Metabolomic Profiling of Children's Brains Undergoing General Anesthesia with Sevoflurane and Propofol

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ABSTRACT

Background: We recently applied proton magnetic resonance spectroscopy (^{1}HMRS) to investigate metabolic consequences of general anesthesia in the rodent brain, and discovered that isoflurane anesthesia was characterized by higher concentrations of lactate, glutamate, and glucose in comparison with propofol. We hypothesized that the metabolomic differences between an inhalant and intravenous anesthetic observed in the rodent brain could be reproduced in the human brain.

Methods: ^{1}HMRS-based metabolomic profiling was applied to characterize the cerebral metabolic status of 59 children undergoing magnetic resonance imaging during anesthesia with either sevoflurane or propofol. ^{1}HMRS scans were acquired in the parietal cortex after approximately 60 min of anesthesia. Upon emergence the children were assessed using the pediatric anesthesia emergence delirium scale.

Results: With sevoflurane anesthesia, the metabolic signature consisted of higher concentrations of lactate and glucose compared with children anesthetized with propofol. Further, a correlation and stepwise regression analysis performed on emergence delirium scores in relation to the metabolic status revealed that lactate and glucose correlated positively and total creatine negatively with the emergence delirium score.

Conclusions: Our results demonstrating higher glucose and lactate with sevoflurane in the human brain compared with propofol could reflect greater neuronal activity with sevofluane resulting in enhanced glutamate-neurotransmitter cycling, increased glycolysis, and lactate shuttling from astrocytes to neurons or mitochondrial dysfunction. Further, the association between emergence delirium and lactate suggests that anesthesia-induced enhanced cortical activity in the unconscious state may interfere with rapid return to "coherent" brain connectivity patterns required for normal cognition upon emergence of anesthesia.

What We Already Know about This Topic

- Metabolomics is a science that studies cellular events by detection of products of metabolism
- Prior work has shown that cerebral metabolomic profiles are different in rodents anesthetized with propofol *versus* isoflurane
- Causal relationships between anesthetics and emergence delirium are undefined

What This Article Tells Us That Is New

- Cerebral metabolomic signatures are different in children anesthetized with sevoflurane *versus* propofol (higher lactate and glucose)
- Brain glucose and lactate concentrations correlated with propensity to exhibit emergence delirium

THE field of metabolomics has been defined as "the systematic study of the unique chemical fingerprints that specific cellular processes leave behind"[1] with the ability to quantify a range of small molecular weight molecules, such as glucose and amino acids, involved in metabolism.[2–4] These metabolites can be measured in the brain in real time with proton magnetic resonance spectroscopy (^{1}HMRS) to track biochemical abnormalities that might precede or be directly associated with pathologic changes.[5–7] During the past two decades, ^{1}HMRS has been widely applied in studies investigating metabolic processes involved in stroke,[8–11] cancer,[12–14] Alzheimer disease,[15–18] Parkinson disease,[19,20] multiple sclerosis[21–24] and cognitive dysfunction.[25,26]

We recently applied *in vivo* ^{1}HMRS to investigate metabolic consequences of general anesthesia with isoflurane and

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propofol administered at equipotent doses in the rodent brain.[27] The analysis revealed that the cerebral metabolic status with isoflurane was characterized by higher concentrations of lactate and glutamate in comparison with propofol.[27] In contrast, the cerebral metabolic signature of propofol anesthesia was a low concentration of glucose.[27] The pathophysiological significance of these findings is still not understood. However, we hypothesized that the higher lactate and glucose with isoflurane compared with propofol administered at the same anesthetic potency could be explained by a relatively lower cerebral metabolic rate of glucose utilization with isoflurane; or that an alternate energy source was utilized by the brain, such as lactate. We also speculated whether the metabolomic differences between the inhalant (isoflurane) and intravenous (propofol) anesthetic observed in the rodent brain could be reproduced in the human brain.

The application of ^{1}HMRS in clinical practice continues to be somewhat limited for several reasons, including difficulties in streamlining spectral acquisitions and processing.[28–30] However, the technical challenges of ^{1}HMRS have been partly overcome with the implementation of higher magnetic field magnetic resonance imaging (MRI) instruments, phased-array radio-frequency coils, and improved volume selective pulse sequences with effective water suppression.[31,32] Thus, it is now possible to routinely acquire single voxel ^{1}HMRS scans in less than 5–10 min on most clinical MRI systems, and several metabolites can be reliably detected including *N*-acetylaspartate (neuronal marker), choline metabolites (which reflect the metabolism of cell membranes), glutamate, creatine, and, in some instances, lactate and glucose (energetics).[33]

Here we applied ^{1}HMRS to characterize the cerebral metabolic status in children undergoing general anesthesia for a routine MRI scan. We selected this patient population because the MRI procedure is noninvasive and surgical pain would therefore not confound the data, and the standard of care at our institution prescribes either sevoflurane or propofol anesthesia for this procedure, which would allow us to randomize the children into an "inhalational" and intravenous propofol group to test our main hypothesis that sevoflurane and propofol anesthesia would be characterized by different metabolomic signatures, as previously observed in the preclinical study. In addition, the pediatric anesthesia literature has reported that sevoflurane anesthesia can be associated with emergence delirium[34,35] and therefore we also explored the potential association between emergence delirium and specific brain metabolites.

Materials and Methods

Subjects

Under an Institutional Review Board (Committees on Research Involving Human Subjects [CORIHS], Stony Brook University, Stony Brook, New York) approved protocol and with parental consent, ^{1}HMRS was performed on children ages 2–7 yr old, scheduled to undergo a clinically indicated MRI during general anesthesia. Consecutive patients seen at the MRI suite were recruited by the attending anesthesiologist. Exclusion criteria were: (1) inability of parents to understand the procedures and to provide consent; (2) inability or contraindication to be randomized to a specific anesthetic regimen (*e.g.*, airway problems, allergies or history of malignant hyperthermia, muscular dystrophy); (3) acute brain trauma, stroke, or hemorrhage, or (4) processes increasing intracranial pressure.

Anesthetic Management

Following parental consent, the children were randomized to undergo either propofol (Group P) or sevoflurane (Group S) anesthesia. All children received an inhalational mask induction, which lasted approximately 1 or 2 min, with sevoflurane. Following induction and establishment of intravenous access, children randomized to propofol anesthesia were immediately converted to an intravenous propofol infusion with oxygen supplementation *via* a nasal cannula. The average propofol infusion rate was 165 ± 17 $mcg^{-1} \cdot kg^{-1} \cdot min^{-1}$ for children in Group P (n = 23). In Group S, the airway was secured using a laryngeal mask airway and sevoflurane titrated to 1 to 1.5 minimum alveolar concentration (3.1%). All children were spontaneously breathing, continuously monitored using standard-of-care monitoring, and received intravenous normal saline (10–20 ml/kg) throughout the procedure. Anesthesia care for all children was provided by the same anesthesiologist (ZJ).

Assessment of Emergence Delirium

At the conclusion of the procedure the children emerged from anesthesia and were transferred to the MRI suite recovery room, where nursing supervision was provided. During emergence from anesthesia, each child was assessed for emergence delirium by a recovery room nurse using the Pediatric Anesthesia Emergence Delirium (PAED) scale.[36] Total recovery time was defined as the time from arrival to the recovery suite until the child met postanesthetic modified Aldrete recovery score criteria[37] for discharge home.

MR Spectroscopy Acquisition and Scan Parameters

All MRI imaging was performed on a Philips Achieva 3T (Philips Healthcare, Andover, MA) MRI instrument using an eight-channel sensitivity-encoding head coil. For each child, ^{1}HMRS spectra were acquired over approximately 10 min and before administration of contrast (if needed). Only one ^{1}HMRS scan per child was performed in parietal cortex to limit anesthesia exposure time. The T1-weighted scans (repetition time = 8 s, echo time = 4 ms, flip angle = 8;

spatial resolution of 0.94 × 0.94 × 1.00 mm^3) acquired as part of the clinical routine were used to position the ^{1}HMRS voxel in the left parietal cortex. The parietal cortex was selected because this region is part of the default mode network[38,39] and indirectly involved in essential cognitive processes such as memory retrieval, visual spatial processing,[40–42] and the state of consciousness,[43] and because high-quality ^{1}HMRS spectra can be acquired consistently and reproducibly in contrast to the frontal cortex or hippocampus, which in our experience can be technically difficult. For ^{1}HMRS, we used a single-voxel position resolved spectroscopy sequence (PRESS) with the following parameters: echo time = 32 ms; repetition time = 2 s; number of points = 2,048; bandwidth = 2,000 Hz; number of averages = 256; and voxel size of 1.5 × 1.5 × 1.5 cm^3. Extra care was taken to avoid cerebrospinal fluid and outer volume lipid contamination when positioning the voxel in the parietal cortex. A water-unsuppressed scan was also acquired for eddy current correction and metabolite quantification.

Spectral Data Analysis

Data analysis of ^{1}HMRS spectra was performed using linear combination modeling (LCModel[44]) with prior knowledge of simulated spectral signatures for the following brain metabolites: alanine, aspartate, creatine, phosphocreatine, γ-aminobutyric acid, glucose, glutamine, glutamate, glutathione, glycerophosphocholine, phosphocholine, myo-inositol, lactate, *N*-acetylaspartate, *N*-acetylaspartylglutamate, phosphorylethanolamine, scyllo-inositol, and taurine, in addition to lipid and macromolecules. No baseline correction, zero-filling, or apodization functions were applied to the *in vivo* data before LCModel analysis. For further details of the analysis procedures, see Makaryus *et al.*[27]

Statistical Analysis

All data are presented as mean ± SD. Statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC) and XLSTAT Version 2011 (Addinsoft, New York, NY). Statistical comparison of mean differences in age and weight were compared using a Student *t* test for independent groups ($P < 0.05$ for significance). The percentage differences between other demographic variables (table 1) were tested using the Fisher exact test. Differences in physiologic parameters and recovery time were assessed using an independent Student *t* test (two-sided, $P < 0.05$ for significance); and differences in PAED scores were assessed by Mann–Whitney U test (two-sided, $P < 0.05$ for significance).

Analysis of covariance (ANCOVA) with age adjustment was employed to examine differences in metabolite concentrations between two anesthesia groups while controlling for multiple comparisons using the false discovery rate (QVALUE package in R software††). This approach is considered less conservative and more powerful compared with conventional Bonferroni correction for multiple comparisons.[45,46] Standardized effect sizes for the main effects (anesthetic) of the ANCOVA were calculated for each of the metabolites. Exploratory stepwise regression was used to identify the specific metabolites that were associated with the severity of emergence delirium, and the α level was set as 0.05 for this analysis.

Table 1. Demographic Data

Variables	Group P (No. = 23)	Group S (No. = 27)	*P* Value
Mean (SD) Age, months	59.2 (21.2)	56.1 (21.2)	0.61 (0.15)
Gender (Male, Female)	13, 10	16, 11	1
ASA Status 1, n (%)	15 (65.2%)	16 (59.3%)	0.77
ASA Status 2, n (%)	8 (34.8%)	11 (40.7%)	
Weight (SD), kg	20.1 (5.0)	19.3 (7.0)	0.67 (0.13)
Seizure disorder, n (%)	6 (26.1%)	13 (48.1%)	0.15
Headaches, n (%)	6 (26.1%)	3 (11.1%)	0.27
Developmental delay, n (%)	2 (8.7%)	3 (11.1%)	1
ADHD, n (%)	2 (8.7%)	1 (3.7%)	0.59
Other, n (%)	7 (30.4%)	7 (25.9%)	0.76

The mean difference in age and weight were compared by Student *t* test for independent groups ($P < 0.05$ for significance). The percentage difference for gender, ASA, and disease status were compared using Fisher exact test.

ADHD = attention deficit hyperactivity disorder; ASA Status = American Society of Anesthesiologists status.

Results

Demographics

A total of 59 children were enrolled for ^{1}HMRS scanning in two phases spanning 1.5 yr. Interim analysis was performed after recruitment of 30 subjects; and the sample size was adjusted because of reviewer criticism after the interim analysis. In the following, only the analysis performed on the final sample size is presented and no effort was made to further adjust the α rate from the interim analysis. Brain spectra from nine children were discarded secondary to artifacts (for further details, see Spectral Data Analysis). Table 1 shows the demographics of the 50 children included in the final analysis, divided into the two different anesthesia exposure groups. The children did not differ in age, weight, or gender between the two groups (table 1). Seizure disorders, headaches, and workup for developmental delay or attention deficit hyperactivity disorder were among

†† http://cran.r-project.org/src/contrib/Archive/qvalue/, Alan Dabney and John D. Storey, Department of Biostatistics, University of Washington, Seattle, Washington. Accessed April 26, 2012.

the most frequent reasons for MRI referral. There were no significant differences between groups in the frequency of indications for brain MRI (table 1).

Brain Pathology by MRI

The anatomical MRI scans acquired as part of the clinical work up were read by a neuroradiologist. For Group P, 20 of the 23 brain scans (87%) were interpreted as "normal"; one child had hydrocephalus and, in two children, nonspecific, punctate hyperintensities of uncertain etiology in frontal cortex and/or hippocampus were noted. For Group S, 23 of the 27 brain scans (85%) were interpreted as normal. In Group S, one child was diagnosed with Chiari 1 malformation (otherwise normal brain) and another child was evaluated for acute disseminated encephalomyelitis displaying punctate hyperintensities. Finally, a MRI scan from a 2-yr-old child anesthetized with sevoflurane who had a hemorrhage as a neonate was read as "resolving hemorrhage" with no new acute changes.

Anesthesia Duration, Hemodynamics, and Recovery

Eight of the children in Group P and three children in Group S received intravenous contrast (gadolinium) as part of their diagnostic MRI workup, however, all ^{1}HMRS scans were acquired before administration of IV contrast (which typically added an additional 10–15 min to the total scan time). Therefore, regardless of IV contrast administration, the average total duration of anesthesia was similar between the two groups (Group P = 65.5 ± 15.5 min *vs.* Group S = 61.3 ± 10.2 min, P = 0.27, effect size = 0.32). The mean arterial pressure was significantly higher (9%) in Group P compared with Group S (68 ± 7 mmHg *vs.* 61 ± 8 mmHg, P = 0.001, effect size = 0.93), whereas the average heart rate was higher in Group S compared with Group P (Group P: 87 ± 14 beats/min *vs.* Group S: 100 ± 13 beats/min, P = 0.001, effect size = 0.96).

All children recovered without complications from anesthesia. Recovery time was shorter for children anesthetized with propofol compared with sevoflurane (Group P: 42 ± 17 min *vs.* Group S: 53 ± 15 min, P = 0.02, effect size = 0.68). The average total PAED score for children anesthetized with sevoflurane was significantly higher compared with Propofol (Group S: 7.0 ± 5.7 *vs.* Group P: 3.9 ± 4.7; P = 0.037, effect size = 0.6, fig. 1), indicating more agitation and delirium with the former anesthetic.

^{1}HMRS Spectra Analysis

Figure 2 shows the anatomical location of the ^{1}HMRS voxel within the parietal cortex and the associated spectral output. Specifically, in figure 2, panel A shows the projected position of the voxel onto a surface reconstructed three-dimensional MRI mask of one of the children, and panel B shows the voxel position on T1-weighted anatomical scans presented in three orthogonal views. A typical ^{1}HMRS spectrum from the parietal cortex of a child anesthetized with propofol analyzed by LCModel is shown in panel C. In general, the ^{1}HMRS

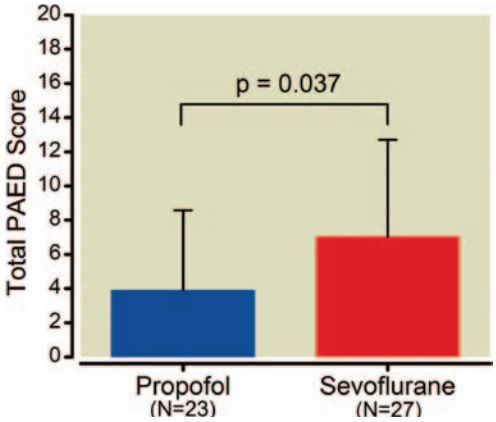

Fig. 1. Quantitative assessment of the average Pediatric Emergence Delirium (PAED) score from children anesthetized with sevoflurane and propofol, demonstrating significantly higher scores with the former anesthetic.

scan quality was excellent, as assessed by the full width half maximum (0.027 to 0.031 ppm) and a signal-to-noise ratio of 15–20 based on the *N*-acetylaspartate peak derived from the LCModel analysis. However, as previously mentioned, nine scans had to be discarded because of either motion artifacts or "lipid" contamination secondary to insufficient outer volume suppression. Accuracy of the quantitative assessment of individual metabolites by LCModel was assessed by their corresponding average Cramér–Rao lower bound (CRLB) values. The following eight metabolites were included in the final data analysis: glucose (CRLB ≃19%), glutamate (CRLB ≃9%), myo-inositol (CRLB ≃6%), lactate (average CRLB 80% or fewer in 80% of the scans), *N*-acetylaspartate (CRLB ≃3%), taurine (CRLB ≃40%), total choline (glycerophosphocholine and phosphocholine, CRLB ≃4%), and total creatine (creatine and phosphocreatine, CRLB ≃3%). Metabolites such as γ-aminobutyric acid (CRLB ≃31%), creatine (CRLB ≃11%), phosphocreatine (CRLB ≃11%), and aspartate (CRLB ≃19%) were excluded from the quantitative analysis as their spectral signatures are highly overlapping and the CRLB is therefore generally not considered sufficient for assessing reliability of the measurement.

Quantitative Analysis of Metabolites

ANCOVA with age adjustment was employed to examine differences in metabolite concentrations between the two anesthesia groups. Assumption of homogeneity for the covariate regression coefficients for the two anesthetics is a prerequisite for the ANCOVA analysis and was first tested. Specifically this test evaluates the interaction between the covariate (age) and the independent variable (anesthesia effect) in the prediction of the dependent variable (metabolite concentrations). If the interaction between the covariate and the independent variable is significant (P < 0.05), the results from the ANCOVA would be noninterpretable. The result of the first analysis demonstrated no significant interaction terms for any of the

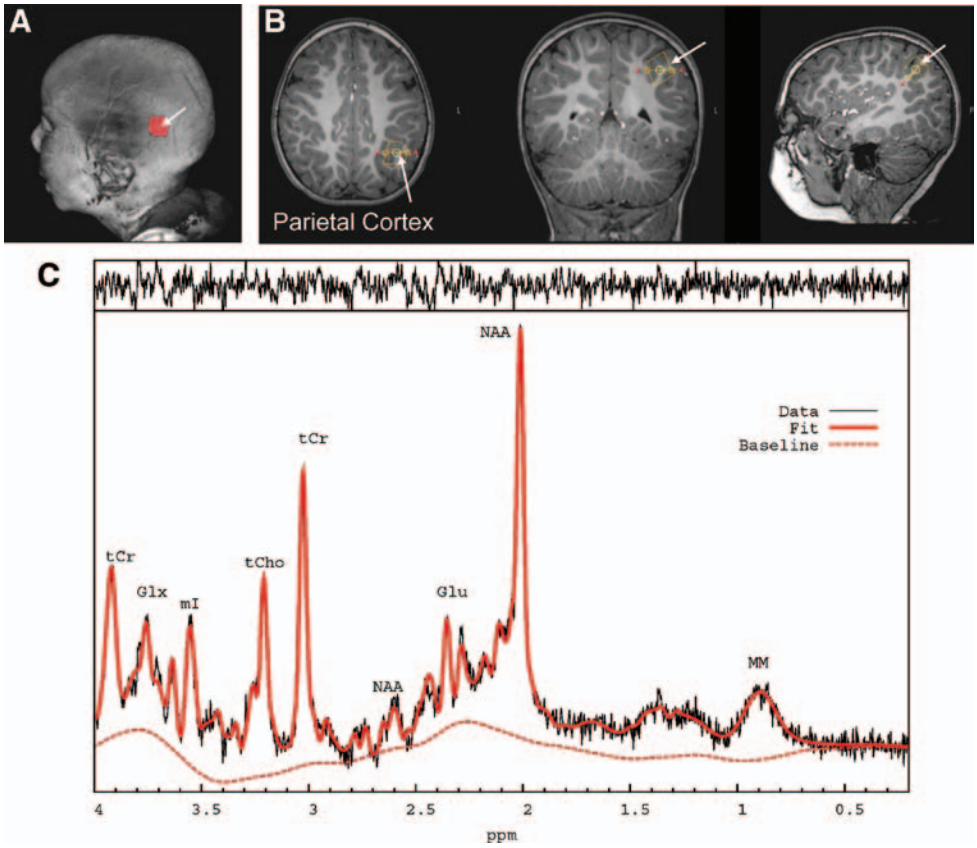

Fig. 2. The projected position of the voxel within the parietal cortex for the proton magnetic resonance spectroscopy (^{1}HMRS) scan is illustrated on a surface reconstructed three-dimensional mask from one of the children (*A*) and on the corresponding three orthogonal views (*B*). The figure also shows the derived ^{1}HMRS spectrum from the parietal cortex of a child anesthetized with sevoflurane analyzed by LCModel (*C*). The spectrum shown in *C* is of high quality with sufficient water suppression and excellent spectral resolution to resolve at least 6–10 metabolites. The raw unsmoothed spectrum is shown (*black*) in addition to the LCModel fitted spectral output (*red solid line*). Glu = glutamate; Glx = glutamate and glutamine; mI = myo-inositol; MM = macromolecules; NAA = *N*-acetylaspartate; tCho = total choline; tCr = total creatine.

selected metabolites, thus the ANCOVA could be performed on all metabolites. The results of the ANCOVA are shown in table 2, and show that when controlling for age, the concentrations of taurine (τ), glucose, and lactate are significantly different between the two anesthetics (false discovery rate method was used as the multiple testing correction method for adjustment of *P* values, with $P < 0.05$ indicating significance). In addition, the analysis also shows that the concentration of *N*-acetylaspartate is age-dependent, as previously reported in the literature.[47] Table 3 shows the actual metabolite concentrations in Group P and Group S and demonstrates that τ, glucose, and lactate are significantly higher in Group S compared with Group P when controlling for age. Specifically, lactate was 2-fold higher and glucose 1.2-fold higher in the parietal cortex of children anesthetized with sevoflurane compared with propofol, in agreement with our previous findings in rodents.[48]

Given that some of the children's brain scans revealed abnormalities, we repeated the quantitative analysis including only data from the children with MRI scans that were read as "normal" by the neuroradiologist. This analysis also demonstrated lower glucose ($P = 0.007$) and lactate ($P = 0.007$) in the propofol-anesthetized children compared with sevoflurane, supporting the main hypothesis that the anesthetics are responsible for the metabolic signatures and not the prior pathologic condition of the child.

Several reports in the literature have documented higher levels of lactate in animal models of generalized epilepsy[49] and in humans with epileptiform foci,[50,51] and there is also evidence that sevoflurane has epileptogenic properties.[52] We therefore compared lactate in children with suspected or documented seizure activity with that observed in children without seizure disorders for each anesthetic. For sevoflurane, this analysis demonstrated no differences in lactate for children with seizure disorder compared with those without; subjects with seizure disorder, n = 13, lactate = 0.28 mM *versus* subjects without seizure disorder, n = 14, lactate = 0.34 mM, $P = 0.283$. Similar negative and nonsignificant results were found for propofol-anesthetized children with and without seizure disorder.

Table 2. *P* Values of F Test on Covariate (Age) and the Main Effect (Anesthetic)

Metabolites	Anesthesia Effect, *P* (Effect Size)	Age Effect, *P* Value
Cr + PCr	0.8970 (0.02)	0.4184
Glucose	0.0119 (0.11)	0.6162
Glutamate	0.8640 (0.02)	0.4034
GPC + PCh	0.3573 (0.003)	0.8436
Ins	0.2315 (0.01)	0.8976
Lac	0.0028 (0.15)	0.2336
NAA	0.1662 (0.02)	0.0144
τ	0.0105 (0.11)	0.2923

Effect size measures the proportion of the adjusted main effect (anesthetic) in the total variation of the dependent variable (metabolite concentration).

Cr = creatine; GPC = glycerophosphocholine; Ins = myo-inositol; Lac = lactate; NAA = *N*-acetylaspartate; PCh = phosphocholine; PCr = phosphocreatine; τ = taurine.

Prediction of PAED Score

An exploratory multiple regression analysis was performed on the PAED score with all the preselected measured metabolites. Stepwise selection was used to select the metabolites which contributed significantly to the total amount of variance accounted for by the model. To correct for multiple comparisons, the α level was set to $P < 0.05$ for this analysis. We first tested the hypothesis that the PAED score would be associated with specific metabolomic profiles regardless of the anesthetic used. This exploratory regression analysis was conducted with all children included in the study and revealed a significant relationship between metabolites and the PAED score (fig. 3). Specifically, the concentration of total creatine (creatine and phosphocreatine = Cr + PCr) in the parietal cortex was negatively related to the PAED score, whereas lactate and glucose showed a positive relationship. The model derived is as follows:

$$\text{PAED} = 22.8 - 4.4\,(\text{Cr} + \text{PCr}) + 9.9\,(\text{lactate}) + 2.8\,(\text{glucose})$$

The R^2 and *P* value for this model was 0.261 and 0.003, respectively; and the model indicates that if Cr + PCr increases 1 mM in a given patient's parietal cortex, the PAED score will decrease by 4.4 points (95% CI: 1.49–7.24). Conversely, if lactate as well as glucose increases by 1 mM, the PAED score will increase by approximately 13 points (95% CI for the regression coefficient of lactate: 1.5–18.2; 95% CI for the regression coefficient of glucose: 0.4–5.2).

We next tested the hypothesis that only for sevoflurane anesthesia would the PAED score be positively associated with brain lactate. The result of this exploratory analysis revealed that lactate was (weakly) positively associated with the magnitude of the PAED score for sevoflurane anesthetized children (R^2 = 0.150; P = 0.056), whereas for propofol there was no association between the two parameters (R^2 = 0.001; P = 0.913).

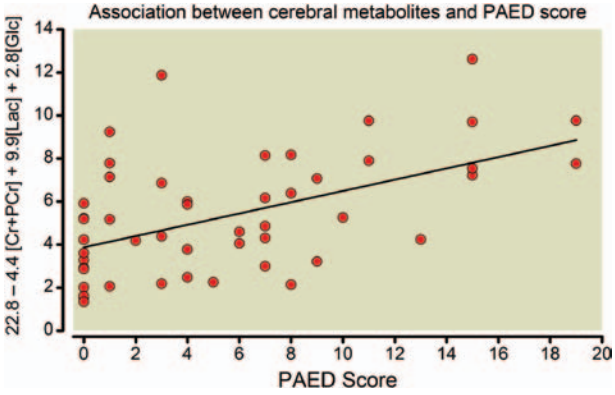

Fig. 3. Prediction of emergence delirium from the concentration of brain metabolites in all anesthetized children. The stepwise selection and exploratory regression analysis demonstrated that the concentration of total creatine (creatine and phosphocreatine, or Cr + PCr) in the parietal cortex was negatively related to the Pediatric Anesthesia Emergence Delirium (PAED) score, whereas the concentrations of lactate and glucose showed a positive relationship. Specifically, the model predicts that if the total creatine increases 1 mM in a given patient's parietal cortex, the PAED score will decrease by 4.4 points. Conversely, if lactate and glucose increase 1 mM, the PAED score will increase by approximately 13 points. The R^2 and *P* value of this model were 0.261 and 0.003, respectively. Cr = creatine; Glc = glucose; Lac = lactate; PCr = phosphocreatine.

Table 3. Metabolite Concentrations of Children Anesthetized with Propofol and Sevoflurane

Metabolites	Group P (No. = 23)	Group S (No. = 27)	*P* Value for ANCOVA Main Effect after FDR Adjustment
τ*	1.12 (0.84)	1.69 (0.64)	0.032
Glc*	2.45 (0.54)	2.90 (0.63)	0.032
Glu	7.77 (0.66)	7.82 (0.96)	0.897
Lac*	0.17 (0.11)	0.30 (0.18)	0.022
NAA	7.65 (0.50)	7.46 (0.42)	0.332
Ins	6.12 (0.64)	6.39 (0.83)	0.370
GPC + PCh	1.22 (0.11)	1.26 (0.17)	0.476
Cr + PCr	6.22 (0.39)	6.23 (0.63)	0.897

Data are presented as mean and (SD).

* Metabolites that are significantly higher in Group S compared with Group P ($P < 0.05$).

Cr + PCr = creatine and phosphocreatine; FDR = false discovery rate; Glc = glucose; Glu = glutamate; GPC + PCh = glycerophosphocholine and phosphocholine; Ins = myo-inositol; Lac = lactate, NAA = *N*-acetylaspartate; τ = taurine.

Discussion

We documented differences in the cerebral metabolic status of children anesthetized with sevoflurane compared with propofol. Sevoflurane's metabolic signature consisted

of higher glucose, lactate, and τ in the parietal cortex compared with propofol. The metabolomic analysis further revealed that lactate contributed most to the separation of the two anesthesia groups. We also observed that the average total PAED score was significantly higher in the children anesthetized with sevoflurane compared with propofol, indicating more agitation and delirium associated with emergence from the former anesthetic, as previously reported.[34] Finally, an exploratory stepwise regression analysis performed on PAED scores from all children showed that lactate and glucose were positively and Cr + PCr negatively correlated with magnitude of the PAED score.

Greater Glucose and Lactate with Sevoflurane Compared with Propofol

We conducted this study to determine if the cerebral metabolomic signature measured by ^{1}HMRS in the human brain anesthetized with an inhalational (sevoflurane) and intravenous (propofol) anesthetic would be different, mirroring our previous metabolomic findings in the rodent brain.[48] In the previous study in the rodent brain, isoflurane and propofol were administered at equipotent dose ranges and the metabolomic data revealed that the cortical glucose and lactate in the isoflurane rats were approximately 2-fold higher compared with propofol-anesthetized rats.[48] In this study, lactate was approximately 2-fold higher and glucose 1.2-fold higher in the parietal cortex of sevoflurane-anesthetized children compared with propofol. The discrepancy in glucose might be related to pharmacodynamic differences pertaining to the inhalational anesthetic (*i.e.*, isoflurane was used in the rodents), anesthetic depth as well as total duration of anesthesia, because this study was only approximately 1 h, whereas 4–6 h were used in our previous study.[48] In addition, it is also important to point out that quantification of glucose in the brain with ^{1}HMRS in general is challenging because of overlapping resonances, especially from myo-inositol and taurine metabolites,[53,54] and will be more difficult when conducted at lower magnetic field strength. In support of this statement, in this study conducted at 3 Tesla, the concentration of taurine was also found to be higher with sevoflurane, and taurine could have interfered with the actual LCModel calculation of glucose.

A moderately increased lactate in the sevoflurane-anesthetized brain compared with propofol could be explained in a number of different ways. One possibility is that there is more overall neuronal activity (glutamatergic) with sevoflurane, which would stimulate glycolysis (either astrocytic or neuronal). Astrocytic lactate (released from astrocytes *via* the so-called "astrocyte-neuron lactate shuttle") would serve as fuel for nearby neurons.[55] Increased neuronal activity with sevoflurane could also up-regulate neuronal glycolysis with rapid lactate release and adenosine triphosphate production, a process which is referred to as "aerobic glycolysis."[55] Alternatively, an increased lactate with the inhalant anesthetic could reflect greater compromised mitochondrial function[56] and the need for increased aerobic glycolysis to produce adenosine triphosphate. There is ample data in the clinical ^{1}HMRS literature demonstrating that lactate is increased in mitochondrial disease states, although lactate in these conditions is reported to be much higher (*i.e.*, 3–11 mM[57]) than lactate reported in our study. Further, in mitochondrial disease states, *N*-acetylaspartate is typically decreased (see Saneto *et al.*[57]). Nevertheless, it is possible that the lactate increase observed with sevoflurane in comparison with propofol can be attributed to disruption of the mitochondrial electron-transport chain and consequent depletion of the coenzymes nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, which will shift metabolism from the tricarboxylic acid cycle to glycolysis.

Increased Levels of Lactate and Implication of Epileptogenic Properties of Sevoflurane

Several studies have documented seizure-like movements or epileptiform electroencephalogram changes during induction with sevoflurane (7 or 8% sevoflurane), although other studies do not support these findings.[52] We performed the ^{1}HMRS scan near the conclusion of the diagnostic MRI exam while the children were maintained with sevoflurane at an average inspired concentration of 3.1%. We could not measure the electroencephalogram of the subjects during the ^{1}HMRS scan, and therefore cannot comment on the electroencephalographic activity patterns of the children. It is possible that sevoflurane might have induced subclinical seizure activity, which could have contributed to the metabolic changes, including the higher levels of lactate in sevoflurane compared with propofol. For example, lactate levels are known to increase during ictal and postictal states[51] and several of the children were diagnosed with seizure disorder (which had prompted the MRI exam). However, when comparing lactate in children with seizure disorder with those without, for either anesthetic, we did not find any differences. These results support our original hypothesis stating that the anesthetic and not the underlying brain pathology of the child is responsible for the metabolomic pattern observed.

The PAED Score Is Associated with Lactate, Glucose and Cr + PCr in the Parietal Cortex

The more intriguing finding of this study was the apparent correlation between the PAED score and specific metabolites including lactate, glucose, and Cr + PCr. Thus, the children (regardless of anesthetic) who emerged with more agitation and cognitive dissociation according to their scores on the PAED scale were characterized by higher lactate, glucose, and lower concentration of total creatine in the parietal cortex. Emergence delirium and cognitive dissociation have been associated with sevoflurane anesthesia in young children and in some studies attributed to rapid emergence with highly insoluble volatile anesthetic compounds and/or pain.[58–61] Because the children in our study did not undergo surgery but only noninvasive MRI, it is assumed that any observable emergence delirium was unrelated to pain.

It is difficult to further interpret the metabolic data observed in this study obtained in one brain region (parietal cortex) in the context of theoretical models and studies of the pathophysiological origin of delirium and/or "cognitive dissociation" involving global brain networks. Classically, delirium has been defined an "acute confusional state characterized by fluctuating consciousness, arousal level and cognitive function,"[62] and several hypotheses including altered brain metabolism have been put forward to explain the altered brain connectivity patterns underlying this phenomenon.[63] Electroencephalographic studies in animal models suggest a functional disconnection between the thalamocortical and limbic systems, so that the higher order executive and attentional networks are relatively depressed in relation to the reticular activating and limbic systems. Other theories highlight changes in inhibitory (GABAergic) tone,[62] ascending arousal systems, or corticoparietal networks[64] We are in the process of expanding our studies to include resting-state functional magnetic resonance imaging and chemical shift imaging, which will allow for global assessment of connectivity changes in parallel to metabolic status in children undergoing general anesthesia with sevoflurane and propofol.

Limitations of the Study

Several limitations of this study can be pointed out, including the narrow range of brain regions which were explored, the inability to quantify anesthetic depth in the two groups, and the fact that the children suffered from a variety of diseases. As mentioned, we are in the process of expanding our study population and implementing different MRI modalities, such as resting-state functional MRI and chemical shift imaging, which will allow for assessment of global neuronal networks involved in emergence delirium in addition to their corresponding metabolomic status changes. However, we find the present findings of sufficient importance to alert other investigators in the field of the feasibility of applying ^{1}HMRS to the study of metabolic consequences of general anesthesia in children. Further, we plan to incorporate measurement of electroencephalogram and blood measurement of propofol concentration to better assess anesthetic depth in the two groups of patients. Finally, although we established that the demographic variables including the frequency of comorbid state in the two groups were identical, a larger sample size will enable thorough analysis with the application of multivariate logistic and linear regression modeling, allowing adjustment for comorbidity, disease etiology, brain development (age), and length of anesthesia, as well as previous exposure to anesthesia and surgery.

In conclusion, our study showed that routine anesthesia with sevoflurane or propofol in children undergoing medically indicated MRI of their brain resulted in different metabolomic signatures. Specifically, in children anesthetized with sevoflurane, the lactate and glucose were significantly higher compared with propofol anesthesia. We also observed an apparent positive relationship between the magnitude of the PAED score upon emergence and lactate, suggesting that an elevation in brain lactate may be predictive of emergence delirium.

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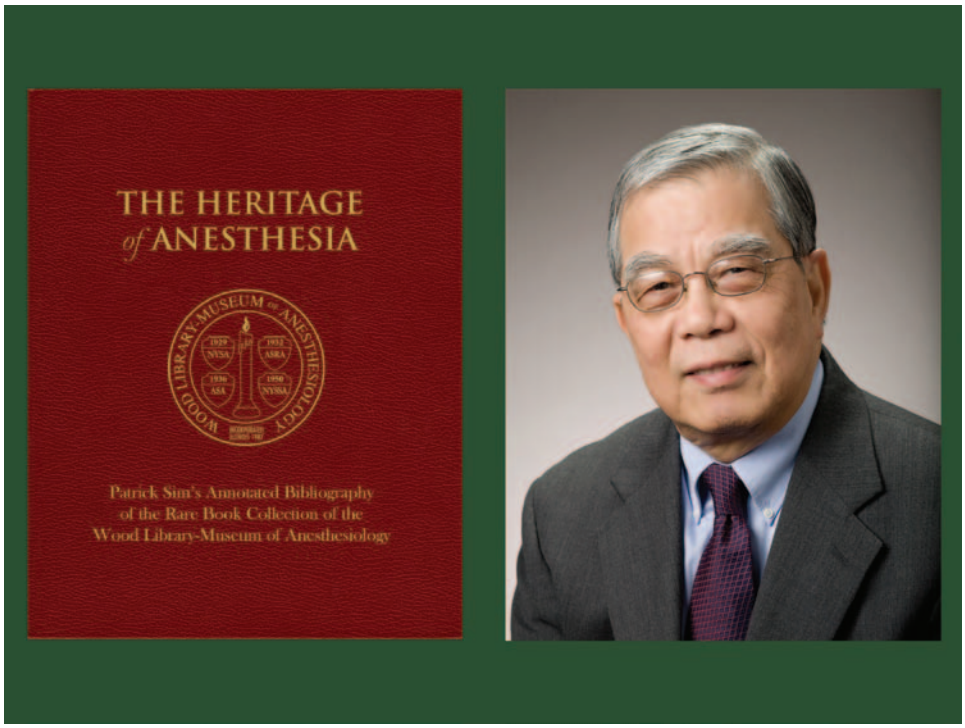

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