Non-invasive MRI measurements of venous oxygenation, oxygen extraction fraction and oxygen consumption in neonates

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ABSTRACT

Background and purpose: Brain oxygen consumption reflects neuronal activity and can therefore be used to investigate brain development or neuronal injury in neonates. In this paper we present the first results of a non-invasive MRI method to evaluate whole brain oxygen consumption in neonates.

Materials and methods: For this study 51 neonates were included. The T1 and T2 of blood in the sagittal sinus were fitted using the ‘T2 prepared tissue relaxation inversion recovery’ pulse sequence (T2-TRIR). From the T1 and the T2 of blood, the venous oxygenation and the oxygen extraction fraction (OEF) were calculated. The cerebral metabolic rate of oxygen (CMRO2) was the resultant of the venous oxygenation and arterial spin labeling whole brain cerebral blood flow (CBF) measurements.

Results: Venous oxygenation was 59 ± 14% (mean ± sd), OEF was 40 ± 14%, CBF was 14 ± 5 ml/100 g/min and CMRO2 was 30 ± 12 μmol/100 g/min. The OEF in preterms at term-equivalent age was higher than in the preterms and in the infants with hypoxic–ischemic encephalopathy (p < 0.01). The OEF, CBF and CMRO2 increased (p < 0.01, < 0.05 and < 0.01, respectively) with postnatal age.

Conclusion: We presented an MRI technique to evaluate whole-brain oxygen consumption in neonates non-invasively. The measured values are in line with reference values found by invasive measurement techniques. Preterms and infants with HIE demonstrated significant lower oxygen extraction fraction than the preterms at term-equivalent age. This could be due to decreased neuronal activity as a reflection of brain development or as a result of tissue damage, increased cerebral blood flow due to immature or impaired autoregulation, or could be caused by differences in postnatal age.

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Abbreviations: ASL, arterial spin labeling; Cm, oxygen carrying capacity of blood; CBF, cerebral blood flow; CMRO2, cerebral metabolic rate of oxygen; Hct, hematocrit; HIE, hypoxic–ischemic encephalopathy; MRI, magnetic resonance imaging; NIRS, near-infrared spectroscopy; OEF, oxygen extraction fraction; PASL, pulsed arterial spin labeling; PMA, postmenstrual age at birth; PMA*, postmenstrual age at the time of MR imaging; PNA, postnatal age; sd, Standard deviation; SIO2, tissue oxygen saturation; T1b, longitudinal relaxation time of blood; T2-TRIR, T2 prepared tissue relaxation inversion recovery; PT-TEA, Preterm at term-equivalent age; TRUST-MRI, T2-relaxation under spin tagging MRI; Ya, arterial oxygen saturation; Yv, venous oxygen saturation.

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Introduction

Brain growth (Kretschmann et al., 1986) and myelinisation are at their maximum postnatal (Bourgeois and Rakic, 1993) which makes this period of brain development most vulnerable. Both myelinisation and increases in capillary density are known to be energy demanding (Altman et al., 1988) and therefore monitoring the energy consumption of the brain can be used to evaluate brain development. Due to technical advances in neonatal intensive care an increasing number of infants are born preterm. Preterm infants are at risk for cerebral injury such as hypoxia–ischemia, stroke and periventricular leukomalacia causing neurological deficits (Kiechl-Kohlendorfer et al., 2009). Hemodynamic instability may be the cause of these injuries necessitating the need for hemodynamic monitoring of the brain. In 2 per 1000 term live births hypoxic–ischemic encephalopathy occurs (Himmelmarmann et al., 2005). The majority of these infants suffer from the reperfusion phenomenon in which autoregulation is lost causing hyperperfusion (Pryds et al.,...
which restores the high energy phosphates and can lead to delayed cell death (Grant and Yu, 2006). Brain hemodynamic evaluation may detect this hyperperfusion stage (Shi et al., 2012) and could then be used to initiate and evaluate neuroprotective therapies. Quantitative estimates of the brain oxygen consumption can be obtained with oxygen-15 positron emission tomography (Mintun et al., 1984). Alternatively, the xenon clearance technique can measure the cerebral blood flow and provides a measurement of the brain oxygen consumption when the oxygenation in the jugular vein is evaluated (Skov et al., 1993). Unfortunately, both techniques are invasive as they require the use of ionizing radiation which limits their usability in neonates. An upcoming non-invasive tool to evaluate brain hemodynamics is near infrared spectroscopy (NIRS). This technique relies on the attenuation of near-infrared light (~650–950 nm) when it permeates biological tissue. Deoxygenated and oxygenated hemoglobin contribute to this attenuation. Therefore, NIRS can be used to estimate changes in deoxygenated hemoglobin and oxyhemoglobin (Wray et al., 1988) allowing for an evaluation of the oxygen saturation of the tissue (StO2). This technique is advantageous as it allows for a non-invasive continuous monitoring at the bedside. However, NIRS is limited to the evaluation of the overall tissue oxygen saturation and not the oxygen extraction fraction, and therefore, the technique does not allow for the evaluation of the oxygen consumption. In addition, it only provides regional evaluation at the location where the probe is positioned and the sensitivity of NIRS is limited to the superficial brain tissue as the penetration depth of near-infrared light is limited (Boas et al., 2004). Non-invasive methods which can evaluate whole brain oxygen consumption are currently under investigation. A combined approach of non-invasive venous oxygenation measurements in the sagittal sinus, using T2-relaxation–under-spin-tagging magnetic resonance imaging (TRUST-MRI) (Lu and Ge, 2008), and flow measurements with phase-contrast MR angiography has been used to evaluate whole brain oxygen consumption in adults (Xu et al., 2009) and the feasibility of this technique within neonates has been investigated (Liu et al., 2014). Another non-invasive MRI technique to evaluate venous oxygenation and oxygen consumption in the brain combines MR susceptibility, to evaluate the venous oxygenation in the sagittal sinus, with phase-contrast MR angiography (Jain et al., 2011). Venous oxygenation and brain oxygen consumption measurements, obtained in neonates with this technique, were shown to correlate well to results obtained by means of diffuse optical and correlation spectroscopies (Jain et al., 2013).

In this paper we present a newly developed non-invasive approach to investigate whole brain oxygen consumption in neonates. The ‘T2 Preparied Tissue Relaxation Inversion Recovery’ (T2-TRIR) MRI pulse sequence (Petersen et al., 2012) is used to measure the transverse and longitudinal relaxation rate of blood (T2b and T1b) in the sagittal sinus. The venous oxygenation is subsequently derived from the T2b and the T1b-derived hematocrit (Lu et al., 2012). Arterial oxygenation is measured with pulse oximetry allowing for a calculation of the oxygen extraction fraction. Pulsed arterial spin labeling (ASL) perfusion MRI is used to measure whole brain cerebral blood flow. By combining information of venous oxygenation, arterial oxygenation and whole brain cerebral blood flow a non-invasive MRI measurement of the brain oxygen consumption is obtained. In order to investigate the validity of this new non-invasive approach we compare the obtained results to previous reported reference values (Altman et al., 1993), investigate if the technique detects changes related to postnatal age, as was earlier found by NIRS studies (Franceschini et al., 2007; Roche-Labarre et al., 2012) and are thought to be related to the decline in hematocrit after birth (Palis and Segel, 1998), and we evaluate if the technique can detect differences in between categories of neonates which can be contributed to differences in neuronal activity. For the latter, results obtained in preterm neonates and in neonates with hypoxic–ischemic encephalopathy are compared to the results of neonates at term-equivalent age.

Materials and methods

Subjects

The present study was approved by our institutional review board and the requirement to obtain written parental informed consent was waived. Fifty-one neonates who underwent MRI for clinical reasons were included: 5 preterm infants, 17 preterms with MR imaging at term-equivalent age (PT-TEA), 19 infants with hypoxic–ischemic encephalopathy (HIE) and 9 infants with another diagnosis (others). Mean postmenstrual age at the time of MR imaging was 29 weeks for preterms (range: 28–29 weeks), 39 weeks for PT-TEA (range: 38–40 weeks), 38 weeks for HIE (range: 34–41 weeks) and 39 weeks for the infants with another diagnosis (range: 33–52 weeks). HIE was diagnosed in infants having altered alertness, abnormal tone, feeding difficulties or seizures with at least three of the following criteria: 1) late decelerations on fetal monitoring or meconium staining, 2) delayed onset of respiration, 3) arterial cord blood pH < 7.10, 4) Apgar scores < 7 at 5 min, and 5) multi-organ failure (van Rooij et al., 2010). The baseline characteristics – postmenstrual age at birth, postmenstrual age at the time of MR imaging, postnatal age and reason for MRI – for all groups are shown in Table 1.

### MR imaging

MR imaging was performed on a 3.0 Tesla Philips Achieva System (Philips Medical Systems, Best, The Netherlands) with a quadrature body coil for transmission and an 8-element phased-array SENSE head coil as a signal receiver. Prior to MR imaging infants were sedated; either by oral administration of chloral hydrate (50 to 60 ml per kilogram body

<table>
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<th>Table 1</th>
<th>Baseline characteristics.</th>
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<td>PMAb (in weeks)</td>
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<tr>
<td>Preterm</td>
<td>5</td>
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<tr>
<td>PT-TEA</td>
<td>18</td>
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<tr>
<td>HIE</td>
<td>19</td>
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</tbody>
</table>

PMAb = postmenstrual age at birth, mean (range); PMAb = postmenstrual age at the time of MR imaging, mean (range); PNA = postnatal age, mean (range); PT-TEA = preterm born infants with MR imaging at term-equivalent age; PHVD = posthemorrhagic ventricular dilatation; HIE = infants with hypoxic–ischemic encephalopathy; US = ultrasound.

The category ‘others’ comprises all infants who did not fit in any of the other categories.
weight), by a combination of intramuscular pethidine (2 mg per kilogram body weight), chlorpromazine (0.5 mg per kilogram body weight), and promethazine (0.5 mg per kilogram body weight) (Groenendaal et al., 1994), or by intravenous administration of morphine (10 μg per kg). Infants were wrapped into a vacuum cushion in order to minimize motion during imaging. Minimuffs (Natus Europe, Münich, Germany) and earmuffs (EM’s 4 kids, Everton Park, Australia) were used to reduce noise. Heart rate (Nonin Pulse Oximetry, Nonin Medical, Plymouth, MN), respiratory rate (Phillips, Best, The Netherlands) and transcutaneous oxygen saturation (Nonin Pulse Oximetry, Nonin Medical, Plymouth, MN) were monitored throughout the examination. A neonatologist was present throughout the examination. The scan protocol consisted of conventional MR imaging, the T2 prepared tissue relaxation inversion recovery (T2-TRIR) pulse sequence (Fig. 1) and pulsed arterial spin labeling (ASL) imaging. A 2D phase contrast MR angiography scan was used as a localizer to plan the imaging plane of the T2-TRIR sequence which was positioned perpendicular to the sagittal sinus. The T2-TRIR sequence enables simultaneous evaluation of the longitudinal and transverse relaxation rate of blood (T1b and T2b) in the sagittal sinus (Petersen et al., 2012). It consists of a presaturation pulse in the imaging plane, followed by an MLEV-T2 preparation scheme (Brittain et al., 1995) and a non-selective inversion pulse. Multiple single-shot echo-planar imaging was used as readout. The MR sequence chart of the T2-TRIR sequence is shown in Fig. 1a. Scan parameters of the T2-TRIR sequence were: repetition time 15 s, echo time 20 ms, ΔTI 140 ms, TI1 20 ms, scan matrix 128 × 128, FOV 160 × 160, flip angle 95°, slice thickness 3 mm, SENSE = 2.5, effective echo time 0, 40, 80 and 160 ms, and total scan time 90 s. Pulsed ASL (PASL) was used to evaluate brain perfusion. A pulsed star labeling of arterial regions pulse sequence (Golay et al., 2005) was followed by a multi-slice, single-shot echo-planar imaging readout. The labeling slab (10 cm) was aligned parallel to the imaging plane with a gap of 1 cm in between. To obtain a finite bolus length a saturation pulse was applied in the imaging region 600 ms after the labeling pulse using the Q2-TIPS technique (T1i = 600 ms) (Luh et al., 1999). Scan parameters of the PASL sequence were: matrix 40 × 40, FOV 160 × 128 mm, SENSE 2.5, voxel size 4 × 4 × 7 mm, TR/TE: 2700/17 ms, flip angle 90°, inversion delay (TI2) 1500 ms, no vascular crushers, 70 averages, and scan time 200 s. Eleven slices were acquired in ascending slice order and planning of the imaging plane was performed based on sagittal T1-weighted images.

Data analysis

All images were analyzed using IDL 6.1 for Windows (ITT Visual Information Solutions, Boulder, CO, U.S.A.). First, an automatic localizer tool was used to identify blood signal in the sagittal sinus on the magnitude reconstructed data of the T2-TRIR sequence (Fig. 1c). Blood signal in the sagittal sinus was detected based on the suppression of the static surrounding tissue at later time points caused by repeated acquisition at high flip angle while the inflowing blood signal in the sagittal sinus was high. From the blood signal four different inversion recovery curves with effective echo times of 0, 40, 80 and 160 ms respectively, were plotted (Fig. 1b). For this, the combination of voxels which resulted in the smallest overall residual error of the fit was chosen from within the initial region-of-interest. From the inversion recovery curves the T1i and the T2b were simultaneously fitted by means of the following formula:

\[ M_b(T1) = M_0b \cdot \left[ 1 - e^{-e_{IE/T2b} \cdot IE} \right] \cdot e^{-T1_i/T1b} \]  

where \( M_b \) is the longitudinal magnetisation of blood at each inversion time (TI), \( M_0b \) is the equilibrium magnetization of blood, eIE the effective echo time and IE the inversion efficiency. Second, hematocrit was derived from the following formula: 1 / T1b = 0.5 · Hct + 0.37 (Varela et al., 2011). Third, from information of both the hematocrit and the T2b, the venous oxygen saturation (Yv) was estimated (Lu et al., 2012).

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**Fig. 1.** The T2-TRIR sequence. a) Sequence chart of the ‘T2 prepared tissue relaxation inversion recovery’ (T2-TRIR) pulse sequence is shown. The image region is presaturated (90° pulse) after which the longitudinal magnetization is T2 prepared using an MLEV preparation scheme. Subsequently, inversion (180° pulse) is followed by multiple readouts at high flip angle. The sequence is repeated 4 times, each with a different T2 preparation, resulting in effective TEs of 0, 40, 80 and 160 ms. b) Acquisition at high flip angle saturates the brain tissue but not the inflowing blood in the sagittal sinus. Four inversion recovery curves with effective echo times of 0, 40, 80 and 160 ms are obtained from the signal within the sagittal sinus. c) The brain tissue is saturated due to the high flip angle readout, only inflowing blood within the sagittal sinus is visible. An automatic localizer tool (red dot) localizes the inflowing blood in the sagittal sinus based on signal intensity.
As arterial oxygen saturation ($Y_a$) was monitored during MR imaging with pulse oximetry the oxygen extraction fraction (OEF) could be calculated:

$$\text{OEF} = \frac{(Y_a - Y_v)/Y_a} \cdot 100. \quad (2)$$

Cerebral blood flow maps were generated by post processing of ASL images. First, ASL images were motion corrected using a two step approach. A 6 parameter affine transformation between (control-label) image pairs were applied followed by a 12 parameter affine transformation to get all control-label volume pairs aligned. Subsequently, the ASL imaging pairs were subtracted to generate $\Delta M$ images.

The mean and standard deviation of the difference signal over the subtracted images pairs was calculated and image pairs with a difference signal larger than 2 standard deviations of the mean were automatically discarded (Oguz et al., 2003). Second, $\Delta M$ images were averaged to create $\Delta M_{\text{total}}$ images. Finally, perfusion was quantified on the $\Delta M_{\text{total}}$ images (Luh et al., 1999; Ostergaard et al., 1996):

$$f = \frac{\lambda \Delta M}{2\alpha M_0 T_1 e^{-\lambda t}}. \quad (3)$$

For this, the inversion efficiency ($\alpha$) and the brain–blood partition coefficient ($\lambda$) were assumed to be 95% and 1.1 mL/g (Herscovitch and Raichle, 1985), respectively. The latter value represents an average whole-brain $\lambda$ value in neonates. The $T_1$ was fitted from the inversion recovery curves obtained with the T2-TRIR sequence as described above. Finally, CBF, $Y_a$ and $Y_v$ were used to calculate the oxygen consumption of the brain or the cerebral metabolic rate of oxygen (CMRO2).

CMRO2 = CBF · (Ya − Yv) · Cs

(4)

where Cs is the oxygen carrying capacity of blood which was corrected for the individual $T_{1b}$-derived hematocrit (Guyton and Hall, 2005; Xu et al., 2009).

**Laboratory analysis**

Hematocrit (Hct) was measured in 22 infants within 24 h of MR imaging. 15 blood samples were capillary-drawn and 7 arterial-drawn. For this, a test cartridge blood analysis system (Abbott i-STAT System, Abbott Laboratories, Abbott Point of Care Inc., Princeton, New Jersey) which estimates the hematocrit based on conductivity measurements was used. Capillary-drawn blood was collected by pricking the skin of the infant heel and arterial-drawn blood was collected via an umbilical line or through a line in the radial artery. The infants were in supine position during blood collection.

**Statistical analysis**

For statistical analysis IBM SPSS statistics (version 19.0.1, SPSS Inc., Chicago, IL) was used. The mean (and standard deviation (sd)) number of voxels used to fit the $T_{1b}$ and the $T_{2b}$ was calculated for each category; the preterms, the PT-TEA, the HIE and the others. For each individual the 95% confidence interval of the $T_{1b}$ and $T_{2b}$ fitting was calculated as the percentage from the individual’s mean. The confidence interval was estimated based on bootstrap resampling of the fitting residuals (Efron, 1979). For each category, the mean lower and upper border of the 95% confidence interval was calculated. The mean (sd) percentage of discarded ASL images was calculated for each category. The mean postnatal age, hematocrit, $T_{1b}$, $T_{2b}$, Y_a, OEF, CBF and CMRO2 across groups were compared with an ANOVA test followed by a post hoc procedure (Tukey’s test). To evaluate the relation of the different parameters with postnatal age linear regression analysis was used. The correlation between the $T_{1b}$-derived hematocrit and clinical-obtained hematocrit was evaluated with a bivariate correlation and the relation between clinical hematocrit and the $T_{1b}$ (= $1/T_{1b}$) was investigated by means of linear regression analysis. In the 22 infants with clinical hematocrit, the $Y_v$, OEF and CMRO2 were calculated based on the clinical hematocrit value. The correlation between the values ($Y_v$, OEF and CMRO2) found when using the $T_{1b}$-derived hematocrit versus the clinical hematocrit was evaluated with a bivariate correlation. A p-value smaller than 0.05 was considered statistically significant.

**Results**

The T2-TRIR sequence was able to fit the $T_2$ of blood and the $T_1$ of blood in 82% of the neonates (42 out of 51 neonates), for this, 6 ± 3 voxels (mean ± sd) were selected on average in the sagittal sinus. In these infants, the $T_1$ of blood was 1903 ± 18 ms (mean ± sd) and the $T_2$ of blood was 83 ± 16 ms. The mean 95% confidence interval of the $T_2$ fitting was [−1.96%, 1.92%]. The mean 95% confidence interval of the $T_2$ fitting was [−5.80%, 5.98%]. The blood $T_1$-derived hematocrit was 32 ± 10%, venous oxygenation was 59 ± 14%, arterial oxygenation was 97 ± 2% and oxygen extraction fraction was 40 ± 14%. Of the 42 neonates with successful $T_1$ and $T_2$ fitting, ASL image quality was good in 22 subjects (52%): in 10 preterm born infants imaged at term-eqivalent age, in 9 infants with hypoxic–ischemic encephalopathy (HIE) and in 3 infants with another diagnosis (others). Therefore, cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO2) could only be measured in 22 subjects. In these 22 subjects, 9.2 ± 3.4% of the images were discarded. Mean CBF was 14 ± 5 ml/100 g/min and mean CMRO2 was 30 ± 12 µmol/100 g/min. Subjects were categorized into preterm infants, preterm born infants with MR imaging at term-equivalent age, infants with HIE and infants with another diagnosis (others). The average number of voxels used for $T_{1b}$ and $T_{2b}$ fitting was 5 ± 2 in the preterms, 5 ± 1 in the PT-TEA, 7 ± 3 in the HIE and 6 ± 3 in the others. Table 2 shows, for each category, mean and standard deviation of the $T_1$ and the $T_2$ of blood, the hematocrit, the venous oxygenation and the oxygen extraction fraction. For the $T_1$ and the $T_2$ of blood, the 95% confidence interval of the fit is shown in between square brackets in Table 2. The median, interquartile range, minimum and maximum values for the venous oxygenation and the oxygen extraction fraction per category are shown by means of boxplots in Fig. 2. The ‘others’ category was excluded from the following analysis. The mean postnatal age, the $T_1$ of blood, the $T_2$ of blood, the hematocrit, the venous oxygenation and the oxygen extraction fraction per category are shown by means of boxplots in Fig. 2.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Venous oxygenation and oxygen extraction fraction.</th>
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<tbody>
<tr>
<td>n</td>
<td>$T_{1b}$</td>
</tr>
<tr>
<td>------</td>
<td>-----------</td>
</tr>
<tr>
<td>Preterm</td>
<td>5</td>
</tr>
<tr>
<td>PT-TEA</td>
<td>17</td>
</tr>
<tr>
<td>HIE</td>
<td>11</td>
</tr>
<tr>
<td>Others</td>
<td>9</td>
</tr>
</tbody>
</table>

$T_{1b}$ = longitudinal relaxation time of blood; $T_{2b}$ = transverse relaxation time of blood; Hct = hematocrit; $Y_a$ = arterial oxygenation; $Y_v$ = venous oxygenation; OEF = oxygen extraction fraction; PT-TEA = preterm born infants with MR imaging at term-equivalent age; HIE = infants with hypoxic–ischemic encephalopathy. The category ‘others’ comprises all infants who did not fit in any of the other categories. The mean ± standard deviation for $T_{1b}$, $T_{2b}$, Hct, $Y_a$, $Y_v$ and OEF are shown for each category. For the $T_{1b}$ and the $T_{2b}$ the mean 95% confidence interval of the fit is shown as a percentage in between square brackets. The $T_{1b}$, the $Y_v$ and the OEF were significantly different in the preterm infants than in the PT-TEA (p < 0.05). The $T_{1b}$, the Hct, the $Y_v$ and the OEF were significantly different in the HIE infants compared to the PT-TEA infants (p < 0.05 and < 0.01). The category ‘others’ comprises all infants with another diagnosis, thus, the infants who did not fit in any of the other categories.
The cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen (CMRO2) were measured in 10 preterms at term-equivalent age (PT-TEA), 9 infants with HIE and 3 infants with another diagnosis. In the PT-TEA 9.5 ± 2.9% of the ASL images were discarded, in the HIE 9.5 ± 3.2% of the ASL images were discarded. The Yv and the OEF in PT-TEA differed significantly from the Yv and the OEF in the preterms and in the HIE infants (p < 0.01). No significant differences in CBF or CMRO2 were found.

Linear regression analysis, performed on the data of all infants, demonstrated a significant decrease in hematocrit with postnatal age (R2 = 0.17, p < 0.01, Hct = −0.1 · PNA + 37), a decrease in venous oxygenation with postnatal age (R2 = 0.25, p < 0.01, Yv = −0.18 · PNA + 66) and an increase in oxygen extraction fraction with postnatal age (R2 = 0.26, p < 0.01, OEF = 0.19 · PNA + 32). A significant increase in CBF with postnatal age was seen (R2 = 0.26, p < 0.05, CBF = 0.07 · PNA + 12) and a significant increase in CMRO2 with postnatal age (R2 = 0.367, p < 0.01, CMRO2 = 0.13 · PNA + 21) (Fig. 3).

The average hematocrit value derived from the T1 of blood was 32 ± 10% (n = 42) and mean clinical hematocrit was 43 ± 7% (n = 22). For each category of neonates, the mean ± sd hematocrit derived from the T1 of blood and the mean clinical hematocrit is shown in Table 4. The Pearson correlation coefficient between the T1 of blood derived hematocrit and clinical hematocrit was 0.65 (p < 0.01). The relation between clinical hematocrit and R1 of blood was; R1b = 0.48 [0.21–0.74] · Hct + 0.34 [0.23–0.46], with the 95% confidence interval between brackets (R2 = 0.414, p < 0.01). The Yv calculated based on the T1b-derived hematocrit was 64 ± 13% while the Yv based on clinical hematocrit was 72 ± 8%, Pearson’s R was 0.72 (p < 0.001). The OEF calculated based on the T1b-derived hematocrit was 34 ± 13% while the OEF based on clinical hematocrit was 26 ± 8%, Pearson’s R was 0.69 (p < 0.001). The CMRO2 calculated based on the T1b-derived hematocrit was 23 ± 10 μmol/100 g/min while the CMRO2 based on clinical hematocrit was 22 ± 12 μmol/100 g/min, Pearson’s R was 0.97 (p < 0.001).

Table 3
Cerebral blood flow and oxygen consumption.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Hct (%)</th>
<th>Yv (%)</th>
<th>CBF (ml/100 g/min)</th>
<th>CMRO2 (μmol/100 g/min)</th>
</tr>
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<tbody>
<tr>
<td>PT-TEA</td>
<td>10</td>
<td>26 ± 8</td>
<td>52 ± 12</td>
<td>14 ± 3</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>HIE</td>
<td>3</td>
<td>33 ± 8</td>
<td>65 ± 13</td>
<td>12 ± 4</td>
<td>24 ± 12</td>
</tr>
<tr>
<td>Others</td>
<td>7</td>
<td>31 ± 16</td>
<td>57 ± 25</td>
<td>17 ± 2</td>
<td>29 ± 6</td>
</tr>
</tbody>
</table>

Hct = hematocrit; Yv = venous oxygenation; CBF = Cerebral Blood Flow; CMRO2 = Cerebral metabolic rate of oxygen; PT-TEA = preterm infants with MR imaging at term-equivalent age; HIE = hypoxic–ischemic encephalopathy. The category ‘others’ comprises all infants who did not fit in any of the other categories. No significant differences were found between the PT-TEA and the HIE infants. Values are displayed as mean ± standard deviation.
weeks after birth when hematocrit declines (Palis and Segel, 1998). Although we demonstrated a linear relation between the oxygen extraction fraction and postnatal age, this may only be true in the first weeks after birth when hematocrit declines (Palis and Segel, 1998).

Discussion

We presented the initial results of a non-invasive MRI method to evaluate venous oxygenation, oxygen extraction fraction, and oxygen consumption in neonates. The values we found are realistic as they compare well to earlier reported reference values. In addition, the technique allowed us to detect differences related to postnatal age and disease state.

We found a mean oxygen extraction fraction of 40% which relates well to earlier reported values. For instance, Altman et al. measured the oxygen extraction fraction with positron emission tomography and found values ranging from 5 to 41% (Altman et al., 1993), and Jain et al. found oxygen extraction fractions, measured with MR susceptometry, between 26 and 42% (Jain et al., 2013). We found oxygen extraction fraction to increase with advancing postnatal age which is in agreement with earlier performed studies (Roche-Labarbe et al., 2012). After birth, the heart and respiratory rate, arterial oxygen saturation and hemoglobin levels alter and may influence the oxygen extraction fraction (de Alarcón and Werner, 2005; Tina et al., 2009). Although we demonstrated a linear relation between the oxygen extraction fraction and postnatal age, this may only be true in the first weeks after birth when hematocrit declines (Palis and Segel, 1998).

Table 4

<table>
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<th>T1 of blood derived hematocrit and clinical hematocrit.</th>
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<tr>
<td>T1 of blood derived hematocrit</td>
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<tr>
<td>n (%)</td>
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<tr>
<td>Preterm 5 36 ± 4</td>
</tr>
<tr>
<td>PT-TEA 17 26 ± 8</td>
</tr>
<tr>
<td>HIE 11 35 ± 9</td>
</tr>
<tr>
<td>Others 9 36 ± 13</td>
</tr>
</tbody>
</table>

PT-TEA = preterm born infants with MR imaging at term-equivalent age; HIE = infants with hypoxic–ischemic encephalopathy. The category ‘others’ comprises all infants who did not fit in any of the other categories. The mean T1 of blood derived hematocrit and the mean clinical hematocrit is shown for all categories. Values are displayed as mean ± standard deviation.

We found a higher oxygen extraction fraction in preterm born infants imaged at term-equivalent age than in the preterms and the infants with hypoxic–ischemic encephalopathy (49% versus 29% and 32%). These differences could be caused by differences in postnatal age between the different categories (Roche-Labarbe et al., 2012), the postnatal age in the preterms at term-equivalent age was significantly higher. Alternatively, lower oxygen consumption in the preterms as a reflection of brain maturation (Altman et al., 1993), or decreased oxygen consumption due to neuronal loss (Shi et al., 2012) may have led to lower oxygen extraction fractions in the preterms and in the infants with hypoxic–ischemic encephalopathy. Another possibility is that immature autoregulation in the preterms (Lemmers et al., 2006) or impaired autoregulation in the infants with hypoxic–ischemic encephalopathy (Prudys et al., 1990) caused abundant cerebral blood flow resulting in decreased oxygen extraction fraction. Though, we do not find support for this in the cerebral blood flow values of the infants with hypoxic–ischemic encephalopathy. In any case, a previous NIRS study demonstrated higher oxygen saturation fractions in neonates with hypoxic–ischemic encephalopathy compared to healthy neonates of the same postnatal age (74.7% and 80.1% versus 66.4%), which suggests another origin of these differences than postnatal age (Zaramella et al., 2007). Unfortunately, due to the nature of our study, a proof of principle study with no clinical aim and without the inclusion of age-matched controls, we cannot exclude postnatal age as the origin of the differences, nor can we allocate impaired autoregulation or lower oxygen consumption as the source. This is because cerebral blood flow and oxygen consumption measurements could only be performed in a subset of infants.

The mean oxygen consumption in the neonates was 30 μmol/100 g/min. This corresponds well to positron emission tomography studies who reported values from 2.7 to 24 μmol/100 g/min in preterm infants and up to 58 μmol/100 g/min for term infants (Altman et al., 1993). In addition, the recently presented non-invasive MRI techniques found similar values; 21.9 μmol/100 g/min in full term infants with congenital heart defects (Jain et al., 2013) and 38.3 μmol/100 g/min in infants with a gestational age ranging from 35 to 42 weeks (Liu et al., 2014). We did not find significant differences between categories of...
infants. Earlier studies did demonstrate lower oxygen consumption after hypoxic–ischemic encephalopathy [Frewen et al., 1991; Shi et al., 2012; Thorngren-Jerneck et al., 2001]. The mean oxygen consumption in our infants with hypoxic–ischemic encephalopathy (24 μmol/100 g/min) was lower than in our preterm born infants imaged at term-equivalent age (30 μmol/100 g/min), though this difference was not significant. The lack of significance may be caused by the smaller sample size on which the oxygen consumption measurements were performed.

As a small digression, we want the reader to note that we did not find differences in cerebral blood flow between infants with hypoxic–ischemic encephalopathy and preterm infants imaged at term-equivalent age. This even though hyperperfusion is known to occur after hypoxic–ischemic encephalopathy (Wintervalk et al., 2011). We can think of two reasons why this is so. First, posthypoxic–ischemic hyperperfusion may only occur in infants with severe hypoxic–ischemic encephalopathy (Prds et al., 1990). Second, hyperperfusion may only occur in the damaged area which is predominantly the basal ganglia and thalami (de Vries and Groenendaal, 2010); studies of Wintervalk et al. have found lower cerebral blood flow in the frontal cortex of infants with severe hypoxic–ischemic encephalopathy (Wintervalk et al., 2014), but, increased cerebral blood flow in the basal ganglia (Wintervalk et al., 2011). As our cerebral blood flow results are whole brain measurements, regional hyperperfusion may have been obscured. It is known from previous positron emission tomography studies that the cerebral blood flow in neonates is lower than in adults with CBF values ranging from 5 to 23 ml/100 g/min in preterms and from 9 to 73 ml/100 g/min in term born neonates (Altman et al., 1988). The whole brain cerebral blood flow results of this study correspond well to the earlier found ASL CBF values of 12 ml/100 g/min (de Vis et al., 2013a) and 21 ml/100 g/min (Miranda et al., 2006) in preterms at term-equivalent age.

This study was limited to the fact that oxygen extraction fraction and oxygen consumption data could only be obtained in part of the infants. Successful fitting of the T1 and T2 of blood by means of the T2-TRIR sequence was achieved in 82% of the infants. Unsuccessful fitting was caused by a too large uncertainty in the fit which may have been caused by image noise or patient motion. Even more infants had to be excluded for the oxygen consumption measurements as a result of bad ASL image quality which was in particular a problem in the preterms and made that we could not measure the cerebral metabolic rate of oxygen in any of these infants. Bad ASL image quality was in part due to inherent problems of ASL imaging in neonates – low cerebral blood flow limiting the signal-to-noise ratio, longer tracer lifetime causing negative perfusion and varying blood velocities influencing the labeling (Wang et al., 2006) – with the worst problems in preterms due to their very low brain perfusion (De Vis et al., 2013a, 2013b; Wang et al., 2006), but, was as well caused by patient motion. Our scans were performed at the end of a clinical imaging protocol and therefore experienced ‘worst case’ motion problems. The success rate of the performed measurements should go up when dealt with the motion issues. In this study a pulsed ASL sequence was used which is known to invert a stack of blood within the labeling region independent of blood velocity. However, pseudocontinuous ASL (pCASL) may perform better as it is possible to generate longer boluses which therefore increases the SNR (De Vis et al., 2013b). Unfortunately, the labeling efficiency of this technique is dependent on the blood velocity which is highly variable in the neonates (Wang et al., 2006) and the increased energy absorption related to pCASL has to be carefully considered when scanning the small neonates. Future studies should focus on the adaptation of pCASL for neonates. It could also be interesting to use a look-locker readout (Gunther et al., 2001) or a QUASAR sequence (Petersen et al., 2006) in this patient population as it provides cerebral blood flow and arterial arrival time information at the same time. However, multiple low flip angle readouts compromise the SNR and are therefore difficult to perform in neonates. Although the T1 of blood generally is longer in the neonates than in the adults, which essentially will make you label bolus last longer, then the signal to noise remains low in the neonates. This is due not only to their intrinsic low perfusion but also to the fact that motion can be a problem when they wake up during the scan. Hardware improvements and optimization of e.g. pCASL for the neonates may improve the situation. However, good strategies for dealing with motion may be even more beneficial in this patient population. For instance, the potential benefits of high signal-to-noise 3D readout strategies with subsequent image registration and prospective motion correction schemes should be investigated in future.

Another limitation of this study is that the quantification of the venous oxygenation was based on earlier obtained relations between the T2 of blood, hematocrit and the venous oxygenation determined on bovine in vitro blood samples (Lu et al., 2012). Although bovine blood has physiologic and MR properties comparable to human blood (Benga and Borza, 1995), lower methemoglobin levels in an in vivo situation may influence the relation (Farahani et al., 1999), the hematocrit range on which the relation was determined was narrower than the one found in our population and fetal hemoglobin present in neonates may influence the relation. Though, fetal hemoglobin only differs from adult hemoglobin in the type of globin chains present, therefore, it is unlikely that the paramagnetism of fetal deoxyhemoglobin is substantially different from that of adult deoxyhemoglobin. We estimated the hematocrit based on an earlier obtained (strong) relation between neonatal blood T1 and hematocrit (Varela et al., 2011). The hematocrit values found with this method reflect the developmental changes during fetal and neonatal hematopoiesis, with lower values in the preterm born infants than in the term born infants with hypoxic–ischemic encephalopathy (Proytcheva, 2009), and lower hematocrit levels in the preterm born infants imaged at term-equivalent age (Palis and Segel, 1998). However, the hematocrit values seem to be in the lower range of earlier reported neonatal values (28–67%) (Christensen, 2000; Geaghan, 2005) and were also lower than the clinical hematocrit values which were available in some infants. Our clinical hematocrit values were comparable to earlier published values; 41% in preterms of 26 to 29 week gestation (Forestier et al., 1991) and 33% in term born infants with a postnatal age of 11 to 12 weeks (Matoh et al., 1971). The underestimation of the hematocrit may be due to the effect of blood oxygen saturation on the T1 of blood, with higher oxygen saturation increasing the T1 of blood (Lu et al., 2004) and thereby decreasing the estimated hematocrit as the formula does not take into account the oxygen saturation (Varela et al., 2011). This may have affected the results we found. However, we did investigate the relation between the clinical hematocrit and the T1o-derived hematocrit and found a strong relationship. As well, the relation between clinical hematocrit and R<sub>1</sub> fell within the confidence interval of Varela’s relation (Varela et al., 2011) and we found very strong relations between the Y<sub>o</sub>, OEF and CMRO<sub>2</sub> measurements calculated using the T1o-derived hematocrit and the clinical hematocrit suggesting that the potential error introduced by our hematocrit estimate may not be too much of a problem.

At last, due to the limited sample size, we could not correct for the influence of sedation, antiepileptic treatment, sleep stage (Bangash et al., 2008), or ventilation modus (Milan et al., 2009) on the brain oxygen consumption. Therefore, further data are needed to determine whether drug-therapy influences the observations found in this study, to investigate if the results of this study can be reproduced in a larger sample size and to investigate the origin of the differences which we found.

To summarize, we presented a non-invasive technique able to evaluate venous oxygenation, oxygen extraction fraction and oxygen consumption. Obtained results were comparable to reference values obtained with invasive techniques and preliminary results obtained with non-invasive MRI techniques. We demonstrated that the technique can be used to detect changes with postnatal age or to detect differences in between patient categories. Differences in oxygen extraction fraction could reflect lower oxygen consumption as a reflection of...
brain maturation or as a result of neuronal loss, or can be caused by increased cerebral blood flow due to immature or impaired autoregulation.

References


