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Mid-trimester amniotic fluid methionine concentrations: a predictor of birth weight and length

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Abstract

The association between body size at birth and risk of later cardiovascular disease is thought to be a consequence of metabolic changes that accompany slow growth in utero. The metabolism of methionine and homocysteine has been investigated in relation to cardiovascular risk and has also been assigned an important role in organogenesis and normal fetal growth. We determined concentrations of cobalamin, folate, methionine, cysteine, cystathionine, and the marker of B-vitamin function, homocysteine, in 625 samples of amniotic fluid obtained in the second trimester from normal pregnancies. Both vitamins and metabolites varied according to gestational age. The most noticeable observation was that methionine in amniotic fluid during gestational weeks 13 to 17 strongly predicted final birth weight and length. Metabolism of methionine may be a critical factor affecting fetal growth.

1. Introduction

Recent studies have associated low birth weight with chronic disease in later life, such as hypertension, coronary artery disease, insulin resistance, and non–insulin-dependent diabetes [1-3]. The association between body size at birth and risk of later disease is believed to be a consequence of metabolic changes/features that accompany slow growth in utero [1]. Both maternal nutrition and the amino acid metabolism in the feto-maternal unit are known to affect fetal organogenesis [4].

Recent animal data suggest that methylation reactions and the micronutrients involved may be important in regulation of the long-term programming of gene expression [5-7]. Methionine metabolism and related B vitamins have been assigned an important role in fetal nutrition, growth, and development [5,8-11]. Abnormalities in methionine, homocysteine, and cysteine metabolism have been associated with adverse pregnancy outcomes, such as placental dysfunction and preeclampsia [12-14].

Remethylation of homocysteine to methionine is in most tissues catalyzed by the enzyme, methionine synthase, which requires cobalamin (vitamin B_{12}) as cofactor and folate as substrate. This explains why plasma total homocysteine (tHcy) is elevated in both cobalamin and folate deficiencies, and is a sensitive indicator of these deficiency states [15]. In addition, homocysteine can be irreversibly converted to cystathionine and further to cysteine by 2 pyridoxal phosphate (B_6)–dependent enzymes, cystathionine β-synthase and cystathionine lyase [16].

The composition of amniotic fluid (AF) is influenced by both maternal and fetal metabolism and provides a rational compartment for studies of fetal nutrition and metabolism [17,18].

We examined methionine, homocysteine, the relevant B vitamins, and the transsulfuration metabolites, cystathionine and cysteine, in 625 samples of AF obtained in the second trimester from pregnancies with a documented normal outcome. Vitamins and metabolites were related to pregnancy outcome and birth size.

2. Materials and methods

2.1. Materials

The AF specimens were taken by trans-abdominal amniocentesis for genetic prenatal diagnoses. The total

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Table 1
Characteristics of the population (N = 625)

Indications for amniocentesis	
Maternal age, n (%)	549 (88)
Previous child with chromosomal aberration, n (%)	47 (8)
Maternal epilepsy, n (%)	13 (2)
Other, n (%)	16 (3)
Maternal age, y, median (total range)	39.0 (24-46)
Gestational age, wk, median (total range)	14.4 (13-17)
Male sex, n (%)	315 (51)
Birth weight, g, mean (SD)	3575 (604)
Birth length, cm, mean (SD)	50 (3)

number of AF samples was 625, and about 120 samples were collected each year from 1993 to early 1998. Because of limited volumes available, all analytes were not measured in all samples.

The gestational age was determined by menstrual dates and checked by ultrasound examination. Information on pregnancy outcome was retrieved from questionnaires that prenatal diagnosis patients routinely return after delivery and linked to the corresponding sample. Samples collected in gestational weeks 13 to 17 from singleton pregnancies with a normal outcome were stripped for identifiers and made available for this study. The procedures for information collection were in accordance with the Revised Helsinki Declaration of 1983, and ethical approval of the protocol was granted by the local committee on medical research ethics.

2.2. Sampling and storage

After amniocentesis, the samples were centrifuged. The supernatants were used for α-fetoprotein determination, and the remaining fluid was stored at −20 °C. All samples were without visible signs of blood contamination. The storage period before analysis of vitamins and metabolites ranged from 1 to 7 years.

2.3. Biochemical analyses

Cobalamin was determined by a *Lactobacillus leichmannii* microbiologic assay [19] and folate by a *Lactobacillus casei* microbiologic assay [20]. Both cobalamin and folate assays were adapted to a microtiter plate format [21] and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz AG, Bonaduz, Switzerland). Concentrations of tHcy, cystathionine, and total cysteine (tCys) in AF were assayed using a gas chromatography–mass spectrometry method based on ethyl chloroformate derivatization [22]. Methionine was determined with a slight modification of a published method [23] based on liquid chromatography/mass spectrometry/mass spectrometry.

2.4. Statistical analysis

Data are presented as mean and SD or 95% confidence interval, and median and total or interquartile range. The concentrations of some vitamins/metabolites (in particular, folate) changed significantly according to storage period, which varied between 1 and 7 years. The concentrations of cobalamin and the metabolites (tHcy and methionine) were essentially stable. The vitamin and metabolite values were therefore adjusted for sample storage time by general linear univariate analysis of variance. Means were compared by Student *t* tests and general linear univariate analysis of variance. Correlation was assessed by Spearman correlation coefficients.

Multiple linear regression models were used to assess the relation between birth weight and the concentration of vitamins and metabolites in AF. Two-sided P values $< .05$ were considered statistically significant. The SPSS statistical package (version 11, SPSS, Chicago, IL) was used for all statistical analyses.

3. Results

3.1. Characteristics of study population

The characteristics of the study population are presented in Table 1. The main indication for amniocentesis was advanced maternal age (≥38 years old at delivery), and only 16% (n = 99) of the pregnant women were 37 years or younger. Twenty-two infants (4%) had a birth weight of less than 2500 g. Fifteen of these were born at term and defined as intrauterine growth retarded. Eleven infants were born prematurely (gestational week <37). There were significant differences in birth weight (3642 vs 3511 g, $P = .001$), but not in birth length (50.5 vs 49.6 cm, $P = .09$) between boys and girls. Apgar data were available for 386 infants (62%).

Table 2
Vitamins and metabolites in AF from normal pregnancies in the second trimester

Parameters	Gestational week					P[a]
	13	14	15	16	17	
n	121	233	78	86	18	
Cobalamin (pmol/L)	675 (588-761)	806 (742-871)	650 (554-746)	722 (617-826)	405 (206-604)	.001
Folate (nmol/L)	4.6 (4.2-5.0)	4.6 (4.3-4.9)	4.3 (3.8-4.7)	3.8 (3.3-4.3)	3.0 (2.0-3.9)	.005
Homocysteine (μmol/L)	1.2 (1.2-1.3)	1.2 (1.1-1.3)	1.4 (1.2-1.5)	1.4 (1.3-1.5)	1.4 (1.2-1.7)	.05
Methionine (μmol/L)	32.2 (31.0-33.4)	30.4 (29.5-31.3)	27.2 (25.9-28.5)	26.0 (24.5-27.5)	23.6 (20.8-26.3)	<.001
Cystathionine (μmol/L)	1.9 (1.8-2.1)	2.0 (1.9-2.1)	1.4 (1.2-1.7)	1.6 (1.3-1.8)	1.5 (1.0-1.9)	.004
Cysteine (μmol/L)	113 (109-116)	110 (108-113)	99 (94-103)	97 (93-101)	97 (89-106)	<.001

The values are given as mean and 95% confidence interval, and have been adjusted for storage period.
[a] Difference according to gestational age, adjusted for storage period, by general linear univariate analysis of variance.

The mean value for Apgar 1 was 8.8 (range, 0-10) and for Apgar 2 was 9.2 (range, 7-10).

3.2. Vitamins and metabolites according to gestational age

Table 2 shows the mean and 95% confidence interval for vitamins and metabolites in AF according to gestational weeks 13 to 17. The values were adjusted for storage time. All the analytes varied in relation to gestational week. Methionine, tCys, cystathionine, folate, and cobalamin concentrations decreased during the 5 weeks period, whereas the concentration of tHcy increased.

3.3. Simple correlations

Spearman correlations for vitamins and metabolites in AF are presented for the total group, as we did not find any differences in correlations according to gestational age (data not shown).

Total homocysteine was inversely, weakly, but significantly correlated with cobalamin ($r = -0.20$, $P < .001$) and folate ($r = -0.25$, $P < .001$), and both vitamins were positively related to methionine ($r = 0.20$, $P < .001$, and $r = 0.24$, $P < .001$, respectively) and to tCys ($r = 0.16$, $P < .01$, and $r = 0.30$, $P < .001$, respectively). The strongest relation was seen between methionine and tCys ($r = 0.52$, $P < .001$), but significant relations were also found between methionine and tHcy ($r = 0.23$, $P < .001$), methionine and cystathionine ($r = 0.35$, $P < .001$), and between tHcy and tCys ($r = 0.43$, $P < .001$).

3.4. Variations in analytes according to final birth weight and length

Vitamins and metabolites in AF obtained in the second trimester were related to measures of final fetal growth, birth weight, and length, by unadjusted correlation (Fig. 1) and multiple linear regression (Table 3). There was a significant increase in birth weight with increasing methionine concentration (Spearman $r = 0.13$, $P = .001$; Fig. 1). Other metabolites or vitamins did not vary significantly according to birth weight by unadjusted correlation.

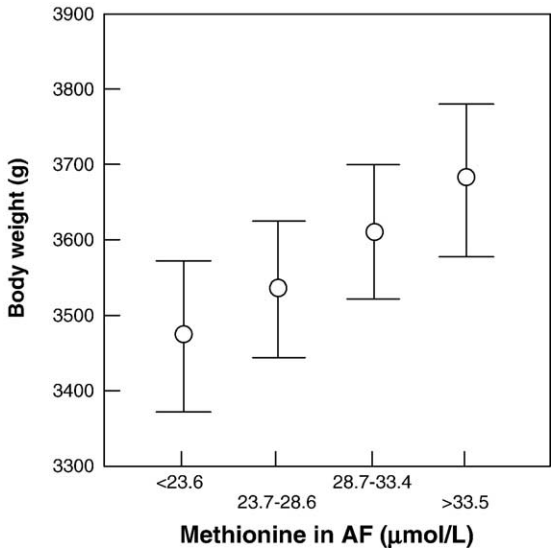

Fig. 1. Relation between methionine concentrations in AF samples and birth weight. There was a significant increase in methionine quartiles with increasing birth weight (Spearman $r = 0.13$, $P = .001$, n = 621). Bars represent 95% confidence interval of the mean.

Table 3
Metabolites in AF as determinants of birth weight and length

Independent variables	Dependent variables			
	Birth weight (g) (n = 533)		Birth length (cm) (n = 474)	
	B^a	P	B^a	P
Methionine	87	.001	0.34	.02
Cysteine	−52	.04	−0.15	.26

Multiple linear regression; the model contains sex, gestational age, and storage period in addition to the parameters listed in the table. The variables are presented in the model as quartiles.

[a] Regression coefficient.

The initial multiple linear regression model included all variables (cobalamin, folate, tHcy, methionine, cystathionine, and tCys) in addition to sex, gestational week, and storage period of the samples. As only methionine and tCys were significant at a level of $P < .1$, the other analytes were excluded from the final model, and essentially the same results were obtained. Methionine was the strongest predictor of both birth weight ($P < .001$) and birth length ($P < .02$), whereas tCys was a significant negative predictor for birth weight ($P < .04$), but with no effect on birth length ($P < .26$) (Table 3).

4. Discussion

We have determined concentrations of folate, cobalamin, and related metabolites involved in methionine metabolism in 625 samples of AF obtained in the second trimester from pregnancies with a normal outcome. Both vitamins and metabolites varied according to gestational age, and a positive correlation between various analyte concentrations was observed. The most noticeable observation was that methionine in AF during gestational weeks 13 to 17 strongly predicted birth weight and length, whereas a weaker, negative association was seen for tCys.

4.1. Study design and limitations

The AF samples were collected and stored on a routine basis through a median period of 4 years (range, 1-7 years). The reduction in concentration as a function of storage time was moderate for some analytes, but marked for folate, which is essentially in accordance with published data on the stability of B vitamins in samples frozen at −20 °C [24]. The vitamin and metabolite concentrations (Table 2) were therefore adjusted for storage time, which was also included in the multiple regression model (Table 3).

Birth weight is known to be influenced by various maternal factors, such as pre-pregnancy weight, height, and

smoking habits [25]. Information of these parameters was not available, which is a limitation of our study.

4.2. Concentrations of B vitamins and metabolites

The concentrations of folate and cobalamin in AF result from placental transfer, fetal renal excretion and diffusion through the nonkeratinized fetal skin [26], and interaction with folate [27] and cobalamin [28] binders, which has been detected in AF. The concentrations of metabolites and amino acids in AF are also affected by placental and fetal metabolism and transportation [29-32]. Protein binding may however be less in AF than in plasma, as the concentration of albumin, the main carrier of homocysteine and cysteine in plasma [33], is low in AF [34].

We detected cobalamin and folate concentrations in AF that are similar to concentrations previously reported by others [11,35-38]. The cobalamin content was substantially higher than in serum from term newborns and mothers [39]. We observed a reduction in both B vitamins according to gestational age, as has previously been reported [38,40].

We measured tCys and methionine in AF at concentrations similar to those published by others [8,41]. Methionine, tCys, and cystathionine decreased as a function of gestational age, as has been consistently reported for several amino acids [41,42]. Notably, tHcy actually increased according to gestational age. Such increase in tHcy concentration in AF has been reported previously [36].

We detected a positive correlation between several metabolites in AF. A strong correlation between neutral amino acids in AF has been described before and interpreted in terms of common transportation systems and metabolic pathways [41]. Total homocysteine was inversely related to both folate and cobalamin, as previously reported by Steegers-Theunissen et al [36]. This suggests that tHcy in AF is influenced by the activity of the folate- and cobalamin-dependent homocysteine remethylation, as has been established for tHcy in serum/plasma [15].

4.3. Birth weight and length

A novel and the most notable finding in this study was the strong relation between AF methionine concentration and birth weight. The relation was observed across the whole range of methionine concentrations and within the range of normal birth weight (Fig. 1, Table 3). The same pattern was seen for birth length, but the association was weaker. In adults, a significant portion of the ingested methionine is converted to cysteine through the transsulfuration pathway catalyzed by the enzyme cystathionine lyase, but this enzyme activity is low or absent in fetal tissue [43-45]. Inefficient transsulfuration in the fetus may conserve methionine for important cellular functions, including DNA methylation, polyamine synthesis, protein synthesis, and cell growth [46].

Both animal and human studies suggest fetal methionine conservation to support cell growth. Studies in rats have demonstrated a positive relation between dietary methionine and body weight [47]. Malinow et al [9] observed a negative relation between birth weight and maternal homocysteine at birth, and an umbilical venous-arterial difference for plasma tHcy, which indicates fetal uptake of homocysteine. Steegers-Theunissen et al [11] reported a high concentration of methionine in coelomic fluid at 8 to 12 weeks of gestation, which exceeds, but is strongly correlated to, the concentration in AF. Thus, the high concentrations of methionine and cobalamin combined with low tHcy in extraembryonic fluids [48] suggest that homocysteine remethylation is an important source of methionine in the fetus.

Smoking is known to be one of the most significant factors adversely affecting fetal growth [49]. In adults, smoking is associated with decreased serum concentrations of cobalamin and folate [50], and elevated plasma tHcy [51]. Both reduced serum cobalamin [52] and increased plasma tHcy concentrations [53] have been reported in infants born to smoking mothers. Consequently, smoking should be considered as a factor responsible for both impaired fetal growth and reduced homocysteine remethylation and thereby low methionine concentration. However, this explanation is not supported by the observations of a positive correlation between methionine and tHcy ($r = 0.23$, $P = .001$), and no association of birth weight with tHcy, folate, or cobalamin.

The lack of cystathionine lyase activity in fetal tissue [43-45] may render cysteine an essential amino acid for the fetus, and cysteine in AF may mainly reflect maternal sources. We observed a weak, negative association between tCys levels in AF and birth weight. This may be a chance finding. However, high maternal tCys levels have also been associated with pregnancy complications and adverse outcomes, including preeclampsia, premature delivery, low birth weight, and stillbirth [13,54].

In conclusion, this study demonstrates a strong association of methionine in AF with birth weight and length. This finding highlights the importance of methionine, but also related metabolites and B vitamins for fetal growth and development.

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