Statistical Analysis Plan (SAP) for the project "Association between the overall inflammatory potential of diet during pregnancy and the child's subsequent risk of type 1 diabetes"

Drafted by Rohina Noorzae, Thorhallur I Halldorsson, Anne Ahrendt Bjerregaard and Sjurdur F Olsen

Study background

A growing body of evidence suggests that diet is a modifiable determinant of low-grade systemic inflammation, as expressed by inflammatory markers. Processed meats, dairy products and refined carbohydrates have been linked to higher levels of pro-inflammatory markers.[1,2] Contrastingly, certain types of fruits and vegetables and whole grains have been associated with lower levels of inflammation and inflammatory diseases.[2–4]

Type 1 diabetes, an autoimmune disease, is due to both genetic and environmental contributions resulting in an immune-mediated destruction of insulin-producing β-cells and leading to lifelong insulin treatment.[5] The incidence of type 1 diabetes is highest in countries following a western lifestyle,[6] and the increasing rate has been faster than can be accounted for by genetic drift, therefore pointing to the importance of environmental factors.[6] As an autoimmune response against the insulin-producing beta cells is a central feature of type 1 diabetes,[2,7,8] external factors that may influence the immune system and inflammatory responses constitute potentially interesting candidate exposures for novel research. Since the immune system establishes and develops in early life and to a certain degree antenatally,[7] there is good reason to explore the role of diet consumed during pregnancy with special focus on dietary components with inflammation- and immuno-regulatory properties. It is possible that such components may alter the immune cell reactivity and inflammatory state of the mother during pregnancy and subsequently in the child.[9,10]

Several validated indexes have been developed to quantify and reflect the overall inflammatory potential of diet by correlating the frequency of consumption of food groups with the concentration of inflammatory markers in the blood.[11,12] Such inflammatory diet scores have been associated with increased risk of cardiovascular disease[13], colorectal cancer[14], and prostate cancer.[15]

Study objective and hypothesis

Our study will address this question: *Is there an association between inflammatory dietary patterns during pregnancy and T1D risk in the offspring.* The hypothesis of this study is that consuming a pro-inflammatory diet during pregnancy increases offspring risk of developing T1D. This will be studied in a prospective cohort design in the Danish National Birth Cohort (DNBC), where we have developed an inflammatory diet index for pregnancy diet using the methodology previously described by Tabung et al. to rank the women with respect to the overall inflammatory potential of the diet they had reported to consume during pregnancy.[16]

Study design

Prospective nationwide birth cohort study in Denmark.

Study period

Enrolment initiated in 1996. Collection of data utilized in the present study was finalized in 2018.

Data source

- The Danish National Birth Cohort (DNBC)[17,18]
- Danish Registry of Childhood and Adolescent Diabetes (DanDiabKids)[19]

Study population

Enrolled: 101,033 pregnancies
Answered food frequency questionnaire (FFQ) in gestational week (GW) 25: 73,010 participants (72.3%)

Inclusion and exclusion criteria

- Only live born children included
- Only singletons included
- Mothers with T1D are excluded
- Implausibly low (<2,500 kJ/day) and high (>25,000 kJ/day) energy intake are excluded

Variables

Exposure: The inflammatory diet index, designated the Empirical Dietary Inflammatory Index (EDII), for DNBC women's diet during pregnancy has been calculated according to the method described by Tabung et al.[16] Data deriving from DNBC's sister cohort, the Norwegian Mother-Father-Child (MoBa) cohort, were used for the calculation of EDII scores for DNBC women's diet during pregnancy.

The EDII calculation carried out on MoBa data is based on 2,999 participants with available blood samples in the Norwegian Environmental Biobank, filled out the FFQ and provided information in lifestyle and health in other MoBa questionnaires during pregnancy. The MoBa FFQ was administered in week 22 of gestation and covered habitual dietary intake from beginning of pregnancy, while CRP was assessed in plasma samples (week 17-18). Mean daily intakes (grams/day) of food groups were calculated from the MoBa pregnancy FFQ.[20–23] Reduced rank regression (RRR) was used to identify foods associated with C-reactive protein . Then Stepwise regression techniques were used to identify food groups that were most important in determining the RRR pattern. The regression coefficients were then used to weight these food groups, and the resulting weighted value was rescaled to derive an inflammatory diet score for each food group in the index. The so-obtained scores for each individual food groups were subsequently transferred to and applied in the context of DNBC nutritional data[18], and used to estimate the inflammatory potential – the EDII – of each individual DNBC woman's diet during pregnancy, on a continuum from anti-inflammatory (represented by a low score) to pro-inflammatory (represented by a high score).

Adapting the EDII calculations from MoBa to DNBC data is justifiable due to similarities 1) between Norwegian and Danish culinary habits, 2) in the overall design of the two cohorts, MoBa and DNBC (including the timing of assessing dietary intake during pregnancy), and 3) in methodology and structure of the dietary instruments (Food Frequency Questionnaires, FFQs) employed to assess pregnancy diet in DNBC and MoBa.[18,23,24]

Outcome: Type 1 diabetes in the child

Covariates potentially included in the analyses, with suggested categorization:

We have selected characteristics that might influence the risk of T1D and include these as potential confounders in our adjusted analyses and construct the model in a similar way as an earlier study focusing on gluten in pregnancy and child's risk of T1D[6].

- Mother's age at childbirth
    - <25, 25-35, ≥35 years, missing
- Maternal parity
    - Primiparous, 1, 2+, missing
- Maternal pre-pregnancy BMI
    - Underweight (<18.5), Normal weight (18.5-24.9), Overweight (25.0-29.9), Obese (≥30.0)
- Maternal smoking during pregnancy, categorized as in an earlier study focusing on smoking in pregnancy and child's risk of T1D[25].
    - No, During the first 12 gestational weeks only, Also after 12 gestational weeks
- Parental socioeconomic status
    - Hjgh/intermediate level proficiency, skilled worker, unskilled worker/unemployed, student, missing
- Breastfeeding duration
    - 0, 1, 2, 3, 4, 5, ≥6 months, missing
- Offspring sex
    - Male, female, missing
- Caesarean section
    - Yes, no, missing
- Gluten intake
- Total energy intake
    - By quintiles

Adjustments will be performed in the following models:

- Model 1: Unadjusted model
- Model 2: Adjusted for:
    - Mothers age at childbirth
    - BMI before pregnancy
    - Parity
    - Smoking during pregnancy
    - Parental socioeconomic status
    - Breastfeeding duration
    - Caesarean section
    - Offspring sex
    - Total energy intake
- Model 3: Adjusted for:
    - Pre-existing maternal type 2 diabetes
    - Suspected gestational diabetes mellitus cases

Statistical analyses

The aim of the analyses is to examine the association between the inflammatory potential of the mother's diet and the child's subsequent risk of developing type 1 diabetes, based on data from DNBC.

Children will be followed from the date of birth until type 1 diabetes diagnosis or the end of follow up (June 01, 2018). If we get the opportunity to have an updated data extraction from DanDiabKids before publishing of the study, we may include these new data and change the follow-up date accordingly.

Multivariable analysis of maternal EDII and childhood-onset T1D will be performed in models 2-4 (see above). To estimate the association of EDII with childhood-onset T1D we will use Cox proportional hazards regression, reporting hazards ratios (HR) and 95% confidence intervals (CI). Missing covariate values will be imputed using multiple imputation.

We will make a table to look at maternal characteristics across the EDII scores (Table1). We will also make a table to look at risk of type 1 diabetes in the offspring according to maternal EDII scores (Table 2).

To assess whether there is evidence in the data for deviation from linearity, we will also run a likelihood ratio test (P curvature, F test) comparing the linear model fit with a model fit based on restricted cubic spline (for spline see Durrleman S, Simon R. Stat Med., 1989, vol. 8, issue 5)**.** If there is evidence for deviation from linearity, we will plot spine-model predictions to visualize the results.

All above analyses will include the above-mentioned standard set of covariates, and the same approach will be used for robustness analyses listed below.

Robustness analyses

In addition to complete case analysis, we will evaluate the influence of adjusting for additional covariates that are thought or known to predict either exposure or endpoint, but not necessarily both. Experience

have shown that reviewers and readers often want to see analyses adjusted for such variables. The planned extra covariates to adjust for include those listed below (coordinated with “Models” above).

1. Complete case analyses
2. Adjust for gluten intake during pregnancy in a separate model
3. Adjust for birth weight in a separate model

Table shells:

Table 1

The distribution of maternal and offspring characteristics across the quartiles of the Empirical Dietary Inflammatory Index (EDII) scores.

	EDII score Q1 (-6 to -4)	EDII score Q2 (-3 to -1)	EDII score Q3 (0 to 2)	EDII score Q4 (3 to 5)
Maternal characteristics				
Total no. (%)				
Age				
BMI				
Smoking during pregnancy				
Alcohol				
Drug use				
Use of non-steroid anti-inflammatory drugs (NSAID) 1. Yes 2. No (B222) 3. Do not know (B222) "In what gestation week did you take pain killer (B220)" "B221_1-45 How many (*answer in B219)* did you take all together in a week?"				
History of type 1 diabetes				
History of metabolic disorder (prior to pregnancy)				
History of metabolic disorder during pregnancy				
Diabetes during pregnancy				
Parity				
Physical activity				
Infertility treatment				
Offspring characteristics				
Male				
Female				
Birthweight, g				
Gestational age, w				
Duration of breastfeeding				
Timing of introduction of solid food, months				
Delivery by cesarean section				
Vaginal birth				

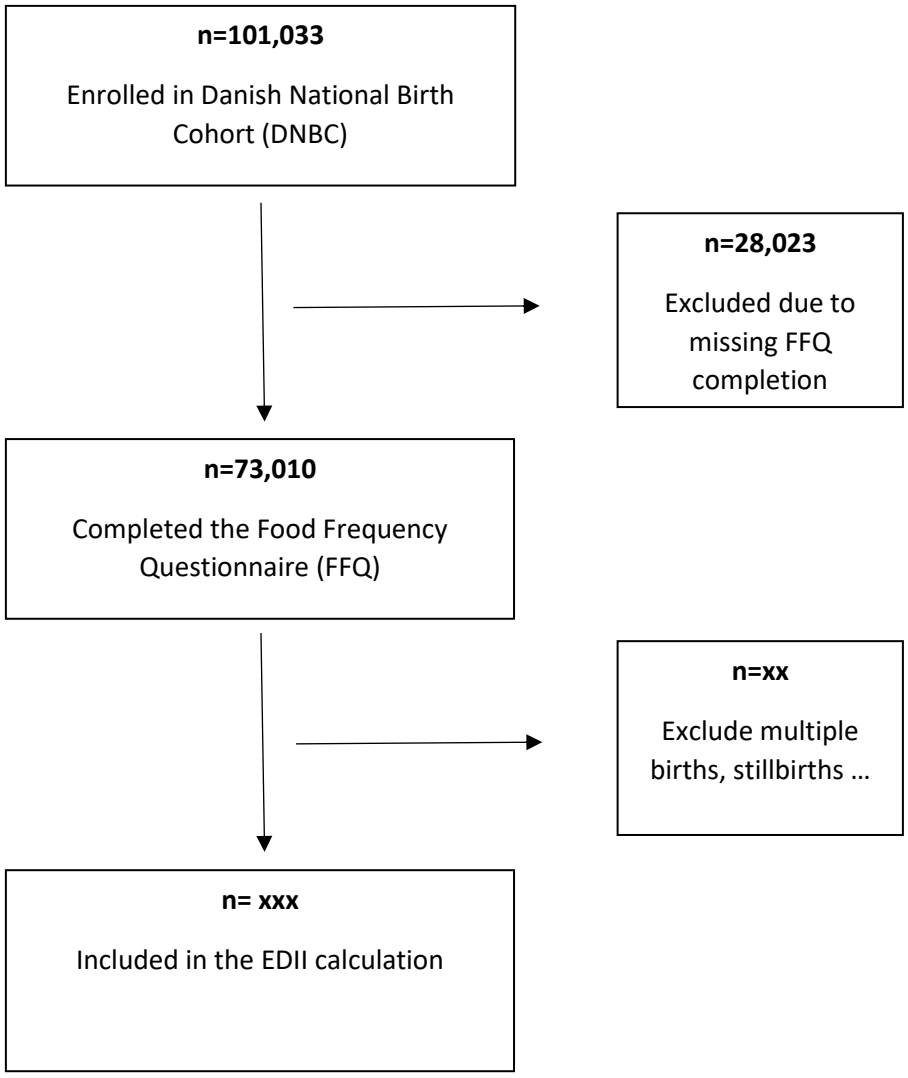
Table 2

Hazard ratio (HR) of type 1 diabetes (T1D) in the offspring according to maternal Empirical Dietary Inflammatory Index (EDII) scores.

	Maternal EDII scores	**Model 1 HR (95% CI)**	**Model 2 HR (95% CI)**	**Etc.**
HR of T1D in the offspring	Q1 (-6 to -4)			
	Q2 (-3 to -1)			
	Q3 (0 to 2)			
	Q4 (3 to 5)			

Figures:

1) Illustration of sample selection in the cohort



References

1. Nettleton, J. A. *et al. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA) 1-3*. *American Journal of Clinical Nutrition* vol. 83 https://academic.oup.com/ajcn/article/83/6/1369/4633049 (2006).

2. Van Woudenbergh, G. J. *et al.* Meat Consumption and Its Association With C-Reactive Protein and Incident Type 2 Diabetes The Rotterdam Study. *Diabetes Care* **35**, 1499–1505 (2012).

3. Holt, E. M. *et al.* Fruit and Vegetable Consumption and Its Relation to Markers of Inflammation and Oxidative Stress in Adolescents. *J. Am. Diet. Assoc.* **109**, 414–421 (2009).

4. Bonaccio, M. *et al.* Mediterranean diet, dietary polyphenols and low grade inflammation: results from the MOLI-SANI study. *Br. J. Clin. Pharmacol.* **83**, 107–113 (2017).

5. Insel, R. A. *et al.* Staging presymptomatic type 1 diabetes: A scientific statement of jdrf, the endocrine society, and the American diabetes association. *Diabetes Care* **38**, 1964–1974 (2015).

6. Antvorskov, J. C. *et al.* Association between maternal gluten intake and type 1 diabetes in offspring: National prospective cohort study in Denmark. *BMJ* **362**, 1–9 (2018).

7. Warner, J. O. The early life origins of asthma and related allergic disorders. *Arch dis child* (2002) doi:10.1136/adc.2002.013029.

8. Bluestone, J. A., Herold, K. & Eisenbarth, G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* **464**, 1293–300 (2010).

9. L.C. Stene, J. Ulriksen, P. Magnus, G. J. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. *Diabetologia* **43**, 1093–1098 (2000).

10. Simopoulos, A. P. Omega-3 Fatty Acids in Inflammation and Autoimmune Diseases. *J. Am. Coll. Nutr.* **21**, 495–505 (2002).

11. Tabung, F. K. *et al.* Development and validation of an empirical dietary inflammatory index. *J. Nutr.* **146**, 1560–70 (2016).

12. Shivappa, N. *et al.* Associations between dietary inflammatory index and inflammatory markers in the Asklepios Study. *Br. J. Nutr.* (2015) doi:10.1017/S000711451400395X.

13. Shivappa, N. *et al.* Dietary inflammatory index and cardiovascular risk and mortality—a meta-analysis. *Nutrients* at https://doi.org/10.3390/nu10020200 (2018).

14. Shivappa, N. *et al.* Dietary inflammatory index and colorectal cancer risk—a meta-analysis. *Nutrients* (2017) doi:10.3390/nu9091043.

15. Zhu, Y., Li, Q. & Xu, X. Dietary inflammatory index and the risk of prostate cancer: a dose-response meta-analysis. *Eur. J. Clin. Nutr.* **74**, 1001–1008 (2019).

16. Tabung F. K., Smith-Warner S. A., Chavarro J. E., Wu K., Fuchs C. S., Hu F. B., Chan A. T., Willett W. C., G. E. L. Development and Validation of an Empirical Dietary Inflammatory Index. *J. Nutr.* **146**, 1560–1570 (2016).

17. Olsen, J. *et al.* The Danish National Birth Cohort--its background, structure and aim. *Scand. J. Public Health* **29**, 300–307 (2001).

18. Olsen, S. F. *et al.* Data collected on maternal dietary exposures in the Danish National Birth Cohort. *Paediatr. Perinat. Epidemiol.* **21**, 76–86 (2007).

19. Svensson, J. *et al.* Danish Registry of Childhood and Adolescent Diabetes. *Clin. Epidemiol.* **8**, 679–683 (2016).

20. Magnus, P. *et al.* Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). *Int. J. Epidemiol.* **45**, 382–388 (2016).

21. Rønningen, K. S. *et al.* The biobank of the Norwegian Mother and Child Cohort Study: a resource for the next 100 years. *Eur. J. Epidemiol.* **21**, 619–625 (2006).

22. Caspersen, I. H. *et al.* Patterns and dietary determinants of essential and toxic elements in blood measured in mid-pregnancy: The Norwegian Environmental Biobank. *Sci. Total Environ.* **671**, 299–308 (2019).

23. Meltzer, H. M., Brantsaeter, A. L., Ydersbond, T. A., Alexander, J. & Haugen, M. Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa). *Matern Child Nutr* **4**, 14–27 (2008).

24. Olsen SF., Eva Birgisdottir, B., Halldorsson IT., Brantsæter AL., Haugen M, Torjusen H, PetersenSB., Strøm M, Meltzer HM. Possibilities and considerations when merging dietary data from the world's two largest pregnancy cohorts: The Danish National Birth Cohort and the Norwegian Mother and Child Cohort Study. *Acta Obstet. Gynecol. Scand.* **93**, 1131–1140 (2014).

25. Maria C Magnus, German Tapia, Sjurdur F Olsen, Charlotta Granstrom, Karl Mårild, Per M Ueland, Øivind Midttun, Jannet Svensson, Jesper Johannesen, Torild Skrivarhaug, Geir Joner, Pål R. Njølstad, Ketil Størdal, L. C. S. Parental Smoking and Risk of Childhood-onset Type 1 Diabetes. *Epidemiology* **29**, 848–856 (2018).