

## Magnetic Resonance Spectroscopy of Diffuse Brain Trauma in the Pig

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### ABSTRACT

The acute metabolic events linked to the evolution of selective axonal pathology in the white matter following diffuse brain injury have not previously been evaluated due to the paucity of relevant experimental models. Here, we utilized a new model of inertial brain injury in the pig that selectively damages axons in the white matter, and applied proton and phosphorous magnetic resonance spectroscopy (MRS) to noninvasively monitor the temporal course of metabolic changes following trauma. Evaluating four pigs with MRS prior to injury, within 1 h and 3 and 7 days postinjury, we found that widespread axonal injury was produced in the absence of changes in pH, PCr/Pi, or the concentrations of ATP, and lactate. However, we did observe an acute 60% loss of intracellular Mg<sup>2+</sup> levels, which gradually resolved by 7 days postinjury. In addition, we found that the levels of the neuron marker, *N*-acetylaspartate (NAA), acutely dropped 20% and remained persistently decreased for at least 7 days postinjury. Moreover, the changes in Mg<sup>2+</sup> and NAA were found with MRS in the absence of abnormalities with conventional magnetic resonance imaging (MRI). These results show that (1) profound alterations in intracellular metabolism occur acutely following diffuse axonal pathology in the white matter, but in the absence of indicators of ischemia, and (2) axonal pathology may be evaluated with high sensitivity utilizing noninvasive MRS techniques.

**Key words:** axonal injury; diffuse brain injury; NAA; phosphorus MRS; proton MRS

### INTRODUCTION

WHILE THE ETIOLOGY OF TRAUMATIC closed-head injury in humans is enormously varied, two major categories have been established: focal and diffuse traumatic brain injury. Focal brain injuries, including cerebral contusion and hematomas, are typically observed following blunt trauma, due to contact loading forces. Diffuse brain injuries are thought occur primarily from

shearing or impulsive forces, commonly resulting from automotive crashes in which the brain is rapidly rotated. These loading conditions may induce disruption of white matter widely throughout the brain, resulting in diffuse axonal injury (DAI). DAI is present in over half of all severely head-injured patients and in more than 80% of the severe head injuries resulting from motor vehicle crashes (Graham et al., 1993; Adams et al., 1989). Although conventional brain imaging techniques are useful

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for revealing macroscopic changes often seen in diffuse brain trauma, such as white matter tears and parenchymal hemorrhage, they cannot easily detect the predominant pathology of DAI, axonal swellings and bulbs. Thus, patients with little macroscopic injury following diffuse brain injury may have normal appearing images of the brain and, as such, DAI is thought to be substantially underdiagnosed (Mittl et al., 1994). Moreover, the precise pathophysiologic mechanisms leading to DAI remain to be established.

The paucity of investigative efforts aimed at determining the pathophysiology of diffuse white matter trauma in the brain may be linked, in part, to the difficulty in developing relevant experimental models. Although we and others have extensively used rodent models of impact brain injury to explore various aspects of axonal injury (Foda and Marmarou, 1994; Pierce et al., 1996; Dixon et al., 1991; Lewen et al., 1995; Heath and Vink, 1995, 1996), it is important to note that rodents have very small lissencephalic brains with relatively sparse pure white matter domains. Due to these limitations, many of the mechanical loading conditions and pathologic features found in human diffuse brain injury cannot be reproduced in rodents. However, nonimpact rotational brain injury in the nonhuman primate has been shown to replicate all the salient characteristics of DAI (Gennarelli et al., 1982). The success of this model has been attributed to the application of inertial loading conditions to the head of an animal with a relatively large gyrencephalic brain. Adapting the same injury apparatus as used for the nonhuman primate studies, we have recently developed a model of rotational acceleration brain injury in young adult miniature swine (Meaney et al., 1993; Smith et al., 1997). This species and strain was chosen due to their large gyrencephalic brains (70–95 g) and low body weight (17–20 kg). We have shown that the swine model of inertial brain injury can produce widely distributed axonal pathology in the white matter in the absence of macroscopic damage. In the present study, we used this pig brain injury model to noninvasively explore the pathophysiologic sequelae of diffuse axonal pathology utilizing proton ( $^1\text{H}$ ) and phosphorus ( $^{31}\text{P}$ ) magnetic resonance spectroscopy (MRS) techniques to evaluate acute and subacute metabolic changes following inertial brain injury. Proton and phosphorus MRS have been extensively utilized to monitor neuronal damage and metabolic dysfunction following experimental focal brain injury (Andersen and Marmarou, 1992; Berry et al., 1986; Inao et al., 1988; McIntosh et al., 1987; Vink et al., 1987, 1988) and widely distributed axonal injury in the rat (Heath and Vink, 1995, 1996). However, no previous studies have applied these tech-

niques to evaluate the acute response of experimental diffuse brain injury in a gyrencephalic animal.

## MATERIALS AND METHODS

### *Preinjury Preparation*

Four miniature swine (Hanford strain), young adult (4 months of age) males and females, 17–20 kg, were used for this study. The animals were fasted for 12 h, following which anesthesia was induced with an initial injection of midazolam (400–600 mg/kg). Once sedated, animals received 2–4% isoflurane via snout mask until they reached a plane of surgical anesthesia. A venous catheter was then inserted in the ear, and the animals were endotracheally intubated and maintained on 1.5–2% isoflurane. Subsequently, two of the animals received sterile placement of an intracranial pressure (ICP) fiberoptic probe housed in a subarachnoid bolt, which was screwed into the parietal bone. In addition, sterile placement of a femoral arterial catheter was also performed. Additional monitoring apparatus included noninvasive ECG electrode leads affixed to the chest and extremities, a pulse oximeter placed on the skin of the tail, a rectal thermometer, and sampling tubes for end tidal  $\text{CO}_2$  measurement attached to the endotracheal tube. Arterial blood gasses were also periodically evaluated prior to injury and following injury. The pigs were continuously monitored, and all data from physiologic monitoring were collected on a computer driven storage system. All incisions were closed and dressed.

### *Brain Injury*

Brain trauma was induced via head rotational acceleration as previously described in detail (Meaney et al., 1993; Smith et al., 1997). Briefly, the animals' heads were secured to a padded snout clamp, which, in turn, is mounted to the linkage assembly of a HYGE device that converts the linear motion to an angular (rotational) motion. For these experiments, the linkage was adjusted to produce a pure impulsive head rotation  $110^\circ$  in the coronal plane, with the center of rotation close to the brain center of mass. Head rotational acceleration was biphasic with a predominant deceleration phase. Triggered release of pressurized nitrogen rotates the linkage assembly the full  $110^\circ$  in 20 msec. Following injury, the animals' heads were released from the clamp. Upon stabilization of vital signs, arterial and venous lines and the ICP bolt were removed, all incisions sutured closed and a topical antibiotic and dressing applied to the wounds and animals were transported to the MRS facility. Upon

withdrawal of isoflurane anesthesia, all animals received buprenorphine (0.1 mg/kg i.m., q 12 h p.r.n) for postoperative analgesia. It is important to note that previous studies with these techniques demonstrated injured animals were awake and ambulatory within 8 h of injury (Smith et al., 1997).

### Imaging Preparation

MRI and MRS procedures were performed at four time points; 1–3 days preinjury, immediately following injury (40–60 min), and 3 and 7 days following injury. For preinjury and 7 days postinjury magnetic resonance (MR) studies, once endotracheal intubation was performed, isoflurane anesthesia was discontinued and the animals received thiopental (i.v. 10–20 mg/kg/h) for transport to the MR facility and during MR examination. An i.v. drip of thiopental was not used due to the exquisitely sensitive nature of miniature swine to barbiturate anesthesia. The ET tube remained in place throughout transport and MR examination. The animals were continuously monitored (corneal reflex, limb withdrawal, ear twitch) and received supplements of thiopental as needed (approximately every 15–30 min). For the acute postinjury procedure, once the animals were considered stable (spontaneously breathing, stabilized heart rate, MAP, and ICP), they received thiopental anesthesia (i.v. 10–20 mg/kg/h to maintain a plane of anesthesia) and were transported to the MR facility and spectroscopic studies were all initiated within 30 min to 1 h following injury. All animals were continuously monitored while in transit and during the MR evaluation.

### MRI

All studies were performed on a General Electric Signa 1.5-T clinical MR scanner equipped with a spectroscopy software package (GE Medical Systems, Milwaukee, WI). A Sagittal T<sub>1</sub>-weighted sequence (TR/TE 600/20 msec, 5-mm thickness, 16-cm FOV, 1 NEX) and a coronal FSE T<sub>2</sub>-weighted and proton density-weighted (TR/2,700/20,80; 256 × 192; 3-mm thickness; 16-cm FOV) were acquired at each study.

### Proton MRS Evaluation

Solvent suppressed localized proton spectra were obtained at an echo delay of 20 msec (mixing time of 10.4 msec) using the STEAM sequence. A cubic region of the brain measuring approximately 1.5 × 1.5 × 1.5 cm was graphically selected from the MR image at the appropriate slice in the hemisphere opposite the ICP probe placement in the pertinent animals. These regions predominantly encompassed white matter domains, but also included gray matter. The homogeneity of the static mag-

netic field was adjusted in two steps. First, the homogeneity was adjusted by an automatic shimming algorithm (linear shims only), which was applied to the entire axial slice. Second, this was followed by an automated shimming algorithm, which adjusted the homogeneity over the selected voxel. The width at half height of the water line after shimming was 3 to 4 Hz. Solvent suppression was achieved by the application of three chemically selective pulses (CHESS) followed by spoiler gradients. The suppression was optimized using an automated algorithm, which adjusted the amplitude of the third CHESS pulse. Localized spectra were acquired using a 2-sec repetition time, 1,000-Hz sweep width, 2,048 complex points, eight-step phase cycling, outer volume spatial saturation, and 256 averages. The spectra were processed using ProNMR (Softpulse Software, Guelph, Ontario, Canada). The processing involved exponential apodization (2-Hz line broadening), zero filling to 4 K points, Fourier transformation, and zero order phase correction. Areas under the peaks were estimated using a Marquardt fitting routine to Lorentzian line shapes in the frequency domain. This fitting resulted in the determination of an area for each peak. From this method, metabolite ratios were determined.

### Phosphorous MRS Evaluation

Spectra were obtained by placing a 3-inch, proton/phosphorous double-tuned surface coil over the head of the animal sampling approximately a 60-cc volume, roughly the size of the brain. The transmitter power was adjusted to be equivalent to a 180-degree flip at the surface of the coil. Spectra were acquired using a 50-μsec pulse, 8-sec repetition time, 2,000-Hz sweep width, 2,048 complex points, and 32 averages. The spectral processing was performed with ProNMR using exponential apodization (10-Hz line broadening), Fourier transformation and zero order phase correction. The pH was calculated from the difference in chemical shift between the PCr and Pi:

$$\text{pH} = \frac{6.66 + \log_{10}(A - 3.074)}{5.57 - A}$$

where  $A$  = chemical shift of the inorganic phosphate peak. Because there is current debate in the literature as to the correct formula to determine changes in intracellular magnesium ( $[\text{Mg}^{2+}]$ ), we utilized the two most common equations:

$$([\text{Mg}^{2+}]_i) = 50 \frac{10.82 - \Delta}{\Delta - 8.35}$$

(based on efforts by Vink et al., 1988) (1)

$$[\text{Mg}^{2+}]_i = 50 \frac{10.759 - \Delta}{\Delta - 8.247}$$

(based on efforts by Williams et al., 1993) (2)

where  $\Delta$  = chemical shift difference between the alpha and beta resonances of ATP, assuming pH = 7.2, the theoretical dissociation constant for Mg ATP,  $K_D = 50 \mu\text{m}$ .

### Histopathology

At 7 days following brain injury, the animals received an overdose of pentobarbital (150 mg/kg i.v.) and were transcardially perfused with 4 L of saline following by 10 L of 4% paraformaldehyde. The brains were removed postfixed in 4% paraformaldehyde for 2 h and stored in phosphate buffer solution and cryoprotected with sucrose. The brains were blocked into 0.5-cm coronal sections intervals for gross examinations and photography. A series of 40- $\mu\text{m}$  frozen sections were cut from the front face of each block and mounted on microscope slides. One set of sections was mounted and stained with Nissl stain cresyl violet, H&E, and immunohistochemical techniques using the following primary antibodies, monoclonal antibodies used included the NR4 antibody, targeting the 68-kD neurofilament subunit (Boehringer Mannheim, Indianapolis, IN, 1:4), NN18 antibody, targeting the 160-kD neurofilament subunit (Sigma 1:40), N52 antibody, targeting the 200-kD neurofilament subunit (Sigma, St Louis, MO, 1:400) SMI-31 antibody, targeting selected epitopes primarily on the 170–200-kD neurofilament subunits and reacting with extensively phosphorylated NF-H and to a lesser extent with NF-M (Sternberger and Meyer, Baltimore, MD, monoclonals 1:5,000). The sections were incubated with primary antibody overnight at 4°C and then incubated at room temperature for 1 h, each with the appropriate secondary antibodies and ABC solution (1:1,000). Peroxidase activity was revealed with 0.025%, 3,3'-diaminobenzidine, 300 mg imidazole, and 0.25%  $\text{H}_2\text{O}_2$  for 10 min. All sections were examined under light microscopy to determine the extent and distribution of axonal damage throughout the brain.

## RESULTS

### Physiology and Behavior

Consistent with a previous report, immediately following trauma, only very subtle and transient increases in ICP and MAP were observed, resolving by 5–10 min postinjury (Smith et al., 1997). In addition, no substantial changes were noted in arterial blood gasses, pulse oximetry, or end tidal  $\text{CO}_2$  following injury. All animals began to awaken within 15 min following injury, but were

anesthetized with thiopental for transport to the MR facility. Following MR evaluation and termination of anesthesia, the animals quickly woke up, ambulated, and drank water, but appeared to have slightly sluggish responses to sensory stimuli. However, by 24 h postinjury, the animals appeared completely normal based on gross neurosensory examination (alert, responsive to stimuli, normal ambulation).

### Conventional MRI

No signal variations were found in the two animals that did not receive placement of an ICP probe. For the animals that were evaluated for ICP changes, hyperintensities were observed only in the region of the ICP probe placement and these changes did not correspond with the overall pattern of axonal pathology.

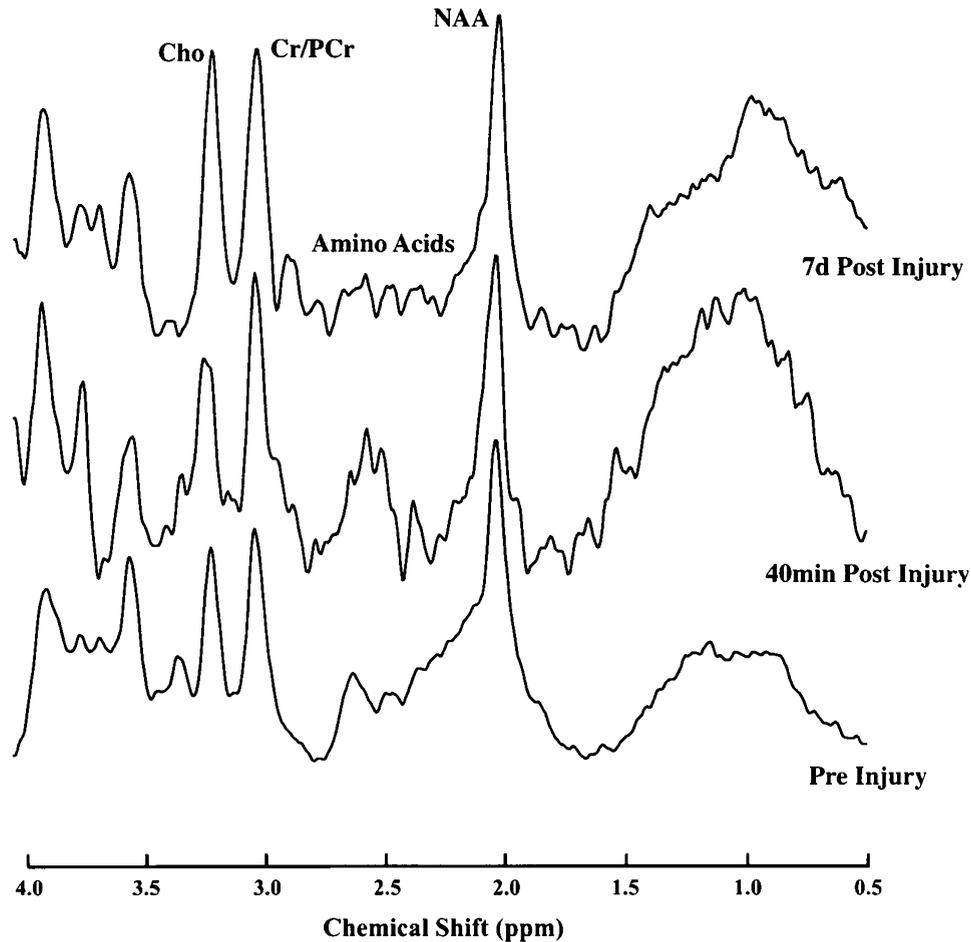
### Proton MRS

We found a consistent 20% decrease in the concentration of free NAA (determined from NAA/Cr ratios) within 1 h postinjury, that persisted for at least 7 days following injury (Figs. 1 and 2). In addition, there was an increase in the combined peaks representing glutamate, glutamine, GABA, and aspartate following injury, but, due to their spectral overlap, it could not be determined which amino acids contributed to this change. No significant changes in lactate concentration choline/Cr or myoinositol/Cr were observed in any animal at any timepoint.

### Phosphorous MRS

A significant 60% decrease in the concentration of intracellular free  $\text{Mg}^{2+}$  was found acutely following injury in all animals according to both formulas utilized. This change appeared to gradually resolve by 1 week following injury in all animals (Figs. 3 and 4). Although the mean value for  $[\text{Mg}^{2+}]_i$  at 3 days postinjury was substantially lower than the preinjury mean and had a relatively small standard error, significance was not determined at this timepoint, since the accuracy of each measure is limited by the precision in the determination of the chemical shift values of the alpha and beta resonances of ATP. We assume that the chemical shift difference is dominated by the digital resolution of the MRS acquisition. Under this assumption, error in the  $\Delta$  in the equations 1 and 2 is 0.035 ppm. Equations 1 and 2 can be arranged to give estimates of error in  $[\text{Mg}^{2+}]_i$  based on the uncertainties in  $\Delta$  at this particular field strength. At 40 min following injury, the mean value greatly exceeded the potential error in the determination of  $[\text{Mg}^{2+}]_i$ , hence, statistical analysis was valid. However, at 3 days postinjury, the error interval in the determina-

## MRS IN DIFFUSE BRAIN TRAUMA



**FIG. 1.** Representative single voxel proton MR spectra of swine brain prior to and following brain trauma. Cho, composite of compounds with choline moiety; Cr/PCr, creatine and phosphocreatine; NAA, *N*-acetylaspartate; d, day. Note the decrease in NAA peak compared to Cr/PCr peak following injury.

tion of  $[Mg^{2+}]$  was almost the same value as the mean, thus invalidating statistical comparisons. Although substantial changes in  $[Mg^{2+}]$ , were found at 40 min postinjury, no alterations in PCr/Pi, pH, or concentrations of ATP were noted at any evaluated timepoint in any animal.

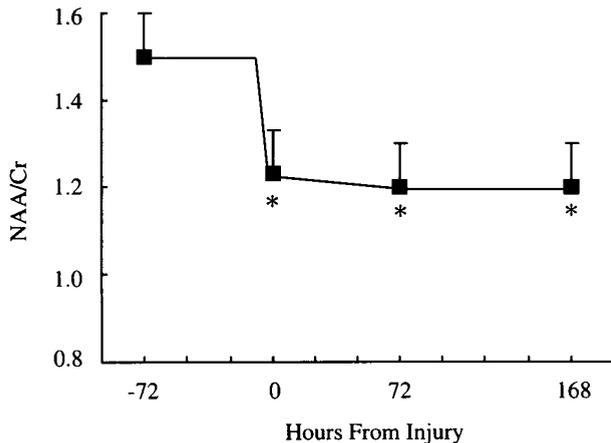
### *Histopathology*

Diffuse, multifocal axonal pathology, including bulbs and swellings, were identified with immunostains for neurofilament and H&E stains and found in an identical distribution as previously described (Smith et al., 1997). Regions affected included the deep white matter at the root of the gyri, the junction of white and gray matter in the frontal, parietal, and temporal lobes, margins of the lateral ventricles, external capsule, cerebral peduncles, and basal ganglia. In addition, pyknotic neurons were found in both hippocampi of all animals (Fig. 5). No con-

tusions, tissue tears, intraparenchymal hemorrhages, or other macroscopic damage was produced by the head rotational acceleration in animals without ICP probe placement. In the animals receiving ICP probe placement, a small 1–2-mm deep laceration was observed at the implantation site in the cortex. Axonal pathology was also found at this site in association with modest hemorrhagic changes extending into the subcortical white matter. It is important to note that the voxel selected for proton MRS evaluation encompassed regions of axonal pathology and the hippocampi in the hemisphere opposite ICP probe placement in the pertinent animals.

## DISCUSSION

Although acute changes in cerebral metabolism have been well documented in several rodent models of im-



**FIG. 2.** Temporal changes in the ratio of *N*-acetylaspartate to Creatine (NAA/Cr) in swine brain prior to and following brain trauma based on single voxel proton MR. Error bars = SEM. \* $p < 0.01$  compared to preinjury baseline measurement.

pact brain injury (McIntosh et al., 1987; Berry et al., 1986; Andersen and Marmarou, 1992; Vink et al., 1987, 1988; Inao et al., 1988; Heath and Vink, 1995, 1996), the present study is the first investigation of the acute metabolic consequences of inertial brain trauma in a gyrencephalic animal using proton and phosphorous MRS. Since head rotational acceleration in the coronal plane of swine produced axonal injury of the white matter as the predominant pathology, decreases in NAA and  $[Mg^{2+}]_i$  observed in this study may primarily be ascribed to reflect metabolic changes during the evolution of traumatic axonal injury.

The present results offer strong evidence that post-traumatic axonal pathology may develop in the absence of ischemic conditions. No acute changes in pH or lactate concentrations were found, ATP concentrations remained stable, and PCr/Pi did not change, even within 1 h following brain trauma. Thus, there was no evidence of cellular energetic stress that would indicate an ischemic condition. Previously, a subacute examination of brain-injured patients, including four subjects with the primary diagnosis of DAI, also revealed no evidence of subacute intracellular acidosis (Rango et al., 1990). This evidence provides support that mechanical loading forces applied to the brain are by themselves sufficient to produce axonal damage.

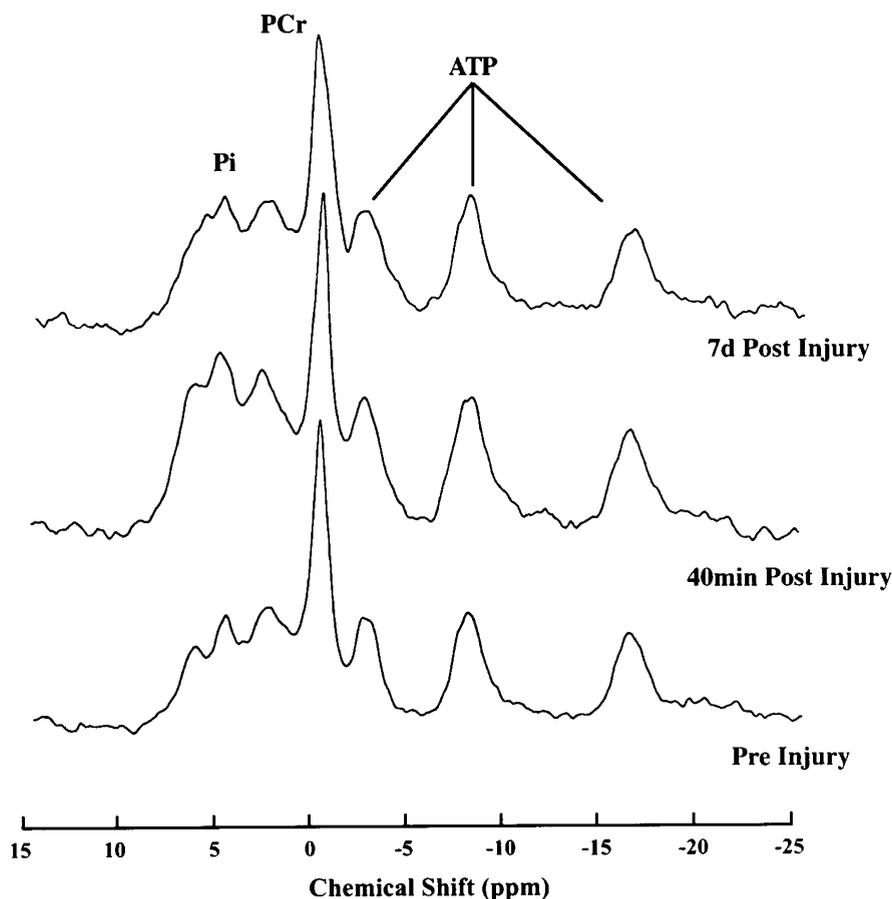
Previously, contusion models of brain trauma have been shown to produce a fall in regional intracellular pH, a decrease in PCr/Pi, and an increase in lactate concentrations (Berry et al., 1986; McIntosh et al., 1987; Vink et al., 1987; Andersen and Marmarou, 1992; Inao et al., 1988). In a model of selective axonal injury the rat (Foda

and Marmarou, 1994), no substantial pH changes were found following head impact, but PCr/Pi was altered (Heath and Vink, 1995, 1996). Therefore, there appear to be substantial differences in posttraumatic metabolic status between models of brain injury that produce different pathologies through different mechanical loading conditions. Taken together, the differences in metabolic response found by MRS examination between models of brain injury may have important clinical implications for both the diagnosis of the type of brain trauma and for the development of targeted therapeutic strategies.

An acute posttraumatic loss of  $[Mg^{2+}]_i$  was the only change found with phosphorous MRS following diffuse brain injury in the pig, and may reflect important metabolic changes. In addition, magnesium's global role in cellular energetics and physiology, it also provides a voltage-dependant block of the neuronal *N*-methyl-D-aspartate (NMDA) receptor. Overexcitation of the NMDA receptor is thought to be an integral component of the pathophysiologic sequelae of brain trauma contributing to secondary damage (for review, see Smith and McIntosh, 1996). Previously, substantial decreases in  $[Mg^{2+}]_i$  and total tissue  $[Mg^{2+}]$  have been characterized following parasagittal fluid-percussion brain injury in the rat that correlated with the severity of injury (Vink et al., 1988) and following isolated traumatic axonal injury in the rat (Heath and Vink, 1995). In addition, treatment with various pharmacologic agents, including NMDA receptor antagonists and magnesium, have been shown to improve behavioral outcome attenuate changes in PCr/Pi, and maintain magnesium homeostasis, both intracellularly and in total tissue following fluid-percussion brain injury in the rat (Faden et al., 1989; McIntosh et al., 1989, 1987, 1989, 1988; Smith et al., 1993). The results from these previous studies suggest that  $[Mg^{2+}]_i$  may be a useful marker for both the severity of focal brain injury and the efficacy of therapeutic modalities. The dramatic loss of  $[Mg^{2+}]_i$  observed in the present study suggests that diffuse brain injury also disrupts bioenergetic processes and may predispose the brain to neurotoxic events linked to NMDA receptor overactivation. However, it is important to note that, in contrast to focal brain injury such as fluid-percussion, diffuse brain injury in the pig produced only modest neuronal injury in regions of NMDA receptors, with the predominant damage in the white matter where few NMDA receptors are found. Therefore, while the global loss of the  $[Mg^{2+}]_i$  suggests profound alterations of cellular metabolism, its effect on NMDA receptor function following diffuse brain injury in the pig cannot be presently determined.

In contrast to changes in  $[Mg^{2+}]_i$ , the loss of NAA/Cr following diffuse brain injury in the pig detected via localized proton MRS, may be linked with more specific

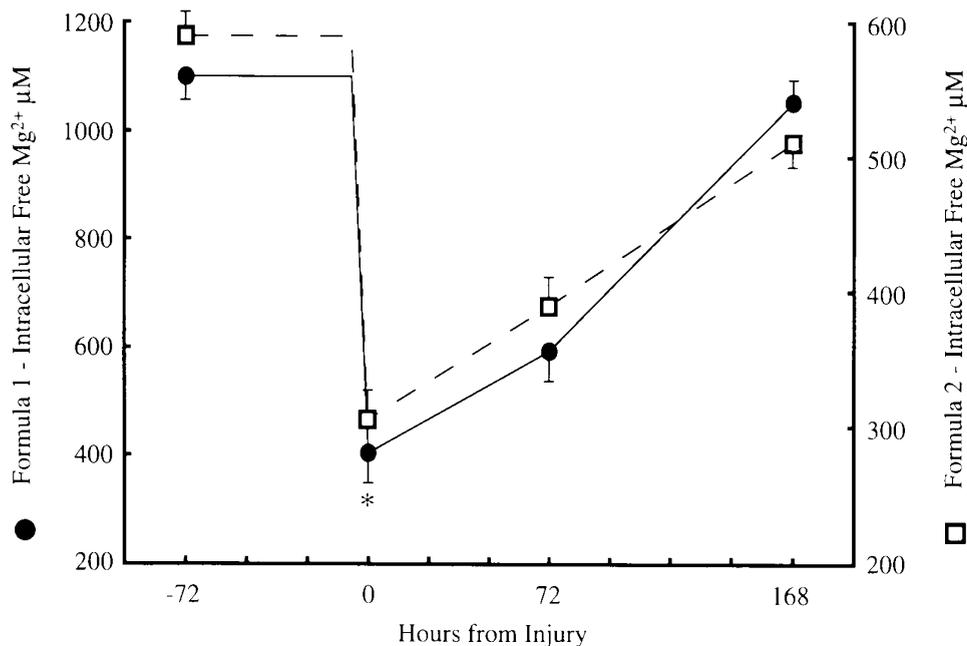
## MRS IN DIFFUSE BRAIN TRAUMA



**FIG. 3.** Representative phosphorous MR spectra of swine brain prior to and following brain trauma. Pi, phosphoinositol; PCr, phosphocreatine; ATP, adenosine triphosphate; d, day.

pathologic processes. NAA is one of the most abundant amino acids in the brain, found at concentrations exceeded only by glutamate (Tallan et al., 1956), but is only found in neurons. Thus, decreases in the brain concentration of NAA is thought to indicate the loss or damage of neurons or neuronal processes. Accordingly, a decrease in the levels of brain NAA has been observed due to aging, neurodegenerative disorders, seizures, and focal brain injury (Rubin et al., 1997; van der Knapp et al., 1990; Klunk et al., 1992; Dunlop et al., 1992; Plaitakis and Constantakakis, 1993; Meyerhoff et al., 1989). Although the specific role of NAA has yet to be elucidated, it has been demonstrated that NAA can act as a major source of acetyl groups used for lipid synthesis (Burri et al., 1991), which is of particular importance in the white matter (Miyake and Kakimoto, 1981). The inability to tap this resource of acetate may be the mechanism that leads to degeneration of white matter tracts in Canavan's disease, where acetate cannot be cleaved from NAA due to the genetic deficiency of the enzyme, NAA aspartoacylase (Austin et al., 1991). Further, during normal brain

development, low NAA levels in the white matter have been ascribed to the high activity of NAA aspartoacylase during lipogenesis (D'Adamo et al., 1973; Truckenmiller et al., 1985). Therefore, a decrease in brain NAA may indicate either an increased demand for lipid synthesis, such as in development, regenerative processes, or damage to neuronal structures involved in its synthesis or storage. In support of this hypothesis, following fluid-percussion brain injury in the rat, using high-resolution proton MRS, we found that NAA/Cr levels fell without a reciprocal rise in acetate, suggesting a rapid utilization of acetate following trauma, which we postulated was due to reparative processes (Rubin et al., 1997). For the present study, we propose that the acute and persistent loss of NAA may reflect several responses that change during the temporal evolution of axonal injury. The acute loss of NAA/Cr following diffuse brain injury in the pig appears prior to overt damage of the axons and, thus, may reflect a disequilibrium of NAA in the white matter due to its utilization for repair. By 1 week following injury, persistently diminished NAA levels may result from con-



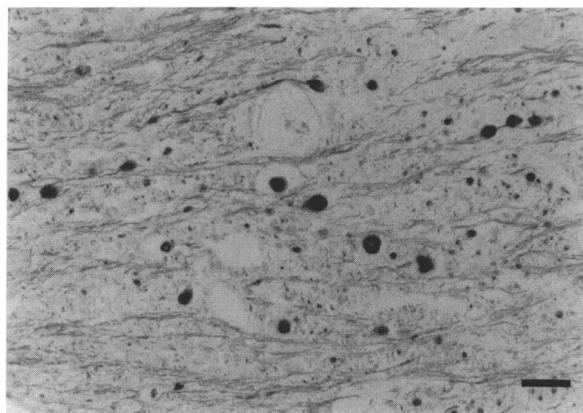
**FIG. 4.** Temporal changes in the concentration of intracellular free magnesium in swine brain prior to and following brain trauma. Data for each timepoint is plotted according to analysis by two different formulas, with respective numerical values listed on separate Y axes. Error bars = SEM. \* $p < 0.01$  compared to preinjury baseline measurement.

tinued disequilibrium of NAA and/or the loss of neural tissue in the white matter resulting from degeneration of axons.

A surprising finding in this study was that both proton and phosphorous MRS proved useful for detecting changes following diffuse brain injury, even in cases where no abnormalities were found with conventional MRI. Therefore, in addition to potentially differentiating diffuse from focal brain injury, MRS techniques may also have enhanced sensitivity over standard MRI for detecting microscopic injury following diffuse brain injury. This observation may be of particular importance for the radiologic evaluation of brain-injured patients, since axonal pathology is thought to be severely underestimated using routine radiologic techniques (Mittl et al., 1994). To pursue this issue, we have recently found that proton MRS evaluation of brain-injured patients also revealed a decrease in the levels of NAA/Cr in regions that appear normal with conventional MRI (Cecil et al., 1998). Therefore, we have developed a model system of MRS evaluation following experimental diffuse brain injury that may be used to corroborate findings in parallel clinical studies.

Although we have found that noninvasive proton and phosphorous MRS may sensitively detect pathologic

changes following diffuse brain injury in the pig, further studies are needed to elucidate the specific roles that decreases in  $[Mg^{2+}]_i$  and NAA levels play in combination with axonal injury. Nonetheless, our present results may have important clinical implications for the evaluation and diagnosis of patients with diffuse brain injury.



**FIG. 5.** Representative photomicrograph of antineurofilament immunostained white matter in swine 1 week following brain trauma. Black profiles represent pathologic axonal bulb formation. Bar = 40  $\mu\text{m}$ .

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