

Cerebral ^{31}P Magnetic Resonance Spectroscopy and Systemic Acid-Base Balance during Hypoxia in Fetal Sheep

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ABSTRACT

The purpose of this study was to investigate cerebral energy metabolism and acid-base homeostasis during impaired oxygen supply in fetal sheep. Systemic acid-base balance was correlated with the sequence in changes of cerebral phosphorus metabolite ratios and intracellular pH. Phosphorus magnetic resonance spectra were obtained from the brain of six fetal sheep simultaneously with repeated measurements of fetal arterial oxygen saturation and acid-base balance. Fetal hypoxia was induced by gradually reducing the oxygen supply to the anesthetized pregnant ewe to establish an intended arterial pH of 7.00 or lower. The ratio of phosphocreatine to inorganic phosphate decreased from 1.08 ± 0.10 (SD) during the control period to 0.77 ± 0.29 at an arterial pH between 7.20 and 7.25. The inorganic phosphate level became significantly increased at an arterial pH between 7.10 and 7.15 compared with control values. With ongoing arterial acidosis, cerebral intracellular pH decreased linearly with the arterial pH. At an arterial pH of 7.00, cerebral intracellular pH was decreased from 7.18 ± 0.03 to 6.71 ± 0.28 , and phosphocreatine and nucleoside triphosphates levels were decreased significantly. In fetal sheep brain, cerebral oxidative phosphorylation (ratio of phosphocreatine to inorganic phosphate) is already affected at a

mild arterial acidosis. At an arterial pH of 7.00 or lower, nucleoside triphosphates disappeared, which almost inevitably was followed by death in fetal sheep. (*Pediatr Res* 54: 747–752, 2003)

Abbreviations

FBP, fetal blood pressure
FHR, fetal heart rate
FiO₂, fraction of oxygen in inspiratory gas
 $^1\text{H-MRS}$, proton magnetic resonance spectroscopy
MR, magnetic resonance
NTP, nucleoside triphosphate
P_i, inorganic phosphate
PCr, phosphocreatine
PDE, phosphodiester
pH_i, intracellular pH
PME, phosphomonoesters
 $^{31}\text{P-MRS}$, phosphorus magnetic resonance spectroscopy
P_{tot}, total phosphorus
Sao₂, arterial oxygen saturation
VOI, volume of interest

Fetal hypoxia is one of the causes of neonatal brain injury, and future neuromotor and intellectual impairment (1, 2). Low blood pH has been proposed as a clinical standard in defining fetal hypoxia during labor (3). On the basis of such a pH value, the decision is made whether to further tolerate an abnormal fetal heart pattern or to intervene. A problem in the prevention

of neurologic morbidity is that the degree of acidosis in the blood is not a good predictor of later neurologic disability (4). Severe intrapartum asphyxia, quantified by an umbilical artery pH < 7.00, is associated with neonatal neurologic complications (5). Although at an arterial pH of 7.00 or lower the relationship with cerebral damage is clear, it is not known how fetal cerebral metabolism changes with mild acidosis. As it becomes more apparent that neurologic damage may also be the result of mild hypoxia, resulting in mild acidosis, this study sets out to obtain insight into the relationship between the degree of acidosis in the blood and the sequence of events in fetal cerebral metabolism.

$^{31}\text{P-MRS}$ can be used to measure phosphorus metabolites that are involved in cerebral energy metabolism, such as PCr, P_i, and ATP. Cerebral pH_i can also be measured with $^{31}\text{P-MRS}$. During oxygen shortage, production of ATP by oxida-

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tive phosphorylation will decrease. The creatine kinase reaction is a buffering mechanism and leads to the replenishment of ATP, causing a decrease in PCr. More severe hypoxia leads to the breakdown of ATP by hydrolysis, producing P_i and proton accumulation (6).

The ratio of PCr to P_i is a direct measure of oxidative phosphorylation potential (7). A significant relationship was shown in human neonates between decreased PCr/ P_i occurring some days after asphyxia during secondary energy failure as measured with ^{31}P -MRS and poor neurodevelopment outcome at 1 y of age (8). Decreased PCr/ P_i was related to increased cerebral echodensities in preterm and term infants, known to be associated with hypoxic-ischemic brain injury (9), although the mechanisms for the PCr/ P_i decrease are quite different. When cerebral ATP was reduced, death was almost inevitable (10).

Experimental studies with ^{31}P -MRS on asphyxiated neonatal or immature animals showed a correlation between ATP levels, phosphorylation potential (PCr/ P_i), and histologic damage (7, 11). Cerebral acidosis appears to be more important in the pathogenesis of brain damage than cerebral lactate accumulation (12, 13). Van Cappellen van Walsum *et al.* (14) investigated cerebral metabolism of hypoxic fetal sheep with ^1H -MRS: at an arterial pH of 7.00 or lower, cerebral lactate levels were strongly elevated, and *N*-acetyl aspartate, a neuronal marker, was decreased. Above an arterial pH of 7.28, no cerebral lactate appeared. Between an arterial pH of 7.28 and 7.00, no changes in ^1H -MRS spectra were observed, except an increase in cerebral lactate. Especially in this pH range, in which clinical decisions are made during labor, cerebral changes in energy metabolism that may precede neuronal damage are expected to appear.

In this study, the protocol of van Cappellen van Walsum *et al.* (14) is used with ^{31}P -MRS instead of ^1H -MRS to monitor the sequence of changes in cerebral phosphorus metabolites and pH_i related to developing systemic metabolic acidosis during gradually decreased Sao_2 in fetal sheep.

METHODS

Surgical procedures. After 48 h fasting, six pregnant ewes of the Dutch Texel breed were operated on at a gestational age of 127–128 d (term, 147 d). Anesthesia was induced with 30 mg/kg pentobarbital and 0.5 mg atropine *i.v.*, and was maintained with 0.5–1.0% Ethrane in a 2:1 mixture of nitrous oxide and oxygen and a continuous *i.v.* infusion of ketamine (4–7 mg·kg⁻¹·h⁻¹) and midazolam (0.1–0.2 mg·kg⁻¹·h⁻¹). Ketamine does not cause a dose-dependent decrease of cerebral metabolism and blood flow (15) and does not impair blood flow in brain, heart, and kidneys in the acidotic fetal lamb (16). Midazolam was added to antagonize the stimulating effects of ketamine on FBP (17).

The fetal sheep was approached by hysterotomy, and the fetal head was lifted out of the uterus while the fetal body remained *in utero*. A polyvinyl catheter (inner diameter 0.8 mm, outer diameter 1.6 mm) was inserted in the left axillary artery to draw fetal blood samples. Fetal arterial blood pressure was recorded via this catheter. The rectal temperature of the ewe was kept constant by means of a thermostatic heating pad

underneath the animal. The temperature of the fetus was measured in the throat.

The experiments were approved by the local ethical committee for animal research.

Transport and MR setting. This procedure was described earlier by van Cappellen van Walsum *et al.* (14). In short, the pregnant ewe was transported after surgery from the animal laboratory to the MR magnet. In the MR magnet, ventilation of the ewe required 12-m-long tubes to avoid magnetic interference. The fetal head, still outside the uterus, was fixed to prevent movements, which may cause artifacts of the MR signal.

Measurements. Fetal blood samples were analyzed within 2 min to assess Sao_2 , pH, Hb, and blood gases (ABL 510, Radiometer, Copenhagen, Denmark). Extracellular fluid base excess was calculated according to the formula used by the blood gas analyzer. FHR was derived from the FBP signal. FBP and FHR data were stored on a personal computer with the Poly program (Physiologic Analysis Package, Inspector Research System, Amsterdam, The Netherlands).

MR imaging and ^{31}P -MRS. MR measurements were performed at 1.5 T (^1H frequency, 63.6 MHz; ^{31}P frequency, 25.7 MHz) on a Siemens Magnetom Vision system (Erlangen, Germany). First, MR images in coronal, sagittal, and transversal directions were recorded. ^{31}P -MRS was performed using a dual-resonant probe consisting of an 8-cm-diameter ^{31}P surface coil and a butterfly type ^1H coil. The center of the ^{31}P coil was fixed directly above the fetal head. ^{31}P -MR spectra were obtained using an adiabatic B1-independent rotation phase-cycled pulse (18) (pulse length, 5.12 ms; 45° nutation) and an 8 × 8 × 8 three-dimensional chemical shift imaging (CSI) sequence (TR = 1000 ms; transmitter amplitude = 95 V). The nominal voxel size was 2.5 × 2.5 × 2.5 cm³, and the VOI covered both hemispheres including parts of the striatum and the thalamus. To improve the signal to noise ratio, ^1H decoupling was applied using a WALTZ 4 sequence (240 ms duration, 6 W power) (19). The time taken to obtain one ^{31}P -MR spectrum was 17 min. The following metabolites were measured (Fig. 1): PME, PDE, P_i , PCr, and NTP.

Design of the experiment. The control period was defined as a period with a fetal arterial pH ≥ 7.30 during 50 min or more when the ewe was situated in the MR magnet. During the control period, three or more ^{31}P -MR spectra had to be recorded. After control measurements the maternal Fio_2 was gradually decreased, and stabilized on each of the intended fetal pH values to obtain the corresponding ^{31}P -MR spectra (one spectrum takes 17 min during which time the pH is in the intended range). The stepwise reduction of fetal oxygenation was continued until a fetal arterial blood pH of 7.00 or lower was reached. Measurements were stopped if blood sampling was impossible because of insufficient FBP. Fetal arterial blood samples were drawn every 5–15 min. FHR, FBP, and temperature were measured continuously during the course of the experiment.

MR data analysis. Postprocessing of the MR spectra was performed using Magnetic Resonance User Interface software (20). After apodization with a Lorentzian filter (4 Hz), the spectra were fitted in the time domain using the Variable

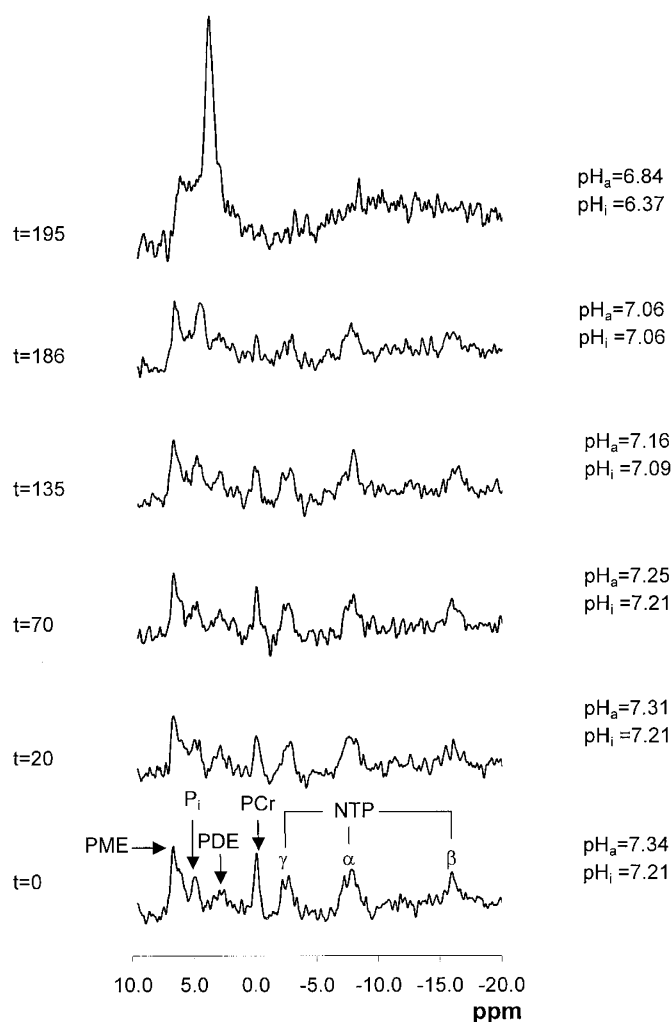


Figure 1. Stack plot of selected ³¹P-MR spectra of sheep 1 during control and hypoxia. t, time (min) after the start of hypoxia. t = 0 represents a spectrum during the control period. Cerebral pH_i and mean arterial pH (pH_a) are indicated for each spectrum.

Projection method (VARPRO) (21). Prior knowledge was used to fit the doublets of α and γ NTP, and the triplet of β NTP. To calculate NTP levels, the γ and β peaks were used. Peaks from phosphates other than ATP, such as nicotinamide adenine nucleotides, may contribute to the α peak of NTP. pH_i was estimated using the Henderson-Hasselbalch relationship:

$$pH_i = 6.77 + \log[(\sigma - 3.29)/(5.68 - \sigma)]$$

where σ represents the chemical shift of P_i referred to that of PCr. This equation is based on an *in vitro* titration curve according to Petroff *et al.* (22). When no PCr peak was present in a ³¹P-MR spectrum, information about the position (in parts per million) of the PCr peak was obtained from the last preceding spectrum recorded in the same experimental session. When there was a doublet structure of the P_i, the mean of the chemical shift of the two P_i peaks was taken.

Chemical shifts were given in parts per million with the PCr signal set at 0 ppm. The values of the metabolites were given as ratios to P_{tot}. P_{tot} was quantified as the peak integrals of PME + PDE + P_i + PCr + γ NTP + α NTP + β NTP. The

values of the blood samples, FBP, FHR, and temperature were averaged during the recording time of one ³¹P-MR spectrum (17 min).

Data analysis and statistics. Blood gas values, acid-base balance, FBP, FHR, Hb, and temperature were compared between the end of the hypoxic period and the control period. Ratios of the cerebral metabolites and pH_i were calculated at several determined pH intervals during the hypoxic period and at the end of the hypoxic period, and compared with control values. Values are expressed as mean (\pm SD). Paired *t* tests are used for statistical analysis.

RESULTS

A total of six fetal sheep were operated on. In the fetal sheep the pH varied from 7.21 to 7.38 during surgery. One of them (no. 2) passed through a moment of decreased SaO₂ (24%). All fetal sheep arrived at the MR unit with a satisfactory metabolic status (pH \geq 7.30). During the control period, when the ewe was situated in a stable condition in the MR bore, the mean arterial pH varied from 7.30 to 7.37 in the six fetal sheep. In four fetal sheep, three to five ³¹P-MR spectra of 17 min were recorded during the control period; in one fetal sheep one spectrum was recorded. In one fetal sheep (no. 6), arterial pH was below 7.30 when the first ³¹P-MR spectrum was measured, and no ³¹P-MR spectrum was available during the control period. Although not all fetal sheep fulfilled the control criteria (pH > 7.30) before the maternal Fio₂ was decreased, for all six fetal sheep the ³¹P-MR spectra did not change and the cerebral pH_i was constant in the period before the Fio₂ was decreased.

The arterial pH decreased during hypoxia in all fetal sheep, and the lowest values varied from 6.71 to 6.99. All fetal sheep were alive when an arterial pH of 7.00 was reached. The mean extracellular fluid base excess decreased from -5.7 ± 2.1 to -19.6 ± 3.7 mM. FBP fell from 52 ± 11 mm Hg during the control period to 23 ± 11 mm Hg at the end of the hypoxic period. Arterial Pco₂ was increased at the end of the experiment. Fetal Hb, FHR, and fetal temperature did not change significantly throughout the experiment.

Table 1 summarizes blood gases, acid-base balance, Hb, FHR, FBP, and temperature of the fetal sheep. Table 2 summarizes ratios of the metabolites measured with ³¹P-MRS and cerebral pH_i, at the following time points: during the control period, when arterial pH was between 7.20 and 7.25, when arterial pH was between 7.10 and 7.15, when an arterial pH of 7.00 was reached, and at the end of the experiment, when the last blood sample was obtained. The interval (minutes) between the moment when SaO₂ dropped below 30% [the moment when anaerobic metabolism starts in fetal sheep (23)] and the events defined as time points for measurements is given in the last row of Table 2. At an arterial pH between 7.20 and 7.25, the average PCr/P_i decreased significantly from 1.08 ± 0.10 during the control period to 0.77 ± 0.29 , and decreased further toward the end of the experiment. The PCr/P_i of all fetal sheep fell below 0.8 at an arterial pH of 7.21 ± 0.08 , which took 0–192 min, counted from the moment when SaO₂ dropped below 30%. Subsequently, at an arterial pH between 7.10 and 7.15, P_i/P_{tot} was increased compared with control values and

Table 1. Fetal arterial blood variables ($n = 6$), temperature, FHR ($n = 5$), and FBP ($n = 5$) during control periods, between an arterial pH of 7.20 and 7.25, between an arterial pH of 7.10 and 7.15, when an arterial pH of 7.00 was reached, and at the end of the hypoxic period

Variable	Control	pH 7.20–7.25	pH 7.10–7.15	pH 7.00	End of hypoxia
Sao ₂ (%)	64.0 ± 15.6	25.5 ± 8.7**	16.9 ± 2.7**	14.2 ± 4.2‡	12.6 ± 5.2‡
pH	7.33 ± 0.03	7.25 ± 0.02**	7.14 ± 0.02**	6.99 ± 0.04‡	6.88 ± 0.10‡
BE _{ecf} (mM)	-5.7 ± 2.1	-8.0 ± 2.7	-13.3 ± 2.8‡	-17.3 ± 2.8‡	-19.6 ± 3.7‡
Pco ₂ (kPa)	4.88 ± 0.68	6.02 ± 0.78	5.86 ± 1.04	7.01 ± 1.02*	8.18 ± 2.52**
Hb (mM)	7.2 ± 2.3	7.1 ± 1.8	6.8 ± 2.0	7.0 ± 2.2	6.7 ± 2.1
FBP (mm Hg)	52 ± 11	53 ± 3	53 ± 13	30 ± 13*	23 ± 11**
FHR (bpm)	144 ± 30	171 ± 30	190 ± 29	153 ± 52	146 ± 36
temp (°C)	38 ± 4	39 ± 2	39 ± 2	39 ± 2	39 ± 1

Values are mean ± SD.

* $p < 0.05$, ** $p < 0.01$, ‡ $p < 0.001$, compared with control value.

Abbreviation used: BE_{ecf}, extracellular fluid base excess.

Table 2. Ratios of phosphorus metabolites and pH_i ($n = 6$)

	Control	pH 7.20–7.25	pH 7.10–7.15	pH 7.00	End of hypoxia
PCr/P _i	1.08 ± 0.10	0.77 ± 0.29**	0.46 ± 0.30*	0.27 ± 0.2*	0.00 ± 0.00‡
PCr/P _{tot}	0.10 ± 0.03	0.12 ± 0.02	0.10 ± 0.03	0.03 ± 0.04*	0.00 ± 0.00‡
P _i /P _{tot}	0.11 ± 0.03	0.14 ± 0.02	0.18 ± 0.04*	0.43 ± 0.25*	0.62 ± 0.22**
NTP/P _{tot}	0.12 ± 0.02	0.13 ± 0.02	0.11 ± 0.04	0.05 ± 0.05*	0.00 ± 0.00‡
pH _i	7.18 ± 0.03	7.20 ± 0.05	7.05 ± 0.13	6.71 ± 0.28*	6.47 ± 0.31**
time		0–192	69–208	104–260	138–278

$n = 5$ for the control period because metabolic acidosis was present when ³¹P-nMRS measurements started in one fetal sheep. Values are presented as mean ± SD. Time (min) is indicated from the moment when Sao₂ dropped below 30% to the specific time points at which measurements were obtained.

* $p < 0.05$, ** $p < 0.01$, ‡ $p < 0.001$, compared with control value.

increased further up to the end of the experiment. At an arterial pH of 7.00, PCr/P_{tot}, NTP/P_{tot}, and pH_i were decreased compared with control values. P_{tot} ($p = 0.004$, paired t test) was decreased only at the end of the hypoxic period (pH 6.88) compared with control values.

Figure 1 shows ³¹P-MR spectra obtained from this VOI during the experiment. The following spectral peaks can be recognized in the spectra during normoxia: PME, P_i, PDE, PCr, and the γ , α , and β phosphorus nuclei of NTP (24). Under hypoxic conditions, PCr decreased, P_i increased, and NTP was depleted at the end of the experiment. The P_i peak has an irregular shape during the first part of the hypoxic period (arterial pH 7.31, 7.25, and 7.16) and shifts upfield indicating brain tissue acidification with ongoing hypoxia.

The relationship between cerebral measurements and the pH of the arterial blood is demonstrated in Figure 2.

DISCUSSION

In fetal sheep, no cerebral lactate increase appeared, as detected by ¹H-MRS, above an arterial pH of 7.28 (14). During sustained decreased Sao₂ and the subsequent systemic acidosis, ³¹P-MRS showed cerebral changes. During the first part of hypoxia, the P_i peak became irregular, which might reflect the presence of variable tissue pH values in the VOI. The brain contains several intracellular and extracellular compartments of P_i (25). This phenomenon may be an early indicator of changes in pH of different compartments in brain tissue during the hypoxic period.

As shown in Table 2, the mean PCr/P_i was decreased less than 0.80 at a mild acidosis with an arterial pH of between 7.20 and 7.25. Hypoxia results in a decrease in ATP production. Replenishment of ATP is facilitated by the creatine kinase

reaction, leading to a decrease in PCr. During severe hypoxia, ATP is hydrolyzed, which results in P_i production. P_i/P_{tot} is significantly increased at pH 7.10–7.15. PCr/P_{tot} is decreased significantly at pH 7.00, a more hypoxic situation as compared with the increase of P_i/P_{tot} at a pH of 7.10–7.15. This can be explained because activation of ATP hydrolysis during hypoxia may precede glycolytic activity, and an increase of P_i might be a relative early event (6). Values for PCr/P_i less than 0.80 in asphyxiated neonates during secondary energy failure were associated with a very bad prognosis for survival and early neurodevelopmental outcome (8). Despite a decreased PCr/P_i, these arterial pH levels in the fetal sheep are not necessarily associated with a poor neurologic outcome because the situation in the fetal sheep and human neonates is not comparable (13). Furthermore, the PCr/P_i reduction in the asphyxiated neonates occurred on the second to ninth days after birth, associated with secondary energy failure. The fall in PCr/P_i in itself at a relatively mild acidosis of an arterial pH between 7.20 and 7.25 in the fetal sheep will probably not lead to cerebral damage, but may precede a cascade of events leading to cerebral damage. Buffering of ATP is provided by processes such as the creatine kinase reaction, which causes a fall in PCr. A mild acidosis (pH 7.25) that is prolonged for several hours leads to increased concentrations of hypoxanthine, which is produced during the breakdown of ATP, in the cerebrospinal fluid of fetal sheep (26). At a pH of 7.20–7.25 during delivery, adaptive processes may also be activated to protect the brain. This insight helps to estimate the condition of the brain at a pH that needs clinical decision making. It has to be kept in mind that the development of brain damage is a combined action of several processes such as fetal adaptive responses and both duration and depth of the oxygen depletion.

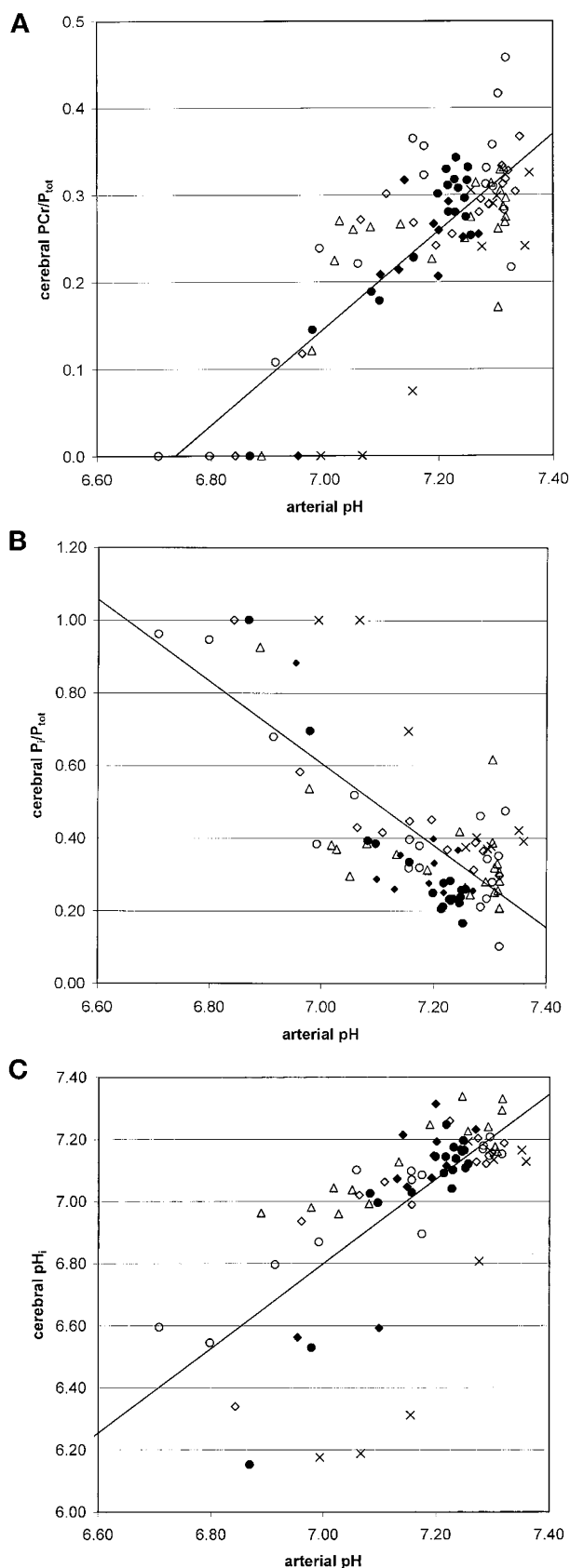


Figure 2. Cerebral $\text{PCr}/\text{P}_{\text{tot}}$ (A), $\text{P}_i/\text{P}_{\text{tot}}$ (B), and cerebral pH_i (C) plotted against arterial pH for six fetal sheep. There was a significant linear correlation between arterial pH and pH_i ($r = 0.70$; $p < 0.0001$). Symbols refer to the following sheep identification numbers: \diamond = sheep no. 1; \times = sheep no. 2; Δ = sheep no. 3; \circ = sheep no. 4; \blacklozenge = sheep no. 5; \bullet = sheep no. 6.

However, to which extent the pH level of 7.20–7.25 is associated with lower cerebral PCr/P_i values in human fetuses remains to be elucidated.

Hydrolysis of ATP causes P_i increase, resulting in a significantly increased $\text{P}_i/\text{P}_{\text{tot}}$ between an arterial pH of 7.10 and 7.15 in the fetal sheep. NTP was decreased while there was still PCr present, which was also demonstrated in the brain of fetal rats (27) and lambs (28). The fact that not all PCr was used as a buffer to maintain ATP levels in the brain points to a heterogeneous metabolic situation in the measured VOI of the brain. Compartmentation of PCr in brain has been suggested by Holtzman *et al.* (29); in the brain of mice, only half of the PCr content could be substituted by creatine analogs, in contrast to skeletal muscle. One PCr compartment is stable, whereas the other compartment is used during hypoxia (30). At the point when cerebral damage is expected to be present (arterial pH < 7.00) (5), the $\text{NTP}/\text{P}_{\text{tot}}$ and pH_i were decreased compared with control values. In hypoxic-ischemic lambs, β -ATP was decreased significantly at an arterial pH of 7.17 (28). Although the experimental protocols were not identical, this difference may be related to the ability of fetal brain to withstand hypoxia better compared with more mature brain.

The arterial pH is linearly correlated to cerebral pH_i (Fig. 2C), a phenomenon also demonstrated in dogs, in which cerebral pH_i decreased in parallel with blood pH after cardiac arrest (31). The relationship between arterial pH and pH_i may be of clinical relevance, as arterial pH can be measured during labor and decreased pH_i is associated with cerebral damage.

The experiments of the present study are performed under anesthesia and during surgery, which may affect the results. Van Cappellen van Walsum *et al.* performed studies with an identical design with respect to the obtained hypoxia-induced acidemia during surgery (26) and in chronically instrumented fetal sheep (32). Comparison of these two studies shows clearly the effects of acute experiments on cerebral metabolism. Lactate in the cerebrospinal fluid was not different at the baseline in the acute experiments compared with the chronic experiments. However, during severe hypoxia lactate increased more in the cerebrospinal fluid of the fetal sheep with anesthesia and surgery, compared with the sheep without anesthesia and surgery in the chronically instrumented fetal sheep. These results may indicate that anaerobic metabolism starts earlier in fetal sheep under anesthesia and surgery, and that at a pH of 7.25 the changes in energy metabolism are not present yet. Still, caution should be considered when mild hypoxia or acidosis is prolonged for some time, as is shown by the study of van Cappellen van Walsum *et al.* (26).

CONCLUSIONS

In conclusion, ^{31}P -MRS of fetal sheep brain clearly demonstrates that oxidative phosphorylation potential was affected at a mild arterial pH between 7.20 and 7.25 compared with control values, followed by a significant P_i increase resulting from NTP hydrolysis at an arterial pH between 7.10 and 7.15. At an arterial pH ≤ 7.00 , cerebral homeostasis was no longer maintained and NTP was depleted, inevitably followed by death in fetal sheep. The present results are clinically valuable

not only because they show the need to prevent mild hypoxia, but they will also help physicians to evaluate brain damage after birth. Only with increasing insight into the mechanism of brain damage can we start to develop tools to prevent prolonged hypoxia during pregnancy and labor. This strategy is needed in the quest to reduce the incidence of mental handicaps and cerebral palsy.

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