Comparison of dynamic brain metabolism during antegrade cerebral perfusion versus deep hypothermic circulatory arrest using proton magnetic resonance spectroscopy

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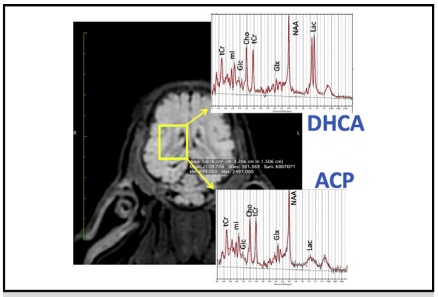

Piglet midbrain MRI slice showing selected voxel and a corresponding proton MRS spectrum.

CENTRAL MESSAGE

Brain metabolism during deep hypothermic arrest is active and abnormal, resulting in a buildup of lactate and loss of energy substrates. Antegrade cerebral perfusion prevents these abnormalities.

See Commentaries on pages e229 and e231.

Details regarding brain metabolism under varying flow conditions during cardiopulmonary bypass (CPB), such as deep hypothermic circulatory arrest (DHCA) and antegrade cerebral perfusion (ACP), remain incompletely understood. Metabolic changes in the brain have been observed under different CPB conditions,[1,2] but which of these are truly causative of neurotoxicity and injury remains unclear. We sought means better to assess dynamic brain metabolic changes during CPB surgery under varying flow conditions (Figure 1). Our studies focus on neonates, because DHCA is most commonly used in that patient subset. Secondarily, it is the neonate in whom the lifelong impact of potential inadvertent brain injury garners its most significant impact. Duration of circulatory arrest is a critical variable. Our studies use a 60-minute arrest period to assess metabolic dynamics in an attempt to capture the full spectrum of clinically relevant DHCA situations.

Proton magnetic resonance spectroscopy (MRS) provides an in vivo profile of brain metabolism that is based on the noninvasive detection of 17 to 20 detectable metabolites, with prominent signals from *N*-acetylaspartate (a neuronal metabolite), creatine (an energy substrate), and choline (a cell membrane constituent), as well as smaller signals from compounds including myoinositol (an osmolyte and glial marker), glutamate and glutamine (involved in neuronal function), lactate (a measure of anaerobic metabolism), glucose (the brain's primary energy substrate), phosphocreatine (an energy substrate), and the antioxidants glutathione and ascorbate.[3] Brain temperature can also be monitored through the chemical shift difference between water and *N*-acetylaspartate.[4]

In this preliminary investigation, we studied the brain metabolism of 5neonatal piglets during CPB, with 3 of the animals undergoing DHCA and 2 undergoing ACP.

Under Stanford University Institutional Animal Care and Use Committee–approved protocol, neonatal piglets underwent median sternotomy and central cannulation for a 60-minute period of CPB at 18°C under either DHCA or ACP, as previously described,[5-8] while placed within the magnetic resonance imaging scanner to assess brain energy metabolite levels during the CPB session. The speed of cooling or rewarming was constantly adjusted to maintain a temperature gradient of less than 8°C between the water temperature and the arterial blood temperature going to the piglet and between the arterial blood temperature going to the piglet and the core temperature. We used pH-stat when cooling down and then transitioned to alpha-stat as rewarming commences, continuing to

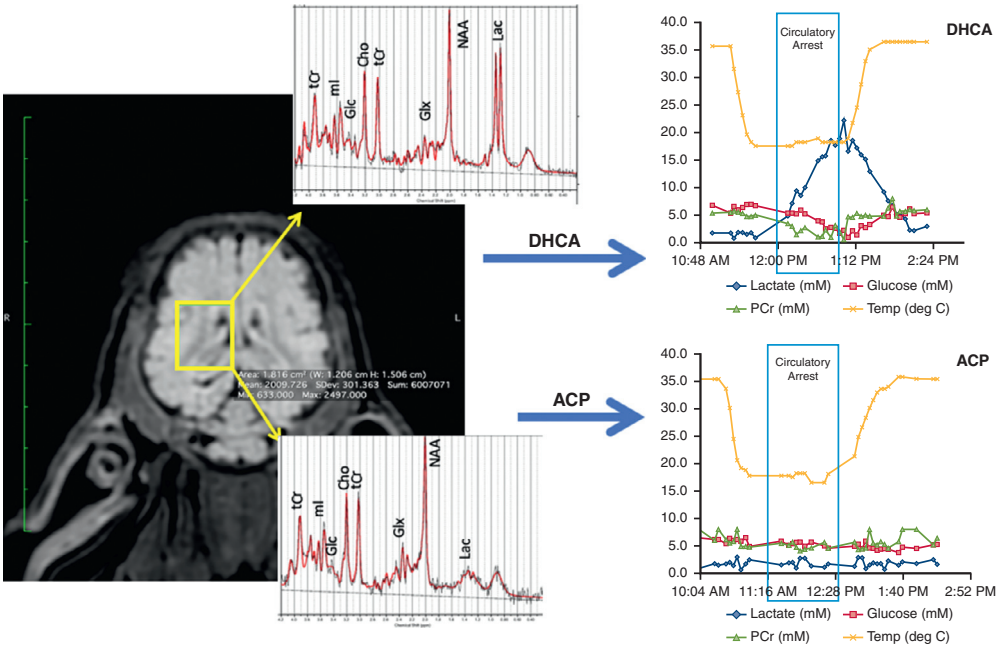

FIGURE 1. Proton magnetic resonance spectroscopy is a highly sensitive approach to measuring neonatal piglet local brain temperature and dynamic metabolic changes occurring during cardiopulmonary bypass using deep hypothermic circulatory arrest (*DHCA*) or antegrade cerebral perfusion (*ACP*). We show right midbrain prescription metabolite level spectra taken in deep hypothermia after 45 minutes of deep hypothermic circulatory arrest (*left upper panel*) or antegrade cerebral perfusion (*left lower panel*) and demonstrate the dynamic plots (*right*) of lactate (*Lac*), glucose (*Glc*), total creatine (*tCr*, creatine plus phosphocreatine [*PCr*]), and temperature (*Temp*) levels during the circulatory arrest period under each of the 2 cardiopulmonary bypass protocols (*right upper* and *lower panels*). *mI*, Myoinostol; *Cho*, choline; *Glx*, glutamate and glutamine; *NAA*, *N*-acetylaspartate.

normothermia. After rewarming to a temperature of 37°C, magnetic resonance imaging and bypass were discontinued, followed by the animal's death. The brain was fixed, processed, and paraffin embedded for histologic examination and then reviewed by a veterinary pathologist to confirm absence of gross pathology.

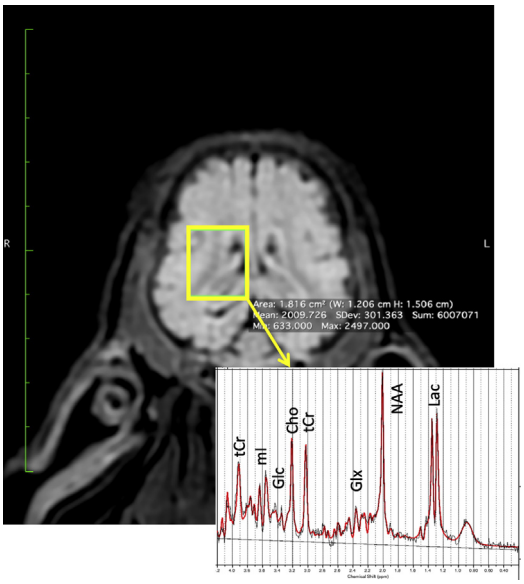

FIGURE 2. Representative selected right midbrain voxel and corresponding magnetic resonance spectroscopy spectra taken in deep hypothermia after 45 minutes of deep hypothermic circulatory arrest (DHCA). *tCr*, Total creatine (creatine plus phosphocreatine); *mI*, myoinostol; *Glc*, glucose; *Cho*, choline; *Glx*, glutamate and glutamine; *NAA*, *N*-acetylaspartate; *Lac*, lactic acid.

MRS spectra were repeatedly collected on a GE MR750 scanner (GE Healthcare, Waukesha, Wis) with a single-voxel PRESS pulse sequence with the following acquisition parameters: TE/TE = 2000/35 ms, 15 mm × 12 mm × 12 mm voxel, 2:56 minute acquisition, acquired with either a 16-channel flex or an 8-channel knee radiofrequency coil. All spectra were analyzed with LCModel fitting software[9] and quantified according to an assumed brain total creatine (tCr; creatine + phosphocreatine) concentration of 8 mmol/L, with a representative selected voxel and corresponding spectrum shown in Figure 2.

Figure 2 shows representative metabolite time curves. In particular, proton MRS of neonatal piglet brains during 60 minutes of 18°C hypothermic circulatory arrest (DHCA) demonstrated an approximate 10-fold elevation of lactate and marked reduction of brain glucose and phosphocreatine (Figure 3, *A*). Upon rewarming, lactate only partially recovered to pre-cooling levels. When ACP was used at 18°C, all 3 metabolites remained at precooling levels (Figure 3, *B*).

The finding of increased lactate during DHCA is consistent with previous reports of high lactate levels as measured by microdialysis[1]; however, the dynamic MRS measurements also allow the measurement of the lactate production

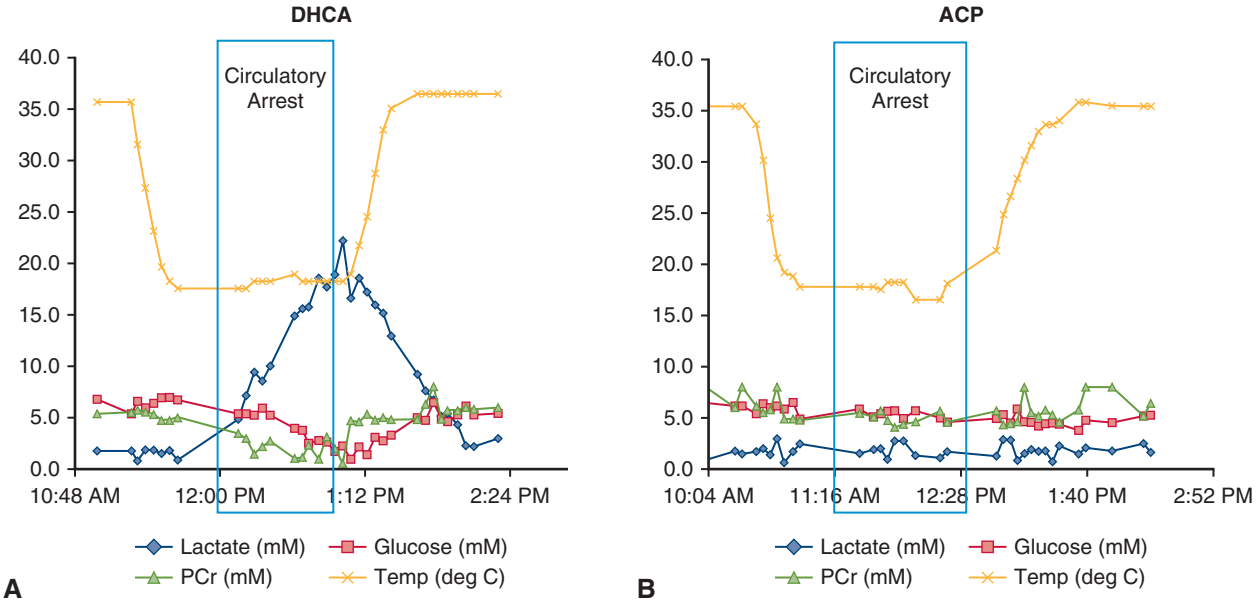

FIGURE 3. A and B, Representative results showing time courses of in vivo changes in brain temperature (*Temp*) and metabolites as measured by proton magnetic resonance spectroscopy in neonatal piglets under deep hypothermic circulatory arrest (*DHCA*; A) and antegrade cerebral perfusion (*ACP*; B) cardiopulmonary bypass conditions. The 60-minute period of whole-body circulatory arrest without or with antegrade cerebral perfusion, respectively, is designated by the *outlined box*. *PCr*, Phosphocreatine.

rate. Reduction of brain glucose is consistent with previous studies; however, the greater than stoichiometric lactate/glucose ratio change observed during DHCA suggests that depletion of brain glycogen may play an important metabolic role.[10] Furthermore, and importantly, as illustrated in the data shown in Figure 3, lactate production rate appears to decrease significantly or even stop as glucose levels reach undetectable concentrations (approximately 50 minutes after circulatory arrest). The time point of this reduced lactate production rate and glucose depletion may represent the critical point of brain injury, because these MRS results suggest that energy production may have become exhausted during this interval.

Proton MRS during bypass provides an unprecedented opportunity to monitor brain cellular metabolism in real time and clearly reveals marked metabolic changes during DHCA relative to ACP. Noninvasive dynamic in vivo mapping of cerebral metabolism with MRS enables a more complete understanding of brain changes during cooling, hypothermia, and the recovery periods, offering significant advantages relative to other, more invasive, techniques.

We are currently refining our spectroscopic data analysis tools to improve quantitation with temperature-dependent spectral basis functions[11] and T_2 relaxation rates. With these improvements, we anticipate more robust measurement of metabolites, including those relevant to adenosine triphosphate synthesis (phosphocreatine), antioxidant defense (glutathione and ascorbate), and neurotoxicity and function (glutamate and glutamine). We intend to explore a range of ACP temperatures going forward to demonstrate the effects of perfusion strategies on brain metabolic dynamics.

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