INTRODUCTION
Perinatal asphyxia affects 1% of newborns worldwide. Six per 1000 neonates suffer from neonatal encephalopathy, resulting in cerebral palsy, mental retardation, learning disabilities, and epilepsy. Early identification of neonates at risk and the presence of biphasic changes in cerebral energy status measured with \(^{31}\)P- and \(^{1}H\)-MRS provides a window for therapeutic intervention. Free radicals play a crucial role in brain cell injury after HI. Xanthine oxidase inhibitors (e.g. allopurinol, ALLO) and non protein bound iron chelators (e.g. deferoxamine, DFO) have been shown to reduce, but not prevent completely the free-radical induced brain injury. During HI free radicals as superoxide react with nitric oxide to form peroxynitrite, which is deleterious to cell membranes. Specific inhibition of neuronal Nitric Oxide Synthase (e.g. 2-Iminobiotin, 2-IB) seems to be promising: the neuronal formation of nitric oxide can be blocked, without the adverse effect of inhibiting the endothelial form of NOS. This leads to a reduction in cerebral perfusion. The purpose of this study was to evaluate the potential beneficial effect of 2-IB, compared to ALLO and DFO, administered at the onset of reperfusion on cerebral energy status and electrical brain activity in a neonatal piglet model, using \(^{31}\)P- and \(^{1}H\)-MRS and amplitude integrated EEG (aEEG).

MATERIAL AND METHODS
Following anesthesia and instrumentation 37 newborn Dutch store piglets (1-3 days old) were subjected to HI by occluding both common carotid arteries with inflatable cuffs and reducing the fraction of inspired oxygen for 60 min. MRS was performed continuously before, during and up to 3 h after start of HI and repeated at 24 h post HI. During hypoxia FiO\(_2\) was reduced ‘online’ until PCr/Pi had decreased to at least 25% of baseline values. Immediately after HI the piglets received either placebo (PLAC; n=10), ALLO (20mg/kg i.v.; n=10), DFO (10 mg/kg i.v.; n=10) or 2-IB (150µM; i.v.; n=7). Before HI and from 3 to 24 h post HI the piglets were monitored using aEEG for electrical brain activity determination. A neurologic scoring system was used ranging from 4 (normal) to 0 (flat trace). For \(^{1}H\)-MRS a PRESS sequence with CHESS water suppression was used to define a 1.7 ml periventricular voxel (TR 6 s, TE 144 ms, and nt=32 or 64). \(^{31}\)P-MRS was done using a 4 cm \(^{1}H\)-MRS and amplitude integrated surface coil for excitation and detection (TR 10 s, nt=32). Peak amplitudes of PCr, Pi, Lac and NAA were determined with time domain fitting procedures (WARPRO). Paired t-tests were used to compare measurements at 24 h versus baseline; repeated ANOVA served to monitor for trends between treatment groups.

RESULTS
Three piglets in the PLAC group and 1 piglet in the ALLO group died due to HI complications at respectively 5, 9 and 19 h and 18 h post HI, 1 piglet died at 13 h in the DFO group because of hypovolemic shock and 1 in the 2-IB group due to sepsis. \(^{31}\)P-MR spectra of a representative PLAC and 2-IB-treated piglet are shown in figure 1 at 24 h post HI. Secondary energy failure, defined as a secondary fall in PCr/Pi is observed in the PLAC, but not in the 2-IB piglet. PCr/Pi as percentage of baseline and Lac/NAA ratios from normoxia to 24 h post HI for all treatment groups are presented in figure 2. For the 2-IB group 24 h post HI values were identical to baseline values of PCr/Pi and Lac/NAA. For PLAC-treated piglets PCr/Pi was significantly decreased and Lac/NAA significantly increased at 24 h post HI. Figure 3 shows the neurologic score for all treatment groups. Using repeated ANOVA a significant difference was demonstrated between PLAC and 2-IB, ALLO and DFO (all p<0.05).

Legend for figures:

![Figure 1](image1.png)  \(^{31}\)P-MR spectra of a representative PLAC treated piglet (A) and a representative 2-IB treated piglet (B) at 24 h post HI.

![Figure 2](image2.png)  PCr/Pi % and Lac/NAA ratios from normoxia until 24 h post HI in PLAC, ALLO, DFO and 2-IB-treated piglets.

![Figure 3](image3.png)  aEEG for PLAC, ALLO, DFO and 2-IB treated piglets before HI, and at 3, 6, 12 and 24 h post HI.

CONCLUSION
Whereas ALLO en DFO prevented partly the reduction in PCr/Pi ratios at 24 h post HI, 2-IB prevented completely cerebral energy status at 24 h post HI and to a lesser degree ALLO and DFO prevented increment of Lac/NAA at 24 h post HI and preserved electrical brain activity. We speculate that the remarkable preservation of the cerebral energy status by 2-IB is due to prevention of the formation of peroxynitrite following hypoxia-ischemia in the newborn piglet.

REFERENCES

Cacha Peeters\(^1\), Wouter Veldhuis\(^2\), Ika Borst\(^3\), Kees Braun\(^2\), Robin de Graaf\(^2\), Klaas Nicolay\(^2\), Floris Groenendaal\(^1\), Wilhelmina Children’s Hospital\(^1\), Dept. Experimental In Vivo NMR, Image Sciences Institute, Utrecht University\(^2\), Utrecht, the Netherlands