2A; islet antigen-2 autoantibodies HLA; Human Leukocyte Antigen, FFQ; food frequency questionnaire

The data in the current study are not publicly available due to the protection of the identity of the study participants and their clinical data, but data is available from the corresponding author on reasonable request.

Abstract

Background: High dietary intake of nitrate and nitrite might increase the risk of type 1 diabetes. No earlier prospective study has explored whether maternal dietary intake of nitrate and nitrite during pregnancy is associated with the risk of type 1 diabetes in the offspring.

Objective: Our aim was to study association between maternal intake of nitrate and nitrite during pregnancy and the risk of islet autoimmunity and type 1 diabetes in the offspring.

Design: Children born between 1997 and 2004 at Oulu and Tampere University Hospitals in Finland and carrying increased HLA-conferred risk for type 1 diabetes were followed in the Type 1 Diabetes Prediction and Prevention (DIPP) Study from 3 months of age. Islet autoantibodies were screened at 3 to 12-month intervals from sera. Out of 4,879 children, 312 developed islet autoimmunity and 178 developed type 1 diabetes during 15-year follow-up. Maternal intake of nitrate and nitrite during the 8th month of pregnancy was assessed after birth using a validated self-administered food-frequency questionnaire. Cox proportional hazards regression was used for the statistical analyses.

Results: Maternal intake of nitrate and nitrite during pregnancy was not associated with the child's risk of islet autoimmunity [(nitrate: HR 0.99; 95% CI 0.88, 1.11) (nitrite: HR 1.03; 95% CI 0.92, 1.15)] or type 1 diabetes [(nitrate: HR 1.02; 95% CI 0.88, 1.17) (nitrite: HR 0.97; 95% CI 0.83, 1.12)] when adjusted for energy (residual method), sex, HLA risk group and family history of diabetes. Further adjustment for dietary antioxidants (vitamin C, vitamin E and selenium) did not change the results.

Conclusion: Maternal dietary intake of nitrate or nitrite during pregnancy is not associated with the risk of islet autoimmunity or type 1 diabetes in the offspring genetically at risk for type 1 diabetes.

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Key words: pregnancy; islet autoimmunity; type 1 diabetes mellitus; cohort; child; diet; nitrate; nitrite; N-nitroso compounds

Introduction

Type 1 diabetes results from the destruction or malfunction of pancreatic beta cells mediated by autoimmune mechanisms (1, 2). Environmental triggers such as diet during early childhood (3, 4) and pregnancy (5, 6) might influence the disease development in childhood. High intake of nitrate and nitrite from the diet could increase their endogenous conversion to N-nitroso compounds such as nitrosamine and nitrosamide (7), which could potentially be toxic to beta cells (8).

Endogenously 5-7 % of total ingested nitrate is reduced to nitrite by salivary bacteria (9). In the stomach, nitrite is further reduced to nitric oxide which has several biological functions such as smooth muscle dilation. However, excess nitrite can also increase the formation of N-nitroso compounds (7).

Nitrate and nitrite are naturally found inorganic compounds. Main dietary sources of nitrate are vegetables such as leafy greens, root vegetables, tubers as well as drinking water. Nitrate's reduced form nitrite, is used as a preservative food additive in various processed meats such as ham, sausages and bacon (10). Besides endogenous formation, N-nitroso compounds also occur in foods such as processed meats and beer (11). In vitro, animal, and observational studies suggest that high intake of nitrate, nitrite and N-nitroso compounds from the diet could increase the risk of type 1 diabetes (8, 12, 13). Ecological surveys and case-control studies in humans assessing intake of nitrate and nitrite from drinking water have given inconsistent results (14). Case-control studies have suggested that high dietary intake of nitrites (15, 16) and N-nitroso compounds (15) in childhood could increase the risk of type 1 diabetes but results are inconsistent (17).

High consumption of red meat and processed meat during childhood could increase the risk of type 1 diabetes due to high intake of nitrite and N-nitroso compounds (18-20). In a case-control study, the risk of type 1 diabetes was higher in children whose intake of both N-nitroso compounds and protein from meat was high in comparison to only high intake of N-nitroso compounds (15). Undigested protein residues in the gut are converted to nitrosatable compounds

such as phenols, indoles, ammonia, amines, and amides via microbial fermentation and in conjunction with nitrosating compounds such as nitrite added in processed meat can enhance the formation of N-nitroso compounds (21).

High maternal intake of nitrite during pregnancy might also increase the risk of type 1 diabetes in the offspring (16) but this has not been assessed in prospective studies. The plasma nitrite level of a newborn infant is lower than that of an adult, but before birth, the maternal and fetal blood nitrite level is similar due to passive exchange of anions across placenta (22). Thus, high maternal nitrate and nitrite intakes could increased maternal plasma nitrite level and expose fetus to high nitrite levels. However, little is still known about the regulation of nitrite exchange via placenta.

In a prospective cohort study, maternal intake of red meat and processed meat products during pregnancy was not associated with islet autoimmunity or type 1 diabetes (23), but whether nitrate and nitrite per se increases the risk has not been explored in prospective studies. Furthermore, whether maternal intake protein modifies the association between maternal nitrate or nitrite intakes and the risk of type 1 diabetes development in offspring has not been studied. As dietary antioxidants such as vitamin C and vitamin E inhibit the formation of N-nitroso compounds from nitrate and nitrite they were used as putative confounders in the analysis (24, 25).

The aim of this prospective birth cohort study was to investigate associations between the maternal intake of dietary nitrate and nitrite during pregnancy and the risk of islet autoimmunity and type 1 diabetes in children genetically susceptible to type 1 diabetes. Furthermore, we explored whether maternal protein intake modifies the association between nitrate and nitrite intakes and type 1 diabetes outcomes.

Subjects and Methods

Subjects

The present study was a part of the Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study. DIPP Nutrition Study is a part of the larger DIPP Study, a multidisciplinary prospective population-based cohort study were all newborn infants from the regions of Oulu, Tampere and Turku University Hospitals in Finland are screened for HLA DQB1-conferred susceptibility to type 1 diabetes using cord blood samples provided that the parents give their informed consent (26). All infants carrying a high or moderate genetic risk are invited to a follow-up study. Follow-up visits were scheduled for 3, 6, 12, 18 and 24 month and thereafter annually up to 15 years of age or at the onset of type 1 diabetes. The present study comprises mothers of children born between October 1997 and September 2004 in the Oulu and Tampere University Hospitals. The present report includes 4,887 children with data on islet autoimmunity and 4,943 children with data on type 1 diabetes. Maternal diet during pregnancy was assessed by a food frequency questionnaire (FFQ) and data were available for 4,879 pregnancies due to twin pregnancies. The flow chart of participation is presented in **Figure 1**. The study adheres to the Declaration of Helsinki, and the local ethics committees approved the study protocol. Families gave their written informed consent for the genetic testing of the newborn infant and for their participation in the follow-up study.

Islet cell antibodies (ICA) were screened at 3 to 12-month intervals up to 15 years of age (27). If a participant was observed to test positive for ICA, all available samples from such a subject were analyzed for insulin (IAA), glutamic acid decarboxylase (GADA) and islet antigen-2 autoantibodies (IA-2A). Islet autoimmunity was defined by repeated positivity for ICA and at least one other autoantibody. Type 1 diabetes was defined according to World Health Organization criteria (28).

Genetic methods

HLA-DQ genotyping using panels of sequence-specific oligonucleotide probes has been described previously (26). The *HLA-DQB1(*02/*03:02)* genotype represent "high" and *HLA-DQB1*03:02/*x (x ≠**02, *03:01, *06:02*) "moderate" risk for type 1 diabetes.

Dietary methods

The mothers completed a validated 181-item semi-quantitative food frequency questionnaire (FFQ) concerning their diet during pregnancy (29). The FFQs were mailed to the mothers after delivery and checked at the child's 3-month follow-up visit. Mothers were asked retrospectively after delivery to describe their diet during the eighth month of pregnancy, which is the last month preceding the maternity leave in Finland (29). The FFQ comprised a list of 181 food items and mixed dishes. Open-frequency categories were used in increasing order: not at all, number of times per month, week or day. The serving sizes chosen for each item were based on commonly used portions identified during earlier Finnish dietary studies, and for some foods (e.g. eggs and beverages), natural units were used. Information about supplement use during whole pregnancy was collected. Mothers were instructed to record the dietary supplements with brand names, manufacturers of the supplements, amounts of supplements per day, week or month and the month of pregnancy when the supplements were used.

Maternal individual nutrient intakes were calculated using the data gathered by FFQs. The calculation was made with the in-house software (Finessi) of the Finnish Institute for Health and Welfare, Finland using the Finnish national food composition database (Fineli) as the source of food composition data (30). Energy from dietary fiber was included in the energy from carbohydrates and in the total energy. FFQs with over 10 missing items were excluded. Questionable values were double-checked on the original FFQ and the database.

Finnish Customs Laboratory and Finnish Food Authority analyzed nitrate and nitrite values of various vegetables and meat products during 2008-2012 (9, 31). The data of these analyses were used to add nitrate and nitrite values to the respective food items in the Fineli database. The nitrate

or nitrite values of foods, which were not analyzed in the above mentioned analyzes were determined from scientific literature (32-35). The latest scientific literature from the year 2000 onwards had the highest priority followed by literature from years 1980-2000. Analytical values representing predominantly European food items were preferred. Values not found from literature were derived from aggregation, recipe calculation or imputation from similar foods.

Sociodemographic characteristics

Information on type 1 diabetes status of the first-degree relatives, child sex, and maternal education were collected from parents after delivery using a structured questionnaire. Information on the gestational age, birth weight and length and maternal smoking during pregnancy was acquired from the medical birth registers of the university hospitals.

Statistical methods

One-factor ANOVA or t-test was used to study the differences in maternal nitrate / nitrite intake and background variables. Maternal age, BMI in early pregnancy and weight gain rate during pregnancy were categorized into quartiles or tertiles (BMI) for the analysis. Maternal intake of nitrate and nitrite were analyzed as continuous variables. Cox proportional hazards regression was applied to estimate associations between maternal intake of nitrates and nitrites and the risk of islet autoimmunity and type 1 diabetes in the offspring. Analyses were adjusted for energy using Willett's residual method (36), maternal education, child's genetic susceptibility to type 1 diabetes and family history of type 1 diabetes. In a second model, maternal intake of dietary antioxidants: vitamin C, vitamin E and selenium were used as additional adjustments. Furthermore, we tested whether protein intake modifies the association between both nitrate and nitrite intakes and the development of type 1 diabetes outcomes including the interaction term protein*nitrate/nitrite in the Cox proportional hazards regression models. SAS software version 9.3 (SAS Institute, Cary, NC, USA) was used in the outcome analyses. Analyzes concerning background characteristics

were done using IBM SPSS Statistics version 25.0 (IBM Corporation, NY, USA). Statistical significance was set at 2-sided $P < 0.05$.

Results

Overall, 312 children (6.8%) developed islet autoimmunity at a median (IQR) age of 3.5 (1.7-6.6) years, and 178 (3.6%) developed type 1 diabetes at a median age at 7.1 (4.3-10.6) years during 15-year follow-up. During the autoantibody follow-up of 4,887 participants, the dropout rates were 279 children (5.7 %) at 1-year and 1,415 children (30 %) at 5-year follow-up. Mean (SD) maternal intake of nitrate was 151 (97.4) mg/day and nitrite 3.00 (1.06) mg/day (**Table 1**). Intake of nitrate was higher in older mothers, non-smokers and well-educated mothers than younger mothers, smokers and mothers with poor education (Table 1). Intake of nitrite was higher in older mothers, well-educated mothers and mothers with high BMI.

The main sources of nitrate were leaf vegetables 78.3 mg/day (51.7 % of total intake), root vegetables 17.9 mg/day (11.8 %) and fruit vegetables 11.9 mg/day (7.9 %). The main sources of nitrite were cereals 1.48 mg/day (49.2 %) followed by processed meat products 0.92 mg/day (30.6 %) (**Table 2**).

Maternal intake of nitrate and nitrite during pregnancy was not associated with child's risk of islet autoimmunity or type 1 diabetes in an unadjusted model (not shown) or in a model adjusted for energy by Willett's residual method, sex, family history of diabetes, and HLA genotype (**Table 3**). The results remained similar in a model, which was further adjusted for the intake of dietary antioxidants (vitamin C, vitamin E, and selenium) (Table 3). Maternal intake of protein during pregnancy did not modify the association between intake of nitrate or nitrite and the risk of islet autoimmunity (nitrate*protein interaction P = 0.23, nitrite*protein interaction P = 0.99) or type 1 diabetes (nitrate*protein interaction P = 0.24, nitrite*protein interaction P = 0.86).

Discussion

In our prospective cohort, the maternal dietary intake of nitrate and nitrite during pregnancy was not associated with the risk of islet autoimmunity or type 1 diabetes in the offspring. Maternal intake of protein did not modify the association between intake of nitrate or nitrite and the risk of islet autoimmunity or type 1 diabetes.

A strength of our study is that it was conducted in a well-defined birth cohort of individuals with increased genetic risk of type 1 diabetes. Our study is, as far as we are aware, the first study to explore prospectively whether maternal intake of nitrate and nitrite during pregnancy is associated with type 1 diabetes development as previous studies have been ecological surveys or case-control studies (14). Furthermore, our study explored the association between maternal nitrate and nitrite intakes and the risk islet autoimmunity, which has not been studied previously. We used a regularly updated national food composition database were nitrate and nitrite contents were updated specifically for the current study. The validation study of the FFQ used in our survey suggested that the FFQ is appropriate for estimation of nitrate and nitrite intakes from food. Intake calculated from FFQ vs. food records showed a correlation of 0.63 for nitrate and 0.79 for nitrite (29). In addition, our study took into account the intake of dietary antioxidants, which could confound association between nitrate and risk of type 1 diabetes-related outcomes (15, 37-39).

A major limitation in our study was the imprecision in the calculation of nitrate content from vegetables. The food composition database used in the current study does not take into account the cooking or food preparation losses for nitrate or nitrite (9). Washing leaf vegetables decreases the nitrate content approximately 10-15% while cooking decreases the nitrate content in vegetables and potatoes approximately 51% depending on cooking method (40). Since nitrate is water soluble, consuming or discarding the cooking liquid also affects the exposure. Our food

composition database also did not included nitrate or nitrite content of drinking water. Another limitation in our study was that the children's diet during infancy was not available for the current study.

In our study, maternal intake of nitrate and nitrite during pregnancy was not associated with the risk of type 1 diabetes development, which is not in line with the previous Childhood Diabetes in Finland (DiMe) case-control study (16). In addition to the different study design, the maternal intake of nitrate and nitrite focused at a different time period and was asked later in life when the offspring had developed type 1 diabetes. In the DiMe study, the consumption frequency of the most important dietary sources of nitrate and nitrite were inquired, whereas the current study included detailed calculation of total nitrate and nitrite intakes using recently updated food composition database. In the DiMe study, maternal intake of nitrate and nitrite from diet focused at the time of conception while the FFQ in the current study represented the dietary intake during the 8^{th} month of pregnancy. Our validation study showed that mothers were able to report their food consumption reliably for the 8th month of pregnancy even if the assessment was done after delivery (29). The 8^{th} month of pregnancy is a very well identifiable time point for Finnish pregnant women as most of the women work and they are requested to start the pregnancy leave 1 month before estimated delivery. For a person, it is difficult to estimate food consumption during periods of changing diet like during the whole pregnancy as e.g. nausea in the beginning of pregnancy is common. We think that this 8^{th} month of pregnancy reflects also earlier pregnancy. Furthermore, it is not known whether there are critical time points during pregnancy that are related to the development of autoimmune diseases in offspring.

The maternal dietary protein intake did not modify the risk between nitrate or nitrite intakes and type 1 diabetes outcomes in our study. Previous Swedish case-control study suggested that the

combination of high intake of N-nitroso compounds and protein from meat in childhood could further increase the risk of type 1 diabetes in comparison to high intake of N-nitroso compounds alone (15). Our study assessed the intake of nitrate and nitrite, not N-nitroso compounds and thus the exposure was different, which might contribute to different outcomes. Furthermore, we explored total protein intake instead protein from specific dietary sources. In the European Food Safety Authority report, the median total exposure to volatile nitrosamines from meat products was 2.5 ng/kg of body weight per day in Finnish children aged 3-9 years, which was above the European median, 2.0 ng/kg (41). In Finnish adult population, the intake was 0.9 ng/kg, which is same as the European median (41). Furthermore, we found no association between maternal consumption of processed meat during pregnancy and the risk of type 1 diabetes outcomes in our previous study (23). Thus, the childhood consumption of processed meat products may play a bigger role than maternal consumption.

In our study, the mean daily nitrite intake was 3 mg and the last measured mean maternal weight during pregnancy was 79 kg, which corresponds to 0.04 mg/kg bw/day. This is similar to recent European Food Safety Authority report of 0.04 mg/kg bw/day in Finnish adult population (41). In our study the mean nitrate intake was rather high (151 mg / day) compared to previous studies in adults. Earlier Finnish study reported nitrate intake of 77 mg / day based on dietary history interview method (42). In a Danish survey, mean nitrate intake was 61 mg / day, and in a Dutch study 80 mg / day measured from duplicate 24-h diet samples (43, 44). Different nitrate intake estimates between studies may be explained by methodological differences.

In our study, the main sources of nitrate were leaf and root vegetables, which contributed to more than three quarters of the daily intake. In our previous study, the maternal consumption of vegetables during pregnancy was not associated with the risk of islet autoimmunity (5), which is

in line with our current study. Surprisingly, the main source of nitrite was cereals comprising almost half of the daily intake followed by processed meat with 31% of the daily intake. Maternal consumption of cereals during pregnancy was not associated with the risk of islet autoimmunity in our previous study (5). However, two recent studies have observed that maternal (45) and childhood (46) intake of gluten containing cereals might increase the risk of type 1 diabetes development. Although the nitrite content in cereals is lower than in processed meats, the proportion of cereals in the diet is higher than of that of meat in DIPP Study mothers (47). Although processed meats are generally suggested as the main source of nitrite (41, 48), cereals have been observed to be the main source of nitrite in the adult population also previously (49). Studies analyzing nitrate and nitrite content in cereals in Europe are rather limited and nitrite content in cereals in Finland has not been analyzed. Therefore, we cannot rule out the possibility of overestimation.

Our study did not include the contents of N-nitroso compounds in foods as the Fineli food composition database used in our study does not contain these values. In addition to endogenous formation, the direct exposure to N-nitroso compounds from diet and drinking water could also influence the risk of type 1 diabetes (15, 19). Food preparation can induce N-nitroso compounds formation in the food before consumption e.g. cooking nitrite-containing bacon at high temperature (50). Thus, future studies should consider both endogenous and exogenous exposure to these compounds. Furthermore, the maternal intake of N-nitroso compounds in association with type 1 diabetes in offspring has not been previously studied.

In our study, we could not consider the maternal use of nitrosatable drugs. These drugs along with nitrosating compounds such as nitrite from the diet may enhance the endogenous formation of N-nitroso compounds. Prenatal use of these drugs has been associated with several unfavorable pregnancy outcomes (51-53), but their association with type 1 diabetes development in offspring

has not been studies. Nitrosatable drugs include e.g. antibiotics and antihistamines. Since these drugs are rather commonly used, their use during pregnancy might be important to consider in future studies.

In conclusion, our prospective study suggests that maternal intake of nitrate and nitrite is not associated with the risk of islet autoimmunity or type 1 diabetes in the offspring.

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TABLE 1. Nitrate and nitrite intakes of mothers during month 8 of pregnancy, stratified by various characteristics, DIPP Nutrition Study, Finland.

Variable	***n***	**Nitrate, mg/day**	**P value[2]**	**Nitrite, mg/day**	**P value[2]**
All mothers	4879	151 ± 97.4[1]		3.00 ± 1.06[1]	
Age, years			<0.001		<0.001
< 24	926	127 ± 90.0		2.93 ± 1.12	
25-29	1700	148 ± 96.4		2.94 ± 1.05	
30-34	1412	159 ± 94.6		3.04 ± 1.04	
≥ 35	841	172 ± 105		3.12 ± 1.04	
Missing	0				
BMI in early pregnancy, kg/m2			0.74		<0.001
< 25	3025	150 ± 94.4		2.95 ± 1.02	
25-29.9	1123	152 ± 90.2		3.07 ± 1.10	
≥ 30	434	154 ± 119		3.13 ± 1.13	
Missing	297				
Weight gain rate, g/week			0.48		0.78
1st quarter <0.33	1136	153 ± 104		3.01 ± 1.11	
2nd quarter 0.33-0.41	1137	154 ± 94.5		2.98 ± 1.06	
3rd quarter 0.42-0.51	1137	149 ± 92.0		2.98 ± 1.01	
4th quarter ≥0.52	1136	148 ± 92.0		3.02 ± 1.04	
Missing	333				
Vocational education[3]			<0.001		0.001
None	294	115 ± 79.7		3.06 ± 1.14	
Vocational School or Course	1291	139 ± 96.0		3.05 ± 1.18	
Secondary Vocational Education	2067	150 ± 93.4		3.02 ± 1.05	
University Studies or Degree	1097	170 ± 104		2.89 ± 0.89	
Missing	130				
Smoking during pregnancy			<0.001		0.91
Yes	467	120 ± 83.8		3.00 ± 1.20	
No	4246	154 ± 98.1		3.00 ± 1.05	
Missing	166				
Diabetes[4]			<0.001		0.003
Yes	164	187 ± 134		3.24 ± 1.18	
No	4611	150 ± 95.4		2.99 ± 1.06	
Missing	103				

[1] Values are means ± SDs

[2] *P* values for difference between groups from one-factor ANOVA or t-test.

[3] at the time of birth.

[4] Data on maternal diabetes from questionnaire completed after birth. Type of diabetes not specified.

BMI, Body Mass Index

TABLE 2 Maternal intake of nitrate and nitrite during pregnancy from food groups, DIPP Nutrition Study, Finland.

Food groups	**Nitrate mg/day[1]**	**% of total**	**Nitrite mg/day[1]**	**% of total**
Fruits and berries[2]	8.63 ± 6.69	5.70	0.05 ± 0.07	1.59
Fruit juices	5.46 ± 6.82	3.61	0 ± 0	0
Other sweetened fruit drinks	0.76 ± 1.39	0.50	0.03 ± 0.06	1.05
Vegetables				
Leaf vegetables	78.3 ± 79.5	51.7	0.05 ± 0.05	1.59
Fruit vegetables	11.9 ± 9.93	7.88	0.12 ± 0.11	3.86
Root vegetables	17.9 ± 17.7	11.8	0.04 ± 0.04	1.23
Other vegetables[3]	8.07 ± 10.1	5.33	0.03 ± 0.04	1.11
Legumes, nuts, seeds and soy products	0.31 ± 1.44	0.21	<0.01 ± 0.01	0.08
Potatoes and potato based products	7.28 ± 3.45	4.81	0.14 ± 0.07	4.70
Dairy products	0.89 ± 0.65	0.59	0.05 ± 0.04	1.65
Cereals	8.46 ± 3.15	5.59	1.48 ± 0.55	49.2
Egg and egg dishes	0 ± 0	0	0 ± 0	0
Fish and fish dishes	0.16 ± 0.27	0.10	0.02 ± 0.03	0.68
Meat and meat dishes (beef, pork, lamb, poultry, game)				
Unprocessed meat	0.45 ± 0.23	0.30	0.06 ± 0.03	2.05
Processed meat[4]	2.19 ± 1.58	1.44	0.92 ± 0.66	30.6
Other foods[5]	0.67 ± 0.55	0.44	0.02 ± 0.01	0.64
All foods	151 ± 97.4	100	3.00 ± 1.06	100

[1] Mean ± SD intake of 4,879 mothers
[2] Including canned and dried fruits and berries
[3] Cabbages, onions, mushrooms, and canned vegetables
[4] Sausages and cured meat products
[5] Fats, oils, beverages, sugars, sweets, condiments, and dietary supplements

TABLE 3. HRs (95% CIs) for the association between maternal intake of nitrate and nitrite during pregnancy and the risk of islet autoimmunity and type 1 diabetes in the offspring[1]

	Islet Autoimmunity		**Type 1 Diabetes**	
	Model 1 *n* = 4706 (305)[2]	**Model 2** *n* = 4706 (305)[2]	**Model 1** *n* = 4757 (174)[2]	**Model 2** *n* = 4757 (174)[2]
Nitrate from diet per 1 SD	0.99 (0.88, 1.11)	1.00 (0.88, 1.14)	1.02 (0.88, 1.17)	1.02 (0.87, 1.20)
Nitrite from diet per 1 SD	1.03 (0.92, 1.15)	1.03 (0.92, 1.16)	0.97 (0.83, 1.12)	0.99 (0.85, 1.16)

[1] Values are HRs (95% CIs) analyzed using Cox proportional hazard regression model. Model 1: adjusted for energy with residual method, sex, family history of diabetes, and HLA genotype. Model 2: like model 1 and further adjusted for the intakes of vitamin C, vitamin E, and selenium

[2] n represents total number of children and number in parenthesis represents numbers of children with the outcome

FIGURE 1. Participant flow chart.