

RESEARCH ARTICLE

# Elevated renal tissue oxygenation in premature fetal growth restricted neonates: An observational study

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## Abstract

### Background

Fetal growth restriction (FGR) is associated with an increased risk for kidney disease in later life. Studies reporting on early signs of renal disturbances in FGR are sparse and mostly include invasive measurements, which limit the possibility for early identification and prevention. We aim to investigate whether renal tissue oxygen saturation (rSO<sub>2</sub>) measured with near-infrared spectroscopy (NIRS) and the derived value fractional tissue oxygen extraction (FTOE) differ between premature FGR and control neonates in the first three days after birth.

### Methods

Nine FGR and seven control neonates born <32 weeks of gestation were included. FGR was defined as biometry <p10 combined with prenatal signs of placental insufficiency. Renal rSO<sub>2</sub> was measured continuously with NIRS for 72 hours. FTOE was calculated as: (arterial saturation-rSO<sub>2</sub>)/arterial saturation. Renal artery blood flow (pulsatility and resistance index) was measured within 24 hours after birth. A linear mixed model approach was used (intercept ± slope = *r*) to analyze the NIRS parameters.

### Results

Renal rSO<sub>2</sub> was higher in FGR neonates compared to controls (94% vs. 83%; *p*<sub>group</sub> = 0.002). During the first three days after birth, renal rSO<sub>2</sub> decreased in FGR neonates and increased in controls (*r* = -0.25 vs. *r* = 0.03; *p*<sub>interaction</sub> = 0.001). Renal FTOE was lower in FGR neonates (0.02 vs. 0.14; *p*<sub>group</sub> = 0.01) and increased slightly during three days after birth, while it remained stable in controls (*r* = 0.003 vs. *r* = -0.0001; *p*<sub>interaction</sub> = 0.001). Renal artery blood flow was similar between groups.

design, data collection and analysis, decision to publish, or preparation of the manuscript

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## Conclusions

FGR neonate kidneys showed higher  $rSO_2$  as measured with NIRS and lower derived values of FTOE in the first three days after birth. We speculate that this was caused by either a reduced oxygen consumption due to impaired renal maturation or increased renal oxygen supply. How these observations correlate with short- and long-term renal function needs further investigation before renal NIRS can be implemented in screening and prevention in clinical practice.

## Introduction

Fetal growth restriction (FGR) is a condition in which the fetus fails to reach its genetic growth potential[1]. It increases the risk of perinatal morbidity and mortality, which is partly due to the higher likelihood of induction of premature delivery[2]. The major cause (70–80%) of FGR is placental insufficiency[3]. The pathophysiology of placental insufficiency is complex, and multiple factors including immunologic, angiogenic, and maternal genetic,—can lead to poor utero-placental perfusion with reduced nutrient and oxygen supply to the fetus resulting in FGR[4].

As a response, blood flow preferentially distributes towards the fetal brain (brain-sparing). This occurs at the expense of perfusion to other organs. Studies report significant differences—however in contradicting direction—in renal blood flow of FGR fetuses compared to controls fetuses[5–7]. An adverse environment can negatively affect nephrogenesis, which completes in utero, and epigenetic programming of renal function[8,9]. This possibly contributes to the increased risk of hypertension and renal disease seen in FGR infants later in life[10].

Unfortunately, measuring renal function in premature neonates is challenging. Early neonatal creatinine and urea levels partly reflect maternal renal function, which limits the validity of these easily measured routine parameters during the first few days after birth. Therefore, studies on early signs of renal disturbances in FGR neonates are sparse and mostly include invasive measurements such as nephron counts or cumbersome measurements such as inulin clearance[11,12].

Near-infrared spectroscopy (NIRS) is a non-invasive bedside technique that is successfully used to continuously measure tissue oxygenation of the neonatal brain during the perinatal transition in preterm neonates. It measures the percentage of oxygenated hemoglobin of arterial, venous, and capillary blood in the tissue region under the sensor ( $rSO_2$ ) by the absorption difference between oxygenated and deoxygenated hemoglobin in the near-infrared spectrum of light. [13–16]

Better understanding of the adaptation of the neonatal kidneys to FGR could contribute to early identification of functional disturbances and prevention of hypertension and renal disease in later life. So far, tissue oxygenation has not yet been measured in FGR neonatal kidneys in comparison to an age-matched control group. The aim of this study is to measure renal tissue oxygen saturation with NIRS and the derived oxygen extraction to assess whether these variables differ between FGR and control neonates during the transitional phase. Either decreased oxygenation due to relatively low perfusion, and therefore low oxygen delivery, or increased oxygenation due to low solute reabsorption, and therefore low oxygen consumption, are conceivable as a mechanism for potentially observed differences.

## Methods

### Study population

This observational study was performed at the Wilhelmina Children's Hospital in preterm neonates (gestational age [GA] <32 weeks) admitted to the neonatal intensive care unit from

July 2016 until April 2017. Nine placental insufficiency-related cases of FGR were compared to seven controls. FGR was defined as estimated fetal weight (EFW) or abdominal circumference (AC) under the 10<sup>th</sup> percentile. Exact percentiles for EFW were determined with the Hadlock formula using GA in weeks and days:  $0.578 + 0.332 * GA - 0.00354 * GA^2$  [17]. Placental insufficiency was defined as abnormal Doppler measurements in fetal middle cerebral artery (maximal velocity >p95), umbilical artery (absent or reversed end-diastolic flow or increased pulsatility index >p95), cerebral-placental ratio (decreased ratio in pulsatility index between umbilical artery and fetal middle cerebral artery <p5), uterine artery (notching or elevated resistance index p>95), or deflecting growth rate in 3 or more consecutive prenatal measurement points. The control group consisted of preterm neonates with normal growth for gestational age. Exclusion criteria were congenital disorders or multiple pregnancies. The Medical Ethical Committee of University Medical Centre Utrecht approved the study on 13-07-2016; protocol number 16-381C. Written informed consent was obtained from parents during pregnancy.

### Study protocol

The study protocol consisted of collecting clinical data and postnatal measurements from the medical records. Maternal comorbidities included cardiovascular disorders, diabetes, thyroid disorders and auto-immune disorders. Exact percentiles for birth weight and head circumference at birth were determined with Intergrowth-21<sup>st</sup> [18]. Neonatal complications included infant respiratory distress syndrome, intraventricular haemorrhage, sepsis and necrotizing enterocolitis. Treatment with mechanical ventilation during the period of admission was defined by intubation. The Score for Neonatal Acute Physiology with Perinatal Extension-II (SNAPPE-II) was calculated during the first 12 hours after birth as a predictor of mortality and morbidity [19]. Mean blood hemoglobin, arterial CO<sub>2</sub> and CRP levels, as well as urine output (per kg body weight) were determined each day.

**NIRS.** Regional cerebral and renal oxygenations (rSO<sub>2</sub>) were measured continuously with a 2-wavelength (730 and 810 nm) NIRS for 72 hours after birth (INVOS 5100C, Medtronic, Boulder, CO). Sensors were placed as soon as possible after birth; a small adult sensor (Soma-Sensor SAFB-SM, Medtronic) was fixed on the forehead (left and right were alternated), and a neonatal sensor (OxyAlert CNN NIRSsensor) on the flank at the level of the left kidney. Sensor positioning was verified concomitantly with renal ultrasound. BedBase (in-house developed software) was used to simultaneously record SaO<sub>2</sub>, heart rate, systolic and diastolic blood pressure (SBP, DBP) by catheter and rSO<sub>2</sub>. One FGR neonate did not receive an intravascular catheter due to good clinical condition. Fractional tissue oxygen extraction (FTOE) was calculated by SignalBase (off-line version of BedBase) with the formula:  $FTOE = (SaO_2 - rSO_2) / SaO_2$ . A decrease in FTOE can represent either an increase in oxygen supply or a decrease in oxygen consumption [20].

Artefacts were defined as 1) physiologically unexplained dips or peaks of at least 30% between two data points in rSO<sub>2</sub> or in one of the accompanied parameters or 2) changes in rSO<sub>2</sub> combined with severe deformed accompanying variables, alternated with missing data points, together suggesting neonatal movement [21]. After manually removing artefacts, 1-hour periods of consecutive high quality renal rSO<sub>2</sub> data per 3-hour interval were selected. Most studies report tissue oxygenation data with 1-hour interval and therefore we also chose this to increase comparability. In cases of similarly high-quality data in a certain interval, the 1-hour period closest to the middle of the interval was selected to optimize time-based comparison between patients.

**Renal ultrasound.** A renal ultrasound of the right kidney was performed in seven FGR and six control neonates within 24 hours after birth (Aplio 400, Toshiba medical systems,

Zoetermeer, The Netherlands, 7 MHz, convex probe). Pulsatility and resistance index in renal artery and renal length were measured 2–3 times and means were subsequently calculated. Renal ultrasound was not conducted in all patients due to unavailability of investigators and in one case due to poor neonatal condition. Most ultrasounds were performed by the same investigator (DCV) with exception of two ultrasounds.

## Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, NY). To compare groups for clinical and ultrasound data, we used an independent t-test for continuous parametric data, Fisher exact test for categorical data, and Mann-Whitney test for continuous non-parametric data. Non-parametric data were expressed as mean  $\pm$  standard error of the mean (s.e.m), counts (percentage), or median with interquartile range (IQR), respectively. Repeated neonatal measurements (hemoglobin, CRP, CO<sub>2</sub>, urine production per body weight) were tested with two-way repeated ANOVA with Bonferroni's post-hoc analysis to compare mean per day. We used a linear mixed-model statistical analysis for postnatal monitoring and NIRS parameters with individuals as a random effect nested within the groups and compound symmetry as (repeated) covariance type. We did not correct for factors or covariates due to the small sample size. The group effect ( $p_{\text{group}}$ ) and whether the groups behaved differently over time ( $r = \text{slope per 3-hour interval}$ ;  $p_{\text{interaction}}$ ) were investigated. A two-sided p-value  $< 0.05$  was considered significant.

## Results

### Baseline characteristics

No maternal comorbidities were present in the control group, while in the FGR group, one pregnancy was complicated by a thyroid disorder and one by pre-existing hypertension (Table 1). Seven of the FGR neonates' mothers experienced pre-eclampsia. More antihypertensive drugs and fewer antibiotics were used in pregnancies complicated by FGR than in controls. Five of the FGR cases were predicted to be severely growth restricted ( $\text{EFW} < p3$ ), two showed asymmetric growth restriction and seven showed an abnormal ratio in pulsatility index between umbilical artery and middle cerebral artery. Neonatal baseline characteristics are presented in Table 2; note that the gestational age was similar between groups at the time that regional oxygenation was measured with NIRS. Hemoglobin levels per day were higher in FGR vs. control neonates, but remained within reference ranges[22]. CRP, CO<sub>2</sub> levels and urine production per body weight did not differ significantly between groups over time.

### Renal and cerebral rSO<sub>2</sub> and FTOE

Renal rSO<sub>2</sub> was higher in FGR vs. control neonates (Fig 1 and Table 3). During the first three days after birth, the groups behaved differently: renal rSO<sub>2</sub> decreased in FGR neonates but increased in controls. Cerebral oxygenation was higher in FGR vs. control neonates. Cerebral rSO<sub>2</sub> in both groups was similarly stable over time.

Renal FTOE was lower in FGR neonates vs. controls (Fig 2). Renal FTOE increased slightly in FGR neonates during the first three days after birth, while it remained stable in controls. Cerebral FTOE was lower in FGR vs. control neonates and both groups remained stable during the first three days after birth. Arterial oxygen saturation was similar between groups (Table 4).

**Table 1. Maternal and pregnancy characteristics.**

	FGR (n = 9)	Control (n = 7)	p-value
<b>Maternal</b>			
Age in years	29.7 ± 1.3	29.7 ± 1.6	0.98
BMI	25.9 ± 1.7	24.0 ± 1.9	0.47
Ethnicity: Caucasian	4 (44.4)	7 (100)*	<b>0.03</b>
Comorbidities	2 (22.2)	0 (0)	0.48
<b>Pregnancy</b>			
Preeclampsia	7 (78)	0 (0)**	< <b>0.01</b>
Smoking	4 (44)	2 (29)	0.63
Caesarean section	9 (100)	4 (57)	0.06
<b>Medication during pregnancy</b>			
MgSO <sub>4</sub>	8 (89)	6 (86)	1.00
Antihypertensive drugs	7 (78)	1 (14)*	<b>0.04</b>
Antidepressant	0 (0)	1 (14)	0.44
Immuno-depressant	1 (0)	2 (29)	0.18
Insulin	0 (0)	1 (14)	0.44
Antibiotics	2 (22)	6 (86)*	<b>0.04</b>
Paracetamol	3 (33)	2 (29)	1.00
<b>Prenatal ultrasound</b>			
GA at ultrasound measurement	29.6 ± 0.6	26.3 ± 0.8**	< <b>0.01</b>
EFW percentile < p3	5 (56)	0 (0)	0.11
Asymmetric FGR	2 (22)	0 (0)	1.00
UA PI	1.70 ± 0.11	N/A	N/A
UA PI > p95	6 (67)	N/A	N/A
MCA V <sub>max</sub> (cm/s)	39.0 ± 1.9	N/A	N/A
MCA V <sub>max</sub> > p95	1 (17)	N/A	N/A
UA/MCA PI ratio > p95	7 (78)	N/A	N/A

Data shown as mean ± s.e.m. or n (%) and analysed with respectively independent t-test or Fisher exact test. Asymmetric FGR is defined as head circumference/abdominal circumference ratio >p95. Abbreviations: BMI, body mass index; EFW, estimated fetal weight; FGR, fetal growth restriction; GA, gestational age; MCA, middle cerebral artery; MgSO<sub>4</sub>, magnesium sulphate; N/A, not available; PI, pulsatility index; UA, umbilical artery; V<sub>max</sub>, maximal velocity.

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### Blood pressure and heart rate

Heart rate was similar between groups but increased significantly over time in FGR only (Fig 3 and Table 4). SBP and DBP were similar between groups and increased to the same extent in both groups over the 72 hours after birth.

### Renal ultrasound in first day after birth

The renal ultrasound was performed at a similar mean time after birth (FGR: 15.0 ± 2.9 hours after birth vs. control 13.9 ± 2.7 hours after birth; p = 0.80). No significant differences were found in pulsatility index and lower resistance index of the renal artery or kidney length in FGR vs. control neonates (Table 5).

### Discussion

The preterm FGR neonates in our study showed higher renal and cerebral rSO<sub>2</sub> with NIRS during the first three days after birth compared to preterm controls. Additionally, we showed

**Table 2. Baseline characteristics of neonates and neonatal measurements.**

	FGR (n = 9)	Control (n = 7)	p-value
<b>Neonate</b>			
GA at birth in weeks	30.6 ± 0.5	29.1 ± 1.0	0.18
Gender: male	7 (78)	4 (57)	0.60
BW in grams	1106 ± 71	1411 ± 176	0.10
BW percentile	8 (3–17)	74 (70–81)	<b>0.001</b>
HC at birth in cm	26.3 ± 0.7	26.8 ± 1.0	0.67
HC at birth percentiles	16.1 ± 6.1	44.9 ± 9.7	<b>0.02</b>
<b>Medication</b>			
Incorporated antenatal steroids (n, %)	8 (89)	7 (100)	1.00
Postnatal steroids (n,%)	0 (0)	1 (14)	0.44
Antibiotics (n,%)	6 (67)	7 (100)	0.21
Surfactant treatment (n,%)	3 (33)	2 (29)	0.60
Ductus arteriosus treatment (n,%)	0 (0)	1 (14)	0.40
Inotropine (n,%)	0 (0)	0 (0)	1.0
<b>Neonatal health</b>			
<i>Umbilical cord blood</i>			
pH arterial	7.26 ± 0.03	7.33 ± 0.03	0.17
Base excess	-2.67 ± 0.88	-6.00 ± 1.53	0.13
<i>Apgar score &lt;5</i>			
1 min	1 (11)	3 (43)	0.26
5 min	0 (0)	3 (43)	0.06
SNAPPE-II score	5 (0–16)	33 (9–40)	0.09
NICU admission in days	13 (10–20)	17 (16–87)	0.08
Mechanical ventilation	2 (22)	4 (57)	0.30
<i>Neonatal complications</i>			
IRDS	1 (11)	4 (57)	0.11
Sepsis	0 (0)	1 (14)	
IVH	0 (0)	2 (29)	
<b>Neonatal measurements</b>			
<i>CRP in mg L<sup>-1</sup></i>			
Day 1	2.4 ± 0.7	1.7 ± 1.2	>0.99
Day 2	4.4 ± 1.2	2.5 ± 1.7	0.92
Day 3	4.8 ± 2.4	1.5 ± 0.5	0.23
<i>Hemoglobin in mmol L<sup>-1</sup></i>			
Day 1	12.0 ± 0.3	9.8 ± 0.4	< <b>0.001</b>
Day 2	11.4 ± 0.3	9.3 ± 0.3	< <b>0.001</b>
Day 3	11.1 ± 0.4	10.1 ± 0.2	0.07
<i>CO<sub>2</sub> in mmHg</i>			
Day 1	42.6 ± 2.8	43.9 ± 3.1	>0.99
Day 2	42.1 ± 1.2	37.5 ± 2.8	0.52
Day 3	41.7 ± 1.5	42.1 ± 1.4	>0.99
<i>Urine output in ml/kg BW</i>			
Day 1	87.5 ± 11.2	92.1 ± 12.5	>0.99
Day 2	103.5 ± 14.8	110.5 ± 5.5	>0.99

(Continued)

Table 2. (Continued)

	FGR (n = 9)	Control (n = 7)	p-value
Day 3	114.2 ± 7.8	141.2 ± 13.0	0.32

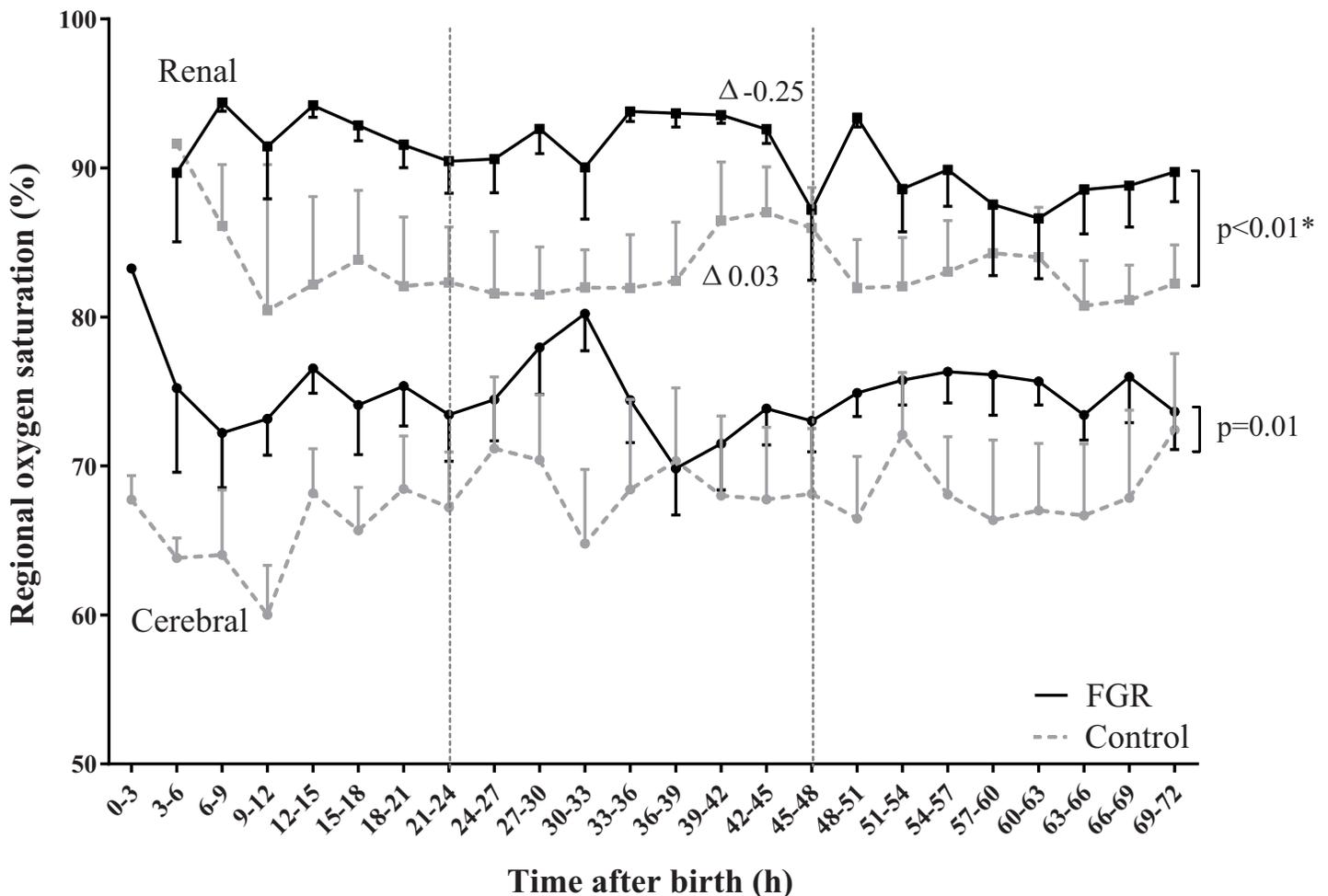
Data shown as mean ± s.e.m., n (%) or median (IQR) and analysed with respectively independent t-test, Fisher exact test, or Mann-Whitney test. The neonatal measurements were tested with two-way repeated measures ANOVA with Bonferroni's post-hoc analysis to compare per day.

Abbreviations: BW, birth weight; CO<sub>2</sub>, carbon dioxide; CRP, C-reactive protein; FGR, fetal growth restriction; GA, gestational age; HC, head circumference; IRDS, infant respiratory distress syndrome; IVH, intraventricular haemorrhage; NICU, neonatal intensive care unit; SNAPPE-II, score for neonatal acute physiology with perinatal extension-II

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that both renal and cerebral FTOE was lower in FGR neonates. Renal artery blood flow did not differ between groups.

To the best of our knowledge, this is the first study in which renal tissue oxygenation was compared between FGR neonates and controls during the transition from intrauterine to extrauterine life. Tanis et al. showed higher renal FTOE in early FGR neonates compared with



**Fig 1. Regional oxygen saturation (rSO<sub>2</sub>).** Renal and cerebral rSO<sub>2</sub> are elevated in fetal growth restriction (FGR) vs. control neonates and over time groups behaved differently in renal rSO<sub>2</sub>. Significant group differences are shown and \* indicates whether the groups behaved differently over time ( $p_{interaction} < 0.05$ ). Δ indicates the slope per 3 hours interval.

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Table 3. Variables measured with or derived from NIRS per neonatal group.

		Intercept	95% CI	P <sub>group</sub>	r	95% CI	P <sub>interaction</sub>
Cerebral rSO <sub>2</sub> (%)	FGR	74.4	70.1; 78.8	0.01	0.04	-0.14; 0.21	0.40
	Control	65.8	61.0; 70.7		0.14	-0.04; 0.34	
Renal rSO <sub>2</sub> (%)	FGR	93.7	88.9; 98.6	0.002	-0.25	-0.38; -0.11	0.01
	Control	82.7	77.7; 87.7		0.03	-0.13; 0.20	
Cerebral FTOE	FGR	0.19	0.13; 0.24	0.005	0.001	-0.001; 0.003	0.10
	Control	0.31	0.25; 0.37		-0.001	-0.003; 0.001	
Renal FTOE	FGR	0.02	-0.02; 0.07	0.004	0.003	0.002; 0.004	0.001
	Control	0.14	0.08; 0.19		-0.0001	-0.003; 0.001	

r = slope per 3 hour interval. \*

Abbreviations: CI, confidence interval; FGR, fetal growth restriction; FTOE, fractional tissue oxygen extraction; NIRS, near-infrared spectroscopy; rSO<sub>2</sub>, regional oxygenation

<https://doi.org/10.1371/journal.pone.0204268.t003>

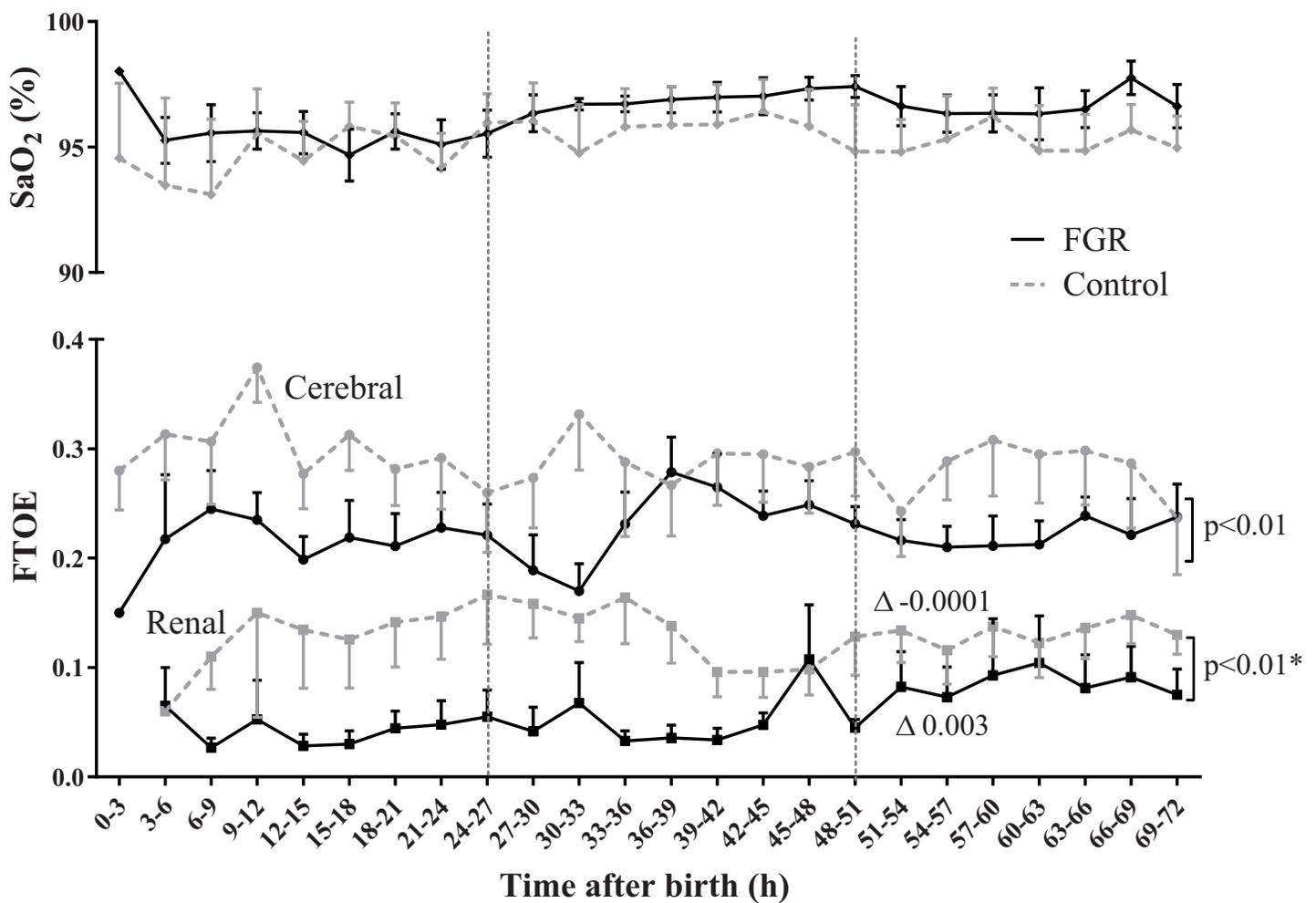


Fig 2. Arterial saturation (SaO<sub>2</sub>) and fractional tissue oxygen extraction (FTOE). Arterial saturation was similar between groups and over time. Renal and cerebral FTOE was lower in FGR compared to controls. Renal FTOE, but not cerebral FTOE, behaved differently over time. Significant group differences are shown and \* indicates whether the groups behaved differently over time (p<sub>interaction</sub><0.05). Δ indicates the slope per 3 hours interval. Data shown as mean ± s.e.m.

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Table 4. Bedside monitoring variables.

		Intercept	95% CI	P <sub>group</sub>	r	95% CI	P <sub>interaction</sub>
HR (bpm)	FGR	135	128; 142	0.07	0.93	0.73; 1.12	<0.01
	Control	145	137; 153		0.09	-0.13; 0.31	
SBP (mmHg)	FGR	45.4	42.1; 48.6	0.48	0.45	0.34; 0.56	0.61
	Control	43.7	40.2; 47.2		0.40	0.28; 0.52	
DBP (mmHg)	FGR	31.9	28.1; 35.8	0.27	0.26	0.16; 0.37	0.25
	Control	28.9	24.7; 33.0		0.17	0.06; 0.29	
SaO <sub>2</sub> (%)	FGR	95.4	93.5; 97.3	0.73	0.07	0.03; 0.11	0.44
	Control	94.9	92.8; 97.0		0.05	0.0004; 0.09	

r = slope per 3-hour interval.

Abbreviations: bpm, beat per minute; FGR, fetal growth restriction; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; SaO<sub>2</sub>, oxygen saturation

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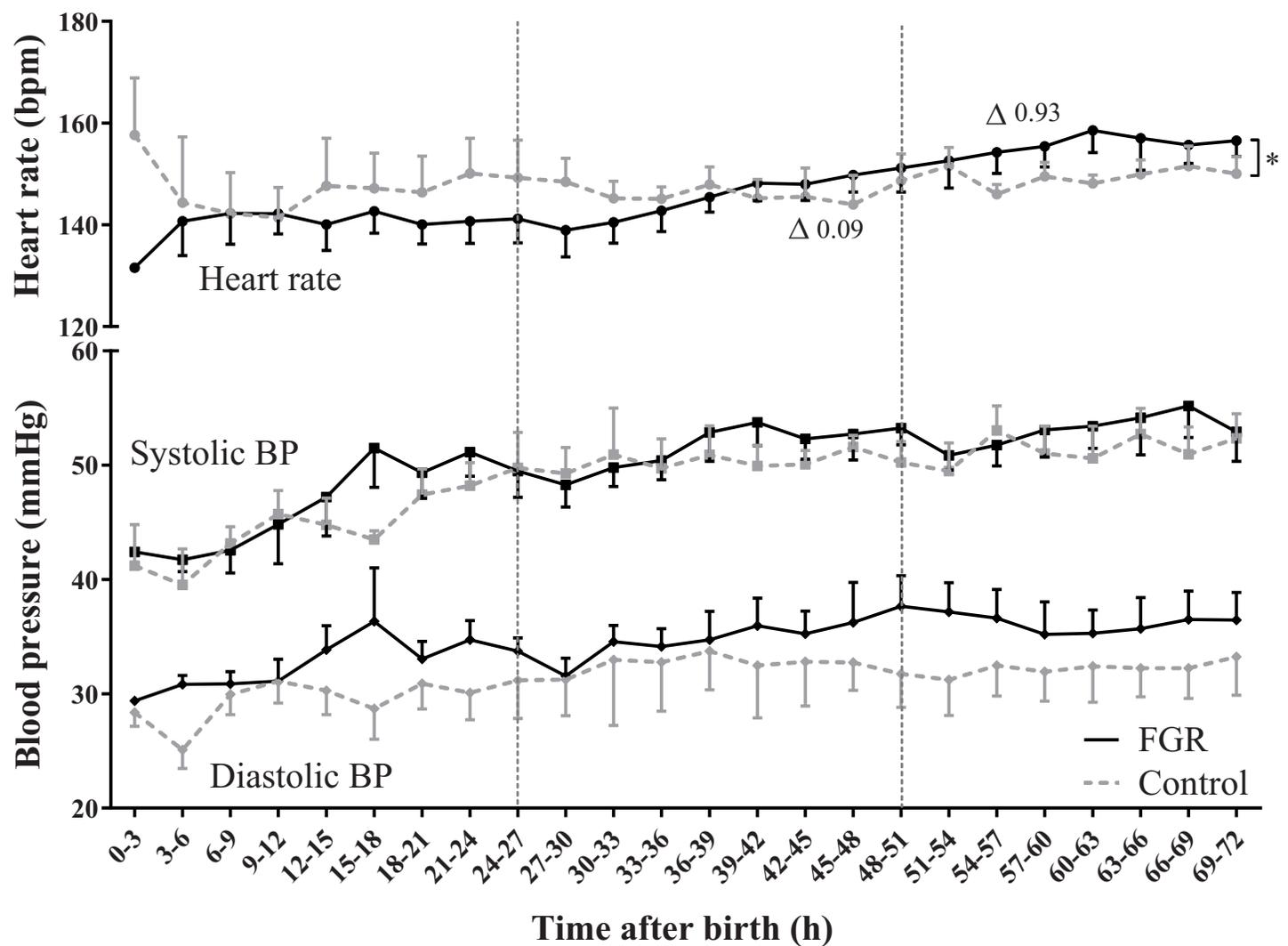


Fig 3. Heart rate and systolic and diastolic blood pressure (SBP and DBP). Heart rate and systolic and diastolic blood pressure were similar between groups. During the three days after birth, only heart rate increased significantly more in FGR compared to controls. Significant group differences are shown and \* indicates whether the groups behaved differently over time ( $p_{interaction} < 0.05$ ).  $\Delta$  indicates the slope per 3 hours interval. Data shown as mean  $\pm$  s.e.m.

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**Table 5. Postnatal ultrasound of renal artery blood flow and kidney length.**

	FGR (n = 7)	Control (n = 6)	p-value
<b>Pulsatility index</b>	2.25 ± 0.39	2.06 ± 0.34	0.71
<b>Resistance index</b>	0.86 ± 0.05	0.93 ± 0.10	0.53
<b>Kidney length (mm)</b>	31.6 ± 1.3	32.5 ± 2.5	0.71

Renal artery blood flow measured within 24 hours after birth.

Data shown as mean ± s.e.m. and analysed with independent t-test. Abbreviation: FGR, fetal growth restriction.

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late FGR neonates (but control neonates were not included in this study) and found that fetal brain-sparing signs were correlated with a low cerebral-renal ratio after birth, supporting the concept of preferential redistribution of blood after birth[16]. Similar to our findings, other studies also failed to detect differences in renal blood flow between the two groups[23,24].

Higher cerebral rSO<sub>2</sub> and lower cerebral FTOE in FGR neonates correspond to earlier findings, confirming the validity of the NIRS technique[25,26]. These differences in cerebral oxygenation have been attributed to an increased cerebral blood flow, caused by vasodilation in order to maintain cerebral oxygen supply—an adaptation to the adverse prenatal FGR environment (as continuation of brain-sparing)[25,26]. Despite similar findings of increased tissue oxygenation in the kidney and the brain, this is not necessarily caused by the same mechanism as characteristics of blood flow regulation in brain and kidney are fundamentally different[27].

Increased renal tissue oxygenation might be caused by decreased renal arteriovenous shunting, decreased oxygen consumption in the kidneys, or by increased renal blood flow, which is most often the case[28]. However, we found that renal blood flow did not significantly differ between groups. Increased oxygen supply could also be caused by systemic vasodilation, increased arterial saturation, elevated levels of hemoglobin or increased cardiac output[28]. However, blood pressure was similar between groups and heart rate was slightly reduced rather than increased during the first three days after birth. While hemoglobin was within the reference range, levels were significantly higher in FGR vs. control neonates, although this difference decreased over time. Erythropoiesis is accelerated in growth restricted fetuses, presumably as a compensation for the decreased placental oxygen supply to the fetus[25,26]. Due to a small sample size, we could not perform a correlation between the renal or cerebral oxygenation and hemoglobin levels in our study. Ishii et al. did not observe a correlation between the level of hemoglobin and cerebral oxygenation in small for gestational age neonates[26]. In addition, fractional fetal hemoglobin is higher in FGR neonates[26]. As fetal hemoglobin has slightly different absorption curves in the near-infrared range, this could also contribute to the higher rSO<sub>2</sub> levels[29]. However, studies have shown that higher fetal hemoglobin levels in preterm neonates did not affect cerebral rSO<sub>2</sub> or FTOE values[30,31]. Whether this also applies to renal rSO<sub>2</sub> is still unknown.

On the other hand, the increased renal tissue oxygenation in FGR neonates that we observed may be caused by decreased oxygen consumption. Renal oxygen consumption depends mainly on sodium reabsorption and indirectly on glomerular filtration rate (GFR), renal blood flow and systemic vasodilation[28]. As the latter two are similar between the groups, we speculate that the decreased oxygen consumption is most likely caused by a decrease in the absolute level of sodium reabsorption, possibly secondary to a low GFR. Correspondingly, an increased fractional sodium excretion has been detected in FGR neonates on the first day after birth[32]. This speculation is in line with the reduced number of nephrons that has been reported following FGR in both human and animal studies[8,11,33–35].

Teleologically, low sodium reabsorption would be necessary to prevent volume overload in the setting of low GFR.

Differences in baseline characteristics may also have influenced renal tissue oxygenation. Antihypertensive drug use in pregnancies complicated by FGR could affect hemodynamic parameters and thereby tissue oxygenation. Maternal beta-blockers intake could result in reduced renin release, reduced heart rate—as observed in this study—and vasodilatation. While theoretically this could result in lower renal or cerebral  $rSO_2$ , Thewissen et al. did not find a correlation between labetalol use and cerebral tissue oxygenation [36].

This study has several strengths. This is the first study comparing renal tissue oxygenation measured with NIRS between preterm FGR and preterm control neonates. NIRS parameters were measured continuously during the first three days after birth in contrast to other studies, in which tissue oxygenation was only measured intermittently with NIRS. Aside from renal NIRS, we studied renal artery blood flow, arterial blood pressure, heart rate, and arterial saturation. In addition, we used prenatal ultrasound measurements to clearly define placental insufficiency phenotype in our study population.

We acknowledge some limitations. The number of participants is limited. Despite the small sample size, a highly significant difference in renal oxygenation was found. However, a type II error might have occurred with renal artery blood flow. As a result, caution regarding the conclusion on renal artery blood flow limits us in leaning towards one of our two speculations on underlying mechanisms. We were also unable to adjust for covariates or different baseline characteristics that might have been of influence in the linear mixed model. Another limitation is that renal NIRS parameters have not yet been linked to renal function parameters, which is difficult due to the lack of renal function measurements especially during the first three days after birth. Persistent ductus arteriosus might have influenced our results, as previous studies report abnormal renal Doppler patterns [37, 38] and reduced cerebral oxygenation around 72 hours after birth in preterm neonates with persistent ductus arteriosus [39,40]. An ultrasound to assess left ventricular cardiac output and persistent ductus arteriosus (with shunt direction) at the time of renal ultrasound would have helped to better interpret the results. In addition, repeat renal artery Doppler measurements during these first three days after birth could have provided additional insight. As the neonatal sensors are relatively large for the FGR neonates, they may also have registered organs adjacent to the kidney, such as adrenal glands [41]. Based on previous literature we selected 1-hour intervals to increase comparability. Mintzer *et al.* showed that shorter selected intervals of tissue oxygenation measured with NIRS reduces baseline variability [42]. Although a different interval than 1 hour might slightly influence our results, it is unlikely that shorter intervals with reduced variability would result in a substantially different outcome of our study. Future studies to the exact influence of different selected time periods would be of interest.

While we speculate that increased renal  $rSO_2$  in FGR is caused by impaired renal maturation, no study has correlated NIRS parameters at birth with renal function at birth or in later life. Unfortunately, the restricted measurements options for renal function in the neonatal period (glomerular filtration rate, urea, and creatinine were not measured in the included neonates during their first three days of admission) limit us in studying this correlation and make fractional sodium excretion the best measurement option. A larger study including interesting renal function measurements—especially fractional electrolyte excretions—is of importance to further explore our speculation on decreased oxygen consumption. Follow-up studies should identify whether renal  $rSO_2$  correlates with renal function in the neonatal period (fractional sodium excretion), and with renal function (GFR, creatinine clearance) and blood pressure in childhood and adulthood.

In conclusion, elevated tissue oxygenation and lower oxygen extraction is observed in kidneys of FGR neonates during the first three days after birth. This can be caused by decreased oxygen consumption in the kidneys and/or increased oxygen supply. To make a better distinction between those two potential underlying mechanisms, this study should be performed with a larger sample size and, in particular, include repeated renal artery Doppler measurements and, although difficult to achieve, fractional electrolyte excretion. How these findings correlate with renal function on the short- and long-term needs further investigation before renal NIRS can be implemented in clinical practice. This applies to both screening (follow-up renal measurements of neonates potentially at risk) and prevention (avoidance of second hits such as nephrotoxic agents or high salt diets; monitoring of potential improvement after in utero strategies to prevent FGR and renal impairment).

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## References

1. Gordijn SJ, Beune IM, Thilaganathan B, Papageorgiou A, Baschat AA, Baker PN, et al. Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound Obstet Gynecol* 2016; 48:333–9. <https://doi.org/10.1002/uog.15884> PMID: 26909664
2. Bamfo JEK, Odibo AO. Diagnosis and management of fetal growth restriction. *J Pregnancy* 2011; 1–15.
3. Sharma D, Shastri S, Farahbakhsh N, Sharma P. Intrauterine growth restriction—part 1. *H Matern Fetal Neonatal Med* 2017; 29:3977–87.
4. Wang A, Rana S, Karumanchi SA. Preeclampsia: The Role of Angiogenic Factors in Its Pathogenesis. *Physiology* 2009; 24:147–58. <https://doi.org/10.1152/physiol.00043.2008> PMID: 19509125
5. Arduini D, Rizzo G. Fetal renal artery velocity waveforms and amniotic fluid volume in growth-retarded and post-term fetuses. *Obstet Gynecol* 1991; 77:370–3. PMID: 1992401
6. El Behery MM, Ibrahim MA. Fetal Renal Volume and Fetal Doppler in Normal and Growth Restricted Fetuses: Is there a Correlation? *Gynecol Obstet* 2012; 2:2–6.
7. Vyas S, Nicolaidis KH, Campbell S. Renal artery flow-velocity waveforms in normal and hypoxemic fetuses. *Am J Obstet Gynecol* 1989; 161:168–72. PMID: 2665495
8. Schreuder MF, Nauta J. Prenatal programming of nephron number and blood pressure. *Kidney Int* 2007; 72:265–8. <https://doi.org/10.1038/sj.ki.5002307> PMID: 17495859
9. Chen M, Zhang L. Epigenetic mechanisms in developmental programming of adult disease. *Drugs Discov Today* 2012; 16:1007–18.
10. Barker D. Adult consequences of fetal growth restriction. *Clin Obs Gynecol* 2006; 49:270–83.
11. Mackenzie HS, Brenner BM. Fewer nephrons at birth: a missing link in the etiology of essential hypertension? *Am J Kidney Dis* 1995; 26:91–8. PMID: 7611275

12. Bauer R, Walter B, Zwiener U. Comparison between inulin clearance and endogenous creatinine clearance in newborn normal weight and growth restricted newborn piglets. *Exp Toxicol Pathol* 2000; 52:367–72. [https://doi.org/10.1016/S0940-2993\(00\)80065-2](https://doi.org/10.1016/S0940-2993(00)80065-2) PMID: 10987193
13. Pellicer A, Bravo C. Near-infrared spectroscopy: A methodology-focused review. *Semin Fetal Neonatal Med* 2011; 16:42–9. <https://doi.org/10.1016/j.siny.2010.05.003> PMID: 20580625
14. Bel F Van, Lemmers P, Naulaers G. Monitoring Neonatal Regional Cerebral Oxygen Saturation in Clinical Practice: Value and Pitfalls. *Neonatology* 2008; 94:237–44.
15. Marin T, Moore J. Understanding Near-Infrared Spectroscopy. *Adv Neonatal Care* 2011; 11:382–8. <https://doi.org/10.1097/ANC.0b013e3182337ebb> PMID: 22123468
16. Tanis JC, Boelen M, Schmitz D, Casarella L, van der Laan ME, Bos AF, et al. Prenatal Doppler flow patterns and neonatal circulation in fetal growth restriction. *Ultrasound Obstet Gynecol* 2015; 45:1–3. <https://doi.org/10.1002/uog.14744>
17. Hadlock FP1, Harrist RB M-PJ. In utero analysis of fetal growth: a sonographic weight standard. *Radiology* 1991; 181:129–33. <https://doi.org/10.1148/radiology.181.1.1887021> PMID: 1887021
18. Anderson NH, Sadler LC, McKinlay CJD, McCowan LME. INTERGROWTH-21st vs customized birth-weight standards for identification of perinatal mortality and morbidity. *Am J Obstet Gynecol* 2016; 214:509.e1–509.e7.
19. Richardson DK, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: Simplified newborn illness severity and mortality risk scores. *J Pediatr* 2001; 138:92–100. PMID: 11148519
20. Naulaers G, Meyns B, Miserez M, Leunens V, Van Huffel S, Casaer P, 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. <https://doi.org/10.1159/000101063> PMID: 17377413
21. Alderliesten T, Dix L, Baerts W, Caiceo A, van Huffel S, Naulaers G et al. Reference values of regional cerebral oxygen saturation during the first 3 days of life in preterm neonates. *Pediatr Res*. 2015; 79:55–64. <https://doi.org/10.1038/pr.2015.186> PMID: 26389823
22. Polin RA, Abman SH, Rowitch DH, Benitz WE, Fox WW. *Fetal and Neonatal Physiology, 2-Volume Set. Chapter 116—Developmental Erythropoiesis. Fifth Edit.* Philadelphia: Elsevier Inc.; 2017.
23. Silver LE, Decamps PJ, Korst LM, Platt LD, Castro L. Intrauterine growth restrictions is accompanied by decreased renal volume in the human fetus. *Am. J. Obstet Gynecol* 2003; 188:1320–5. PMID: 12748506
24. Kolarovszka H, Zibolen M, Stavel M, Matasova K, Kolarovszki B, Ciljak M, et al. Assessment of renal circulation in small for gestational age and appropriate for gestational age term newborns: a prospective study. *J Clin Ultrasound* 2008; 36:200–3. <https://doi.org/10.1002/jcu.20421> PMID: 17960824
25. Cohen E, Baerts W, Alderliesten T, Derks J, Lemmers P, van Bel F. Growth restriction and gender influence cerebral oxygenation in preterm neonates. *Arch Dis Child—Fetal Neonatal Ed* 2016; 101:F156–61. <https://doi.org/10.1136/archdischild-2015-308843> PMID: 26311070
26. Ishii H, Takami T, Fujioka T, Mizukaki N, Kondo A, Sunohara D, et al. Comparison of changes in cerebral and systemic perfusion between appropriate- and small-for-gestational-age infants during the first three days after birth. *Brain Dev* 2014; 36:380–7. <https://doi.org/10.1016/j.braindev.2013.06.006> PMID: 23838311
27. Sparks H V, Belloni FL. The peripheral circulation: local regulation. *Annu Rev Physiol* 1978; 40:67–92. <https://doi.org/10.1146/annurev.ph.40.030178.000435> PMID: 416746
28. Evans RG, Ince C, Joles JA, Smith DW, May CN, O'Connor PM, et al. Haemodynamic influences on kidney oxygenation: Clinical implications of integrative physiology. *Clin Exp Pharmacol Physiol* 2013; 40:106–22. <https://doi.org/10.1111/1440-1681.12031> PMID: 23167537
29. Cochran-Black D, Cowan L, Neas B. The relation between newborn hemoglobin F fractions and risk factors for sudden infant death syndrome. *Arch Pathol Lab Med* 2001; 125:211–7. [https://doi.org/10.1043/0003-9985\(2001\)125<0211:TRBNHF>2.0.CO;2](https://doi.org/10.1043/0003-9985(2001)125<0211:TRBNHF>2.0.CO;2) PMID: 11175637
30. Wickramasinghe YABD, Palmer KS, Houston R, Spencer SA, Rolfe P, Thorniley MS, et al. Effect of Fetal Hemoglobin on the Determination of Neonatal Cerebral Oxygenation by Near-Infrared Spectroscopy. *Pediatr Res* 1993; 34:15–7. <https://doi.org/10.1203/00006450-199307000-00004> PMID: 7689196
31. Wardle SP, Yoxall CW, Weindling AM. Determinants of Cerebral Fractional Oxygen Extraction Using Near Infrared Spectroscopy in Preterm Neonates. *J Cereb Blood Flow Metab* 2000; 20:272–9. <https://doi.org/10.1097/00004647-200002000-00008> PMID: 10698064
32. Robinson D, Weiner CP, Nakamura KT, Robillard JE. Effect of intrauterine growth retardation on renal function on day one of life. *Am J Perinatol* 1990; 7:343–6. <https://doi.org/10.1055/s-2007-999519> PMID: 2222625

33. Manalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: A histomorphometric study. *Kidney Int* 2000; 58:770–3. <https://doi.org/10.1046/j.1523-1755.2000.00225.x> PMID: 10916101
34. Woods LL, Weeks DA, Rasch R. Programming of adult blood pressure by maternal protein restriction: Role of nephrogenesis. *Kidney Int* 2004; 65:1339–48. <https://doi.org/10.1111/j.1523-1755.2004.00511.x> PMID: 15086473
35. Boubred F, Daniel L, Buffat C, Tsimaratos M, Oliver C, Lelièvre-Pégorier M, et al. The magnitude of nephron number reduction mediates intrauterine growth-restriction-induced long term chronic renal disease in the rat. A comparative study in two experimental models. *J Transl Med* 2016; 14:331. <https://doi.org/10.1186/s12967-016-1086-3> PMID: 27899104
36. Thewissen L, Pistorius L, Baerts W, Nauleurs G, Van Bel F, Lemmers P. Neonatal haemodynamic effects following foetal exposure to labetalol in hypertensive disorders of pregnancy Neonatal haemodynamic effects following foetal exposure to labetalol in hypertensive disorders of pregnancy. *J Matern Fetal Neonatal Med* 2017;7058.
37. Bomelburg T, Jorch G. Abnormal blood flow patterns in renal arteries of small preterm infants with patent ductus arteriosus detected by Doppler ultrasonography. *Eur J Pediatr*. 1989; 148:660–4. PMID: 2526019
38. Wong SN, Lo RN, Hui PW. Abnormal renal and splanchnic arterial Doppler pattern in premature babies with symptomatic patent ductus arteriosus. *J Ultrasound Med*. 1990; 9:125–30. PMID: 2407859
39. 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. <https://doi.org/10.1542/peds.2007-0925> PMID: 18166568
40. Cohen E, Dix L, Baerts W, Alderliesten T, Lemmers P, van Bel F. Reduction in cerebral oxygenation due to patent ductus arteriosus is pronounced in small-for-gestational-age neonates. *Neonatology*. 2017; 111:126–132. <https://doi.org/10.1159/000448873> PMID: 27756065
41. Heese S, Hammer K, Möllers M, Köster HA, Falkenberg MK, Eveslage M, et al. Adrenal gland size in growth restricted fetuses. *J Perinat Med* 2018 Mar 15; Epub.
42. Mintzer JP, Parvez B, Chelala M, Alpan G, LaGamma EF. Quiescent variability of cerebral, renal and splanchnic regional tissue oxygenation in very low birth weight neonates. *J Neonatal Perinatal Med*. 2014; 7: 199–206. <https://doi.org/10.3233/NPM-14814035> PMID: 25322996