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ORIGINAL CONTRIBUTION

Dietary intake of B-vitamins in mothers born a child with a congenital heart defect

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Abstract *Background* Periconceptional use of multivitamins reduces the risk of a child with a congenital heart defect (CHD). Data on the impact of maternal diet, however, are lacking. *Aim of the study* We investigated the association between the maternal dietary intake of B-vitamins and having a child with a CHD. *Methods* A case–control study was performed in 192 mothers of a child with a CHD and 216 mothers of a healthy child. Mothers filled out food frequency questionnaires covering the current dietary intake, and general questionnaires at 17 months after the index-pregnancy. Maternal blood samples were taken to determine B-vitamin and plasma total homocysteine (tHcy) concentrations as nutritional biomarkers. Pregnant and lactating mothers and those with another diet compared with the preconceptional period were excluded for analysis. Case-mothers and controls were compared using the Mann–Whitney *U* test and logistic regression. *Results* The dietary intake of macronutrients and B-vitamins was comparable between both groups, but all mothers had a substantially lower median folate intake (cases 161 μg, controls 175 μg) than the Dutch recommended dietary allowance of 300 μg. Within the case-group, the intake of proteins and vitamin B_6 and the concentrations of serum vitamin B_{12} and folate were significantly lower in hyperhomocysteinemics (tHcy ≥ 14.5 μmol/l) than in normohomocysteinemics. The maternal educational level was positively associated with B-vitamin intake, except for vitamin B_{12} in controls. Low educated case-mothers showed a significantly lower median vitamin B_{12} intake than controls (2.8 μg and 3.8 μg, $P = 0.01$). The CHD risk doubled if vitamin B_{12} intake in these mothers reduced by 50% (OR 2.0; 95% CI: 1.1–3.5). *Conclusions* A diet low in vitamin B_{12} is associated with an increased risk of a child with a CHD, especially in low educated women. A disbalance in the maternal intake of proteins and low folate intake may play a role as well, but needs further investigation. As hyperhomocysteinemia is a strong risk factor for adult cardiovascular disease, these data may imply that the hyperhomocysteinemic mothers and their children should be targeted for nutritional interventions.

Key words food – heart defects – congenital – vitamin B_{12} – pyridoxine – homocysteine

Introduction

Worldwide, 1 million children per year are born with a congenital heart defect (CHD) [1]. These complex malformations are responsible for a high infant mortality and morbidity rate and go together with substantial health care costs [2, 3]. Both genetic and environmental factors, such as nutrition and lifestyle, are implicated in the pathogenesis of CHDs. The mother serves as the environment of the child during embryogenesis, whereby the maternal dietary intake plays an important role.

After the Second World War epidemics of congenital malformations and miscarriages were found in all European cities that were affected by the famine [4]. Recently, low maternal intakes of nutrients, such as vegetable proteins, polysaccharides, dietary fibers, iron, and magnesium, were associated with an increased risk of a child with a spina bifida or orofacial cleft [5, 6]. B-vitamin intakes were also significantly lower in mothers of a child with an orofacial cleft than in controls [7, 8]. Several epidemiological studies demonstrated the preventive effect of periconceptional folic acid supplementation against the development of CHDs [9–11]. Among non-vitamin using mothers, the daily intake of folic acid fortified cereals significantly reduced the risk of conotruncal heart defects by 83% [11].

Kapusta et al. firstly reported of the association between a mild maternal hyperhomocysteinemia and CHD risk, which was recently confirmed by others [12, 13]. The main cause of mild hyperhomocysteinemia is a low intake of B-vitamins. Folate and vitamin B_{12} are involved in the remethylation of homocysteine and donation of one-carbon groups to proteins, lipids and nucleotides, whereas vitamin B_6 is important in the transsulphuration of homocysteine [14]. Insufficient intake of B-vitamins results in biochemical derangements leading to hyperhomocysteinemia and DNA hypomethylation that may contribute to the development of CHDs [15]. Interestingly, DNA hypomethylation is minimised at intake levels in excess of current recommended dietary folate and vitamin B_{12} intakes [16].

Therefore, we hypothesise that low maternal B-vitamin intakes detrimentally affect the embryonic cardiovascular development. We investigated the maternal dietary intake in a case–control study conducted in the Netherlands.

Materials and methods

Recruitment of subjects

The Dutch HAVEN study, acronym for the study of heart anomalies and the role of genetic and nutritional factors, is an ongoing case–control study designed to identify environmental and genetic factors in the pathogenesis of CHDs. The study has been performed at the Department of Obstetrics and Gynaecology of Erasmus MC in Rotterdam in close collaboration with the Centres of Paediatric Cardiology of the same hospital, and of Leiden University Medical Centre in Leiden, VU University Medical Centre and Academic Medical Centre in Amsterdam and with the child health centres of 'Thuiszorg Nieuwe Waterweg Noord' in the Rotterdam region. Eligible families are children with a CHD and healthy children with both parents living in the Western part of the Netherlands.

The paediatric cardiologists of the aforementioned Centres of Paediatric Cardiology diagnose and recruit the case-children and their parents in collaboration with the research team of the HAVEN study. The selected diagnoses comprise transposition of the great arteries, tetralogy of Fallot, atrioventricular or perimembranous ventricular septal defect, aortic valve stenosis, pulmonary valve stenosis, coarctation of the aorta, and hypoplastic left heart syndrome. Healthy control-children and both parents are enrolled in cooperation with the physicians of the child health centres. Control-children do not have congenital malformations or chromosomal defects according to the medical record and regular health checks by the physician of the child health centre. Invited case and control-children are between 11 and 18 months of age. Case and control-families are not related, and speak, read and write the Dutch language.

We obtained questionnaire and biochemical data of 247 case and 266 control-mothers that were collected during the hospital visit at Erasmus MC in Rotterdam between October 2003 and July 2005. Mothers who were pregnant, lactating, or those who reported a changed diet compared with the preconceptional period, were excluded for analysis. This resulted in a dataset of 192 case and 216 control-mothers. The Central Committee of Research in Human and the Medical Ethical Committees of the participating hospitals reviewed and approved the study protocol. Prior to participation, written informed consent was obtained from both parents.

Study design

Dietary habits are rather stable and do not change except for increased needs because of breastfeeding and pregnancy, and episodes of illnesses and dieting [17–19]. In addition, most congenital malformations are detected during the first year of life. Therefore, we carried out a standardised investigation between 11 and 18 months after the index-pregnancy under the

assumption that these data reflect the maternal nutritional status in the preconceptional period according to our previous studies [5–8].

At home mothers filled out the food frequency questionnaire (FFQ) that covered the intake of the previous 4 weeks, and the general questionnaire. During the study visit we performed maternal anthropometry and obtained maternal blood samples as biomarkers of nutritional intake. We checked the questionnaire data for completeness and consistency.

Data collection

We estimated daily habitual energy, macronutrient, and micronutrient intakes using a modified version of the semiquantitative FFQ of Feunekes et al. [20]. This FFQ has been updated twice based on data of Dutch national food consumption surveys in 1992 and 1998 [21, 22]. Additionally, this FFQ has been modified for the estimation of dietary B-vitamin intakes. Food items rich in B-vitamins were added to the food list when they contributed more than 0.1% to the intake of each of the nutrients of interest according to the food consumption survey of 1998 [22]. Thus, the FFQ covers the daily intake of each nutrient or food of interest for at least 90% of the population mean intake.

The FFQ consists of 121 items and is structured according to a meal pattern. Participants report the intake of foods used during the previous month. Questions about preparation methods, portion sizes and additions are also included. The average daily nutrient intake was calculated using the 2001 electronic version of the Dutch food composition table [23]. We evaluated the existence of under-reporting. The mean basal metabolic rate (BMR) was estimated according to the Schofield equations [24]. The physical activity level was calculated by the ratio of the reported energy intake (EI)/BMR [25].

Extracted data of the general questionnaire included maternal age, time after index-pregnancy, body mass index (BMI), educational level, ethnicity, smoking, use of alcohol, oral contraceptives and vitamin supplements at the study moment. Mothers were considered smokers or alcohol drinkers when any smoking or alcohol consumption was reported. Educational level and ethnicity were classified according to the definitions of Statistics Netherlands [26]. Educational level was categorised into low (primary/lower vocational/intermediate secondary), intermediate (intermediate vocational/higher secondary) and high education (higher vocational/university). Mothers were classified as Dutch natives, Western or non-Western immigrants.

We performed standardised maternal measurements of weight (weighing scale, SECA, Germany) with 0.5 kg accuracy and height (anthropometric rod, SECA, Germany) up to 0.1 cm accuracy. Venous blood samples were drawn from all mothers to measure concentrations of red blood cell (RBC) and serum folate, serum vitamin B_{12} and plasma total homocysteine (tHcy) as nutritional biomarkers as described before [27].

Immediately after blood sampling 0.1 ml EDTA whole blood was haemolysed with 0.9 ml freshly prepared 1.0% ascorbic acid. Subsequently, the haematocrit of the EDTA whole blood was measured (ADVIA 120 Haematology Analyzer, Bayer Diagnostics, Germany). Another EDTA-tube was put on ice and centrifuged immediately after blood sampling for measurement of the tHcy concentration. Blood samples were centrifuged at $4{,}000 \times g$ for 10 min at 4°C and separated within 1 h after blood sampling. Folate and vitamin B_{12} concentrations were routinely determined by immunoelectrochemiluminescence assay (Roche Modular E170, Roche Diagnostics GmbH, Germany). Shortly before the folate measurement the haemolysate was centrifuged at $1{,}000 \times g$ for 5 min at 18°C. The folate concentration in the haemolysate was recalculated in RBC folate using the following formula: (nM haemolysate folate*10/haematocrit) − (nM serum folate*{1−haematocrit}/haematocrit) = nM RBC folate. The tHcy concentration was routinely measured by high performance liquid chromatography with reverse phase separation and fluorescence detection [27].

The inter-assay coefficients of variation (CV) for vitamin B_{12} was 5.1% at 125 pmol/l and 2.9% at 753 pmol/l; for folate these CV were 9.5% at 8.3 nmol/l and 3.2% at 20.2 nmol/l and for tHcy 5.9% at 15.3 μmol/l and 3.4% at 39.3 μmol/l. Until measurement, all sera and plasma were stored at −80°C. Some biomarkers were missing due to failures in blood sampling or laboratory testing. All laboratory analyses were performed anonymously in batches within 3 months after collection.

Statistical analysis

Differences in the distributions of categorical variables were tested by the Chi-square test. The dietary intakes were approximately normally distributed except for vitamin B_{12} and folate intake. These two variables were log-transformed. The nutritional biomarkers showed skewed distributions even after transformation. Therefore, all data are presented as medians with interquartile range and differences between cases and controls were evaluted by the Mann–Whitney U test. We compared the data with the Dutch dietary rec-

ommended intakes (DRIs) for non-pregnant women to check the appropriateness of the dietary intakes of our study population [28, 29]. Moreover, Pearson correlation coefficients were computed to investigate the associations between the B-vitamin intakes and the corresponding biomarkers. The B-vitamin intakes were compared between cases and controls stratified for educational level using ANOVA.

The mean dietary B-vitamin intakes were adjusted for total energy intake using the residual method [30]. Shortly, the B-vitamin intakes were regressed on the total energy intake and the predicted mean B-vitamin intake was calculated for the mean total energy intake of the study population. The energy-adjusted B-vitamin intake was calculated by adding the individual residuals to the predicted mean B-vitamin intake.

We assessed the association between maternal dietary intake of B-vitamins and CHD risk for the crude and energy-adjusted data. Because of the complex pathogenesis of CHDs, it is most likely that a low dietary intake of B-vitamins might be a risk factor in a subgroup of cases only. Therefore, we created the 10th percentile of vitamin B_6, vitamin B_{12} and folate intake based on the control-data and estimated the CHD risk using odds ratios (OR) and 95% confidence intervals (CI) in a logistic regression model. In addition, we performed a logistic regression analysis of B-vitamin intake stratified for educational level. *P*-values <0.05 were considered statistically significant. All analyses were performed using SPSS-software package version 11.0 (SPSS Inc, Chicago, IL).

Results

The median maternal age of cases was slightly higher than that of controls (Table 1). The distributions of ethnicity and educational level were comparable in case and control-mothers. There were no significant differences in BMI, use of vitamin supplements, alcohol and cigarettes at the standardised study moment. All CHDs were pooled because subgroup analyses did not reveal significant differences in demographics, biomarkers and dietary intakes.

As shown in Table 2, all mothers had a substantially lower folate intake than the Dutch DRI. The daily dietary intake of macronutrients and B-vitamins was comparable between cases and controls. The ratio EI/BMR was 1.46 for cases and 1.44 for controls. Among the biomarkers, only the median tHcy concentration was significantly higher in case-mothers (10.4 μmol/l) than in controls (10.0 μmol/l). Within the case-group, the intake of proteins and vitamin B_6 and the concentrations of serum vitamin B_{12} and folate were significantly lower in hyperhomocysteinemics (tHcy $\geq$ 14.5 μmol/l) than in normohomocysteinemics (Table 3). Hyperhomocysteinemic controls had a significantly lower intake of proteins and vitamin B_{12} and significantly lower biomarker concentrations. The correlation coefficient between the intake and serum vitamin B_{12} concentration in all mothers was 0.27 ($P < 0.001$). The correlations between dietary folate intake and the serum and RBC folate concentration were 0.12 ($P < 0.03$) and 0.17 ($P = 0.002$), respectively.

Overall, a low maternal dietary intake of vitamin B_{12} was associated with an increased risk of a child with a CHD. The risk increased approximately 2-fold (OR 1.9, 95% CI: 1.03–3.4) at the 10th percentile of vitamin B_{12} intake (Table 4). The energy-adjusted vitamin B_{12} intake did not show a significant association nor did vitamin B_6 or folate intake. In both groups, educational level was positively associated with B-vitamin intake, except for vitamin B_{12} intake in controls (Fig. 1). The associations were significant for cases only. Low educated case-mothers showed a significantly lower median vitamin B_{12} intake of 2.8 μg per day than controls (3.8 μg). The CHD risk doubled if their vitamin B_{12} intake reduced by 50% (OR 2.0, 95% CI 1.1–3.5, $P_{trend} = 0.01$) (Table 5). Median daily intakes of vitamin B_6 (1.5 mg and 1.3 mg) and folate (169 μg and 143 μg) were not significantly lower in low educated cases than in low educated controls. The corresponding risk estimates

Table 1 General characteristics of mothers of a child with a CHD and controls at the study moment

	Cases $n = 192$	Controls $n = 216$
Maternal age (years)	33.3 (29.9–36.7)[a]	32.6 (29.0–34.9)[b]
Time after index-pregnancy (months)	17.0 (15.3–20.7)	16.8 (15.2–18.6)
BMI (kg/m^2)	24.1 (22.0–27.4)	24.1 (22.0–27.8)
Educational level[c] [*n* (%)]		
Low	58 (30)	53 (25)
Intermediate	88 (46)	108 (50)
High	46 (24)	55 (25)
Ethnicity[d] [*n* (%)]		
Dutch native	155 (81)	176 (81)
Western immigrants	14 (7)	12 (6)
Non-Western immigrants	23 (12)	28 (13)
Use of [*n* (%)]		
Alcohol	98 (51)	125 (58)
Cigarettes	39 (20)	43 (20)
Oral contraceptives	78 (41)	102 (47)
Vitamin supplements	35 (18)	41 (19)

[a]Values are median (interquartile range)
[b]$P < 0.05$ (Mann–Whitney *U* test)
[c]Categorised as low (primary/lower vocational/intermediate secondary education), intermediate (higher secondary/intermediate vocational education) or high (higher vocational/university education) [26]
[d]Classified according to the definitions of Statistics Netherlands [26]

Table 2 Dietary intake and biomarkers of nutritional intake of mothers of a child with a CHD and controls at the study moment

Daily intake of nutrients	Cases $n = 192$	Controls $n = 216$	DRI[a]
Energy (MJ)	8.5 (7.3–10.3)[b]	8.7 (7.4–10.4)	9.7–10.2
Fats (g)	82.0 (69.5–105.3)	83.4 (68.2–104.7)	
(en%)[c]	36.5 (33.7–40.6)	36.8 (32.5–39.4)	20–40
Proteins (g)	75.1 (62.2–85.1)	76.0 (64.4–87.0)	50–52
(en%)	14.5 (12.8–16.2)	14.5 (13.0–15.7)	9–25
Carbohydrates (g)	238 (203–290)	250 (209–293)	
(en%)	47.0 (43.5–51.0)	48.3 (44.4–51.3)	40
Vitamin B_6 (mg)	1.5 (1.3–1.8)	1.6 (1.4–1.9)	1.5
Vitamin B_{12} (μg)	3.6 (2.5–4.5)	3.6 (2.8–4.4)	2.8
Folate (μg)	161 (122–206)	175 (135–210)	300
Biomarkers[d]			
tHcy, plasma (μmol/l)	10.4 (8.9–12.9)	10.0 (8.4–12.2)[e]	
Vitamin B_{12}, serum (pmol/l)	272 (211–359)	247 (200–349)	
Folate, serum (nmol/l)	15.0 (12.3–18.6)	14.6 (12.1–19.4)	
Folate, RBC (nmol/l)	666 (535–793)	674 (537–855)	

[a]Dutch dietary reference intakes (DRI) for non-pregnant women aged 19–50 years [28, 29]
[b]Values are median (interquartile range)
[c]en%, percentage of total energy intake
[d]Homocysteine, 151 cases and 209 controls. Serum vitamin B_{12} and folate, 150 cases and 210 controls. RBC folate, 149 cases and 210 controls
[e]$P < 0.05$ (Mann–Whitney U test)

were 1.8 (0.8–3.8) per unit decrease of vitamin B_6 intake and 1.5 (0.7–3.1) per 50% reduction of the folate intake.

Discussion

In this study we demonstrate for the first time that a low maternal dietary vitamin B_{12} intake is associated with an approximately twofold increased risk of a child with a CHD. So far, associations between maternal vitamin B_{12} intake and a child with other malformations have not been found [5, 8]. Our findings are in line with other reports showing an association between a low blood vitamin B_{12} concentration and an increased risk of neural tube defects [31] and orofacial clefts [32]. A low vitamin B_{12} intake results in low blood vitamin B_{12} concentrations and increased homocysteine concentrations. Therefore, our finding is consistent with previous

Table 3 Daily nutrient intakes of the case and control-mothers after stratification for the tHcy concentration[a]

			Case-mothers		Control-mothers	
Nutrients	Unit	DRI[b]	Normal tHcy, $n = 125$	High tHcy, $n = 26$	Normal tHcy, $n = 188$	High tHcy, $n = 21$
Energy	MJ	9.7–10.2	8.4 (7.2–9.9)[c]	8.8 (7.4–11.1)	8.7 (7.4–10.4)	8.7 (7.3–9.5)
Fats	g	–	80.2 (70.2–98.8)	84.9 (64.4–123.1)	84.1 (67.5–105.9)	80.0 (71.9–90.5)
	en%[d]	20–40	36.5 (33.3–39.9)	35.9 (32.4–40.5)	36.8 (32.6–39.8)	36.8 (32.5–38.0)
Proteins	g	50–52	75.3 (63.8–83.9)	68.5 (54.2–81.0)	76.1 (65.7–88.1)	65.5 (55.1–82.3)[e]
	en%	9–25	14.8 (13.0–16.5)	12.7 (11.4–14.7)[e]	14.6 (13.2–15.9)	13.1 (12.0–14.9)[e]
Carbohydrates	g	–	232 (202–290)	262 (221–299)	246 (208–293)	267 (221–291)
	en%	40	47.1 (43.7–51.2)	47.7 (44.1–52.7)	47.8 (44.2–51.1)	49.2 (46.6–55.1)
Vitamin B_6	mg	1.5	1.5 (1.3–1.8)	1.3 (1.1–1.7)[e]	1.6 (1.4–1.9)	1.5 (1.3–1.8)
Vitamin B_{12}	μg	2.8	3.8 (2.5–4.6)	3.4 (2.3–4.0)	3.6 (2.9–4.5)	3.1 (2.3–3.8)[e]
Folate	μg	300	165 (122–210)	138 (120–174)	176 (137–210)	156 (106–202)
Vitamin B_{12}, serum[f]	pmol/l	–	285 (229–372)	175 (152–273)[e]	255 (207–358)	200 (150–258)[e]
Folate, serum[f]	nmol/l	–	15.4 (12.9–19.4)	11.1 (10.0–15.1)[e]	14.9 (12.3–19.7)	12.4 (10.6–14.9)[e]
Folate, RBC[f]	nmol/l	–	666 (572–790)	627 (466–842)	679 (547–892)	538 (467–716)[e]

[a]Stratification based on the 90th percentile (i.e., 14.5 μmol/l) of plasma tHcy concentrations of the controls
[b]Dutch dietary reference intakes for non-pregnant women aged 19–50 years [28, 29]
[c]Values are median (interquartile range)
[d]en%, percentage of total energy intake
[e]Reflects the comparison of hyper- and normohomocysteinemics within the case and within the control-group, $P < 0.05$ (Mann–Whitney U test)
[f]Hyperhomocysteinemic cases: serum vitamin B_{12} and folate, $n = 124$; RBC folate, $n = 123$. Normohomocysteinemic controls: $n = 187$

Table 4 Risk estimates for the association between maternal dietary B-vitamin intake per day[a] and CHDs

	Cut-off value[b]	Case/control (n = 192/216)	OR (95% CI)	Cut-off value[c]	Case/control (n = 192/216)	OR (95% CI)[c]
Vitamin B_6 (mg)	<1.1	25/21	1.39 (0.75–2.57)	<1.3	27/21	1.52 (0.83–2.79)
Vitamin B_{12} (μg)	<2.1	32/21	1.86 (1.03–3.35)	<2.2	28/21	1.59 (0.87–2.90)
Folate (μg)	<103.6	22/21	1.20 (0.64–2.26)	<117.6	26/21	1.45 (0.79–2.68)

[a]Data of vitamin B_{12} and folate intake were log-transformed
[b]Cut-off values are based on the lowest 10th percentile of the control vitamin intake
[c]Cut-off values and OR (95% CI) of energy-adjusted dietary intake [30]

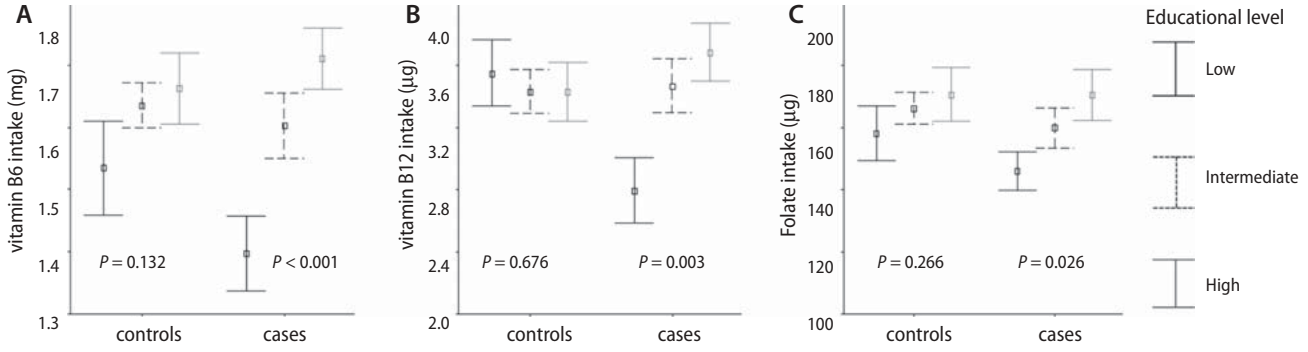

Fig. 1 Vitamin B_6 (**A**), vitamin B_{12} (**B**) and folate (**C**) intake in 216 mothers of children with a CHD and 192 control-mothers stratified for educational level. Data shown are mean ± SEM for vitamin B_6 and geometric mean ± SEM for folate and vitamin B_{12}. ANOVA P-values <0.05 indicate a significant trend over the educational levels

reports that showed an association between maternal hyperhomocysteinemia and having a child with a CHD [12, 13]. The significant correlation between dietary vitamin B_{12} intake and the corresponding biomarker supports this finding. Moreover, experimental studies substantiate the finding of the teratogenic effect of mild hyperhomocysteinemia [33].

Our data suggest that hyperhomocysteinemia in the subgroup of case-mothers can partly be explained by the significantly lower vitamin B_6 intake and low folate and vitamin B_{12} status. In contrast, hyperhomocysteinemic controls did not show a lower vitamin B_6 intake but their protein intake was lower than in normohomocysteinemic controls. This illustrates the heterogeneous aetiology of hyperhomocysteinemia [34].

The overall diet met the Dutch DRI in both cases and controls except for the low folate intake. The protein intake has to be in balance with the B-vitamin intakes. Dietary protein intake is the main source of methionine, which is the sole precursor of homocysteine. A high-protein diet increases tHcy concentrations throughout the day [35], and particularly folate is an important substrate for the remethylation of homocysteine. Therefore, a disbalance in the maternal intake of proteins and folate deranges the homocysteine metabolism and easily leads to mild hyperhomocysteinemia. The finding of low median folate intakes is supported by other Dutch studies and strengthens the recommendation to use a folic acid supplement to achieve an optimal periconceptional folate status [5, 36].

Low educated women more often demonstrated a low dietary vitamin B_{12} intake that was associated with a higher risk of CHDs than women with a higher educational level. This risk was substantiated by a significant dose–response relation within these women. This supports the findings of others that people with a low education and low income are more likely to engage in poor dietary practice than their wealthier and higher educated counterparts [37]. Dietary vita-

Table 5 Risk estimates for the association between CHDs and maternal dietary vitamin B_{12} intake per day stratified for educational level[a]

Educational level[b]	Case/control (n = 192/216)	OR (95% CI)	OR (95% CI)[c]
Low	58/53	1.96 (1.11–3.45)	1.86 (1.05–3.29)
Intermediate	88/108	0.96 (0.63–1.49)	1.01 (0.63–1.61)
High	46/55	0.69 (0.33–1.47)	0.64 (0.18–1.48)

[a]Data of vitamin B_{12} intake were log-transformed (base 2)
[b]Low (primary/lower vocational/intermediate secondary), intermediate (higher secondary/intermediate vocational) or high education (higher vocational/university) [26]
[c]OR (95% CI) for energy-adjusted dietary intake [30]

min B_{12} is only available from animal sources like fish and red meat. Therefore, low educated people may not have enough income for these more expensive foods or they are not correctly informed to buy and prepare healthy food.

Interestingly, hyperhomocysteinemia is also a strong risk factor for adult cardiovascular disease [38]. Our experimental data suggest that prenatal exposure to hyperhomocysteinemia induces the first features of atherosclerosis that are associated with cardiovascular diseases in later life [39]. Together with our current results this may imply that the hyperhomocysteinemic mothers and their children are both at risk for the development of cardiovascular disease in adulthood. Since B-vitamin intake is an important determinant of hyperhomocysteinemia this group should, therefore, be targeted for nutritional interventions.

We address some issues of our study design. Dietary assessment methods have a strong bias towards underestimation of habitual energy intake. Some under-reporting may equally be present in both our case- and control-group according to the cut-off value of 1.55 that allows measurement imprecision arising from day-to-day variability [25]. However, the FFQ covered a 4 weeks period and therefore, the day-to-day variability of food intake is minimised. Moreover, the ratio was representative of long-term habitual intake according to the cut-off value of 1.35 [25]. Furthermore, adjustment for energy intake or expression of macronutrient intake as percentages of total energy intake minimises the bias generated by under-reporting [40]. We excluded some mothers from analysis because of conditions that affect the nutritional intake, such as pregnancy, lactation or the use of another diet compared with the preconceptional period. Moreover, multivitamin users may be more aware of the importance of a healthy diet than non-users. However, exclusion of the multivitamin users did not significantly affect the results.

The case–control study is the usually used epidemiological study design for congenital defects due to the relatively low birth prevalence rates. Although, recall bias is not frequently present in case–control studies on congenital malformations [41], we have chosen for one investigation at a fixed moment shortly after pregnancy to reduce the potential for recall bias. A single investigation increases the feasibility of the study and compliance of participants and decreases the potential for selection bias as well. An earlier study moment after birth would imply a significant interference of the maternal physiology and endocrinology with the biomarkers as well as some misclassification of the cases and controls, because most malformations are detected and completely diagnosed during the first postnatal year. Moreover, this study moment is around 2 years after conception of the index-pregnancy, and equals the season of the pre- and periconceptional period. Thus, the seasonal influences on food intake are comparable in both groups. Therefore, after exclusion of pregnant and breastfeeding women and those who changed their diet during the preconceptional period, a standardised investigation between 11 and 18 months after the index-pregnancy in both cases and controls is the best moment to mimic the maternal nutritional status in the preconceptional period and to minimise the risk of misclassification of CHDs. This is substantiated by the concentrations of tHcy, folate and vitamin B_{12} in our study that were comparable to the concentrations measured in the preconceptional period by Cikot et al. [19]. To homogenise the CHD group, we only included mothers of patients with a type of CHD that has been associated with folic acid or other environmental factors, because periconceptional use of multivitamins containing folic acid is suggested to reduce the occurrence of cardiac outflow tract anomalies [9].

Although, the correlation coefficients between folate and vitamin B_{12} intake, and their blood levels were statistically significant, they were rather low. This was not due to time delay in data collection, because the FFQ covered the 4 weeks before the study moment and blood sampling was performed directly thereafter. Blood samples were centrifuged and separated within 1 h after blood sampling and stored at $-80^{\circ}C$ until measurement. All laboratory measurements were performed within 3 months after blood sampling. Therefore, it is very unlikely that this procedure has affected the blood levels. In addition, the B-vitamin biomarkers are not only determined by intake, but also by absorption, metabolism, clearance, and genetic polymorphisms encoding enzymes in the folate and homocysteine metabolism.

In conclusion, we show that a low maternal dietary vitamin B_{12} intake is associated with an increased risk of a child with a CHD, especially in low educated women. A disbalance in the maternal intake of proteins and folate may play a role as well, but needs further investigation. If future studies confirm our findings, women who are planning a pregnancy should use vitamin supplements containing both folic acid and vitamin B_{12}.

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