0031-3998/02/5102-0215
PEDIATRIC RESEARCH
Vol. 51, No. 2, 2002
Printed in U.S.A.

Circulating S100β Protein Is Increased in Intrauterine Growth-Retarded Fetuses

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ABSTRACT

To determine whether S100β, an acidic calcium-binding protein previously demonstrated as a reliable indicator of a brain lesion, could be helpful in the detection of brain distress in intrauterine growth-retarded (IUGR) fetuses, we studied, by a case-control study, the correlation between S100B protein and the degree of fetoplacental blood flow impairment. Maternal and umbilical blood samples and placental tissue specimens were collected at delivery from IUGR pregnancies with normal ($n = 10$) or abnormal ($n = 10$) umbilical artery Doppler findings and from 40 uncomplicated pregnancies. S100β protein levels were measured by means of a specific RIA, and flow velocimetry waveforms were recorded from uterine, umbilical, and fetal middle cerebral arteries. Overall mean S100β proteins in umbilical plasma levels were higher ($p < 0.05$) in IUGR patients (121.8 ± 70.4 fmol/mL) than in control patients (54.7 ± 21.9 fmol/mL). IUGR fetuses with redistribution of blood flow showed the higher concentration of the protein (163.7 ± 55.2 fmol/mL). Fetal S100β concentrations correlated with middle cerebral artery pulsatility index ($r = -0.536$, $p < 0.03$) and with umbilical artery pulsatility index to middle cerebral artery pulsatility index ratio ($r = 0.469$, $p < 0.03$). No difference in the localization or intensity of S100β staining in the placental tissues or cord between uncomplicated and IUGR pregnancies was found. This study provides evidence that circulating S100β protein is increased in IUGR fetuses and correlates with cerebral hemodynamics, suggesting that it may represent an index of cerebral cell damage in the perinatal period. **(*Pediatr Res* 51: 215–219, 2002)**

Abbreviations
FVWs, flow velocity waveforms
IUGR, intrauterine growth retardation
PI, pulsatility index
RI, resistance index

The S100 family of calcium-binding proteins, first isolated in 1965 by Moore (1) in a subcellular fraction from bovine brain, contains approximately 16 members, each of which exhibits a unique pattern of tissue- or cell type–specific expression. Although the distribution of these proteins is not restricted to the nervous system, the involvement of several members of this family in nervous system development, function, and disease has sparked new interest in these proteins. S100β, one of the original two members of this family, is an acidic calcium-binding protein with a molecular weight of 21 kD. It is present extracellularly, intracellularly, and in the cytosol; its half-life is approximately 2 h, and it is mainly eliminated by the kidney (2). S100β is present in CNS and is concentrated in the glial cells, astrocytes, Schwann cells, and neurons. It regulates several cellular functions (cell-cell communication, cell growth, cell structure, energy metabolism, contraction, and intracellular signal transduction). Elevated plasma levels are found in patients with brain damage (3). Abnormal S100β levels have been associated with neurobehavioral abnormalities and microcephaly caused by *in utero* cocaine exposure (4), and abnormal S100β immunoreactivity cells in anencephalic fetuses have been shown (5).

The S100β concentration in blood and in cerebrospinal fluid is increased as result of brain damage in adults and infants (6–8). Although S100β is detectable in the umbilical cord blood of preterm and term fetuses (9), increased circulating protein levels have been related to the occurrence of intraventricular hemorrhage in preterm infants (10).

IUGR is commonly accepted as an expression of persistent suppression of genetic growth potential caused by decreased oxygen and substrate supply. IUGR is generally associated with uteroplacental blood flow insufficiency (up to 50%) because of impaired trophoblast invasion of spiral arteries, which are not transformed to low resistance vessels (11, 12). Fetoplacental insufficiency, and subsequent fetal hypoxia, activates

Received May 11, 2000; accepted May 10, 2001.
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This work was supported in part by Italian CNR and MURST (F.M. and D.G.).

a cascade of pathophysiologic events leading to brain damage in which vasoactive agents and calcium-mediated effects are involved (13).

In light of these facts, we investigated whether circulating S100β is increased in IUGR fetuses and is correlated with hemodynamic findings, to evaluate its potential role as an indicator of cerebral cell damage.

METHODS

Patients. We studied 20 women with singleton pregnancies complicated by IUGR between 28 and 39 wk of gestation. Gestational age was determined by clinical data and by a first trimester ultrasound scan. IUGR was defined by the presence of ultrasonographic signs (biparietal diameter below the 10th percentile and abdominal circumference below the 5th percentile) according to the nomograms of Campbell and Thoms (14), and a fall in the percentile of fetal sizes was recorded between the first scan after referral and the final scan before delivery.

FVWs of the main branch of the uterine artery bilaterally, umbilical artery, and fetal middle cerebral artery were recorded by means of a duplex pulsed color Doppler ultrasound (Aloka, SSD-2000, Tokyo, Japan) with a convex 3.5-MHz transducer, and the RI (peak systolic velocity − end-diastolic velocity / peak systolic velocity) and PI (peak systolic velocity − end-diastolic velocity / mean velocity) were calculated automatically by the built-in software. A spatial peak temporal average <100 mW/cm^2 was used for blood flow measurements in the middle cerebral artery. A 100-Hz high-pass filter was used, and Doppler waveforms were obtained in the absence of fetal body or breathing movements by a single observer (D.G.). In every record, three to five consecutive cardiac cycles were examined, and the mean of at least three values from each vessel was used for subsequent analysis. Abnormal RI for uterine artery or PI for umbilical artery was defined as >95th percentile for gestational age for uncomplicated pregnancies (15). Similarly, an abnormal middle cerebral artery PI <5th percentile for gestational age for uncomplicated pregnancies and an umbilical artery PI to middle cerebral artery PI ratio >1 were considered as indexes of redistribution of fetal blood flow (16).

The control group consisted of 40 normal fetuses matched for gestational age at sampling (range, 32–40 wk of gestation) and birth weights between the 10th and 90th percentiles (two controls for each IUGR fetus). In these pregnancies the FVWs of the fetal middle cerebral arteries were not recorded because velocimetry waveforms in the uterine and umbilical arteries were normal.

Sample collection. All subjects were delivered by elective cesarean section, performed within 1 h after FVW recording. Maternal plasma was collected from cubital vein before induction of anesthesia. At delivery, the umbilical cord was clamped before any signs of breathing were seen, and blood was drawn from the umbilical vein. None of the patients experienced uterine contractility before cesarean section. Indications for elective cesarean section in the controls included breech presentation, previous cesarean section, placenta praevia, and maternal cardiac disease.

At delivery placental tissues and cord specimens were collected from all pregnant women. The local ethics committee approved this study, and informed consent was obtained from all participants.

S100β measurement. Heparin-treated blood samples taken at birth were immediately centrifuged at $900 \times g$ for 10 min, and the supernatants stored at −70°C. The S100β concentration was measured in all samples using a commercially available RIA kit (Sangtec 100, AD Sangtec Medical, Bromma, Sweden), specific to the β-subunit of the protein, which is known to predominate (80–96%) in the human brain, as reported (3). Each measurement was performed in duplicate and the averages were reported. The sensitivity of the assay was 0.2 μg/L (fmol/mL). The inter- and intrassay coefficient of variation was 10% and <5%, respectively. The assay detects exclusively S100β protein and no cross-reactivity has been found with other S100 proteins.

Immunohistochemistry. Specimens of placental tissues (placenta, fetal membranes, and cord) collected at delivery were fixed in 4% paraformaldehyde–0.2% glutaraldehyde, washed, and embedded in paraffin. The presence of S100β was sought by immunohistochemistry on 5-μm paraffin sections. The sections were stained using the avidin-biotin peroxidase technique (Vector ABC, Vector Laboratories, Burlingame CA, U.S.A.) and incubated with polyclonal antibody raised in rabbits against purified S100β subunit (Sigma Chemical Co., St. Louis, MO, U.S.A.) at a dilution of 1:100. Negative controls were conducted on placental tissue incubated with either nonimmune rabbit serum or antibody dilution buffer.

The number of positive cells was quantified using a quantitative system (field = 0.175 mm^2 at ×250 magnification). Ten randomly selected fields were independently counted by three different examiners (R.D.I., E.M., F.M.) by visual examination, and the proportion of stained cells was expressed as a percentage of the total cells (stained and unstained). Positively stained cells were assessed when a brown granular staining of the cytoplasm was revealed at low-power magnification (×10).

Statistical Analysis

S100β concentrations are expressed as mean ± SD. Statistical analysis was performed with determination of Spearman rank order correlation and comparison between groups by Kruskal-Wallis one-way ANOVA and Mann-Whitney U test when data were not normally distributed. Clinical characteristics of women are expressed as mean ± SD and were compared by Mann-Whitney U two-sided test. To compare proportions between groups, Fisher's exact test was used, whereas to determine whether there was a difference in the intensity of the staining among groups, contingency table analysis of the score was performed. Statistical significance was set at $p < 0.05$.

RESULTS

Characteristics of studied groups are shown in Table 1. As expected, birth weight was lower in IUGR group than in control patients. Twelve of 20 IUGR pregnancies had an abnormal uterine artery RI or umbilical artery PI; in 10 of these the fetal middle cerebral artery PI was <5th percentile and the

Table 1. *Characteristics of pregnant women studied*

Characteristic	Controls ($n = 40$)	IUGR ($n = 20$)
Maternal age (y)	25.7 ± 4.1	24.8 ± 3.9
Gestational age at sampling (wk)	34.6 ± 3	33.6 ± 2.6
Birth weight (g)	2.226 ± 421	1.782 ± 525*
Placental weight (g)	448.2 ± 121.3	332.3 ± 113.6*
Apgar 1 min <7	3/20	1/40
Apgar 5 min <7	2/20	0/40
Uterine artery RI	0.56 ± 0.09	0.65 ± 0.03*
Uterine artery RI > 95th percentile	0/40	12/20*
Umbilical artery PI	0.9 ± 0.19	1.41 ± 0.22*
Umbilical artery PI >95th percentile	0/40	10/20*
MCA PI	not recorded	1.42 ± 0.25
MCA PI <5th percentile	not recorded	10/20
Umbilical artery PI/MCA PI	not calculated	1.01 ± 0.34
Umbilical artery PI/MCA PI >1	not calculated	10/20
Maternal plasma S100β (fmol/mL)	undetectable	undetectable

Data are shown as mean ± SD.
* $p < 0.01$.
Abbreviation: MCA = middle cerebral artery.

umbilical artery PI to middle cerebral artery PI ratio was >1. Five of these patients had unilateral early diastolic notch in the uterine artery, but none showed bilateral notch in the uterine arteries or absent or reverse diastolic flow in the umbilical artery.

The FVWs in uterine and umbilical arteries for all fetuses in the control group were appropriate for gestational age.

In both groups, none of the infants showed neurologic abnormalities at the time of discharge from the hospital, and no overt neurologic syndromes were observed during recovery. Isolated and transient symptoms in 10 IUGR infants, including hypertonia-hypotonia ($n = 4$), dystonia ($n = 3$), and hyperexcitability ($n = 3$), have been shown.

Maternal S100β concentrations were under the limit of detection in both control and IUGR groups. In contrast, mean fetal S100β levels in the IUGR group (121.8 ± 70.4 fmol/mL) were significantly higher ($p < 0.05$) than those in the control group (54.7 ± 21.9 fmol/mL) and correlated with middle cerebral artery PI ($r = -0.536$, $p < 0.03$; Fig. 1, upper panel) and with the umbilical artery PI to middle cerebral artery PI ratio ($r = 0.493$, $p < 0.03$; Fig. 1, bottom panel). Although S100β levels were higher at greater umbilical artery PI, this correlation was not statistically significant ($r = 0.33$; $p = 0.14$). When IUGR fetuses were grouped according to the presence of redistribution of fetal blood flow (middle cerebral artery PI <5th percentile and the umbilical artery PI to middle cerebral artery PI ratio >1), S100β levels were significantly higher ($p < 0.05$) only in those fetuses with redistribution of fetal circulation (163.7 ± 55.2 fmol/mL). In IUGR fetuses without this brain-sparing effect, S100β concentrations (80.0 ± 59.5 fmol/mL) were not different from those found in control patients (54.7 ± 21.9 fmol/mL; $p = 0.52$; Fig. 2).

In the control group, in accordance to previous observations (10), a negative significant correlation between S100β and gestational age was found ($r = -0.84$; $p < 0.001$), whereas this correlation was lost in the IUGR group. No correlation was found between S100β and birth weight in any group.

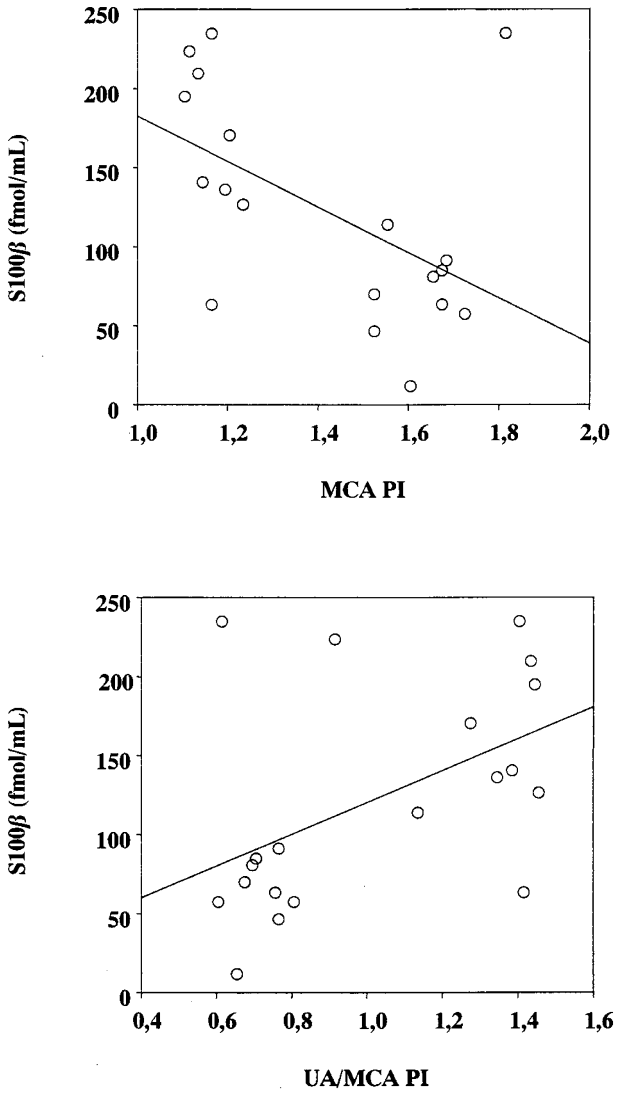

Figure 1. Correlation analysis of fetal S100β concentrations and FVWs in IUGR patients. There was a negative correlation with middle cerebral artery PI (*Top*) and a positive correlation with umbilical artery (UA) to middle cerebral artery (MCA) PI ratio (*Bottom*).

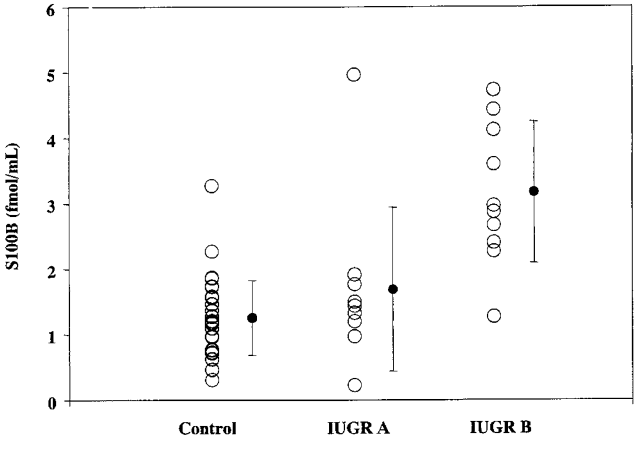

Figure 2. Scatter distribution of individual values of S100β protein in umbilical plasma in control and IUGR groups without (*A*) or with (*B*) redistribution of blood flow. (●) and vertical lines represent the mean value and SD. S100β protein values were significantly higher ($p < 0.05$) in umbilical plasma from IUGR with redistribution of cardiac output than in IUGR with normal Doppler findings or in controls.

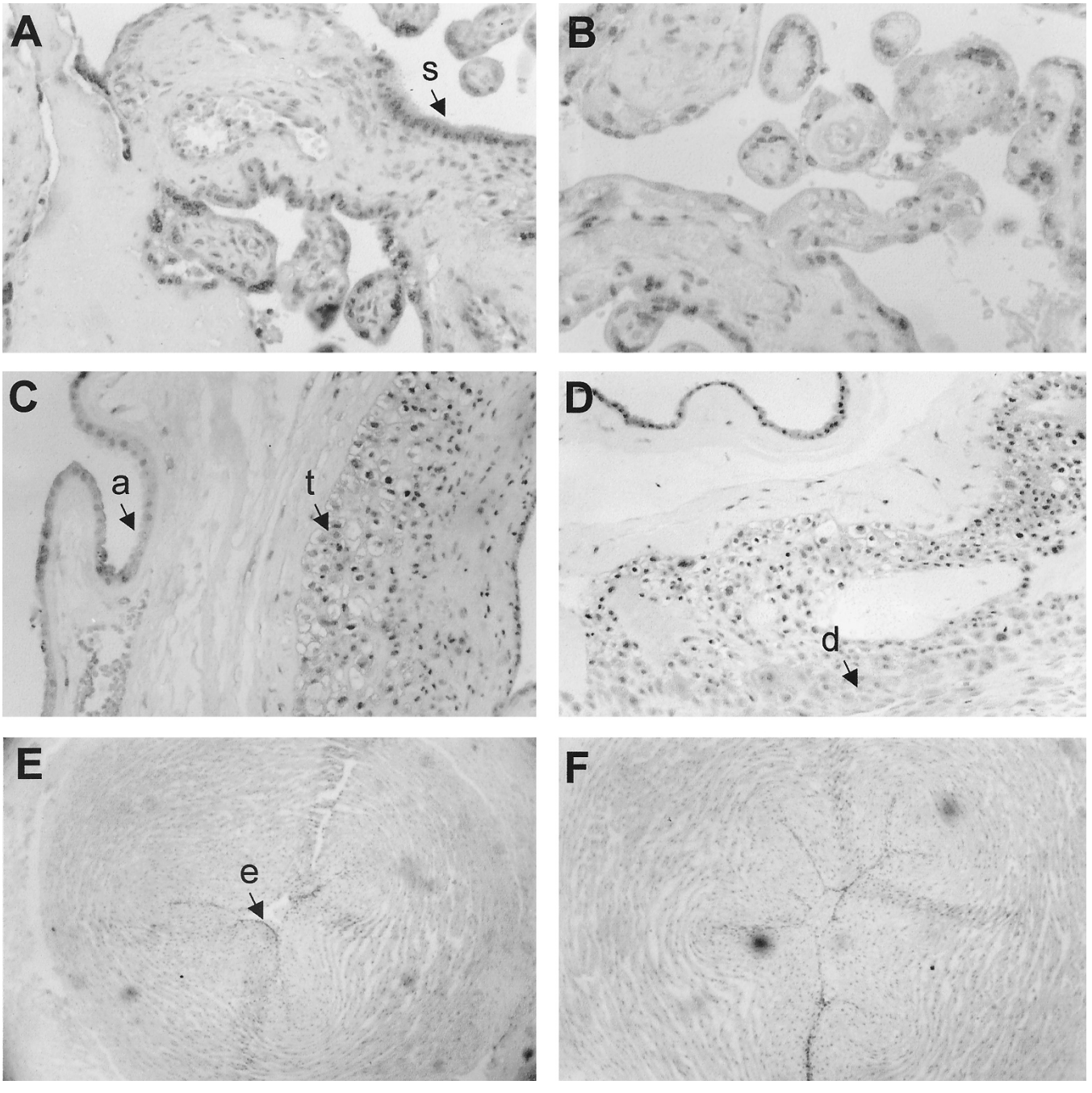

Figure 3. Immunohistochemistry of S100β in the placenta (magnification $\times$40), fetal membranes ($\times$40), and cord ($\times$10) of control (*A, C, E*) and IUGR with redistribution of blood flow (*B, D, F*) pregnancies. S100β was localized in syncytiotrophoblast cells (*s*) of the placenta; in amnion (*a*), trophoblast cells of chorion (*t*) and decidua (*d*) in the fetal membranes; and in endothelial cells (*e*) of cord vessels. No difference was found in intensity or prevalence of the staining between IUGR and control groups.

Strong immunostaining for S100β was localized in the syncytiotrophoblast cells of chorionic villi, in the amnion, in trophoblast cells of chorion and the decidua of fetal membranes, and in endothelial cells of the umbilical arteries and vein. No difference in the prevalence of positive cells or distribution or intensity of the staining was found in either the placental tissues or cord specimens between uncomplicated and IUGR pregnancies with or without redistribution of blood flow (Fig. 3).

DISCUSSION

As already reported we found that S100β is present in considerable amount in the fetal circulation, whereas it is undetectable in plasma of pregnant women as demonstrated in healthy adults. In our study we showed that S100β concentration was higher in growth-retarded fetuses with redistribution of blood flow and correlated with the degree of fetal hemodynamic impairment as indicated by middle cerebral artery PI and umbilical artery to

middle cerebral artery PI ratio. In contrast, IUGR fetuses without redistribution of blood flow showed S100β levels similar to those found in fetuses with normal growth. Thus impairment of fetal growth *per se* does not affect S100β concentrations in the fetal circulation, as also demonstrated by lack of correlation between protein levels and birth weight.

Although in our series neurologic outcome, at least at the time of hospital discharge, was normal, we hypothesize that increased S100β levels in IUGR fetuses with redistribution of fetal circulation may reflect fetal cellular brain damage owing to chronic hypoxia. In the last decade, several studies demonstrated that Doppler findings, and particularly the ratios of Doppler FVWs in cerebral and peripheral vessels, are reliable indices of redistribution of fetal blood flow and are correlated with the degree of fetal hypoxia and perinatal outcome (17–19). In IUGR pregnancies with impaired placental perfusion, transfer of oxygen and nutrients from the mother to the fetus is reduced, leading to a cardiovascular response characterized by a redistribution of cardiac output to maintain oxygen supply to the brain, heart, and adrenal at the expense of visceral organs to preserve their function. However, despite this hemodynamic mechanism, adverse effects of hypoxemia on brain maturation have been demonstrated in clinical and histologic studies. Disturbances in the development of fetal behavioral states, defined as expression of brain injury, have been reported (20–22), and ultrastructural studies in human and animal have shown that chronic intrauterine stress affected the functional maturation of various organ systems, including fetal brain (23). Alterations were also observed in developing neuronal peripheral tissues of growth-retarded fetuses regarding axons and Schwann cells (23), which express S100β (3). In our study the loss of the correlation between gestational age and S100β in growth-retarded fetuses supports the hypothesis of an alteration in the normal process of development and maturation of the brain in this condition.

On the other hand, we cannot exclude the possibility that increased S100β concentration in peripheral blood of growth-retarded fetuses with decreased cerebral vessel resistance may derive from an increased leaking of the protein. Redistribution of fetal circulation is related to hypoxemia-mediated excessive release of vasoactive agents (24, 25), which, in turn, may alter the permeability of the blood–brain barrier, accounting for increased S100β protein transfer from the tissue to the systemic circulation.

Last, the question of whether high S100β cord levels are the result of the release of S100β primarily from brain tissue or from other organs (placenta, adipose tissue) is an issue that cannot be solved definitively at the present time. Lack of differences in S100β protein in the placental tissues and the low levels detected in the maternal plasma in IUGR pregnancies argues, at least, against a placental origin.

In conclusion, our study shows that circulating S100β is increased in growth-retarded fetuses and correlates with fetal FVWs. Although the appearance in the blood of preterm infants with neurologic sequelae of fetal hypoxia markers such as nucleated erythrocytes and uric acid has been already reported (26, 27), to our knowledge the release in the systemic circulation of brain constituents such as S100β, which is a direct indicator of active cell damage in the nervous system, has not been reported and thus may be promising. The mechanism that gives rise to increased S100β and its relevance in the monitoring of IUGR fetuses, however, remains to be established.

Acknowledgment. The authors thank Sangtec Medical, Bromma, Sweden, for its technical support.

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