Low ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine is associated with vitamin deficiency in Brazilian pregnant women and newborns[1–3]

Elvira M Guerra-Shinohara, Olga E Morita, Sabrina Peres, Regina A Pagliusi, Luiz F Sampaio Neto, Vânia D'Almeida, Silvia P Irazusta, Robert H Allen, and Sally P Stabler

ABSTRACT

Background: Pregnant women with low cobalamin concentrations are unable to provide the necessary amount of cobalamin to their fetuses. The effect of low maternal cobalamin concentrations on transmethylation metabolism in pregnant women and their newborns is unknown.

Objective: We investigated the relation between maternal and neonatal cobalamin concentrations and changes in total homocysteine (tHcy), *S*-adenosylmethionine (SAM), and *S*-adenosylhomocysteine (SAH).

Design: Hematologic data and concentrations of cobalamin, red blood cell folate, serum folate, tHcy, methylmalonic acid, SAM, SAH, and other metabolites were measured in 119 serum specimens from pregnant Brazilian women (gestational age: 37–42 wk) and their newborns' placental veins at the time of delivery.

Results: The tHcy concentrations were higher in placental vein serum from newborns whose mothers had low cobalamin. Serum SAH concentrations were elevated and serum SAM and methionine concentrations were decreased in pregnant women with lower cobalamin concentrations. SAM:SAH was significantly decreased in both cobalamin-deficient pregnant women and their newborns.

Conclusions: Lower maternal cobalamin concentrations are associated with higher tHcy and lower SAM:SAH in newborns. Because SAM:SAH is closely linked with the activity of numerous enzymatic methylation reactions, these results suggest that methylation could be impaired in cobalamin-deficient pregnant women and their newborns. *Am J Clin Nutr* 2004;80:1312–21.

KEY WORDS Cobalamin, folate, homocysteine, methylmalonic acid, *S*-adenosylhomocysteine, *S*-adenosylmethionine, pregnant women, newborns

INTRODUCTION

Cobalamin and folate are necessary for DNA synthesis and thus for cell division. Fetuses and pregnant women are in a state of rapid turnover that requires a high rate of DNA synthesis (1).

Methylcobalamin is a required coenzyme in the synthesis of methionine from homocysteine. This pathway involves the enzymatic transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine by methionine synthase. In the absence of cobalamin, folate is "trapped" and cannot be recycled into the folate pool. Serum concentrations of total homocysteine (tHcy) are elevated in cobalamin and folate deficiency (2). It was theorized that impaired synthesis of methionine and therefore of *S*-adenosylmethionine (SAM), the most important methyl donor, plays a role in the pathophysiology of cobalamin deficiency. After donating a methyl group, SAM is converted to *S*-adenosylhomocysteine (SAH), which is hydrolyzed to homocysteine. Elevated concentrations of SAH can inhibit transmethylation reactions (3), which could also be deleterious in cobalamin deficiency. We showed previously that patients with severe cobalamin-deficient megaloblastic anemia have elevated serum SAH, which is corrected with cobalamin therapy (4).

Cobalamin is also a cofactor in the enzymatic conversion of L-methylmalonyl-coenzyme A to succinyl-coenzyme A. Impaired cobalamin status causes accumulation of serum methylmalonic acid (MMA; 2).

During pregnancy, the cell multiplication resulting from the enlargement of the uterus, placental development, and fetal growth increases cobalamin and folate requirements. It is well known that maternal serum cobalamin and folate concentrations decrease during pregnancy (5–8). Bruinse and van den Berg (6) reported that the cobalamin serum concentration fell gradually by ≈75–100 pmol/L during pregnancy. It is not known why cobalamin or folate decreases during gestation, but several factors, such as hemodilution, hormonal influences, and nutritional deficiency, could be responsible. The serum

[1] From the Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, São Paulo, Brazil (EMGS and SP); Programa de Pós-Graduação da Coordenadoria dos Institutos de Pesquisas da Secretaria de Saúde do Estado de São Paulo, São Paulo, Brazil (OEM); Instituto Adolfo Lutz, Laboratório de São José do Rio Preto, São José do Rio Preto, Brazil (RAP); Faculdade de Medicina, Pontificia Universidade Católica de Sao Paulo, São Paulo, Brazil (LFSN); Departamento de Pediatria, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil (VDA); Serviço de Patologia Clínica do Conjunto Hospitalar de Sorocaba, Secretaria da Saúde do Estado de São Paulo, São Paulo, Brazil (SPI); and the Department of Medicine, University of Colorado Health Sciences Center, Denver (RHA and SPS).

[2] Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil (Proc. 00/12467-0), and by the National Institute on Aging (AG-09834; to SPS). SP was the recipient of a fellowship from CNPq, Brazil.

[3] Address reprint requests to EM Guerra-Shinohara, Department of Clinical Chemistry and Toxicology, Faculty of Pharmaceutical Science, University of Sao Paulo, Av. Prof. Lineu Prestes, 580, CEP 05508-900, Sao Paulo, SP, Brazil. E-mail: emguerra@usp.br.

Received August 22, 2003.

Accepted for publication April 14, 2004.

cortisol concentrations were an important variable for explaining the variance in cobalamin concentrations at the 34th wk of pregnancy (6). Cortisol, progesterone, and prolactin were associated with decreasing folate concentrations during pregnancy (6). Many studies showed relations between low folate concentrations, elevated tHcy, and pregnancy complications or adverse neonatal outcomes from neural tube defects (NTDs) and low birth weight (9–17).

Cobalamin deficiency was also related to the incidence of NTDs and birth complications. Thame et al (18) found an 11.8% prevalence of cobalamin deficiency (cobalamin <110.67 pmol/L) in Brazilian women with NTD-affected pregnancy. In addition, Adams et al (19) observed higher MMA concentrations in women with NTD-affected pregnancy than in women with unaffected pregnancy. However, the midtrimester MMA and tHcy values in their subjects were 25% and 34% lower than in female blood donors, which suggests that these metabolites, like the vitamin concentrations, decrease during normal gestation (19). A recent study showed that preterm birth was related to both hyperhomocysteinemia and cobalamin deficiency (20). The aim of this study was to investigate the relation between maternal and neonatal cobalamin and folate status by analyzing the vitamin-dependent metabolites, including SAM and SAH, in pregnant women and in placental vein blood from their newborns.

SUBJECTS AND METHODS

Subjects

Blood was collected from 119 pregnant women at 2 public hospitals in Sorocaba City, Brazil, after admission for labor and up to 12 h before delivery. After expulsion of the placenta, the blood from placental veins was collected.

All deliveries occurred within the gestational age range of 37–42 wk. Women with a clinical diagnosis of metabolic diseases, renal insufficiency, increased serum liver enzymes, multiple gestations, and complications during delivery, including the birth of a premature newborn or a newborn with congenital malformation and anoxia, were excluded from the study.

Socioeconomic data (monthly per capita income, schooling, and occupation), obstetric status, and supplemental vitamin intake were assessed by questionnaire. Neonatal birth weight and Apgar score were collected. The protocol was approved by the local ethics committees (Brazil) and by the investigational review board at the University of Colorado, and written informed consent was obtained from all pregnant women before participation.

Blood sampling

Peripheral venous maternal blood and samples from placental veins were drawn into tubes with EDTA for measurements of the hemogram and whole blood folate concentration. Serum was obtained for measurements of concentrations of cobalamin, folate, tHcy, cystathionine, cysteine, methionine, SAM, SAH, MMA, 2-methylcitric acid, serine, glycine, *N*,*N*-dimethylglycine, and *N*-methylglycine. The clot tubes were cooled immediately at 4 °C and centrifuged 1 h after blood collection. The serum was frozen and sent to the United States frozen on dry ice and assayed within 1 y of collection.

Biochemical measurements

Red blood cell (RBC), leukocyte, and platelet counts; hemoglobin concentration; and hematocrit were measured on all samples with use of an electronic cell counter (Coulter STKS; Coulter Corp, Hialeah, FL). Serum folate concentrations were measured by using the ion capture method (IMx System; Abbott Laboratories, Abbott Park, IL). This method is based on binding by a specific folate-binding protein. RBC folate was measured with use of the same procedure after 1:20 dilution of a whole blood aliquot with lysis solution (Folate Lysis Reagent No. 9C13-60; Abbott Laboratories). RBC folate concentration was calculated as follows: folate content (in nmol/L)/hematocrit × dilution factor.

The serum cobalamin concentration was measured by 2 methods: method 1 used a totally automated kit (IMx System; Abbott Laboratories) with no boil pretreatment, and method 2 used a semiautomated kit (Immulite; Diagnostic Products Corp, Los Angeles), which involved sample pretreatment in a boil bath. Both methods use porcine intrinsic factor as a ligand.

Indexes of kidney and liver function were investigated with measurements of serum creatinine, aspartate aminotransferase, and alanine aminotransferase obtained by using kits (Dimension AR; Dade Behringer, Newark, DE). Pregnant women with abnormalities in these indexes were excluded from our study.

The measurements of tHcy, MMA, and other amino acids were performed with the use of stable isotope dilution capillary gas chromatography–mass spectrometry (21–23). SAM and SAH were measured with use of a newly developed stable isotope dilution liquid chromatography–mass spectrometry method (24). The CV for intraassay precision (within-run) was 5% for SAM and 17% for SAH. The CV for interassay precision (run-to-run) was 7.4% for SAM and 22% for SAH (24).

Statistical analysis

All statistical analyses were carried out with use of SAS for WINDOWS software (version 6.12; SAS Institute Inc, Cary, NC), with the level of significance set at $P < 0.05$. The correlation between the concentrations of cobalamin measured by the 2 methods was assessed with use of the Pearson coefficient. The Bland-Altman analysis was used to study the agreement between methods of measuring cobalamin concentrations (25).

To establish a cobalamin deficiency cutoff in pregnant women and newborn groups, we used the highest quartile of maternal tHcy (>7.8 μmol/L), maternal MMA (>270 nmol/L), maternal SAH (>27 nmol/L), neonatal tHcy (>6.8 μmol/L), neonatal MMA (>384 nmol/L), and neonatal SAH (>67 nmol/L) and the lowest quartile of maternal (<2.6) and neonatal (<2.8) SAM:SAH. The maternal or neonatal variables were used alone or in combinations of variables as criteria of cobalamin deficiency in these groups.

The correlation between the concentrations of cobalamin and those of tHcy, MMA, and other metabolites in pregnant women and in newborns independently and the correlations of the concentrations between pregnant women and their respective newborns were determined by the Pearson coefficient. This coefficient was also used to measure the correlation between neonatal birth weight and maternal and neonatal biochemical measurements. The biochemical variables (ie, cobalamin, RBC folate, serum folate, tHcy, MMA, SAM, SAH, SAM:SAH, methionine,

cystathionine, 2-methycitric acid, cysteine, glycine, serine, *N*,*N*-dimethylglycine, and *N*-methylglycine), hematologic variables (RBC count and hemoglobin concentration), neonatal birth weight, and per capita income data were not consistent with a normal distribution and were transformed in a logarithmic scale for the statistical analysis. The maternal age data were not log transformed.

The paired Student's *t* test was used to compare the mean of log-transformed data on the limits evaluated in pregnant women and newborn groups. One-way analysis of variance was used to compare the maternal and neonatal biochemical log-transformed data from groups formed according to quartiles of maternal cobalamin concentrations. When significant differences among groups were observed, Tukey's post hoc test was performed to identify the significantly different group means.

Multiple linear and multiple logistic regression analysis

To assess the simultaneous relations between the various predictors of tHcy, MMA, and SAM:SAH in the pregnant women and the newborns (as dependent variables), several models of stepwise multiple linear regression analysis were used. For all models, the biochemical variables were log transformed before the analysis. The models were adjusted by covariates: maternal age, racial group (whites compared with blacks or mixed groups), and parity.

In model 1, the dependent variables were maternal tHcy, MMA, and SAM:SAH. The independent variables included cobalamin, RBC folate, serum folate, creatinine, SAM:SAH, and tHcy for the dependent variables tHcy and SAM:SAH. The independent variables for MMA were cobalamin, RBC folate, serum folate, and creatinine. In model 2, the dependent variables were neonatal tHcy, MMA, and SAM:SAH, and the independent variables were the same maternal variables as in model 1. In model 3, the dependent variables were the same as those in model 2, and the independent variables are the same as those in model 1 but were from the newborns. In model 4, the dependent variables were the same as those in models 2 and 3, and the independent variables were those significant maternal and neonatal variables in models 2 and 3, separately, for tHcy, MMA, and SAM:SAH.

A multivariate logistic regression with stepwise analysis was done with the same independent variables as were used in models 1–4 from the multiple linear regression analysis. The outcomes were the following: maternal tHcy, >7.8 μmol/L; maternal MMA, >270 nmol/L; maternal SAM:SAH, <2.6; neonatal tHcy, >6.8 μmol/L; neonatal MMA, >384 nmol/L; and neonatal SAM:SAH, <2.8. These covariates of maternal age (year), racial group (whites compared with blacks plus mixed racial group), and parity were used in all of the multivariate logistic regression analyses. The cutoff values for maternal and neonatal outcomes were the highest quartiles for tHcy and MMA and the lowest quartiles for SAM:SAH.

Receiver operator characteristic curve

The receiver operator characteristic (ROC) curve was used for establishing the cobalamin cutoff indicators of metabolic cobalamin deficiency (26). The values of sensitivity and specificity for each biochemical variable (tHcy, MMA, SAH, and SAM:SAH or combinations of variables) were obtained by using contingency tables that crossed the criteria and the number of subjects below and above the cutoff value set in the ROC curves to maximize both sensitivity and specificity. When ≥1 limit was used as the criterion, it was considered altered if ≥1 of the limits was altered. The area under the ROC curve tends to be near 1.0 for a sensitive and specific diagnostic method (26).

RESULTS

Population characteristics

The mean (±SD) age of the pregnant women was 25.5 ± 6.7 y (range: 15–44 y), gestational age was 39.4 ± 1.1 wk (range: 37–42 wk), and parity was 2.6 ± 1.7, including the current pregnancy. The pregnant women were generally of low socioeconomic status without an occupation (74.8%) and without basic schooling (42.4%). The mean (±SD) per capita income was US$86.50 ± 65.20/mo. The cohort's racial composition was 53.9% white ($n = 64$), 6.7% black ($n = 8$), 38.7% mixed black and white ($n = 46$), and 0.8% Asian ($n = 1$).

Cobalamin assays

We measured the cobalamin concentrations by using 2 methods. Strong and significant correlations were found between the 2 methods in both the pregnant ($r = 0.933$, $P < 0.001$) and the newborn ($r = 0.963$, $P < 0.001$) subjects (**Figure 1**). For the statistical evaluations, we used concentrations measured by using method 1.

Simple correlations between vitamins and metabolites

Maternal biochemical values

The geometric means and 95% CIs of cobalamin, folate, MMA, tHcy, and other metabolites of methionine metabolism from maternal subjects are shown in **Table 1**. Mean serum cobalamin concentrations were low, and mean MMA and cystathionine concentrations were relatively high in this population. There were weak but significant correlations between maternal cobalamin concentrations and tHcy ($r = -0.186$, $P = 0.043$), SAM ($r = 0.232$, $P = 0.013$), and SAH ($r = -0.248$, $P = 0.007$) concentrations and SAM:SAH ($r = 0.293$, $P = 0.002$). No significant correlation was observed between maternal cobalamin and MMA ($r = -0106$, $P = 0.251$). There were inverse correlations between RBC folate and tHcy ($r = -0.337$, $P < 0.001$) and between serum folate and tHcy ($r = -0.485$, $P < 0.001$).

No correlation was found between tHcy and cystathionine concentrations in the pregnant subjects ($r = 0.055$, $P = 0.553$). However, significant correlations were found between tHcy and cysteine concentrations in the pregnant subjects ($r = 0.245$, $P = 0.007$). No significant correlations were observed between birth weight and maternal cobalamin, RBC folate, or serum folate concentrations.

Placental vein biochemical values

The geometric means and 95% CIs of cobalamin, folate, MMA, tHcy, and other biochemical variables from the placental vein samples are shown in Table 1. Significant relations were found between cobalamin concentrations and tHcy ($r = -0.321$, $P < 0.001$) and MMA ($r = -0.338$, $P < 0.001$) in the newborns. In contrast, no significant correlations were observed between RBC folate and tHcy ($r = -0.148$, $P = 0.123$) or between serum folate and tHcy ($r = -0.154$, $P = 0.114$).

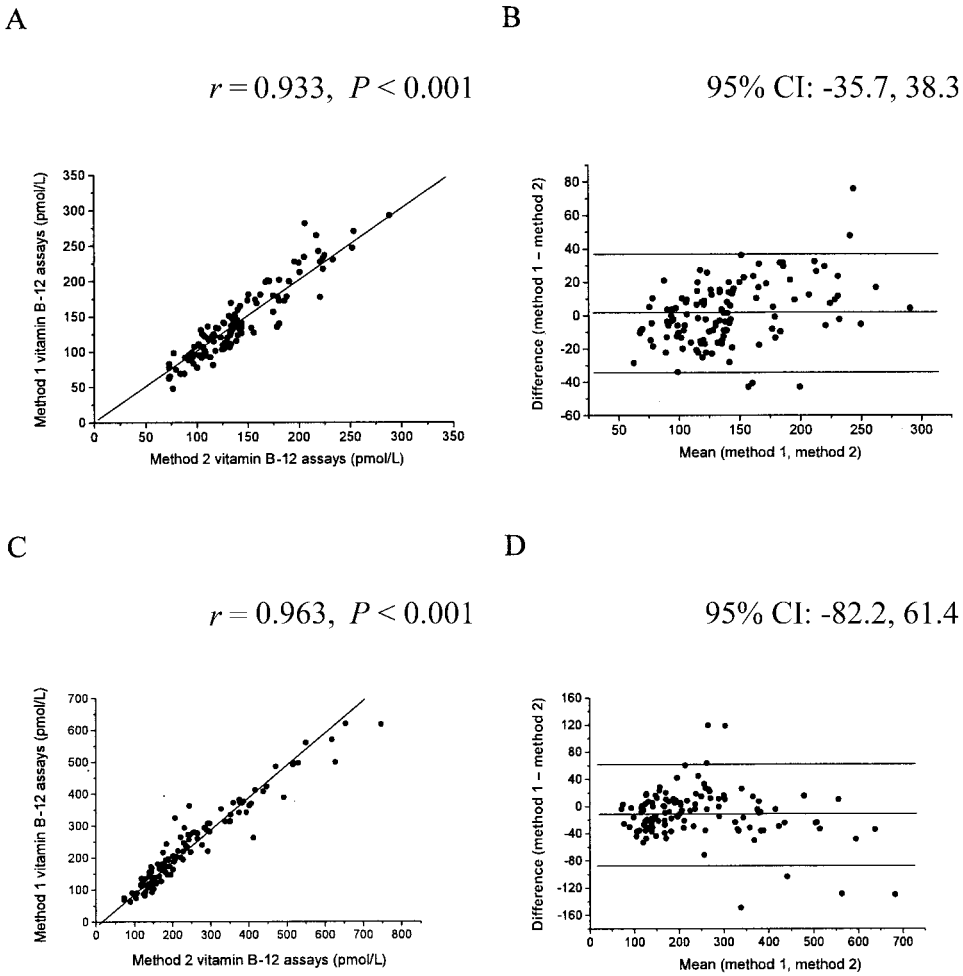

FIGURE 1. Relation between serum cobalamin concentrations measured by method 1 and method 2. Regression analyses (A and C) and Bland-Altman analyses (B and D) are shown for pregnant subjects (n = 119) and placental vein samples (n = 118).

There was no correlation between tHcy and cystathionine concentrations in the neonatal samples (r = 0.056, P = 0.558). However, a significant correlation was found between tHcy and cysteine concentrations (r = 0.414, $P < 0.001$). No significant correlations were observed between birth weight and neonatal cobalamin, RBC folate, or serum folate concentrations.

Maternal and placental vein biochemical values

SAM, SAH, MMA, methionine, cystathionine, 2-methylcitric acid, glycine, serine, *N*,*N*-dimethylglycine, and *N*-methylglycine concentrations were significantly higher in the placental samples. No significant difference was observed between maternal and neonatal SAM:SAH (P = 0.605). However, the neonatal tHcy and cysteine concentrations were significantly lower than were the maternal concentrations. Strong associations were observed between the maternal and neonatal variables for cobalamin, tHcy, MMA, cystathionine, *N*,*N*-dimethylglycine, and *N*-methylglycine (Table 1). Weaker relations were observed between maternal and neonatal SAM, maternal and neonatal SAH, and maternal and neonatal SAM:SAH (Table 1).

Effect of maternal cobalamin concentrations on metabolites

Data from pregnant women and placental vein samples were divided into quartiles according to maternal cobalamin concentrations: quartile 1 (group 1), cobalamin ≤ 102 pmol/L (n = 29); quartile 2 (group2), cobalamin ≤128 pmol/L (n = 29); quartile 3 (group 3), cobalamin ≤162 pmol/L (n = 30); and quartile 4 (group 4), cobalamin > 162 pmol/L (n = 30), as shown in **Table 2**.

Maternal age was not significantly different across different cobalamin quartiles. The pregnant women from the lowest quartile (group 1) had lower mean RBC counts (3.82×10^9/L) and hemoglobin concentration (109.7 g/L) than did pregnant women in the highest quartile (group 4) (4.15×10^9/L and 119.1 g/L, respectively). However, no significant difference was found in RBC counts or hemoglobin concentrations between the respective newborn groups.

Serum folate but not RBC folate concentration was lower in women from group 1 than in women from group 4. The tHcy concentrations did not increase significantly in women with the lowest cobalamin values (P = 0.112). The placental vein cobalamin

TABLE 1
Geometric means, 95% CIs, and Pearson correlations between maternal and neonatal placental vein data[1]

Variables	Pregnant subjects[2]	Neonatal placental vein[3]	Pearson correlation coefficient[4] (r)	P
Cobalamin, pmol/L (n = 117)	130 (122, 138)	205 (186, 225)	0.570	<0.001
Red blood cell folate, nmol/L (n = 116)	643 (591, 701)	1108 (1033, 1188)	0.488	<0.001
Serum folate, nmol/L (n = 112)	12.9 (12.0, 14.0)	30.9 (29.8, 32.1)	0.409	<0.001
Total homocysteine, μmol/L (n = 110)	6.5 (6.1, 6.9)	5.8 (5.4, 6.1)	0.753	<0.001
MMA, nmol/L (n = 110)	200 (185, 216)	308 (289, 328)	0.693	<0.001
SAM, nmol/L (n = 105)	75 (70, 81)	176 (164, 190)	0.219	0.024
SAH, nmol/L (n = 107)	22 (20, 23)	52 (48, 57)	0.219	0.023
Ratio of SAM to SAH (n = 105)	3.5 (3.1, 3.9)	3.4 (3.1, 3.7)	0.288	<0.001
Methionine, μmol/L (n = 110)	16.9 (16.2, 17.5)	26.3 (25.4, 27.3)	0.394	<0.001
Cystathionine, nmol/L (n = 110)	197 (183, 212)	314 (290, 340)	0.728	<0.001
2-Methylcitric acid, nmol/L (n = 109)	115 (109, 121)	177 (170, 185)	0.478	<0.001
Cysteine, μmol/L (n = 110)	191 (185, 196)	181 (176, 187)	0.432	<0.001
Glycine, μmol/L (n = 110)	190 (182, 199)	281 (271, 292)	0.474	<0.001
Serine, μmol/L (n = 110)	136 (131, 141)	155 (151, 160)	0.417	<0.001
N,N-dimethylglycine, μmol/L (n = 110)	2.8 (2.5, 3.1)	3.7 (3.4, 4.1)	0.936	<0.001
N-methylglycine, μmol/L (n = 110)	0.6 (0.6, 0.7)	0.9 (0.9, 1.0)	0.826	<0.001

[1] All values are geometric $\bar{x}$; 95% CIs in parentheses. MMA, methylmalonic acid; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.
[2] n = 113–119 for the different variables.
[3] n = 111–117 for the different variables.
[4] Calculated on the log-transformed variables.

concentrations from maternal groups 1, 2, and 3 were significantly lower than those from maternal group 4 (highest cobalamin quartile; Table 2), whereas there were no changes in folate concentrations between groups. Neonatal tHcy concentrations were higher in groups 1 and 2 than in group 4. Neonatal MMA tended to be higher (P = 0.081) in the groups with lower maternal cobalamin. Serum SAM and methionine concentrations were decreased, and serum SAH concentrations were elevated in pregnant women with lower cobalamin concentrations. SAM:SAH was significantly decreased in both pregnant subjects and placental vein samples in the lowest group. Maternal and neonatal cysteine concentrations were not different according to the quartile of maternal cobalamin.

TABLE 2
Maternal and placental vein biochemical variables divided by maternal cobalamin quartile[1]

	Quartile of maternal serum cobalamin[2]				
Variables	1	2	3	4	p[3]
Maternal cobalamin (pmol/L)	84 (79, 90)	116 (113, 119)	141 (138, 144)	206 (192, 220)	—[4]
Neonatal cobalamin (pmol/L)	149 (127, 174)^{a}	181 (153, 214)^{a,b}	215 (180, 257)^{b}	303 (247, 371)^{c}	<0.001
Maternal RBC folate (nmol/L)	614 (505, 745)^{a}	606 (528, 694)^{a}	626 (522, 749)^{a}	734 (609, 885)^{a}	0.353
Neonatal RBC folate (nmol/L)	995 (874, 1133)^{a}	1185 (1057, 1328)^{a}	1064 (906, 1251)^{a}	1208 (1030, 1418)^{a}	0.168
Maternal serum folate (nmol/L)	10.7 (9.2, 12.4)^{a}	11.9 (10.6, 13.5)^{a,b}	14.4 (12.4, 16.6)^{b}	15.2 (12.7, 18.0)^{b}	0.003
Neonatal serum folate (nmol/L)	30.3 (27.8, 33.2)^{a}	30.4 (28.6, 32.2)^{a}	31.5 (29.3, 33.9)^{a}	31.5 (29.0, 34.1)^{a}	0.810
Maternal tHcv (μmol/L)	7.1 (6.0, 8.3)^{a}	7.0 (6.3, 7.7)^{a}	6.2 (5.6, 6.9)^{a}	5.9 (5.2, 6.7)^{a}	0.112
Neonatal tHcy (μmol/L)	7.0 (6.0, 8.1)^{a}	6.4 (5.7, 7.1)^{a,b}	5.4 (4.9, 5.9)^{b,c}	4.7 (4.2, 5.3)^{c}	<0.001
Maternal MMA (nmol/L)	199 (169, 233)^{a}	233 (205, 265)^{a}	180 (153, 211)^{a}	193 (161, 231)^{a}	0.129
Neonatal MMA (nmol/L)	319 (284, 359)^{a}	347 (307, 393)^{a}	294 (256, 337)^{a}	280 (247, 318)^{a}	0.081
Maternal SAM (nmol/L)	63 (55, 73)^{a}	77 (68, 87)^{a,b}	85 (75, 95)^{b}	79 (67, 93)^{a,b}	0.021
Neonatal SAM (nmol/L)	161 (131, 199)^{a}	168 (140, 202)^{a}	194 (177, 213)^{a}	183 (163, 206)^{a}	0.305
Maternal SAH (nmol/L)	26 (22, 30)^{a}	22 (20, 25)^{a,b}	21 (18, 24)^{a,b}	19 (16, 22)^{b}	0.013
Neonatal SAH (nmol/L)	59 (47, 74)^{a}	55 (46, 66)^{a}	47 (41, 54)^{a}	49 (41, 59)^{a}	0.280
Maternal SAM:SAH	2.5 (2.0, 3.1)^{a}	3.4 (2.9, 4.1)^{a,b}	4.1 (3.4, 5.0)^{b}	4.1 (3.2, 5.4)^{b}	0.003
Neonatal SAM:SAH	2.7 (2.4, 3.1)^{a}	3.0 (2.2, 4.1)^{a,b}	4.1 (3.6, 4.8)^{b}	3.7 (3.2, 4.3)^{a,b}	0.007
Maternal methionine (μmol/L)	15.5 (14.2, 16.8)^{a}	17.5 (16.4, 18.7)^{a,b}	18.1 (16.9, 19.3)^{b}	16.6 (15.2, 18.1)^{a,b}	0.022
Neonatal methionine (μmol/L)	27.1 (25.1, 29.4)^{a}	25.9 (24.2, 27.8)^{a}	26.7 (24.8, 28.7)^{a}	25.7 (23.8, 27.8)^{a}	0.699
Maternal cysteine (μmol/L)	181 (171, 192)^{a}	195 (185, 205)^{a}	192 (180, 204)^{a}	196 (183, 210)^{a}	0.236
Neonatal cysteine (μmol/L)	186 (173, 199)^{a}	187 (178, 196)^{a}	180 (170, 190)^{a}	175 (164, 186)^{a}	0.318

[1] All values are geometric $\bar{x}$; 95% CI in parentheses. RBC, red blood cell; tHcy, total homocysteine; MMA, methylmalonic acid; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine. Values in a row with different superscript letters are significantly different, $P < 0.05$ (Tukey's test).
[2] Quartile 1, ≤102 pmol/L; quartile 2, 103–128 pmol/L; quartile 3, 129–162 pmol/L; quartile 4, ≥163 pmol/L.
[3] ANOVA performed on the log-transformed variables.
[4] Statistical analysis was not evaluated for this variable.

TABLE 3
Association between maternal and neonatal biochemical variables and total homocysteine (tHcy), methylmalonic acid (MMA), and the ratio of *S*-adenosylmethionine (SAM) to *S*-adenosylhomocysteine (SAH) in mothers and children by stepwise multiple linear regression analysis[1]

Model[2] and dependent variable	Step	Independent variables	β	Partial R^2	P
1					
Maternal tHcy	1	Maternal SF	−0.373	0.207	<0.001
Maternal MMA	1	Maternal SC	0.609	0.074	0.003
Maternal SAM:SAH	1	Maternal cobalamin	0.504	0.089	0.001
2					
Neonatal tHcy	1	Maternal cobalamin	−0.357	0.205	<0.001
	2	Maternal SF	−0.211	0.055	<0.001
Neonatal MMA	1	Maternal cobalamin	−0.221	0.066	0.015
	2	Maternal SC	0.353	0.044	0.026
Neonatal SAM:SAH	1	Maternal cobalamin	0.271	0.035	0.053
3					
Neonatal tHcy	1	Neonatal cobalamin	−0.195	0.094	<0.001
	2	Neonatal SAM:SAH	−0.128	0.038	0.033
Neonatal MMA	1	Neonatal cobalamin	−0.227	0.129	<0.001
Neonatal SAM:SAH	1	Neonatal SF	−0.853	0.076	<0.001
	2	Neonatal tHcy	−0.381	0.057	0.001
4					
Neonatal tHcy	1	Maternal cobalamin	−0.345	0.194	<0.001
	2	Maternal SF	−0.194	0.046	0.012
Neonatal MMA	1	Neonatal cobalamin	−0.225	0.132	<0.001
Neonatal SAM:SAH	1	Neonatal SF	−0.853	0.076	<0.001
	2	Neonatal tHcy	−0.381	0.057	0.011

[1] All variables were log transformed. The regression models were adjusted by maternal age, racial group, and parity. The regression parameter estimate (β), partial R^2, and P values are for the independent variables within each model. SF, serum folate; SC, serum creatinine.

[2] Model 1: Maternal variables are used as determinants of maternal tHcy, MMA, and SAM:SAH. Independent variables for tHcy and SAM:SAH include maternal RBC and serum concentrations of folate, cobalamin, creatinine, tHcy, and SAM:SAH. For MMA, the independent variables are maternal RBC and serum concentrations of folate, cobalamin, and creatinine. Model 2: Maternal variables are used as determinants of neonatal tHcy, MMA, and SAM:SAH. Independent variables are the same as in model 1. Model 3: Neonatal variables are used as determinants of neonatal tHcy, MMA, and SAM:SAH. Independent variables are the same as in model 1, but from the newborn. Model 4: Neonatal and maternal variables that were significant in model 2 or model 3 are used as determinants of neonatal tHcy, MMA, and SAM:SAH.

Predictors of metabolite variables

The association between various maternal and neonatal biochemical variables and the outcomes for tHcy, MMA, and SAM:SAH in mothers and newborns by stepwise multiple linear regression analysis is shown in **Table 3**. The odds ratio for elevated tHcy and MMA or low SAM:SAH in mothers and newborns by stepwise multiple logistic regression analysis is shown in **Table 4**. Two categories for independent variables were used in multivariate logistic regression analysis.

In both linear and logistic regression analyses, we tested maternal determinants of maternal MMA, tHcy, and SAM:SAH (model 1); maternal determinants of neonatal MMA, tHcy, and SAM:SAH (model 2); neonatal determinants of neonatal MMA, tHcy, and SAM:SAH (model 3); and the combined neonatal and maternal determinants of neonatal MMA, tHcy, and SAM:SAH (model 4). Maternal serum folate concentration was an independent inverse predictor of maternal tHcy concentrations. The partial R^2 for this regression indicated that maternal serum folate concentrations explained 20.7% of the variance in maternal tHcy concentrations (Table 3). Pregnant women with low maternal serum folate (<9.3 nmol/L) had a 5.8-fold greater risk of higher tHcy concentrations than did pregnant women with higher serum folate concentrations (Table 4). The maternal MMA concentrations were predicted only by maternal serum creatinine (partial $R^2 = 0.0740$; Table 3). The odds ratio for elevated maternal MMA for pregnant women with serum creatinine ≥ 0.7 mg/dL was 4.8 (95% CI: 1.3, 17.8; Table 4). Maternal SAM:SAH was predicted by maternal cobalamin concentration (Table 3). The pregnant women with lower cobalamin concentrations had a 7.2-fold greater risk of lower maternal SAM:SAH than did pregnant women with higher serum cobalamin concentrations (Table 4).

The maternal cobalamin and maternal serum folate concentrations inversely predicted the neonatal tHcy concentrations (model 2). The partial R^2 values for these regressions indicate that maternal cobalamin accounts for 20.5% of the variance in neonatal tHcy concentrations, and maternal serum folate accounts for only 5.5% of the variance in neonatal tHcy concentrations. In model 3, which tested only neonatal independent variables, the neonatal cobalamin concentration and SAM:SAH were inversely associated with neonatal tHcy concentrations. In contrast to the maternal cobalamin, the neonatal cobalamin and neonatal SAM:SAH explained less of the variance in neonatal tHcy. Model 4 explores the significant maternal and neonatal independent variables from models 2 and 3. Model 4 showed that there was no increase in the summed value of partial R^2 when model 2 was compared with model 4 (26.0% and 24%, respectively). The same determinant variables from model 2 were selected (maternal cobalamin and maternal serum folate), and they were inversely associated with neonatal tHcy concentrations in model 4 (Table 3).

The predictors of neonatal MMA concentrations were maternal cobalamin and maternal serum creatinine in model 2, neonatal cobalamin in model 3, and neonatal cobalamin in model 4. The

TABLE 4
Odds ratio (OR) for elevated total homocysteine (tHcy) and methylmalonic acid (MMA) and a low ratio of *S*-adenosylmethionine (SAM) to *S*-adenosylhomocysteine (SAH) in mothers and children by stepwise multiple logistic regression analysis[1]

Model[2] and dependent variable	Step	Independent variable	OR (95% CI)	*P*
1				
Maternal tHcy >7.8 μmol/L	1	Maternal SF <9.3 nmol/L	5.8 (2.1, 16.0)	0.001
Maternal MMA >270 nmol/L	1	Maternal SC ≥0.7 mg/dL	4.8 (1.3, 17.8)	0.019
	2	Maternal RBC folate <472 nmol/L	3.3 (1.2, 9.3)	0.023
Maternal SAM:SAH <2.6	1	Maternal cobalamin <102 pmol/L	7.2 (2.6, 20.2)	0.001
2				
Neonatal tHcy >6.8 μmol/L	1	Maternal cobalamin <102 pmol/L	6.9 (2.2, 22.2)	0.001
	2	Maternal RBC folate <472 nmol/L	3.4 (1.1, 10.5)	0.035
Neonatal MMA >384 nmol/L		—[3]		
Neonatal SAM:SAH <2.8	1	Maternal cobalamin <102 pmol/L	5.0 (1.8, 13.9)	0.002
3				
Neonatal tHcy >6.8 μmol/L	1	Neonatal SAM:SAH <2.8	3.3 (1.2, 9.6)	0.024
	2	Neonatal cobalamin <135 pmol/L	2.9 (1.0, 8.0)	0.045
Neonatal MMA >384 nmol/L	1	Neonatal cobalamin <135 pmol/L	3.7 (1.4, 9.7)	0.009
Neonatal SAM:SAH <2.8	1	Neonatal tHcy >6.8 μmol/L	3.2 (1.2, 8.9)	0.023
4				
Neonatal tHcy >6.8 μmol/L	1	Maternal cobalamin <102 pmol/L	3.7 (1.3, 10.5)	0.013
Neonatal MMA >384 nmol/L	1	Neonatal cobalamin <135 pmol/L	3.6 (1.4, 9.4)	0.010
Neonatal SAM:SAH <2.8	1	Maternal cobalamin <102 pmol/L	5.0 (1.8, 13.6)	0.002

[1] The multiple logistic regression models were adjusted by maternal age, racial group, and parity. SF, serum folate; SC, serum creatinine; RBC, red blood cell.

[2] Models are as described in Table 3, except that both the dependent and independent variables were dichotomized by using thresholds corresponding to the 75th percentile for the metabolites and creatinine and the 25th percentile for the vitamins and SAM:SAH. Reference population for the independent variables is subjects with values in the 3 lowest (for tHcy and MMA) or 3 highest (for cobalamin, RBC folate, serum folate, and SAM:SAH) quartiles.

[3] No variable selected at the 0.05 significance level.

maternal cobalamin concentrations explained 3.5% of the variance in neonatal SAM:SAH in model 2 (Table 3), and pregnant women with lower cobalamin concentrations (<102 pmol/L) had a 5.0-fold greater risk of lower neonatal SAM:SAH than did pregnant women with higher serum cobalamin concentrations (Table 4). The neonatal serum folate and neonatal tHcy concentrations were inversely associated with neonatal SAM:SAH in models 3 and 4, and these variables explained 7.6% and 5.7% of the variance, respectively, in neonatal SAM:SAH (Table 3). There were weak but significant inverse correlations between SAM:SAH and serum folate ($r = -0.272$, $P = 0.005$) and tHcy ($r = -0.226$, $P = 0.017$) in the newborns. The higher value of the regression parameter estimate ($\beta = -0.853$) for serum folate in models 3 and 4 of SAM:SAH as dependent variable could be explained by values in a particular range of folate distribution.

Newborns with higher tHcy concentrations (>6.8 μmol/L) had a 3.2-fold greater risk of lower SAM:SAH than did newborns with lower tHcy concentrations, and the pregnant women with lower cobalamin concentrations had a 5.0-fold greater risk of delivery of a newborn with a lower SAM:SAH than did those pregnant women with higher cobalamin concentrations (Table 4).

Establishment of cobalamin deficiency cutoffs in pregnant women and newborn groups

We used ROC curves to establish a cobalamin deficiency cutoff in pregnant women and newborn groups (26). In pregnant women, the combination of tHcy concentrations and SAM:SAH was the best criteria (as shown by a combination of higher sensitivity and specificity) for detection of cobalamin deficiency (**Table 5**). When used as a single factor, tHcy had the best combination of sensitivity and specificity and showed the same cobalamin cutoff (<132 pmol/L) as was obtained in the combination of tHcy and SAM:SAH. According to this cutoff, 63 (52.9%) pregnant women in our study had cobalamin deficiency.

No significant differences were found among the values of area under ROC curves for all criteria used in the newborn group. The combination of tHcy and MMA concentrations showed the highest value of area under ROC curve (0.675; 95% CI: 0.567, 0.782) and a good sensitivity (69.0%) and specificity (62.7%). When used as single limits, tHcy and MMA also showed a good sensitivity and specificity. However, the cobalamin cutoff for MMA alone as the limit was lower than the cutoff values for tHcy alone and the combination of tHcy and MMA concentrations. By using the cobalamin cutoff (<194 pmol/L) for newborns, we found 54 (49.5%) newborns in our study who had cobalamin deficiency.

DISCUSSION

We studied the cobalamin and folate status of a group of socioeconomically disadvantaged Brazilian women in labor who likely were at risk of vitamin deficiency because of infrequent vitamin supplementation and the low animal protein content of their daily diet. We found that the mean maternal serum cobalamin concentration was much lower than values reported from the United States and Europe (6–8, 19, 27–30) and similar to a value from Ireland (31). Most of the serum cobalamin concentrations detected in our study were <180 pmol/L, the value cited by Bruinse and van den Berg (6) to indicate marginal or deficient vitamin stores. The lower cobalamin concentrations were found by using 2 separate methods of quantitation, which correlated

TABLE 5
Cobalamin cutoffs for pregnant women and newborns obtained by using the total homocysteine (tHcy), methylmalonic acid (MMA), and *S*-adenosylhomocysteine (SAH) concentrations and the ratio of *S*-adenosylmethionine (SAM) to SAH as indicators of cobalamin deficiency[1]

Criteria	Area under ROC curve	Sensitivity, specificity	Cobalamin cutoffs
Pregnant subjects		*%*	*pmol/L*
tHcy	0.598 (0.483, 0.714)[a,b2]	75.0, 54.4	132
MMA	0.497 (0.380, 0.614)[a]	48.3, 66.3	116
SAH	0.597 (0.472, 0.722)[a,b]	78.6, 42.5	141
SAM:SAH	0.724 (0.613, 0.836)[b]	78.6, 40.0	128
tHcy and MMA	0.544 (0.439, 0.650)[a,b]	64.6, 54.3	133
tHcy and SAM:SAH	0.682 (0.583, 0.782)[a,b]	75.0, 62.9	132
tHcy, MMA, and SAM:SAH	0.659 (0.559, 0.760)[a,b]	68.8, 64.8	133
tHcy, SAH, and SAM:SAH	0.661 (0.561, 0.761)[a,b]	72.4, 60.0	135
tHcy, MMA, SAH, and SAM:SAH	0.649 (0.545, 0.752)[a,b]	66.7, 65.2	134
Newborns			
tHcy	0.645 (0.522, 0.768)[a]	72.0, 59.5	191
MMA	0.649 (0.523, 0.774)[a]	61.5, 71.1	164
SAH	0.528 (0.397, 0.659)[a]	64.0, 53.6	195
SAM:SAH	0.496 (0.360, 0.632)[a]	48.0, 64.3	167
tHcy and MMA	0.675 (0.567, 0.782)[a]	69.0, 62.7	194
tHcy and SAM:SAH	0.580 (0.464, 0.696)[a]	62.5, 60.9	191
tHcy, MMA, and SAM:SAH	0.614 (0.506, 0.722)[a]	52.8, 75.0	167
tHcy, SAH, and SAM:SAH	0.600 (0.490, 0.710)[a]	67.4, 61.9	195
tHcy, MMA, SAH, and SAM:SAH	0.622 (0.516, 0.729)[a]	64.3, 64.2	195

[1] ROC, receiver operator characteristic. When ≥1 limit was used in the criteria, it was considered altered if ≥1 of the limits was altered. Values with different superscript letters are significantly different, $P < 0.05$ (ROC curves comparison test).
[2] $\bar{x}$; 95% CI in parentheses (all such values).

strongly. Thus, the lower values we found probably are not due to methodologic differences from previous studies. The mean maternal serum cobalamin and folate values were virtually the same as reported 30 y ago (32) and were 100 pmol/L and 14 nmol/L, respectively, less than the values in Brazilian mothers who received vitamin supplements (33). There are difficulties in interpreting these low cobalamin values because it is well known that serum cobalamin values decrease during pregnancy (5–8). In addition, the geometric mean MMA was 200 nmol/L, which was much higher than the value in healthy women blood donors, and in particular was higher than midtrimester values seen in a pregnant population in the United States (19). Interpreting values for the cobalamin-dependent metabolites, MMA and tHcy, is also problematic during pregnancy because their values decrease during gestation (7, 8, 19). We did not find that MMA was associated with lower cobalamin values, which is consistent with the findings of other studies (34–36). The interpretation of tHcy values is further complicated by the fact that tHcy is also a marker for inadequate folate status, and it appears that our subjects in the lowest quartile of cobalamin values also had significantly lower serum folate than did subjects in the other quartiles. Therefore, elevated tHcy in our population probably was related to both poor folate and poor cobalamin status.

The mean maternal tHcy concentration in the current study was similar to values obtained in previous French (29), Spanish (30), Canadian (37), and US (38) investigations. Studies from Ireland (31) and the Netherlands (39) found much higher mean tHcy and lower folate values than we found. Therefore, we conclude that the folate status of the Brazilian mothers in the current study was probably adequate, unlike their cobalamin status.

For the first time, we showed that maternal SAM is lower, SAH is higher, and SAM:SAH is lower in women in the lowest cobalamin quartile than in those in the other quartiles. Because these subjects also had lower serum folate, it is possible that a combined deficiency exacerbated the abnormalities, although the multiple linear regression model showed that only the maternal cobalamin was a predictor of the maternal SAM:SAH. Because we previously showed that SAH is increased and SAM:SAH is decreased in Brazilian patients with severe cobalamin-deficient megaloblastic anemia (4), we interpret these changes in the pregnant subjects as showing biochemically significant cobalamin deficiency. This maternal cobalamin deficiency apparently causes neonatal cobalamin deficiency, because the neonatal cobalamin values were strongly dependent on the maternal values. The tHcy, MMA, and SAM:SAH were also abnormal in the newborns of mothers in the lowest cobalamin quartile. The inverse relations between neonatal tHcy, MMA, cobalamin, and serum folate concentrations and SAM:SAH and the maternal cobalamin and serum folate concentrations confirm that there were metabolic consequences of deficiency for the newborns. Cobalamin was shown to be an important determinant of neonatal tHcy in other studies (28, 31).

It appears that the analysis of SAM, SAH, and SAM:SAH might be of diagnostic utility in assessing vitamin deficiency in pregnancy. We were very careful to prepare the samples in a uniform manner and without allowing room-temperature storage or incubation at any time from phlebotomy to analysis. This care is important because we showed that SAH increases and SAM decreases after relatively short intervals of room-temperature or 4 °C incubation of samples (24). Further investigations of SAM and SAH after stringent sample preparation and storage conditions should be pursued in other populations with poor intake of cobalamin and folate.

In this study, we drew blood from placental veins, which is a noninvasive method of obtaining blood from the neonatal circulation. The values obtained in this investigation were very similar to those previously obtained from studies of cord blood (40). The elevated serum SAH and low SAM:SAH found in the newborns and the mothers with the lowest cobalamin values could reflect an abnormal methylation status in the tissues (41). The mothers in the lowest quartile of cobalamin values were more severely anemic than were the rest of the mothers, but there was no apparent effect of vitamin status on the neonatal weight or other limits. Future studies should be directed toward following the development and cognitive abilities of these children. Children raised on macrobiotic diets, although admittedly much more severely cobalamin deficient than the subjects in this study, have been shown to have growth and cognitive impairment (42). DNA hypomethylation associated with elevated SAH was reported in vascular disease (43, 44). Such studies should be pursued in vitamin-deficient mother-newborn pairs.

The serum SAM:SAH is greatly dependent on the renal status of the person (24, 45). We previously showed that the urinary fractional excretion of SAM is close to 100% and SAH is ≈40% in healthy nonpregnant subjects (24). We excluded subjects with known renal disease from the current investigation. However, the serum creatinine was the only predictor of elevated maternal MMA. In addition, we did not find that the creatinine was an independent predictor of the maternal SAM:SAH, in contrast to the maternal cobalamin value. Thus, there was probably little confounding of the interpretation of low SAM:SAH by renal insufficiency in this cohort.

For reasons that have not yet been studied, newborns have much higher values for SAM and SAH than do their mothers. The tHcy is lower and cystathionine and methionine are higher in the newborns, which could imply differences in the balance of methylation and transsulfuration in newborns and adults.

Higher concentrations of tHcy, MMA, cystathionine, 2-methylcitric acid, and *N*,*N*-dimethylglycine were observed in pregnant Brazilian women than in pregnant US women, albeit the latter were studied earlier in the pregnancy (19). Relative deficiencies of either cobalamin or folate, or both, would explain these differences (2, 21–23).

We studied women attending public hospitals who had poor socioeconomic status and who likely consumed diets low in animal protein, which is the dietary source of cobalamin. Because this vitamin is not present in the plant protein sources (beans and grains) commonly eaten by this cohort, its members are likely cobalamin deficient on a dietary basis, although we did not access intake. Parasitic or bacterial infections could also play a role in cobalamin deficiency in this population. In addition, the pregnant women with low cobalamin concentrations had low serum folate concentrations, also probably because of low intake. However, lower folate concentrations could also result from the fact that cobalamin deficiency can interfere with folate metabolism. In the absence of cobalamin, methylfolate is trapped and cannot be recycled into the folate pool.

Cobalamin deficiency might prove to be an important public health problem in areas of the world where animal protein foods are not widely consumed for reasons of expense and scarcity, because lower serum cobalamin concentrations in pregnant women seem to have metabolic effects on their newborns at birth. Studies should be performed to show whether routine supplementation of vitamins that include both cobalamin and folic acid would be beneficial in such populations in improving maternal and child health.

We thank Helymar C Machado for statistical analysis.

EMG-S was the coordinator of this study in Brazil and was responsible for all of its phases (planning, collecting of the samples, interviews, analysis of the data, interpretation of the values, and preparation of the manuscript). OM, SP, and RAP conducted the collecting of blood samples (from pregnant women and from placenta veins), interviewed the pregnant women, and processed the blood samples right after blood collection. LFSN was the obstetrician who participated in planning and executing this study in the Hospital Regional and in Santa Lucinda Hospital. VD'A participated in the preparation of the manuscript. SPI supervised and coordinated sample analysis. SP and OEM measured the concentrations of cobalamin, folate, ferritin, and hemogram at the hematology laboratory of the Clinical and Toxicology Analyses Department of the University of São Paulo. The Brazilian authors have no conflicts of interest. SPS and RHA were responsible for the assays of the amino acids, SAM, and SAH in their laboratories and also provided interpretation of the values and participated in the preparation of the manuscript. The University of Colorado and SPS and RHA hold patents on various aspects of the use of homocysteine and methylmalonic acid in the diagnosis of cobalamin or folate deficiency. A company was formed at the University of Colorado to perform the assays.

REFERENCES

1. Shojania AM. Folic acid and vitamin B12 deficiency in pregnancy and in the neonatal period. Clin Perinatol 1984;11:433–59.
2. Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. Am J Med 1994;96:239–46.
3. Finkelstein JD, Kyle WE, Harris BJ. Methionine metabolism in mammals: regulatory effects of *S*-adenosylhomocysteine. Arch Biochem Biophys 1974;165:774–9.
4. Guerra-Shinohara EM, Morita OE, Pagliusi RA, Blaia-D'Avila VL, Allen RH, Stabler SP. Elevation of serum *S*-adenosyhomocysteine in severe cobalamin deficient megaloblastic anemia. Blood 2001;98:13a (abstr).
5. Green R, Colman N, Metz J. Comparison of results of microbiological and radioisotopic assays for serum vitamin B_{12} during pregnancy. Am J Obstet Gynecol 1975;122:21–4.
6. Bruinse HW, van den Berg H. Changes of some vitamin levels during and after normal pregnancy. Eur J Obstet Gynecol Reprod Biol 1995; 61:31–7.
7. Walker MC, Smith GN, Perkins SL, Keely EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. Am J Obstet Gynecol 1999;180:660–4.
8. Cikot RJ, Steegers-Theunissen RP, Thomas CM, de Boo TM, Merkus HM, Steegers EA. Longitudinal vitamin and homocysteine levels in normal pregnancy. Br J Nutr 2001;85:49–58.
9. Alperin JB, Haggard ME, McGanity WJ. Folic acid, pregnancy, and abruptio placentae. Am J Clin Nutr 1969;22:1354–61.
10. Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. Placenta 1999;20:519–29.
11. Nelen WL, Blom HJ, Steegers EA, den Heijer M, Thomas CM, Eskes TK. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol 2000;95:519–24.
12. Refsum H. Folate, vitamin B12 and homocysteine in relation to birth defects and pregnancy outcome. Br J Nutr 2001;85(suppl 2):S109–13.
13. Bower C, Stanley FJ. Dietary folate as a risk for neural tube defects: evidence from a case–control study in Western Australia. Med J Aust 1989;150:613–9.
14. Chanarin I. Megaloblastic anemia, cobalamin and folate. J Clin Pathol 1987;40:978–84.
15. Kirke P, Weir DG, Scott JM. Preconception nutrition and prevention of neural tube defects. In: Sadler MJ, Strain JJ, Caballero B. Encyclopedia of human nutrition. Vol 3. New York: Academic Press, 1998:1609–19.
16. Vollset SE, Refsum H, Irgens LM, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocyteine Study. Am J Clin Nutr 2000;71:962–8.
17. Kumar KS, Govindaiah V, Naushad SE, Devi RR, Jyothy A. Plasma

homocysteine levels correlated to interactions between folate status and methylene tetrahydrofolate reductase gene mutation in women with unexplained recurrent pregnancy loss. J Obstet Gynaecol 2003;23:55–8.
18. Thame G, Guerra-Shinohara EM, Santos JG, Moron AF. Folato, vitamina B12, ferritina sérica e os Defeitos do Tubo Neural (DTN). [Folate, vitamin B12, serum ferritin and the neural tube defects (NTD).] Rev Bras Ginecol Obstet 1998;20:449–53 (in Portuguese).
19. Adams MJ Jr, Khoury MJ, Scanlon KS, et al. Elevated midtrimester serum methylmalonic acid levels as a risk factor for neural tube defects. Teratology 1995;51:311–7.
20. Ronnenberg AG, Goldman MB, Chen D, et al. Preconception homocysteine and B vitamin status and birth outcomes in Chinese women. Am J Clin Nutr 2002;76:1385–91.
21. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Elevation of 2-methylcitric acid I and II levels in serum, urine and cerebrospinal fluid of patients with cobalamin deficiency. Metabolism 1993;42:978–88.
22. Allen RH, Stabler SP, Lindenbaum J. Serum betaine, N-N-dimethylglycine and N-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. Metabolism 1993;42:1448–60.
23. Stabler SP, Lindenbaum J, Savage DG, Allen RH. Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. Blood 1993;81:3104–13.
24. Stabler SP, Allen RH. Quantification of serum and urinary S-adenosylmethionine and S-adenosylhomocysteine by stable isotope dilution liquid chromatography–mass spectrometry. Clin Chem 2004;50:365–72.
25. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;307–11.
26. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–77.
27. Malinow MR, Rajkovic A, Duell PB, Hess DL, Upson BM. The relationship between maternal and neonatal umbilical cord plasma homocyst(e)ine suggests a potential role for maternal homocyst(e)ine in fetal metabolism. Am J Obstet Gynecol 1998;178:228–33.
28. Bjorke Monsen AL, Ueland PM, Vollset SE, et al. Determinants of cobalamin status in newborns. Pediatrics 2001;108:624–30.
29. Chery C, Barbe F, Lequere C, et al. Hyperhomocysteinemia is related to a decreased blood level of vitamin B12 in the second and third trimester of normal pregnancy. Clin Chem Lab Med 2002;40:1105–8.
30. Lopez-Quesada E, Vilaseca MA, Lailla JM. Plasma total homocysteine in uncomplicated pregnancy and in preeclampsia. Eur J Obstet Gynecol Reprod Biol 2003;108:45–9.
31. Molloy AM, Mills JL, McPartlin J, Kirke PN, Scott JM, Daly S. Maternal and fetal plasma homocysteine concentrations at birth: the influence of folate, vitamin B12, and the 5, 10-methylenetetrahydrofolate reductase 677C→T variant. Am J Obstet Gynecol 2002;186:499–503.
32. Cook JD, Alvarado J, Gutnisky A, et al. Nutritional deficiency and anemia in Latin America: a collaborative study. Blood 1971;38:591–603.
33. Donangelo CM, Trugo NMF, Koury JC, et al. Iron, zinc, folate and vitamin B12 nutritional status and milk composition of low-income Brazilian mothers. Eur J Clin Nutr 1989;43:253–66.
34. Metz J, Mcgrath K, Bennett M, Hyland K, Bottiglieri T. Biochemical indices of vitamin B_{12} nutrition in pregnant patients with subnormal serum vitamin B_{12} levels. Am J Hematol 1995;48:251–5.
35. Pardo J, Peled Y, Bar J, et al. Evaluation of low serum vitamin B12 in the non-anemic pregnant women. Hum Reprod 2000;15:224–6.
36. McMullin MF, Young PB, Bailie KE, Savage GA, Lappin TR, White R. Homocysteine and methylmalonic acid as indicators of folate and vitamin B_{12} deficiency in pregnancy. Clin Lab Haematol 2001;23:161–5.
37. Infante-Rivard C, Etienne Rivard G, Yotov WV, Theoret Y. Perinatal reference intervals for plasma homocysteine and factors influencing its concentration. Clin Chem 2002;48:1100–2.
38. Powers RW, Dunbar MS, Gallaher MJ, Roberts JM. The 677 C-T methylenetetrahydrofolate reductase mutation does not predict increased maternal homocysteine during pregnancy. Obstet Gynecol 2003;101:762–6.
39. Raijmakers MTM, Roes EM, Steegers EAP, et al. Umbilical cord and maternal plasma thiol concentrations in normal pregnancy. Clin Chem 2001;47:749–51.
40. Guerra-Shinohara EM, Paiva AA, Rondo PH, Yamasaki K, Terzi CA, D'Almeida V. Relationship between total homocysteine and folate levels in pregnant women and their newborn babies according to maternal serum levels of vitamin B12. Br J Obstet Gynaecol 2002;109:784–91.
41. Clarke S, Banfield K. S-adenosylmethionine dependent methyltransferases. In: Carmel R, Jacobsen DW, eds. Homocysteine in health and disease. Cambridge, United Kingdom: Cambridge University Press, 2001:63–78.
42. Dagnelie PC, van Staveren WA, Vergote FJ, et al. Nutritional status of infants aged 4 to 18 months on macrobiotic diets and matched omnivorous control infants: a population-based mixed-longitudinal study. II. Growth and psychomotor development. Eur J Clin Nutr 1989;43:325–38.
43. Yi P, Melnyk S, Pogribna M, et al. Increase in plasma homocysteine associated with parallel increases in plasma *S*-adenosylhomocysteine and lymphocyte DNA hypomethylation. J Biol Chem 2000;275:29318–23.
44. Castro R, Rivera I, Struys EA, et al. Increased homocysteine and *S*-adenosylhomocysteine concentrations and DNA hypomethylation in vascular disease. Clin Chem 2003;49:1292–6.
45. Loehrer FMT, Angst CP, Brunner FP, et al. Evidence for disturbed *S*-adenosylmethionine: *S*-adenosylhomocysteine ratio in patients with end stage renal failure: a cause for disturbed methylation reactions? Nephrol Dial Transplant 1998;13:656–61.