Effect of Verapamil Administration on Hypoxic Human Fetal Brain after Lactate detection by 1H Magnetic Resonance Spectroscopy (1HMRS)

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Consignado el 31 de Diciembre del 2000 a la Revista Vitae Academia Biomédica Digital.

SUMMARY

The purpose of this study was to evaluate the effect of verapamil administration on Human Fetuses in High Risk Pregnancies (FHRP) by Proton Magnetic Resonance Spectroscopy (1HMRS) after Lactate identification. Two 1HMRS studies were performed on a 1.5 Tesla system using the body RF coil: the first one was done between 30-31 weeks of gestation and the second between 34-35 weeks after verapamil administration. The results obtained in the second 1HMRS study show a significant decrease in Lactate signal in FHRP compared with the first study. This reduction could be related to a more oxygen availability due to the verapamil vasodilator effect in FHRP.

KEY WORDS: Proton Magnetic Resonance Spectroscopy, Fetal Brain, Lactate, Hypoxia, Verapamil.

INTRODUCTION

The major cause of perinatal brain injury is the acute cerebral hypoxia-ischemia, which mostly occurs by impaired intrapartum gas exchange (1). Cerebral hypoxia in the fetus and newborn increase neonatal morbidity and mortality (2). The most frequently sequelae are mental retardation, cerebral palsy, seizure disorders (1-2), and attention deficit disorder (3) among others. 1HMRS allows noninvasive observations of cerebral Lactate (Lac), Creatine and phosphocreatine (Cr), Choline (Cho) and N-Acetylaspartate (NAA) in newborn infants with and without hypoxic-ischemic brain injury (4-9). Previous investigators have provided data suggesting that Lac is undetectable in the normal neonatal brain at term, but may be found in the brains of both preterm infants and infants who are small for his gestational age (5). 1HMRS is recognized as a noninvasive approach to monitor the human fetal brain since 1994 (10), and has been successful in detecting Lac on lambs fetal brain (11-12) and newborn piglet (15) during hypoxia. In a previous research (14-15), we reported Lac on the Brains of Human Fetuses in High Risk Pregnancies (FHRP) by 1HMRS which brought the possibility of prediction intrauterine hypoxia before the labor. The purpose of the present work was to evaluate the ability of vasodilator to reduce Lac upon administration to mothers with special emphasis on the improvement of the anaerobic pathway observed in FHRP.

Abbreviations:

^{1}HMRS: Proton Magnetic Resonance Spectroscopy
MRI: Magnetic Resonance Imaging
Cho: Choline
Cr: Creatine and phosphocreatine
NAA: N-Acetylaspartate
Lac: Lactate
TE: Echo time
TR: Repetition time
RF: Radiofrequency
VOI: Volume of interest
FHRP: Human Fetuses in High Risk Pregnancies
Pi: inorganic phosphorus
ATP: Adenosine Triphosphate

Acknowledgements:
Physicians (Radiologists): H. Ortíz, M. Torcat, G. Zapata.
Anaesthetics: L.Díaz, M. Alliegro, J. Yungano, J. Morales.
MRI & MRS technicians: A. Guitan, J.Guitan, F. Paseta, A. Robaina, N. Isava, R. Cedeño, E. Contreras and M.V. Itriago.
Secretarial assistance: Y. Nazal, R. Velásquez, Y. Soares (CAIBCO)

METHODS

Six FHRP were studied with the informed consent from the expectant mothers. Magnetic Resonance Imaging (MRI) and ^{1}HMRS was performed on a 1.5 Tesla system (Symphony, Siemens Erlangen®). The standard body coil was used for radiofrequency (RF) excitation and the surface coil was positionated close to the fetal head for signal reception. A fast spin-echo sequence was used for scout and MRI localization with the following parameters: Repetition Time (TR) = 15 msec, Echo Time (TE) = 6 msec and acquisition time of 16 seconds. An alternative single-shot turbo spin-echo sequence (HASTE) was used to obtain MR images of the fetal brain in saggital and coronal orientations. Those images allowed us to select a nominal volume of interest (VOI). We performed two ^{1}HMRS: the first one was done between 30-31 weeks of gestation and the second between 34-35 weeks. After Lac detection on FHRP in the first ^{1}HMRS, we began verapamil administration (20 mg every 8 hours) to the mothers for four weeks. Single voxel was located between the two cerebral hemispheres from a VOI of 2.5 cm x 2.5 cm x 2.5 cm = 15.63 cm^{3}. ^{1}HMRS data sets were acquired with a 90°-180°-180° Spin-Echo Sequence (TR = 1500 msec, TE = 135 msec, 256 acquisitions were averaged) with CHESS for water suppression. Voxel placement for ^{1}HMRS is demonstrated in Figure 1.

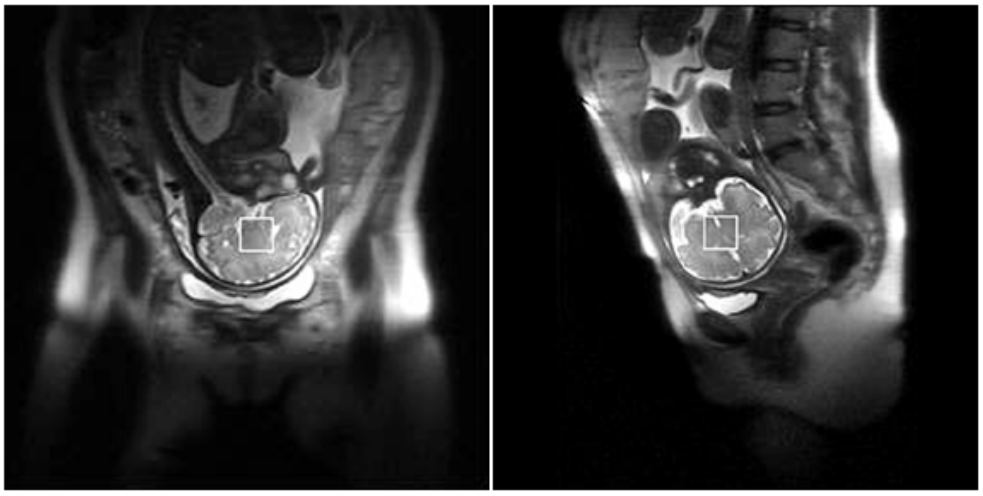

Figure 1. MRI in sagittal and coronal orientations of a fetus at 34 weeks gestational age. A VOI (15.63 cm^3) of fetal brain tissue was selected for ^{1}HMRS

NAA, Cho and Cr intensity signals were detected and NAA/(Cho+Cr), NAA/Cr and Cho/Cr ratios were calculated. Data postprocessing was performed with the software supplied by the manufacturer (NUMARIS®, Siemens Medical Systems®). The raw data were processed with a Gaussian filter in the chemical shift domain before Fourier transformation. The water peak was set to 4.7 ppm in chemical shift.

The results were analyzed with repeated measured Analysis of Variance (ANOVA) and Student-Newman-Keuls for comparison among ratio means.

RESULTS

Figure 2 shows the ^{1}HMRS spectra demonstrating Lac detection at 30 weeks of gestation in FHRP # 2. Lac is identified as an inverted peak represented by a duplet at 1.33 ppm (Lac signal integral = - 241.29). Negative notation is typical of a J-coupling integration of the two composed peaks in the second quadrant of the Cartesian axis. NAA/Col ratio for FHRP (0,30 ± 0,07) obtained previous to verapamil administration was significantly less ($p< 0,004$) than our reference value (0,53 ± 0,09) for Fetuses in Healthy Pregnancies for a similar gestational age as previously reported (14-16).

NAA/Col+Cr ratios in FHRP also showed a lower value (0,22 ± 0,08) compared with our reference value (0,37 ± 0,06) ($p< 0,01$). According with previous reports, the signal intensity for Cho decreased while NAA signal increased during perinatal and neonatal stages during brain maturity process (17-19).

Figure 3 shows FHRP # 2 after four weeks of verapamil administration (34 weeks of gestation). We clearly noted the Lac reduction and physiological evolution of metabolites ratios when compared with Figure 2. The increase in NAA/Cho and NAA/Cho+Cr ratios between the two studies agreed with previously published data (20-22).

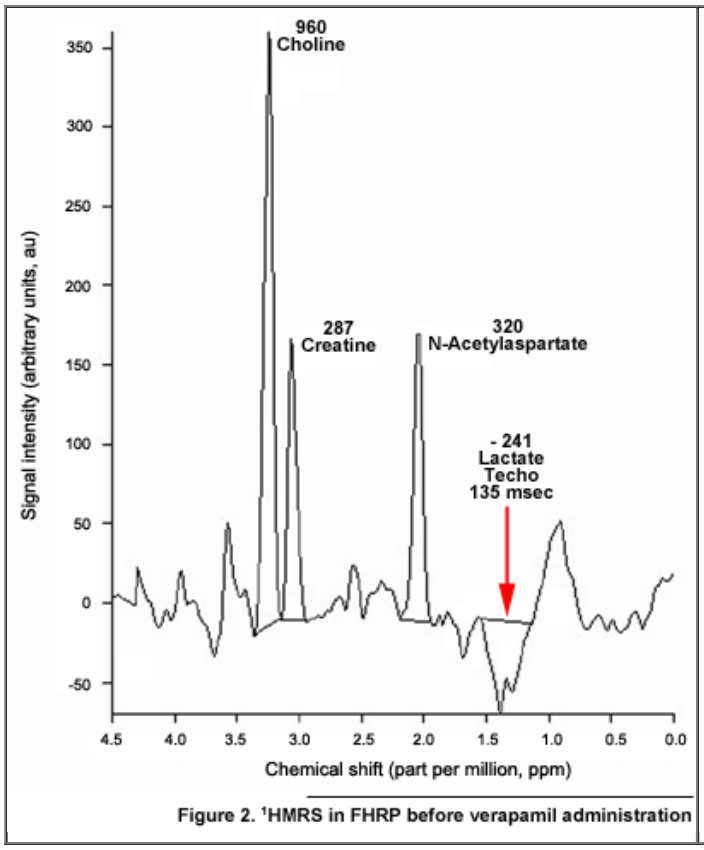

Figure 2. ^{1}HMRS in FHRP before verapamil administration

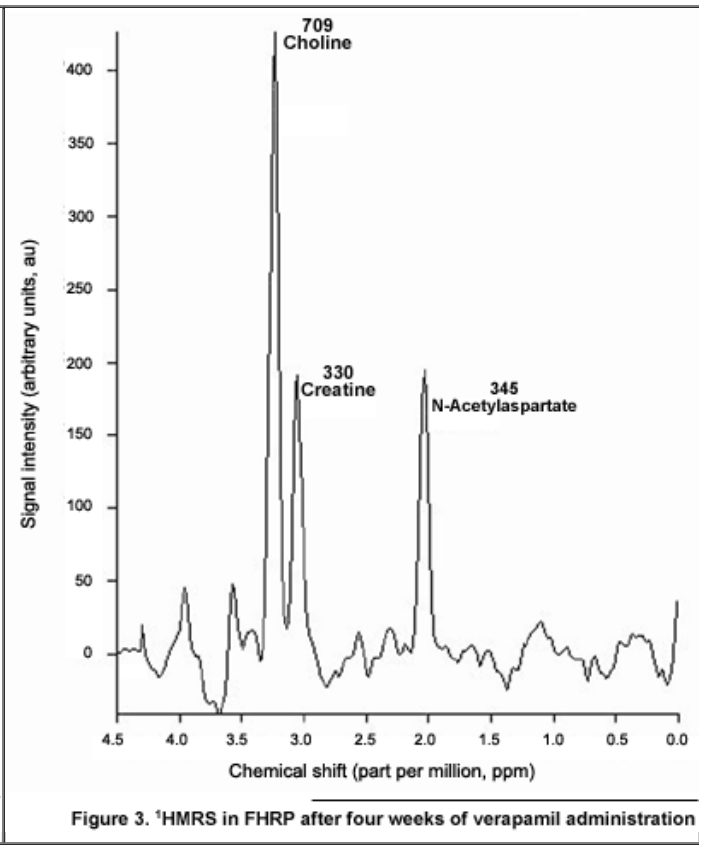

Figure 3. ^{1}HMRS in FHRP after four weeks of verapamil administration

DISCUSSION

The present study clearly demonstrated that by using a method as ^{1}HMRS we are capable to identify in the early stages, the hypoxia development in fetal brain. Little information is known about hypoxic fetal brain. Previous studies have shown changes in the cerebral metabolites of human neonates (4-7) and animals (11-13, 23-24) following hypoxia-ischemia. Brain Lac increment, which under normal circumstances is present in very small amounts in the neonatal brain at term age predicts a poor outcome (5). The results obtained clearly demonstrated that hypoxia could be developed during the intrauterine life of the fetus. Therefore, the metabolic changes developed during the perinatal or neonatal period of life in brain fetuses with hypoxia, could begin before the labor if the pharmacological intervention is not applied. Lac accumulation is associated with the development of intra and extracellular acidosis, formation of cytotoxic and vasogenic edema, changes in Na^{+}/H^{+} and $Cl^{-}/HCO3^{-}$ ion-exchange mechanism, further inhibition of oxidative phosphorylation, accelerated calcium influx and free radical injury (25). In the developing brain, determinants of susceptibility to hypoxia might include changes on lipid composition of the brain cell membrane, the rate of lipid peroxidation, the presence of antioxidant defenses, and the development and modulation of excitatory aminoacid neurotransmitter receptors; the N-methyl-D-aspartate (NMDA) receptor and intracellular Ca^{++} and intranuclear Ca^{++}-dependent mechanisms. Recent reports suggest that hypoxia-induced modification of the NMDA receptor leading to augmentation of intracellular Ca^{++} resulted in free radical generation and cell injury, indicating that during hypoxia the increased intracellular Ca^{++} may lead to a rise of the intranuclear Ca^{++} concentration and altered nuclear events including transcription of specific apoptotic genes and activation of endonucleases, resulting finally in programmed cell death (26) .

Studies realized in several species of mammals using ^{1}HMRS and ^{31}PMRS have described a characteristics biphasic pattern of cerebral metabolic abnormality after cerebral hypoxia-ischemia. It has been observed that intracerebral [PCr] / [Pi] ratio falls, internal pH falls, Lac increases and ATP declines(27). But although these temporary changes in metabolites concentration return to undetectable levels, prompt resuscitation causes all these metabolites to quick turn to normal values. Again, some hours later [PCr] / [Pi] ratio declines and Lac increases, although pHi becomes alkaline (28). There is a dose-response relationship between the severity of the hypoxic-ischemic insult, the magnitude of the secondary changes in cerebral energy metabolism, and the extent

of histological injury. Additionally, hypoxic ischemic injury can increase the intracellular calcium and lead to successive neuronal damage (29). According to the previously exposed, we tested a hypothesis considering the vasodilator effect of the verapamil supplying more oxygen to the fetal brain when it is administered to the mother. Previous reports have successfully demonstrated the beneficial effects of verapamil administration to fetuses throughout his mothers (30-32). The fetuses presented increased resistance of the middle cerebral arteries, increased resistance with notching, and absent end diastolic velocity or reverse diastolic flow (30-32). The disappearance of Lac after four week of verapamil administration might be related with a possible increase in the blood flow to the fetal brain due to the vasodilator effect of this calcium antagonist (33-35). This effect could minimize the redistribution of the blood flow to the heart, liver, adrenals and other organs observed during perinatal asphyxia. Restitution to normal blood flow can increase the brain oxygen concentration and return to the aerobic oxidation glucose pathway minimizing the Lac production in the fetal brain. All these evidences enhance the potential of ^{1}HMRS and ^{31}PMRS to assess the main key metabolites in human fetal brain in utero (36-39).

In conclusion, ^{1}HMRS is a useful tool in detecting Lac and predicting intrauterine hypoxia before the labor. Verapamil administration could be used without secondary effects to increase fetal brain blood flow and minimize the metabolic changes that cause the presence of Lac.

REFERENCES

1. **Penrice J, Cady E, Lorek A, Wylezinska M, Amess P, Aldridge R.et. al.** Proton Magnetic Resonance Spectroscopy of the Brain in Normal Preterm and Term Infants, and Early Changes after Perinatal Hypoxia-Ischemia. Pediatr. Res. 1996; 40: 6-14.
2. **Edwards A and Azzopardi D.** Perinatal hypoxia-ischemia and brain injury. Pediatr. Res. 2000; 47: 431-432.
3. **Toft P.** Prenatal and perinatal striatal injury: a hypothetical cause of attention-deficit-hyperactivity disorder?. Pediatr. Neurol. 1999; 21: 602-610.
4. **Barkovich A, Baranski K, Vigneron D, Partridge J, Hallam D, Latal B et. al.** Proton MR Spectroscopy for the evaluation of brain injury in asphyxiated, term neonates. Am. J. Neuroradiol. 1999; 20: 1399-1405.
5. **Zarifi M, Astrakas L, Poussaint T, du Plessis A, Zurakowski D and Tzika A.** Prediction of adverse outcome with cerebral lactate level and apparent diffusion coefficient in infants with perinatal asphyxia. Radiology 2002; 225: 859-870.
6. **Borowska-Matwiecjzuk K, Lemancewicz A, Tarasów E, Urban J, Urban R, Walecki W et. al.** Assessment of fetal distress based on magnetic resonance examinations: preliminary report. Acad. Radiol. 2003;10:1274-1282.
7. **Borowska-Matwiecjzuk K, Lemancewicz A, Tarasów E, Urban J, Urban R, Walecki W et. al.** The assessment of fetus in distress using MRI and ^{1}HMRS based on performed observation. Pol. J. Radiol. 2003; 68:5-12.
8. **Roelants-Van Rijn A, Van Der Grond J, De Vries L and Groenendaal F.** Value of ^{1}HMRS using different echo times in neonates with cerebral hypoxia-ischemia. Pediatr. Res. 2001;49: 356-362.
9. **Leth H, Toft P, Pryds O, Peitersen B, Lou H and Henriksen O.** Brain lactate in preterm and growth-retarded neonates. Acta Paediatr. 1995; 84:495-499.
10. **Heerschap A and van den Berg, P.** Proton Magnetic Resonance Spectroscopy of Human Fetal Brain. Am. J. Obstet. Gynecol. 1994; 170: 1150-1151.
11. **van Cappellen A, Heerschap A, Nijhuis J, Oeseburg H and Jongsma.** Proton magnetic resonance spectroscopy of fetal lamb brain during hypoxia. Am. J. Obstet. Gynecol. 1998; 179: 756-757.
12. **van Capellen A, Heerschap A, Nijhuis J, Oeseburg B and Jongsma H.** Hypoxia, the subsequent systemic metabolic acidosis, and their relationship with cerebral metabolite concentrations: An in vivo study in fetal lambs with proton magnetic resonance spectroscopy. Am. J. Obstet. Gynecol 1999; 181:1537-1545.
13. **Penrice J, Lorek A, Cady E, Amess P, Wylezinska M, Cooper C. et.al.** Proton magnetic resonance spectroscopy of the brain during acute hypoxia-ischemia and delayed cerebral energy failure in the newborn piglet. Pediatr. Res. 1997; 41: 795-802.
14. **Alvarado A, Ortega R, Mayobre F, Itriago P, Hernández N and Itriago S.** ^{1}H Magnetic Resonance Spectroscopy (MRS) and Doppler Ultrasound (US) on the Brains of Human Fetuses in High-Risk Pregnancies. Radiology 2000; 217 (Suppl 1): 477.
15. **Alvarado A, Ortega R, Mayobre F, Itriago P, Hernández N and Itriago S.** Estudio Bioquímico del Cerebro Humano Fetal mediante Espectroscopia por Resonancia Magnética (ERM) y Ultrasonido Doppler. Acta

Científica Venezolana 2000; 51: (Supl.2),160.
16. Alvarado A, Ortega R, Mayobre F, Hernández N, Martín M, Guevara F, Siurana S, Paseta F y Guitan J. Effect of verapamil administration on Human Brain Fetuses in High Risk Pregnancy after Lactate detection by 1H Magnetic Resonance Spectroscopy. Proc. Intl. Soc. Mag. Reson. Med.2002; (10): 2547.
17. Kreis R, Hofmann L, Kuhlmann B, Boesch C, Bossi E and Huppi P. Brain metabolite composition during early human brain development as measured by quantitative in vivo 1H magnetic resonance spectroscopy. Magn. Reson. Med. 2002; 48 : 949-958.
18. Kok R, van den Bergh A, Heerschap A, Nijland A and van den Berg P. Metabolic information from the human fetal brain obtained with proton magnetic resonance spectroscopy. Am. J. Obstet. Gynecol. 2001; 185: 1011-1015.
19. Kok R, van der Berg P, van den Bergh A, Nijland R and Heershap A. Maturation of the Human Fetal Brain as observed by 1H MR Spectroscopy. Magn. Reson. Med. 2002; 48: 611-616.
20. Heerschap A, van den Bergh, van den Boogert, Kok R and van den Berg P. Proton MR Spectroscopy of the Human Fetal Brain in utero. Proc. Intl. Soc. Magn. Reson. Med. 1999; (7): 337.
21. van den Berg A, Heerschap A, Kok R and van den Berg. Fetal Brain Development as reflected in metabolite ratio recorded by 1H MRS. Proc. Intl. Soc. Magn. Reson. Med. 2000;(8): 588.
22. Chin-Shoou L, Fenton B, Macedonia C, Schellinger D and Ascher S. In vivo Volume-selected Proton MR Spectroscopy of the Fetus in Utero- Initial Experience. Proc. Intl. Soc. Magn. Reson. Med. 2000; (8):1915.
23. van Cappellen A, Jongsma H, Wevers R, Nijhuis J, Crevels J, Engelke U et. al. Hypoxia in fetal lambs: a study with 1H-NMR spectroscopy of cerebrospinal fluid. Pediatr. Res. 2001; 49: 698-704.
24. van Cappellen A, Jongsma H, Wevers R, Nijhuis J, Crevels J, Engelke U et. al. 1H-NMR spectroscopy of cerebrospinal fluid of fetal sheep during hypoxia-induced acidemia and recovery. Pediatr. Res. 2002; 52: 56-63.
25. Ashwal S, Holshouser B, Tomasi L, Shu S, Perkin R, Nystrom G et.al. 1H-magnetic resonance spectroscopy-determined cerebral lactate and poor neurological outcomes in children with central nervous system disease. Ann. Neurol. 1997;41:470-481.
26. Delivoria-Papadopoulos M and Mishra O. Mechanisms of perinatal cerebral injury in fetus and newborn. Ann. N. Y. Acad. Sci. 2000; 900:159-168.
27. Cooper C and Wyatt J. NMR spectroscopy and imaging of the neonatal brain. Biochem. Soc. Trans. 2000; 28 :121-126.
28. Delivoria-Papadopoulos M and DiGiacomo J. ^{31}P Nuclear magnetic resonance spectroscopy in the human neonatal brain.Semin. Perinatol 1990; 14:248-257.
29. Dilmen U, Mete E, Varoglu E, Akyüz M, Akin Y and Örs R. The effect of verapamil and magnesium sulfate on regional cerebral blood flow in a pup model of perinatal asphyxia. J.Islam. Acad. Sci. 1995; 8 : 1-7.
30. Ortega R, Clarembaux J, Hernandez C and Gil E. El verapamil en las resistencias fetales altas. Rev. Mex. Pueril. Ped. 1995; 3: 38-44.
31. Ortega R, Clarembaux J, Salazar E, Guevara F, Urbina D and Hernández A. Efectos de aspirina y verapamil sobre resistencias materno fetales en primigestas con riesgo de preeclampsia. Rev. Obstet. Ginecol. Venez. 1993; 53: 17-22.
32. Ortega R, Clarembaux J, Guevara F, Ortega J and Weisinger K. Resistencias fetales altas. Uso de vasodilatadores por vía materna. Rev. Obstet. Ginecol. Venez. 1992; 52: 35-41.
33. Isla M and Dyer D. Vasodilatory effects of nifedipine, methoxyverapamil and sodium nitropruside on contractile response of the ewe uterine artery at term pregnancy. Am. J. Obstet. Gynecol. 1990; 163: 1337-1344.
34. Dubiel M, Gunnarsson G and Gudmundsson S. Blood redistribution in the fetal brain during chronic hypoxia. Ultras. Obstet. Gynecol. 2002; 20:117
35. Saliba E, Barantin L, Akoka S, Tranquart F, Pourcelot L, Gold F et.al. Circulation and cerebral metabolism in neonatal hypoxia-ischemia. J. Gynecol. Obstet. Biol. Reprod. 1997; 26:465-469.
36. Kato T, Nishina M, Matsushita K, Hori E, Mito T and Takashima S. Neuronal maturation and N-acetyl-L-aspartic acid development in human fetal and child brains. Brain Dev. 1997;19:131-133.
37. Fenton B, Chin-Shoou L, Macedonia C, Schellinger D and Ascher S. The fetus at term: in utero volume-selected proton MR Spectroscopy with a breath-hold technique-A feasibility study. Radiology 2001; 219: 563-566.
38. van Cappellen A, Rijpkema M, Heerschap A, Oeseburg B, Nijhuis J and Jongsma H. Cerebral (31) P magnetic resonance spectroscopy and systemic acid-base balance during hypoxia in fetal sheep. Pediatr. Res.

2003;54:747-752.
39. Heerschap A, Kok R and van den Berg P. Antenatal proton MR spectroscopy of the human brain in vivo. Childs Nerv. Sys. 2003;19:418-421.