Eur J Nutr (2004) 43 : 7–14
DOI 10.1007/s00394-004-0433-y

ORIGINAL CONTRIBUTION

Maternal dietary B vitamin intake, other than folate, and the association with orofacial cleft in the offspring

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Received: 10 December 2002
Accepted: 19 May 2003
Published online: 6 January 2004

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Amsterdam, The Netherlands, 1997

Summary *Background* Periconceptional folic acid supplementation is suggested to prevent orofacial clefts (OFCs). Other B vitamins however may be beneficial as well. *Aim of the study* To investigate the maternal periconceptional dietary intake of thiamine, riboflavin, niacin, pyridoxine and cobalamin in association with the occurrence of OFC. *Methods* Two hundred and six mothers of a child with nonsyndromic OFC and 203 control mothers filled out a general questionnaire and a food frequency questionnaire around 14 months postpartum as a proxy for periconceptional intake. After exclusion of known pregnant and lactating mothers, those who reported to have altered their diet compared to the periconceptional period, and mothers with incidental folic acid supplement use periconceptionally, data of 182 OFC mothers and 173 controls were analysed. After logarithmic transformation, geometric means (P5-P95) were calculated and compared between the groups. After subsequent adjustment for energy, quintiles of dietary B vitamin intake were created. *Results* The periconceptional intake of thiamine, niacin and pyridoxine was significantly lower in mothers of an OFC child. A trend towards risk reduction for OFC with increasing dietary intake was demonstrated for thiamine ($p = 0.04$) and pyridoxine ($p = 0.03$). Risk reductions were only demonstrated in women using folic acid supplements periconceptionally. Supplement users tended to consume a diet richer in B vitamins. *Conclusions* Periconceptional intake of thiamine, niacin and pyridoxine seems to contribute to the prevention of OFC.

Key words pyridoxine – thiamine – niacin – cleft lip – cleft palate

Introduction

Orofacial clefts (OFCs) are common congenital abnormalities in humans occurring in 1–2 per 1000 Caucasian new-borns. Its prevalence varies with geographic region, ethnic background and socio-economic status [1, 2]. The majority of OFCs are isolated malformations. The aetiology of nonsyndromic OFC is multifactorial, in which both genetic and environmental aspects, such as nutrition are involved. The pathogenesis of OFC though still remains unravelled due to its complexity and heterogeneity [3, 4].

Evidence is accumulating that inadequate maternal nutrition during pregnancy, in particular of vitamins, could be a risk factor for the occurrence of congenital

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malformations [5]. Research on OFC has mainly been focused on maternal use of multivitamin supplements containing folic acid and several studies reported a protective effect on the occurrence and recurrence rate of OFC [6–9]. Recently, van Rooij et al. demonstrated a beneficial effect for the periconceptional intake of a folic acid supplement and/or folate intake on OFC risk [10]. Less is known about the association between OFC and thiamine, riboflavin, niacin, pyridoxine and cobalamin intake. Beneficial effects of these vitamins on facial development are mainly derived from animal studies and one case reported recurrent cleft lip and palate in siblings of a patient with a malabsorption syndrome, of which folic acid and riboflavin deficiency may be plausible causative factors [11–16].

These B vitamins are involved in several metabolisms that are important for normal DNA and RNA synthesis and thus for normal development and growth. Thiamine plays an important role in the carbohydrate and amino acid metabolism [17, 18]. Riboflavin is involved in the metabolisms of fat, carbohydrate, proteins and is an important cofactor in folate metabolism [19]. Niacin is essential for the biosynthesis of pentose, steroids, red blood cells, fatty acids and is involved in glycolysis, protein, carbohydrate and fat metabolism and DNA repair [17, 18]. Furthermore, the metabolism of certain drugs and toxicants is altered by niacin. Pyridoxine acts as a cofactor in the metabolism of carbohydrates, lipids, amino acids, glycogen and in the transsulphuration of homocysteine to cysteine [17, 18]. It also regulates the activity of hormones that bind to the nuclear receptor, thereby influencing transcription and gene expression [12]. The coenzyme cobalamin is important in DNA/RNA, protein, fat, homocysteine and folate metabolism and as such is essential in central and peripheral nervous system functioning [17, 18].

We postulate that cellular nutrient concentration, determined in persons by food intake, affect the regulation of developmental genes. Therefore, decreased intake of B vitamins and the resulting increased homocysteine concentrations interfere with the genetically controlled development of the lip and palate. To investigate this hypothesis, we studied the maternal dietary intake of thiamine, riboflavin, niacin, pyridoxine and cobalamin in the periconceptional period and the risk for OFC offspring in humans.

Materials and methods

We performed a case-control study in the Netherlands in the period 1998–2001 of which the design has been described in detail by Van Rooij et al. [10]. Two hundred and six mothers of a child with nonsyndromic OFC were recruited in collaboration with the nine largest cleft palate centres at around 14 months after the delivery of the child. The control mothers were women with a non-affected child of the same age, recruited in the population domain of the case group, through acquaintances from mothers of an OFC child (59 %) or nurseries and infant welfare centres (41 %). Known pregnant and lactating mothers as those mothers who altered their diet compared to the periconceptional period were excluded. Incidental folic acid supplement users were also excluded from our calculations through which 182 OFC mothers and 172 control mothers revealed for analysis. All mothers were Dutch Caucasians. The Medical Ethical Committees of all participating hospitals approved the study protocol and a written informed consent was obtained from every participant.

All mothers filled out a general questionnaire from which data such as age, education level, pregnancy nausea, maternal periconceptional alcohol consumption, smoking and the periconceptional use of vitamins were extracted. From the mothers who visited the hospital for the study, maternal length and weight was recorded. Their body mass index was defined as weight divided by the quadrate of the length. Educational level was categorised into low education (primary/lower vocational/intermediate/intermediate vocational education) and high education (higher secondary/higher vocational or university education). Nausea was characterised by duration, period, and seriousness. Because of the potential influence on the diet, extreme nausea is defined as nausea starting after the first week of pregnancy with excessive vomiting or nausea resulting in a changed or decreased food intake. Women were considered to be drinking alcohol or smokers when any alcohol consumption or smoking was reported in the periconceptional period. Data on vitamin supplements comprised the dosage, contents of the tablets (folic acid only or multivitamins containing folic acid, thiamine, riboflavin, niacin, pyridoxine and cobalamin), frequency of intake and specification in which weeks the supplements were taken before and during pregnancy. Periconceptional use of vitamin supplements was defined as daily intake from four weeks before through eight weeks after conception.

Furthermore, all participants filled out a validated Food Frequency Questionnaire (FFQ), developed for the Dutch cohorts of the European Prospective Investigation into Cancer and Nutrition study (EPIC) which provided information on the dietary intake of the B vitamins, thiamine, riboflavin, niacin, pyridoxine and cobalamin [20]. The FFQ was mailed to the subjects and filled out at home. During a hospital visit scheduled for this study or through a telephone interview we checked the FFQ in a standard way for completeness and consistency. The FFQ accounted for at least 90 % of the population mean intake of food groups and nutrients of interest. In the FFQ, subjects could indicate their answers in frequency per day, per week, month, year or never. For

several food items additional questions were asked about the consumption frequency for different subitems, preparation methods or additions. The amount eaten was estimated in commonly used units, by using household measures or coloured photographs of foods showing different portion sizes. Average daily nutrient intake was estimated by multiplying the frequency of consumption of the food items by the portion size and the nutrient content per gram. Total energy intake and the intake of thiamine, riboflavin, niacin and pyridoxine were calculated by using the 1993 Dutch food-composition table; folate and cobalamin intake was calculated using that of 2001 [21, 22]. A validation study demonstrated that the reproducibility and validity of foods groups predominantly contributing to the intake of B vitamins for this questionnaire were acceptable and comparable to other FFQs [20, 23]. We collected our information on the dietary pattern around 14 months after delivery, as Devine et al. demonstrated that this period is adequate to reflect the dietary pattern of 24 months before, covering the periconceptional period of the index child [24]. Moreover the assumption of the validity of the data is strengthened because the season in which the periconceptional period took place and the moment at which the FFQ was filled out was comparable.

Statistics

Mothers of OFC children and controls were compared with respect to age at delivery, age of the child at the time of study and body mass index by using a Student's T-test. Differences in maternal education, frequency of extreme nausea in the first trimester of pregnancy, lifestyle factors in the periconceptional period such as alcohol consumption, smoking and vitamin supplement use were evaluated by chi-square tests.

As the distribution of the dietary intake of the B vitamins was skewed, we applied natural logarithmic transformations and presented the dietary intakes of B vitamins as geometric means (P5–P95). The differences in B vitamin intake between the groups was evaluated by a Student's T-test. The association between maternal intake of B vitamins and the risk for OFC was assessed after adjustment for total energy intake [25]. The energy-independent residuals of this analysis were standardised to the predicted B vitamin intake at the average energy intake (9186 KJ/day) in our population. Quintiles of the dietary intake of thiamine, riboflavin, niacin, pyridoxine and cobalamin were created based on the control group in order to derive odds ratios for arbitrary dose categories. The risk for OFC was estimated using Odds Ratios (ORs) and 95 % Confidence Intervals (CIs) for each quintile of vitamin intake with the lowest quintile as a reference in an unconditional logistic regression model. Trends across the quintiles were evaluated, in which quintiles were modelled as continuous variables.

As vitamin supplement use and especially folic acid supplementation is suggested to reduce the occurrence of midline defects such as neural tube defects and OFC, we constructed a multivariate model with a term of interaction of folic acid supplement use with dietary B vitamin intake. Pearson coefficients of correlation were computed to assess the correlations between the intake of energy, thiamine, riboflavin, niacin, pyridoxine, folate and cobalamin.

To investigate the adequacy of the dietary intake of our population, comparisons were made with the Dutch Recommended Daily Allowances of most recent date. For all B vitamins, the RDA from 2000 was used, with the exception of pyridoxine and cobalamin, which were originating from 2003 [26, 27]. The RDA for energy was extracted from 2001 [28]. The RDAs for non-pregnant women in the reproductive age (19–50 years of age) were used because we investigated the maternal dietary intake around 14 months after delivery and assumed that the intake in this period is comparable to the periconceptional period as the need for specific nutrients is not likely to be significantly raised that early in pregnancy.

Significance was defined as $p \leq 0.05$. All analyses were performed using SAS Statistical Analysis System version 6.12 (SAS institute Inc, Cary, NC).

Results

The characteristics of the study population presented in Table 1 are comparable between the mothers of OFC children and controls with the exception of the significantly higher body mass index and significantly lower educational level in mothers of OFC children compared to controls. Control mothers tended to use more folic acid supplements periconceptionally compared to OFC mothers ($p = 0.07$).

The geometric mean dietary intake of energy and B vitamins was lower in mothers of an OFC child compared to control mothers, reaching significance for the intake of energy, thiamine, niacin and pyridoxine (Table 2). All dietary intakes conformed to the Recommended Daily Allowances. Adjustment for energy resulted in a non-significant difference for niacin, and a tendency of a difference for thiamine and pyridoxine (both $p = 0.06$) between the groups.

The dietary intake of thiamine and pyridoxine demonstrated a trend towards a reduced risk for OFC with increasing dietary intake (Table 3). This trend for thiamine intake became apparent above a dietary intake of 1.08 mg/d, reducing OFC risk by 21–64 %. A decreased risk for OFC of 29–65 % became apparent for dietary pyridoxine intake above 1.51 mg/d. Riboflavin, niacin and cobalamin intake did not demonstrate significant

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Table 1 Characteristics of mothers and their children with orofacial clefts (OFCs) and control mothers and their children.

	OFC group n = 182	Control group n = 173	P-value
Maternal age at delivery in years, mean (SD)	31.0 (3.9)	31.5 (3.6)	0.22
Child age at time of study in months, mean (SD)	14.6 (2.8)	14.5 (4.7)	0.78
Maternal body mass index (kg/m^2), mean (SD)[1]	25.2 (4.4)	23.7 (4.4)	0.04
Low maternal educational level, n (%)[2]	107 (59.1)	82 (47.4)	0.03
Extreme nausea in first trimester, n (%)	25 (13.7)	33 (19.1)	0.17
Alcohol consumption periconceptionally, n (%)	66 (36.3)	73 (42.2)	0.25
Cigarettes smoking periconceptionally, n (%)	57 (31.3)	43 (24.9)	0.18
Periconceptional use of, n (%)			
Multivitamin supplements[3]	4 (2.2)	6 (3.5)	0.47
Folic acid supplements[3]	61 (33.5)	74 (42.8)	0.07

[1] Determined on 83 OFC and 81 control mothers visiting the hospital for this study
[2] Low education: primary/lower vocational/intermediate secondary/intermediate vocational education
[3] Daily use from four weeks before until eight weeks after conception

Table 2 Periconceptional dietary intake of B vitamins and energy for mothers of orofacial cleft (OFC) children and control mothers

Dietary intake of		RDA	Mothers of OFC children	Control mothers	P-value
			Geom. mean (P5–P95)	Geom. mean (P5–P95)	
Energy	(kJ/d)	10000	8331 (5865–12330)	9190 (6359–12708)	0.04
Thiamine	(mg/d)	1.1	1.05 (0.69–1.50)	1.12 (0.71–1.62)	0.007
Riboflavin	(mg/d)	1.1	1.55 (0.79–2.63)	1.66 (1.02–2.74)	0.07
Niacin	(mg/d)	13.0	16.1 (9.8–23.5)	17.1 (11.0–23.9)	0.01
Pyridoxine	(mg/d)	1.5	1.58 (1.10–2.27)	1.68 (1.16–2.36)	0.007
Cobalamin	(µg/d)	2.8	5.12 (2.49–9.12)	5.37 (2.85–8.49)	0.24

RDA Recommended daily allowances [26.27]

risk reductions for the occurrence of OFC. A greater percentage of mothers of OFC children were present in the lowest quintiles of dietary intake of all B vitamins. The results did not significantly change after separate adjustment for maternal age, maternal education, extreme nausea in the first trimester and folic acid supplement use. Adjustment for dietary folate intake in quintiles did not significantly affect the associations (Table 3).

Ten women used multivitamins containing B vitamins through which they will have higher B vitamin intakes than non-users. After classification of multivitamin supplement users in the fifth quintile of dietary intake, the association between B vitamins and OFC risk was strengthened and the risk estimate of the highest quintile of niacin intake became significant (OR (95 % CI): 0.51 (0.27–0.98)). The dietary intakes of thiamine, riboflavin, niacin, pyridoxine and cobalamin are positively correlated among each other and significant Pearson coefficients of correlation varied from 0.47 for the intake of riboflavin and niacin to 0.86 for the intake of thiamine and pyridoxine. After adjustment for energy, these correlations decreased and varied from 0.14 for the intake of pyridoxine and cobalamin to 0.68 for the intake of thiamine and pyridoxine.

Thiamine, niacin and pyridoxine only exerted their protective effects in periconceptional folic acid supplement users (Table 4). Adjustment for maternal education only marginally altered our results. Significant risk estimates were observed in the highest quintile of dietary niacin after adjustment for maternal education. After adjustment for dietary folate intake, pyridoxine intake and surprisingly the first quintile of niacin intake remained beneficial factors in OFC risk reduction (data not shown). Folic acid supplement users and non-users were comparable with respect to demographic and pregnancy characteristics. The overall B vitamins intakes tended to be lower in non-folic acid supplement users (Table 5). Energy adjustment did not change the conclusions.

Discussion

This study demonstrates that the periconceptional dietary intake of thiamine, niacin and pyridoxine was significantly lower in mothers of an OFC child compared to controls. A diet rich in thiamine and pyridoxine significantly reduced the risk of an OFC child. The protective effect of these B vitamins however could only be demonstrated in periconceptional folic acid supplement users.

Due to the high correlations between the B vitamins, the identification of one B vitamin with a predominant

Table 3 Periconceptional maternal dietary intake of B vitamins in association with orofacial cleft (OFC) risk in offspring

Dietary intake[1] of	OFC/Controls n/n	OR (95 % CI)	OR (95 %CI)[2]
Thiamine (mg/d)			
0.58–0.96	47/35	1.0 (ref)	1.0 (ref)
0.96–1.08	54/35	1.15 (0.62–2.12)	1.21 (0.65–2.27)
1.08–1.16	28/34	0.61 (0.32–1.19)	0.69 (0.34–1.39)
1.16–1.25	17/35	0.36 (0.18–0.75)	0.42 (0.19–0.91)
1.25–1.53	36/34	0.79 (0.42–1.50)	0.93 (0.45–1.90)
P for trend		0.04	0.21
Riboflavin (mg/d)			
0.54–1.34	42/35	1.0 (ref)	1.0 (ref)
1.36–1.56	41/35	0.98 (0.52–1.84)	1.05 (0.55–2.00)
1.56–1.73	22/34	0.54 (0.27–1.09)	0.60 (0.29–1.22)
1.73–1.90	26/35	0.62 (0.31–1.22)	0.72 (0.36–1.46)
1.90–3.41	51/34	1.25 (0.67–2.33)	1.53 (0.79–2.96)
P for trend		0.86	0.39
Niacin (mg/d)			
8.4–14.9	48/35	1.0 (ref)	1.0 (ref)
14.9–16.4	38/35	0.79 (0.42–1.49)	0.77 (0.40–1.45)
16.5–17.6	31/34	0.67 (0.35–1.28)	0.69 (0.36–1.34)
17.6–19.6	38/35	0.79 (0.42–1.49)	0.85 (0.45–1.62)
19.6–25.9	27/34	0.58 (0.30–1.13)	0.65 (0.33–1.29)
P for trend		0.15	0.32
Pyridoxine (mg/d)			
1.07–1.51	55/35	1.0 (ref)	1.0 (ref)
1.51–1.62	39/35	0.71 (0.38–1.32)	0.76 (0.39–1.48)
1.62–1.72	36/34	0.67 (0.36–1.27)	0.74 (0.37–1.45)
1.72–1.84	19/35	0.35 (0.17–0.70)	0.39 (0.18–0.83)
1.84–2.42	33/34	0.62 (0.33–1.17)	0.74 (0.35–1.54)
P for trend		0.03	0.16
Cobalamin (µg/d)			
2.20–4.13	33/35	1.0 (ref)	1.0 (ref)
4.14–4.92	28/35	0.85 (0.43–1.69)	0.88 (0.44–1.76)
4.92–5.53	40/34	1.25 (0.65–2.41)	1.30 (0.66–2.54)
5.53–6.24	35/35	1.06 (0.54–2.07)	1.18 (0.60–2.35)
6.27–42.92	46/34	1.44 (0.75–2.75)	1.79 (0.90–3.56)
P for trend		0.20	0.06

[1] energy-adjusted dietary intake per quintile
[2] adjustment for dietary folate in quintiles

role in the pathogenesis of OFC is not feasible. The different B vitamins are often simultaneously present in several products, making it difficult to distinguish a separate effect of one B vitamin. In addition, the FFQ may lack power to adequately differentiate between B vitamins. Intervention studies with different B vitamins separately and a study in populations where the dietary intake of B vitamins is less correlated could help elucidate this issue. Furthermore, the question remains whether the adjustment for dietary folate intake or other B vitamins is appropriate as foods are always highly correlated and a great variety of errors and contradictory results may emerge from multivariate analyses [29].

The protective effect of thiamine, niacin and pyridoxine could only be demonstrated in the group of women using folic acid supplements. Although it is most probable that the periconceptional use of these supplements is of major importance in the prevention of OFC, its use and the dietary intake of thiamine, niacin and pyridoxine could also be a reflection of a healthier lifestyle and thus of a more adequate nutrition as main dietary sources of B vitamins are products such as bread, fruits, vegetables, grains, fish and meat. Folic acid supplement users did differ from non-users in diet composition as they reported higher dietary intakes of energy and of all investigated B vitamins. None of these differences reached significance though, which could result from our assessment of folic acid supplement use in our study, being restricted to the daily use of these supplements from four weeks before until eight weeks after conception. This could have underestimated the actual number of women using nutritional supplements in this period.

We speculate that a misbalance between the intake of macronutrients and the micronutrients thiamine, niacin and pyridoxine contributes to OFC risk. Our western diet is known to be rich in macronutrients such as fat and proteins and relatively poor in micronutrients such as vitamins. Thiamine, niacin and pyridoxine are known to be essential for the metabolic breakdown of proteins, fats and carbohydrates for energy supply to the cells. A diet rich in these macronutrients and relatively poor in micronutrients could possibly adversely affect the development of the lip and/or palate.

The use of a FFQ in dietary assessment is subject to errors as it gives a relative rather than an absolute reflection of the actual daily intake. Possible lower coefficients of correlation between the FFQ and the actual dietary intake of certain nutrients could lead to misclassification or over- or underestimation of our risk estimates. Measurement errors due to the FFQ could also be the explanation for the significant OFC risk reductions in the fourth quintile of dietary thiamine and pyridoxine intake rather than the expected highest one. Furthermore, it was remarkable that all median dietary B vitamin intakes conformed to RDA values. Our results can be extrapolated to the Dietary Reference Intakes used in the US as similar conclusions could be drawn after these comparisons [30]. These results should be interpreted with care though as the FFQ has been developed to categorise individuals in levels of low or high dietary intake respectively and is less suitable for comparisons to absolute values. Nevertheless, our FFQ has been described to be acceptable and comparable to other FFQs as the validity of foods contributing to B vitamin intake was considered generally to be good [20, 23]. This finding was supported by reports of Melse et al. demonstrating a positive association between the dietary intake of folate recorded by the same FFQ and plasma folate levels [31].

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Table 4 Periconceptional maternal dietary intake of B vitamins in association with orofacial cleft (OFC) risk in offspring by folic acid supplement use

Supplement users N = 135			Non-supplement users N = 220		
Dietary intake of[1]	OFC/ Controls n/n	OR (95 % CI)	Dietary intake of[1]	OFC/ Controls n/n	OR (95 % CI)
Thiamine (mg/d)					
0.58–0.96	13/11	1.0 (ref)	0.72–0.96	34/24	1.0 (ref)
0.96–1.08	25/13	1.63 (0.57–4.63)	0.97–1.08	29/22	0.93 (0.43–1.99)
1.09–1.16	10/14	0.60 (0.19–1.89)	1.08–1.16	18/20	0.64 (0.28–1.45)
1.17–1.25	3/15	0.17 (0.04–0.74)	1.16–1.25	14/20	0.49 (0.21–1.17)
1.25–1.53	10/21	0.40 (0.13–1.21)	1.25–1.51	26/13	1.41 (0.61–3.29)
P for trend		0.003			0.94
Riboflavin (mg/d)					
0.76–1.33	13/16	1.0 (ref)	1.19–1.33	29/19	1.0 (ref)
1.38–1.55	12/12	1.23 (0.42–3.64)	1.36–1.56	29/23	0.83 (0.37–1.83)
1.56–1.73	8/14	0.70 (0.23–2.19)	1.56–1.73	14/20	0.46 (0.19–1.12)
1.75–1.90	10/16	0.77 (0.26–2.26)	1.73–1.89	16/19	0.55 (0.23–1.33)
1.91–3.36	18/16	1.39 (0.51–3.74)	1.90–3.41	33/18	1.20 (0.53–2.71)
P for trend		0.76			0.92
Niacin (mg/d)					
9.6–14.8	19/11	1.0 (ref)	8.4–14.9	29/24	1.0 (ref)
14.9–16.4	10/17	0.34 (0.12–1.00)	14.9–16.4	28/18	1.29 (0.58–2.87)
16.5–17.6	12/13	0.53 (0.18–1.57)	16.5–17.6	19/21	0.75 (0.33–1.71)
17.8–19.5	10/13	0.45 (0.15–1.35)	17.6–19.6	28/22	1.05 (0.48–2.29)
19.6–25.9	10/20	0.29 (0.10–0.84)	19.6–25.1	17/14	1.00 (0.41–2.45)
P for trend		0.058			0.85
Pyridoxine (mg/d)					
1.07–1.51	22/12	1.0 (ref)	1.12–1.51	33/23	1.0 (ref)
1.52–1.61	14/11	0.69 (0.24–1.99)	1.52–1.62	25/24	0.73 (0.34–1.57)
1.62–1.72	9/10	0.49 (0.16–1.54)	1.62–1.72	27/24	0.78 (0.37–1.69)
1.72–1.84	7/19	0.20 (0.07–0.61)	1.72–1.84	12/16	0.52 (0.21–1.31)
1.84–2.42	9/22	0.22 (0.08–0.64)	1.84–2.33	24/12	1.39 (0.58–3.34)
P for trend		0.0006			0.80
Cobalamin (µg/d)					
2.68–4.08	10/12	1.0 (ref)	2.20–4.13	23/23	1.0 (ref)
4.14–4.91	9/16	0.68 (0.21–2.18)	4.16–4.92	19/19	1.00 (0.42–2.36)
4.92–5.51	16/15	1.28 (0.43–3.83)	4.92–5.53	24/19	1.26 (0.55–2.91)
5.53–6.24	10/18	0.67 (0.21–2.09)	5.53–6.21	25/17	1.47 (0.63–3.42)
6.38–10.93	16/13	1.48 (0.49–4.49)	6.27–42.92	30/21	1.43 (0.64–3.19)
		0.50			0.25

[1] Energy adjusted dietary intake per quintile

Table 5 Dietary energy and B vitamin intake for mothers of orofacial cleft (OFC) children and control mothers by periconceptional folic acid supplement use

		Supplement users n = 135	Non-supplement users n = 220	P-value
		Geom. mean (P5–P95)	Geom. mean (P5–P95)	
Energy	(kJ)	9029 (5731–12749)	8921 (5908–12574)	0.62
Thiamine	(mg)	1.11 (0.71–1.59)	1.07 (0.69–1.54)	0.18
Riboflavin	(mg)	1.63 (0.88–2.62)	1.59 (0.83–2.74)	0.44
Niacin	(mg)	17.0 (10.8–24.2)	16.3 (9.9–23.5)	0.14
Pyridoxine	(mg)	1.66 (1.09–2.32)	1.61 (1.12–2.38)	0.21
Cobalamin	(µg)	5.35 (2.71–9.08)	5.17 (2.61–8.62)	0.39

Maternal education, considered as a proxy for socio-economic status, was significantly lower in mothers of OFC children compared to controls. Studies on dietary intake assessment have demonstrated that a lower education is more subject to underreporting of energy in comparison to people with a higher educational back-

ground [32]. This would overestimate our results. A possible selection of controls is not expected to have distorted our findings since underreporting is minimised by adjustment for energy and the ORs only changed marginally after adjustment of maternal education. Information bias is unlikely to have occurred, as none of the participants knew in advance that the study focussed on B vitamins.

In conclusion, this study demonstrates a lower intake of energy and B vitamins in mothers with OFC children compared to controls and suggests that a periconceptional diet rich in thiamine, niacin, pyridoxine is important in reducing OFC risk.

Acknowledgement We thank the participating cleft teams and their co-ordinators (Rijnstate Hospital Arnhem, Dr. W. Brussel; Free University Hospital Amsterdam and Erasmus UMC Rotterdam, Prof. Dr. B. Prahl-Andersen; University Hospital Groningen, Prof. Dr. S. M. Goorhuis-Brouwer; Medical Centre Leeuwarden, Dr. J. J. van der Biezen; St. Elisabeth Hospital Tilburg, Dr. J. G. Daggers; University Medical Centre Utrecht, Drs. T. F. J. M.C. Specken and Sophia Hospital Zwolle, Dr. P. Houpt). We acknowledge the nurseries and infant welfare centres for their participation in the recruitment of control mothers. We are very grateful to Ms M. van der Doelen and Dr. C. van Oostrom, chief nurse officer and head of the outpatient paediatric clinic University Medical Centre Nijmegen, respectively, for their assistance, Dr. C. N. Vermeij-Keers for the opportunity to use the registration system of the Dutch Association for Cleft Palate and Craniofacial Anomalies, Ms A. Pellegrino and Ms L. Lemmens for data-entry, Mr W. Lemmens for data-management, Prof. Dr. C. E. West for reviewing this article and Mr H. Straatman, Ms R. Bretveld and Ms N. Peer for their help and suggestions on the statistical analysis.

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