Neural tube defects and elevated homocysteine levels in amniotic fluid

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OBJECTIVE: Our purpose was to study maternal blood and amniotic fluid concentrations of homocysteine and relevant vitamins in relation to neural tube defects.

STUDY DESIGN: Concentrations of total homocysteine, folate, and vitamins B_{12} and B_6 were measured in maternal blood and amniotic fluid of 27 women carrying a fetus with a neural tube defect and 31 control women carrying a healthy fetus.

RESULTS: The mean total homocysteine concentration in amniotic fluid of the study group was significantly higher than that of the control group. The mean concentrations of total homocysteine in blood and the vitamins folate, B_{12}, and B_6 in, respectively, blood and amniotic fluid were not significantly different between the groups. The mean concentrations of homocysteine and vitamin B_6 were significantly lower in amniotic fluid than in blood in both groups, whereas vitamin B_{12} in amniotic fluid was higher than in blood.

CONCLUSION: These results support the hypothesis that at least the cause of a subset of neural tube defects could reside in a primary or secondary maternal or fetal derangement of homocysteine metabolism. (Am J Obstet Gynecol 1995;172:1436-41.)

Key words: Neural tube defects, amniotic fluid, maternal blood, homocysteine, vitamins

Decreased levels of folate and vitamin B_{12} in maternal blood have been associated with the pathogenesis of neural tube defects.[1, 2] Periconceptional folate treatment of the mother is very likely to prevent neural tube defects.[3, 4] Recently we reported an association between a history of offspring with a neural tube defect and maternal mild hyperhomocysteinemia in the fasting state and after methionine loading.[5, 6] However, it is unclear what biochemical mechanism is involved, but probably the underlying defect is located in the remethylation pathway of homocysteine metabolism.

5-Methyltetrahydrofolate and B_{12} as methylcobalamin are involved in the remethylation of homocysteine to methionine. Vitamin B_6 as pyridoxal phosphate is involved in the competing transsulfuration pathway, which degrades homocysteine by cystathionine into cysteine. Malnutrition or a deranged metabolism of folate or vitamin B_{12} impairs the remethylation to methionine and results in hyperhomocysteinemia.[7-9] The most frequently encountered inherited enzymatic defects in homocysteine metabolism are deficiencies of the enzymes cystathionine synthase and 5,10-methylenetetrahydrofolate reductase.[9] Noteworthy is the recently reported decreased enzyme activity of a thermolabile form of 5,10-methylenetetrahydrofolate reductase, which may result in mild hyperhomocysteinemia in 5% of the general population.[10] Increased homocysteine blood levels can be efficiently lowered by high-dose folate or vitamin B_6.[11, 12]

In view of the previously reported association of maternal hyperhomocysteinemia and offspring with a neural tube defect, it would be intriguing to study the homocysteine exposure of the conceptus during the period that neural tube defects develop (i.e., third to fourth week after conception). However, it is not feasible to assess the homocysteine concentrations in the yolk sac, in the neural tissue, or even in amniotic fluid during that early stage of human pregnancy. Therefore we studied both maternal blood and midtrimester amniotic fluid concentrations of total homocysteine, folate, and vitamins B_{12} and B_6 in women carrying a fetus with a neural tube defect and in a control group of women carrying a normal, healthy fetus.

Material and methods

Fifty-eight women were investigated after written informed consent was obtained. Exclusion criteria were gastrointestinal and endocrine disorders, dietary re-

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Table I. Characteristics of populations

	Group 1 (NTD) (n = 27)	*Group 2 (controls) (n = 31)*	*Significance*
Age (yr) (mean and SD)	30.5 (4.2)	37.8 (1.3)	$p < 0.01$
Nulliparous	9	7	NS
Gestational age (wk) (mean and SD)*	22.3 (8.8)	15.9 (0.7)	$p < 0.01$

NTD, Neural tube defect; *NS,* not significant.
*At amniocentesis.

Table II. Mean and SD amniotic fluid and maternal blood concentrations of total homocysteine and vitamins in women carrying a fetus with a neural tube defect (group 1) and in women with a healthy fetus (group 2)

	Group 1 (NTD) (n = 27)	*Group 2 (controls) (n = 31)*	*Significance between groups*
Total homocysteine (μmol/L)			
Plasma	7.7 (3.2)	7.2 (1.2)	NS
Amniotic fluid	2.6 (1.6)	1.5 (0.4)	$p < 0.01$
Significance within group	$p < 0.01$	$p < 0.01$	
Folate (nmol/L)			
Serum	15 (6)	15 (6)	NS
Amniotic fluid	14 (8)	13 (5)	NS
Significance within group	NS	$p < 0.01$	
Red blood cells	646 (324)	644 (170)	NS
Vitamin B_{12} (pmol/L)			
Serum	219 (67)	238 (66)	NS
Amniotic fluid	379 (298)	481 (278)	NS
Significance within group	$p < 0.05$	$p < 0.01$	
Vitamin B_6 (nmol/L)			
Whole blood	41 (11)	41 (14)	NS
Amniotic fluid	20 (19)	14 (13)	NS
Significance within group	$p < 0.01$	$p < 0.01$	

NTD, Neural tube defect; *NS,* not significant.

strictions, and the use of vitamin preparations or other pharmacologic agents immediately before or during the current pregnancy. Twenty-seven of the women carried a fetus with a neural tube defect (i.e., meningo[myelo]cele [n = 15] or anencephaly [n = 12] (group 1) as concluded from α-fetoprotein determinations in amniotic fluid and from ultrasonographic examination and confirmed by the pregnancy outcome. The control group (group two) consisted of 31 women in whom amniocentesis was performed because of high maternal age (36 to 40 years). All control women gave birth to a healthy child. All pregnancies were accurately dated by the last menstrual period and by first-trimester ultrasonographic investigation. The characteristics of both groups are presented in Table I.

Venous blood samples for the determination of plasma total homocysteine, serum and red blood cell folate, serum vitamin B_{12}, and whole blood vitamin B_6 as pyridoxal phosphate were taken within 10 minutes after amniocentesis. Amniotic fluid samples were obtained by routine transabdominal amniocentesis and collected in dry tubes of 10 ml. All amniotic fluid samples were free from blood contamination. The samples were immediately centrifuged for 10 minutes at 3000 *g*, stored at −20° C, and assayed within 3 months after collection. The analytic methods used in the determination of vitamins in blood and amniotic fluid have been reported before.[13] Total homocysteine was determined essentially according to Fiskerstrand et al.[14]

Results are expressed as mean (SD). To obtain normality, blood and amnion parameters were log-transformed. Possible differences between the groups were then evaluated with Welch's *t* test, whereas differences between paired observations were validated with the Student *t* test. Pearson's partial correlation coefficients (with the group effect partialed out) were calculated to study the relationship between the various variables. A p value < 0.05 was considered statistically significant.

Results

Amniocentesis was performed at a significantly more advanced gestational age in group 1 compared with group 2, and the women in group 2 were significantly older than those in group 1 (Table I).

The mean concentrations of total homocysteine, folate, and vitamins B_{12} and B_6 in maternal blood and amniotic fluid as found in groups 1 and 2 are given in Table II. The mean amniotic fluid concentration of total homocysteine in group 1 was significantly higher than that of group 2 ($p < 0.01$), whereas these concen-

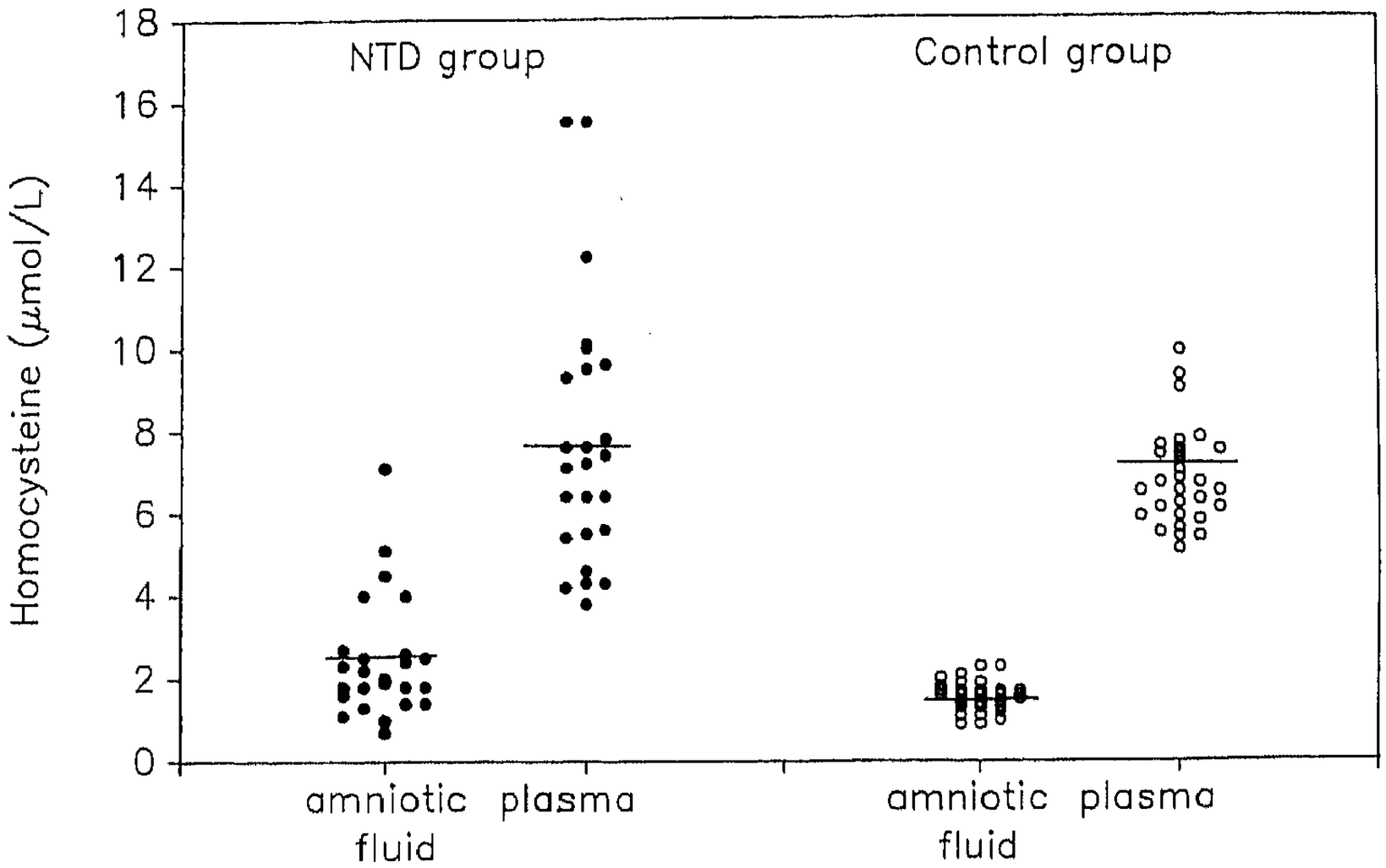

Fig. 1. Individual plasma and amniotic fluid levels of homocysteine in women carrying a fetus with a neural tube defect *(NTD)* (group 1) and in those carrying a healthy fetus (group 2). *Horizontal rule,* Mean.

Table III. Partial correlation coefficients of concentrations of total homocysteine in both plasma and amniotic fluid with concentrations of folate, vitamin B_{12}, vitamin B_6, and gestational and maternal age

	Total homocysteine			
	Plasma	*Significance*	*Amniotic fluid*	*Significance*
Maternal age	−0.07	NS	−0.04	NS
Gestational age	−0.35	$p < 0.05$	−0.05	NS
Total homocysteine				
Plasma	—		0.45	$p < 0.01$
Folate				
Serum	−0.46	$p < 0.01$	−0.36	$p < 0.01$
Red blood cells	−0.55	$p < 0.01$	−0.30	$p < 0.05$
Amniotic fluid	−0.19	NS	−0.43	$p < 0.01$
Vitamin B_{12}				
Serum	−0.21	NS	−0.16	NS
Amniotic fluid	−0.34	$p < 0.05$	−0.23	NS
Vitamin B_6				
Whole blood	0.16	NS	−0.002	NS
Amniotic fluid	0.06	NS	−0.06	NS

NS, Not significant.

trations in plasma did not differ significantly. Twelve of 27 cases of the study group demonstrated total homocysteine concentrations in amniotic fluid exceeding the mean plus twice the SD of the control group (i.e., >2.3 μmol/L). The individual total homocysteine concentrations in amniotic fluid and plasma in both groups are shown in Fig. 1.

The mean amniotic fluid concentrations of folate and vitamins B_{12} and B_6 did not significantly differ between the two groups (Table II). Similarly, none of the vitamins determined in blood were significantly different between the two groups.

In both groups the mean total plasma homocysteine and whole blood vitamin B_6 concentrations were significantly higher than those of the corresponding amniotic fluid samples. Only in group 2 was a significantly lower mean amniotic fluid folate concentration found compared with the mean serum folate value. In groups 1 and 2 the mean vitamin B_{12} levels of amniotic fluid were significantly higher than those in serum. There was no significant difference in the mean vitamin B_6 concentration in whole blood or amniotic fluid between groups 1 and 2. The mean vitamin B_6 concentrations in amniotic fluid and maternal blood did differ significantly within the groups.

Pearson's partial coefficients of correlation were cal-

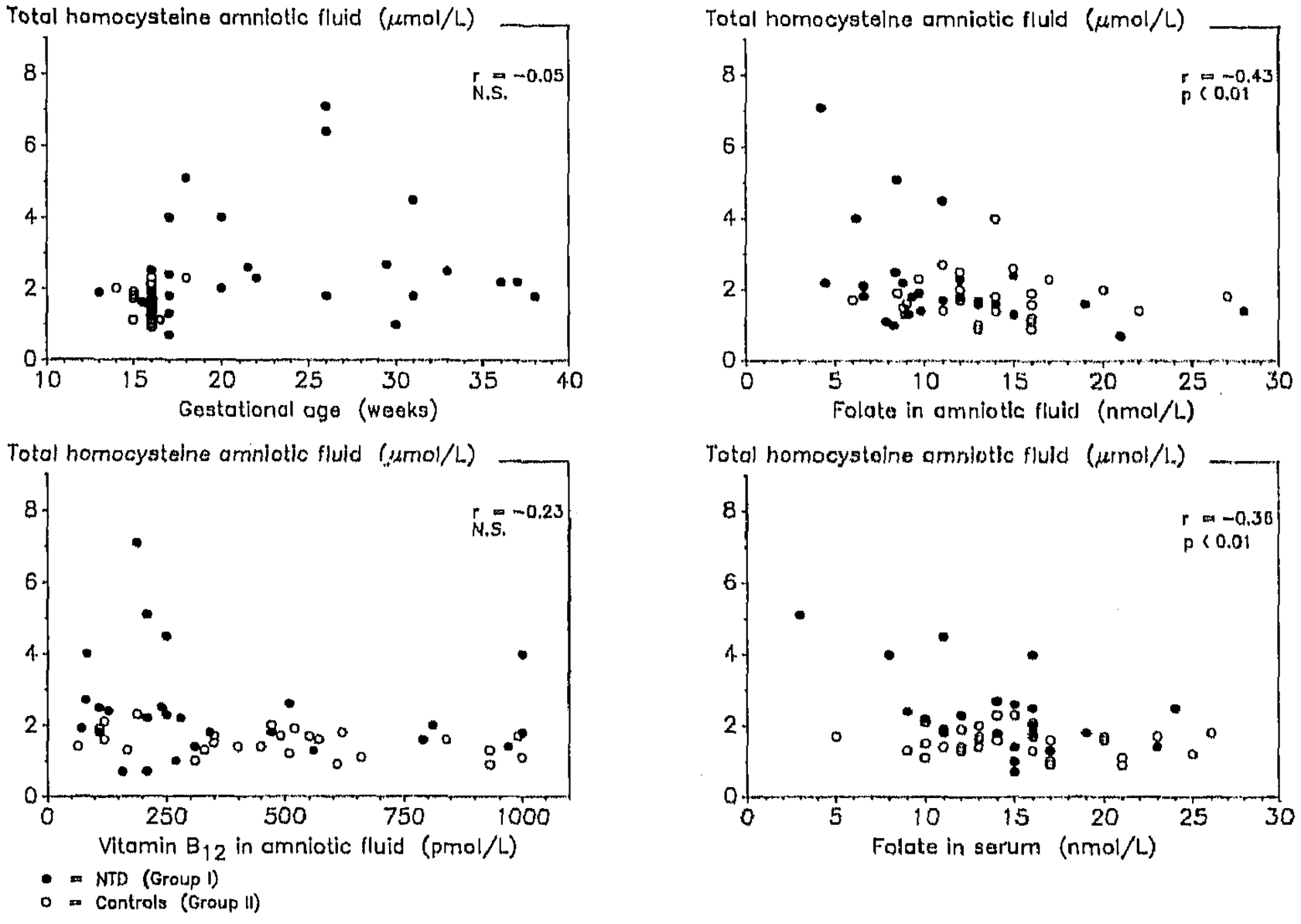

Fig. 2. Correlations of concentrations of total homocysteine in amniotic fluid with gestational age and vitamin B_{12} in amniotic fluid and with folate in amniotic fluid and serum. *NTD*, Neural tube defect.

culated between total homocysteine, the various vitamin levels, and maternal and gestational age. Because the correlations appeared not to be significantly different between the groups and regression lines were parallel, correlation coefficients were calculated for both groups combined. The correlations are shown in Table III and Fig. 2. The total homocysteine concentrations in plasma and amniotic fluid were not significantly correlated with maternal age. Only plasma, not amniotic fluid total homocysteine concentrations, showed a significant inverse correlation with gestational age. Folate levels in maternal serum and red blood cells were significantly inversely correlated with total homocysteine in amniotic fluid. Also, vitamin B_{12} in amniotic fluid and maternal plasma homocysteine levels did show a significant inverse correlation.

Comment

This study demonstrates that pregnant women carrying a fetus with a neural tube defect (group 1) have significantly higher total homocysteine levels in amniotic fluid than do controls (group 2). However, plasma total homocysteine levels did not differ significantly between groups. Plasma levels of either group were lower than those of nonpregnant women (7.2 μmol/L in pregnant control women vs 9.8 μmol/L in nonpregnant women[6]), which is in accordance with other studies.[15, 16] This finding might be related to hemodilution, increased remethylation of homocysteine induced by enhanced demands for methionine by the fetus or to the impact of increased sex hormone levels during pregnancy on homocysteine metabolism.[17, 18]

The total homocysteine concentrations in amniotic fluid of both groups were significantly lower in amniotic fluid than in plasma, as has been demonstrated previously in normal pregnancies.[15] In the current study a significant positive correlation could be established between the total homocysteine concentrations in the two compartments (i.e., maternal plasma and amniotic fluid). The transplacental transfer of homocysteine may be an important determinant of the level of homocysteine in amniotic fluid. The report of Kurczynski et al.,[19] who first described an untreated homocystinuric pregnant woman with dramatically increased homocysteine concentration in amniotic fluid, is in accordance with this observation. However, it is not excluded that the increased homocysteine concentration in amniotic fluid is caused by a defective homocysteine metabolism or by leakage from the neural tube defect of the fetus into the amniotic fluid.

There was no significant correlation between total homocysteine in plasma or amniotic fluid and gestational or maternal age, with the exception of the inverse correlation between plasma total homocysteine and gestational age. Furthermore, it revealed from the study of Andersson et al.[20] that total plasma homocysteine is directly correlated with age. Therefore it is very unlikely that the significantly higher total homocysteine levels in

amniotic fluid of fetuses with a neural tube defect compared with healthy controls are a consequence of the higher gestational age or the lower maternal age at which amniocentesis took place for the study group.

Shojania[21] reported a progressive decline of serum and red blood cell folate and serum vitamin B_{12} concentrations in pregnancy and hypothesized that this might be caused by increased fetal demands, blood volume expansion, and increased urinary excretion and by changes in maternal folate absorption and metabolism. In particular, changes in vitamin B_{12} binders may contribute to the physiologic decrease of this vitamin in pregnancy.[21] Decreased blood levels of vitamin B_6 during pregnancy have also been described.[22]

Folate or vitamin B_{12} deficiency results in increased homocysteine concentrations in blood and urine.[8, 9] A significantly negative correlation between plasma total homocysteine and serum and red blood cell folate was observed in this study, which is consistent with previous studies.[6, 8, 11] The mean folate concentrations in maternal serum and red blood cells and amniotic fluid of group 1 were not significantly different from those in group 2. Therefore the increased homocysteine levels in amniotic fluid of group 1 cannot solely be explained on the basis of lower folate concentrations.

Also serum vitamin B_{12} concentrations were comparable in the neural tube defect group and control women and were revealed to be within the normal ranges as given earlier for women of comparable age.[13, 23] In both groups vitamin B_{12} concentrations in amniotic fluid were significantly higher than those in serum. This is in line with the results of previous studies suggesting that there is an active transplacental transfer of this vitamin against a concentration gradient.[13]

Lower vitamin B_{12} levels in amniotic fluid of normal pregnancies in women who previously had an infant with a neural tube defect have been determined in relation to increased amniotic fluid transcobalamin levels.[24, 25] This phenomenon is thought to be an indication of a genetic factor in the predisposition of neural tube defects. However, in our study the mean vitamin B_{12} concentration in amniotic fluid was not significantly lower in the neural tube defect group compared with the control group.

To our knowledge, the presence of vitamin B_6 in human amniotic fluid has not been demonstrated before. In both groups the vitamin B_6 levels were significantly lower than in maternal blood. Its mean concentrations in maternal blood and amniotic fluid of the neural tube defect group were similar to the corresponding values of controls. No significant correlation between the concentrations of total homocysteine and vitamin B_6 in blood or amniotic fluid was found.

In conclusion, a significantly higher mean total homocysteine concentration was observed in amniotic fluid of a pregnancy in which the fetus was affected with a neural tube defect compared with a control group. This might be the result of a primary or secondary derangement in maternal or fetal homocysteine metabolism, which is in line with our findings and earlier findings of others.[5, 6, 26]

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A new placental enzyme in the metabolism of cocaine: An in vitro animal model

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OBJECTIVE: The aim of this study was to analyze placental metabolism in a genetically controlled in vitro animal model.
STUDY DESIGN: Placentas from Sprague-Dawley rats were centrifuged, and microsomes were isolated. Four treatment groups were incubated with cocaine over four time periods: placental microsomes + cocaine, placental microsomes + diisopropyl fluorophosphate (an anticholinesterase) + cocaine, placental microsomes + cocaine + butyrylcholinesterase, and a blank (cocaine only). Gas chromatography was used to quantify cocaine (Limit of quantitation = 19 ng/ml) and metabolites. Gas chromatography/mass spectrometry was used to verify the identity of the metabolites.
RESULTS: *Butyrylcholinesterase enhanced cocaine metabolism to ecgonine methyl ester.* More than 40% of cocaine was metabolized to norcocaine by rat placenta when diisopropyl fluorophosphate suppressed cocaine. Norcocaine is produced by hepatic N-demethylase action on methyl-bearing nitrogen in cocaine, suggesting that placenta and liver have this capacity. Gas chromatography/mass spectrometry was essential to the identification of norcocaine, because norcocaine is frequently not identified.
CONCLUSIONS: This biotransformation of cocaine to norcocaine may be a primary metabolic pathway induced in the cholinesterase-deficient placenta. This has clinical implications because norcocaine is ninefold more active physiologically than cocaine or ecgononinе methylesterase. (Am J Obstet Gynecol 1995;172:1441-5.)

Key words: N-demethylase, cholinesterase, cocaine, placenta, metabolism

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