vulva was excised. A radical vulvectomy was performed through three incisions or by en bloc technique, starting laterally at the labial-crural folds and removing the entire vulva.

Did we check the margins at the time of surgery? No, we did not. We attempted to get 2 to 3 cm of normal tissue at the margin of the specimen. I am not aware of any samples in the series that had positive margins at the initial operation.

The depth of invasion was determined on the preoperative biopsy specimen. We decided whether the patient could undergo a modified vulvectomy on the basis of this information. The final depth of invasion was determined on the pathologic specimen. We didn't experience skin bridge recurrences. Generally, if there are palpable or suspicious lymph nodes in the groin, we don't leave a skin bridge; if the lymph nodes are clinically negative, a skin bridge might be left.

I believe the recurrences that have been reported have been in patients who had palpable nodes in the groin.

Did radiation therapy help? We don't know. We didn't separate out those patients specifically, and that is obviously something we need to do.

The fetal concentrating index as a gestational age–independent measure of placental dysfunction in intrauterine growth retardation

Isa Bernardini, MS,[a] Mark I. Evans, MD,[b] Kypros H. Nicolaides, MD,[c]
Demetrious L. Economides, MD,[c] and William A. Gahl, MD, PhD[a]

Bethesda, Maryland, Detroit, Michigan, and London, England

In previous work we have shown, using cordocentesis to obtain fetal blood, that fetuses with intrauterine growth retardation are hypoxic and suffer from in utero starvation of nutrients. In this study we have developed gestational age curves for fetal blood amino acids and carnitine that now allow the development of a new parameter, the fetal concentrating index, which is the numeric mean of the fetal/maternal ratio of six essential and nonessential concentrated amino acids. Our data have shown that this index does not vary with gestation in either normal pregnancies (1.83 ± 0.42, mean ± SD) or pregnancies with intrauterine growth retardation (1.46 ± 0.38), but the index is markedly reduced in intrauterine growth retardation ($p < 0.001$). These results suggest that, because cordocentesis has become very safe in experienced hands, cordocentesis to obtain the fetal concentrating index might ethically be obtained in cases of fetuses at risk for intrauterine growth retardation, to devise strategies for intervention before the onset of severe hypoxia and morphometric changes. (AM J OBSTET GYNECOL 1991;164:1481-90.)

Key words: Fetal concentrating index, intrauterine growth retardation, amino acids, carnitine

In previous work we and others have measured maternal and fetal plasma amino acid concentrations.[1-3] These results have indicated that some amino acids, especially the essential ones, are concentrated in the human fetal circulation, whereas others are not.[3] The concentrating process requires energy, and causes of impaired energy production, e.g., starvation and placental insufficiency, reduce the capacity of the fetus to concentrate essential amino acids, such as valine.[1] [5] We specifically investigated the relationship of maternal and fetal amino acids in appropriate-for-gestational age

Table I. Plasma amino acid and carnitine concentrations in normal control fetuses in different gestational age groups

	Gestational age		
	16-24 wk (n = 38) (μmol/L)	25-32 wk (n = 25) (μmol/L)	33-36 wk (n = 13) (μmol/L)
Taurine	119 ± 36 (44-230)	113 ± 42 (75-256)	117 ± 49 (66-221)
Urea	2530 ± 732 (1050-3870)	2457 ± 622 (1394-4112)	2467 ± 705 (1034-3431)
Aspartic acid	10 ± 5 (trace-25)	16 ± 20 (4-82)	11 ± 7 (3-24)
Threonine	230 ± 45 (149-338)	271 ± 61 (161-418)	260 ± 40 (176-302)
Serine	126 ± 25 (84-176)	138 ± 38 (63-217)	130 ± 16 (93-148)
Asparagine	61 ± 24 (7-160)	108 ± 50 (39-203)	111 ± 41 (41-173)
Glutamine	750 ± 287 (126-1550)	621 ± 227 (206-1143)	474 ± 230 (133-1038)
Proline	196 ± 56 (90-396)	203 ± 58 (89-363)	182 ± 32 (136-260)
Glycine	163 ± 49 (88-376)	169 ± 46 (86-353)	177 ± 26 (125-212)
Alanine	293 ± 112 (142-726)	358 ± 154 (146-827)	284 ± 90 (135-498)
Citrulline	16 ± 6 (5-40)	13 ± 5 (6-22)	13 ± 5 (8-27)
Valine	226 ± 48 (138-355)	208 ± 45 (127-286)	200 ± 35 (136-280)
Methionine	32 ± 10 (15-61)	33 ± 10 (19-62)	30 ± 5 (22-39)
Isoleucine	65 ± 17 (32-107)	58 ± 18 (18-78)	59 ± 9 (41-72)
Leucine	110 ± 25 (42-166)	104 ± 26 (62-151)	101 ± 15 (73-118)
Tyrosine	63 ± 17 (35-107)	70 ± 17 (42-103)	72 ± 16 (49-100)
Phenylalanine	64 ± 16 (25-104)	71 ± 14 (55-121)	72 ± 11 (58-93)
Ammonia	98 ± 38 (29-183)	97 ± 58 (30-286)	96 ± 53 (30-185)
Ornithine	122 ± 41 (54-244)	109 ± 36 (51-197)	100 ± 28 (64-156)
Lysine	351 ± 66 (248-485)	347 ± 65 (251-468)	312 ± 61 (163-397)
Histidine	111 ± 27 (59-198)	109 ± 17 (79-137)	112 ± 14 (94-142)
Arginine	80 ± 27 (23-122)	88 ± 31 (31-145)	94 ± 28 (38-136)
Carnitine			
Free	20 ± 7 (7-35) (n = 19)	18 ± 9 (5-36) (n = 17)	19 ± 8 (10-33) (n = 12)
Total	37 ± 15 (18-68) (n = 9)	36 ± 12 (21-57) (n = 7)	31 ± 9 (20-42) (n = 6)

Values are mean ± SD with range shown in parentheses.

(AGA) and small-for-gestational-age (SGA) fetuses and found differences in the amino acid profiles between these groups.[3] The plasma fetal/maternal ratio of a compound indicates whether it is concentrated in the fetal circulation. A substance that is not concentrated has a ratio of approximately 1.0; a highly concentrated substance exhibits a ratio of 2.0 to 3.0. In SGA fetuses the ratio of essential, but not nonessential, amino acids correlated inversely with the changes in umbilical venous Po_2 of fetuses.[1]

Unfortunately, the gestational age variation in the fetal/maternal ratio differs markedly for every amino acid. Moreover, individual amino acids, or separating the essential ones from the nonessential ones, did not by themselves provide a means to prospectively assess impending growth retardation. Therefore we have extended our original observations to include carnitine, which is integral to mitochondrial fatty acid oxygenation and have developed a coordinated parameter, i.e., the fetal concentrating index, to describe quantitatively the ability of the placenta to extract circulating maternal amino acids. This index provides a more powerful measure of fetal concentrating ability than that offered by any single amino acid's fetal/maternal ratio.

Methods

Umbilical venous blood was obtained by cordocentesis at 16 to 36 weeks' gestation from 62 AGA fetuses and 31 SGA fetuses. In all cases gestational age was determined by Nägele's rule and confirmed by an ultrasonographic scan in early pregnancy. In both groups maternal blood was sampled from an antecubital vein immediately before cordocentesis. All the mothers were healthy, had negative screening results for antinuclear factor, and did not show serologic evidence of recent toxoplasmosis, rubella, cytomegalovirus, or syphilis infection. For 75 control pregnancies the indications were: prenatal diagnosis of blood disorders (n = 6), fetal infection (n = 3), α-antitrypsin disease (n = 1), elective termination (n = 4), karyotyping for amniotic fluid culture failure (n = 4), amniotic fluid mosaicism (n = 4), late booking (n = 4), and anatomic defects (n = 40). Patients with encephaloceles, hydrops, or chromosomal defects that could potentially alter amino acids were excluded from the control group.

Of the control pregnancies studied, 16 were terminated, and four were lost to follow-up. The remaining 55 were delivered at 34 to 40 weeks' gestation. Birth weights, available for 52 of these, were in every

Table II. Fetal/maternal ratios of plasma amino acid and carnitine concentrations in normal control pregnancies of different gestational age groups

	Gestational age		
	16-24 wk (n = 37) (μmol/L)	25-32 wk (n = 25) (μmol/L)	33-36 wk (n = 13) (μmol/L)
Taurine	1.86 ± 0.82 (0.37-3.74)	2.09 ± 0.81 (0.50-3.58)	1.54 ± 0.59 (0.69-2.75)
Urea	1.18 ± 0.26 (0.68-2.02)	1.02 ± 0.15 (0.68-1.36)	0.97 ± 0.13 (0.69-1.14)
Aspartic acid	0.98 ± 0.48 (0.40-2.50)	1.06 ± 0.84 (0.11-4.55)	0.68 ± 0.40 (0.25-1.75)
Threonine	1.71 ± 0.27 (1.09-2.45)	1.67 ± 0.27 (1.17-2.15)	1.50 ± 0.25 (0.98-1.88)
Serine	1.41 ± 0.35 (0.78-2.27)	1.41 ± 0.35 (0.63-1.89)	1.33 ± 0.35 (0.82-2.13)
Asparagine	1.08 ± 0.33 (0.22-2.11)	1.05 ± 0.26 (0.28-1.47)	1.01 ± 0.21 (0.66-1.52)
Glutamine	1.14 ± 0.39 (0.26-2.08)	1.09 ± 0.20 (0.78-1.56)	0.90 ± 0.33 (0.22-1.32)
Proline	1.57 ± 0.48 (0.61-2.68)	1.35 ± 0.27 (0.79-2.12)	1.14 ± 0.21 (0.92-1.58)
Glycine	1.28 ± 0.36 (0.82-2.67)	1.22 ± 0.27 (0.61-1.70)	1.43 ± 0.37 (0.90-2.23)
Alanine	1.37 ± 0.29 (0.83-2.32)	1.38 ± 0.31 (0.78-2.17)	1.04 ± 0.32 (0.59-1.71)
Valine	1.64 ± 0.31 (0.77-2.22)	1.60 ± 0.31 (1.13-2.07)	1.48 ± 0.30 (1.02-1.98)
Methionine	1.84 ± 0.70 (0.75-3.78)	1.72 ± 0.52 (1.04-2.95)	1.46 ± 0.47 (1.00-2.78)
Isoleucine	1.46 ± 0.36 (0.85-2.17)	1.32 ± 0.39 (0.49-1.97)	1.44 ± 0.33 (0.89-1.97)
Leucine	1.32 ± 0.41 (0.56-2.22)	1.30 ± 0.41 (0.64-2.02)	1.34 ± 0.38 (0.78-1.85)
Tyrosine	2.08 ± 0.42 (1.21-2.83)	1.84 ± 0.33 (1.10-2.47)	1.76 ± 0.30 (1.19-2.49)
Phenylalanine	1.59 ± 0.45 (0.69-2.85)	1.66 ± 0.34 (1.10-2.44)	1.55 ± 0.38 (0.93-2.38)
Ammonia	1.53 ± 0.48 (0.80-3.03)	1.54 ± 0.65 (0.45-3.13)	1.65 ± 0.64 (0.82-2.83)
Ornithine	2.46 ± 0.66 (1.26-3.76)	2.39 ± 0.57 (1.10-3.67)	2.49 ± 0.88 (0.97-4.14)
Lysine	2.41 ± 0.41 (1.41-3.15)	2.48 ± 0.63 (0.94-3.56)	2.45 ± 0.83 (1.08-4.33)
Histidine	1.12 ± 0.36 (0.37-2.05)	1.20 ± 0.34 (0.40-1.80)	1.17 ± 0.28 (0.69-1.64)
Arginine	1.64 ± 0.89 (0.49-5.50)	2.12 ± 1.35 (0.53-7.11)	2.30 ± 1.17 (0.64-3.88)
Carnitine			
Free	0.82 ± 0.39 (0.37-1.59) (n = 17)	0.64 ± 0.25 (0.28-1.12) (n = 16)	0.91 ± 0.51 (0.43-2.27) (n = 12)
Total	1.04 ± 0.34 (0.56-1.39) (n = 7)	0.85 ± 0.17 (0.65-1.09) (n = 6)	0.92 ± 0.25 (0.50-1.24) (n = 6)

Values are mean ± SD with range shown in parentheses.

case greater than the 5th percentile for gestational age.

The 38 patients in the SGA group were referred for fetal karyotyping and blood gas analysis because of ultrasonographic evidence of severe intrauterine growth retardation (IUGR). The fetal abdominal circumference was <25th percentile of our reference range, which was constructed from the study of 1610 normal pregnancies.[6,7] Three fetuses showed chromosomal abnormalities (one 47,XY +18 and two 69,XXX) and were excluded from this study. In 29 cases a subsequent birth weight was available, which helped confirm the diagnosis (birth weight ≤10th percentile). The gestational age at birth ranged from 22 to 39 weeks. The numbers of cases for differing measures vary somewhat because of the small volume of material available, sometimes requiring that some parameters not be analyzed on a given specimen.

Cordocentesis was performed as an outpatient procedure without maternal fasting or sedation or fetal paralysis and was achieved in every case. Fetal and maternal blood samples (300 μl) were collected in heparized syringes, placed into lithium-heparin tubes, and centrifuged for 10 minutes at 2000 rpm. The plasma was collected and stored at −70° C. Plasma amino acids were measured with an LKB 4151 "alpha plus" amino

acid analyzer (LKB Biochrom Ltd., Cambridge). The intraassay coefficient of variation was 6%.

A specific parameter, the fetal concentrating index, was devised to provide a measure of the overall concentrating ability of the fetus. Six normally concentrated amino acids (threonine, valine, methionine, tyrosine, phenylalanine, and lysine) were selected, and the numeric mean of their fetal/maternal ratios was calculated; this constitutes the fetal concentrating index. The choice of these six amino acids, including both essential and nonessential amino acids in which transplacental passage is mediated by differing mechanisms, is somewhat empiric. However, by using the amino acids that are normally the most concentrated and therefore presumably require the most energy for transport, we believe we can develop the system that is most likely to reflect energy changes in placental function.

Approval from the Hospital Ethics Committee was obtained both for the indication of cordocentesis and for the extra blood taken from mothers and fetuses for this study.

Results

Plasma amino acids from 75 normal control fetuses showed very little variation when grouped between 16
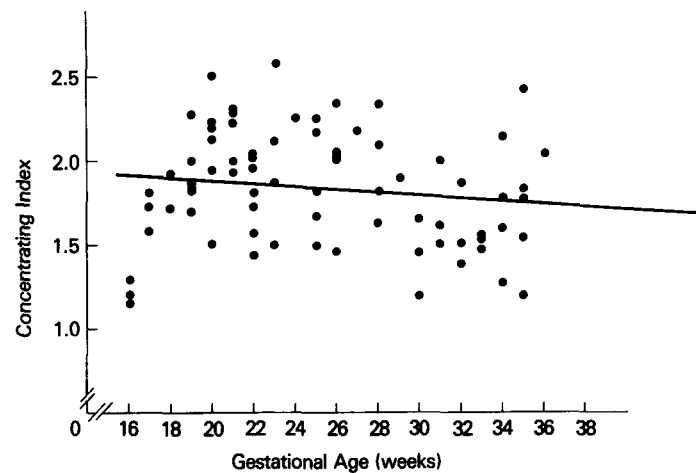
Fig. 1. Amino acid concentrating index as function of gestational age for 75 pregnancies with AGA fetuses. Concentrating index is defined in text. Regression line follows equation $y = -0.0086x + 2.05$. Its correlation coefficient was -0.15.
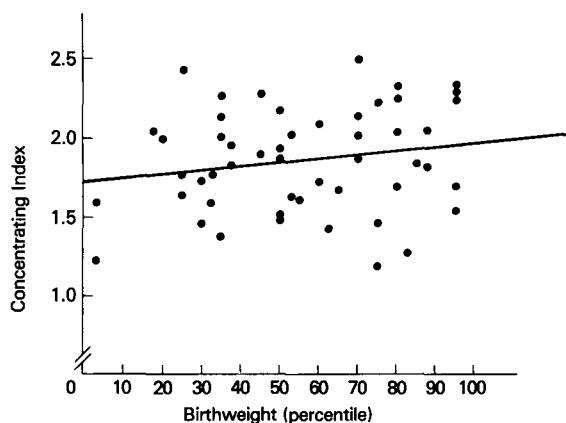
Fig. 2. Amino acid concentrating index as function of birth weight percentile for 51 pregnancies with AGA fetuses. Equation of regression line was $y = 0.0025x + 1.72$. Correlation coefficient was 0.19.

and 36 weeks' gestation (Table I). Two exceptions were asparagine, which was much lower in the second trimester than in the third trimester, and glutamine, whose mean plasma concentration fell with advancing gestational age. Fetal plasma free and total carnitine concentrations did not vary with gestational age (Table I).

Several essential amino acids such as threonine, valine, methionine, and lysine, as well as the presumably nonessential amino acids tyrosine, arginine, and ornithine, exhibited elevated fetal/maternal ratios in all gestational age groups (Table II). These amino acids are apparently concentrated by the fetus. Most nonessential amino acids, as well as free and total carnitine, are not

concentrated in the fetal circulation. For most amino acids, as well as free and total carnitine, the fetal/maternal ratio did not vary with gestational age (Table II). The fetal/maternal ratio for valine, threonine, proline, and tyrosine decreased with advancing gestational age, whereas that for arginine increased.

With the fetal concentrating index, as defined in the Methods section, comparisons could be made between the growth-retarded and control fetuses. For normal control pregnancies the fetal concentrating index was 1.83 ± 0.42 (mean $\pm$ SD) and did not vary significantly with gestational age (Fig. 1) or birth weight percentile (Fig. 2).

To ascertain the potential contribution of mitochondrial fatty acid oxidation to energy production in developing fetuses, carnitine's concentrating ability was compared with amino acid's concentrating ability. Thus the fetal/maternal ratio of free carnitine was plotted against the fetal concentrating index for normal pregnancies (Fig. 3, A). If free carnitine were concentrated in the fetal circulation, a positive correlation would be expected. No such correlation was observed, indicating that the very same fetoplacental units that concentrated several amino acids nevertheless failed to concentrate free carnitine. There was also no correlation between the fetal/maternal ratio for free carnitine and the fetal concentrating index in 24 pregnancies with SGA fetuses (Fig. 3, B). Similar results were obtained when total rather than free carnitine ratios were studied in either AGA or SGA fetoplacental units (data not shown).

Pregnancies with AGA and SGA fetuses were
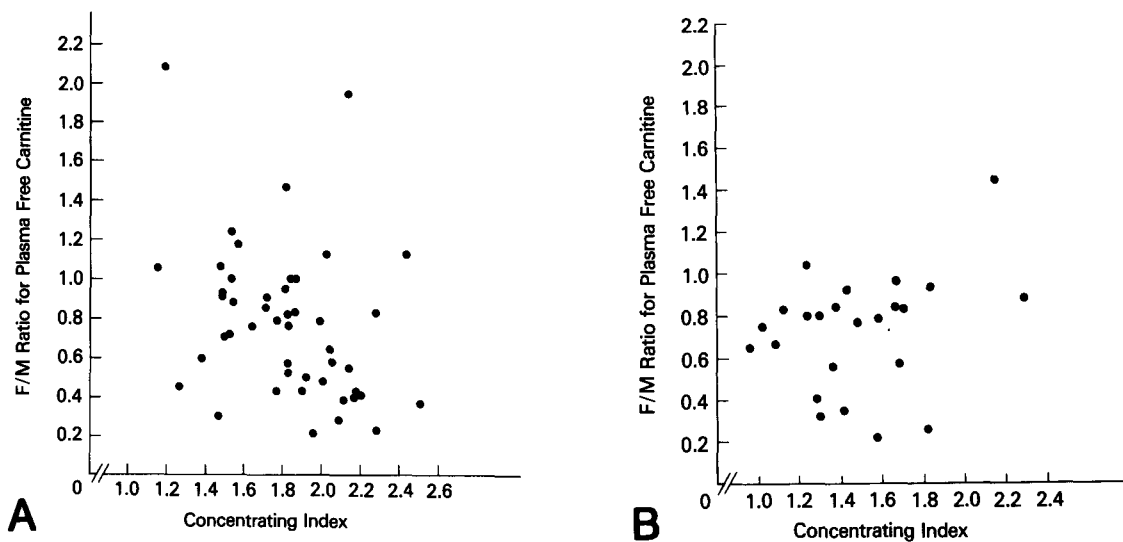
Fig. 3. Fetal/maternal ratio of plasma free carnitine plotted against concentrating index for 47 AGA
(A) and 24 SGA **(B)** fetomaternal units. No correlation was noted for either group.

Table III. Fetal/maternal ratios of free and total carnitine concentrations and concentrating index for pregnancies with AGA and SGA fetuses

	AGA (mean ± SD)	SGA (mean ± SD)	p*
Free carnitine fetal/maternal ratio	0.78 ± 0.37 (n = 45)	0.73 ± 0.27 (n = 24)	NS
Total carnitine fetal/maternal ratio	0.94 ± 0.25 (n = 19)	0.82 ± 0.23 (n = 14)	NS
Concentrating index	1.83 ± 0.42 (n = 74)	1.46 ± 0.38 (n = 41)	<0.001

NS, Not significant at $p = 0.05$.
*Two-tailed Student's t test.

grouped and compared with respect to their fetal/maternal ratios for amino acids and carnitine. Predictably, the ratios for free and total carnitine in pregnancies with SGA fetuses did not differ from normal ones (Table III). In contrast, however, the fetal concentrating index was significantly reduced in the pregnancies with SGA fetuses (1.46 ± 0.38), indicating impaired ability to concentrate certain amino acids in the IUGR fetuses. In fact, seven of 38 pregnancies with SGA fetuses had a fetal concentrating index <1.10, compared with none of 75 normal fetomaternal pairs (χ^2, 18.54; $p < 0.00001$). The fetal concentrating index for 38 pregnancies with SGA fetuses did not vary with gestational age (Fig. 4). A sensitivity of the fetal concentrating index for SGA fetuses was 100% with a specificity of 71%.

Comment

Previous expressions of fetal plasma amino acid concentrations have been in terms of a mean for 26 weeks' gestation and a slope to express variation with gestational age. Grouping by gestational age (Table I) allows

ready determination of the normalcy or abnormalcy of an individual fetal amino acid concentration, determined at a particular gestational age, especially when the normal values change with gestational age. The grouping of fetal plasma amino acid values provides more accessible control values for comparison with those of fetuses at risk for aminoacidopathies and also can be used to monitor maternal dietary control in disorders such as phenylketonuria. The values also provide a reference table for comparison with amniotic fluid,[8, 9] cord blood,[8, 10] and plasma from infants,[8, 11] children,[8] and adults.[8, 12]

The fetal circulation does not concentrate carnitine, or β-hydroxy-δ-trimethylaminobutyric acid. This small molecule transports long-chain fatty acids into the mitochondria for oxidation to produce energy.[13] Apparently, the maternal concentration of free carnitine, which is lower than in nonpregnant women,[14-16] is sufficient to serve the fetal needs for fatty acid oxidation throughout pregnancy. In fact, no fetus with IUGR had a markedly decreased plasma carnitine level as a putative cause of growth retardation. The reason for re-
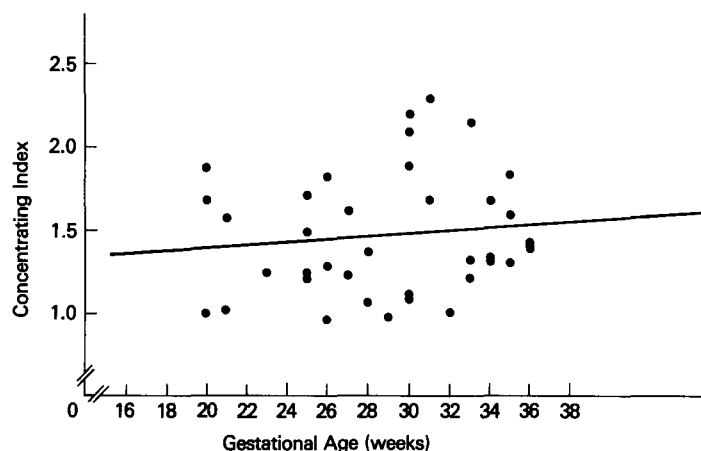
Fig. 4. Amino acid concentrating index as function of gestational age for 38 pregnancies with SGA fetuses. Regression line follows equation $y = 0.0091x + 1.195$. Correlation coefficient was 0.12.

duced maternal plasma carnitine in pregnancy remains enigmatic but may be related to an enhanced volume of distribution without increased hepatic synthesis or intestinal absorption of carnitine.

Our main focus, however, is the introduction of the fetal concentrating index as a more powerful measure of fetal amino acid's concentrating ability. Unlike measurements of individual amino acids that need to be adjusted for gestational age and as a whole do not differ between AGA and SGA fetuses, the fetal concentrating index is very significantly reduced in SGA fetuses compared with normal controls (Table III). Thus in the future the fetal concentrating index might be used as a quantitative gauge of the integrity of the fetoplacental unit when factors causing placental insufficiency are being investigated.

One major advantage of the fetal concentrating index is its gestational age independence; uncertainties about gestational age are rendered irrelevant. Furthermore, since a reduction can be observed even in early gestation in fetuses who develop IUGR, it may be possible to predict the cause of IUGR on the basis of this index. As cordocentesis has become very safe in experienced hands, it may now be ethical to use the fetal concentrating index to predict impending placental compromise, with consideration of early intervention in at-risk pregnancies. Amino acid determinations performed in an experienced laboratory and fetal concentrating index calculations can be offered in attempts to make this parameter part of a practical approach to IUGR diagnosis and potential intervention.

The choice of the six amino acids used in the fetal concentrating index is certainly arbitrary. Conceivably with more experience the choice, number, or relative weighing of components might change. Until a better understanding is obtained, we will continue to collect all parameters. Similarly, further work is needed to correlate these data with other diagnostic parameters such as biophysical and biochemical measurements.

REFERENCES

1. Lindblad BS, Baldesden A. The normal plasma free amino acid levels of non-pregnant women and of mother and child during delivery. Acta Paediatr Scand 1976; 56:37-8.
2. Liechty EA, Lemons JA. Changes in bovine fetal hindlimb amino acid metabolism during maternal fasting. Am J Physiol 1984;246:E459-66.
3. Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Evans MI. Plasma amino acids in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol 1989; 161:1219-27.
4. Arroyave G. Comparative sensitivity of specific amino acid ratios versus "essential to nonessential" amino acid ratio. Am J Clin Nutr 1970;23:703-6.
5. Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Bottoms S, Evans M. Cordocentesis in the diagnosis of intrauterine starvation. Am J Obstet Gynecol 1989; 161:1004-8.
6. Soothill PW, Nicolaides KH, Campbell S. Prenatal asphyxia, hyperlacticaemia, hypoglycaemia, and erythroblastosis in growth retarded fetuses. Br Med J 1987; 1:1051-3.
7. Nicolaides KH, Thorpe-Beeston JG, Noble P. Cordocentesis. In: Eden RD, Boehm FH, eds. Assessment and care of the fetus: physiological, clinical, and medicolegal principles. East Norwalk, Connecticut: Appleton & Lange, 1990:291-306.
8. Bremer HJ, Duran M, Kamerling JP, Przyrembel H, Wadman SK. Disturbances of amino acid metabolism: clinical chemistry and diagnosis. Baltimore: Urban & Schwarzenberg, 1981:203-27.
9. Mesavage WC, Suchy SF, Weiner DL, Nance CS, Flannery DB, Wolf B. Amino acids in amniotic fluid in the second trimester of gestation. Pediatr Res 1985;19:1021-4.
10. Boersma ER, Blom W, Massawe AW. Free amino acid levels of mother and child immediately after delivery in Dar es Salaam, Tanzania. Biol Neonate 1980;37:22-31.
11. Rigo J, Verloes A, Senterre J. Plasma amino acid concentrations in term infants fed human milk, a whey-predominant formula, or a whey hydrolysate formula. J Pediatr 1989;115:752-5.

12. Scriver CR, Gregory DM, Sovetts D, Tisenbaum G. Normal plasma free amino acid values in adults: the influence of some common physiological variables. Metabolism 1985;34:868-73.
13. Broquist HP. Carnitine biosynthesis and function. Fed Proc 1982;41:2840-2.
14. Bargen-Lockner C, Hahn P, Wittmann B. Plasma carnitine in pregnancy. AM J OBSTET GYNECOL 1981;140: 412-4.
15. Cederblad G, Fiahraeus L, Lindgren K. Plasma carnitine and renal-carnitine clearance during pregnancy. Am J Clin Nutr 1986;44:379-83.
16. Cederblad G, Niklasson A, Rydgren B, Albertsson-Wikland K, Olegard R. Carnitine in maternal and neonatal plasma. Acta Paediatr Scand 1985;74:500-4.

Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. JACQUES F. ROUX, Jackson, Wyoming. In the past 20 years maternal and fetal plasma amino acid profiles and concentrations have been determined in umbilical cord blood of normal deliveries and cesarean sections.[1, 2] In recent years, with the use of ultrasonography, cord blood is obtained in utero. This procedure is called cordocentesis or percutaneous blood sampling. It has to be noted that Cetin et al.[3] in 1988 and Economides et al.[4] in 1990 used a similar research protocol to determine amino acid concentration. They observed a decrease in amino acid concentration in the cord blood of SGA fetuses. Carnitine concentrations in fetal blood were measured, and no major comments need to be made about this substrate of fatty acid oxidation.

In this article the data have been broken down by gestational ages, including single amino acid concentration and fetal versus maternal level of amino acid. The results are expressed as a fetal/maternal ratio. When the ratio is >1, there is active transport of maternal amino acids to the fetal compartment. I have difficulty accepting some of the statements without a statistical evaluation of the data, especially those on asparagine and glutamine. Each standard deviation is very large and differs for each amino acid. This indicates that each substrate has its own metabolic pathway and that, the concentration differences not being significant among age groups, the data can be pooled. The authors should study one amino acid at a time in well-defined metabolic circumstances. For instance, asparagine and glutamine might be of interest since they show changes that seem to be statistically significant. Glutamine is the precursor of acetoacetate, a citric acid substrate, necessary to the Krebs cycle to provide energy to the fetal cells in the form of adenosine 5′-triphosphate. Asparagine, like alanine, goes to pyruvate and then to glucose and is a precursor of glycoprotein, a component of fetal membranes. Therefore the measurement of these two amino acids would provide interesting information on the fetal-placental unit, and

the results might be more meaningful to the clinician than an indiscriminate screen of amino acids.

The present results are compared with maternal blood obtained in the upper extremity. The maternal concentration of amino acids could be misleading since muscles release various amounts of this substrate in response to food intake, amount of time after ingestion, and exercise. Furthermore, the circadian rhythm for amino acids is characterized by peak level, between 12 and 8 o'clock in the evening and between 4 and 8 o'clock in the morning.[5] These observations give little support to the use of brachial venous blood without a well-defined time of testing. Intervillous blood would be the best standard of comparison.

The computation of the fetal/maternal ratio indicates an active amino acid transport system. The large variability of the standard deviation of this ratio makes it difficult to interpret the data, except that the fetal/maternal ratio of valine, threonine, proline, and tyrosine decreases with advancing age, but there is no statistical study of the data. The results could be due to an increase in maternal amino acid concentration with gestational age. This would increase the denominator of the fetal/maternal fraction and produce a decreased ratio.

The authors stated that they had difficulty in developing a meaningful clinical test for amino acids. They decided to average the fetal/maternal ratio of six amino acids showing a high transport rate. They called it the fetal concentrating index. The idea was an excellent one, if one wanted to focus on transport and as long as one accepted that each amino acid has its own metabolic pathway, as is the case. The authors show that the fetal concentrating index for the SGA fetus is lower than that of the control. They then conclude that the SGA fetus has a deficiency in active placental transport of the six amino acids because of placental asphyxia. Two strong objections can be made to these statements. First, the difference between the fetal concentrating index means is 20%. Clinically this is meaningless because the statistical significance is mainly due to the number of determinations. Therefore, from the clinical point of view, it may be true that in a large population of SGA fetuses the fetal concentrating index will be low; however, on a case-to-case basis it will be difficult to consistently demonstrate a significant variation among samples. A normal physiologic variation can be as large as 15% to 20%. Second, in this clinical sampling one does not know how placental asphyxia was evaluated. In each case fetal acid-base status should have been determined and the data correlated with the fetal/maternal ratio. In this regard determinations on intervillous blood samples would be rewarding. Without this information the authors' conclusions are open to question because they are not verified empirically.

From a theoretical point of view the metabolism of amino acids supports the observations of the authors. In adults amino acids are transported actively through membranes by glutamyl transpeptidase.[6] This is an

energy-dependent mechanism. Therefore a decreased fetal/maternal ratio could be explained by a lack of adenosine 5'-triphosphate generation, as a secondary result of a low Po_2.

In man, muscle releases alanine, which is transported to the liver, which converts it to glucose.[7, 8] Therefore, if the amino acid–glucose cycle is active in the placenta, a decrease in placental blood amino acid concentrations could reduce fetal amino acid availability. It is known that human placental tissue actively transports amino acids in vitro and in vivo. It uses them for its own metabolic needs and releases them in the fetal circulation. The mechanism of release was studied by Schneider et al.[9] He showed that placental tissue released only 26% to 47% of its amino acid content in an amino acid–free medium. It is then possible that the classic amino acid–glucose cycle observed in muscle and liver is different in the placenta. For instance, the decrease in amino acid level in the fetus could be due to a change in turnover of substrates in the fetal blood, the liver extracting a greater amount in the SGA fetus than in the normal fetus. Another possibility is that the SGA fetus releases less amino acid into the circulation from the placenta, muscle, and liver. This is independent of an abnormal amino acid transport. I strongly suggest to the authors that they expand their investigation to placental tissue and fetal-maternal blood obtained under ultrasonographic guidance. Blood glucose, pyruvate, amino acids, glucagon, insulin, and α-ketoglutarate concentration should be determined under controlled conditions to avoid biologic variations. These data should then be correlated with the maternal-fetal Po_2 and the acid-base balance in each case. A placental membrane preparation should be investigated for the presence of glutamyl transpeptidase in the SGA and normal fetus.

In the meantime, the present data interpretation is doubtful and the clinical applications are very limited. This study helps us to formalize new work hypotheses and to have a set-of-data basis. It opens new vistas on fetal biochemistry, a field that has been somewhat neglected in the last 10 to 15 years.

REFERENCES

1. Cockburn F, Blagden A, Michie EA, Forfor JO. The influence of preeclampsia and diabetes mellitus on plasma free amino acids in maternal umbilical vein and infant blood. J Obstet Gynaecol Br Commonw 1971;78:215-31.
2. Kerr GR. The free amino acids of serum during development of Macaca mulatta during pregnancy and fetal life. Pediatr Res 1968;2:493-500.
3. Cetin I, Corbetta C, Sereni LP, et al. Umbilical amino acid concentrations in normal and growth-retarded fetuses sampled in utero by cordocentesis. AM J OBSTET GYNECOL 1990;162:253-61.
4. Economides DL, Nikolaides KH, Gahl WA, Bernardini I, Evans MI. Plasma amino acids in appropriate- and small-for-gestational-age fetuses. AM J OBSTET GYNECOL 1989; 161:1219-27.
5. Feigin RD, Klainer AS, Beisel WR. Factors affecting circadian periodicity of blood amino acids in man. Metabolism 1968;17:764-75.
6. Meister A. On the enzymology of amino acid transport. Science 1973;180:33-9.
7. Felig P, Wahren J. Protein turnover and amino acid metabolism in the regulation of gluconeogenesis. Fed Proc 1974;33:1092-7.
8. Felig G, Marliss E, Pozefsky T, Cahill GF. Amino acid metabolism in the regulation of gluconeogenesis in man. Am J Clin Nutr 1970;23:986-92.
9. Schneider H, Mohlen KH, Dancis J. Transfer of amino acids across the in vitro perfused human placenta. Pediatr Res 1979;13:136-240.

DR. RALPH K. TAMURA, Chicago, Illinois. With this study Bernardini et al. are extending and elaborating on significant prior work done in this area. Having previously published data on fetal plasma amino acid profiles in SGA and AGA fetuses, they now report on the usefulness of adding carnitine levels, and they have developed a coordinated parameter that they refer to as the fetal concentrating index to quantitatively describe the ability of the placenta to extract circulating amino acids. It appears to me that the patient data used for this study are identical to those previously reported by the authors,[1, 2] except of course for carnitine levels and for the fetal concentrating index. The following are queries that hopefully may clarify and strengthen this paper:

1. For the control pregnancies studied, could the fetal disease in any way affect the amino acid levels of the umbilical venous blood?

2. Why were fetuses with encephaloceles, hydrops, or chromosomal defects excluded? What are fetal concentrating indexes in these fetuses?

3. Regarding the number of control pregnancies, 75 minus 20 (16 terminations, 4 lost to follow-up) equals 55, not 57.

4. Regarding the cordocentesis procedure, how many attempts were performed per patient to obtain an adequate sample?

5. Why are there 80 control fetuses in the Results section but 75 in the Methods section?

6. Should not mention of the fetal concentrating index be placed in the Methods section?

7. Have these investigators analyzed the effect of using different individual amino acids for different gestational ages? That is, might not a different set of amino acids improve the predictive value of the fetal concentrating index in different trimesters?

8. Why were ornithine and taurine (high fetal concentrating index values) not used as part of the fetal concentrating index?

9. In the Methods section, 31 SGA fetuses were studied. Why were there 38 SGA pregnancies in the Results section?

10. May we asume that, on the basis of the data provided, the predictive value of the fetal concentrating index for SGA fetuses is 7 of 38 (18%) with a fetal concentrating index cutoff value of 1.10? What are sensitivity and specificity values?

This is an extremely interesting study in which basic and clinical research appear to have coalesced hope-

fully to provide some meaningful insights into the understanding of IUGR and SGA fetuses. To be used as part of the clinical armamentarium, however, correlations will need to be made between the fetal concentrating index and other antenatal diagnostic tests (i.e., biophysical profile, nonstress testing, and Doppler velocimetry, all of which are noninvasive).

REFERENCES

1. Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Evans MI. Plasma amino acids in appropriate- and small-for-gestational-age fetuses. AM J OBSTET GYNECOL 1989; 161:1219-27.
2. Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Bottoms S, Evans M. Cordocentesis in the diagnosis of intrauterine starvation. AM J OBSTET GYNECOL 1989;161: 1004-8.

DR. KENNETH J. MOISE, JR., Houston, Texas. I was wondering if you tried to correlate the ultrasonographic weights with the fetal concentrating index, both in the normal population and in the IUGR population? You appeared to be talking about babies after they were born as being ranked in the 5th percentile. Was there any correlation between the fetal concentrating index and the actual ultrasonographic weight at the time of the fetal blood sampling and the same question for IUGR?

DR. RICHARD P. PERKINS, Omaha, Nebraska. I am trying to decide what the importance of this is. First, it depends on whether you are of the school of thought that to detect it very early is a good thing. If one pursues that logic to its conclusion, one would say that cordocentesis is a procedure that is essentially without risk in the most experienced hands, that the objective therefore is to do it at the earliest point possible, and that we should have all of our cordocenteses done by only one person.

DR. CHIN-CHU LIN, Chicago, Illinois. I enjoyed the study very much. Briefly, in fetal growth retardation one of the mechanisms is thought to be a placental malfunction of the transport mechanism in the placenta for essential nutrients across the membranes.

My question is, have you made any correlation with the concentration of fetal-to-maternal rate gradient with respect to simple measures, such as placental morphologic type, either grossly or histologically?

DR. CHESTER B. MARTIN, Madison, Wisconsin. To follow up on the last question, were there any differences in an outcome variable between those SGA fetuses with a fetal concentrating index <1.1 and >1.1? And how many inadvertent or unplanned interventions were carried out as a consequence of the cordocentesis?

DR. PETER F. FEHR, St. Paul, Minnesota. One of the problems experienced with patients who had intravascular transfusions is fetal-maternal bleeding. I'm wondering if the authors measured these and whether they discovered, as our areas discovered, that synthesis is occurring in these patients at a higher rate than in the

normal population. How would they justify this in terms of maternal informed consent before they do a cordocentesis in these patients?

DR. WILLIAM J. OTT, St. Louis, Missouri. I have a number of questions, but I will limit them to two. In your Methods section, how did you differentiate between the SGA infant who suffers from IUGR and those infants that are small for gestational age but are so because they are constitutionally small but otherwise totally normal? Second, how did you differentiate between the AGA fetuses whose birth weights may be above the 5th percentile but still have not met their inherent growth potential?

The second question deals with the clinical relevance of the study. Although cordocentesis may provide important biochemical data, it is an extremely operator-intense procedure. Our institution has been using the technique for approximately 2½ to 3 years, and we find that it significantly raises catecholamine levels in both the mother and the physician. I wonder whether the information obtained from cordocentesis provides any additional information over that obtained from noninvasive procedures, such as fetal heart rate monitoring or Doppler blood flow studies?

DR. EVANS (Closing). I thank all of the discussants and questioners for their thoughtful comments.

Probably the most important concept I would like to reemphasize is that this is a pilot study. We felt it was necessary to do these determinations on patients who were having cordocentesis for other indications and use extra blood to see if we could define a parameter that would help us. I think we have to some degree.

I agree with Dr. Roux that there are a number of different possible explanations for the specific differences seen. It is also not clear that these six amino acids that we have chosen through our first pass will end up being the ultimate parameters that are used, if any, as this data set continues to go on. The major thing we tried to accomplish with this article was to show optimism that we might have a useful parameter and to make it ethically appropriate to attempt to study this prospectively.

None of the management of patients was affected by this, because these tests were conducted long after the pregnancies were concluded. As a matter of fact, they were run on different sides of the Atlantic from where the patients actually were, so it would be hard to have an exact intervention on the basis of the results.

In terms of what Dr. Roux said about where we draw the blood, it might be better scientifically to get the blood out of the femoral artery. I think politically it would be much less acceptable and much less likely to be allowed to be used.

Dr. Tamura, as far as numbers are concerned, obviously I'm not as good at the abacus as you are. There are a couple of differences mainly because we did not have all the data for some of the patients because there was a variable amount of blood available for running the 30 or so different parameters. So for some patients it turns out we had enough blood to run for the pa-

rameters that ultimately became the fetal concentrating index but not enough for other parameters.

A 1.1 cutoff was arbitrarily defined but did pick up, as Dr. Tamura said, in seven out of 38 cases. Whether that will be the ultimate cutoff will depend on how many more numbers are necessary to have an index.

As for Dr. Moise's question, the patients were categorized at the time of the cordocentesis as to the ultrasonographic findings, and the results were confirmed post partum; only patients who fell into the same category twice were used.

Dr. Lin asked about discordant twins. We have a couple of samples from those in the freezer, but we haven't run them yet.

Dr. Ott also asked about distinguishing between IUGR and SGA fetuses; it is true that we can't distinguish the exact cause in all those patients, but again we're trying to work on it.

There were no complications from cordocentesis in this particular series. Certainly, depending on the published series, when complications have been as high as 5% to 7% or as low as 0.5% to 1%, I think that as experience becomes greater the complication rates will go down to a point where a number of people will be able to do this procedure with safety.

I think that the issue of fetal-maternal bleeding is certainly one issue that needs to be addressed and one that needs to be looked at. We did not look at it nor were there any particular questions.

Another question was asked regarding control. The control patients were mostly patients who were being tested for diseases such as hemoglobinopathies but were found not to be affected. Anatomic defects such as limb reduction defects were felt not to be likely to affect the plasma amino acids.

Patients who had more serious internal disorders, such as hydrops, encephalocele, etc., for whom we did have a serious concern but certainly no data that there might be some impact on the circulating amino acids, were not included in this study for precisely those reasons.

As the numbers build up over time, we can probably begin to subcategorize patients by the different areas, but at this point the quickest thing to do was merely to categorize patients as easily as possible.