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The maternal Mediterranean dietary pattern is associated with a reduced risk of spina bifida in the offspring

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Objective The objective of this study was to test the hypothesis whether a maternal dietary pattern is associated with the risk of spina bifida (SB) in the offspring.

Design Case–control study.

Setting Eight clinic sites in the Netherlands, 1999–2001.

Sample A total of 50 mothers of children with SB and 81 control mothers.

Methods Maternal food intakes were obtained by food frequency questionnaires at the standardised study moment of 14 months after the birth of the index child. Principal component factor analysis (PCA) and reduced rank regression (RRR) were used to identify dietary patterns.

Main outcome measures Maternal biomarkers were used as response measures in the RRR analysis and composed of serum and red blood cell (RBC) folate, serum vitamin B12 and total plasma homocysteine. The strength of the use of the dietary pattern in association with SB risk was estimated by odds ratios and 95% CI with the highest quartiles of the dietary pattern as reference.

Results A predominantly Mediterranean dietary pattern was identified by both PCA and RRR. Those dietary patterns were highly correlated ($r = 0.51$, $P < 0.001$) and characterised by joint intakes of fruit, vegetables, vegetable oil, alcohol, fish, legumes and cereals and low intakes of potatoes and sweets. We observed a significantly increased risk of SB offspring in mothers with a weak use of the Mediterranean dietary pattern, OR 2.7 (95% CI 1.2–6.1) and OR 3.5 (95% CI 1.5–7.9). The Mediterranean dietary pattern was correlated with higher levels of serum and RBC folate, serum vitamin B12 and lower plasma homocysteine.

Conclusion The Mediterranean dietary pattern seems to be associated with reduction in the risk of offspring being affected by SB.

Keywords Factor analysis, folate, Mediterranean diet, neural tube defect, reduced rank regression.

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Introduction

Spina bifida (SB) belongs to the neural tube defects and is a severe malformation of the central nervous system. In most cases, SB is considered to have a multifactorial origin, and during the past decades, the role of maternal nutrition—as an environmental factor—has been emphasised, in particular that of the B-vitamin folate.[1,2] The discovery that maternal folic acid supplementation protects against SB has stimulated the introduction of folic acid fortification and supplementation programs in several countries.[3–7] It has also been shown that low maternal dietary intakes of other nutrients, such as a vitamin B12, iron, magnesium and niacin, are associated with SB offspring.[8,9] Furthermore, there is a large variation in the occurrences of SB in regions and countries worldwide. The prevalence is generally lower in Southern European countries and in countries with mandatory folic acid fortification.[10] This leads to the hypothesis that the overall maternal diet may confer supplementary protection against maldevelopment of the neural tube.

Nutrients from natural food sources are generally consumed in meals and not as isolated components. They serve

as substrates, transcription factors and modifiers of gene expression and thereby influence complex biological pathways involved in embryogenesis. Dietary patterns have been derived successfully from food frequency questionnaires (FFQs) by data-driven dimension reduction techniques, such as principal component factor analysis (PCA)[11] and reduced rank regression (RRR).[12] To date, dietary patterns have been associated with biomarker concentrations and related to complex diseases, such as cardiovascular disease.[13–15] Therefore, the aims of this study were to identify dietary patterns in case and control mothers and to examine whether associations exist between these dietary patterns and the risk of SB risk in the offspring. This new approach is important because it could result in recommendations for preconception counselling and dietary interventions.

Methods

A case–control study was conducted between 1999 and 2001 in which 77 mothers of children with SB and 151 control mothers have been included as has been described previously.[8,9] In summary, Dutch Caucasian mothers and their child with a nonsyndromic meningo(myelo)cele were eligible to participate at around 15 months after the index pregnancy. The meningo(myelo)cele, referred to as SB, was diagnosed by a neuropaediatrician at birth. Controls were Dutch Caucasian mothers of children without congenital abnormalities of the same age as the cases. Exclusion criteria were (1) pregnancy at the study moment, (2) consanguinity, (3) a familial relationship between the case and the control families and (4) maternal diabetes mellitus. Informed consent was obtained from every subject, and the study was approved by the Central Committee on Research in Human and the Medical Ethics Committees of all participating hospitals.

Questionnaires were filled out at home at the study moment for information on current lifestyle, demographic factors and other. From the questionnaire, data were extracted concerning age, body weight, education and lifestyle factors, such as smoking and vitamin intake. The maternal body mass index (BMI) was calculated as the ratio of weight per height squared (kg/m^2). Education was categorised into low (primary/lower vocational/intermediate or secondary/intermediate vocational), intermediate (higher secondary) and high education (higher vocational or university). Mothers were considered smokers when any smoking (cigarettes, cigars or pipe) was reported. Data on vitamin intake comprised information on the contents (folic acid only or multivitamins), dosage and frequency of intake.

Food frequency questionnaire

For quantification of the diet, mothers filled out validated FFQs developed for the Dutch cohorts of the European Prospective Investigation into Cancer and Nutrition study at around 14 months after the index pregnancy covering the nutrient intake 3 months before the study moment.[16,17] The frequency with which mothers used the foods could be indicated per day, week, month, year or never. They were also asked about the methods of preparation, additions and portion sizes. All FFQs were individually checked for completeness and consistency at the hospital or by telephone by the researcher. The average daily nutrient intakes were estimated by multiplying the frequency of consumption of food items by the portion size and nutrient content per gram based on the 1998 electronic version of the Dutch food composition table. The exceptions to this were folate and cobalamin, which were based on the 2001 electronic version.[18,19] As stated by the expert nutritionist Willett and confirmed by others, in general, the individual dietary pattern is rather constant and is influenced only by episodes of temporary dieting, illnesses, nausea and increased needs due to excessive growth, such as during pregnancy and breastfeeding.[20–23] Therefore, in this analysis, mothers who reported (1) to be pregnant, (2) to give breastfeeding, (3) a changed diet at the study moment compared with the periconception period, (4) to have had severe nausea and/or vomiting starting after the first week of pregnancy, resulting in a changed or decreased food intake and (5) mothers whose nutritional intake data and/or biomarkers were incomplete or lacking were excluded.

The periconception period was defined as the period from 4 weeks before through 8 weeks after the conception of the index pregnancy. Periconception multivitamin supplement intake including folic acid or a folic acid supplement and mild nausea and/or vomiting during the first trimester of pregnancy were included in the analysis. The periconception intake of supplements was specified according to which weeks the supplements were taken on a daily basis before and during pregnancy.

Assays of biomarkers

Appointments were made at the University Medical Center in Nijmegen, the Netherlands, for blood sampling. Mothers fasted from 10 p.m. the day before the blood sampling. Venous blood of the mother was drawn into anticoagulant-free vacutainer tubes for folate and vitamin B12 measurements according to the procedures of Kuemmerle *et al.*[24] and Molloy and Scott.[25] The intra-assay and interassay coefficients of variation for folate and vitamin B12 were 6.1 and 10.2% and 5.7 and 6.3%, respectively. For vitamin B6 determination, venous blood was drawn into lithium heparin vacutainer tubes and routinely analysed according to the methods of Schrijver *et al.*[26] The intra-assay and interassay coefficients of variation were 1.4 and 7.3%, respectively. Venous blood was drawn into vacutainer tubes that contained ethylenediaminetetraacetic acid for the measurement of total homocysteine (tHcy). The tubes were immediately placed on ice and processed within 2 hours after blood sampling and

analysed according to routine methods of Te Poele-Pothoff *et al.*[27] Both intra-assay and interassay coefficients of variation were <6.5%. The remaining blood was used to determine the haematocrit level. All laboratory analyses were performed without knowledge of the identity of the participants. Not all blood parameters were available for every participant. Missing results occurred in approximately 5% of the participants because of failures in blood sampling or laboratory testing.

Statistical and food frequency analyses

A detailed description of the PCA and RRR procedure for extracting dietary patterns from food consumption data can be found elsewhere.[12] In short, PCA works only by extracting successive linear combinations of the predicting variables to explain a maximal variance occurring within these predicting variables. The RRR method also extracts linear combinations (called factors) from predicting variables; however, the goal of this method is to maximise the variance explained within a set of response measures. The predicting variables are the food groups, and biomarkers are the response measures.

In summary, all FFQ data of case and control mothers were pooled into one data set, and factor analysis was performed on all mothers. This enabled us to examine whether the strengths of the intake of one specific diet differed between cases and controls in association with SB risk and biomarker levels. The 200 food items from the FFQ were reduced to 16 predefined food groups based on similar nutrient content, which are comparable with grouping schemes in the literature.[28] The food groups were adjusted for the total intake of energy.[29] The first factor, for example dietary pattern, was extracted from the PCA solution. In the RRR method, the 16 predefined food groups were chosen as predicting variables and the maternal biomarkers as response measures. The overall *P* value for the explained variance of the factor solution was tested with the permutation method. Each mother received a personal score for PCA as well as RRR to describe the agreement of her diet with that of the extracted factor. PCA was performed with SPSS software, release 11.0.1, (SPSS Inc., Chicago, IL, USA) and RRR analysis with CANOCO (Canonical Community Ordination), version 4.5 (Microcomputer Power, Ithaca, NY, USA) for Windows.[30]

Differences in baseline characteristics between case and control mothers were tested with analysis of variance for maternal age and BMI and Mann–Whitney *U* test for the remaining continuous variables. Dietary characteristics were obtained using Pearson *r* correlation coefficients between maternal factor scores and food groups. Subsequently, cases and controls were pooled and divided into quartiles according to their factor score. Maternal age at the study moment and BMI are presented as mean with SD, and energy intake and biomarkers are presented as median with range per quartile of each dietary pattern. The associations between diet and continuous variables were evaluated with a linear regression model. Differences in educational level, smoking, alcohol use and vitamin supplement intake between the quartiles were tested using the Chi-square test for linear association. Differences in biomarker concentrations between quartiles of the dietary patterns were evaluated in a linear regression model, adjusted for the periconception use of folic acid and/or multivitamins. The percentiles were also used for risk estimation by odds ratios and 95% CI. The distribution of case and control mothers in the highest quartiles (Q2, Q3 and Q4) of the diet was used as a reference. The estimates were adjusted for potential confounders in a logistic regression model based on significant baseline periconception characteristics between cases and control and significant characteristics of each dietary pattern ($P \leq 0.05$).

Results

Fifty case mothers and 81 control mothers were included in this study. Eighteen of the 77 case mothers and 38 of 151 control mothers were excluded due to excessive vomiting and/or a reported change in nutritional intake in the periconception period compared with the study moment. In addition, four case mothers and four controls were excluded because information on excessive vomiting in the periconception period and/or change in nutritional pattern was lacking. Finally, 5 case mothers and 28 control mothers were excluded because of incomplete FFQs.

The characteristics of case and control mothers are shown in Table 1. At the study moment, case mothers had a slightly higher BMI ($P = 0.08$), lower education ($P < 0.001$) and used less folic acid supplements ($P = 0.03$) and alcohol ($P = 0.01$). Alcohol use of case mothers was also lower in the periconception period ($P = 0.04$).

The first factor quantified the most prominent diet in the study group as a continuous variable. It explained 14.7% of the total variance and was labelled the Mediterranean dietary pattern comprising high intakes of vegetables, fruit, legumes, vegetable oil, cereal products, alcohol and fish and low intakes of potatoes and sugar and confectionary. A high maternal Mediterranean dietary pattern score from PCA was significantly associated with higher concentrations of maternal serum vitamin B12 ($P < 0.01$), serum ($P = 0.01$) and red blood cell (RBC) folate ($P = 0.04$) and lower levels of tHcy ($P = 0.051$) (Table 3). This dietary pattern was characterised by a higher maternal age at birth of the index child, higher education and more alcohol consumption in the periconception period and at the study moment ($P < 0.001$ for all comparisons) (Table 3).

The first factor extracted by the RRR method explained 13.0% of the total variance between food groups and 71.1% of the variance in biomarker variables. The overall *P* value for the factor solution was 0.03, which points out the significant

Table 1. Characteristics of mothers of children with SB and controls

	SB (n = 50)	Controls (n = 81)	P value
Age at delivery (years), mean (SD)	30.3 (3.9)	31.7 (3.5)	0.09
BMI (kg/m²), mean (SD)	25.4 (5.3)	22.9 (4.5)	<0.01
High education, *n* (%)	8 (1.0)	44 (54.3)	<0.001
Male child, *n* (%)	19 (3.0)	38 (46.9)	0.32
Periconception			
Use of folate, *n* (%)	19 (3.0)	38 (47.5)	0.29
Smoking, *n* (%)	11 (2.0)	15 (18.5)	0.63
Alcohol, *n* (%)	18 (3.0)	44 (54.3)	0.04
At 14 months postpartum			
Use of folate, *n* (%)	11 (2.0)	7 (8.6)	0.03
Smoking, *n* (%)	12 (2.0)	15 (18.5)	0.45
Alcohol, *n* (%)	26 (5.0)	60 (74.1)	0.01
Energy intake (kJ/day), median (range)	8826 (6191–15 750)	9076 (5200–13 615)	0.70

reproducibility between the food groups and the biomarkers. The factor corresponds with a Mediterranean dietary pattern as well as it comprises of high intakes of fruit, vegetable oil, vegetables, legumes, dairy products, grains, alcohol and fish and low intakes of potatoes, sugar and confectionary and sauces and condiments. The maternal Mediterranean diet from RRR is negatively associated with tHcy ($P < 0.001$) and shows positive associations with serum vitamin B12 ($P < 0.001$) and folate in RBC ($P < 0.001$) and serum ($P < 0.01$) (Table 3). Mothers with a high Mediterranean diet score according to RRR were generally higher educated and consumed more alcohol in the periconception period ($P < 0.01$ for both characteristics) (Table 3).

Table 2. The factor loadings of first major dietary pattern from PCA and RRR and the Pearson correlation coefficients with food groups*

	Mediterranean diet**			
	PCA (n = 50)	P value	RRR (n = 81)	P value
Food group				
Alcohol	0.25	<0.01	0.23	0.02
Vegetables	0.68	<0.01	0.29	<0.01
Vegetable oil	0.71	<0.01	0.36	<0.01
Sugars	−0.37	<0.01	−0.26	0.01
Sauces and condiments	0.10	0.24	−0.35	<0.01
Potatoes	−0.37	<0.01	−0.60	<0.01
Drinks	−0.14	0.10	−0.28	<0.01
Meat	−0.47	<0.01	−0.02	0.82
Margarine	0.11	0.21	−0.08	0.46
Legumes	0.30	<0.01	0.27	0.01
Fruits	0.50	<0.01	0.49	<0.01
Fish	0.31	<0.01	0.20	0.04
Eggs	−0.06	0.53	−0.04	0.69
Dairy products	0.03	0.76	0.39	<0.01
Cereal products	0.55	<0.01	0.36	<0.01
Butter	0.23	0.01	−0.11	0.26

*Food group intake data are adjusted for total energy intake.
**The Pearson r correlation coefficients between factor loadings and food groups are presented with P values.

We used maternal dietary patterns to assess SB risk in the offspring. Table 4 reveals that the mothers in the lowest quartile of the Mediterranean dietary pattern, that is weakest use, extracted by both PCA and RRR, had a significantly increased SB risk compared with those in the higher quartiles, OR 2.7 (95% CI 1.2–6.1) and OR 3.5 (95% CI 1.5–7.9), respectively. Adjustment for confounders including maternal BMI, age at the index pregnancy and periconception folic acid supplement use did not significantly affect the risk estimates. Thus, mothers who were weak users of the Mediterranean dietary pattern in the periconception period were over two times as likely to give birth to SB offspring when compared with strong users of the Mediterranean diet.

Discussion

For the first time, a maternal dietary pattern is associated with the risk of having SB offspring independent of periconception folic acid supplementation. This is considered to be clinically relevant because low maternal intake of vegetable oil, vegetables, fruits, fish and whole grains was associated with a more than two-fold increased SB risk. This observation may suggest that a healthy diet contains enough natural folate to reach a similar protective effect as supplementation with a synthetic folic acid tablet.[4] Latest findings in literature have reported higher bioavailability of folate in foods than previously was suggested.[31] Of interest is that the results also provide a plausible explanation for the relatively low occurrence of SB in Southern European countries where the Mediterranean diet is

Table 3. Characteristics of mothers (pooled) stratified in quartiles from low (Q1) to high (Q4) dietary pattern scores*

	Mediterranean diet				*P* value
	Q1 (*n* = 33)	Q2 (*n* = 33)	Q3 (*n* = 33)	Q4 (*n* = 32)	
PCA					
Age at delivery (years)	29.1 (3.4)	31.1 (3.5)	31.8 (3.1)	33.0 (3.7)	<0.001
BMI (kg/m^2)	26.0 (6.3)	25.2 (5.0)	25.0 (4.7)	22.9 (3.0)	0.08
High education, *n* (%)	3 (9.4)	11 (33.3)	16 (48.5)	22 (66.7)	<0.001
Male child, *n* (%)	16 (5.0)	10 (30.3)	18 (54.5)	13 (39.4)	0.86
Use of folate**, *n* (%)	15 (46.9)	12 (36.4)	19 (57.6)	13 (40.6)	0.94
Smoking**, *n* (%)	10 (31.3)	4 (12.1)	6 (18.2)	6 (18.2)	0.30
Alcohol**, *n* (%)	3 (9.4)	16 (48.5)	20 (60.6)	23 (69.7)	<0.001
Energy intake*** (kJ/day)	9328 (5651–15 750)	8469 (5515–13 205)	8921 (5200–13 913)	9193 (6191–13 659)	0.86
Biomarkers****					
Plasma tHcy	11.7 (7.5–85.8)	11.9 (3.7–59.3)	10.3 (4.5–18.7)	10.3 (4.9–22.3)	0.05
Serum folate	12.7 (3.4–183.2)	11.0 (5.0–36.5)	15.9 (2.4–178.7)	19.9 (6.6–679.5)	0.01
RBC folate	600 (139–1758)	617 (345–1545)	629 (147–3143)	815 (352–3999)	0.04
Whole blood vitamin B6	51 (35–90)	57 (35–140)	60 (35–225)	60 (37–140)	0.16
Serum vitamin B12	219 (66–426)	239 (131–449)	281 (54–741)	320 (48–680)	<0.01
RRR					
Age at delivery (years)	30.5 (3.7)	31.0 (3.2)	33.1 (3.9)	30.4 (3.3)	0.09
BMI (kg/m^2)	25.4 (6.1)	25.0 (4.7)	24.5 (4.8)	24.2 (4.3)	0.76
High education, *n* (%)	6 (18)	12 (36)	21 (64)	13 (41)	<0.01
Male child, *n* (%)	9 (27)	18 (55)	15 (46)	15 (47)	0.20
Use of folate**, *n* (%)	20 (61)	24 (73)	15 (46)	14 (45)	0.16
Smoking**, *n* (%)	9 (27)	5 (15)	6 (18)	6 (19)	0.47
Alcohol**, *n* (%)	10 (30)	11 (33)	22 (67)	19 (59)	<0.01
Energy intake*** (kJ/day)	8625 (5515–13 615)	8897 (5200–15 750)	8824 (5517–12 182)	9265 (6191–13 913)	0.67
Biomarkers****					
Plasma tHcy	12.8 (5.2–85.8)	10.3 (8.2–16.3)	11.0 (6.9–16.7)	9.9 (3.7–59.3)	<0.001
Serum folate	11.7 (2.36–183.2)	13.8 (4.5–37.2)	17.9 (4.6–178.7)	19.2 (5.9–679.5)	<0.01
RBC folate	564 (139–1757)	587 (159–1498)	706 (291–3142)	834 (394–3998)	<0.001
Whole blood vitamin B6	50 (35–120)	58 (35–225)	55 (37–100)	61 (36–140)	0.43
Serum vitamin B12	221 (48–433)	240 (54–550)	284 (138–680)	304.5 (182–741)	<0.001

*Quartiles were calculated by summation of intake food groups weighted by their factor loadings. Data are presented as mean (SD) or median 2(range) unless stated otherwise.

**The maternal use refers to the periconception period.

***Maternal energy intakes are reported at 14 months postpartum.

****P values for biomarker concentrations have been adjusted for folic acid and/or multivitamin supplement use.

commonly used.[10] Nevertheless, this association should be interpreted cautiously, and further studies are needed to substantiate this finding.

The PCA and RRR methods have identified a comparable dietary pattern, which was labelled 'Mediterranean' as it was characterised by high intakes of fruits, vegetables, vegetable oils, legumes, fish, alcohol and cereal products and low intakes of potatoes and sweets.[32] The dietary pattern from PCA differs by the higher intakes of butter and lower intakes of meat. The diet from RRR contained more dairy products but less drinks, sauces and condiments. Alcohol intake was strongly correlated with the Mediterranean diet. The general alcohol intake was very low among all mothers in the periconception period, and the intake was usually restricted to one or occasionally two glasses of wine per week. Nevertheless, the observed association may be explained by the well-known relationship between educational level and alcohol intake in the Netherlands. The drinking of wine is part of social behaviour of socio-economic privileged households, in which there is also much interest in the Mediterranean style of food preparation and cooking. This is substantiated by the significantly lower education in case mothers compared with controls. Education is a known risk factor for SB and could be considered a proxy for the quality of the dietary pattern and lifestyles. This is also in agreement with the higher BMI observed in case mothers.

There was also a strong association between education and use of the Mediterranean diet. This was the rationale

Table 4. Low (<p25) versus high (≥p25) percentiles of the maternal dietary pattern and the association with SB risk*

Mediterranean diet	SB (n = 50)	Controls (n = 81)	Crude OR (95% CI)	Adjusted OR (95% CI)**
PCA				
<p25, *n* (%)	18 (36.0)	14 (17.3)	2.7 (1.2–6.1)	2.3 (0.9–5.6)
≥p25, *n* (%)	32 (64.0)	67 (82.7)	1.0 (reference)	1.0 (reference)
RRR				
<p25, *n* (%)	20 (40.0)	13 (16.0)	3.5 (1.5–7.9)	3.5 (1.5–8.2)
≥p25, *n* (%)	30 (60.0)	68 (84.0)	1.0 (reference)	1.0 (reference)

*Calculated from the summation of intake food groups weighted by their factor loadings.
**Odds ratios for SB according to PCA factor percentiles have been adjusted for maternal age, periconception folic acid/multivitamin supplement use and BMI in a logistic regression model. Subsequently, odds ratios according to RRR factor quartiles have been adjusted for periconception folic acid/multivitamin supplement use and BMI.

for not adjusting the odds ratios for educational level. Finally, the Mediterranean dietary pattern was also associated with higher maternal age and folic acid supplementation, which may give rise to the future identification of a maternal lifestyle and thus phenotype with regard to the risk for SB offspring.

It is important to note that the Mediterranean dietary pattern revealed positive associations with blood concentrations of folate and vitamin B12 and an inverse association with tHcy. This may suggest that the Mediterranean diet reduces hyperhomocysteinaemia and related oxidative stress. Oxidative stress is also involved in cardiovascular disease and, not surprisingly, the Mediterranean diet has been associated with a decreased risk of cardiovascular diseases.[14,15] These findings are in line with the fact that intrauterine nutritional programming has long-term consequences and is associated with adult diseases in adulthood.[33]

The use of dietary pattern analysis is commonly accepted as it provides essential and complementary insights into the overall dietary behaviour and, to date, has successfully been related to disease risk. The PCA analysis describes interactions between a set of correlated predicting variables, for example maternal food intakes, independent of a certain outcome measure. Therefore, it is possible that certain combinations of food intakes are overlooked that may be better related to disease status. Hoffmann *et al.*[12] addressed this issue by introducing RRR, in which predicting variables (food groups) are used to predict certain response measures, such as the biomarkers that are known to be linked to a disease or phenotype. However, the use of specific biomarkers as response measures also has some disadvantages because biomarkers serve as intermediary variables in biochemical pathways that may not be important for all individuals. Other pathways that may be involved in the chain from diet to disease have, however, not been considered. We addressed these issues by performing both PCA and RRR. Both statistical methods have identified a comparable Mediterranean dietary pattern predominantly present in the study group.

Our study was conducted at a fixed study moment of 14 months after the birth of the index child to reflect the maternal dietary intake and lifestyle during periconception period. While a prospective study focused on maternal exposures during periconception period would be a preferable study design, this would require enormous financial and logistic resources. Therefore, we standardised the study design as much as possible and excluded women from analysis if their dietary intake might have been different between the periconception period and the time of the study due to illnesses, a reported change in diet, pregnancy or lactation. This is in agreement with the rational of Willet[20] and others that the individual dietary intake is rather stable during life, except for periods of dieting, breastfeeding and extreme growth. The use of such strict exclusion criteria may potentially result to a degree of selection bias. However, the additional analysis showed that there were almost no differences in the general characteristics, biomarkers and micronutrient and macronutrient intakes of the included and excluded mothers, which makes such bias unlikely. Nevertheless, our findings should be carefully interpreted, and future studies with larger samples sizes are needed for further validation.

We considered the power of the study by performing an additional power calculation based on estimated odds ratios between 2.5 and 3.0, type I error of 0.05 and a power of 80%. It revealed that the sample sizes needed are between 47 to 68 cases and 75 to 108 controls. This means that with the 50 cases and 81 controls, we had sufficient power to detect significant effects of a weak use of the dietary Mediterranean intake.

Conclusions

The identification of the Mediterranean dietary pattern in association with SB risk is valuable in understanding how an overall diet can be linked to biomarkers, metabolic pathways and risk estimates. In future, this may lead to opportunities for more natural and flavoursome strategies for the target group in addition to current pharmacological

interventions. Encouraging women to use a Mediterranean diet will not only protect against cardiovascular diseases but may be beneficial for reproductive outcome as well.

Disclosure of interests

None.

Contribution to authorship

M.V., R.P.S.-T and C.W.L. were primarily responsible for analysing the data from the study. All authors assisted in the interpretation of the study results and critical revision of the manuscript. All authors participated in the design, implementation and writing of the manuscript, and all have seen and approved the final version. R.P.S.-T. is the guarantor.

Details of ethics approval

This study was approved by the Central Committee on Research in Human and the Medical Ethics Committees of all participating hospitals in the Netherlands.

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