

Phenobarbital and neonatal seizures affect cerebral oxygen metabolism: a near-infrared spectroscopy study

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BACKGROUND: Near-infrared spectroscopy (NIRS) measures oxygen metabolism and is increasingly used for monitoring critically ill neonates. The implications of NIRS-recorded data in this population are poorly understood. We evaluated NIRS monitoring for neonates with seizures.

METHODS: In neonates monitored with video-electroencephalography, NIRS-measured cerebral regional oxygen saturation (rSO₂) and systemic O₂ saturation were recorded every 5 s. Mean rSO₂ was extracted for 1-h blocks before, during, and after phenobarbital doses. For each electrographic seizure, mean rSO₂ was extracted for a period of three times the duration of the seizure before and after the ictal pattern, as well as during the seizure. Linear mixed models were developed to assess the impact of phenobarbital administration and of seizures on rSO₂ and fractional tissue oxygen extraction.

RESULTS: For 20 neonates (estimated gestational age: 39.6 ± 1.5 wk), 61 phenobarbital doses and 40 seizures were analyzed. Cerebral rSO₂ rose ($P = 0.005$), and fractional tissue oxygen extraction declined ($P = 0.018$) with increasing phenobarbital doses. rSO₂ declined during seizures, compared with baseline and postictal phases (baseline 81.2 vs. ictal 77.7 vs. postictal 79.4; $P = 0.004$). Fractional tissue oxygen extraction was highest during seizures ($P = 0.002$).

CONCLUSIONS: Cerebral oxygen metabolism decreases after phenobarbital administration and increases during seizures. These small, but clear, changes in cerebral oxygen metabolism merit assessment for potential clinical impact.

Seizures are common in neonates and often associated with adverse outcomes (1). Whether seizures themselves harm the developing brain or are simply manifestations of abnormal cerebral physiology remains an active debate. Animal data suggest that seizures amplify brain injury (2). Neonates with hypoxic-ischemic encephalopathy (HIE) and high seizure burden show more significant brain injury on magnetic resonance imaging and have worse outcomes than those who are seizure-free (3–5). High seizure burden in critically ill neonates and older children has also been independently associated with a higher probability and magnitude of neurological decline (6).

Long-term outcome studies of neonates with seizures demonstrate that a majority suffer significant neurodevelopmental abnormalities (7).

Phenobarbital is typically used as an initial treatment for neonatal seizures, despite incomplete efficacy (7–9), and concerns that this medication could induce abnormal neuronal apoptosis and cognitive impairment (10). Furthermore, the safety of loading dose administration in low-birth-weight and preterm neonates has been questioned, with currently recommended doses leading to higher than anticipated drug levels (11).

The gold standard for seizure diagnosis in neonates is electroencephalography (EEG) monitoring (12). Recently, additional cerebral monitoring has been advocated. Among clinically available noninvasive devices, near-infrared spectroscopy (NIRS) has been suggested as a potentially helpful instrument for monitoring brain functional integrity (13,14). Disturbances in cerebral oxygen metabolism and blood flow, measured by a transcranial doppler, have been linked to electrographic seizures (15,16). The NIRS has been shown to reflect cerebral blood flow (17), but its utility as a bedside monitor for infants at risk for neonatal seizures remains uncertain. We hypothesized that this device could provide data on the pathophysiology of neonatal seizures and consequences of their treatment. We aimed to assess the use of NIRS monitoring for neonatal seizures and to determine whether NIRS reveals physiological changes associated with doses of phenobarbital. We hypothesized that cerebral oxygen metabolism would peak during neonatal seizures. We further theorized that phenobarbital administration would be associated with a decline in brain tissue oxygen extraction.

RESULTS

There were 61 doses of phenobarbital administered to 20 patients (mean gestational age: 39.55 ± 1.47 wk), with the majority of neonates receiving more than one dose. There were 40 individual seizures recorded on 11 of the patients. The seizure etiologies included HIE ($N = 7$), neonatal epilepsy syndromes ($N = 5$), arterial ischemic stroke ($N = 2$), sepsis ($N = 2$), benign neonatal seizures ($N = 1$), sinovenous thrombosis

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($N = 1$), intracranial hemorrhage ($N = 1$), and uncertain etiology ($N = 1$). The demographic data are presented in **Table 1**.

Phenobarbital Models

The phenobarbital doses ranged from 2.1 to 20.3 mg/kg, with 49 maintenance and 12 bolus doses. The absolute changes in regional oxygen saturation (rSO₂) and fractional tissue oxygen extraction (FTOE) associated with phenobarbital dosing were small (**Table 2**). The estimated mean left-cerebral rSO₂ was higher at baseline compared with the hour after phenobarbital

administration (75.8 ± 2.84 vs. 74.9 ± 2.80 ; Bonferroni-adjusted $P = 0.049$). In these analyses, there was no change in MAP, systemic O₂ saturation (SaO₂), or systemic rSO₂ associated with phenobarbital dosing.

The models refit to include an interaction between time period (1-h before, during, and after the dose) and weight-adjusted doses (mg/kg) were significant. For bolus doses, the cerebral rSO₂ was significantly higher in the hour after the dose, compared with the predose baseline (83.4 ± 2.4 vs. 79.3 ± 3.5 , $P = 0.005$; **Figure 1a**), while rSO₂ was lower in the hour after maintenance doses (81.1 ± 1.69 vs. 82.9 ± 1.53 , $P = 0.012$). The cerebral rSO₂ during the hour of phenobarbital administration did not differ significantly from baseline levels for either maintenance or bolus doses.

Systemic O₂ saturation (SaO₂) was slightly higher during the hour of phenobarbital bolus dose administration, compared with baseline ($96.9 \pm 0.51\%$ vs. $95.4 \pm 0.77\%$, $P = 0.014$), and returned to baseline in the subsequent hour. The maintenance doses were not associated with significant changes in SaO₂.

The models of FTOE, as derived from rSO₂ and SaO₂, predicted reduced cerebral FTOE, by 3.6% on average, after phenobarbital bolus dose administration, compared with baseline ($13.5 \pm 2.66\%$ vs. $15.81 \pm 3.68\%$, $P = 0.018$; **Figure 1b**). There was no significant difference in FTOE associated with maintenance doses.

Seizure Models

For data that included all 40 seizures, both right- and left-cerebral rSO₂ declined during seizures, compared with baseline and postictal phases (right: 81.2 ± 0.98 vs. 77.7 ± 2.57 vs. 79.4 ± 3.28 , $P = 0.005$; left: 77.6 ± 1.35 vs. 74.9 ± 1.96 vs. 75.8 ± 2.79 , $P = 0.004$; **Table 3**). The right and left FTOE were highest during seizures (right: 23.7 ± 1.64 vs. 26.9 ± 2.81 vs. 23.9 ± 2.91 , $P = 0.002$; left: 19.9 ± 1.90 vs. 22.6 ± 2.27 vs. 20.0 ± 3.19 , $P = 0.002$), compared with the baseline and the postictal periods (**Table 3**). There was no change in SaO₂ or systemic NIRS variables (rSO₂ or FTOE) associated with seizures. The duration of seizures did not affect the results.

The model diagnostics demonstrated one subject to be highly influential in many of the results, based on Cook's distance

Table 1. Demographics of 20 neonates monitored with simultaneous conventional EEG and NIRS

Gender	N (%)
Male	10 (50.0)
Female	10 (50.0)
	Mean (SD)
Birth weight (g)	3,308 (481)
Gestational age (wk)	39.6 (1.5)
Phenobarbital dose (mg)	17.4 (13.8; range: 6.0–69.0)
Dose/birth weight (mg/kg)	5.2 (4.1; range: 2.1–20.3)
Seizure duration (s)	120.0 (180.4; range: 16.0–510.0)
Phenobarbital doses per patient	N (%)
1	5 (25.0)
2	2 (10.0)
3	6 (30.0)
4	1 (5.0)
5	6 (30.0)
Seizures per patient	N (%)
0	9 (45.0)
1	1 (5.0)
2	2 (10.0)
4	5 (25.0)
5	3 (15.0)

EEG, electroencephalography; NIRS, near-infrared spectroscopy; SD, standard deviation.

Table 2. Phenobarbital administration model results, controlling for dose^a

Outcome: average	Predose, estimated mean (95% CI)	During dose, estimated mean (95% CI)	After dose, estimated mean (95% CI)	P-value
Right-cerebral rSO ₂ , $n = 56$	79.7 (74.4, 85.0)	79.4 (74.1, 84.7)	79.0 (73.7, 84.3)	0.57
Left-cerebral rSO ₂ , $n = 59$	75.8 (69.1, 82.5)	75.1 (68.4, 81.7)	74.9 (68.2, 81.6)	0.04
Systemic rSO ₂ , $n = 61$	77.8 (74.0, 81.6)	77.6 (73.8, 81.4)	79.5 (75.7, 83.3)	0.16
SaO ₂ , $n = 61$	96.7 (95.9, 97.6)	96.7 (95.8, 97.5)	96.4 (95.6, 97.3)	0.52
MAP, $n = 54$	53.5 (49.8, 57.2)	54.3 (50.6, 58.0)	53.1 (49.4, 56.8)	0.22
Left-cerebral FTOE, $n = 59$	22.0 (15.0, 28.9)	22.5 (15.6, 29.4)	22.3 (15.3, 29.2)	0.42
Right-cerebral FTOE, $n = 56$	17.8 (12.2, 23.3)	18.0 (12.5, 23.6)	18.0 (12.4, 23.5)	0.91
Systemic FTOE, $n = 61$	19.6 (15.7, 23.5)	19.7 (15.8, 23.6)	17.5 (13.6, 21.4)	0.10

CI, confidence interval; FTOE, fractional tissue oxygen extraction; rSO₂, regional oxygen saturation; SaO₂, systemic O₂ saturation.

^aEstimated marginal means from linear mixed models with random intercepts for patient and dose event with ratio of dose to birth weight (mg/kg) set to the average value of 5.24 (mg/kg). P-value is from an overall Wald χ^2 test for inclusion of time as a predictor in the model. Sample size represents the number of dose events included in the model.

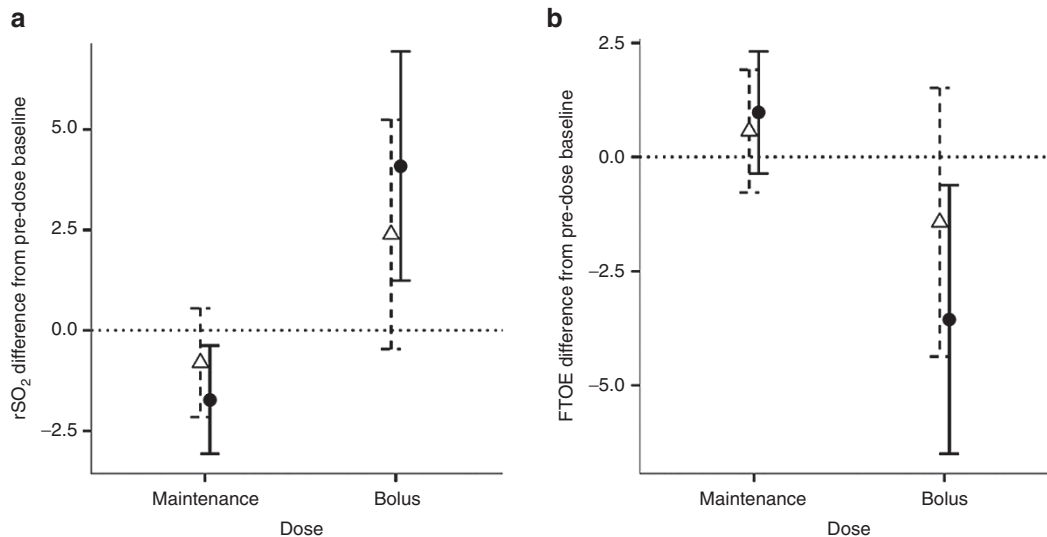


Figure 1. Changes in cerebral oxygen metabolism associated with phenobarbital administration. Linear mixed models with interactions were developed to assess the impact of phenobarbital administration on regional oxygen saturation (rSO₂) (a) and fractional tissue oxygen extraction (FTOE) (b). The estimated marginal means and SEs are presented for the difference in rSO₂ and FTOE values measured at baseline vs. during phenobarbital dose administration (white triangles), and at baseline vs. 1 h after phenobarbital maintenance (<10 mg/kg) and bolus (≥10 mg/kg) doses (dark circles).

Table 3. Seizure model results^a

	Preseizure, estimated mean (95% CI)	During seizure, estimated mean (95% CI)	After seizure, estimated mean (95% CI)	P-value
Right-cerebral rSO ₂ , n = 36	81.2 (72.0, 90.3)	77.7 (68.5, 86.8)	79.4 (70.3, 88.6)	0.005
Left-cerebral rSO ₂ , n = 40	77.6 (66.6, 88.6)	74.9 (64.0, 85.9)	75.8 (64.8, 86.7)	0.004
Systemic rSO ₂ , n = 40	70.2 (63.1, 77.4)	69.6 (62.5, 76.7)	67.6 (60.4, 74.7)	0.11
SaO ₂ , n = 40	96.7 (94.7, 98.6)	96.1 (94.2, 98.1)	95.6 (93.6, 97.6)	0.37
MAP, n = 22	51.7 (47.5, 56.0)	50.2 (45.9, 54.5)	51.7 (47.5, 55.9)	0.31
Left-cerebral FTOE, n = 40	19.9 (8.6, 31.3)	22.6 (11.2, 34.0)	20.0 (8.6, 31.4)	0.002
Right-cerebral FTOE, n = 37	23.7 (6.9, 40.5)	26.9 (10.1, 43.7)	23.9 (7.1, 40.7)	0.002
Systemic FTOE, n = 40	27.2 (20.1, 34.3)	27.9 (20.8, 35.0)	28.6 (21.6, 35.7)	0.51

CI, confidence interval; FTOE, fractional tissue oxygen extraction; rSO₂, regional oxygen saturation; SaO₂, systemic O₂ saturation.

^aEstimated marginal means from linear mixed models with random intercepts for patient with time length set to the average value of 178.7 s. P-value is from an overall Wald χ^2 test for inclusion of time as a predictor in the model. Sample size represents the number of seizure events included in each model.

values. This infant's seizures were related to HIE, and the cerebral rSO₂ declined more dramatically during seizures than the other subjects'. Removing this infant's data changed the statistical significance of the findings (**Supplementary Table S1** online). The mean left-cerebral FTOE remained higher during seizures, compared with baseline (Bonferroni-adjusted $P = 0.033$). The previously significant mean cerebral rSO₂ rose (to $P = 0.07$ – 0.09), just above the usual significance threshold.

DISCUSSION

This novel use of NIRS monitoring with concurrent conventional EEG to confirm neonatal seizures demonstrates that treatment with loading doses (≥10 mg/kg) of phenobarbital is associated with reduced cerebral oxygen metabolism (increased rSO₂ and decreased FTOE) that persists for at least an hour after the dose is administered. EEG-confirmed neonatal seizures may, conversely, be associated with increased cerebral oxygen metabolism. The changes in oxygen metabolism

were statistically robust, but the absolute differences in rSO₂ were small and the rSO₂ values always remained within the normal range. Thus, NIRS reveals compelling pathophysiological information, but assessment of potential clinical impact will require further study.

To our knowledge, we are the first to report changes in NIRS-measured cerebral oxygen metabolism associated with phenobarbital dosing for neonatal seizures. We found that the brain oxygen metabolism decreased after phenobarbital loading doses. This change was independent of seizure burden, since the seizures analyzed during this study were at separate times from the phenobarbital doses. Notably, the cerebral NIRS changes associated with phenobarbital and with seizures were also independent of systemic NIRS or MAP.

The published reports of NIRS changes associated with neonatal seizures have been confined to case studies. For a 39-wk male infant with HIE treated with phenobarbital and phenytoin, prolonged (>7 min) subclinical seizures were associated

with decreased cerebral tissue oxygenation index (analogous to rSO_2) (14). This was similar to our finding of lower cerebral rSO_2 values during seizures.

Two other case reports of infants monitored by NIRS and EEG demonstrated rSO_2 fluctuations during seizures (18,19). During a 30-min epoch, the rSO_2 of a 2-mo-old preterm infant with cortical dysplasia abruptly declined 16 times, 11 of which were coincident with EEG-confirmed seizures (18). The authors noted that the NIRS patterns varied across individual seizures, a finding that is mirrored in our study, in which there was one individual whose seizure data were outliers. In another case study of a 2.5-mo-old infant with congenital heart disease, a repetitive spike pattern in NIRS-measured cerebral oxygenation was associated with subclinical status epilepticus (19). In all three case studies, and in our results, systemic oxygen saturation remained constant during the seizures.

None of the case studies reported FTOE changes associated with seizures or their treatment, but we showed that FTOE increased during seizures, even after an outlier was excluded. These findings echo previous work that described increased cerebral blood flow during electrographic and electroclinical neonatal seizures (15), and suggest that brain oxygen metabolism increases during neonatal seizures. Our data suggest that seizures place metabolic demands on the newborn brain. However, based solely on our results, we cannot be sure that these metabolic demands cause harm. The rSO_2 was always within the expected physiological range and so the changes associated with seizures would not be expected to induce injury.

Few studies have differentiated between left- and right-cerebral NIRS measurements. In one study of very-preterm neonates, interhemispheric rSO_2 and FTOE differences were noted during periods of unstable systemic oxygenation (20). In one of the above case studies, the right- and left-cerebral NIRS measures were recorded for a 30-min epoch. After the administration of pancuronium and subsequent doses of fosphenytoin, a decoupling of right- and left-cerebral NIRS data occurred (18). No physiological explanation was offered, only that the values did not appear to be artifact. Our study results also reflected differences between right- and left-cerebral NIRS measures, seen, e.g., in the mean rSO_2 changes associated with phenobarbital across time (right: $P = 0.57$ vs. left: $P = 0.04$). The placement of NIRS sensors in clinical practice, on the forehead vs. over the parietal regions, may influence the test results and should be taken into consideration when data are interpreted.

Our study has some limitations. Although 20 infants were included, not all infants experienced seizures during the simultaneous NIRS-EEG monitoring. We attempted to avert erroneous conclusions related to outliers by limiting the data to a maximum of five seizures or phenobarbital doses per individual subject, and adjusting for subject in the models. There was one outlier subject in our dataset, who had substantial rSO_2 changes during seizures. There were no obvious clues in the clinical or EEG data to suggest why that subject's NIRS patterns differed from the others'. The subject was one of seven with HIE, and the seizure duration was not different from the

others'. This subject's results dampened our conclusions about cerebral oxygen metabolism during seizures. However, the results also suggest that there may be some neonates for whom seizures can produce a clinically meaningful change in cerebral oxygen metabolism. We found no such outliers in the phenobarbital dosing analyses.

Although our findings regarding cerebral oxygen metabolism changes associated with phenobarbital loading doses are compelling, we only analyzed data from 1 h after medication administration. Thus, we are unable to comment on how long the documented changes in cerebral rSO_2 were sustained. Also, we cannot comment on the effect of phenobarbital blood levels on NIRS results, since most infants did not have clinically indicated drug levels at the time of monitoring. However, higher doses of phenobarbital were associated with more significant changes in cerebral oxygen metabolism.

Our study subjects had seizures due to a range of etiologies. Due to the small subgroup sample sizes, we did not attempt to determine whether the NIRS changes associated with seizures or phenobarbital differed according to the underlying seizure etiology or gestational age. Additionally, we excluded moderate-preterm and very-preterm infants because their small head size precludes simultaneous monitoring of conventional EEG along with bilateral cerebral NIRS. Therefore, these data may not be directly applicable to one of the populations most commonly monitored with NIRS (16).

Most neonates in this study did not have invasive blood pressure monitoring. Artifact and missing data from noninvasive blood pressure measurements limited potential analysis. We are unable to comment on the possibility that abnormal cerebrovascular autoregulation could have contributed to the NIRS changes observed after phenobarbital administration. However, clinically significant hypotension was not noted for any of these infants.

There were significant strengths to this research. We provide real-world bedside physiological monitoring data, recorded with readily available clinical NIRS and EEG equipment, on late-preterm and term neonates with a variety of seizure etiologies. Our dataset, while limited, is notably larger than that of previous studies and provides insight for both the physiology of neonatal seizures and their treatment with phenobarbital. All analyzed seizures were identified using the gold standard multichannel conventional EEG. Since neonatal seizures are defined according to their EEG patterns, we did not classify seizures according to the presence or absence of associated clinical signs.

We utilized United States Food and Drug Administration (FDA)-approved neonatal NIRS sensors, which are the sensors most likely to be used in clinical practice in the United States. Others have reported NIRS data gathered with different sensor types. This methodological detail must be accounted for in data interpretation, since neonatal sensors' rSO_2 results are typically higher than the pediatric sensors' results (21). We also studied rSO_2 recorded over the parietal regions, rather than the typical frontal recording. This provided independent bihemispheric cerebral NIRS data, which were recorded from

the cerebrovascular watershed regions and co-localized with the most common sites of origin for neonatal seizures (22). We also measured systemic rSO_2 , pulse oximetry, and, whenever possible, mean arterial blood pressure, in order to exclude changes in systemic physiology as causes for the cerebral oxygen metabolism variation associated with phenobarbital dosing and seizures. Notably, these systemic variables did not fluctuate with seizures or phenobarbital doses.

Conclusions

Typical phenobarbital loading doses are accompanied by prolonged declines in cerebral oxygen extraction, and neonatal seizures may be associated with transient increases in oxygen metabolism. Whether these small but statistically significant changes, measured by NIRS, have clinical consequences remains to be determined. Therefore, although NIRS may provide scientifically interesting data, our results alone do not provide rationale for routine clinical NIRS monitoring among neonates with seizures.

METHODS

The University of Michigan Institutional Review Board approved this study and the parent of each infant provided written informed consent. Each infant's chart was systematically reviewed for demographic and clinical details. Infants who required video-EEG monitoring due to clinical suspicion for seizures were included. Premature infants (≤ 35 -wk gestation) and those with multiple congenital anomalies were excluded. Infants were not excluded based on any other seizure etiology.

Near-Infrared Spectroscopy (NIRS)

The late-preterm and full-term neonates monitored with conventional video-EEG due to suspected seizures were simultaneously monitored with NIRS. The neonatal NIRS sensors (Invos 5100c, Somanetics Corp, Troy, MI) were placed over the bilateral parietal regions (the cerebrovascular watershed areas) and the thigh (systemic control). The NIRS-measured cerebral rSO_2 and SaO_2 (measured via pulse oximetry) were recorded every 5 s and downloaded to a research computer for post hoc analyses. The FTOE was calculated ($FTOE = (SaO_2 - rSO_2)/SaO_2$).

Phenobarbital

The infants' medical records were systematically reviewed for phenobarbital dosing (mg/kg/dose) and administration data. A loading dose (≥ 10 mg/kg) of phenobarbital is typically administered over 20 min. The mean rSO_2 was extracted for 1-h epochs before (baseline), during, and after each phenobarbital dose. To avoid erroneous conclusions based on overrepresentation of individual subjects, a maximum of five phenobarbital doses were analyzed per study subject, and statistical models took subject into account. The time periods for phenobarbital dose data did not overlap with the seizure data (see below). Linear mixed models with interactions were developed to assess the impact of phenobarbital administration on rSO_2 and FTOE, adjusted for dose (mg/kg).

Seizures

The electrographic seizures were defined as abnormal EEG events greater than 10 s in duration, with a clear evolution of frequency, morphology, and/or location, and a minimum of 2- μ V peak-to-peak amplitude (23). For each electrographic seizure, the mean and SD of rSO_2 values were extracted for a period of three times the duration of the seizure before and after the ictal pattern, as well as during the seizure. This duration was selected in order to account for potential effects of variation in seizure duration (e.g., a prolonged seizure might result in a more prolonged postictal change in cerebral metabolism than a brief seizure). A maximum of five seizures were analyzed for

each individual subject. The seizure times did not overlap with the above phenobarbital administration times. Linear mixed models and post hoc pairwise comparisons were employed to evaluate the seizure data, adjusted for seizure duration. Bonferroni-adjusted *P*-values were presented.

Statistical Models

Most neonates had more than one dose of phenobarbital or more than one seizure. Furthermore, each individual administration of medication or seizure produced three measurements, one for each time period (before, during, and after the seizure or phenobarbital dose). To account for this, the linear mixed models with random intercepts for patient and phenobarbital dose or seizure event were employed. The models were run separately for phenobarbital and seizure data, including weight-adjusted dosage (mg/kg) in phenobarbital models and length of seizure in seizure models as covariates. The significance of the effect of time period on the NIRS data was determined by a Wald χ^2 test on the inclusion of time period coefficients in the models. Due to the small amount of missingness combined with the relatively small sample size, listwise deletion was used in the presence of missing data. All analyses were performed using Stata version 13.1 (StataCorp, College Station, TX).

To investigate whether weight-adjusted phenobarbital dose affected NIRS data, a second set of models was developed to include an interaction between time period and weight-adjusted dosage (mg/kg). The dose was categorized into bolus (≥ 10 mg/kg) or maintenance (< 10 mg/kg) for a clinically meaningful comparison of time effect differences across dose values. The significance of interaction terms was determined through an overall Wald χ^2 test. The effects of phenobarbital across the three time points were investigated through plots of estimated marginal means and differences between time periods for the categories of weight-adjusted doses (bolus vs. maintenance).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

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