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Is Dietary Intake of Methionine Associated With a Reduction in Risk for Neural Tube Defect-Affected Pregnancies?

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ABSTRACT Results from experimental animals and other laboratory data have suggested a role for methionine, an essential amino acid, in normal closure of the neural tube. We hypothesized that women who had higher dietary intakes of methionine would be at lower risk for neural tube defect (NTD)-affected pregnancies. Data were derived from a population-based case-control study of fetuses and liveborn infants with NTDs among a 1989–1991 California birth cohort. Interviews, which included a 100-item food frequency questionnaire, were conducted with mothers of 424 NTD cases and 440 nonmalformed controls. Risk for having an NTD-affected pregnancy was estimated according to quartiles (established from intakes among control mothers) of average daily maternal dietary intake of methionine in the 3 months before conception. We observed an approximately 30–40% reduction in NTD-affected pregnancies among women whose average daily dietary intake of methionine was above the lowest quartile of intake (>1,341.86 mg/day). These reductions in NTD risk were observed for both anencephaly and spina bifida; remained after adjustment for maternal race/ethnicity and education; and were observed irrespective of maternal level of folate intake. Although we were unable to establish whether the observed reductions in NTD risk were attributable to maternal periconceptional methionine intake or to another highly correlated nutrient, these data add to the growing body of evidence that maternal diet plays a role in neural tube closure. *Teratology 56:295–299, 1997.* © 1997 Wiley-Liss, Inc.

Maternal nutritional status has been implicated in the complex etiology of neural tube defects (NTDs). Substantial epidemiologic evidence is available to demonstrate that periconceptional multivitamin supplementation with folic acid reduces the risk of women having NTD-affected pregnancies (Medical Research Council Vitamin Study Research Group, '91; Czeizel and Dudas, '92). The underlying mechanisms by which folic acid contributes to NTD risk reduction, however, are unknown.

A diet insufficient in folate could result in insufficient methyltetrahydrofolate, the methyl donor, for conversion of homocysteine to methionine. In such a scenario, methionine, an essential amino acid in humans, becomes a limiting nutrient. Animal studies and other experimental data have suggested that methionine contributes to normal closure of the neural tube (Coelho et al., '89; Coelho and Klein, '90; Nosel and Klein, '92; Essein and Wannberg et al., '93). For example, studies of cultured rat embryos suggest that methylation of amino acids in the contractile proteins of the microfilaments of the neural tube plays a key role in neural tube closure (Coelho and Klein, '90).

We hypothesized that women who had higher dietary intakes of methionine would be at lower risk for NTD-affected pregnancies. Thus, we examined data from a large population-based case-control study to investigate a possible association between maternal periconceptional dietary intake of methionine and having a NTD-affected pregnancy. The role of methionine was assessed considering maternal intake of both supplemental and dietary sources of folate.

MATERIALS AND METHODS

Details of the population-based case-control study used in this analysis have been described (Shaw et al., '95). Briefly, infants or fetuses with an NTD (anencephaly, spina bifida cystica, craniorachischisis, or iniencephaly) were ascertained by reviewing medical records, including ultrasonography, at all hospitals and genetic clinics for those infants/fetuses delivered in select California counties, and whose mothers gave their residence as California. Singleton liveborn infants and fetuses (including those prenatally diagnosed and electively terminated between February 1989 and January 1991 with NTDs) among the cohort of 708,129 births (including fetal deaths) between June 1989 and May 1991 were eligible; 653 singleton infants/fetuses with an eligible NTD diagnosis were ascertained. Controls were randomly selected from each area hospital in proportion to the hospital's estimated contribution to the total population of infants born alive in a given

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month from June 1989 to May 1991. Six hundred forty-four singleton infants who were born alive without a reportable congenital anomaly (Croen et al., '91) and whose mother was a California resident were ascertained.

Women who only spoke languages other than English or Spanish, or who had had a previous NTD-affected pregnancy were excluded, leaving 613 cases and 611 controls. In-person interviews were completed with mothers of 538 (87.8%) cases and 539 (88.2%) controls an average of 4.9 months for cases and 4.6 months for controls after the actual or projected data of term delivery.

A 100-item food frequency questionnaire was used to assess nutrient intake from diet (Block et al., '86). Study women completed the questionnaire (English or Spanish) with interviewers present to assist. Each woman was instructed to estimate her usual frequency and portion size of the food items she consumed during the 3 months before conception. Average daily intake of various nutrients, including methionine, was computed using analytic software developed for the survey instrument (Block et al., '86). Methionine and cysteine values for each food item, provided to us by another group of investigators (Dr. Elaine Flagg, personal communication) (Jones et al., '92), were incorporated into the nutrient data base. Of the 1,077 women who completed an interviewer-administered questionnaire, 1,007 completed a food frequency questionnaire. Of these, 916 revealed suitable data based on error checks built into the analytic software. An additional 52 women who reported the use of food supplements were excluded because of inadequate information to assess the methionine content of these supplements. The remaining 864 women comprised the study's analytic base. Among these 864, 424 were NTD case mothers and 440 were control mothers. The 424 cases included 161 with anencephaly, 242 with spina bifida, and 21 other (combined anencephaly and spina bifida, craniorachischisis, and iniencephaly).

Levels of dietary intake of methionine in the control group were used to establish quartile categories of intake. Odds ratios (ORs) and their 95% confidence intervals (CIs) were computed to estimate risk using as reference the lowest quartile of methionine intake. We added daily folic acid intake from vitamin supplements in the period 3 months before conception to usual daily folate intake from diet also for the period 3 months before conception to estimate the total folate intake from both sources. Levels of total folate intake in the control group were used to establish quartile categories of intake. A similar approach was used to estimate daily intake of zinc and calcium as well as cysteine and total protein. In addition to maternal folate, zinc, calcium, cysteine, and total protein intake, maternal race/ethnicity (Latina, foreign-born; Latina, U.S.-born; White, non-Hispanic; Black; other), education (<high school graduate; high school graduate; college graduate), and periconceptional alcohol intake (none; <1 drink/day; ≥1 drink/day) were considered as covariates for analyses of methionine intake.

RESULTS

Compared with control mothers, case mothers were more likely to be foreign-born Latinas or to be <25 years of age, and case mothers were less likely to have graduated from college, to have consumed alcohol either in the period 3 months before conception or during the first trimester, or to have consumed vitamin supplements containing folic acid in the 3 months before or after conception (Table 1).

Estimated risks for having an NTD-affected pregnancy according to quartile of average daily maternal dietary intake of methionine in the 3 months before conception are shown in Table 2. There was an approximately 30–40% reduction in NTD risk among women whose average daily dietary intake of methionine was more than 1,341.86 mg/day. These reduced risks were 1) observed for both spina bifida and anencephaly analyzed separately, and 2) similar across maternal race/ethnicity, education, and periconceptional alcohol intake strata (although data were sparse in the stratum of women whose alcohol intake was ≥1 drink/day). The ORs associated with the 3 quartiles of methionine intake greater than 1,341.86, simultaneously adjusted for maternal race/ethnicity and education, were similar to the crude risk estimates. The adjusted ORs were 0.67 (0.46–0.98), 0.66 (0.45–0.96), and 0.50 (0.34–0.73) for the 3 increasing quartiles of methionine intake.

Considering as the reference population women who were categorized as having the lowest intake of methionine and having the lowest daily total intake of folate (both supplements and diet combined) revealed that intakes of methionine above the lowest quartile were associated with lower NTD risks irrespective of level of folate intake (Table 3). Similar NTD risk reductions were observed for maternal methionine intakes above the lowest quartile when considered in the context of intakes of several other nutrients (above their lowest quartile of intake) highly correlated with methionine intake, namely zinc, calcium, cysteine, and total protein. However, because these nutrients were highly correlated, data were too sparse in many of the intake level comparisons to adequately assess risks.

DISCUSSION

These data reveal a reduction in NTD risk associated with maternal periconceptional dietary intake of methionine. This reduction in risk did not appear to be substantially influenced by maternal periconceptional intake of folate. To our knowledge, these are the first epidemiologic data to specifically estimate both maternal intake of the essential amino acid methionine and risk for having an NTD-affected pregnancy. However, other studies have suggested results that could be interpreted as consistent with our study.

In the case-control study by Choi et al. ('72), although lacking specific details on the nutritional data collected,

TABLE 1. Characteristics of interviewed mothers of cases and controls

	Cases (N = 424)		Controls (N = 440)	
	No.	%[1]	No.	%[1]
Race/ethnicity				
Latina, foreign-born	151	35.6	92	20.9
Latina, U.S.-born	49	11.6	62	14.1
White, non-Hispanic	181	42.7	235	53.4
Black	19	4.5	18	4.1
Other	24	5.7	32	7.3
Age (years)				
<20	56	13.2	37	8.4
20–24	115	27.1	101	23.0
25–29	126	29.7	139	31.6
30–34	93	21.9	114	25.9
≥35	34	8.0	49	11.1
Education				
<High school graduate	164	38.7	114	25.9
High school graduate	184	43.4	191	43.4
College graduate	75	17.7	134	30.5
Gravidity				
1	101	23.8	91	20.7
2	111	26.2	135	30.7
3	93	21.9	90	20.5
≥4	119	28.1	124	28.2
Alcohol use in first trimester				
None	304	71.6	289	65.7
<1 drink/day	112	26.4	145	33.0
≥1 drink/day	8	1.9	6	1.4
Alcohol use 3 months before conception				
None	200	47.2	183	41.7
<1 drink/day	197	46.5	221	50.3
≥1 drink/day	27	6.4	35	7.8
Used vitamins containing folic acid in 3 months before to 3 months after conception				
No	166	39.2	121	27.5
Yes	64	15.1	70	15.9
Began use in first trimester	179	42.2	235	53.4

[1]May not add to 100 due to missing information or rounding.

TABLE 2. ORs (relative risks) of NTD-affected pregnancies according to quartile of average daily maternal dietary intake of methionine in 3 months before conception, assessed from food frequency questionnaire

Quartile of methionine (mg/day)	Cases (N = 424)	Controls (N = 440)	OR	95% CI
<1,341.87	147	110	Reference	—
1,341.87–1,750.35	98	110	0.67	0.46–0.96
1,750.36–2,347.61	97	110	0.66	0.46–0.95
>2,347.61	82	110	0.56	0.38–0.81

TABLE 3. ORs (relative risks) of NTD-affected pregnancies according to quartile of average daily maternal dietary intake of methionine and folate in 3 months before conception, assessed from food frequency questionnaire and maternal report of vitamin use

Quartile of methionine (mg/day)	Quartile of folate (μg/day)			
	≤255.48	255.49–368.86	368.87–578.50	≥578.5
<1,341.87				
Cases/controls	80/60	26/28	22/10	18/12
OR	Reference	0.70	1.6	1.1
95% CI		0.37–1.4	0.73–3.7	0.50–2.5
1,341.87–1,750.35				
Cases/controls	26/27	27/29	22/23	22/27
OR	0.72	0.70	0.72	0.61
95% CI	0.38–1.4	0.37–1.3	0.37–1.4	0.32–1.2
1,750.36–2,347.61				
Cases/controls	18/15	24/33	35/36	19/24
OR	0.90	0.55	0.73	0.59
95% CI	0.42–1.9	0.29–1.0	0.41–1.3	0.30–1.2
>2,347.61				
Cases/controls	1/6	17/18	24/39	39/45
OR	0.13	0.71	0.46	0.65
95% CI	0.01–1.1	0.34–1.5	0.25–0.85	0.38–1.1

they observed that control mothers were more likely than case mothers to consume 4 food items: liver, eggs, cheese, and milk. The investigators suggested that such a consumption pattern might have reflected calcium deficiency among case mothers, a suggestion that contrasts with the increased risk for central nervous system anomalies observed among infants whose mothers consumed calcium compounds in the Collaborative Perinatal Project (Heinonen et al., '77). The intake pattern described by Choi et al. ('72) also could have reflected a lower methionine intake among case mothers. That is, milk, eggs, and cheese were among the major methionine contributors in our population. Further, a small case-control study conducted in Newfoundland also reported that NTD case mothers were less likely, compared to control mothers, to consume dairy products (Friel et al., '95). Our study also revealed that NTD risk decreased with increased daily servings of dairy products (not shown). That is, based on quartiles (established among controls) of servings of dairy products (milk, yogurt, and cheese), ORs were 0.80 (0.55–1.1), 0.73 (0.50–1.1), and 0.52 (0.35–0.77) for the 3 increasing intake quartiles relative to the lowest quartile of <1.7 servings/day.

Other human evidence to implicate methionine includes studies that have demonstrated an association between NTD risk and potential abnormalities in maternal homocysteine metabolism leading to higher circulating levels of homocysteine (Steegers-Theunissen et al., '94; Mills et al., '95; Steegers-Theunissen et al., '95). Such abnormalities in homocysteine metabolism could conceivably lead to lower maternal circulating levels of methionine.

In addition to the human evidence supporting a role of methionine in NTD risk, results from experimental animals and other laboratory data have demonstrated the role of methionine in normal closure of the neural tube. In vitro studies in the rat have revealed that a deficiency specific to methionine in the culture medium was associated with development of NTDs in the embryo. Methionine when added to the culture medium also has been found to reduce the probability of resorptions in rats linked with valproic acid, a human terato-

gen for NTDs (Nosel and Klein, '92). Methionine supplements have also been found to reduce the incidence of NTDs in a mouse mutant strain, Axd mutant mouse (Essein and Wannberg, '93). The reduction in incidence was not altered in that study by maternal animals being supplemented with folinic acid or vitamin B-12. However, in these various experimental studies, the underlying molecular role of methionine in neural tube closure has not been specifically established. Thus, how methionine would contribute to a reduction in NTD risk is unknown.

In humans, it has been hypothesized that a combination of low dietary methionine, low dietary folate, and a mutation of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene might lead to a circumstance that results in NTDs (Lucock et al., '95). MTHFR converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the latter being the primary methyl donor for the remethylation of homocysteine to methionine. Four studies have recently reported an association between homozygosity for the C677T mutation of the MTHFR gene and risk for NTD-affected pregnancies (van der Put et al., '95; Whitehead et al., '95; Kirke et al., '96; Ou et al., '96). Because some of the infants with spina bifida and control infants in our study were previously genotyped for C677T genotypes (Shaw et al., submitted), we explored whether C677T homozygosity was more common among case and control infants whose mothers' intakes of both methionine and folate were low compared to case and control infants whose mothers' intakes were not low. Among mothers whose intake of both methionine and folate were low (80 case and 60 control mothers), only 2 case and 5 control infants were C677T homozygous (5 case and 8 control infants were heterozygous), too few to adequately assess this potential gene-nutrient interaction. However, the small number of infants homozygous for C677T among the maternal low methionine/folate group does indicate that this particular mutation among infants is probably not a large contributor to the NTD/methionine association observed in this study.

Our study's large size, population-based ascertainment of cases (including elective terminations) and controls, and relatively short period for maternal recall between periconceptional event of interest and interview, were among its advantages. Nevertheless, our study had several limitations. First, it estimated risk associated with intake level of methionine and not tissue-dose level of methionine. Given individual variation in gut absorption and maternal-fetal exchange, we do not know how well maternal intake approximates the level of methionine that reaches the developing fetus. Second, because methionine in foods is highly correlated with several other nutrient parameters, e.g., other essential amino acids, zinc, cysteine, calcium, and total protein, we could not fully establish whether the observed reduction in risk was attributable to methionine or to another highly correlated nutrient. Third, these data relied upon a food frequency questionnaire to assess methionine intake. Limitations of this type of instrument have been described (Willett, '90; Block, '82). Although the instrument we used was not internally validated, validation studies have revealed that it provides reasonable estimates of usual dietary intake for diets consumed by women even in the distant past (Block et al., '90, '92; Mares-Perlman et al., '93). In addition, the questionnaire has been used in surveys with Hispanic populations and has produced dietary findings that are comparable to data collected using other intake methods (Block and Subar, '92). A fourth potential limitation of this study was the lower percentage of all eligible case and control mothers for whom we had dietary information about methionine. It is unknown whether the lack of this information distorted the observed risk estimates in this study.

Although our findings will need to be replicated to determine whether these or other limitations alternatively explain the observed association with methionine, these data add to the growing body of evidence that maternal diet plays a key role in neural tube closure.

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