

**The relation of S100beta and metabolic and endocrine responses to acute fetal hypoxemia**

**Avnesh S Thakor<sup>1</sup>, Diego Gazzolo<sup>2,3</sup>, Rosanna Frulio<sup>2</sup>, Dino A Giussani<sup>1</sup>**

<sup>1</sup>*Department of Physiology University of Cambridge, Cambridge, UK*, <sup>2</sup>*Department of Pediatrics and Neuroscience, Giannina Gaslini Children's University Hospital, Genoa I-16147, Italy*, <sup>3</sup>*Department of Maternal Fetal and Neonatal Health, G. Garibaldi Hospital Catania, Italy*

**TABLE OF CONTENTS**

1. *Abstract*
2. *Introduction*
3. *Materials and methods*
  - 3.1. *Surgical preparation*
  - 3.2. *Experimental protocol*
    - 3.2.1. *Blood sampling regimen*
    - 3.2.2. *Hormone analyses*
      - 3.2.2.1. *S100beta assay*
      - 3.2.2.2. *ACTH assay*
      - 3.2.2.3. *Cortisol assay*
      - 3.2.2.4. *NPY assay*
      - 3.2.2.5. *Catecholamine assay*
  - 3.3. *Data and statistical analyses*
  4. *Results*
    - 4.1. *Fetal arterial blood gas and metabolic status*
    - 4.2. *Fetal endocrine status*
    - 4.3. *Plasma S100beta during acute hypoxemia*
    - 4.4. *The relationship between plasma S100beta and stress hormone concentrations*
    - 4.5. *The relationship between plasma S100beta and blood glucose and lactate concentrations*
  5. *Discussion*
  6. *Acknowledgements*
  7. *References*

**1. ABSTRACT**

Elevations in S100beta protein in umbilical cord blood have been proposed as a reproducible marker of fetal stress, leading to cell damage within the central nervous system. However, it remains unknown whether fetal S100beta concentrations correlate with established endocrine and metabolic indices of fetal distress. Hence, in the late gestation ovine fetus, plasma concentrations of S100beta, adrenocorticotrophic hormone (ACTH), cortisol, neuropeptide Y (NPY), and catecholamines and blood concentrations of glucose and lactate were measured during acute hypoxemia. Under general anesthesia, 5 sheep fetuses were chronically instrumented with catheters and subjected 5 days later to 1h normoxia, 0.5h hypoxemia and 1h recovery. Plasma samples were taken during each experimental period. Hypoxemia induced significant falls in  $P_aO_2$  with increases in fetal plasma concentrations of ACTH, cortisol, catecholamines and NPY, and elevations in blood glucose and lactate, all of which showed significant positive relationships with fetal plasma S100beta concentrations. Hence, evaluation of S100beta may provide a valuable clinical tool in the assessment of fetal well-being in suspected complicated pregnancies.

**2. INTRODUCTION**

Clinical tools for fetal surveillance during pregnancy have relied heavily upon fetal heart rate monitoring and Doppler blood flow velocimetry (1-4). Since their introduction, surprisingly, few additional techniques have been implemented in routine perinatal clinical practice. A potential tool for improving the surveillance of fetal health in compromised pregnancy is S100beta. This protein is a member of a multigenic family of calcium-binding proteins characterized by a helix-loop-helix structure. The family was first discovered in 1965 following the identification of a protein fraction, from brain extracts, which was soluble in a 100 % saturated solution of ammonium sulfate (5). S100beta has a low molecular weight (~10,000 Da) and a half-life in the circulation of about an hour (6). Results from recent studies have shown that S100beta concentrations are increased in cord blood of fetuses with brain damage [for review, see Michetti and Gazzolo (2003) (7)] and in pregnancies complicated by intrauterine growth retardation (IUGR; 8-9). Hence, elevations in umbilical blood of the protein have been suggested to be a reliable marker of stress to the central nervous system (CNS) during the

perinatal period in complicated pregnancies. However, accumulating evidence indicates that fetal S100beta concentrations can also be elevated during the stress of routine vaginal delivery (10) or during acute hypoxic stress of the type and magnitude that can occur during normal labor (11). Hence, elevations in S100beta concentrations may provide the obstetrician with an early marker of fetal hypoxic/ischemic distress, prior to permanent compromise of the fetal CNS, rather than a marker of irreversible damage to the fetal CNS. From this important point of view, assessment of S100beta may be a valuable clinical tool in the early assessment of fetal well being in suspected complicated pregnancy. Previously, we have reported that fetal exposure to an episode of acute hypoxia does elicit significant elevations in fetal plasma S100beta concentration that correlate significantly with hemodynamic changes indicative of fetal hypoxia (11). However, whether elevations in S100beta concentrations actually correlate with established endocrine and metabolic indices of fetal distress is still to be determined. The focus of this manuscript is therefore an extension of our previous work to address this specific aim.

The hypothalamo-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) constitute the efferent limbs of the stress-system (12), and have been shown to be functional in the late gestation fetus. One of the most common stressful conditions that the fetus faces during pregnancy is an episode of hypoxemia. Such a challenge is known to increase plasma concentrations of adrenocorticotrophic hormone (ACTH), cortisol (13-14), neuropeptide Y (NPY) (15) and catecholamines (16), demonstrating activation of the HPA axis and the SNS, respectively. In addition, the rise in fetal plasma catecholamines contributes to the stimulation of fetal metabolic responses during acute hypoxemia (17). These include hyperglycemia, which increases glucose availability to the fetal tissues (18), and lactic acidemia, which allows lactate to be utilized as a metabolic substrate by the fetal myocardium and liver (19-20).

In the present study, we have tested the hypothesis that S100beta correlates with such endocrine and metabolic indices of fetal distress during fetal exposure to acute hypoxemia. The hypothesis was tested using the chronically instrumented ovine fetus during late gestation, surgically prepared for long-term recording in unanesthetized conditions as our experimental model. This avoided confounding problems in the interpretation of data common to acute preparations, such as the stresses of surgery and anesthesia.

### **3. MATERIALS AND METHODS**

#### **3.1. Surgical preparation**

All procedures were performed under the UK Animals (Scientific Procedures) Act 1986 and were approved by the Ethical Review Committee of the University of Cambridge. Five Welsh Mountain sheep fetuses were surgically instrumented with vascular catheters for long-term recording at 121±1 days of gestation (term is *ca.* 145 days) using strict aseptic

conditions as previously described in detail (11). During recovery, ewes were housed in individual pens in rooms with a 12 h : 12 h / light : dark cycle where they had free access to hay and water and were fed concentrates twice daily (100 g sheep nuts no. 6; H & C Beart Ltd, Kings Lynn, UK). Antibiotics were administered daily to the ewe (0.20-0.25 mg.kg<sup>-1</sup> IM Depocillin; Mycofarm, Cambridge, UK) and fetus IV and into the amniotic cavity (150 mg.kg<sup>-1</sup> Penbritin; SmithKline Beecham Animal Health, Welwyn Garden City, Hertfordshire, UK). The ewes also received 2 days of post-operative analgesia (10-20 mg.kg<sup>-1</sup> oral Phenylbutazone; Equipalozone paste, Arnolds Veterinary Products Ltd, Shropshire, UK). Generally, normal feeding patterns were restored within 48 h of recovery. Following 72 h of post-operative recovery, ewes were transferred to metabolic crates where they were housed for the remainder of the protocol. Whilst on the metabolic crates, the patency of the fetal catheters was maintained by a slow continuous infusion of heparinized saline (80 IU heparin.ml<sup>-1</sup> at 0.1 ml.h<sup>-1</sup> in 0.9 % NaCl) containing antibiotic (1 mg.ml<sup>-1</sup> benzylpenicillin; Crystapen, Schering-Plough, Animal Health Division, Welwyn Garden City, UK).

#### **3.2. Experimental protocol**

Following at least 5 days of post-operative recovery, all fetuses were subjected to an experimental protocol which consisted of a 2.5 h period divided into: 1 h normoxia, 0.5 h hypoxemia and 1 h recovery. Acute hypoxemia in the fetus was induced by maternal inhalational hypoxia. In brief, a large transparent respiratory hood was placed over the ewes' head into which air was passed at a rate of *ca.* 50 l.min<sup>-1</sup> for the 1 h period of normoxia. Following this control period, acute fetal hypoxemia was induced for 30 min by changing the concentrations of gases breathed by the ewe to 6 % O<sub>2</sub> in N<sub>2</sub> with small amounts of CO<sub>2</sub> (15 l.min<sup>-1</sup> air : 35 l.min<sup>-1</sup> N<sub>2</sub> : 1.5-2.5 l.min<sup>-1</sup> CO<sub>2</sub>). This mixture was designed to reduce fetal P<sub>a</sub>O<sub>2</sub> to *ca.* 10 mmHg whilst maintaining P<sub>a</sub>CO<sub>2</sub>. Following the 0.5 h period of hypoxemia, the ewe was returned to breathing air for the 1 h recovery period. At the end of the experimental protocol, the ewes and fetuses were humanely killed using a lethal dose of sodium pentobarbitone (200 mg.kg<sup>-1</sup> I.V. Pentoject; Animal Ltd, York, UK). Post-mortems were carried out at 130±1 days of gestation during which time the positions of the implanted catheters were confirmed.

#### **3.2.1. Blood sampling regimen**

During the acute hypoxemia protocol, descending aortic blood samples (0.3 ml) were taken using sterile techniques from the fetus at set time intervals to determine arterial blood gas status (ABL5 Blood Gas Analyzer, Radiometer, Copenhagen, Denmark; measurements corrected to 39.5°C). Blood glucose and lactate concentrations were measured using an automated analyzer (Yellow Springs 2300 Stat Plus Glucose / Lactate Analyzer; YSI Ltd, Farnborough, UK). An additional 4 ml of arterial blood was withdrawn at set intervals for hormone analyses. These samples were collected under sterile conditions into chilled heparin tubes (2 ml Li<sup>+</sup> / heparin tubes; L. I. P. Ltd, Shipley, West Yorkshire, UK) containing reduced glutathione (4 nmol per tube; G-4251;

Sigma Chemicals, UK) and EGTA (5 nmol per tube; E-4378; Sigma Chemicals, UK) for catecholamines analysis or into chilled EDTA tubes (2 ml K<sup>+</sup> / EDTA; L. I. P. Ltd., Shipley, West Yorkshire, UK) for ACTH, cortisol, NPY or S100beta analyses. All samples were then centrifuged at 4480g for 4 min at 4°C. The plasma obtained was then dispensed into pre-labelled tubes and the samples were stored at -80°C until analysis. All hormone measurements were performed within 2 months of sample collection.

### **3.2.2. Hormone analyses**

#### **3.2.2.1. S100beta assay**

Fetal plasma concentrations of S100beta were measured in all samples by an immunoluminometric assay (Liaison Sangtec 100, AB Sangtec Medical, Bromma, Sweden). According to the manufacturer's instructions, this assay is specific for the beta subunit of the S100 protein as defined by the three monoclonal antibodies SMST 12, SMSK 25 and SMSK 28 (21). Each measurement was performed in duplicate according to the manufacturer's recommendations and the averages were reported. The sensitivity of the assay ( $B_0 \pm 3SD$ ) was 0.02  $\mu\text{g.l}^{-1}$ , and the intra-assay and inter-assay coefficients of variation were 5.5 % and 10.1 %, respectively for concentrations ranging between 0.3 and 4.2  $\mu\text{g.l}^{-1}$ .

#### **3.2.2.2. ACTH assay**

Fetal plasma ACTH concentrations were measured using commercially available double antibody <sup>125</sup>I RIA kit (DiaSorin Inc., Stillwater, Minnesota, USA) as previously described (22). Bound and free ACTH fractions were separated by immuno-precipitation with the second antibody-precipitating complex (500  $\mu\text{l}$ ). The lower limit of detection of the assay (90 % bound:free<sup>-1</sup>) was between 10-25  $\text{pg.ml}^{-1}$ . The intra-assay coefficients of variation for 3 plasma pools (mean concentration: 69, 281 and 600  $\text{pg.ml}^{-1}$ ) were 12.5, 6.3 and 8.1 %, respectively. The inter-assay coefficients of variation for 2 plasma pools (mean concentration: 36 and 136  $\text{pg.ml}^{-1}$ ) were 6.0 and 6.7 %, respectively. The anti-ACTH antiserum showed less than 0.01% cross-reactivity against  $\alpha$ -MSH, beta-endorphin, beta-lipotropin, leucine enkephalin, methionine enkephalin, bombesin, calcitonin, parathyroid hormone, follicle stimulating hormone, arginine vasopressin, oxytocin and substance P.

#### **3.2.2.3. Cortisol assay**

Fetal plasma cortisol concentrations were measured by RIA using tritium-labelled cortisol as tracer, as previously described (23). Duplicate 20  $\mu\text{l}$  plasma samples were taken from previously unthawed K<sup>+</sup>/EDTA-treated sample aliquots and were extracted twice in 300  $\mu\text{l}$  ethanol. Bound and free cortisol fractions were separated using stirred dextran-coated charcoal suspension (300  $\mu\text{l}$ ; 0.075 g.% dextran and 0.75 g.% activated charcoal in PBS). Recoveries averaged 90 %. A standard curve was produced by the computer using a spline curve fit, and cortisol concentrations were calculated for each sample. The lower limit of detection of the assay was between 1.0-1.5  $\text{ng.ml}^{-1}$ . The intra-assay coefficient of variation was 5.3 % for a mean value of 13  $\text{ng.ml}^{-1}$ . The inter-assay coefficients of variation for 2 plasma pools (mean concentration: 13 and 28  $\text{ng.ml}^{-1}$ )

were 13.6 and 11.4 %, respectively. The cross-reactivities of the antiserum at 50 % binding with other cortisol-related compounds were: 0.5 % cortisone; 2.3 % corticosterone; 0.3 % progesterone; 4.6 % deoxycortisol.

#### **3.2.2.4. NPY assay**

Fetal plasma NPY concentrations were measured by radioimmunoassay as previously described in detail (15,24). All samples were analysed in duplicate at the same time. The assay used rabbit antiserum (produced in horse) and <sup>125</sup>I-labelled porcine peptide. Separation of the free and bound fractions was performed with dextran-coated charcoal. The assay was validated for use with ovine plasma using stripped sheep plasma. The assay could detect less than 1 pmol.l<sup>-1</sup> (95 % confidence interval). The interassay coefficient of variation was 6.8 %. There was no detectable cross-reactivity of the anti-NPY antiserum with the peptide YY.

#### **3.2.2.5. Catecholamine assay**

Fetal plasma epinephrine and norepinephrine concentrations were measured by high-performance liquid chromatography (HPLC) using electrochemical detection as previously described in detail (22). The samples were prepared by absorption of 250  $\mu\text{l}$  of plasma onto acid-washed alumina and 20  $\mu\text{l}$  aliquots of the 100  $\mu\text{l}$  perchloric acid elutes were injected onto the column. Dihydroxybenzylamine was added as the internal standard to each plasma sample before absorption. The limit of sensitivity for the assay was 20  $\text{pg.ml}^{-1}$  for epinephrine and norepinephrine. Recovery ranged from 63 % to 97 % and all catecholamine values were corrected for their respective recovery. The interassay coefficients of variation for epinephrine and norepinephrine were 7.3 % and 6.2 %.

### **3.3. Data and statistical analyses**

Values for all arterial blood gas, metabolic and endocrine variables are expressed as mean $\pm$ S.E.M. at 0 (N0) and 45 (N45) min of normoxia, 15 (H15) and 30 (H30) min of hypoxemia, and at 30 (R30) and 60 (R60) min of recovery. All measured variables were first analyzed for normality of distribution, and then assessed using One-way ANOVA with Repeated Measures (RM; Sigma-Stat; SPSS Inc., Chicago, IL, USA) comparing the effect of time (normoxia vs. hypoxemia / recovery). Where a significant effect was indicated, the *post hoc* Tukey test was used to isolate the statistical differences. In all individual fetuses, the relationships between parallel measurements of plasma concentrations of S100beta and plasma concentrations of ACTH, cortisol, NPY and catecholamines, and blood concentrations of glucose and lactate, were assessed using the Pearson Product Moment correlation (r). For all comparisons, statistical significance was accepted when P < 0.05.

## **4. RESULTS**

### **4.1. Fetal arterial blood gas and metabolic status**

Basal values for arterial blood gas and metabolic status were with normal range for Welsh Mountain sheep fetuses at *ca.* 125 days of gestation (Table 1). In all fetuses, acute hypoxemia induced significant falls in P<sub>a</sub>O<sub>2</sub>

**Table 1.** Fetal arterial blood gas and metabolic status

	Normoxia		Acute Hypoxaemia		Recovery	
	N0	N45	H15	H30	R30	R60
pH <sub>a</sub> (mmHg)	7.35±0.01 54.3±1.5	7.35±0.01 54.6±1.9	7.36±0.01 52.2±1.4	7.21±0.02* 54.2±0.9	7.24±0.02* 52.3±1.2	7.30±0.02* 52.7±1.4
P <sub>a</sub> O <sub>2</sub> (mmHg)	20.5±0.8	20.5±0.9	9.7±1.0*	10.0±0.8*	20.3±2.2	19.2±0.9
[Blood Glucose] (mM)	0.77±0.09	0.81±0.08	1.40±0.13*	1.56±0.20*	1.52±0.32*	1.42±0.35*
[Blood Lactate] (mM)	0.92±0.08	0.98±0.09	3.52±0.54*	5.08±0.82*	5.62±0.90*	4.99±1.11*

Values represent the mean±S.E.M. at 0 (N0) and 45 (N45) min of normoxia, at 15 (H15) and 30 (H30) min of hypoxemia, and at 30 (R30) and 60 (R60) min of recovery for fetuses exposed to 0.5 hour of hypoxemia (n=5). Significant differences: \*P < 0.05, normoxia vs. hypoxemia or recovery (One-way RM ANOVA with *post hoc* Tukey test). pH<sub>a</sub>, arterial pH; P<sub>a</sub>CO<sub>2</sub>, arterial partial pressure of CO<sub>2</sub>; P<sub>a</sub>O<sub>2</sub>, arterial partial pressure of O<sub>2</sub>; [Blood Glucose], blood glucose concentration; [Blood Lactate], blood lactate concentration.

**Table 2.** Fetal endocrine status

	Normoxia		Acute Hypoxemia		Recovery	
	N0	N45	H15	H30	R30	R60
ACTH(pg.ml <sup>-1</sup> )	22±1	26±4	786±120*	992±187*	329±56*	211±48
Cortisol(ng.ml <sup>-1</sup> )	14±2	14±2	42±7*	50±9*	39±10*	27±6
Neuropeptide Y(pmol.l <sup>-1</sup> )	39±6	45±7	82±13*	88±13*	64±9	55±5
Adrenaline (pg.ml <sup>-1</sup> )	89±41	84±32	1511±879*	2930±1396*	688±495	189±73
Norepinephrine(pg.ml <sup>-1</sup> )	1305±634	1167±405	4426±2186*	3614±1095*	1538±560	1633±603

Values represent the mean±S.E.M. at 0 (N0) and 45 (N45) min of normoxia, at 15 (H15) and 30 (H30) min of hypoxemia, and at 30 (R30) and 60 (R60) min of recovery for fetuses exposed to 0.5 hour of hypoxemia (n=5). Significant differences: \*P < 0.05, normoxia vs. hypoxemia or recovery (One-way RM ANOVA with *post hoc* Tukey test). [ACTH], plasma ACTH concentration; [Cortisol], plasma cortisol concentration; [NPY], plasma neuropeptide Y concentration; [Epinephrine], plasma epinephrine concentration; [Norepinephrine], plasma norepinephrine concentration.

and pH<sub>a</sub>, without any alteration to P<sub>a</sub>CO<sub>2</sub>. In addition, blood glucose and lactate concentrations significantly increased during the challenge (P < 0.05; Table 1). During recovery, P<sub>a</sub>O<sub>2</sub> returned to basal values while pH<sub>a</sub> remained significantly depressed and blood glucose and lactate concentrations remained elevated from basal values (Table 1).

#### 4.2. Fetal endocrine status

Basal values for plasma concentrations of ACTH, cortisol, NPY and catecholamines were with normal range for Welsh Mountain sheep fetuses at *ca.* 125 days of gestation. In all fetuses, acute hypoxemia significantly increased plasma concentrations of ACTH, cortisol, NPY, epinephrine and norepinephrine (P < 0.05; Table 2). By the end of recovery, plasma concentrations of all hormones returned towards basal values (Table 2).

#### 4.3. Plasma S100beta during acute hypoxemia

Basal fetal plasma S100beta concentrations averaged 9.6±2.8 µg.l<sup>-1</sup>. During acute hypoxemia, S100beta concentrations increased significantly by 24.9±2.9 % (P < 0.05; Figure 1). This elevation in S100beta concentration persisted until the end of the experimental protocol, with values remaining significantly elevated from baseline by 21.9±4.1 % (P < 0.05; Figure 1).

#### 4.4. The relationship between plasma S100beta and stress hormone concentrations

Correlation analysis for all values from all individual fetuses obtained during normoxic and hypoxic conditions revealed significant positive relationships between the percent change in plasma S100beta concentration and the percent increment in

plasma levels of ACTH (r=0.68, n=20, P<0.05; Figure 2), cortisol (r=0.75, n=20, P<0.05; Figure 2), NPY (r=0.61, n=20, P<0.05; Figure 3) epinephrine (r=0.57, n=20, P<0.05; Figure 3) and norepinephrine (r=0.54, n=20, P<0.05; Figure 3).

#### 4.5. The relationship between plasma S100beta and blood glucose and lactate concentrations

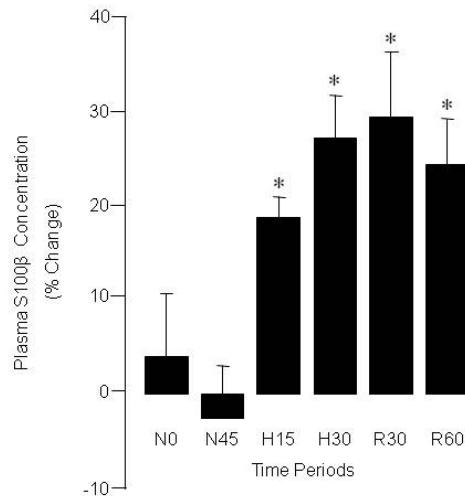
Correlation analysis for all individual fetuses during all values obtained during normoxic and hypoxic conditions between the percentage change in S100beta concentration and the percentage change in blood glucose (r=0.65, n=20, P<0.05; Figure 4) and lactate (r=0.87, n=20, P<0.05; Figure 4) concentrations revealed significant positive relationships.

## 5. DISCUSSION

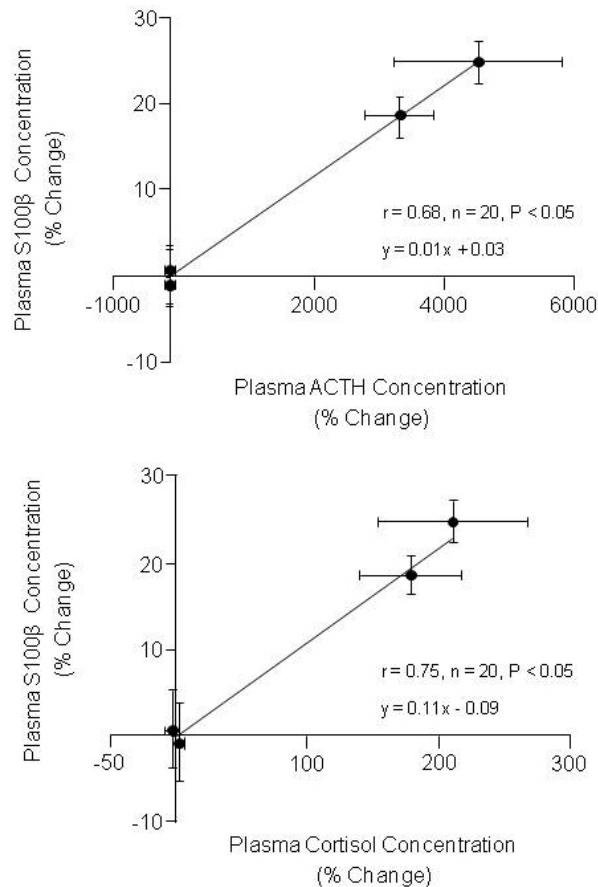
These data extend our previous study (11) to show that increases in plasma S100beta concentrations during acute hypoxia in the fetus are significantly related to fetal plasma ACTH, cortisol, NPY and catecholamines, in addition to fetal blood concentrations of glucose and lactate. The data from this study therefore support the hypothesis tested that S100beta concentrations correlate with endocrine and metabolic indices of fetal distress, in particular with those which primarily result from activation of both limbs of the stress system.

The fetal defense to acute (25-26) or chronic (27-28) stress involves the activation of the stress system. The main central components of the stress system are the corticotrophin-releasing hormone (CRH) neurones and the locus coeruleus-norepinephrine autonomic complex [LC/NE;

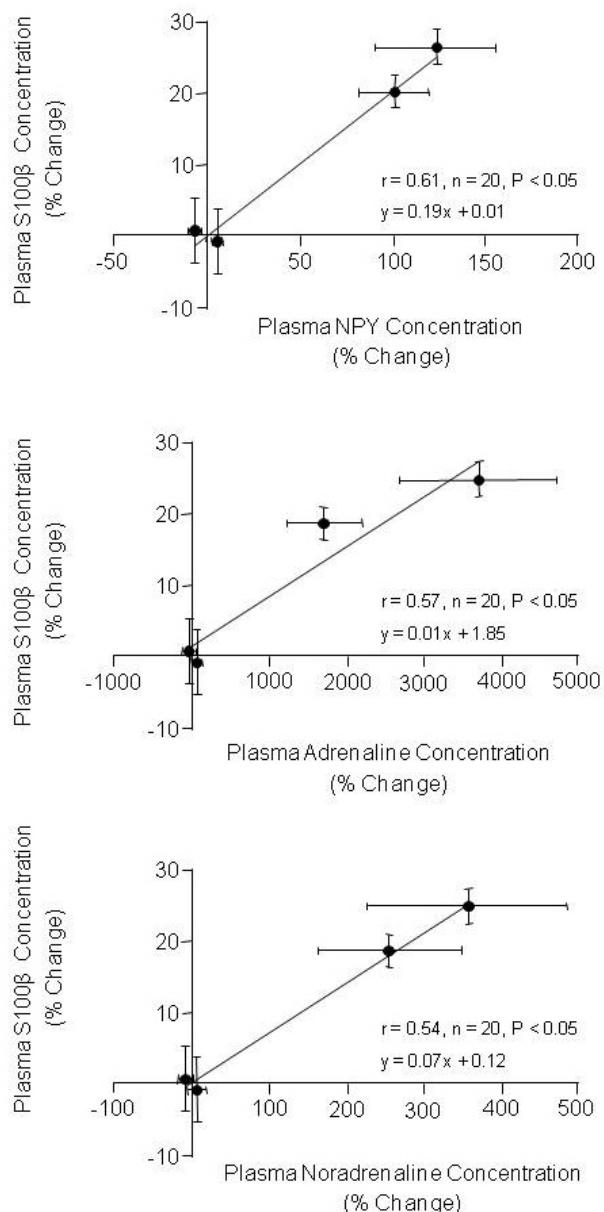
## S100beta and fetal indices of hypoxic stress



**Figure 1.** Plasma S100beta concentrations in response to acute hypoxemia. Bars represent the mean $\pm$ S.E.M. for the percentage change from baseline in plasma S100beta concentration at 0 (N0) and 45 (N45) min of normoxia, 15 (H15) and 30 (H30) min of hypoxemia, and at 30 (R30) and 60 (R60) min of recovery for fetuses exposed to 0.5 hour hypoxemia (n=5). Significant differences: \*P < 0.05, for normoxia vs. hypoxemia or recovery (One-way RM ANOVA with *post hoc* Tukey test).



**Figure 2.** The relationship between plasma S100beta and HPA axis hormones. Values represent the mean $\pm$ S.E.M. (x and y) for all paired plasma S100beta and plasma ACTH and cortisol concentrations (n=20) from all individual fetuses obtained during basal and hypoxic conditions (n=5). Each linear relationship is described by the Pearson Product Moment correlation coefficient (r). In the equations 'n' represents the overall number of values, which were correlated for all individual fetuses.

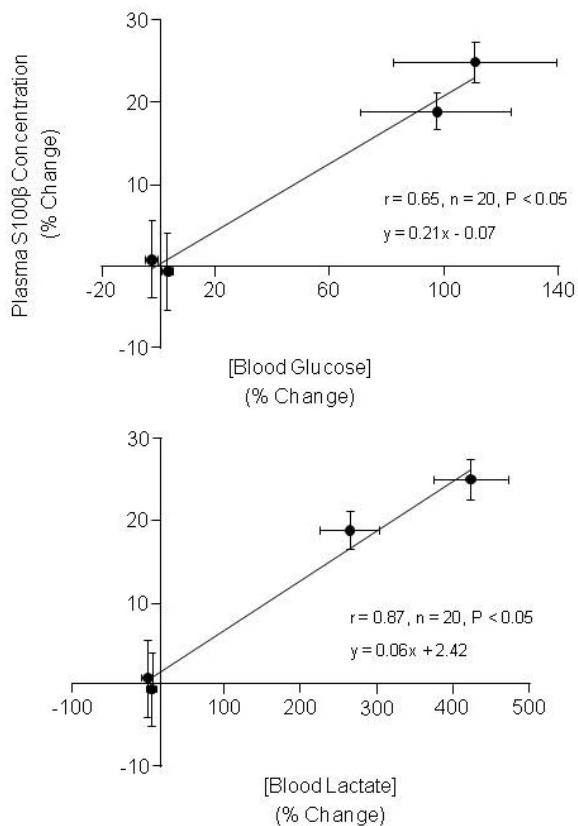


**Figure 3.** The relationship between plasma S100beta and SNS hormones. Values represent the mean $\pm$ S.E.M. (x and y) for all paired plasma S100beta and NPY, epinephrine and norepinephrine concentrations (n=20) from all individual fetuses obtained during basal and hypoxic conditions (n=5). Each linear relationship is described by the Pearson Product Moment correlation coefficient (r). In the equations 'n' represents the overall number of values, which were correlated for all individual fetuses.

(12)]. The pituitary-adrenocortical and sympathoadrenomedullary axes represent the peripheral efferent limbs of the stress-response system, respectively. Both limbs have been shown to be functional in the ovine fetus in late gestation (14-25), and their neuroendocrine responses have been well documented in this animal model during episodes of acute hypoxemia (29-30).

During acute hypoxemia, the release of ACTH from the anterior pituitary gland in late gestation fetal sheep is known to be the result of hypothalamic stimulation since hypothalamo-pituitary disconnection abolishes the plasma

ACTH response (30). This is further supported by evidence showing an increase in the levels of CRH mRNA in the hypothalamus and pro-opiomelanocortin (POMC) mRNA in the pituitary gland of the mature ovine fetus during acute hypoxemia (31). Stimulation of the HPA axis during hypoxia serves to promote an increase in fetal plasma cortisol (25,29,32). The increased concentrations of the steroid facilitate fetal survival during episodes of oxygen deprivation by amplifying the actions of the sympathetic nervous system (12), thereby targeting the delivery of oxygen and nutrients to essential circulations, such as those perfusing the fetal heart and brain. Endogenous



**Figure 4.** The relationship between plasma S100beta and metabolic indices. Values represent the mean $\pm$ S.E.M. (x and y) for all paired plasma S100beta and blood glucose and lactate concentrations (n=20) from all individual fetuses obtained during basal and hypoxic conditions (n=5). Each linear relationship is described by the Pearson Product Moment correlation coefficient (r). In the equations 'n' represents the overall number of values, which were correlated for all individual fetuses.

glucocorticoids, such as cortisol, are synthesised in the zona fasciculata of the adrenal cortex by the conversion of cholesterol to 21 C steroid hormones. Increased output of cortisol by the fetal adrenal gland results from both an increased drive to the fetal zona fasciculata by increased plasma concentrations of ACTH, and increased fetal adrenal responsiveness to ACTH (33).

During acute hypoxemia plasma concentrations of catecholamines (16) and NPY (15) are increased in the fetal circulation. The origin of the increase in fetal plasma catecholamines during acute hypoxemia has been shown to be primarily from the fetal adrenal gland since adrenal demedullation completely abolished the rise in plasma epinephrine concentration and reduced the norepinephrine response to 10 % of normal (34). Hence, an elevation in the plasma concentration of catecholamines is a good index of SNS activation during stimulated conditions. NPY is co-localised with norepinephrine in sympathetic nerve terminals and it is also present in the adrenal medulla in mammals (35). At the synaptic terminal, NPY is stored in large dense

vesicles and it may be co-released with norepinephrine from sympathetic varicosities depending on the frequency and pattern of nerve stimulation (36). Elevations of plasma concentrations of NPY in the present study not only confirm SNS activation, but they provide an even better measure of increased SNS activity, since unlike norepinephrine, the peptide lacks a synaptic reuptake mechanism (37).

This activation of the SNS is also largely responsible for the metabolic responses observed in the late gestation fetus during the acute hypoxic challenge. Increased sympathetic outflow has been shown to inhibit insulin release from the fetal pancreas, thereby decreasing glucose uptake and utilization by the fetal tissues (18). In addition, catecholamines are also known to mobilize and release glucose from glycogen stores in the fetal liver (38), further contributing to the increase in circulating blood glucose concentrations. The fetal lactic acidemia results from anaerobic metabolism of glucose in hypoxic fetal tissues, particularly in the periphery where blood flow and oxygen delivery are markedly declined as a consequence of fetal blood flow redistribution (32). Therefore, the hyperglycaemia and lactic acidemia measured in the present also provide an indirect index of the activation of the SNS.

Michetti & Gazzolo (7) reported that the expression of S100beta increases in biological fluids in the fetus during complicated intrauterine conditions, at times when other clinical assessments miss the adverse diagnosis. Infants with moderate and severe hypoxic ischemic encephalopathy after perinatal asphyxia also have significantly higher serum S100beta levels on the first two postnatal days relative to healthier infants (39). Thus, elevations in S100beta have been proposed to represent an index of susceptibility to hypoxic/ischemic brain damage during the perinatal period in complicated pregnancy. The results of the present study extend this view to show that significant elevation in S100 beta protein can occur in fetal plasma following the onset of an acute hypoxic challenge of short duration and of the magnitude that may occur during the actual processes of labor and delivery in normal uncomplicated pregnancy. Human clinical studies also report that plasma concentrations of S100beta are elevated in healthy newborns who were delivered vaginally compared to those that were delivered by Cesarean section (10). Combined, these observations therefore indicate that elevations in plasma concentrations of S100beta may be a useful tool in obstetric practice for the early detection of acute fetal distress that involves activation of the fetal HPA and sympathoadrenomedullary axes, such as hypoxemia. This may provide an important window for clinical intervention and prevent sustained hypoxic/ischemic distress to the fetus, prior to permanent compromise of the fetal CNS.

In conclusion, this study reports that during a controlled period of acute fetal hypoxemia, there is an immediate and sustained increase in plasma S100beta concentrations, which correlates strongly with endocrine and metabolic indices of fetal distress. Evaluation of changes in fetal S100beta concentrations may provide a valuable clinical tool in the early assessment of fetal well being in suspected complicated pregnancy.

## 6. ACKNOWLEDGMENTS

Authors Thakor, Gazzolo, Frulio and Giussani equally contributed to this article. We are thankful to Mr. Scott Gentle and Mrs. Sue Nicholls for their invaluable help with the maintenance of the animals. We are thankful for the financial support for this research from: The International Journal of Experimental Pathology, The Lister Institute for Preventive Medicine and The Stella Cometa Foundation, Rome, Italy.

## 7. REFERENCES

1. Z. Hajek: Foetal ECG in the prediction of intrapartum hypoxia. *Cas Lek Cesk* 144, 168-171 (2005)
2. J.A. Spencer and P. Johnson: Fetal heart rate variability changes and fetal behavioural cycles during labour. *Br J Obstet Gynaecol* 93, 314-321 (1986)
3. Z. Alfirevic, D. Devane and G. M. Gyte: Continuous cardiotocography (CTG) as a form of electric fetal monitoring (EFM) for fetal assessment during labour. *Cochrane Database Syst Rev* 19, 3CD006066 (2006)
4. S. Miyadahira, R. P. Francisco and M. Zugaib: How to use the Doppler velocimetry for the screening of fetal welfare in pre-parturient period. *Rev Assoc Med Bras* 51, 126 (2005)
5. B. W. Moore: A soluble protein characteristic of the nervous system. *Biochem Biophys Res Commun* 19, 739-744 (1965)
6. H. Jonsson, P. Johnsson, P. Hoglund, C. Alling and S. Blomquist: Elimination of S100B and renal function after cardiac surgery. *J Cardiothorac Vasc Anesth* 14, 698-701 (2000)
7. F. Michetti and D. Gazzolo: S100B testing in pregnancy. *Clin Chim Acta* 335, 1-7 (2003)
8. D. Gazzolo, E. Marinoni, R. di Iorio, M. Lituani, P.L. Bruschettini and F. Michetti: Circulating S100beta protein is increased in intrauterine growth-retarded fetuses. *Pediatr Res* 51, 215-219 (2002)
9. D. Gazzolo, E. Marinoni, R. di Iorio, M. Lituania, M. Marras, P. L. Bruschettini, R. Frulio, F. Michetti, F. Petraglia and P. Florio: High maternal blood S100B concentrations in pregnancies complicated by intrauterine growth restriction and intraventricular hemorrhage. *Clin Chem* 52, 819-826 (2006)
10. J.W. Wirds, A. E. Duyn, S. D. Geraerts, E. Preijer, J. A. Diemen-Steenvoorde, J. H. Van Leeuwen, F. J. Haas, W. B. Gerritsen, A. De Boer and J. A. Leusink: S100 protein content of umbilical cord blood in healthy newborns in relation to mode of delivery. *Arch Dis Child Fetal Neonatal Ed* 88, F67-F69 (2003)
11. D. A. Giussani, A. S. Thakor, R. Frulio and D. Gazzolo: Acute hypoxia increases S100 $\beta$  protein in association with blood flow redistribution away from peripheral circulations in fetal sheep. *Pediatr Res* 58, 179-184 (2005)
12. C. Tsigos C and G. P. Chrousos: Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 53, 865-871 (2002)
13. K. Akagi and J. R. Challis: Hormonal and biophysical responses to acute hypoxemia in fetal sheep at 0.7-0.8 gestation: *Can J Physiol Pharmacol* 68, 1527-1532 (1990)
14. C. T. Jones, K. Boddy, J. S. Robinson and J. G. Ratcliffe: Developmental changes in the responses of the adrenal glands of foetal sheep to endogenous adrenocorticotrophin, as indicated by hormone responses to hypoxaemia. *J Endocrinol* 72, 279-292 (1977)
15. A. J. Fletcher, C. M. Edwards, D. S. Gardner, A. L. Fowden and D. A. Giussani: Neuropeptide Y in the sheep fetus: effects of acute hypoxemia and dexamethasone during late gestation. *Endocrinology* 141, 3976-3982 (2000)
16. C. T. Jones and R. O. Robinson: Plasma catecholamines in foetal and adult sheep. *J Physiol* 248, 15-33 (1975)
17. C.T. Jones and J. W. Ritchie: Endocrine and metabolic changes associated with periods of spontaneous hypoxia in fetal sheep. *Biol Neonate* 29, 286-293 (1976)
18. C.T. Jones, J. W. Ritchie and D. Walker: The effects of hypoxia on glucose turnover in the fetal sheep. *J Dev Physiol* 5, 223-235 (1983)
19. D. J. Fisher, M. A. Heymann and A. M. Rudolph: Myocardial oxygen and carbohydrate consumption in fetal lambs in utero and in adult sheep. *Am J Physiol* 238, H399-H405 (1980)
20. C. A. Gleason, C. D. Rudolph, J. Bristow, J. Itsikovitz, A. M. Rudolph: Lactate uptake by the fetal sheep liver. *J Dev Physiol* 7, 177-183 (1985)
21. D. Gazzolo, E. Marinoni, R. di Iorio, M. Lituania, P.L. Bruschettini and F. Michetti: Circulating S100beta protein is increased in intrauterine growth-retarded fetuses. *Pediatr Res* 51, 215-219 (2002)
22. D.S. Gardner, A. J. Fletcher, M. R. Bloomfield, A. L. Fowden and D. A. Giussani: Effects of prevailing hypoxaemia, acidaemia or hypoglycaemia upon the cardiovascular, endocrine and metabolic responses to acute hypoxaemia in the ovine fetus. *J Physiol* 540, 351-366 (2002)
23. P. M. Robinson, R. S. Comline, A. L. Fowden and M. Silver: Adrenal cortex of fetal lamb: changes after hypophysectomy and effects of Synacthen on cytoarchitecture and secretory activity. *Q J Exp Physiol* 68, 15-27. (1983)
24. J. M. Allen, J. C. Yeats, T. E. Adrian and S. R. Bloom: Radioimmunoassay of neuropeptide Y. *Regul Pept* 8, 61-70 (1984)

25. K. Akagi and J. R. Challis: Hormonal and biophysical responses to acute hypoxemia in fetal sheep at 0.7-0.8 gestation. *Can J Physiol Pharmacol* 68, 1527-1532 (1990)

26. C. E. Wood, L. C. Keil and A. M. Rudolph: Hormonal and hemodynamic responses to vena caval obstruction in fetal sheep. *Am J Physiol* 243, E278-E286 (1982)

27. A. D. Bocking, I. C. McMillen, R. Harding R and G. D. Thorburn: Effect of reduced uterine blood flow on fetal and maternal cortisol. *J Dev Physiol* 8, 237-45 (1986)

28. J. Murotsuki, R. Gagnon, S. G. Matthews and J. R. Challis: Effects of long-term hypoxemia on pituitary-adrenal function in fetal sheep. *Am J Physiol* 271, E678-E685 (1996)

29. K. Boddy, C. T. Jones, C. Mantell, J. G. Ratcliffe and J. S. Robinson: Changes in plasma ACTH and corticosteroid of the maternal and fetal sheep during hypoxia. *Endocrinology* 94, 588-591 (1974)

30. I. Z. Ozolins, I. R. Young and I. C. McMillen: Surgical disconnection of the hypothalamus from the fetal pituitary abolishes the corticotrophic response to intrauterine hypoglycemia or hypoxemia in the sheep during late gestation. *Endocrinology* 130, 2438-2445 (1992)

31. S. G. Matthews and J. R. Challis: Levels of proopiomelanocortin and prolactin mRNA in the fetal sheep pituitary following hypoxaemia and glucocorticoid treatment in late gestation. *J Endocrinol* 147, 139-146 (1995)

32. D. A. Giussani, J. A. Spencer, P. J. Moore, L. Bennet and M. A. Hanson: Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *J Physiol* 461, 431-449 (1993)

33. J. R. Challis and A. N. Brooks: Maturation and activation of hypothalamic-pituitary adrenal function in fetal sheep. *Endocr Rev* 10, 182-204 (1989)

34. C. T. Jones, M. M. Roebuck, D. W. Walker and B. M. Johnston: The role of the adrenal medulla and peripheral sympathetic nerves in the physiological responses of the fetal sheep to hypoxia. *J Dev Physiol* 10, 17-36 (1988)

35. J. M. Lundberg, G. Fried, J. Pernow and E. Theodorsson-Norheim: Co-release of neuropeptide Y and catecholamines upon adrenal activation in the cat. *Acta Physiol Scand* 126, 231-238 (1986)

36. S. R. Bloom, A. V. Edwards and C. T. Jones: The adrenal contribution to the neuroendocrine responses to splanchnic nerve stimulation in conscious calves. *J Physiol* 397, 513-526 (1998)

37. J. M. Lundberg: Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. *Pharmacol Rev* 48, 113-178 (1996)

38. R. S. Apatu and R. J. Barnes: Release of glucose from the liver of fetal and postnatal sheep by portal vein infusion of catecholamines or glucagon. *J Physiol* 436, 449-468 (1991)

39. K. Thorngren-Jerneck, C. Alling, A. Herbst, I. Amer-Wahlin and K. Marsal: S100 protein in serum as a prognostic marker for cerebral injury in term newborn infants with hypoxic ischemic encephalopathy. *Pediatr Res* 55, 406-412 (2004)

**Abbreviations:** IUGR: intrauterine growth retardation; CNS: central nervous system; HPA: hypothalamo-pituitary-adrenal; SNS: sympathetic nervous system; ACTH: adrenocorticotrophic hormone; NPY: neuropeptide Y; UK: United Kingdom; IM: intramuscular; IV: intravenous; IU: international units; RIA: radioimmunoassay; HPLC: high-performance liquid chromatography;

**Key Words:** S100beta, Fetus, Hypoxia, Asphyxia

**Send correspondence to:** Dino Giussani, Department of Physiology University of Cambridge, Downing Street, Cambridge, UK, CB2 3EG, Tel: 441223-333894, Fax: 441223-333899, E-mail: dag26@cam.ac.uk

<http://www.bioscience.org/current/vol2E.htm>