Cerebral metabolic rate of oxygen and amplitude-integrated electroencephalography during early reperfusion after hypoxia-ischemia in piglets

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Tichauer KM, Elliott JT, Hadway JA, Lee T, St. Lawrence KS. Cerebral metabolic rate of oxygen and amplitude-integrated electroencephalography during early reperfusion after hypoxia-ischemia in piglets. J Appl Physiol 106: 1506–1512, 2009.—The therapeutic window following perinatal hypoxia-ischemia is brief, and early clinical signs of injury can be subtle. Electroencephalography (EEG) represents the most promising early diagnostic of hypoxia-ischemia; however, some studies have questioned the sensitivity and specificity of EEG. The present study investigated the use of both near-infrared spectroscopy (NIRS) measurements of the cerebral metabolic rate of oxygen (CMRO2) and amplitude-integrated EEG (aEEG) to detect the severity of hypoxia-ischemia after 1 h of reperfusion in newborn piglets (10 insult, 3 control). The CMRO2 was measured before and after 1 h of reperfusion from hypoxia-ischemia, the duration of which was varied from piglet to piglet with a range of 3–24 min, under fentanyl/nitrous oxide anesthesia to mimic awake-like levels of cerebral metabolism. EEG data were collected throughout the study. On average, the CMRO2 and mean aEEG background signals were significantly depressed following the insult (P < 0.05). Mean CMRO2 and mean aEEG background were 2.61 ± 0.11 ml O2·min−1·100 g−1 and 20.4 ± 2.7 μV before the insult and 1.58 ± 0.09 ml O2·min−1·100 g−1 and 11.8 ± 2.9 μV after 1 h of reperfusion, respectively. Both CMRO2 and aEEG displayed statistically significant correlations with duration of ischemia (P < 0.05; r = 0.71 and r = 0.89, respectively); however, only CMRO2 was sensitive to milder injuries (<5 min). This study highlights the potential for combining NIRS measures of CMRO2 with EEG in the neonatal intensive care unit to improve early detection of perinatal hypoxia-ischemia.

near-infrared spectroscopy; piglet; hypoxia-ischemia; electroencephalography

PERINATAL HYPOXIC-ISCHEMIC encephalopathy is a substantial cause of infant mortality and morbidity. It remains a detrimental factor in roughly 1 of every 500 live term births in developed nations, leading to long-term neurological and developmental disabilities (20). Recent clinical trials have demonstrated that the incidence of death and disability from hypoxia-ischemia in newborns can be significantly reduced by initiating treatment strategies, hypothermia, for example, after birth and within a 6-h therapeutic window (9, 11, 14, 32). Due to the brevity of the therapeutic window, early detection of injury and early determination of those infants who are likely candidates for treatment are crucial (15). In this regard, traditional early indicators of brain injury, including Apgar scores, umbilical artery acidosis, and fetal heart rate monitoring, suffer from poor specificity (7, 31), and more specific indicators of injury, such as magnetic resonance imaging and spectroscopy, are insensitive or difficult to implement within the therapeutic window (6, 21).

To this point, the most promising and clinically feasible early indicator of hypoxia-ischemia after birth has been amplitude-integrated electroencephalography (aEEG) due to its ease of use, its noninvasive nature, and its high prognostic value as early as 3 h after birth (1, 40). However, recent studies have questioned the sensitivity of aEEG to detect infants with milder injuries who could still benefit from treatment (30).

Another potentially promising early indicator is the measurement of the cerebral metabolic rate of oxygen (CMRO2), made clinically feasible in the last decade with recent technical advances in near-infrared spectroscopy (NIRS) (44). With the use of continuous-wave, broadband spectrum NIRS, the absolute concentration of cerebral deoxyhemoglobin ([HHb]) (23) and cerebral blood flow (CBF) (4, 26, 35) can be accurately determined. By combining these measurements, our group has developed a bedside NIRS system capable of rapidly measuring the CMRO2 that was validated in healthy piglets over a range of metabolic states (3, 38). Furthermore, our group observed early, persistent reductions in the CMRO2 of piglets subjected to transient hypoxia-ischemia (37). When the duration of ischemia was varied, a statistically significant correlation was found between the CMRO2 and the duration of ischemia by 8 h of reperfusion (39).

The purpose of the present study was to investigate and compare early changes in cerebral metabolism and electrocortical activity following various durations of hypoxia-ischemia in the piglet under awake-like conditions. This was accomplished by comparing the duration of hypoxia-ischemia with the clinically relevant aEEG and NIRS-measured CMRO2 collected after 1 h of reperfusion under fentanyl/nitrous oxide (N2O) anesthesia [an anesthetic regime known to generate cerebral metabolic rates and basal electrocortical activity that are representative of the awake state (33, 45)].

MATERIALS AND METHODS

Animal preparation. The study was approved by the Animal Use Subcommittee of the Canadian Council on Animal Care at the University of Western Ontario. Newborn Duroc piglets (n = 13) were delivered from a local supplier on the morning of the experiment. Anesthesia was induced, and surgery was performed using 3% isoflurane. The surgical procedure has been presented in detail elsewhere (37). Briefly, piglets were tracheotomized and mechanically venti-
lated. Vascular occluders (In Vivo Metric, Healdsburg, CA) were placed around both carotid arteries just proximal to the carotid bifurcation. Cannulas were inserted into an ear and belly vein for injection of the NIRS-sensitive, intravascular contrast agent indocyanine green (ICG) and for fentanyl infusion, respectively. Another cannula was inserted into a femoral artery for continuous monitoring of blood pressure and to allow the collection of arterial blood samples for gas and glucose analysis. Following surgery, isoflurane was reduced to 1.75%, and piglets were allowed to stabilize for 1 h before the anesthetic agent was switched to a 0.02 mg·kg\(^{-1}\)·h\(^{-1}\) infusion of fentanyl, combined with inspiration of a 30% oxygen/70% N\(_2\)O gas mixture. The experiment was commenced after a further 30 min of acclimation (from experience, the emergence time for isoflurane in the mixture). The experiment was commenced after a further 30 min of acclimation (from experience, the emergence time for isoflurane in the mixture). The experiment was commenced after a further 30 min of acclimation (from experience, the emergence time for isoflurane in the mixture).

Experimental procedure. Piglets were randomly divided into two groups: an insult group (n = 10), and a sham-operated control group (n = 3). Two NIRS measurements of CMRO\(_2\) were collected at an interval of 15 min during baseline. Following baseline, isoflurane anesthesia was reinstated for 45 min, and insult group animals were subjected to randomly selected durations of hypoxia-occlusion (10–30 min), induced by clamping both carotid arteries and reducing the fraction of inspired oxygen to 7%. The purpose of reinstating isoflurane was to produce an insult comparable to previous studies conducted by our group (37, 39). For each piglet, the duration of ischemia was defined as the amount of time the mean arterial pressure was <70% of baseline during the insult, a threshold validated in a previous study (39). Following the insult, the carotid clamps were released, and fraction of inspired oxygen was returned to baseline levels. After 30 min of reperfusion, fentanyl/N\(_2\)O anesthesia was reinstated, and, after another 30 min of acclimation, a further two measurements of CMRO\(_2\) were collected. EEG data were collected continuously throughout each study.

At all times during the experiment (excluding the insult), arterial PO\(_2\) was maintained between 38–42 Torr by adjusting the respiratory rate, while arterial PO\(_2\) was maintained at 100–150 Torr by adjusting the ratio of oxygen to medical air. Blood glucose was kept between 3 and 8 mmol/l by intermittent 0.3-ml injections of a 25% dextrose solution into an ear vein. A water-heating blanket was used to maintain a rectal temperature between 38 and 39°C. Arterial pH and heart rate were also monitored.

NIRS. NIRS data were collected with a continuous-wave, broadband (600–1,000 nm) system with a single emission fiber-optic bundle and a single detection bundle (4). An end from each bundle was placed 3 cm apart, parasagittally on the head, proximal to the widest part of the brain.

The method used to calculate CMRO\(_2\) with NIRS has been discussed in detail previously (3). Measurements of CMRO\(_2\) were based on the Fick principle:

\[
\text{CMRO}_2 = \text{CBF}([\text{O}_2\text{a}] - [\text{O}_2\text{v}])
\]

where [O\(_2\text{a}\)] is the oxygen concentration of the arteries feeding the cerebral tissue, and [O\(_2\text{v}\)] is the oxygen concentration of the vessels draining the cerebral tissue. The calculation of CBF required an intravenous 0.1 mg/kg bolus injection of ICG and relied on deconvolution of the subsequent arterial and tissue concentration-time curves of the tracer (4). The main advantage of this technique over others (13, 46) is the increased precision of the CBF measurements gained by using ICG as a tracer (26). A further benefit of the technique is an increased precision by permitting data outside of the first pass of the bolus to be included, which also allowed cerebral blood volume (CBV) to be calculated (4, 8, 47). The [O\(_2\text{a}\)] was determined using a pulse oximeter attached to a foot. Measurements of [HHb\)] were quantified using the second-derivative technique (23) and used to indirectly determine the [O\(_2\text{a}\)] by normalizing the brain [HHb\)] to the CBV and assuming a distribution of 75% venous and 25% arterial blood within the volume (25).

\text{aEEG.} To assess electrocortical brain activity, gold-plated electrode disks were placed on P\(_3\) and P\(_4\) positions, locations that correspond to left and right parietal regions of the brain, according to the 10–20 International System. The signal was recorded at a sampling rate of 250 Hz after being amplified and filtered (0.1–100 Hz) using a high-performance AC preamplifier (model PS111, Grass Technologies Product Group, Astro-Med, West Warwick, RI). Using the PolyView software (Grass Technologies), the raw EEG signal was saved to be converted to aEEG and analyzed post hoc.

To convert the EEG information to an aEEG signal, an algorithm was developed using MATLAB (The MathWorks, Natick, MA). Data were filtered, rectified, and integrated over a window of 100 ms. To eliminate nonphysiological signal components and to slightly attenuate the dominant delta-wave patterns, a series of filters were applied to mimic those used in the Cerebral Function Monitor (24). The final signal was plotted logarithmically, and the resulting aEEG was analyzed by two different methods: a quantitative mean aEEG background technique, and a qualitative neural activity score technique.

\text{Mean aEEG background.} To measure the mean aEEG background signal, a line was drawn through the upper and lower margins of the aEEG band, as described by al Naqeeb et al. (1). In this way, individual spikes separated from the aEEG band are excluded from the measurement. The median line between the upper and lower margins was taken to be the mean amplitude.

\text{Neural activity score.} To measure the neural activity score, intended to reflect the functional state of the brain, background voltage as well as pattern recognition techniques were used. Background voltage was recorded as normal, low, or extremely low. The background pattern was classified as either continuous or discontinuous, and the presence of seizure activity or burst-suppression pattern was noted. A summary of the scoring method is presented in Table 1 based on the analysis of de Vries et al. (10). The aEEG data from each experiment were scored in 10-min intervals by an analyst blinded to the duration of hypoxia-ischemia. The scores were then grouped so that mean neural activity score could be calculated during baseline and following the injury.

\text{Statistical analysis.} SPSS 16.0 (SPSS, Chicago, IL) was used for all statistical analyses. A repeated-measures, mixed ANOVA was used to compare measurements of CMRO\(_2\) between insult and control groups, with time as the within-subjects variable. Time effects were further analyzed with a one-way ANOVA. All correlations of parametric data were analyzed with linear regression. For nonparametric

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**Table 1. Neural activity scoring system**

<table>
<thead>
<tr>
<th>Neural Activity Score</th>
<th>Relative Voltage</th>
<th>Characteristic Patterns</th>
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</thead>
<tbody>
<tr>
<td>4: CNV</td>
<td>Normal voltage (10–25 μV)</td>
<td>Continuous, normal waveforms</td>
</tr>
<tr>
<td>3: DNV</td>
<td>Normal voltage with periods of decreased voltage</td>
<td>Discontinuous pattern</td>
</tr>
<tr>
<td>2: BS</td>
<td>Low background voltage (&lt;5 μV)</td>
<td>Periods of low activity interrupted by brief bursts of activity, including status epilepticus</td>
</tr>
<tr>
<td>1: CLV</td>
<td>Low background voltage</td>
<td>Continuous waveforms</td>
</tr>
<tr>
<td>0: FT</td>
<td>Low background voltage</td>
<td>Inactive, isoelectric pattern</td>
</tr>
</tbody>
</table>

CNV, continuous normal voltage; DNV, discontinuous normal voltage; BS, burst suppression; CLV, continuous extremely low voltage; FT, flat trace.
data, such as the neural activity scores, Spearman’s ρ rank test was used, and differences between pre- and postinsult effects were uncovered using a Wilcoxon signed-ranks test. Statistical significance for all tests was based on a P < 0.05. All values are presented as means ± SE.

RESULTS

A total of 13 piglets (5 male, 8 female) were studied (average age: 13 ± 1 h, weight: 1.67 ± 0.06 kg). Table 2 summarizes the results of the physiological parameters for the insult and control groups at baseline and at 1 h of reperfusion. There were no statistically significant differences between control and insult groups for any measured physiological parameter and no significant correlations were found between any parameter and the duration of ischemia.

Table 3 summarizes the results of the hemodynamic and metabolic measurements made in the study. The average CMRO$_2$ was found to be significantly lower postinsult compared with baseline and controls (P < 0.05), while the average postinsult CBF was significantly lower than baseline but not controls; there was no significant effect of the insult on CBV. When the individual measurements of CMRO$_2$ after 1 h of reperfusion were plotted against the duration of ischemia (Fig. 1), a statistically significant correlation was found (r = 0.714, slope = 0.03 ml O$_2$·min$^{-1}$·100 g$^{-1}$·min of ischemia$^{-1}$, P < 0.05). No significant correlations were found between the duration of ischemia and CBF or CBV.

EEG signal was successfully collected from 9 of the 10 insult group piglets (in 1 piglet, excessive electrical noise made it impossible to extract meaningful EEG signal) and all 3 controls. Figure 2 displays representative aEEG time plots during baseline, hypoxia-ischemia, and reperfusion, with example signals representative of each observed neural activity score (Table 1). Figure 3 summarizes the effect of ischemia duration on the mean level of the aEEG signal (A) and on the neural activity score (B) collected postinsult. A significant correlation was found between the duration of ischemia and both mean aEEG background (r = 0.89, P < 0.01) and neural activity score (r = 0.906, P < 0.01); however, mean aEEG backgrounds of the three piglets with the mildest injuries were within the standard error of baseline levels. The average mean aEEG background signal after hypoxia-ischemia (11.8 ± 2.9 μV) was statistically lower than baseline (20.4 ± 2.7 μV) (P < 0.05). This effect was also observed for neural activity score [2.25 (range: 1.5–3.25) after hypoxia-ischemia compared with 4 (range: 4–4) at baseline]. In controls, the aEEG signal collected at time points comparable to postinsult periods in the insult group was indiscernible from baseline.

Figure 4 displays correlations between coincident measurements of CMRO$_2$ and mean aEEG background before (A) and after (B) hypoxia-ischemia and between CMRO$_2$ and neural activity score after hypoxia-ischemia (C) (a correlation between CMRO$_2$ and neural activity score before hypoxia-ischemia is not provided, since all animals scored a value of 4 during baseline). The correlation between CMRO$_2$ and mean aEEG background before the insult was not significant, although a slight positive trend did exist (r = 0.31, slope = 0.01 ml O$_2$·min$^{-1}$·100 g$^{-1}$·μV$^{-1}$, nonsignificant). Following the insult, the correlation between CMRO$_2$ and mean aEEG background did exhibit a significant positive trend (r = 0.67, ***P < 0.001***).

### Table 2. Physiological parameters before (baseline) and after 1 h of reperfusion (postinsult) for control and insult groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Postinsult</th>
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<tbody>
<tr>
<td>Arterial Pco$_2$, Torr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39.1 ± 1.7</td>
<td>39.8 ± 0.4</td>
</tr>
<tr>
<td>Insult</td>
<td>38.5 ± 0.4</td>
<td>38.4 ± 0.7</td>
</tr>
<tr>
<td>Mean arterial pressure, Torr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>75.0 ± 3.5</td>
<td>68.7 ± 3.2</td>
</tr>
<tr>
<td>Insult</td>
<td>69.6 ± 3.8</td>
<td>63.4 ± 4.4</td>
</tr>
<tr>
<td>Arterial pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.45 ± 0.02</td>
<td>7.46 ± 0.01</td>
</tr>
<tr>
<td>Insult</td>
<td>7.46 ± 0.01</td>
<td>7.43 ± 0.01</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>187 ± 24</td>
<td>207 ± 24</td>
</tr>
<tr>
<td>Insult</td>
<td>192 ± 14</td>
<td>214 ± 11</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

### Table 3. Hemodynamic and metabolic parameters before (baseline) and after 1 h of reperfusion (postinsult) for control and insult groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Postinsult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral blood flow, ml·min$^{-1}$·100 g$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>44.8 ± 3.4</td>
<td>39.4 ± 7.4</td>
</tr>
<tr>
<td>Insult</td>
<td>46.8 ± 3.9</td>
<td>34.0 ± 4.7*</td>
</tr>
<tr>
<td>Cerebral blood volume, ml/100 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.11 ± 0.14</td>
<td>3.43 ± 0.69</td>
</tr>
<tr>
<td>Insult</td>
<td>2.98 ± 0.23</td>
<td>3.16 ± 0.27</td>
</tr>
<tr>
<td>Cerebral metabolic rate of oxygen, ml O$_2$·min$^{-1}$·100 g$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.48 ± 0.24</td>
<td>2.27 ± 0.16</td>
</tr>
<tr>
<td>Insult</td>
<td>2.61 ± 0.11</td>
<td>1.58 ± 0.09†</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *P < 0.05 compared with baseline. †P < 0.05 compared with control.
slope = 0.02 ml O₂·min⁻¹·100 g⁻¹·μV⁻¹, P < 0.05). There also existed a positive trend between CMRO₂ and neural activity score postinsult; however, the trend was not significant (p = 0.40, slope = 0.21 ml O₂·min⁻¹·100 g⁻¹·score value⁻¹, nonsignificant).

DISCUSSION

The brevity of the therapeutic window in newborns suspected of having sustained hypoxia-ischemia during birth makes early detection and prognosis essential. Consequently, much effort has been invested in the search for an ideal indicator of compromised brain function that can be utilized to study newborns immediately after birth. Both NIRS and, to a greater extent, EEG are increasingly being used to monitor brain function in the neonate (16, 44), since they are essentially risk free and can be employed at the bedside. Furthermore, the two techniques are ideally suited for use in concert: while NIRS offers information on cerebral hemodynamics (4, 12, 26), oxygenation (41), and metabolism (3, 13, 46), EEG offers information on electrocortical brain function and seizure activity (16). The purpose of the present study was to compare the influence of acute hypoxia-ischemia on aEEG and on NIRS measured CMRO₂ acquired after 1 h of reperfusion for a range of insult durations. The comparison was made under fentanyl/ N₂O anesthesia in newborn piglets. Anesthesia with fentanyl/ N₂O is facilitated by disorganizing the excitation pattern of neurons, which interferes with neuronal networking causing unconsciousness (33); therefore, it results in minimal changes in electrocortical activity and basal cerebral metabolism from the awake state (45), allowing the results of the study to be more comparable to the clinical situation.

The salient finding of this study was that both the CMRO₂ measurements and mean aEEG displayed statistically significant correlations with the duration of ischemia after only 1 h of reperfusion. In view of the fact that anesthesia with fentanyl/ N₂O represents awake levels of cerebral metabolism (45), we believe the sensitivity of both aEEG and NIRS to insult severity observed as early as 1 h postinsult is of significant clinical interest. This result was somewhat incongruous with previous results obtained by our group. In a recent study in which CMRO₂ was monitored out to 24 h after various durations of hypoxia-ischemia in piglets, CMRO₂ was depressed immediately after the insult; however, a correlation between CMRO₂ and ischemia duration was not observed until 8 h of reperfusion (39). The only change in animal treatment from our laboratory’s previous study to the present was the choice of anesthesia. In our laboratory’s previous study, piglets were

Fig. 2. Amplitude-integrated electroencephalograms (aEEG) of electrocortical activity before, during, and after hypoxia-ischemia. Examples of aEEG from individual piglets are displayed at different chronological segments of the experiment. The traces are displayed semilogarithmically, and the time scale is indicated in the bottom lefthand corner of A. Various background patterns can be observed during different periods of the experiment: A: representative aEEG signal at baseline and during hypoxia-ischemia (HI). Initially, while under fentanyl/nitrous oxide anesthesia, the signal is continuous normal voltage, which then becomes discontinuous normal voltage following a change in anesthetic to isoflurane (denoted by “ISO” arrow). During the insult, a continuously low-voltage signal was observed. B: example of postinsult aEEG at the onset of the restoration of fentanyl anesthesia (denoted by “Fent” arrow); in this case, a discontinuous normal voltage pattern is the result. C: example of postinsult aEEG for which the pattern was scored as burst suppression. D: example of postinsult aEEG that starts as a continuous low-voltage pattern and is interrupted by epileptic activity ("sawtooth pattern").

Fig. 3. Effects of insult duration on cerebral electrophysiology. Individual aEEG background means (A) and neural activity scores (B) taken after 1 h of reperfusion are plotted against duration of ischemia (n = 9). Solid lines represent linear regressions of best fit (slopes: −1.1 μV/min of ischemia and −0.08 neural activity scores/min of ischemia). The dashed line and dotted lines in A represent the average mean aEEG background at baseline ± SE, respectively, for comparison. The neural activity score at baseline for all piglets was 4.
found a significant correlation between background EEG amplitude at 1 h postinsult and pathological score at 48 h, and Björkman et al. (2) found that the minimum EEG amplitude at 1 h postinsult was significantly lower in severely injured animals compared with animals with minor injuries. They also reported that the average minimum EEG amplitude of moderately injured animals fell in between mild and severely injured groups, and, while it was not significantly different from either severe or mild injuries, the trend suggested a correlation between EEG amplitude and insult severity.

From the results of the present study alone, the mechanism of the significant correlation between postinsult CMRO₂ and the duration of ischemia is unclear. The statistically significant correlation observed between CMRO₂ and mean aEEG background postinsult (Fig. 4B), in addition to the significant correlation between mean aEEG signal and duration of ischemia, suggests the response of CMRO₂ to insult severity is coupled to changes in electrocortical activity. This interpretation is supported by recent evidence showing that the release of adenosine following ischemia leads to cerebral metabolic depression due to the inhibition of electrocortical activity (22). However, it was also found that the postinsult mean aEEG background signals of the three piglets with the mildest injuries were not discernible from baseline (Fig. 3A), while corresponding CMRO₂ measurements were considerably lower than baseline (1.75 ± 0.18 compared with 2.61 ± 0.11 ml O₂·min⁻¹·100 g⁻¹). These results suggest that the reductions in CMRO₂ cannot be explained solely by reductions in electrocortical activity and may imply that basic cellular mechanisms, such as protein synthesis, may be downregulated following hypoxia-ischemia (34).

A second potential explanation of reduced metabolism could be that, after hypoxia-ischemia, the metabolic demand of the brain exceeded the mitochondrial capacity to metabolize ATP. Mitochondria extracted from brain tissue that is made ischemic exhibit a reduced capacity to uptake oxygen that is accentuated with increased durations of ischemia (28), possibly due to ischemic damage or inhibition by nitric oxide (5). However, if energy demand exceeds supply, energy failure would be expected. In a previous study involving phosphorous magnetic resonance spectroscopy, we found phosphocreatine to return rapidly to baseline levels after 30 min of hypoxia-ischemia in piglets anesthetized with isoflurane (43). In the neonate, secondary energy failure (a delayed reduction in high-energy phosphates) is rarely observed before 24 h after birth (6). One thing to consider, however, is that previous piglet studies were conducted under general anesthesia, which inhibits the occurrence of seizures, and, in neonates, seizures are generally suppressed pharmaco logically. In the present study, considerable seizure activity was observed following hypoxia-ischemia, which may have resulted in metabolic increases exceeding the capacity of impaired mitochondria.

A secondary finding of this study was that the average CBF of the insult group was slightly depressed following hypoxia-ischemia compared with baseline levels. This effect was expected, considering the significant changes in cerebral metabolism observed following the insult. However, there was no correlation of CBF with the duration of ischemia, nor was the depression significantly different compared with average CBF taken at a corresponding time point in control animals, sug-

kept anesthetized by isoflurane at all times. Isoflurane is known to depress metabolism, and its effects may have been magnified during early reperfusion, if prolonged hypoxia affected the clearance rate of the agent (29).

To the best of our knowledge, this is the first study comparing CMRO₂ during very early reperfusion with insult severity under awake-like levels of cerebral metabolism. At least two previous studies have compared aEEG signal following hypoxia-ischemia to insult severity in piglets, each with findings similar to those reported in our study. Thoresen et al. (36)

![CMRO₂ and aEEG Correlation](image)

**Fig. 4.** Correlation between cerebral metabolism and electrophysiology. Individual CMRO₂ measurements at baseline (A) and after 1 h of reperfusion (B) are plotted against contemporaneous aEEG mean background signals (n = 9). C: individual CMRO₂ measurements are also plotted against neural activity score after 1 h of reperfusion. Solid lines represent linear regressions of best fit [slopes: A, 0.01 ml O₂·min⁻¹·100 g⁻¹·µV⁻¹·µV⁻¹; B, 0.02 ml O₂·min⁻¹·100 g⁻¹·µV⁻¹; C, 0.21 ml O₂·min⁻¹·100 g⁻¹·neural activity score⁻¹].
suggesting that CBF would not make a sensitive marker of insult severity in the clinic.

aEEG is rapidly becoming a commonplace tool for monitoring brain function in the neonatal intensive care unit. In addition to its being noninvasive and applicable at the bedside, it can be continuously monitored by staff and interpreted without a high degree of training, while providing a similar level of specificity and sensitivity to traditional EEG in assessing neonatal neuropathology (17). To investigate the ability of aEEG to detect varying degrees of injury, we developed an algorithm based on the Cerebral Function Monitor, which is employed clinically, to convert EEG signal recorded continuously for the duration of the experiment to an aEEG signal. As mentioned previously, aEEG background signal has shown sensitivity to the severity of hypoxia-ischemia in a number of animal experiments (2, 36, 42), and, more importantly, it has also been effective in the clinical setting (1, 17, 40). Despite these benefits, there are a number of studies questioning absolute reliance on aEEG to diagnose hypoxia-ischemia. Sarkar et al. (30) demonstrated that aEEG recordings within 6 h of birth were associated with a low negative predictive value; that is, some infants with milder injuries who would have benefited from treatment could not be recognized by aEEG. Conversely, Pezzani et al. (27) found that some infants can present an isoelectric EEG signal in the first hours after birth and continue to develop normally.

In the present study, it was impossible to discern the mean aEEG background signal of piglets with the mildest injuries from baseline (Fig. 3A). However, by applying a neural activity score to the data, it was possible to discern the mildest injuries from the aEEG data (Fig. 3B). In a previous study that investigated a similar range of insult severities, we observed very little histological evidence of injury for piglets with insult durations shorter than 5 min (39). On the other hand, that study was conducted under continuous isoflurane anesthesia, which is known to have neuroprotective effects (19) and may have resulted in correspondingly less severe insults than those in the present study.

The aEEG signal scoring method employed in this study was based on the techniques of de Vries et al. (10). In other studies, the weights of 1 and 2, given to continuous low-voltage and burst suppression activity, respectively, are switched, since seizure activity is particularly destructive and is of the utmost importance for treatment. For the purposes of the present study, we were interested in using the scoring system to characterize energy demands of the injured brain, so seizure activity, which would result in higher energy demand, was scored higher than continuous low voltage. When the data from the present study were rescoring using the alternate scoring system, the correlation with duration of ischemia was lost.

To analyze potential benefits of combining NIRS with EEG, a multivariate regression analysis was applied to aEEG and CMRO₂ data with respect to insult duration. The Pearson correlation for the product of mean background aEEG and CMRO₂ was 0.846 (P < 0.01), which was not significantly stronger than that for CMRO₂ or aEEG alone (−0.720, P < 0.05 and −0.857, P < 0.01, respectively). Despite this result, seemingly no added benefit to combining NIRS to EEG, the additional finding that CMRO₂ was more sensitive to mild injuries than aEEG maintains the hypothesis that the inclusion of CMRO₂ measurements could add significantly to the accurate diagnosis and prognosis of hypoxic-ischemic encephalopathy, if used in conjunction with EEG. This is emboldened by results from clinical trials of selective head cooling, which have suggested that the most significant improvements in clinical outcome are seen in infants with less severe aEEG changes (14). An early, more sensitive method of diagnosis, to which CMRO₂ could contribute, may afford therapeutic intervention for those that stand to gain the most from it, the moderately injured infant. Future studies are warranted to determine how the relationship between CMRO₂ and EEG develops throughout the recovery period and with the onset of delayed brain injury.

In summary, the present study demonstrated that, in a piglet model of perinatal hypoxia-ischemia, both NIRS-measured CMRO₂ and aEEG signal could be used to delineate different insult severities after 1 h of reperfusion from hypoxia-ischemia. Since both techniques can be applied simultaneously at the bedside of the sick newborn, the results highlight the potential of using these techniques in conjunction to improve diagnosis and to monitor treatment of hypoxic-ischemic encephalopathy within the therapeutic window.

GRANTS

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REFERENCES
