Reference values of regional cerebral oxygen saturation during the first 3 days of life in preterm neonates

Thomas Alderliesten[1], Laura Dix[1], Wim Baerts[1], Alexander Caicedo[2,3], Sabine van Huffel[2,3], Gunnar Naulaers[4], Floris Groenendaal[1], Frank van Bel[1] and Petra Lemmers[1]

BACKGROUND: Currently, reliable reference values of regional cerebral oxygen saturation ($rScO_2$) for different gestational age (GA) groups are lacking, which hampers the implementation of near-infrared spectroscopy (NIRS) alongside monitoring arterial oxygen saturation (SaO_2) and blood pressure in neonatal intensive care. The aim of this study was to provide reference values for $rScO_2$ and cerebral fractional tissue oxygen extraction (cFTOE; (SaO_2 – $rScO_2$)/SaO_2) for small adult and neonatal NIRS sensors.

METHODS: In this study, 999 infants born preterm (GA <32 wk) were monitored with NIRS during the first 72 h of life. Mixed modeling was used to generate reference curves grouped per 2 wk of GA. In addition, the influence of a hemodynamically significant patent ductus arteriosus, gender, and birth weight were explored.

RESULTS: Average $rScO_2$ was ~65% at admission, increased with GA (1% per week) and followed a parabolic curve in relation to postnatal age with a peak at ~36 h. The cFTOE showed similar but inverse effects. On average, the neonatal sensor measured 10% higher than the adult sensor.

CONCLUSION: $rScO_2$ and cFTOE reference curves are provided for the first 72 h of life in preterm infants, which might support the broader implementation of NIRS in neonatal intensive care.

Despite advances in neonatal intensive care that have led to a decline in morbidity, preterm birth is still associated with neurological sequelae (1). Brain injury in preterm infants is often caused by disturbances in cerebral blood flow (CBF) and oxygenation (2–4). Evidence is accumulating that monitoring blood pressure alone is not enough to ensure adequate (cerebral) perfusion and oxygenation (5,6).

Near-infrared spectroscopy (NIRS) is a technique that can be used to monitor regional cerebral oxygen saturation ($rScO_2$), being both a measure of cerebral oxygenation as well as a surrogate of CBF. NIRS monitoring can be applied for prolonged periods of time, even in the most vulnerable infants (7). It uses multiple wavelengths of NIR light and relies on the distinct absorption spectra of oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin to calculate relative concentrations of O_2Hb and HHb, which are then used to calculate the $rScO_2$ (O_2Hb/(O_2Hb + HHb)). Where pulse-oximetry only measures the oxygen saturation in arterial blood (SaO_2), NIRS makes no distinction between different (cerebral) blood volume compartments; therefore, the $rScO_2$ represents the oxygen saturation in a mixed arterial–capillary–venous compartment in an approximate 20:5:75 distribution (8).

NIRS is increasingly being used as a trend monitor of cerebral oxygen supply in neonates admitted to the neonatal intensive care unit (NICU). Readily interpretable reference values could provide another way of using NIRS in neonates by identifying neonates at risk. In other words, to identify neonates whose $rScO_2$ resides at the outskirts (high or low) of what is considered "normal." Furthermore, reliable reference values could benefit NIRS research by suggesting thresholds that should be explored in relation to interventions and (neurodevelopmental) outcome. However, current literature is quite heterogeneous, with different age groups, small sample sizes, different onsets and durations of measurement, and the use of different devices and sensors (9–15). Therefore, the aim of this study was to construct gestational age (GA)–specific reference curves during the first 72 h of life for $rScO_2$ and its derived cerebral fractional tissue oxygen extraction (cFTOE (SaO_2 – $rScO_2$)/SaO_2) in a large group of neonates who were measured with a small adult NIRS sensor (SomaSensor SAFB-SM using INVOS 4100 or 5100c monitors) (16). The second aim was to provide a conversion model to convert $rScO_2$ values obtained by a neonatal sensor (OxyAlert CNN cerebral NIRsensor; Covidien) to the adult sensor equivalent.

RESULTS

Out of the 1,059 participating infants, 41 were excluded because of technical problems during data collection (e.g., data corruption, missing cable connections, or electrical interference). An additional 19 infants were excluded for having cardiac malformations (n = 8), chromosomal or severe genetic abnormalities (n = 6), or severe congenital malformations (n = 5). **Table 1** summarizes the clinical characteristics of the study population.

[1]Department of Neonatology, Wilhelmina Children's Hospital/University Medical Center Utrecht, Utrecht, The Netherlands; [2]Department of Electrical Engineering (ESAT), Division SCD, KU Leuven, Leuven, Belgium; [3]iMinds-KU Leuven Future Health Department, Leuven, Belgium; [4]Department of Neonatology, University Hospital The Gasthuisberg, Leuven, Belgium. Correspondence: Thomas Alderliesten (t.alderliesten-2@umcutrecht.nl)

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Table 1. Baseline characteristics of the study population

	Total	24–25 wk	26–27 wk	28–29 wk	30–31 wk
Male/female, *n*	539/460	57/50	128/111	171/143	183/156
Gestational age (weeks), mean (SD)	28.7 (1.96)	25.1 (0.57)	27.0 (0.59)	28.9 (0.57)	30.8 (0.55)
Birth weight (grams), mean (SD)	1,150 (330)	770 (110)	930 (180)	1,193 (244)	1,385 (318)
Birth weight *z*-score, mean (SD)	0.02 (0.90)	0.40 (0.84)	0.04 (0.83)	0.12 (0.88)	−0.21 (0.91)
Birth weight <−1 SD, *n* (%)	142 (14.2)	5 (4.7)	29 (12.1)	40 (12.7)	68 (20.1)
Apgar score 1 min, median (IQR)	7 (5–8)	5 (3–6)	6 (4–7)	7 (5–8)	7 (6–8)
Apgar score 5 min, median (IQR)	8 (7–9)	7 (6–8)	8 (7–9)	8 (8–9)	9 (8–9)
Head circumference (cm), mean (SD)	26.1 (2.3)	22.8 (1.4)	24.7 (1.6)	26.4 (1.6)	27.8 (1.9)
aCCS full course, *n* (%)	725 (72.6)	78 (72.9)	172 (72)	236 (75.2)	239 (70.5)
*hs*PDA, *n* (%)	294 (29.4)	78 (72.9)	127 (46.9)	75 (23.9)	29 (8.6)
PA at *hs*PDA diagnosis (hours), median (IQR)	55 (29–91)	58 (38–113)	59 (28–81)	52 (25–86)	62 (28–82)
PIVH, *n* (%)					
None	713 (71.4)	54 (50.5)	156 (65.3)	231 (73.6)	272 (80.2)
Grade 1	88 (8.8)	6 (5.6)	18 (7.5)	34 (10.8)	30 (8.8)
Grade 2	110 (11.0)	28 (26.2)	25 (10.5)	35 (11.1)	22 (6.5)
Grade 3	59 (5.9)	16 (15.0)	32 (13.4)	10 (3.2)	1 (0.3)
Grade 4	29 (2.9)	3 (2.8)	8 (3.3)	4 (1.3)	14 (4.1)
Hospital mortality, *n* (%)	65 (6.5)	23 (21.5)	25 (10.5)	13 (4.1)	4 (1.2)
CRIB II score, median (IQR)	9 (7–11)	14 (13–16)	12 (10–13)	8 (7–9)	6 (5–7)

	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Spontaneous breathing, *n* (%)	535 (53.6)	547 (54.8)	573 (57.5)	20 (18.7)	22 (20.6)	26 (24.3)	69 (28.9)	74 (31.0)	84 (35.4)	188 (59.9)	189 (60.2)	194 (61.8)	258 (76.1)	262 (77.3)	269 (79.4)

aCCS, antenatal corticosteroids; CRIB, clinical risk index for babies; *hs*PDA, hemodynamically significant patent ductus arteriosus; IQR, interquartile range; PA, postnatal age; PIVH, periventricular/intraventricular hemorrhage.

A total of 59,135 1-h periods (median: 873; interquartile range: 817–905 per 1-h period) were available for analysis. Although 1-h periods were used for modeling, **Figure 1** displays the raw $rScO_2$ and cFTOE data in 6-h averages for the first 24 h and 12-h averages for the 48 h thereafter to yield an easily interpretable figure.

Figures 2 and **3** display the main results: the $rScO_2$ and cFTOE reference curves for four different GA groups. Depending on GA and postnatal age (PA), the mean $rScO_2$ during the first 72 h of life ranges from 62 to 71%, with a positive association between $rScO_2$ and GA. A single SD in $rScO_2$ is ~7%, which provides a ±2 SD bandwidth of ~30%. Likewise, mean cFTOE ranges from 0.25 to 0.34 during the first 72 h of life, and a single SD is 0.08, which provides a ±2 SD bandwidth of 0.32.

Table 2 lists the coefficients of the $rScO_2$ and cFTOE models. For example, mean $rScO_2$ increases 0.9% per week of GA. In the models, PA is also represented by the square of PA (PA-sq) to model the parabolic relationship that $rScO_2$ and cFTOE have with PA. The PA, PA-sq, and interactions with PA and PA-sq determine the location of the vertex (i.e., maximum for $rScO_2$ and minimum for cFTOE) and the curvature of the parabola. **Figure 4** is a graphical representation of the main effects and interactions of being born with a birth weight (BW) <−1 SD (i.e., small for gestational age, SGA) and having a hemodynamically significant patent ductus arteriosus (*hs*PDA) as reported in **Table 2**. Infants who developed a *hs*PDA <84 h of life had a lower $rScO_2$ and demonstrated a sharper decline after ~24 h. Infants born SGA started off with a higher $rScO_2$ and had slightly lower values at 72 h compared to those at 1 h PA, whereas infants born appropriate for gestational age (AGA) had higher values at 72 h than at 1 h PA. This difference between infants born SGA and those born AGA diminishes over time (i.e., SGA and AGA lines in **Figure 4** converge) but is still present at 72 h PA. No significant differences were found between data obtained before or after June 2012 (i.e., Poly 5 software + INVOS 4100 vs. BedBase software + INVOS 5100c).

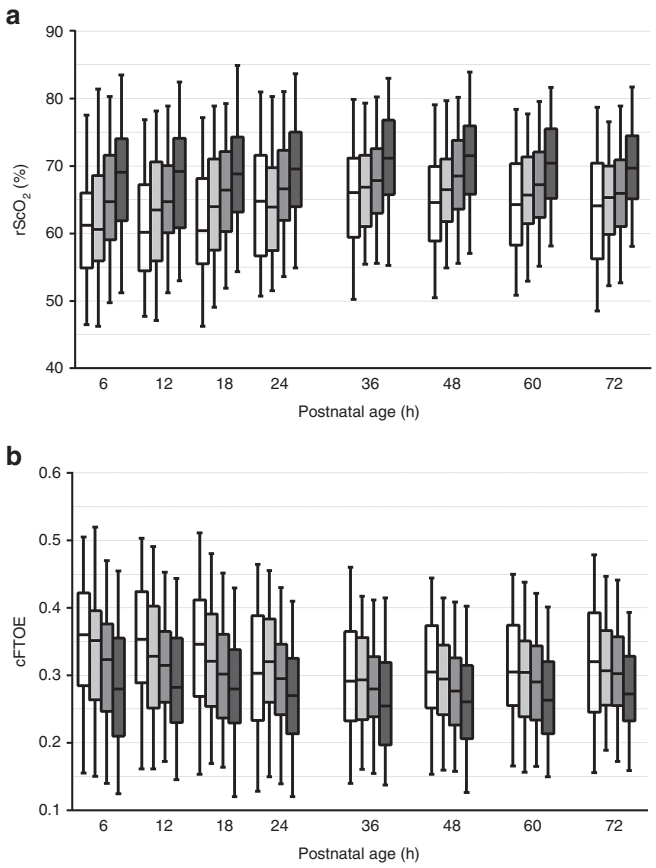

Figure 1. Boxplots of the raw data are displayed for four gestational age groups: white boxes indicate 24–25 wk, light gray boxes indicate 26–27 wk, dark gray boxes indicate 28–29 wk, and black boxes indicate 30–31 wk for (**a**) regional cerebral oxygen saturation ($rScO_2$) and (**b**) cerebral fractional tissue oxygen extraction (cFTOE). Data are displayed in 6-h periods for 0–24 h after birth and in 12-h periods for 24–72 h after birth.

Conversion Diagrams

A strict linear model provided the best fit to convert data obtained by the SAFB-SM (adult) sensor to the CNN (neonatal) sensor: $rScO_{2\text{-neo}} = 0.8481 * rScO_{2\text{-adult}} + 19.11$, $R^2 = 0.65$. **Figure 5** is a conversion of **Figure 2** by using this equation.

DISCUSSION

This is the first study to report reference values of $rScO_2$ and cFTOE obtained using NIRS during the first 72 h of life in a large cohort of preterm neonates born at a GA <32 wk.

Four factors should be taken into account when comparing the work reported here to work of others: (i) GA, (ii) PA, (iii) sample size, and (iv) the sensor and device that were used (see discussion below). Values found in the literature agree quite well with the values reported here (**Table 3**, mean difference: −0.9%). The differences are likely explained by the characteristics of the reported populations (e.g., GA, PA, specific morbidity), duration of measurements, and small sample sizes (9–11,17–20). It seems likely that the $rScO_2$ will either stabilize or may even increase again after 72 h (12,13,15,21). Note that van Hoften *et al.* collected data with a pediatric sensor and Pocivalnik *et al.* and Pichler *et al.* with a neonatal sensor (15,20,21).

It is noteworthy how close the −2 SD bands (i.e., p2.3) are to the $rScO_2$ threshold (i.e., 33–44%) reported to be associated with functional impairment of the brain (22,23). A lower CBF, either regional or global, in infants with a lower GA is the most plausible explanation for the positive association between GA and $rScO_2$. Roche-Labarbe *et al.*, while using a frequency domain NIRS system, also demonstrated lower levels of cerebral oxygenation during the first 7 wk of life in infants with a GA <31 wk compared to infants with a GA >31 wk (24). Furthermore, their data show that infants with a GA of 24–27 wk have the lowest blood flow index, supporting lower CBF as an explanation for lower cerebral oxygenation in younger infants. No associations were found between head circumference and $rScO_2$, and SaO_2 and GA. This makes the influence of head circumference (i.e., different curvature of the head influencing NIRS from a technical point of view) or SaO_2 unlikely. Furthermore, a similar (inverse) association was found between GA and cFTOE. An increased metabolic demand in neonates of lower GA seems unlikely as cerebral activity increases with GA (25).

Female neonates had lower $rScO_2$ as compared to male neonates. This gender difference was also observed by the Pichler *et al.* during transition from fetal to neonatal life (personal communication, data not published). Again, this could not be explained by a difference in SaO_2 or head circumference. Therefore, possible explanations are a higher (regional) CBF or lower metabolic demand. A *hs*PDA can cause a ductal steal phenomenon with a surplus of pulmonary flow at the cost of systemic perfusion and thus CBF (26). Although notably increasing with PA, the effect of a *hs*PDA seems rather limited during the first 3 d of life. The most plausible explanation for this is the fact that most *hs*PDAs become clinically apparent from day 3 onward. In addition, an objectively present *hs*PDA (i.e., confirmed by cardiac ultrasound) does not necessarily decrease CBF, and thus $rScO_2$, as the magnitude of systemic steal depends on shunt volume and left ventricular output. Moreover, in this study, the PA at diagnosis was dichotomized (i.e., ≤84 h); therefore, the exact PA of the individual at diagnosis and start of treatment was not taken into account. In previous publications, we took a different approach with case–control designs and the start of indomethacin or surgery as time reference, at median postnatal days 2 and 7, respectively (26,27).

Higher $rScO_2$ values in infants born SGA demonstrate the brain-sparing effect with a compensatory higher CBF. This has been demonstrated previously with other techniques (28). The difference in $rScO_2$ between SGA and AGA infants diminishes over time, suggesting that the CBF returns to normal after day 3. Interestingly, unlike in AGA infants, $rScO_2$ values in SGA infants were slightly lower at 72-h PA compared to $rScO_2$ values shortly after birth. This suggests downregulation of compensatory mechanisms instead of a relative lack of hemodynamic development in SGA infants as an explanation for values converging toward AGA values. The limited number

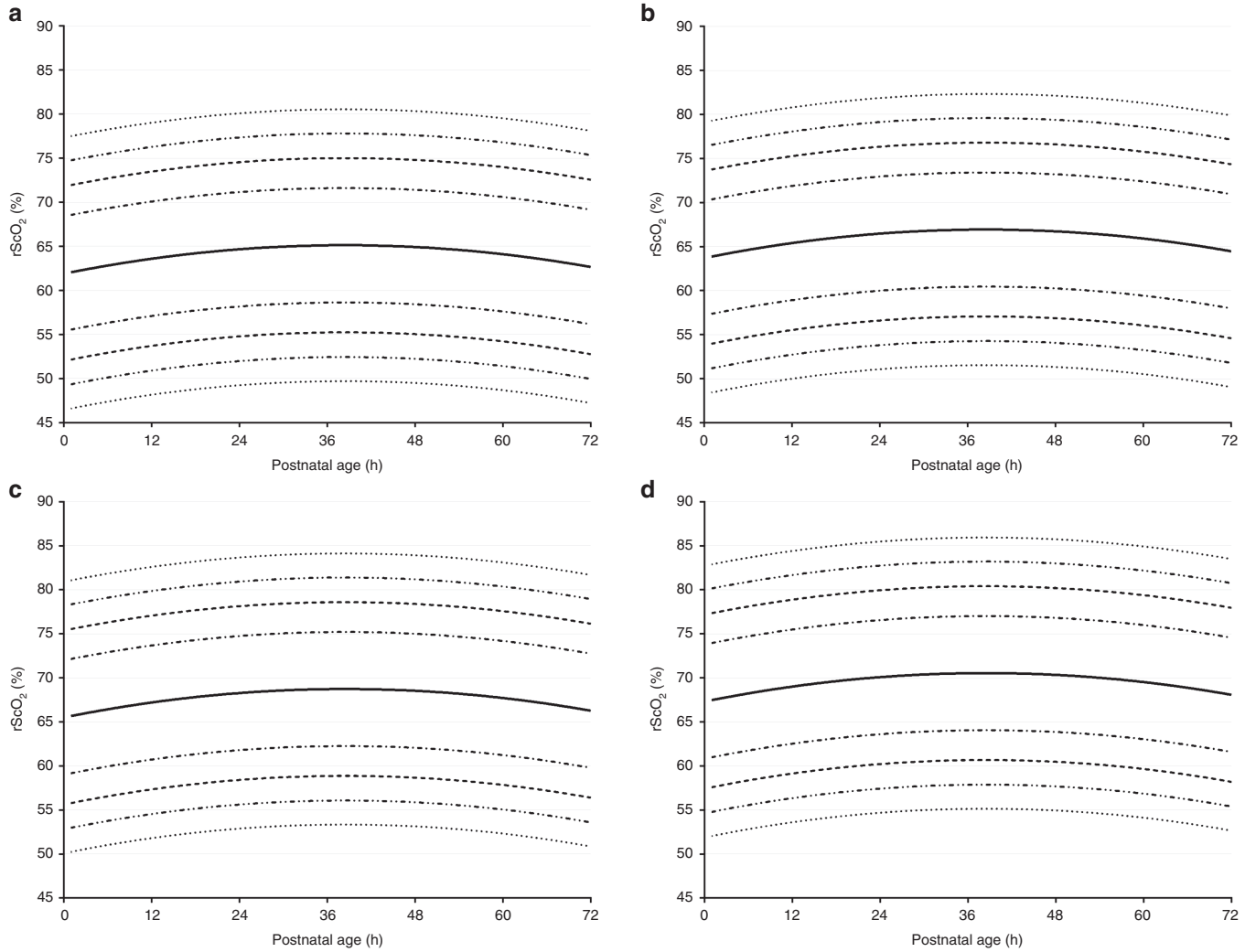

Figure 2. $rScO_2$ reference value curves for neonates of (**a**) 24–25 wk GA, (**b**) 26–27 wk GA, (**c**) 28–29 wk GA, and (**d**) 30–31 wk GA. The line patterns depict different percentiles: dotted lines indicate p2.3 and p97.7, dash dot dot dash lines indicate p5 and p95, dashed lines indicate p10 and p90, dash dot dash lines indicate p20 and p80, and solid lines indicate p50. GA, gestational age.

of severe SGA cases (i.e., BW *z*-score <−2, $n = 18$) prevented statistical significance of the effect that being severely SGA has on $rScO_2$ and cFTOE.

We preferred to present "overall" reference curves over presenting numerous curves according to different morbidities. Therefore, the reference curves are valid for a population of preterm infants admitted to the NICU, with inherent morbidity. The generalized additive models for location, scale, and shape (GAMLSS) results were similar to the mixed-model approach. We chose to report the mixed-model procedure because results are easier to interpret and there is more extensive expertise in mixed-model procedures in our institution.

As mentioned before, substantial differences exist between different NIRS sensors (14,15). The correlation between values obtained with the adult and neonatal sensor is not perfect ($R^2 = 0.65$), which is probably caused by the *in vivo* nature of our experiment. However, the relation is clearly linear, which has also been demonstrated *in vitro* (29). Although the difference between the neonatal and adult sensor can be as high as 15% (mean: 10%), trend monitoring is still possible in a way similar to adult sensors. Moreover, the ±2 SD limits provide a "bandwidth" of ~30% which makes the maximum 15% difference less stressing. However, awareness of a possible offset between sensors is crucial when comparing data between patients, institutions, or devices. For example, a $rScO_2$ of 55% seems low but acceptable when using an adult sensor (**Figure 2**) but is below the −2 SD threshold in all GA groups when using a neonatal sensor (**Figure 5**). A $rScO_2$ of 55% with the neonatal sensor converts to an adult sensor value of ~40%, which is close to or below the thresholds (33–50%) reported to be associated with neuronal damage and adverse neurodevelopmental outcome (5,22,23). Two of these thresholds were established in piglet studies by using devices that are not commercially available (22,23). Therefore, these thresholds should be used with caution, especially in preterm neonates. Also, higher values pose pitfalls, as most devices have an upper detection limit of 95%. Any value >85% obtained by an adult sensor would register as 95% (i.e., 85 + 10%) with a neonatal sensor, losing the ability

to monitor the $rScO_2$ trend. This is particularly relevant for the prognostic value of the $rScO_2$, for example, in asphyxiated neonates (30,31).

A possible limitation of this study is the generalizability of the results as participants were admitted to a single level III NICU. However, neonatal intensive care in the Netherlands takes place in 10 NICUs and admissions are purely based on geography. Equipment availability was the only factor to prohibit data collection. As unavailability was supposedly random, this influence should be minimal. Moreover, the number of recording setups substantially increased over the years, now ensuring round-the-clock availability. The second limitation is the restriction to the first 72 h of life. This choice was made to encompass the most vulnerable period of life and to limit strain on nursing staff at the same time. The final limitation is clinical practice in our unit regarding the used SaO_2 thresholds (i.e., 85–92%), which might differ from other institutions. The $rScO_2$ results were not corrected for SaO_2 to avoid overly complicating results and because model coefficients (**Table 2**) changed less than 5% when correcting to a SaO_2 of 90%. Moreover, the cFTOE curves already provide a form of SaO_2 correction. Altogether, we feel confident that the current results are generalizable to other populations of preterm neonates with a GA <32 wk during the first 72 h of life.

Currently, the core application of NIRS on the NICU lies in trend monitoring. For the inexperienced user, we suggest plotting an infants' $rScO_2$ in the appropriate GA-specific reference curve. In case of sudden changes in $rScO_2$ ≥7% (i.e., 1 SD), we recommend evaluation of clinical parameters (e.g., ventilator settings, hemoglobin levels, presence of a *hs*PDA, medication, perform a cranial ultrasound), but only after ensuring that the measurement setup has not changed (e.g., sensor displacement). Likewise, absolute $rScO_2$ values close to or outside the ±2 SD bands, should trigger an evaluation, similar to what has been done during the SafeBoosC

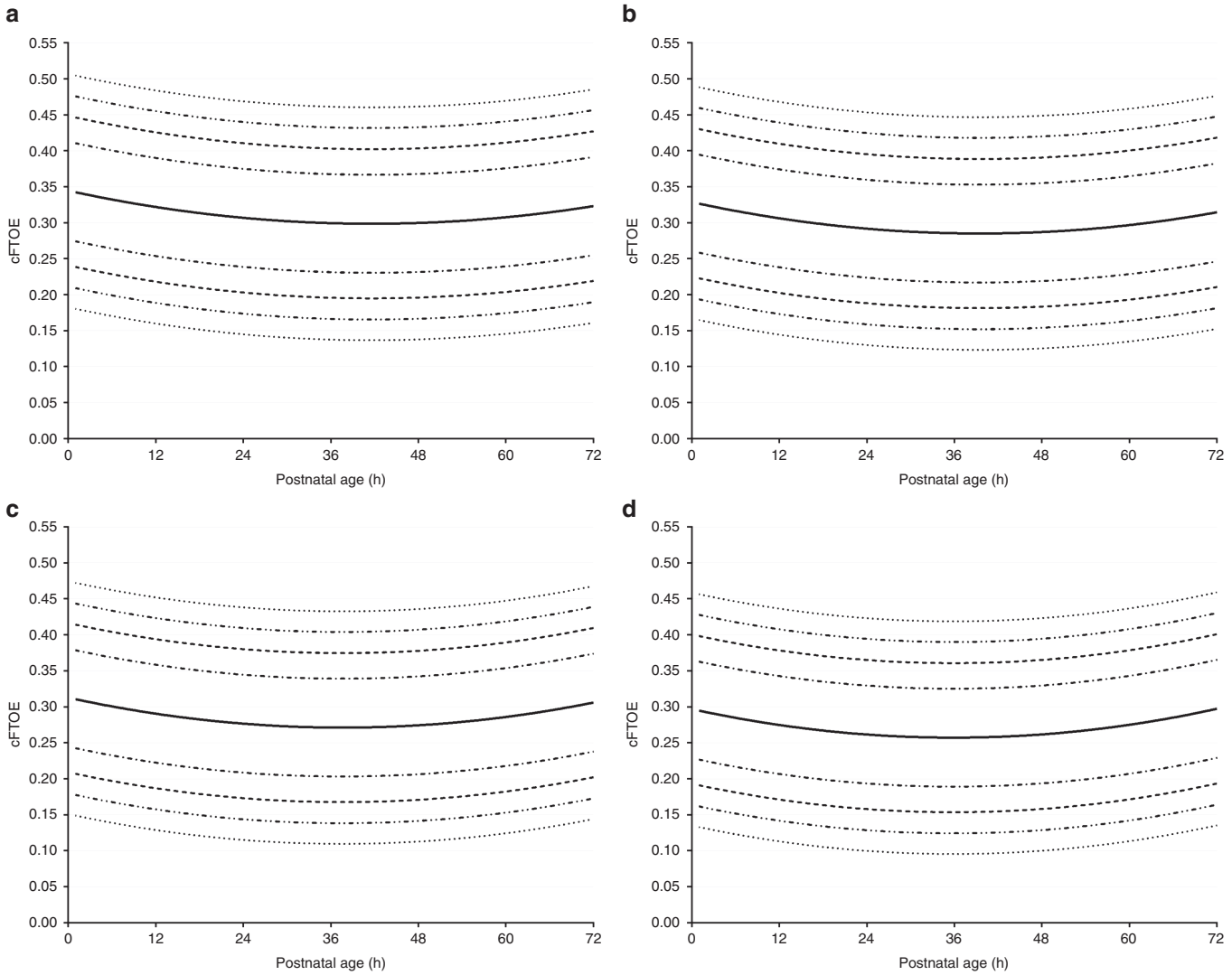

Figure 3. cFTOE reference value curves for neonates of (**a**) 24–25 wk GA, (**b**) 26–27 wk GA, (**c**) 28–29 wk GA, and (**d**) 30–31 wk GA. The line patterns depict different percentiles: dotted lines indicate p2.3 and p97.7, dash dot dot dash lines indicate p5 and p95, dashed lines indicate p10 and p90, dash dot dash lines indicate p20 and p80, and solid lines indicate p50. GA, gestational age.

Table 2. Final model coefficients for the $rScO_2$ and cFTOE

	$rScO_2$		cFTOE	
	Coefficient	95% CI	Coefficient[c]	95% CI
Intercept	59.415	58.138; 60.693***	36.033	34.581; 37.486***
Main effects				
PA (h)	0.240	0.207; 0.273***	−0.221	−0.255; −0.188***
PA-sq[a]	−0.003	−0.003; −0.002***	0.0023	0.002; 0.003***
*hs*PDA ≤ 84 h	−0.581	−1.652; 0.490	−0.113	−1.263; 1.036
GA[b]	0.904	0.699; 1.108***	−0.795	−1.039; 0.552***
Female gender	−1.565	−2.308; −0.822***	1.729	0.946; 2.512***
BW ≤ −1 SD	6.172	4.371; 7.973***	−3.960	−5.243; −2.677***
Interactions				
PA: BW ≤ −1 SD	−0.1431	−0.2325; −0.0536**	n/a	—
PA-sq: *hs*PDA ≤ 84 h	−0.00029	−0.00052; −0.00005*	0.00034	0.00007; 0.00061*
PA-sq: BW ≤ −1 SD	0.0014	0.0002; 0.0025*	0.00040	0.00008; 0.00071*
PA-sq: GA	n/a	—	0.00007	0.00001; 0.00013*

BW, birth weight; CI, confidence interval; GA, gestational age; *hs*PDA, hemodynamically significant patent ductus arteriosus; n/a, not applicable; PA, postnatal age.
[a]PA-sq, postnatal age squared to enable a squared model. [b]GA-24, to make 24 wk of gestation the reference point to yield a interpretable intercept. [c] $(SaO_2 - rScO_2)/SaO_2 \times 100$, to obtain coefficients with the same effect size as for the $rScO_2$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

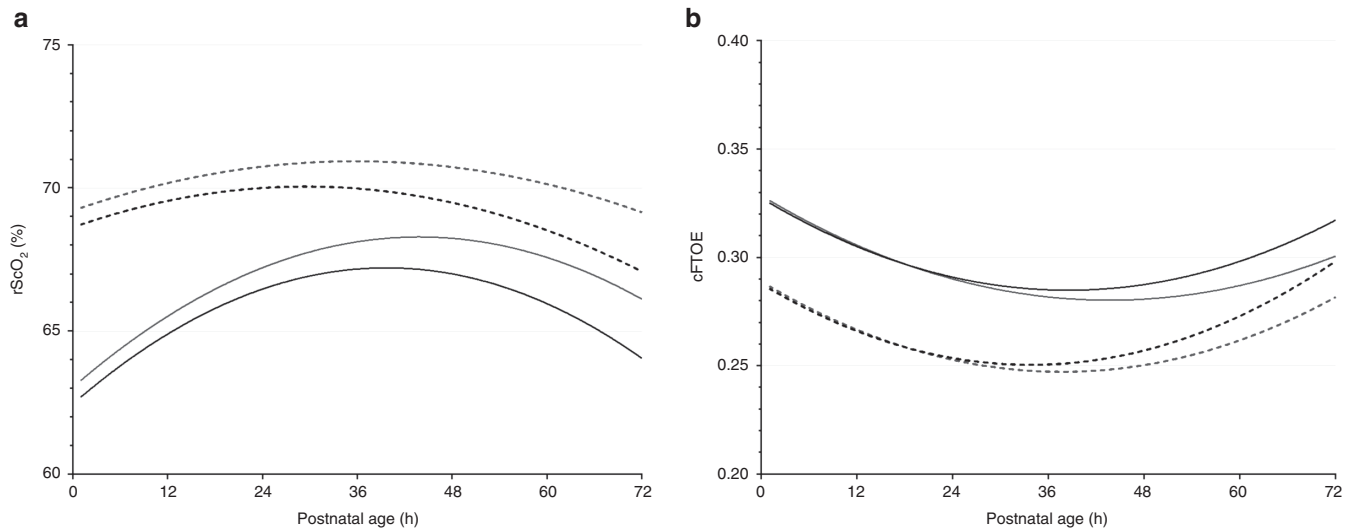

Figure 4. Graphic representation of the interactions of *hs*PDA and SGA with the representatives of postnatal age in the model for (**a**) $rScO_2$ and (**b**) cFTOE. Black solid lines indicate AGA with *hs*PDA, gray solid lines indicate AGA without *hs*PDA, black dashed lines indicate SGA with *hs*PDA, and gray dashed lines indicate SGA without *hs*PDA. AGA, appropriate for gestational age; cFTOE, cerebral fractional tissue oxygen extraction; *hs*PDA, hemodynamically significant patent ductus arteriosus; $rScO_2$, regional cerebral oxygen saturation; SGA, small for gestational age.

trial (Safeguarding the Brains of our smallest Children) (32). For a more detailed discussion on which parameters to evaluate in case of either low or high levels or $rScO_2$, we refer to the treatment guideline published by the "SafeBoosC" research group (33).

Conclusion and Future Research Directives

This study provides reference values for $rScO_2$ and cFTOE measured by NIRS during the first 72 h of life in premature infants. Both $rScO_2$ and cFTOE are influenced by GA, PA, *hs*PDA, gender, and being born SGA. Furthermore, an equation is provided to extend the results to $rScO_2$ values obtained with a neonatal NIRS sensor. These data provide an additional way for applying NIRS on the NICU, on top of trend monitoring. Furthermore, reliable reference data can be useful in future studies. Indices of cerebral oxygenation have only been suggested to be related to outcome (5,22). Future research should focus on developing robust indices of cerebral oxygenation that are related to (long-term) outcome and can be used to guide interventions. Our results suggest that GA- and PA-specific thresholds are worth exploring in this regard. The SafeBoosC research group already reported that NIRS can be used to stabilize the cerebral oxygenation in preterm infants (32).

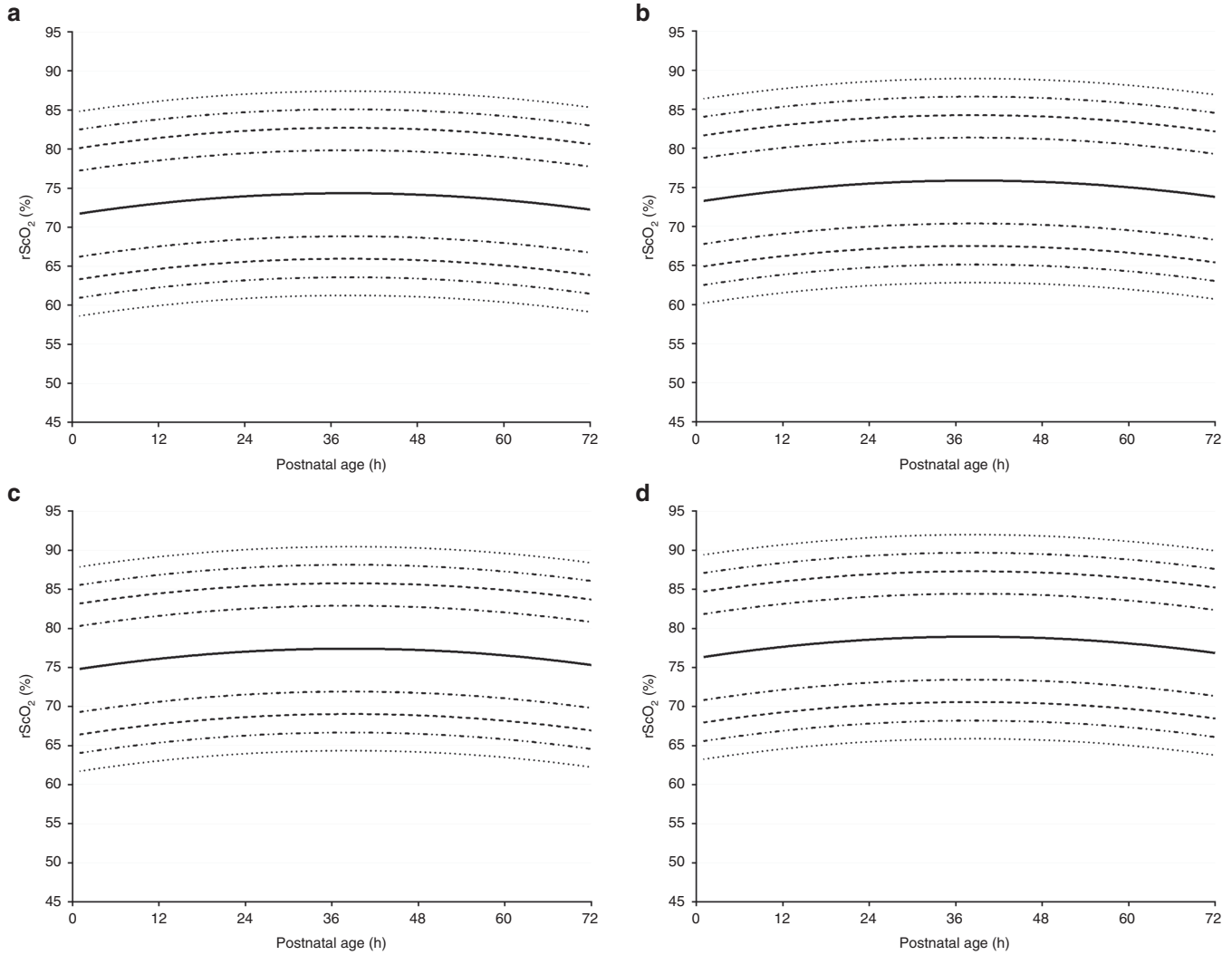

Figure 5. $rScO_2$ reference value curves obtained with the neonatal sensor for neonates of (**a**) 24–25 wk GA, (**b**) 26–27 wk GA, (**c**) 28–29 wk GA, and (**d**) 30–31 wk GA. The line patterns depict different percentiles: dotted lines indicate p2.3 and p97.7, dash dot dot dash lines indicate p5 and p95, dashed lines indicate p10 and p90, dash dot dash lines indicate p20 and p80, and solid lines indicate p50. Note: the neonatal probe (CNN) $rScO_2$ values were obtained by using a conversion from the adult (SAFB-SM) probe values: $rScO_{2\text{-neo}} = 0.8481\ rScO_{2\text{-adult}} + 19.11$. GA, gestational age; $rScO_2$, regional cerebral oxygen saturation.

METHODS

Patients

This study is part of an ongoing prospective observational cohort study which aims to record physiological parameters during at least the first 72 h of life in all infants born with a GA <32 wk who are admitted to the NICU of the Wilhelmina Children's Hospital, Utrecht, The Netherlands. The medical ethical board of the University Medical Center Utrecht approved this study. Informed parental consent was obtained in all cases. Data collection was attempted in 1,059 infants between January 2005 and September 2013.

Data Collection

Obstetrical, intrapartum, and neonatal data were collected from the hospital records. Peri- and intra-ventricular hemorrhages (PIVH) were graded according to the classification of Papile *et al.* (34). The presence of a *hs*PDA was defined as a PDA confirmed to be hemodynamically significant on cardiac ultrasound and either treated with indomethacin or surgically closed (2).

Standard physiological parameters were monitored using a patient monitor (IntelliVue MP70, Philips, Best, The Netherlands): SaO_2 using a pulse-oximetry probe, arterial blood pressure by means of an indwelling catheter (e.g., umbilical, radial, or tibial artery), and heart rate by gel electrodes. In general, the pulse-oximetry probe was placed on one of the lower limbs. In case of a *hs*PDA, the probe was placed on the right hand (i.e., pre-ductal). $rScO_2$ was monitored by using a two-wavelength (i.e., 730 and 810 nm) NIRS monitor (INVOS 4100 or 5100(c)); Covidien, Mansfield, MA) in combination with a small adult sensor (SomaSensor SAFB-SM, Covidien). An elastic bandage was used for sensor fixation. Until June 2012, data were recorded with Poly 5 (Inspector Research Systems, Amsterdam, The Netherlands) at a sample rate of 1 Hz using an INVOS 4100 monitor. Thereafter, in-house developed software (BedBase, University Medical Center Utrecht, Utrecht, The Netherlands) was used to record data from the patient monitor and an INVOS 5100c NIRS monitor at a sample rate of 0.4 Hz.

Data Processing

Data were analyzed with the offline version of the BedBase software (SignalBase, University Medical Center Utrecht, Utrecht, The Netherlands). Before analysis, artifacts were removed manually. Artifacts were defined as: changes in $rScO_2$ that could not be physiologically explained (e.g., a 30% step change between two subsequent data points) or changes that were accompanied by severe distortion in the other parameters suggesting infant movement or handling. Thereafter, 1-h periods were selected during the first 72 h of life and counted in reference to a patient's birth date and time (i.e., PA). Periods with short drops in SaO_2 (i.e., <85%) were not

included in the analysis. In case of SaO_2 drops where additional O_2 was given to assist recovery, the duration of the associated increase in SaO_2 and $rScO_2$ over baseline conditions was also excluded from analysis (35).

Statistical Analysis

Before statistical analysis, 1-h periods containing less than 10 min of data were rejected. Mean values of the 1-h periods were used for analysis. A mixed-model approach was performed using R for Windows 64-bit, version 3.1.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) with the *nlme* package. This approach can handle missing data and obsoletes correcting for multiple comparisons.

The decision was made, *a priori*, to investigate four variables: GA (in weeks), BW *z*-score, gender, and the presence of a *hs*PDA. The BW *z*-score was based on recently published Dutch reference curves and explored both as a continuous variable and dichotomized at −1 SD and −2 SD (36).

The time of diagnosis of a *hs*PDA was expressed as PA at time of the cardiac ultrasound. This PA at diagnosis was dichotomized at different cutoffs, ranging from 60 to 132 h with 12-h increments.

Linear, squared, and polynomial models of time (i.e., PA) were explored to find the best fit to the data. Either the $rScO_2$ or cFTOE was selected as the dependent variable, with the individual subject as a random factor. Both main effects and interactions with PA were explored. It was decided *a priori* that interactions with PA would be included into the final model when the interaction was statistically significant and caused at least a 10% coefficient change with respect to the coefficient for PA in the model with only main effects.

The final model was used to create $rScO_2$ and cFTOE reference curves by generating predictions based on a new set of predictors (i.e., PA, GA, *hs*PDA, gender, and BW). This new set was generated as follows: PA ranging 1–72 h with 0.2-h increments (e.g., 1.0, 1.2, 1.4 h, etc.), GA ranging 24–32 with 1-wk increments, *hs*PDA yes/no, female gender yes/no, and BW <−1 SD yes/no. The SEs of these predictions were calculated and used to create percentile plots based on a normal distribution.

To assess the robustness of the results, analysis was also performed using GAMLSS in R with the *GAMLSS* package (37).

Unless specified otherwise, data are presented as mean with SD for parametric data, median with interquartile range for nonparametric data, and counts (%) for categorical data. A P value < 0.05 was considered statistically significant.

Conversion Models for Neonatal NIRS Sensor

In a subset of infants (n = 16, GA 30 ± 3 wk), $rScO_2$ was recorded bilateral by using the neonatal (OxyAlert CNN cerebral NIRsensor) and small adult sensor (SAFB-SM, as described above), as reported previously (14). Both sensors use a single LED light source, two distant detectors (30 and 40 mm), and two wavelengths (i.e., 730 and 810 nm). After at least 1 h of stable recordings (e.g., free of care,

Table 3. $rScO_2$ values obtained from the literature compared to $rScO_2$ reference values established in this study

	Literature values					Current study		Difference
Author	Time	Measure	GA (wk)	*N*	$rScO_2$/TOI (%)	Ref. GA group	$rScO_2$ (%)	Literature − current
0–72 h of life								
Naulaers *et al.* (9)	day 1	TOI	28 (25–30)	15	57 (54–66)	28–29 (12 h)	67.2	10.2
	day 2	—	—	—	66 (62–82)	28-29 (36 h)	68.7	2.7
	day 3	—	—	—	76 (68–80)	28–29 (60 h)	67.7	−8.3
Lemmers *et al.* (17)	6–12 h	$rScO_2$	29.3 (1.7)	20	70 (61–77)	28–29 (12 h)	67.2	−2.8
	18–24 h	—	—	—	68 (63–75]	28–29 (24 h)	68.3	0.3
	36–48 h	—	—	—	73 (65–84)	28–29 (48 h)	68.5	4.5
	60–72 h	—	—	—	71 (64–75)	28–29 (72 h)	66.3	−4.7
Sorensen *et al.* (11)	19 h (6)	TOI	27.6 (23.9–33)	37	74.6 (8.5)	26–27 (18 h)	66.5	−8.1
Moran *et al.* (19)	day 1	TOI	29 (25.3–31.5)	27	68.1 (7.9)	28–29 (12 h)	67.2	−0.9
Pichler *et al.* (20)	<1 h	$rScO_2$	34.9 (1.4)	27	80 (62–92)	34–35 (1 h)[a]	79.5[ab]	−0.5
Sirc *et al.* (18)	6 h	TOI	25.9 (1.7)	22	65.2 (10)	24–25 (6 h)	62.8	−2.4
	12 h	—	—	—	63.9 (5.9)	24–25 (12 h)	63.6	−0.3
	24 h	—	—	—	68.8 (5.7)	24–25 (24 h)	64.7	−4.1
	48 h	—	—	—	67.2 (7.2)	24–25 (48 h)	64.9	−2.3
Hyttel-Sørensen *et al.* (10)	Average 3 d	$rScO_2$	26.3 (–)	10	64.2 (4.5)	26–27 (36 h)	66.9	2.7
Average difference								−0.9
>7 d of life								
van Hoften *et al.* (21)	17 d (1–93 d)	$rScO_2$	27.3 (25–34)	33	71 (65–96)	26–27 (72 h)	74.7[b]	3.7
Petrova *et al.* (12)	>7 d	$rScO_2$	(24–32)	20	66 (8.8)	28–29 (72 h)	66.3	0.3
Petrova *et al.* (13)	~5 wk	$rScO_2$	26 (2.4)	10	68.5 (4.6)	26–27 (72 h)	66.3	−2.2
Pocivalnik *et al.* (15)	3.9 d (4.8)	$rScO_2$	35.2 (3.0)	37	84.1 (6.4)	34–35 (72 h)[a]	80.0[a,b]	−4.1
	—	TOI	—	—	72.2 (6.0)	34–35 (72 h)[a]	71.3[a]	−0.9

GA, gestational age; rScO2, regional cerebral oxygen saturation; TOI, tissue oxygenation index.
[a]Model fit was obtained in subjects ≤32 wk GA; therefore, data >32 wk GA was extrapolated. [b]Study used a neonatal sensor; for comparison, the following conversion was used: $rScO_{2\text{-neo}} = 0.8481 \times rScO_{2\text{-adult}} + 19.11$.

feedings, and interventions), the sensors were switched to the contralateral side, and data collection continued for another hour. Data analysis was performed in MATLAB (vR2011b; The Mathworks, Natick, MA), and artifacts were removed manually. A linear model, polynomial models, and a piecewise linear model were examined to find the best fit (i.e., lowest residuals and highest R_2) in order to convert data obtained by the neonatal sensor to small adult sensor–equivalent values and *vice versa*.

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REFERENCES

1. van Haastert IC, Groenendaal F, Uiterwaal CS, et al. Decreasing incidence and severity of cerebral palsy in prematurely born children. J Pediatr 2011;159:86–91.e1.
2. Alderliesten T, Lemmers PM, Smarius JJ, van de Vosse RE, Baerts W, van Bel F. Cerebral oxygenation, extraction, and autoregulation in very preterm infants who develop peri-intraventricular hemorrhage. J Pediatr 2013;162:698–704.e2.
3. Van Bel F, Van de Bor M, Stijnen T, Baan J, Ruys JH. Aetiological rôle of cerebral blood-flow alterations in development and extension of peri-intraventricular haemorrhage. Dev Med Child Neurol 1987;29:601–14.
4. Børch K, Lou HC, Greisen G. Cerebral white matter blood flow and arterial blood pressure in preterm infants. Acta Paediatr 2010;99:1489–92.
5. Alderliesten T, Lemmers PM, van Haastert IC, et al. Hypotension in preterm neonates: low blood pressure alone does not affect neurodevelopmental outcome. J Pediatr 2014;164:986–91.
6. Logan JW, O'Shea TM, Allred EN, et al.; ELGAN Study Investigators. Early postnatal hypotension is not associated with indicators of white matter damage or cerebral palsy in extremely low gestational age newborns. J Perinatol 2011;31:524–34.
7. van Bel F, Lemmers P, Naulaers G. Monitoring neonatal regional cerebral oxygen saturation in clinical practice: value and pitfalls. Neonatology 2008;94:237–44.
8. Watzman HM, Kurth CD, Montenegro LM, Rome J, Steven JM, Nicolson SC. Arterial and venous contributions to near-infrared cerebral oximetry. Anesthesiology 2000;93:947–53.
9. Naulaers G, Morren G, Van Huffel S, Casaer P, Devlieger H. Cerebral tissue oxygenation index in very premature infants. Arch Dis Child Fetal Neonatal Ed 2002;87:F189–92.
10. Hyttel-Sørensen S, Austin T, van Bel F, et al. Clinical use of cerebral oximetry in extremely preterm infants is feasible. Dan Med J 2013;60:A4533.
11. Sorensen LC, Greisen G. Precision of measurement of cerebral tissue oxygenation index using near-infrared spectroscopy in preterm neonates. J Biomed Opt 2006;11:054005.
12. Petrova A, Mehta R. Near-infrared spectroscopy in the detection of regional tissue oxygenation during hypoxic events in preterm infants undergoing critical care. Pediatr Crit Care Med 2006;7:449–54.
13. Petrova A, Mehta R. Regional tissue oxygenation in association with duration of hypoxaemia and haemodynamic variability in preterm neonates. Arch Dis Child Fetal Neonatal Ed 2010;95:F213–9.
14. Dix LM, van Bel F, Baerts W, Lemmers PM. Comparing near-infrared spectroscopy devices and their sensors for monitoring regional cerebral oxygen saturation in the neonate. Pediatr Res 2013;74:557–63.
15. Pocivalnik M, Pichler G, Zotter H, Tax N, Müller W, Urlesberger B. Regional tissue oxygen saturation: comparability and reproducibility of different devices. J Biomed Opt 2011;16:057004.
16. Naulaers G, Meyns B, Miserez M, et al. Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation. A validation study in piglets. Neonatology 2007;92:120–6.
17. Lemmers PM, Toet M, van Schelven LJ, van Bel F. Cerebral oxygenation and cerebral oxygen extraction in the preterm infant: the impact of respiratory distress syndrome. Exp Brain Res 2006;173:458–67.
18. Sirc J, Dempsey EM, Miletin J. Cerebral tissue oxygenation index, cardiac output and superior vena cava flow in infants with birth weight less than 1250 grams in the first 48 hours of life. Early Hum Dev 2013;89:449–52.
19. Moran M, Miletin J, Pichova K, Dempsey EM. Cerebral tissue oxygenation index and superior vena cava blood flow in the very low birth weight infant. Acta Paediatr 2009;98:43–6.
20. Pichler G, Binder C, Avian A, Beckenbach E, Schmölzer GM, Urlesberger B. Reference ranges for regional cerebral tissue oxygen saturation and fractional oxygen extraction in neonates during immediate transition after birth. J Pediatr 2013;163:1558–63.
21. van Hoften JC, Verhagen EA, Keating P, ter Horst HJ, Bos AF. Cerebral tissue oxygen saturation and extraction in preterm infants before and after blood transfusion. Arch Dis Child Fetal Neonatal Ed 2010;95:F352–8.
22. Kurth CD, Levy WJ, McCann J. Near-infrared spectroscopy cerebral oxygen saturation thresholds for hypoxia-ischemia in piglets. J Cereb Blood Flow Metab 2002;22:335–41.
23. Hou X, Ding H, Teng Y, et al. Research on the relationship between brain anoxia at different regional oxygen saturations and brain damage using near-infrared spectroscopy. Physiol Meas 2007;28:1251–65.
24. Roche-Labarbe N, Fenoglio A, Aggarwal A, et al. Near-infrared spectroscopy assessment of cerebral oxygen metabolism in the developing premature brain. J Cereb Blood Flow Metab 2012;32:481–8.
25. Hahn JS, Monyer H, Tharp BR. Interburst interval measurements in the EEGs of premature infants with normal neurological outcome. Electroencephalogr Clin Neurophysiol 1989;73:410–8.
26. Lemmers PM, Toet MC, van Bel F. Impact of patent ductus arteriosus and subsequent therapy with indomethacin on cerebral oxygenation in preterm infants. Pediatrics 2008;121:142–7.
27. Lemmers PM, Molenschot MC, Evens J, Toet MC, van Bel F. Is cerebral oxygen supply compromised in preterm infants undergoing surgical closure for patent ductus arteriosus? Arch Dis Child Fetal Neonatal Ed 2010;95:F429–34.
28. van Bel F, van de Bor M, Stijnen T, Ruys JH. Decreased cerebrovascular resistance in small for gestational age infants. Eur J Obstet Gynecol Reprod Biol 1986;23:137–44.
29. Hyttel-Sorensen S, Kleiser S, Wolf M, Greisen G. Calibration of a prototype NIRS oximeter against two commercial devices on a blood-lipid phantom. Biomed Opt Express 2013;4:1662–72.
30. Lemmers PM, Zwanenburg RJ, Benders MJ, et al. Cerebral oxygenation and brain activity after perinatal asphyxia: does hypothermia change their prognostic value? Pediatr Res 2013;74:180–5.
31. Toet MC, Lemmers PM, van Schelven LJ, van Bel F. Cerebral oxygenation and electrical activity after birth asphyxia: their relation to outcome. Pediatrics 2006;117:333–9.
32. Hyttel-Sorensen S, Pellicer A, Alderliesten T, et al. Cerebral near infrared spectroscopy oximetry in extremely preterm infants: phase II randomised clinical trial. BMJ 2015;350:g7635.
33. Pellicer A, Greisen G, Benders M, et al. The SafeBoosC phase II randomised clinical trial: a treatment guideline for targeted near-

infrared-derived cerebral tissue oxygenation versus standard treatment in extremely preterm infants. Neonatology 2013;104:171–8.
34. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr 1978;92:529–34.
35. Baerts W, Lemmers PM, van Bel F. Cerebral oxygenation and oxygen extraction in the preterm infant during desaturation: effects of increasing FiO(2) to assist recovery. Neonatology 2011;99:65–72.
36. Visser GH, Eilers PH, Elferink-Stinkens PM, Merkus HM, Wit JM. New Dutch reference curves for birthweight by gestational age. Early Hum Dev 2009;85:737–44.
37. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape (with discussion). J R Stat Soc Ser C (Applied Stat 2005;54:507–54.