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Maternal intake of fat, riboflavin and nicotinamide and the risk of having offspring with congenital heart defects

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Abstract *Background* With the exception of studies on folic acid, little evidence is available concerning other nutrients in the pathogenesis of congenital heart defects (CHDs). Fatty acids play a central role in embryonic development, and the B-vitamins riboflavin and nicotinamide are co-enzymes in lipid metabolism. *Aim of the study* To investigate associations between the maternal dietary intake of fats, riboflavin and nicotinamide, and CHD risk in the offspring. *Methods* A case-control family study was conducted in 276 mothers of a child with a CHD comprising of 190 outflow tract defects (OTD) and 86 non-outflow tract defects (non-OTD) and 324 control mothers of a non-malformed child. Mothers filled out general and food frequency questionnaires at 16 months after the index-pregnancy, as a proxy of the habitual food intake in the preconception period. Nutrient intakes (medians) were compared between cases and controls by Mann–Whitney *U* test. Odds ratios (OR) for the association between CHDs and nutrient intakes were estimated in a logistic regression model. *Results* Case mothers, in particular mothers of a child with OTD, had higher dietary intakes of saturated fat, 30.9 vs. 29.8 g/d; $P < 0.05$. Dietary intakes of riboflavin and nicotinamide were lower in mothers of a child with an OTD than in controls (1.32 vs. 1.41 mg/d; $P < 0.05$ and 14.6 vs. 15.1 mg/d; $P < 0.05$, respectively). Energy, unsaturated fat, cholesterol and folate intakes were comparable between the groups. Low dietary intakes of both riboflavin (<1.20 mg/d) and nicotinamide (<13.5 mg/d) increased more than two-fold the risk of a child with an OTD, especially in mothers who did not use vitamin supplements in the periconceptional period (OR 2.4, 95%CI 1.4–4.0). Increasing intakes of nicotinamide (OR 0.8, 95%CI 0.7–1.001, per unit standard deviation increase) decreased CHD risk independent of dietary folate intake. *Conclusions* A maternal diet high in saturated fats and low

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in riboflavin and nicotinamide seems to contribute to CHD risk, in particular OTDs.

Key words congenital heart anomaly – saturated fat – B-vitamins – risk factors – prevention

Introduction

Cardiovascular malformations affect about six to eight infants per 1,000 live births per year and are a leading cause of infant deaths due to congenital anomalies [2, 14]. Despite the increasing number of studies focusing on the aetiology of congenital heart defects (CHDs), currently a cause can be identified only in 15% of the cases. Complex interactions between genetic and lifestyle factors have been suggested in which maternal nutrition plays a significant role in the majority of cases [2]. Best known is the preventive effect of periconceptional use of folic acid containing multivitamin supplements on CHD risk [3]. Shaw et al. [30] showed that maternal use of these vitamins during the sensitive period of heart development reduced the risk of conotruncal heart defects in particular. Furthermore, our group recently demonstrated that a low dietary intake of vitamin B12 is an independent risk factor for CHDs [34]. The B-vitamins folate and vitamin B12 are involved in the remethylation of homocysteine into methionine. Insufficient folate supply impairs the methylation of DNA, lipids and proteins. Furthermore, a compromised folate and/or vitamin B12 status also results in a mild hyperhomocysteinemia.

Our group and others showed that maternal hyperhomocysteinemia increases three- to ten-fold the risk of a child with CHD [13, 32]. Maternal diabetes has been associated with CHDs [24] and a clear link is shown between glycemic control during organogenesis and congenital malformations [26]. A number of studies have demonstrated a positive association between maternal obesity and CHDs as well [36, 38], although others reported contradictory results. There is no doubt that obesity is a phenotype of unhealthy lifestyle factors such as an excessive consumption of energy-dense foods. Thus, we became interested in other nutrients not previously studied that may be associated with CHDs, i.e., dietary fats and related B-vitamins. During early embryogenesis, fatty acids play a crucial role in membrane lipids and act as ligands for receptors and transcription factors that regulate gene expression [15]. Oxidation of fatty acids is dependent on adequate concentrations of the B-vitamins riboflavin and nicotinamide. Besides its role in fat metabolism, riboflavin acts as a cofactor in the folate pathway [25, 27]. Nicotinamide is involved in both the synthesis and oxidation of fatty acids and also plays a role in the metabolism of certain drugs and toxicants [18]. Of interest is that the strong maternal use of the western dietary pattern, e.g., high saturated fats and low B-vitamins including riboflavin, has been associated with an almost two-fold increased risk of orofacial clefts [35]. Furthermore, low dietary intakes of riboflavin and nicotinamide have been associated with an increased risk of orofacial clefts [29] and spina bifida [11], respectively. Neural tube defects and orofacial clefts share many similarities in the pathogenesis of CHDs as these birth defects originate from disturbances in neural crest cell behaviour by for example hyperhomocysteinemia and low folate [1, 4, 28]. Therefore, it is conceivable that nutritional factors implicated in the pathogenesis of neural tube defects and orofacial clefts also apply to CHDs.

Currently, research on dietary intake of fatty acids, related B-vitamins other than folate and vitamin B12, and CHD risk is lacking. Therefore, we aimed to investigate whether the maternal dietary intakes of fatty acids and B-vitamins riboflavin and nicotinamide differ between mothers of a child with a CHD and control mothers. This may provide new insights into the optimal balance and quality of the diet in women during the reproductive period.

Subjects and methods

Study population

The HAVEN study, a Dutch acronym for the study of heart anomalies and the role of genetic and nutritional factors, is a case-control family study designed to investigate determinants in the pathogenesis and prevention of CHDs. From June 2003 onwards, the HAVEN Study was carried out at the Department of Obstetrics and Gynaecology of Erasmus MC in Rotterdam, The Netherlands. The study design was previously described in detail [32, 34]. In summary, children with a CHD were diagnosed in the Sophia Children's Hospital/Erasmus MC in Rotterdam, Leiden University Medical Centre in Leiden, VU University Medical Centre and Emma Children's hospital/AMC in Amsterdam and were recruited with both parents in

collaboration with two paediatric cardiologists trained in the same hospital. Medical charts were thoroughly screened by the child health centre physicians and any control infant with a congenital anomaly was excluded. All diagnoses were confirmed by ultrasound and/or cardiac catheterisation and/or surgery. The CHD phenotypes were categorized into outflow tract defects (OTDs), comprising of tetralogy of Fallot (n = 38), atrioventricular septal defects (n = 27), perimembranous ventricular septal defect (n = 77), aortic valve stenosis (n = 6), pulmonary valve stenosis (n = 42), and non-outflow tract defects (non-OTDs) including coarctation of the aorta (n = 27), transposition of the great vessels (n = 34), hypoplastic left heart syndrome (n = 13) and miscellaneous (n = 12). Case and control children were all singletons. Control children were enrolled in collaboration with the public child health care centres in the surroundings of Rotterdam and were unaffected by any major birth defect. All parents were Dutch speaking. Maternal pre-existing diabetes was present in two case mothers and four controls. Between October 2003 and December 2006, we collected questionnaire data on 351 case mothers and 406 control mothers. Pregnant (cases n = 38, controls n = 32) or lactating (cases n = 15, controls n = 16) mothers were excluded. We also excluded mothers who reported an altered diet compared with the periconception period, e.g. slimming or vegetarian diet (cases n = 22, controls n = 34). This resulted in the final evaluation of 276 case mothers and 324 control mothers. The study protocol was reviewed and approved by the Central Committee of Research in Human and the Medical Ethics Committees of the participating hospitals. A written informed consent was obtained from every parent.

Data collection

We collected the dietary intake data at 16 months after the index-pregnancy. At that time, the diagnosis of the CHD phenotypes had been confirmed. Dietary intake was assessed using a modified version of the semiquantitative food frequency questionnaire (FFQ) of Feunekes et al. [8] covering the previous 4 weeks. This for fats validated FFQ has been updated twice based on data of Dutch national food consumption surveys in 1992 and 1998 and was also specifically validated for B vitamin intakes [21, 22, 33]. The FFQ consisted of 195 items, structured according to a meal pattern. Questions about preparation methods, portion sizes and additions were included. We used the 2001 electronic version of the Dutch food composition table to calculate average daily intake of nutrients [23]. Further details on the FFQ are described elsewhere [8, 33].

Nutritional habits are in general rather constant, with the exception of periods of dieting and increased needs during pregnancy and lactation [5, 7, 39]. During the first critical pregnancy weeks, maternofetal nutrient transfer is largely determined by the maternal dietary intake of the previous preconception weeks. Our assumption is that the nutrient data from the FFQ filled out 24 months after conception reflect the maternal nutritional status in the preconception period. Moreover, it resembles the same season of the year. The value of this approach has been demonstrated by studies before [19, 34]. The FFQs were filled out at home and verified by the researcher using a standardized checklist during the hospital visit or occasionally during a telephone interview. During the hospital visit at the Erasmus MC, standardized anthropometric measurements of the mothers were performed. Weight (weighing scale, SECA, Hamburg, Germany) was measured with 0.5 kg accuracy and height (anthropometric rod, SECA, Hamburg, Germany) up to 0.1 cm accuracy. Body mass index was defined as weight divided by height squared.

All mothers completed a questionnaire concerning lifestyle behaviours and demographic data at 16 months after the index-pregnancy and at the periconception period, defined as four weeks prior to conception until 8 weeks after conception. Extracted data included maternal age, time interval after the index-pregnancy, pre-existing diabetes, educational level, ethnicity and use of alcohol, cigarettes and vitamin supplements. Periconception vitamin supplement use was defined as daily intake in the entire periconception period. Data on vitamin supplements included information on the contents (folic acid only or multivitamin supplement) and frequency of intake. Mothers were considered to use alcohol or cigarettes when any consumption in the questioned periods was reported. Mothers were classified by educational level based on definitions used by Statistics Netherlands [31]. Primary/lower vocational/intermediate secondary level of education was defined as low, intermediate vocational/higher secondary as intermediate, and higher vocational/university level as high education. Ethnicity was categorized as Dutch natives (both parents are of European origin and born in the Netherlands), European others (one of the parents is born in a European country or is of European origin and living in the USA, Australia or Indonesia), or non-European others (one of the parents is of non-European origin) [20].

Statistical analysis

General characteristics were compared between case and control mothers. The continuous variables

Table 1 General characteristics of the study population of case and control mothers

Mothers	Total CHD group (n = 276)	Outflow tract defects (n = 190)	Non-outflow tract defects (n = 86)	Control group (n = 324)
16 months after the index-pregnancy				
Maternal age (years)	33.1 (25.1–41.6)[a]	33.2 (24.4–42.3)[a]	32.9 (25.5–39.0)	32.7 (24.5–39.8)
Time after index-pregnancy (months)	16.2 (13.9–26.6)	16.1 (13.8–27.3)	16.5 (13.8–25.5)	16.1 (14.0–21.9)
BMI (kg/m^2)	24.1 (19.2–34.1)	24.3 (19.1–34.2)	23.5 (19.5–32.1)	24.1 (19.6–34.6)
Ethnicity[b]				
Dutch native	221 (80)	146 (77)	25 (87)[e]	258 (80)
European others	14 (5)	8 (4)	6 (7)	15 (5)
Non-European others	41 (15)	36 (19)	5 (6)	51 (16)
Educational level[c]				
Low	74 (27)	56 (29)	18 (21)	77 (24)
Intermediate	129 (47)	85 (45)	44 (51)	165 (51)
High	73 (26)	49 (26)	24 (28)	82 (25)
Use of [n (%)]				
Alcohol	139 (50)	91 (48)[d]	48 (56)	185 (57)
Cigarettes	55 (20)	37 (20)	18 (21)	63 (19)
Folic acid (multi)vitamins	55 (20)	32 (17)	23 (27)	67 (21)
Periconception				
Use of [n (%)]				
Alcohol	94 (34)	62 (33)	32 (37)	107 (33)
Cigarettes	51 (19)	34 (18)	17 (20)	71 (22)
Folic acid (multi)vitamins	135 (49)	93 (49)	42 (49)	163 (50)

Values are given in median (P5–P95) or number (percentage). BMI: n = 2 missing
[a] P = 0.01, significance tested by the Mann–Whitney U test
[b] Dutch natives: Both parents are from European origin and born in the Netherlands. European others: one of the parents is born in a European country or is from European origin and living in the USA, Australia or Indonesia. Non-European others: one of the parents is from non-european origin [20]
[c] Low (primary/lower vocational/intermediate secondary), intermediate (higher secondary/intermediate vocational) or high (higher vocational/university education) [31]
[d] $P < 0.05$, significance tested by X_2 test
[e] $P = 0.05$, significance tested by X_2 test

maternal age, time after index-pregnancy and BMI are presented as medians with 5th and 95th percentiles and tested using the Mann–Whitney U test because of skewed distributions. The categorical variables ethnicity and educational level and the dichotomous variables use of alcohol, cigarettes and folic acid containing (multi) vitamin supplements are presented as numbers with percentages and tested using the χ^2 test. The residual method was used to calculate energy adjusted nutrient intakes [40]. Except for cholesterol, folate, riboflavin and nicotinamide, the distributions of all nutrients were positively skewed and therefore log-transformed. The logarithmic transformed nutrient intakes of the individuals were regressed on their logarithmic transformed total intake of energy in MJ. This regression equation was used to calculate the predicted mean nutrient intake at the average energy intake of the total study population. By adding the predicted mean nutrient intake to the individual residuals, the nutrient intakes were energy adjusted. Energy adjusted nutrient intakes are presented as medians with 5th and 95th percentiles and compared between cases and controls by the Mann–Whitney U test. Spearman's correlation coefficients were computed to assess the correlations between the nutrients.

For each nutrient, univariate and multivariate logistic regression analyses were performed. We created the 25th percentile of dietary nutrient intakes based on the distribution in control mothers and estimated the risk for a CHD affected pregnancy by calculating odds ratios (OR) and their respective 95% confidence intervals (CI). Furthermore, multivariable logistic regression analysis with the B-vitamins folate, riboflavin and nicotinamide as continuous variables was performed to assess the independent relationship of B-vitamin intake via the diet and CHD risk. To adjust for potential confounding factors, we fitted a backward stepwise multiple logistic model. The presence or absence of CHD was the dependent variable. Covariates were maternal age, diabetes, BMI, ethnicity, educational level, periconception folic acid containing supplement, cigarette or alcohol use. The ORs were adjusted for maternal age and ethnicity, as only these predictors remained significant in the logistic model with P values of <0.05. Because mothers who used vitamin supplements in the periconception period had higher total B vitamin intakes than non-users, we performed a stratified analysis for periconception use of vitamins, supplements containing folic acid only, and non-supplement use. Probability values

Table 2 Energy adjusted nutrient intakes for case mothers and controls

	FCS[a]	Total case group (n = 276)	Outflow tract defects (n = 190)	Non-outflow tract defects (n = 86)	Control group (n = 324)
Energy (MJ)	8.5	8.7 (5.8–14.0)	8.7 (5.8–13.9)	8.8 (5.7–14.2)	8.8 (5.7–13.2)
Macronutrients					
Total fat (g)	85	83.6 (62.8–107.9)	84.0 (62.4–108.0)	82.3 (65.1–107.7)	82.8 (63.1–103.6)
Saturated fat (g)	33	30.7 (21.8–42.3)[b]	30.9 (21.6–42.4)[c]	30.3 (22.3–42.5)	29.8 (21.2–40.5)
Monounsaturated fat (g)	30	26.4 (19.2–36.4)	26.4 (18.7–37.4)	26.5 (19.7–33.7)	26.2 (19.3–34.1)
Polyunsaturated fat (g)	15	17.7 (11.2–27.0)	17.9 (11.2–28.1)	16.9 (10.7–26.6)	17.5 (12.0–26.5)
Linoleic acid (g)	–	13.6 (8.6–22.4)	13.9 (8.6–22.8)	13.0 (8.3–21.0)	14.0 (9.2–21.9)
ALA (g)	–	1.14 (0.66–1.86)	1.13 (0.64–1.87)	1.16 (0.66–1.91)	1.07 (0.74–2.01)
EPA (g)	–	0.03 (0.00–0.19)	0.03 (0.00–0.16)	0.04 (0.00–0.25)	0.03 (0.00–0.14)
DHA (g)	–	0.06 (0.01–0.29)	0.06 (0.01–0.23)	0.07 (0.01–0.36)	0.07 (0.01–0.21)
Cholesterol (mg)	200	171 (107–267)	177 (102–283)	179 (124–262)	176 (111–281)
Micronutrients					
Folate (μg)	153	191 (118–284)	189 (118–276)	195 (119–323)	199 (130–287)
Riboflavin (mg)	1.4	1.34 (0.74–1.08)	1.32 (0.72–2.09)[c]	1.38 (0.93–2.11)	1.41 (0.86–2.00)
Nicotinamide (mg)	–	14.7 (9.90–19.7)[c]	14.6 (9.6–20.1)[c]	14.9 (10.7–19.1)	15.1 (10.9–20.1)

Energy-adjusted intakes are medians (P5–P95)
ALA α-linolenic acid, *EPA* eicosapentanoic acid, *DHA* docosahexanoic acid
[a]Food Consumption Survey [22] based on women aged 22–50 years. Significance tested by Mann–Whitney *U* test
[b]$P = 0.05$, significance tested by the Mann–Whitney *U* test
[c]$P < 0.05$, significance tested by the Mann–Whitney *U* test

≤0.05 were considered statistically significant. All analyses were performed with SPSS for Windows software (version 15.0; SPSS Inc, Chicago, IL, USA).

Results

Table 1 summarizes the general characteristics in the total group of case mothers, stratified for OTDs and non-OTDs, and control mothers. Overall, case mothers were slightly older than controls with a mean difference of 0.4 years. Mothers of offspring with non-OTDs were more often from Dutch origin than the control group ($P = 0.05$). The median time after the index-pregnancy was around 16.1 months and comparable between case and control mothers. At 16 months after the index-pregnancy and at the periconception period all lifestyle behaviours were comparable between the groups.

The food frequency analyses showed that mothers of a child with a CHD had a significantly higher intake of saturated fat and a lower intake of nicotinamide (Table 2). These results are most pronounced in mothers of a child with an OTD. Furthermore, this group also showed significantly lower riboflavin intake. The median dietary intakes of energy, unsaturated fats, cholesterol and folate were comparable between the total CHD and stratified groups compared with controls. The dietary intakes of the fats were positively correlated. Spearman's correlation coefficients varied from 0.34 for the intake of cholesterol and saturated fat to 0.53 for the intake of monounsaturated and saturated fats. B-vitamins were correlated with Spearman's correlation coefficients varying from 0.23 for riboflavin and nicotinamide, 0.29 for folate and nicotinamide, to 0.35 for folate and riboflavin.

A maternal dietary intake of monounsaturated fats below the 25th percentile of controls, i.e., 23.1 mg/d, was associated with a reduced risk of CHD, particularly OTDs (OR 0.6, 95%CI 0.4–0.99) (Table 3). A dietary intake of cholesterol below the 25th percentile of intake in controls was associated with a reduced risk of having a child with a non-OTD (OR 0.5, 95%CI 0.3–0.95). The risk of offspring with OTDs was significantly higher with dietary intakes of riboflavin and nicotinamide below the 25th percentile of intakes in control mothers (OR 1.7, 95%CI 1.1–2.5 and OR 1.6, 95%CI 1.06–2.5, respectively). A multivariable model with the three B-vitamins folate, riboflavin and nicotinamide was used to determine the independent associations with CHD risk (Table 4). We demonstrated a dose-response relationship with increasing intakes of nicotinamide and decreasing CHD risk (OR 0.8, 95%CI 0.7–1.001, per unit standard deviation increase). This association was independent of dietary folate and riboflavin intake.

Stratification for periconception use of vitamins, supplements containing folic acid only, and non-supplement use revealed that the association between a dietary intake of riboflavin below 1.20 mg/d and OTD risk was strengthened, but only significant in non-supplement users (OR 2.4, 95%CI 1.4–4.0). A maternal dietary intake of nicotinamide below the 25th percentile of controls, i.e., 13.5 mg/d, was also associated with a higher risk of having a child with an

Table 3 Energy adjusted nutrient intakes and the risk of congenital heart defects

	Cut off levels	Total group cases/controls (n = 276/324)	OR (95%CI)	OTDs vs controls cases/controls (n = 190/324)	OR (95%CI)	Non-OTDs vs. controls cases/controls (n = 86/324)	OR (95%CI)
Macronutrients							
Total fat	<74.9 g/d	55/81	0.8 (0.5–1.2)	32/81	0.6 (0.4–0.97)	23/81	1.2 (0.7–2.1)
Saturated fat	<26.9 g/d	64/81	1.0 (0.7–1.4)	43/81	0.9 (0.6–1.4)	21/81	1.1 (0.6–1.9)
Monounsaturated fat	<23.1 g/d	50/81	0.7 (0.4–0.99)	34/81	0.6 (0.4–0.99)	16/81	0.8 (0.4–1.4)
Polyunsaturated fat	<15.0 g/d	75/81	1.1 (0.8–1.6)	54/81	1.2 (0.8–1.8)	21/81	1.0 (0.6–1.7)
Linoleic acid	<11.9 g/d	77/81	1.2 (0.8–1.7)	52/81	1.1 (0.7–1.7)	25/81	1.2 (0.7–2.1)
ALA	<0.89 g/d	53/81	0.7 (0.5–1.1)	37/81	0.8 (0.5–1.2)	16/81	0.7 (0.4–1.2)
EPA	<0.01 g/d	68/81	1.1 (0.7–1.6)	49/81	1.2 (0.8–1.9)	19/81	0.9 (0.5–1.5)
DHA	<0.03 g/d	74/81	1.2 (0.8–1.8)	54/81	1.1 (0.9–2.1)	20/81	0.9 (0.5–1.6)
Cholesterol	<145 mg/d	60/81	0.9 (0.6–1.3)	48/81	1.1 (0.7–1.6)	12/81	0.5 (0.3–0.95)
Micronutrients							
Folate	<165 μg/d	81/80	1.3 (0.9–1.9)	58/81	1.5 (0.97–2.2)	22/81	1.0 (0.6–1.8)
Riboflavin	<1.20 mg/d	89/81	1.6 (1.1–2.3)	65/81	1.7 (1.1–2.5)	24/81	1.3 (0.8–2.3)
Nicotinamide	<13.5 mg/d	89/81	1.6 (1.1–2.2)	62/81	1.6 (1.1–2.4)	27/81	1.5 (0.9–2.5)

Cut off values were based on the lowest quartile of intake of the control mothers. The reference category was a dietary intake above the cut off value. ORs were adjusted for maternal age and ethnicity

OR odds ratio, *CI* confidence interval, *OTD* outflow tract defect, *ALA* α-linolenic acid, *EPA* eicosapentanoic acid, *DHA* docosahexanoic acid

OTD but only in non-supplement users (OR 1.99, 95%CI 1.14–3.46).

Discussion

This study demonstrates that dietary intakes of saturated fat were slightly but significantly higher in mothers of a child with a CHD than in controls. On the other hand, dietary intakes of fat-related B-vitamins, i.e., riboflavin and nicotinamide, were significantly lower in case mothers, in particular mothers of a child with an OTD, compared with controls. A dietary intake of riboflavin and nicotinamide below 1.20 and 13.5 mg/d, respectively, more than 2-fold increased the risk of CHDs, especially in mothers who did not use vitamin supplements in the periconceptional period. CHD risk decreased with increasing intakes of nicotinamide, which seemed to be independent of dietary folate and riboflavin intake. Median nutrient intakes were similar to those of the Dutch National food consumption survey (FCS) [22], indicating that dietary intakes of case and control mothers generally reflect the intakes of Dutch non-pregnant women aged 22–50 years. Besides this study, research on the associations between dietary fat, riboflavin and nicotinamide is lacking and the underlying mechanisms are unknown. Nevertheless, our findings are in line with the associations demonstrated between maternal dietary intake of nicotinamide and riboflavin and the risk of a child with an orofacial cleft or spina bifida [11, 19]. CHDs share similarities in the pathogenesis of spina bifida and orofacial clefts, because of the involvement of neural crest cells which are very sensitive to exposures of folate and homocysteine [1, 4, 28]. Riboflavin and

Table 4 Multivariable regression analysis of the association between dietary intake of B-vitamins and the risk of congenital heart defects

	Total group cases/controls (n = 276/324)		OTDs vs. controls Cases/controls (n = 190/324)		Non-OTDs vs. controls cases/controls (n = 86/324)	
	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
Model[a]						
Folate	1.0 (0.8–1.2)	0.63	1.1 (1.0–1.1)	0.23	1.1 (0.9–1.5)	0.42
Riboflavin	0.9 (0.8–1.1)	0.32	0.9 (0.7–1.1)	0.25	1.0 (0.7–1.3)	0.98
Nicotinamide	0.8 (0.7–1.001)	0.051	0.8 (0.7–1.02)	0.08	0.8 (0.6–1.1)	0.13

The results of the logistic regression analysis are presented as odds ratio (OR) of CHD risk for one-unit-standard deviation increase in B-vitamins. Folate, 1 SD = 52.3 μg/d. Riboflavin, 1 SD = 0.36 mg/d. Nicotinamide, 1 SD = 3.11 mg/d. ORs were adjusted for maternal age and ethnicity

CI confidence interval, *OTD* outflow tract defects, *Non-OTD* non-outflow tract defects

[a]B-vitamins adjusted for each other

nicotinamide are coenzymes in fat metabolism and are important in molecular biological processes crucial for normal embryonic heart development, such as reduction of lipid peroxides [25, 27], cholesterol and steroid synthesis, glycolysis, regulation of numerous oxidoreductases and DNA repair mechanisms [18]. Riboflavin is also a co-factor in the folate pathway, and might exert its effect via both folate and fat metabolism.

We have shown a higher CHD risk for low dietary intakes of riboflavin and nicotinamide, particularly in mothers who did not use vitamin supplements in the periconceptional period. Thirty-six case mothers and thirty-nine controls used also a B-vitamin supplement. The dosage of the B-vitamins in supplements is much higher than in food. This may explain why we could not assess a higher CHD risk of low dietary intake of B-vitamins in this group. Periconception supplement use was defined as the daily use of vitamin supplements from four weeks before until eighth weeks after conception. Some mothers categorized as non-supplement users were in fact irregular users, which may have underestimated the OR of low dietary riboflavin and nicotinamide intake in this group. Maternal education, as a proxy for socio-economic status, was lower in mothers who did not use vitamin supplements in the periconception period. Furthermore, it is known that maternal education is correlated with periconception supplement use [6]. Therefore, the association between B-vitamin intake and CHD risk may be confounded by education. Adjustment for education, however, showed that it only marginally affected the ORs without consequences for the conclusions.

Animal studies have suggested that in utero exposure to a maternal diet rich in fat may lead to an increased risk of cardiovascular disease in the offspring [16]. Nutrition plays an important role in epigenetic modification of genes, referring to all changes in the genes other than the DNA sequence itself [37]. Mechanisms of epigenetic inheritance include methylation of DNA, modification of histones, binding of transcription factors to chromatin, and the timing of DNA replication. We hypothesize that inadequate maternal nutrition during the periconception period, e.g. excess of fatty acids and deficiencies of the B-vitamins riboflavin and nicotinamide might cause epigenetic changes in the DNA of the developing embryo resulting in an increased vulnerability for an embryo to develop a CHD.

Some strengths and limitations of this study have to be considered. Because of the relatively low prevalence rates of CHDs, we used a case-control study design with a standardized investigation at 16 months after the index-pregnancy. We consider this standardized investigation, of which the value was shown in our previous studies [11, 19, 34] as one of the main strengths. The investigation occurred relatively short after pregnancy to minimize recall bias regarding periconception lifestyle behaviours and to increase compliance of the participants. Furthermore, most CHD are diagnosed during the first year of life and therefore misclassification of cases and controls is avoided. Another strength is the detailed characterisation of CHD phenotypes. To further homogenize our case group in terms of pathogenesis, we clustered the OTDs, which have been reported to result from derangements in folate/homocysteine metabolism [9, 17]. Finally, with regard to nutrition, the study design considers several measures to reduce bias in nutritional intake. Importantly, we used a validated FFQ [8], covering the previous four weeks whereby day-to-day variability of food intake is minimized. Moreover, the standardized investigation at 16 months after the index-pregnancy is 2 years after conception and equals the season of the periconception period. Thus, the seasonal influences on food intake are comparable between the groups. Devine et al. [7] support our approach and state that, in general, no difference occurs in dietary patterns between the beginning of pregnancy and at least 1 year postpartum. Furthermore, the famous nutrition epidemiologist Walter Willet states that dietary patterns are rather stable [39]. We excluded mothers because of conditions that affect nutritional intake, such as pregnancy, lactation or an altered diet compared with the preconception period. Thirdly, energy adjustment also minimized possible underreporting bias. The ratio of energy intake (EI) and basal metabolic rate (BMR) was comparable between cases and controls (OTDs = 1.41; non-OTDs = 1.43; controls = 1.42). In a separate analysis, the EI: BMR ratio was 1.41 for Dutch natives, 1.43 for European others and 1.32 for non-Europeans. The EI: BMI ratio of the total study population was representative of long-term habitual intake according to the cut-off value of 1.35 [10]. However, there may be some underreporting in mothers from non-European origin.

A limitation of our study is the lack of biomarker data, because B-vitamin concentrations are not only determined by intake, but also by absorption, metabolism, clearance and genetic polymorphisms.

Furthermore, we cannot completely rule out the possibility that the observed associations result from residual confounding. However, the multiple logistic regression analysis revealed that maternal diabetes, BMI, educational level, periconception folic acid containing supplement use and periconception use of cigarettes or alcohol did not confound the associations. Due to the positive correlations among the B-vitamins, the identification of one B-vitamin with a predominant role in the pathogenesis of CHD is very

difficult. Nevertheless, nicotinamide intake seemed to be associated with CHD risk, independent of dietary folate and riboflavin. The different B-vitamins are frequently simultaneously present in foods, making it difficult to distinguish between the separate effects of one B-vitamin. Experimental animal studies with administration of separate B-vitamins could elucidate this issue further.

Our findings raise questions on the adequacy of the dietary intake among women of reproductive age, particularly with regard to riboflavin and nicotinamide intakes. Our study population showed adequate dietary intakes of riboflavin and nicotinamide according to the Dutch recommended dietary allowances [12]. This held even for the majority of mothers categorized in the lowest quartile of intake based on the controls. Considering our findings, one may argue whether the recommendations for riboflavin and nicotinamide intake are sufficient for women in reproductive ages and might need to be adapted. Moreover, acquiring evidence on the safety threshold for dietary fat intake is a high priority because of the increasing prevalence of a Western diet rich in fat even among women of childbearing age. In conclusion, our results suggest that a maternal diet high in saturated fat but low in riboflavin and nicotinamide contributes to a higher risk of having a child with a CHD.

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