

Regional brain blood flow and cerebral hemispheric oxygen consumption during acute hypoxaemia in the llama fetus

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Unlike fetal animals of lowland species, the llama fetus does not increase its cerebral blood flow during an episode of acute hypoxaemia. This study tested the hypothesis that the fetal llama brain maintains cerebral hemispheric O₂ consumption by increasing cerebral O₂ extraction rather than decreasing cerebral oxygen utilisation during acute hypoxaemia. Six llama fetuses were surgically instrumented under general anaesthesia at 217 days of gestation (term *ca* 350 days) with vascular and amniotic catheters in order to carry out cardiorespiratory studies. Following a control period of 1 h, the llama fetuses underwent 3 × 20 min episodes of progressive hypoxaemia, induced by maternal inhalational hypoxia. During basal conditions and during each of the 20 min of hypoxaemia, fetal cerebral blood flow was measured with radioactive microspheres, cerebral oxygen extraction was calculated, and fetal cerebral hemispheric O₂ consumption was determined by the modified Fick principle. During hypoxaemia, fetal arterial O₂ tension and fetal pH decreased progressively from 24 ± 1 to 20 ± 1 Torr and from 7.36 ± 0.01 to 7.33 ± 0.01, respectively, during the first 20 min episode, to 16 ± 1 Torr and 7.25 ± 0.05 during the second 20 min episode and to 14 ± 1 Torr and 7.21 ± 0.04 during the final 20 min episode. Fetal arterial partial pressure of CO₂ (*P*_{a,CO₂}, 42 ± 2 Torr) remained unaltered from baseline throughout the experiment. Fetal cerebral hemispheric blood flow and cerebral hemispheric oxygen extraction were unaltered from baseline during progressive hypoxaemia. In contrast, a progressive fall in fetal cerebral hemispheric oxygen consumption occurred during the hypoxaemic challenge. In conclusion, these data do not support the hypothesis that the fetal llama brain maintains cerebral hemispheric O₂ consumption by increasing cerebral hemispheric O₂ extraction. Rather, the data show that in the llama fetus, a reduction in cerebral hemispheric metabolism occurs during acute hypoxaemia.

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The mammalian brain has a high metabolic rate and is considered to be more vulnerable to oxygen limitation than other organs. During pregnancy, the fetus is at greater risk of hypoxia than the adult since it is dependent not only on maternal cardiorespiratory but also on placental function for adequate oxygen delivery. Amongst the fetal strategies that minimise the adverse effects of oxygen deprivation, the most powerful for maintaining oxygen delivery to, and consumption by, the fetal brain, is a pronounced increase in cerebral blood flow (Cohn *et al.* 1974; Jones *et al.* 1981). In fact, in the sheep fetus cerebral blood flow matches the oxygen extraction in such a way that cerebral O₂ consumption remains constant until relatively severe degrees of hypoxaemia, such as those associated with an ascending aortic oxygen content of *ca* 2.2 ml dl⁻¹ (Field *et al.* 1990). In the sheep fetus,

hypoxaemia below this level leads to a progressive fall in fetal cerebral oxygen consumption (Field *et al.* 1990) and values persistently below ~50% of basal cerebral O₂ consumption result in cerebral damage, as evidenced by seizure activity (Ball *et al.* 1994) and/or neuronal death (Gunn *et al.* 1992; Parer, 1998).

The llama (*Lama glama*) is a species which has adapted to chronic hypobaric hypoxia and a number of physiological strategies enable it to thrive at altitudes surpassing 4000 m above sea level. Such strategies are genetically determined since they persist in llamas born and living at sea level, they include high affinity of the blood for oxygen (Moraga *et al.* 1996), efficient O₂ extraction by the tissues (Banchemo *et al.* 1971) and small red blood cells to increase surface area for O₂ exchange (Lewis, 1976). Our previous studies have

reported that during fetal life, unlike other animals such as the monkey (Jackson *et al.* 1987), the sheep (Cohn *et al.* 1974) and even the chicken (Mulder *et al.* 1998), the llama fetus does not show an increase in cerebral blood flow during an episode of acute hypoxaemia (Benavides *et al.* 1988; Llanos *et al.* 1993, 1995; Giussani *et al.* 1996, 1999; Llanos *et al.* 1998). Thus, in order to maintain cerebral O_2 consumption during acute hypoxaemia, an increase in cerebral O_2 extraction must occur in the llama fetus or a decrease in cerebral oxygen utilisation would be observed.

In this study cerebral hemispheric O_2 extraction and consumption were calculated during basal and graded hypoxaemic conditions in the llama fetus to test the hypothesis that the fetal llama brain maintains cerebral hemispheric O_2 consumption by increasing cerebral O_2 extraction. In addition, blood flow distribution within the brain was measured to determine detailed regional differences in oxygen delivery to the fetal llama brain.

METHODS

Surgical preparation

Six pregnant llamas, born and raised in Santiago (586 m above sea level and third generation removed from the highlands), were studied in the Laboratory for Developmental Physiology and Pathophysiology at the Universidad de Chile at 217 days of gestation (2.86 ± 0.22 kg, mean \pm s.e.m., where term is *ca* 350 days of gestation and 10.7 ± 0.7 kg, Fowler, 1989). The llamas were housed in an open yard with access to food and water *ad libitum*. The animals were familiarised with the study metabolic cage and laboratory conditions for 1–2 weeks prior to surgery.

After food and water deprivation for 24 h, polyvinyl catheters (i.d., 1.3 mm) were inserted into the maternal descending aorta and the inferior vena cava via a hindlimb artery and vein, respectively. This procedure was performed under ketamine anaesthesia (10 mg kg^{-1} i.m.; Ketostop, Drag Pharma-Investig, Chile), with local infiltration of lidocaine (lignocaine) (1% Dimecaina, Laboratorio Beta, Chile). The following day the fetuses were surgically prepared under maternal general anaesthesia (1 g sodium thiopentone, Tiopental Sódico, Laboratorio Astorga, Chile for induction and 1% halothane in 50/50 O_2 and N_2O for maintenance). Following lower mid-line laparotomy, a fetal hindlimb was exposed through a small hysterotomy. Polyvinyl catheters (i.d. 0.8 mm) were inserted into the fetal descending aorta via a hindlimb artery and into the inferior vena cava via a hindlimb vein. A fetal forelimb was exposed through a second uterine incision and a catheter was inserted into the ascending aorta via the brachial artery. A sagittal venous sinus catheter was also inserted through a craniotomy (Field *et al.* 1990). Finally, a catheter was placed in the amniotic cavity for zero pressure reference and the uterine and abdominal incisions were closed. All vascular catheters were filled with heparinised (1000 IU ml^{-1}) saline (0.9% NaCl), plugged with a copper pin, exteriorised through an incision in the maternal flank and kept in a pouch sewn onto the maternal skin.

During surgery all animals were continuously hydrated with warm 0.9% NaCl solution ($15\text{--}20 \text{ ml kg}^{-1} \text{ h}^{-1}$) to compensate for any fluid loss. At the end of surgery, and daily after surgery for 5 days, 1 million units of penicillin (Penicilina G Sódica,

Laboratorio Chile, Chile) and 500 mg kanamycin (Canamicina Sulfato, Laboratorio Chile, Chile) were administered in the amniotic fluid via the amniotic catheter. After surgery the animals were returned to the yard and an analgesic (Metamizol, 1 g i.m., Dipirona, Laboratorio Chile, Chile) was administered every 12 h for the next 2 days. At least 4 days of post-operative recovery were allowed before the beginning of the experiments. Vascular catheters were maintained patent by daily flushing with heparinised (200 IU ml^{-1}) saline.

All experimental protocols were reviewed and approved by the Faculty of Medicine Animal Ethics Committee from the University of Chile. Furthermore, all animal procedures, maintenance and experimentation were conducted following the recommendations in the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society, and the British Animals (Scientific Procedures) Act, 1986.

Experimental protocol

All experiments were based on a 2 h protocol: 1 h normoxaemia followed by 3×20 min periods of graded and progressive degrees of hypoxaemia. Following 1 h of the llama breathing room air, fetal hypoxaemia was induced by decreasing the maternal fraction of inspired O_2 (F_{I,O_2}) to reduce the haemoglobin saturation in the fetal ascending aorta to between 45 and 35% for the first 20 min period (H20), to between 35 and 25% for the second 20 min period (H40), and to $< 25\%$ for the final 20 min period (H60). Carbon dioxide (3–5%) was added to the maternal inspired gas mixture during hypoxaemia to maintain the maternal and fetal arterial partial pressure of CO_2 (P_{a,CO_2}) relatively constant. After 1 h graded fetal hypoxaemia, the F_{I,O_2} was returned to room air.

At 20 min intervals during the experimental period ascending aortic and sagittal sinus blood samples were taken from the fetus, and arterial blood samples were taken from the mother to measure pH, P_{O_2} and P_{CO_2} (BMS 3 Mk2 Blood Microsystem and PHM 73 pH/Blood Gas Monitor, Radiometer, Copenhagen, determinations measured at 39°C), percentage saturation of haemoglobin (%sat Hb) and haemoglobin concentration (Hb) (OSM2 Hemoximeter, Radiometer, Copenhagen). Fetal and maternal arterial and venous pressures and amniotic fluid pressure were measured with strain gauge transducers (Statham P23Db, Hato Rey, Puerto Rico) and fetal and maternal blood pressures and heart rates were recorded continuously throughout the experiment on a Gilson ICM-5 polygraph (Gilson, Emeryville, CA, USA).

Experimental techniques

The fetal combined ventricular output and its distribution was measured during normoxaemia, and at H20, H40 and H60 using the radionuclide-labelled microsphere technique (Heymann *et al.* 1977). Beads of $15 \mu\text{m}$ diameter were used labelled with ^{57}Co , ^{113}Sn , ^{46}Sc and ^{103}Ru (New England Nuclear, Boston, MA, USA). The beads were injected into the fetal inferior vena cava while reference samples were obtained from the ascending and descending aorta. The rate of withdrawal of the reference samples was 3.2 ml min^{-1} for 1.5 min. This method allows blood flow determination to all organs except the lungs (Heymann *et al.* 1977). A total of approximately 1×10^6 beads were injected at each measurement. On completion of the experiment the llama was anaesthetised with 1 g sodium thiopentone i.v. (Tiopental Sódico, Laboratorio Chile). A Caesarean section was performed, the fetus removed and injected with (1 g) sodium thiopentone i.v. Both mother and fetus were killed with saturated KCl injected i.v. whilst under anaesthesia.

At post-mortem the tip of the sagittal sinus catheter was found inside the vessel in all fetuses. No sagittal sinus thrombosis or cerebral cortex haemorrhage was found in any of the fetuses. The sagittal sinus was used for sampling cerebral venous drainage because contamination with venous blood from extra-cerebral tissues is negligible (Purves & James, 1969). As the sagittal sinus drains primarily the cerebral hemispheres, measurements made in the present study represent mainly cerebral hemispheric, instead of total cerebral, O_2 extraction and consumption (Purves & James, 1969). The fetal brain and individual organs were removed and weighed. The fetal brain was separated into cerebral hemispheres (which included the midbrain and diencephalon), pons, medulla and cerebellum. All removed tissues were carbonised and radioactivity counted with a multi-channel gamma pulse height analyser (Minaxi 5000, Packard, Canberra, Australia). To ascertain adequate mixing of the microspheres, right and left cerebral hemispheres and right and left kidneys were counted separately. Microsphere mixing was considered adequate when the percentage differences in calculated blood flow to the right and left cerebral hemispheres and kidneys were $< 10\%$. Similarly, in order to attain adequate accuracy in the measurement of the regional blood flows, the number of microspheres in each organ or any area counted was > 400 (Heymann *et al.* 1977).

Blood flow to each region of the fetal brain was calculated by comparing the organ radioactivity with the flow rate and radioactivity of the ascending aortic reference sample. Blood flow to all other organs was calculated by comparing the organ radioactivity with the activity and flow rate of the appropriate reference sample (ascending aorta for upper body organs and descending aorta for lower body organs). Fetal combined ventricular output was calculated as the sum of blood flow to all organs except the lungs (Heymann *et al.* 1977) using the following equation:

$$\dot{Q}_{\text{organ}} = C_{\text{organ}} \times \dot{Q}_{\text{reference}} / C_{\text{reference}}$$

where \dot{Q} is blood flow (expressed in ml min^{-1}), and C is the radioactivity (measured in c.p.m.), and subscripts organ and reference denote the values for organs and references, respectively.

Vascular resistance in the cerebral hemispheres was calculated by dividing perfusion pressure (ascending arterial minus sagittal sinus venous pressure) by cerebral hemispheric blood flow. In addition, blood oxygen content (O_2 content, expressed in $\text{ml } O_2 \text{ dl}^{-1}$), cerebral hemispheric oxygen delivery (O_2 delivery, expressed in $\text{ml } O_2 \text{ min}^{-1} (100 \text{ g})^{-1}$), cerebral hemispheric oxygen extraction (O_2 extraction, expressed in %) and cerebral hemispheric O_2 consumption (\dot{V}_{O_2} cerebral hemispheres, expressed in $\text{ml } O_2 \text{ min}^{-1} (100 \text{ g})^{-1}$) were calculated using the following equations:

$$O_2 \text{ content} = [\text{Hb}] \times \% \text{sat Hb} \times 100^{-1} \times 1.34 (\text{ml } O_2 \text{ g Hb}^{-1}),$$

$$O_2 \text{ delivery} = (O_2 \text{ content}/100) \times \dot{Q} \text{ of cerebral hemispheres,}$$

$$O_2 \text{ extraction} = O_2 \text{ content (ascending aorta - sagittal sinus)} \\ \times 100 / O_2 \text{ content of ascending aorta,}$$

$$\dot{V}_{O_2} \text{ cerebral hemispheres} = (O_2 \text{ content of ascending aorta} \\ - \text{sagittal sinus}/100) \times \dot{Q} \text{ of cerebral hemispheres,}$$

where $[\text{Hb}]$ is measured in g dl^{-1} and \dot{Q} of cerebral hemispheres is measured in $\text{ml min}^{-1} (100 \text{ g})^{-1}$.

Statistical analysis

Values are expressed as means \pm s.e.m. Changes in any measured variable during the experiment were assessed for statistical significance using ANOVA for repeated measures, followed by the Student-Newman-Keuls test. Differences were considered significant when $P < 0.05$ (Zar, 1984).

RESULTS

Maternal and fetal blood gas status

During hypoxaemia, maternal arterial O_2 tension, %sat Hb and O_2 content decreased progressively during H20 (from 100 ± 8 to 60 ± 8 Torr, 99 ± 1 to $93 \pm 4\%$ and 15.8 ± 0.7 to $13.9 \pm 0.5 \text{ ml dl}^{-1}$, respectively), H40 (to 39 ± 5 Torr, $79 \pm 7\%$ and to $13.2 \pm 0.5 \text{ ml dl}^{-1}$, respectively) and H60 (to 30 ± 5 Torr, to $64 \pm 7\%$ and to $11.2 \pm 0.8 \text{ ml dl}^{-1}$, respectively; all $P < 0.05$). Maternal arterial pH (7.44 ± 0.01) and P_{CO_2} (35 ± 3 Torr) did not change significantly from baseline throughout the experiment.

Fetal blood gas and acid base status in the ascending aorta and sagittal venous sinus are shown in Table 1. The values in arterial blood in the basal period are similar to those obtained previously in chronically catheterised fetal llamas (Llanos *et al.* 1995). In the llama fetus there were significant progressive decreases in arterial and venous P_{O_2} , O_2 content, %sat Hb and pH during the experiment (Table 1). While an increase in arterial levels of haemoglobin occurred, venous haemoglobin concentrations remained unchanged from baseline by the end of the hypoxaemia protocol in the llama fetus.

The arterio-venous oxygen differences across the fetal cerebral circulation decreased from 2.8 ± 0.4 to $1.4 \pm 0.3 \text{ ml } O_2 \text{ dl}^{-1}$ after 60 min hypoxaemia ($P < 0.05$, Table 1).

Maternal and fetal cardiovascular responses

During hypoxaemia, there was a pronounced increase in maternal mean systemic arterial blood pressure (from 127 ± 6 to 160 ± 7 mmHg at H60, $P < 0.05$) and in maternal heart rate (from 45 ± 4 to 83 ± 9 beats min^{-1} at H60, $P < 0.05$). In the llama fetus, cardiac output and mean systemic arterial pressure, remained unaltered from baseline during the hypoxaemic protocol (Table 2). Although there was a tendency for heart rate to decrease, and for total vascular resistance to increase during acute hypoxaemia, these differences were not significant (Table 2).

Regional blood flow, resistance and oxygen delivery in the fetal llama brain

Blood flow and vascular resistance in the fetal brain (total), cerebellum and pons remained unchanged from baseline during each of the 20 min episodes of the hypoxaemia protocol (Table 3). In contrast, an increase in blood flow and a fall in vascular resistance occurred in the fetal medulla, which remained maintained during the entire hypoxaemia protocol ($P < 0.05$, Table 3). While a fall in

Table 1. Systemic and sagittal sinus blood gas status in the fetal llama during graded hypoxaemia

	Basal	H20	H40	H60
Systemic arterial blood gases				
pH	7.36 ± 0.01	7.33 ± 0.01	7.25 ± 0.05*†	7.21 ± 0.04*†
P _{O₂} (mmHg)	24 ± 1§	20 ± 1§	16 ± 1	14 ± 1
P _{CO₂} (mmHg)	42 ± 2	40 ± 1	41 ± 2	40 ± 1
Hb saturation (%)	59 ± 4§	43 ± 2§	31 ± 3	19 ± 2
Hb (g dl ⁻¹)	10.4 ± 0.5	11.1 ± 0.5	10.9 ± 0.6	11.9 ± 0.4§
O ₂ content (ml O ₂ dl ⁻¹)	8.2 ± 0.6§	6.3 ± 0.4§	4.5 ± 0.4	3.0 ± 0.4
Sagittal sinus blood gases				
pH	7.31 ± 0.01	7.31 ± 0.01	7.23 ± 0.04	7.17 ± 0.04*†
P _{O₂} (mmHg)	20 ± 1§	16 ± 1§	13 ± 1§	10 ± 1
P _{CO₂} (mmHg)	44 ± 1	42 ± 1	42 ± 2	44 ± 2
Hb saturation (%)	39 ± 3§	24 ± 2	16 ± 2	11 ± 2
Hb (g dl ⁻¹)	10.6 ± 0.5	11.1 ± 0.6	10.8 ± 0.6	11.7 ± 0.5
O ₂ content (ml O ₂ dl ⁻¹)	5.5 ± 0.3§	3.5 ± 0.4§	2.2 ± 0.2	1.7 ± 0.3
Sagittal sinus arterio-venous difference in O ₂ content (Aa–Ss) O ₂ content (ml O ₂ dl ⁻¹)				
	2.8 ± 0.4	2.8 ± 0.3	2.3 ± 0.5	1.4 ± 0.3*†

Hb, haemoglobin; (Aa–Ss), ascending aorta – sagittal sinus. Values are shown as means ± s.e.m. Measurements were made after 20 (H20), 40 (H40) and 60 (H60) min of hypoxaemia. Significant differences ($P < 0.05$) are: * vs. Basal; † vs. H20; § vs. all.

Table 2. Cardiovascular responses to graded hypoxaemia in the fetal llama

	Basal	H20	H40	H60
Mean arterial pressure (mmHg)	48 ± 2	47 ± 1	47 ± 3	49 ± 3
Heart rate (beats min ⁻¹)	126 ± 3	113 ± 12	118 ± 9	108 ± 6
Cardiac output (ml min ⁻¹ kg ⁻¹)	250 ± 13	252 ± 23	209 ± 7	199 ± 30
Total vascular resistance (mmHg min kg ml ⁻¹)	0.19 ± 0.02	0.19 ± 0.02	0.23 ± 0.02	0.28 ± 0.05

Values are shown as means ± s.e.m. Measurements were made after 20 (H20), 40 (H40) and 60 (H60) min of hypoxaemia.

Table 3. Total and regional cerebral blood flows and oxygen deliveries in the fetal llama during graded hypoxaemia

	Basal	H20	H40	H60
Blood flow (ml min ⁻¹ (100 g) ⁻¹)				
Total brain	83 ± 16	120 ± 14	106 ± 14	93 ± 16
Cerebellum	113 ± 21	157 ± 17	150 ± 17	144 ± 22
Pons	135 ± 26	193 ± 18	187 ± 25	174 ± 20
Medulla	147 ± 28	225 ± 22*	224 ± 24*	228 ± 35*
Vascular resistance (mmHg min (100 g) ml ⁻¹)				
Total brain	0.64 ± 0.11	0.41 ± 0.05	0.48 ± 0.06	0.59 ± 0.11
Cerebellum	0.46 ± 0.08	0.31 ± 0.04	0.33 ± 0.03	0.37 ± 0.07
Pons	0.38 ± 0.05	0.26 ± 0.03	0.28 ± 0.04	0.29 ± 0.05
Medulla	0.35 ± 0.06	0.22 ± 0.03*	0.22 ± 0.02*	0.23 ± 0.04*
Oxygen delivery (ml O ₂ min ⁻¹ (100 g) ⁻¹)				
Total brain	6.5 ± 0.9	7.5 ± 1.0	4.8 ± 0.6§	2.7 ± 0.4§
Cerebellum	8.8 ± 0.9	9.9 ± 1.2	6.7 ± 0.8	4.1 ± 0.5§
Pons	10.4 ± 1.1	12.1 ± 1.3	8.3 ± 1.1	5.1 ± 0.6§
Medulla	11.5 ± 1.3	14.1 ± 1.5	10.0 ± 1.1	6.5 ± 0.8§

Values are shown as means ± s.e.m. Measurements were made after 20 (H20), 40 (H40) and 60 (H60) min of hypoxaemia. Significant differences ($P < 0.05$) are: * vs. Basal; § vs. all.

oxygen delivery occurred in the fetal brain (total) by H40 and H60 ($P < 0.05$, Table 3), in the cerebellum, pons and medulla oxygen delivery was more or less maintained during the hypoxaemic protocol, falling significantly only at H60 ($P < 0.05$, Table 3).

Measurements in the fetal cerebral hemispheres

Cerebral hemispheric blood flow and vascular resistance remained unaltered from baseline through each of the 20 min episodes of progressive hypoxaemia in the llama fetus (Fig. 1). The fall in oxygen delivery to the fetal cerebral hemispheres calculated by H40 and H60 ($P < 0.05$) was accompanied by a significant fall in cerebral hemispheric oxygen consumption at H60 (Fig. 2). However, cerebral oxygen extraction remained unaltered from baseline throughout each of the hypoxaemic periods in the llama fetus (Fig. 2).

A plot of all individual values from all fetuses for cerebral hemispheric oxygen uptake against carotid oxygen content revealed a curvilinear relationship (\dot{V}_{O_2} cerebral = $-0.0412x^2 + 0.6584x - 0.5188$; $r = 0.7$, $P < 0.05$). The graph shows that below *ca* 7 ml O₂ dl⁻¹ in carotid oxygen content, a progressive fall in cerebral oxygen consumption occurs in the fetal llama cerebral hemispheres.

DISCUSSION

This study tested the hypothesis that the fetal llama brain maintains cerebral hemispheric O₂ consumption by increasing cerebral hemispheric O₂ extraction rather than decreasing cerebral hemispheric metabolism during acute hypoxaemia. The data presented do not support this hypothesis and show that cerebral oxygen extraction is maintained but a fall in cerebral hemispheric oxygen consumption occurs in the llama fetus during progressive

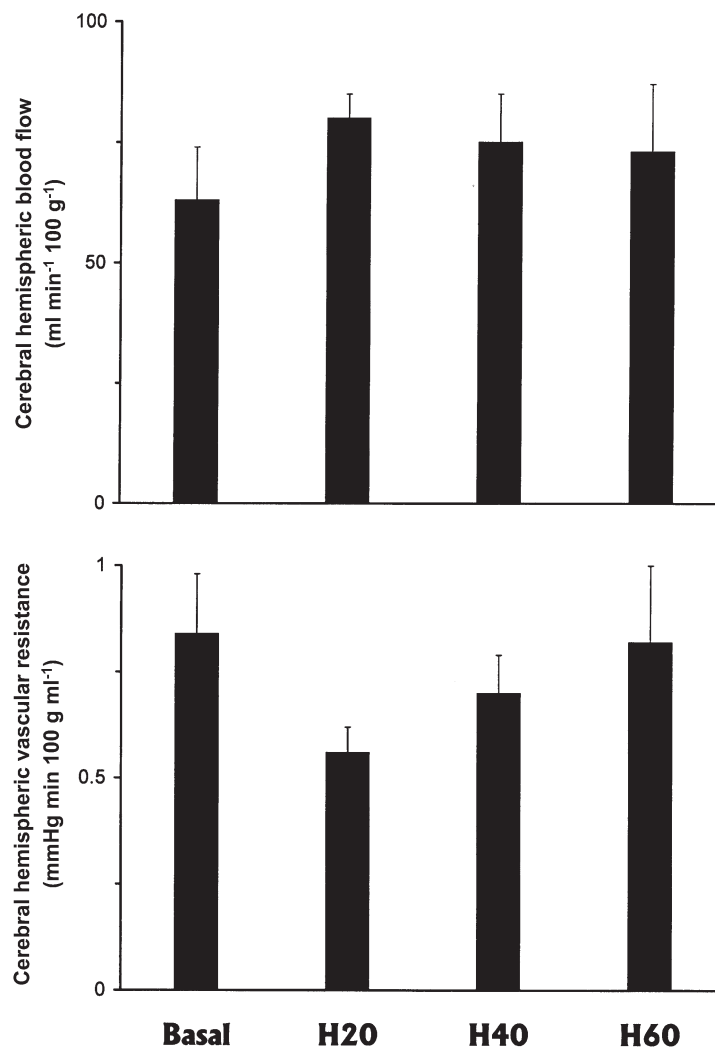


Figure 1. Cerebral haemodynamic responses to progressive hypoxaemia in the llama fetus

Cerebral hemispheric blood flow (upper panel) and vascular resistance (lower panel) in the fetal llama during progressive hypoxaemia (means \pm S.E.M., $n = 6$). Measurements were made after 20 (H20), 40 (H40), and 60 (H60) min of hypoxaemia.

episodes of hypoxaemia. Additional data presented in the study show that a pronounced redistribution of blood flow occurs in favour of the medulla within the fetal llama brain during hypoxaemia, and that cerebral hemispheric oxygen uptake cannot be maintained below *ca* 7 ml O₂ dl⁻¹ in carotid oxygen content in the llama fetus.

The mechanism by which fetal llamas withstand episodes of hypoxaemia differs from that found in fetal animals of

lowland species, such as the sheep, in which cerebral oxygen consumption is maintained during hypoxaemia by an up to 3-fold augmentation in total cerebral blood flow in late gestation (Jones *et al.* 1977). An increase in cerebral blood flow, albeit to a lower extent (1.7-fold), also occurs at 0.68 gestation (Iwamoto *et al.* 1989). The increase in brain blood flow in the fetal sheep during acute hypoxaemia is general, such that a pronounced increase in blood flow

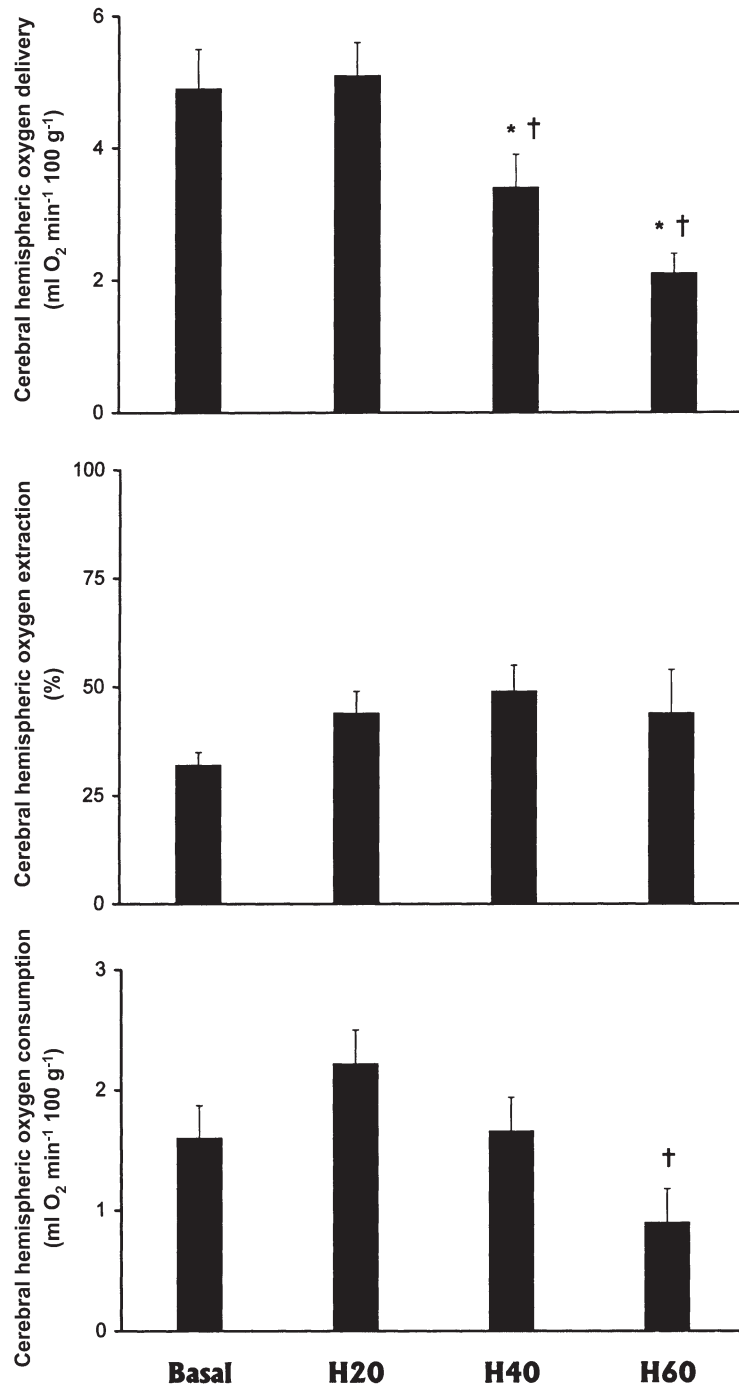


Figure 2. Cerebral metabolic responses to progressive hypoxaemia in the llama fetus

Cerebral hemispheric oxygen delivery, oxygen extraction and oxygen consumption in the fetal llama during progressive hypoxaemia (means \pm s.e.m., $n = 6$). Measurements were made after 20 (H20), 40 (H40) and 60 (H60) min hypoxaemia. Significant differences ($P < 0.05$) are: * vs. Basal; † vs. H20.

occurs to all regions of the fetal sheep brain (Richardson *et al.* 1989). The pronounced increase in cerebral blood flow during acute hypoxaemia in the sheep fetus allows cerebral oxygen uptake to be maintained until ascending aortic blood oxygen content is approximately 2.2 ml dl⁻¹ at 0.85 gestation (Field *et al.* 1990) and 3.3 ml dl⁻¹ at 0.63 gestation (Gleason *et al.* 1990). Below this level of oxygenation, cerebral oxygen uptake falls in the fetal sheep brain. In marked contrast, cerebral blood flow does not increase in response to acute hypoxaemia in the fetal llama. This result was observed in both llama fetuses whose mothers were recently brought down from highland areas and in fetuses whose mothers were the third or fourth generation reared in lowland areas (Llanos *et al.* 1993, 1995, 1998; Giussani *et al.* 1996, 1999). The threshold for maintenance of cerebral hemispheric oxygen uptake appears much higher in the fetal llama brain than in the fetal sheep brain, as cerebral oxygen uptake in the llama brain could not be maintained below approximately 7 ml O₂ dl⁻¹ in the ascending aorta. The pronounced redistribution of blood flow within the fetal llama brain favouring the medulla during the greater part of the hypoxaemic challenge suggests that this area is important in cardiovascular control during acute hypoxaemia in this species.

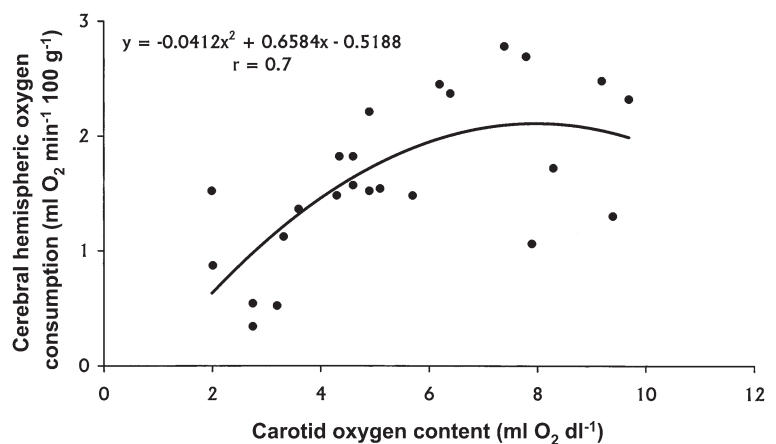
In fetuses of lowland mammalian species (Cohn *et al.* 1974; Jones *et al.* 1981; Jackson *et al.* 1987) during late gestation, and in the chick embryo (Mulder *et al.* 1998) during late incubation, the increase in cerebral blood flow during acute episodes of hypoxaemia is mediated by a fall in cerebral vascular resistance. The fall in cerebral vascular resistance in most species studied to date results from a number of mechanisms (see Longo & Pierce, 1991) including decreased tissue oxygen (Jones *et al.* 1981) and increased tissue carbon dioxide (Rosenberg *et al.* 1982) levels, an increase in prostaglandins (Leffler *et al.* 1985), adenosine (Laudignon *et al.* 1990), arginine vasopressin (Pérez *et al.* 1989) and nitric oxide (Van Bel *et al.* 1995), and activation of potassium channels (Kleppisch & Nelson, 1995). In the llama fetus, all studies so far have reported that there is a differential contribution of the

adrenergic, nitrenergic and vasopressinergic systems to the maintenance of cerebral blood flow during normoxic and hypoxaemic conditions. While treatment of the llama fetus with a selective vasopressin V₁ receptor antagonist did not alter blood flow or vascular resistance in the carotid circulation or in the cerebral hemispheres during basal or hypoxaemic conditions (Giussani *et al.* 1999; Herrera *et al.* 2000), treatment of the llama fetus with the α -adrenergic receptor antagonist phentolamine led to a pronounced fall in carotid blood flow and a pronounced increase in carotid vascular resistance even during basal conditions. Furthermore, treatment of the fetal llama with phentolamine during hypoxaemia led to severe systemic hypotension, circulatory collapse and fetal death (Giussani *et al.* 1999). When L-NAME, a nitric oxide synthase inhibitor was administered to llama fetuses during normoxia, there was a substantial decrease in the carotid artery blood flow and a pronounced increase in the carotid artery vascular resistance (Riquelme *et al.* 1998). Treatment of the fetal llama with L-NAME during hypoxaemia led to no further modifications in carotid blood flow or carotid vascular resistance, but the elevated carotid vascular resistance was maintained high even 1 h after the end of the hypoxaemic episode (Riquelme *et al.* 1998). Combined, these results suggest that in the llama fetus the influence of AVP in regulating the cerebral blood flow is negligible, but that there are important adrenergic and nitrenergic contributions to the maintenance of cerebral blood flow during normoxia and hypoxaemia in this species.

It could be argued that the lack of a cerebral vasodilator response to acute hypoxaemia reported in this study in the llama fetus may be attributed to the early gestational age at which these experiments were performed. However, a significant increase in cerebral blood flow during acute hypoxaemia in 0.58–0.68 gestation fetal sheep has been previously reported (Iwamoto *et al.* 1989), and it is known that an increase in cerebral blood flow and in cerebral oxygen extraction allow both immature and mature fetal sheep to maintain cerebral oxygen consumption during hypoxaemia (Gleason *et al.* 1990; Field *et al.* 1990).

Figure 3. Relationship between cerebral hemispheres oxygen consumption and carotid oxygen content during progressive hypoxaemia in the llama fetus

A plot of all individual values from all fetuses revealed a curvilinear relationship: cerebral $\dot{V}_{O_2} = -0.0412x^2 + 0.6584x - 0.5188$; $r = 0.7$, $P < 0.05$.



In all species studied to date, cerebral metabolic rate and cerebral perfusion are closely coupled. For example, during fetal life, the increase in brain blood flow is closely associated with the increase in fetal brain growth during development (Szymonowicz *et al.* 1988; Richardson *et al.* 1989). Furthermore, cerebral blood flow is known to increase during REM-like low voltage states of electrocortical (ECoG) activity in fetal sheep, when an increase in cerebral oxidative metabolism occurs (Richardson *et al.* 1985). A previous study in the llama fetus reported close coupling between instantaneous changes in carotid blood flow and electrocortical states in the llama fetus, such that a switch in ECoG to low voltage was associated with a marked increase in carotid blood flow (Blanco *et al.* 1997). The intimate relationship between low voltage ECoG activity and vasodilatation in the carotid circulation in the llama fetus suggests that, as in other species, cerebral oxidative metabolism is also increased during REM-like electrocortical states, and that changes in carotid blood flow and in cerebral blood flow are coupled to cerebral metabolism in this species. Therefore, the fall in cerebral oxidative metabolism that occurs in the llama fetus during episodes of acute hypoxaemia reported in this study matches with the lack of an increase in cerebral blood flow during acute hypoxaemia in this species. These results also suggest that cerebral metabolic rate and cerebral perfusion are closely coupled under conditions of hypoxaemia in the llama fetus.

The fall in oxygen consumption in the fetal llama cerebral hemispheres during acute hypoxaemia reported in the present study may either reflect that the fetal llama is decompensating to the progressive degrees of hypoxaemia or that this species has evolved alternative strategies to withstand the period of cerebral oxygen deprivation. Physiological decompensation seems unlikely with carotid arterial P_{O_2} values of *ca* 14 mmHg, as this level of arterial oxygenation is slightly lower than the P_{O_2} found in llama fetuses in its usual habitat, at 4400 m above sea level (Llanos *et al.* unpublished observations). Combined, past and present data therefore favour the interpretation that the fetal llama brain undergoes hypometabolism under episodes of acute hypoxaemia. Such an adaptation has been previously reported in turtles (Pék-Scott & Lutz, 1998; Buck & Bickler, 1998), which, unlike mammals, are able to survive remarkably long periods of anoxia, and surprisingly also in Quechua Indians, indigenous people of South America with a prolonged residence ancestry at high altitude (Hochachka *et al.* 1994).

In conclusion, the data presented in this study do not support the hypothesis that the fetal llama brain maintains cerebral hemispheric O_2 consumption by increasing cerebral hemispheric O_2 extraction. Rather the data show that in the llama fetus, a reduction in cerebral hemispheric metabolism occurs during acute hypoxaemia. In addition,

the data show that there is partial redistribution of blood flow within the fetal llama brain which favours the medulla during episodes of hypoxaemia. These responses reflect alternative strategies that the fetal llama has developed to avoid cerebral hypoxic damage, demonstrating powerful adaptations evolved in response to the chronic stimulus provided by the sustained hypobaric hypoxia of life at extreme altitude.

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