Foetal growth, birth transition, enteral nutrition and brain light scattering

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If the brain structure is assessed at neonatal intensive care units, covert clinical events related with subtle brain injury might be identified. The reduced scattering coefficient of near-infrared light ($\mu'_S$) obtained using time-resolved near-infrared spectroscopy from the forehead of infants is associated with gestational age, body weight and Apgar scores, presumably reflecting subtle changes of the brain related to foetal growth and birth transition. One hundred twenty-eight preterm and term infants were studied to test whether $\mu'_S$ obtained from the head at term-equivalent age is associated with foetal growth, birth transition and nutritional status after birth, which are key independent variables of developmental outcomes. As potential independent variables of $\mu'_S$, birth weight, Apgar scores, age at full enteral feeding and post-conceptional age at the study were assessed to represent foetal growth, birth transition and nutritional status after birth. Subsequently, higher $\mu'_S$ values were associated with higher Apgar scores ($p = 0.003$) and earlier establishment of enteral feeding ($p < 0.001$). The scattering property of near-infrared light within the neonatal brain might reflect changes associated with birth transition and nutritional status thereafter, which might be used as a non-invasive biomarker to identify covert independent variables of brain injury in preterm infants.

Advances in neonatal intensive care have significantly improved the survival rate of preterm infants12. However, a considerable fraction of extremely preterm infants develop cognitive impairments even in the absence of major cerebral lesions, such as intracranial haemorrhage and periventricular leukomalacia3,4. Magnetic resonance imaging (MRI) studies in preterm infants have demonstrated the relationship between subtle brain lesions at term equivalent age and long-term cognitive impairments5–7. However, because of the cost, time and safety associated with the scan, MRI is usually performed only once before discharge from the hospital, causing difficulty in identification of the upstream events associated with subtle brain lesions. Reliable tools for the assessment of subtle change of the brain structure, which can be assessed before and after clinical events at the cot-side, may help distinguish the upstream events responsible for subtle cerebral lesions and cognitive impairments in preterm infants.

Near-infrared spectroscopy (NIRS) is a handy, non-invasive tool, which has been used to analyse the tissue oxygen metabolism in the brains of newborn infants8–11. Near-infrared light penetrates the intact scalp, skull and cerebral tissue more efficiently than visible light, and is mainly absorbed by blood haemoglobin, the level of which depends on the binding of haemoglobin to oxygen12. Thus, fractions of oxygenated and deoxygenated haemoglobin are calculated using light absorption coefficient ($\mu_a$) obtained from the near-infrared light of different wavelengths13. Time-resolved near-infrared spectroscopy (TR-NIRS) is a relatively new technique, which enables simultaneous quantification of $\mu_a$ and reduced scattering coefficient ($\mu'_S$)14,15. Unlike $\mu_a$, predominantly provides information regarding tissue oxygenation, $\mu'_S$ is an index of light scattering, which is theoretically determined by the structural complexity of tissue15. When preterm infants were studied shortly after birth, $\mu'_S$ values

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obtained from the forehead showed a positive linear correlation with gestational age\textsuperscript{16}. Our study in preterm and term infants further confirmed that μ\textsubscript{S}' values obtained shortly after birth were associated with variables, such as antenatal glucocorticoid, emergency delivery, gestational age, body size, Apgar scores, requirement for mechanical ventilation and blood gas data at birth, suggesting the possibility that μ\textsubscript{S}' might reflect subtle structural changes in the brain associated with antenatal growth, peripartum stress and birth transition\textsuperscript{17}. However, little is known regarding the relationship between μ\textsubscript{S}' values obtained from the head of newborn infants and their downstream clinical outcomes.

The aim of this study was to test the association of μ\textsubscript{S}' measured at term-equivalent period with intrauterine growth, birth transition and nutrition after birth, which are short-term surrogate markers for neurodevelopmental outcomes of hospitalised newborn infants\textsuperscript{18–21}.

### Results

Four infants, who developed grade III/IV intraventricular haemorrhage, and one infant, who developed hypoxic-ischaemic encephalopathy, were excluded, leaving 128 infants within the final study cohort (Fig. 1). These infants had a gestation period of 32.0 ± 4.2 weeks and weighed 1564 ± 688 g at birth, and were studied on 44.8 ± 28.3 days of age or 38.6 ± 2.1 weeks post-conceptional age (Table 1).

For the left and right temporal regions and the posterior region, data were not obtained for 8, 8 and 21 infants, respectively, because of insufficient signals from the head (n = 15), poor probe contact (n = 4) and the use of a cap device for non-invasive respiratory support (n = 2). No further data were excluded because of their poor quality or reproducibility. The mean μ\textsubscript{a} and μ\textsubscript{S}' values for all wavelengths and head positions were 0.126 ± 0.025 cm\textsuperscript{-1} and 6.453 ± 1.416 cm\textsuperscript{-1}, respectively.

#### Dependence of μ\textsubscript{a} and μ\textsubscript{S}' on wavelengths and head positions.

The wavelength of 836 nm was associated with higher μ\textsubscript{a} values, whereas the wavelength of 791 nm was associated with lower μ\textsubscript{a} values compared to those of 761 nm (both \(p < 0.001\)) (Table 2). The right temporal and posterior regions of the head were associated with higher μ\textsubscript{a} values compared to those of the anterior region (both \(p < 0.001\)).

The wavelengths of 791 and 836 nm were associated with lower μ\textsubscript{S}' values compared to those of 761 nm (both \(p < 0.001\)). The left and right temporal and posterior regions of the head were associated with higher μ\textsubscript{S}' values compared to the anterior region (all \(p < 0.001\)).

#### Dependence of μ\textsubscript{a} and μ\textsubscript{S}' on clinical variables: univariate analysis.

The higher μ\textsubscript{a} values were positively associated with gestational age (\(p = 0.001\)), body weight at birth (\(p < 0.001\)), blood haemoglobin level at study (\(p < 0.001\)) and μ\textsubscript{S}' values (\(p < 0.001\)), and negatively associated with antenatal glucocorticoid (\(p < 0.001\)), cord blood pH (\(p = 0.003\)) and postnatal age at study (\(p = 0.001\)); relationships with multiple pregnancy (\(p = 0.016\)), head circumference at birth (\(p = 0.005\)) and body weight at study (\(p = 0.036\)) were lost after correction for multiple comparisons (all adjusted for the wavelengths and head positions; Table 2 and Fig. 2).

The μ\textsubscript{S}' level was positively associated with gestational age (\(p < 0.001\)) and μ\textsubscript{a} values (\(p < 0.001\)), and negatively associated with indomethacin for patent ductus arteriosus (\(p < 0.001\)) and postnatal age to achieve full enteral feeding (\(p < 0.001\)); relationships with antenatal glucocorticoid (\(p = 0.013\)), body weight and head circumference at birth (both \(p = 0.012\)), Apgar scores at 1 and 5 min (\(p = 0.039\) and 0.029, respectively) and postnatal age at study (\(p = 0.003\)) were lost after correction for multiple comparisons (all adjusted for the wavelengths and head positions; Table 2). See Online Supplemental Tables S1–S3 for findings from analyses performed for each wavelength.

#### Dependence of μ\textsubscript{a} and μ\textsubscript{S}' on clinical variables: multivariate analysis.

Higher μ\textsubscript{a} values were associated with greater age to achieve full enteral feeding (\(p = 0.049\)), greater post-conceptional age at study...
(p = 0.015), higher blood haemoglobin levels at study (p < 0.001) and higher μS’ values (p < 0.001) (Table 3). Higher μS’ values were associated with higher Apgar scores at 5 min (p = 0.003), smaller age to achieve full enteral feeding (p < 0.001) and higher μa values (p < 0.001). See Online Supplemental Tables S4–S6 for findings from analyses performed for each wavelength.

Discussion
Building on previous studies of TR-NIRS, which suggested that the light scattering within the brain shortly after birth is dependent on variables related to foetal growth, antenatal stress and birth transition, we have demonstrated that higher μS’ values obtained at term-equivalent age were associated with higher Apgar scores and earlier establishment of enteral nutrition. μS’ can be a unique and clinically useful biomarker of subtle changes in the brains of newborn infants with respect to antenatal stress, birth transition and nutritional status after birth. Light scattering within a tissue theoretically increases with relatively more complex microstructures due to increased reflection and path length of near-infrared light14. Thus μS’ has a potential to provide microstructural information of the brain. Ijichi and colleagues first reported that μS’ values of near-infrared light obtained shortly after birth from the foreheads of newborn infants with a gestation age of 30–41 weeks depended on gestational age16. Our previous study confirmed that μS’ values obtained from the foreheads of preterm and term infants assessed shortly after birth were dependent on body size and Apgar scores, as well as on gestational age. These findings suggest the possible utility of μS’ values as a non-invasive marker to evaluate subtle differences in the brain subsequent to foetal maturation, antenatal stress and birth transition17. Our previous study confirmed that μS’ values obtained from the foreheads of preterm and term infants assessed shortly after birth were dependent on body size and Apgar scores, as well as on gestational age. These findings suggest the possible utility of μS’ values as a non-invasive marker to evaluate subtle differences in the brain subsequent to foetal maturation, antenatal stress and birth transition17. Our current study further verified that the μS’ value obtained at term equivalent period is associated with both clinical variables at birth and those related to the nutritional status of the infant after birth. Intrauterine growth and maturation, intrapartum stress and response and postpartum nutrition and growth constitute key independent variables of the neurodevelopmental outcomes of the infant18–21. If the consequence of the intrinsic maturity, extrinsic stress, birth transition and nutritional status of the infant can be assessed using μS’ values obtained from the heads of newborn infants, along with other substantiations, μS’ might serve as a clinically useful biomarker of cerebral maturation and a

| Maternal and antenatal variables          | Value |
|-------------------------------------------|-------|
| Antenatal glucocorticoid                   | 61 (47.7%) |
| Multiple pregnancy                         | 39 (30.5%) |
| Emergency caesarean delivery               | 55 (43.0%) |

| Variables at birth                        | Value     |
|-------------------------------------------|-----------|
| Gestational age (week)                    | 32.0 ± 4.2 |
| Body weight at birth (g)                  | 1564 ± 688 |
| Z-score of above                          | −0.9 ± 1.3 |
| Head circumference at birth (cm)          | 28.3 ± 3.5 |
| Z-score of above                          | −0.2 ± 1.1 |
| Male sex                                  | 68 (53.1%) |
| Cord blood pH                             | 7.299 ± 0.146 |
| Apgar score (1 min)                       | 7 (4, 8)  |
| Apgar score (5 min)                       | 8 (7, 9)  |
| Hypoglycaemia < 48 h of birth             | 8 (6.3%)  |

| Variables during hospital stay             | Value     |
|-------------------------------------------|-----------|
| Indomethacin for patent duc tus arteriosus | 38 (29.7%) |
| Surgical closure of patent duc tus arteriosus | 1 (0.8%)   |
| Grade I/II intraventricular haemorrhage   | 6 (4.7%)  |
| Perventricular leukomalacia                | 1 (0.8%)  |
| Full enteral feeding ≥ 100 mL/kg/d (day)   | 7.7 ± 5.3 |
| Days on invasive ventilation              | 10.3 ± 18.4 |
| Chronic lung disease*                     | 22 (17.2%) |

| Variables at study                        | Value     |
|-------------------------------------------|-----------|
| Post-conceptional age (week)              | 38.6 ± 2.1 |
| Postnatal age (day)                       | 44.8 ± 28.3 |
| Body weight (g)                           | 2775 ± 408 |
| Blood haemoglobin (g/dL)                  | 12.6 ± 2.4 |
| μa (cm⁻¹)                                 | 0.126 ± 0.025 |
| μS’ (cm⁻¹)                                | 6.453 ± 1.416 |

Table 1. Background characteristics of 128 infants within the study cohort. Values are number (%), mean ± standard deviation or median (lower/upper quartiles). μa, absorption coefficient, μS’ reduced scattering coefficient. *Assessed at 36 weeks post-conceptional age (or on day 28 for those born later than 32 weeks gestation).
damage. Future studies need to address the contribution of other potential independent variables of light scattering as measured from the scalp, such as the gyration of the brain and developmental changes in the layer of cerebrospinal fluid.

With regard to the absorption of near-infrared light, only modest relationships were observed between higher $\mu_a$ values and longer time to achieve full enteral feeding and greater post-conceptional age at the time of the study; robust correlations were only observed between $\mu_a$ values and priori covariates of the wavelengths of light, head position and blood haemoglobin concentration at the time of the study. Given that absorption of near-infrared light within the range of 750–850 nm is primarily determined by the tissue haemoglobin concentrations\(^{14,15}\), $\mu_a$ values might reflect the maturation of the cerebral tissue via increased complexity of the cerebral vessels and subsequent blood volume. Progression of anaemia and increase in the cerebral blood flow and volume with increasing postnatal age might also affect the dependence of $\mu_a$ values on clinical variables\(^{12}\).

**Strengths and limitations.** We were able to elucidate the clinical variables potentially determining the property of light absorption and scattering within the brain in a relatively large cohort of newborn infants. However, we were unable to present a direct association between $\mu_s'$ values and microstructure of the brain. As described in the previous section, the observed relationships between $\mu_a$, $\mu_s'$ and clinical variables can be affected by a range of clinical biases. For example, extremely preterm infants are relatively anaemic at birth and the anaemia progresses with postnatal age without transfusion, potentially leading to lower blood haemoglobin and $\mu_a$

| Independent variables | Correlation with $\mu_a$ | Correlation with $\mu_s'$ |
|-----------------------|-------------------------|-------------------------|
|                       | B  | 95% CI     | $p$       | B  | 95% CI     | $p$         |
| Wavelength (vs. 761 nm)\(^1\) |     |            |           |     |            |           |
| 836 nm                | 0.663 | 0.530   | 0.797   | <0.001 | −0.337 | −0.375 | −0.300 | <0.001 |
| 791 nm                | −1.508 | −1.634 | −1.382 | <0.001 | −0.169 | −0.227 | −0.112 | <0.001 |
| Position (vs. anterior)\(^2\) |     |            |           |     |            |           |
| Posterior             | 3.140 | 2.809   | 3.471   | <0.001 | 1.414 | 1.129 | 1.699 | <0.001 |
| Right                 | 0.449 | 0.199   | 0.699   | <0.001 | 1.554 | 1.302 | 1.807 | <0.001 |
| Left                  | 0.196 | −0.015 | 0.406   | 0.068 | 0.909 | 0.667 | 1.152 | <0.001 |
| Maternal and antenatal variables\(^3\) |     |            |           |     |            |           |
| Male sex              | −0.530 | −1.144 | 0.083   | 0.090 | −0.210 | −0.528 | 0.107 | 0.195 |
| Multiple pregnancy    | −0.656 | −1.190 | −0.121  | 0.016 | −0.241 | −0.627 | 0.144 | 0.220 |
| Antenatal glucocorticoid | −1.274 | −1.829 | −0.719  | <0.001 | −0.392 | −0.702 | −0.083 | 0.013 |
| Hypoglycaemia < 48 h of birth | 0.746 | −1.385 | 2.878   | 0.492 | 0.649 | −0.233 | 1.531 | 0.149 |
| Variables at birth\(^4\) |     |            |           |     |            |           |
| Indomethacin for patent ductus arteriosus | −0.481 | −1.084 | 0.122   | 0.118 | −0.519 | −0.810 | −0.228 | <0.001 |
| Emergency caesarean delivery | −0.468 | −1.049 | 0.113   | 0.114 | −0.296 | −0.603 | 0.101 | 0.058 |
| Chronic lung disease* | −0.484 | −1.059 | 0.092   | 0.099 | −0.345 | −0.711 | 0.022 | 0.065 |
| Intraventricular haemorrhage | −0.254 | −0.742 | 0.235   | 0.309 | 0.302 | −0.396 | 1.000 | 0.396 |
| Gestational age (week) | 0.167 | 0.066   | 0.268   | 0.001 | 0.074 | 0.035  | 0.112 | <0.001 |
| Body weight (kg)      | 0.124 | 0.072   | 0.176   | <0.001 | 0.034 | 0.007  | 0.060 | 0.012 |
| Z-score of above      | 0.129 | −0.076 | 0.334   | 0.218 | −0.041 | −0.157 | 0.074 | 0.484 |
| Head circumference (cm) | 0.142 | 0.043   | 0.240   | 0.005 | 0.060 | 0.013  | 0.106 | 0.012 |
| Z-score of above      | −0.039 | −0.382 | 0.305   | 0.824 | −0.140 | −0.280 | 0.000 | 0.050 |
| Cord blood pH per 0.1 change | −0.280 | −0.466 | −0.093  | 0.003 | −0.054 | −0.142 | 0.035 | 0.233 |
| Apgar score (1 min)   | 0.009 | −0.128 | 0.146   | 0.899 | 0.064 | 0.003  | 0.125 | 0.039 |
| Apgar score (5 min)   | −0.097 | −0.314 | 0.120   | 0.382 | 0.088 | 0.009  | 0.168 | 0.029 |
| Variables at study\(^5\) |     |            |           |     |            |           |
| Postnatal age (day)   | −0.021 | −0.033 | −0.008  | 0.001 | −0.008 | −0.014 | −0.003 | 0.003 |
| Post-conceptional age (week) | 0.131 | −0.048 | 0.310   | 0.151 | 0.066 | −0.013 | 0.145 | 0.104 |
| Body weight (kg)      | 0.065 | 0.004   | 0.126   | 0.036 | −0.006 | −0.044 | 0.032 | 0.753 |
| Blood haemoglobin (g/dL) | 0.568 | 0.462   | 0.675   | <0.001 | 0.057 | −0.018 | 0.133 | 0.135 |
| Full enteral feeding ≥ 100 mL/kg/day | −0.027 | −0.077 | 0.022   | 0.278 | −0.052 | −0.077 | −0.027 | <0.001 |
| $\mu_s'$ (cm\(^{-1}\)) | 0.573 | 0.314   | 0.833   | <0.001 | Not applicable

| $\mu_a$ (cm\(^{-1}\)) | Not applicable | 22.521 | 16.167 | 28.875 | <0.001 |

Table 2. Dependence of $\mu_a$ and $\mu_s'$ on clinical variables: univariate analysis. B regression coefficient, CI confidence interval, $\mu_a$ absorption coefficient, $\mu_s'$ reduced scattering coefficient. *Assessed at 36 weeks post-conceptional age (or on day 28 for those born later than 32 weeks gestation). Findings are adjusted for the wavelengths of near-infrared light** and position of the head\(^1\).
Figure 2. Dependence of $\mu_s'$ on $\mu_a$ in four head regions. Scatter plots demonstrating relationships between $\mu_a$ and $\mu_s'$ obtained from the anterior (A), left-temporal (B), right-temporal (C) and posterior (D) head regions for wavelengths of 761 nm (circle), 791 nm (triangle) and 836 nm (cross). $\mu_a$, absorption coefficient. $\mu_s'$, reduced scattering coefficient.

Table 3. Dependence of $\mu_a$ and $\mu_s'$ on clinical variables: multivariate analysis. The model is also adjusted for the wavelengths of near-infrared light and position of the head. $B$ regression coefficient, CI confidence interval, $\mu_a$, absorption coefficient, $\mu_s'$, reduced scattering coefficient.

| Correlation with $\mu_a \cdot 10^2$ | Correlation with $\mu_s'$ |
|-----------------------------------|---------------------------|
| **Independent variables**         | **B** | **95% CI** | **Upper** | **P** | **B** | **95% CI** | **Upper** | **p** |
| Body weight at birth (per 100 g)  | $-0.015$ | $-0.053$ | $0.022$ | $0.420$ | $-0.021$ | $-0.049$ | $0.007$ | $0.136$ |
| Apgar score (5 min)               | $-0.011$ | $-0.165$ | $0.143$ | $0.890$ | $0.096$ | $0.033$ | $0.159$ | $0.003$ |
| Full enteral feeding $\geq 100$ mL/kg/d (day) | $0.027$ | $0.000$ | $0.053$ | $0.049$ | $-0.051$ | $-0.077$ | $-0.026$ | $<0.001$ |
| Post-conceptional age at study (week) | $0.141$ | $0.028$ | $0.255$ | $0.015$ | $0.055$ | $-0.009$ | $0.119$ | $0.093$ |
| **Covariates**                    |       |           |         |       |       |           |         |       |
| Antenatal glucocorticoid          | $-0.156$ | $-0.525$ | $0.214$ | $0.409$ | $-0.035$ | $-0.335$ | $0.265$ | $0.820$ |
| Multiple pregnancy                | $-0.254$ | $-0.587$ | $0.079$ | $0.135$ | $-0.153$ | $-0.486$ | $0.180$ | $0.369$ |
| Male sex                          | $-0.080$ | $-0.436$ | $0.277$ | $0.662$ | $-0.225$ | $-0.496$ | $0.046$ | $0.103$ |
| Blood haemoglobin (g/dL)          | $0.569$ | $0.466$ | $0.671$ | $<0.001$ Not involved |       |           |         |       |
| $\mu_a$ (cm$^{-1}$)               | $0.450$ | $0.303$ | $0.596$ | $<0.001$ Not applicable |       |           |         |       |
| $\mu_s'$ (cm$^{-1}$)              | Not applicable |       |       |       |       |       |           |         |       |
| $\mu_s'$ (cm$^{-1}$)              | $22.692$ | $15.952$ | $29.432$ | $<0.001$ |       |           |         |       |
levels with greater gestational age at birth and greater postnatal age at the time of TR-NIRS study. Although we 
carefully selected independent variables and covariates to minimise the bias, the findings might still be affected 
by the bias derived from the collinearity between the variables. Our study cohort comprised newborn infants, 
who were hospitalised at a tertiary neonatal intensive care unit. Although the observed \( \mu_a \) and \( \mu_s' \) values were 
comparable to those reported in healthy newborn infants\(^\text{25} \), extrapolation of our findings into physiological 
transition and growth in healthy newborn infants must be done cautiously. Finally, the longitudinal follow-up 
study of the study population is still underway, resulting in the lack of outcome information in association with 
the light absorption and scattering properties.

Conclusions
The \( \mu_s' \) values of the near-infrared light obtained at term-equivalent period from the heads of newborn infants 
were associated with Apgar scores and postnatal age when full enteral feeding was achieved, suggesting a cor-
relation between the light scattering property and stress-response at birth and nutritional status of the infant 
thereafter. With further validations, \( \mu_s' \) might serve as a biomarker to distinguish the variation of the microstruc-
tural complexity of the brain tissue subsequent to different maturational stage, antenatal stress, tissue damage 
and repair, nutritional status and growth. Associations between the \( \mu_s' \) values and detailed clinical courses, 
macro- and microstructural MRI findings and neuro-developmental outcomes need to be addressed to assess 
the clinical utility of this non-invasive cot-side tool.

Materials and methods
This study was conducted in compliance with the Declaration of Helsinki under the approval of the Ethics 
Committee of Kurume University School of Medicine (reference number: 12128). Informed parental consent 
was obtained for each participating newborn infant. All methods were carried out in accordance with relevant 
guidelines and regulations.

Study population.
This study was performed as a secondary analysis of a prospective longitudinal study, 
which was performed between June 2009 and January 2015 to serially collected the TR-NIRS data of pre-
term and term infants hospitalised at a tertiary neonatal intensive care centre of Kurume University Hospital 
(Kurume, Fukuoka, Japan). Independent variables of \( \mu_s' \) values obtained shortly after birth from a part (n = 60) 
of the current cohort have been reported in a previous study\(^\text{17} \). Of 136 newborn infants within the original study 
cohort, 132 infants, who had TR-NIRS data obtained between 34 and 42 weeks postconceptional age, were con-
sidered. Infants with chromosomal aberration, malformation syndrome, grade III/IV intraventricular haemor-
rhage, hypoxic-ischaemic encephalopathy, congenital hydrocephalus and other major cerebral anomalies were 
excluded.

Data collection.
The \( \mu_a \) and \( \mu_s' \) values were obtained from the heads of the infants for three wavelengths, 
761, 791 and 836 nm, using a TR-NIRS system (TRS-10, Hamamatsu Photonics K.K., Hamamatsu, Shizuoka, 
Japan)\(^\text{37} \). This system employs the time-correlated single photon counting method to create time response pro-
files of pulsed laser light penetrating an object. The time response profiles were then fitted into a photon diffusion 
equation using the nonlinear least square fitting method to obtain \( \mu_a \) and \( \mu_s' \) for each wavelength\(^\text{14} \). Although 
the data acquisition for the original study was repeated with intervals of approximately 1 week from birth to 
discharge, for the current study, a particular value obtained between 34 and 42 weeks of post-conceptional age 
(closest to 40 weeks gestation if there were multiple records) was used to represent each infant.

Data were acquired when the infant was clinically stable and asleep or calmly awake. The TR-NIRS probes 
were inserted into a rubber holder, with an inter-optode distance of 3 cm, and was applied to a relatively flat part 
of the head. Data acquisition (10 s) was repeated five times for each of the frontal, left and right tempo-parietal 
and occipital regions by repositioning the probe each time. In our previous study, which acquired TR-NIRS 
data using the same protocol to the current one\(^\text{17} \), standard deviations of \( \mu_a \) and \( \mu_s' \) values for five successively 
obtained data within the same head position and infant were, in average, 2.4% and 2.7%, respectively. Based on 
these small intra-individual and intra-regional differences in \( \mu_a \) and \( \mu_s' \) values, five readings each of \( \mu_a \) and \( \mu_s' \) 
were averaged for each brain region. We confirmed the degree of fit to the photon diffusion equation using the 
conversion chi-square value index of between 0.8 and 1.2\(^\text{22} \). Data were not collected for brain regions with poor 
probe contact (typically due to the lack of flat surfaces or use of cap devices for non-invasive respiratory support), 
poor fit to the photon diffusion equation or insufficient signal-to-noise ratio with the count rate < 100 K counts/s 
or relative dark- to peak-count ratio of > 0.1. The data were retrospectively assessed to identify those with poor 
quality or intra-regional reproducibility before being processed for further analysis.

Clinical information.
The clinical background information was obtained from the electronic records of the patients, including (1) maternal and antenatal variables (antenatal glucocorticoid, multiple pregnancies and 
emergency caesarean delivery), (2) variables at and shortly after birth (sex, cord blood pH, Apgar scores at 1 min 
and 5 min, gestational age, body weight, head circumference, hypoglycaemia within 48 h of birth, indomethacin 
for the treatment of the patent ductus arteriosus, grade I/II intraventricular haemorrhage and periventricular 
leukomalacia, (3) variables associated with clinical variables of infants after the transitional period (body 
weight on the day of study, age when full enteral feeding of > 100 ml/kg/d was achieved and chronic lung disease 
assessed 36 weeks post-conceptional age or on day 28, whichever was later). In order to assess the influence of 
intratraume growth on \( \mu_s' \) values, body weight and head circumference at birth were expressed as z-scores in 
accordance with the New Japanese Neonatal Anthropometric Charts for Gestational Age at Birth\(^\text{24} \).
Data analysis. To minimise biases owing to missing data, multiple imputation of the missing values of less than 10% (excluding for \( \mu_a \) and \( \mu_S' \)) was performed (n = 5 imputations), based on the correlation between variables with missing values and other characteristics of the participants (SPSS version 22.0, IBM, Armonk, NY, U.S.A.). Although the property of \( \mu_S' \) was out of our study scope, independent variables of both \( \mu_a \) and \( \mu_S' \) were assessed to clarify the possible influence of light absorption to the relationship between \( \mu_a \) and \( \mu_S' \) values and clinical variables. The generalised estimating equation with a linear model was used to account for repeated sampling of TR-NIRS data for three near-infrared light wavelengths and four head regions. Although the influence of the wavelength is much greater on \( \mu_a \) than on \( \mu_S' \), the three wavelengths were incorporated within the model for consistency in the analytical procedure. Crude effects of clinical variables on \( \mu_a \) and \( \mu_S' \) values were assessed using the univariate model adjusting for the wavelengths and head positions. \( p \) values < 0.002 were assumed to be significant, correcting multiple comparisons of 25 variables. The final models to explain \( \mu_a \) and \( \mu_S' \) values were developed based on our hypothesis, which employed the body weight at birth, Apgar scores at 5 min, age to achieve full enteral feeding and post-conceptional age at study; the model was also adjusted for priori covariates, which were known independent variables of clinical outcomes (antenatal glucocorticoid, multiple pregnancies and sex), \( \mu_a \) (wavelength, position of the head and \( \mu_S' \)) and \( \mu_S' \) (wavelength, position of the head and \( \mu_a \)). Data were presented as mean ± standard deviation unless specified otherwise.

Received: 20 March 2021; Accepted: 14 October 2021
Published online: 29 October 2021

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Acknowledgements
The authors thank the newborn infants and their families for their participation and cooperation with the study, and Ms. Chiho Yoshii and Chiaki Ueno for their consistent support. This work was supported by the Japan Society for the Promotion of Science (Grants-in-Aid for Scientific Research 20H00102, 16K09005, 18K07795 and 18K15722).
Author contributions
O.I., S.I., T.K., M.O. and K.T. designed the study. S.I., T.K., K.T., K.K., S.T., M.K., M.S. and Y.A. participated in the patient recruitment and data collection. S.I., Y.C.L. and Y.A. performed the statistical analyses. O.I., S.I., T.K., K.T., E.O. and S.S. contributed to the interpretation of the findings. O.I. and S.I. drafted the manuscript, which was critically reviewed by T.K., K.T., K.K., M.K., Y.C.L., M.S., Y.A., S.T., E.O. and S.S. O.I., S.I., T.K., K.T., K.K., M.K., Y.C.L., M.S., Y.A., S.T., E.O. and S.S. approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-00624-9.

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