Multisite measurement of regional oxygen saturation in Fontan patients with and without protein-losing enteropathy at rest and during exercise

Simon Schröer[1], Fabian B. Fahlbusch[2], Frank Münch[3], Muhannad Alkassar[1], Hakan R. Toka[4], Manfred Rauh[2], André Rüffer[3], Robert Cesnjevar[3], Sven Dittrich[1], Okan Toka[5] and Julia Moosmann[1]

BACKGROUND: Protein-losing enteropathy (PLE) is a severe complication of Fontan circulation with increased risk of end-organ dysfunction. We evaluated tissue oxygenation via near-infrared spectroscopy (NIRS) at different exercise levels in Fontan patients.
METHODS: Assessment of multisite NIRS during cycle ergometer exercise and daily activities in three groups: Fontan patients with PLE; without PLE; patients with dextro-transposition of the great arteries (d-TGA); comparing univentricular with biventricular circulation and Fontan with/without PLE. Renal threshold analysis (<65%;<55%;<45%) of regional oxygen saturation (rSO2) was performed.
RESULTS: Fontan patients showed reduced rSO2 ($p < 0.05$) in their quadriceps femoris muscle compared with biventricular d-TGA patients at all time points. rSO2 in renal tissue was reduced at baseline ($p = 0.002$), exercise ($p = 0.0062$), and daily activities ($p = 0.03$) in Fontan patients with PLE. Renal threshold analysis identified critically low renal rSO2 (rSO2 < 65%) in Fontan patients with PLE during exercise (95% of monitoring time below threshold) and daily activities (83.7% time below threshold).
CONCLUSION: Fontan circulation is associated with decreased rSO2 values in skeletal muscle and hypoxemia of renal tissue solely in patients with PLE. Reduced rSO2 already during activities of daily life, might contribute to comorbidities in patients with Fontan circulation, including PLE and renal failure.

INTRODUCTION

The Fontan procedure was first described in 1971 by Fontan and Baudet as a palliative procedure for all patients with inborn heart malformations of the single-ventricle type (SV).[1] Since 1985, cardiovascular surgical techniques and postoperative care have improved, resulting in increased long-term survival rates of up to 85%, 20 years after Fontan surgery.[2–4] Simultaneously, the rate of late complications and comorbidities associated with univentricular circulation increased.[2,5] The Fontan circulation presents with an elevation of central venous pressure, a non-pulsatile pulmonary blood flow, and reduced cardiac output.[6,7] Midterm follow-up data indicated an increased risk of liver fibrosis, renal dysfunction, impaired lymphatic drainage, increasing pulmonary vascular resistance, and altered bone density.[8–10] Of all Fontan patients, 3–15% develop a protein-losing enteropathy (PLE) leading to increased morbidity and mortality with an estimated 5-year survival rate of ~50%.[5] PLE is a clinical diagnosis with gradual onset of symptoms, including hypoalbuminemia, hypogammaglobulinemia, diarrhea, and dysregulation of the extracellular sodium balance resulting in peripheral edema, ascites, and pleural effusions.[8] While the pathomechanisms of PLE are barely understood, its clinical course is characterized by significant morbidity and mortality due to end-organ dysfunction.[10,11]

Near-infrared spectroscopy (NIRS) was established for non-invasive measurement of regional oxygen saturation (rSO2). Technically based on the Beer–Lambert law equation, NIRS detects the differences between oxygenated and deoxygenated hemoglobin on the tissue level via light absorption, reflection, and dispersion.[12–16]

In tissues of interest, rSO2 measurements show a positive correlation to hemodynamic changes. Disturbances in tissue perfusion are regionally reflected by altered oxygen supply-and-demand ratios, e.g., secondary to changes in microcirculation or vascular resistance.[12,17] In pediatric patients, NIRS is primarily used for neurologic monitoring of critically ill patients in intensive care units (ICU) or during cardiothoracic surgery with cardiopulmonary bypass.[14,17–19] The use of multisite NIRS monitoring has been evaluated in healthy populations to predict global cardiac output distribution trends during exercise testing.[15]

In patients with congenital heart disease (CHD), Navaratnam et al.[20] have recently employed multisite NIRS to investigate dynamic exercise responses in a small cohort ($n = 10$) of stable Fontan patients without PLE (mean age of 26.4 years, range 19–31 years) in comparison with healthy controls ($n = 9$) to evaluate exercise-induced changes in systemic venous pressure (SVP, monitored in the right upper limb) and renal and cerebral tissue

[1]Department of Pediatric Cardiology, Friedrich-Alexander-University of Erlangen-Nuernberg (FAU), Loschgestraße 15, 91054 Erlangen, Germany; [2]Department of Pediatrics and Adolescent Medicine, Friedrich-Alexander-University of Erlangen-Nuernberg (FAU), Loschgestraße 15, 91054 Erlangen, Germany; [3]Department of Pediatric Cardiac Surgery, Friedrich-Alexander-University of Erlangen-Nuernberg (FAU), Loschgestraße 15, 91054 Erlangen, Germany; [4]Department of Graduate Medical Education, Manatee Memorial Hospital, Bradenton, FL, USA and [5]Medical Care Center for Children's and Adolescence Medicine, Bahnhofplatz 6, 90762 Fürth, Germany
Correspondence: Julia Moosmann (Julia.moosmann@uk-erlangen.de)

oxygenation (NIRS of the brain and kidney). High-intensity cardiopulmonary exercise was associated with systemic venous hypertension and reduced oxygen delivery. It was concluded that these pathophysiologic alterations might potentially contribute to end-organ injury in Fontan patients.[20]

Based on these results, we set out to use multisite NIRS to investigate the prevalence and degree of impaired tissue oxygenation in Fontan patients with PLE at rest, during exercise, and especially during activities of daily life. We compared these results with NIRS measurements obtained from stable Fontan patients without PLE and with patients who underwent neonatal correction of dextro-transposition of the great arteries (d-TGA) without residual heart defects, as biventricular controls.

Despite great efforts regarding the management of PLE in Fontan patients, the risk for end-organ dysfunction, especially renal failure, remains high. The exact pathomechanism of PLE itself, however, remains unclear. Our study addresses the potential association of local oxygenation changes with the development of PLE in Fontan patients. Improved monitoring of PLE might ultimately help to prevent end-organ injury.

Table 1. Cardiac anatomy of Fontan patients

Cardiac anatomy	Fontan with PLE	Fontan without PLE	
Age at Fontan surgery (years)	3.9 ± 2.5	3.5 ± 2.4	
Systemic ventricle			
Left	$n = 4$	$n = 15$	
Right	$n = 3$	$n = 7$	
Tricuspid atresia (TA)	$n = 2$	$n = 7$	
Pulmonary atresia with intact ventricular septum (PA-IVS)	$n = 1$	$n = 1$	
Unbalanced atrioventicular canal (ub-AVSD)	$n = 1$		
Hypoplastic left heart syndrome (HLHS)	$n = 2$	$n = 1$	
Interrupted aortic arch, hypoplastic left ventricle	$n = 1$	$n = 1$	
Single ventricle (SV)		$n = 7$	
Double-inlet left ventricle (DILV)		$n = 3$	
Double-outlet right ventricle (DORV), mitral atresia		$n = 1$	
Double-outlet right ventricle (DORV), criss-cross heart		$n = 1$	
Fenestration	$n = 1$	$n = 2$	
AV insufficiency			
I°	$n = 3$	$n = 11$	
II°		$n = 3$	
Cardiac rhythm			
Sinus rhythm	$n = 5$	$n = 19$	
Pacemaker	$n = 1$	$n = 1$	
Other (e.g., atrial rhythm, AV-block I)	$n = 1$	$n = 2$	
Cardiac MRI			
End-diastolic volume	82.8 ± 38.4	104 ± 53.1	$p = 0.266$
End-systolic volume	38.2 ± 20.9	49.7 ± 28.1	$p = 0.456$
Ejection fraction of a single ventricle	52.3 ± 22.3	52.6 ± 17.7	$p = 0.466$

Age at Fontan surgery and MRI parameters are expressed as mean ± SD
PLE protein-losing enteropathy

MATERIALS AND METHODS

Patients

Outpatients visiting our clinic at the Department of Pediatric Cardiology (between June 2013 and June 2014), University of Erlangen, Germany were identified via a clinical database search. Individuals with infections or surgical interventions within the last 6 months were excluded from the study. A minimum body height of 130 cm was a prerequisite for ergometry testing. All Fontan patients with PLE currently receiving care in our institution were recruited. Our single-center cohort included $n = 38$ individuals ($n =$ 22 Fontan patients without PLE, $n = 7$ Fontan patients with PLE, and $n = 9$ d-TGA patients). Patients with d-TGA were chosen to compare univentricular with biventricular circulation. All d-TGA patients had undergone successful arterial switch operations and were otherwise healthy without pathological findings in long-term follow-up. Details on the cardiac anatomy at the time of the study are given in Table 1. An overview of the adjunctive medication in our cohort is presented in Table 2. Demographic data were collected and percentiles were calculated for patients < 18 years according to Kromeyer-Hauschild et al.[21]. The respective data are shown in Table 3. Blood and stool samples of all participants were analyzed to determine the presence of PLE, which was defined by a combination of mild-to-profound diarrhea, third space volume retention (i.e., ascites, edema, or pleural effusions), elevated fecal alpha-1 antitrypsin (AT) (>400 µg/g), serum (s) total protein <50 g/l, hypoalbuminemia (<30 g/l), hyponatremia (<135 mmol/l), and hypogammaglobulinemia (IgG) (<4 g/l), as described by others.[8]

Table 2. Adjunctive medication in Fontan and d-TGA patients

	Fontan with PLE	Fontan without PLE	d-TGA
Diuretic therapy (*n*)	7/7	1/22	0/9
Furosemide (mg/kg/d)	1.21 ± 0.7 ($n = 7$)	0.18 ± 0.0 (n=1)	None
Eplerenone (mg/kg/d)	0.83 ± 0.4 ($n = 3$)	0.45 ± 0.0 (n=1)	None
Hydrochlorothiazide (mg/kg/d)	0.80 ± 0.5 ($n = 3$)	None	None
Spironolactone (mg/kg/d)	0.87 ± 0.4 ($n = 3$)	None	None
Tolvaptan (mg/kg/d)	0.95 ± 0.5 ($n = 2$)	None	None
Quadruple	$n = 2$	None	None
Triple	$n = 1$	None	None
Double	$n = 3$	$n = 1$	None
Single	$n = 1$	None	None
ACE inhibitors (*n*)	1/7	2/22	0/9
Captopril (mg/kg/d)	0.96 ± 0.0 ($n = 1$)	0.39 ± 0.0 ($n = 1$)	None
Enalapril (mg/kg/d)	None	0.25 ± 0.0 $n = 1$	None
Anticoagulation (*n*)	7/7	22/22	0/9
ASS	$n = 2$	$n = 20$	None
Phenprocoumon	$n = 3$	$n = 1$	None
Clopidogrel	$n = 1$	$n = 3$	None
Warfarin	$n = 1$		None
Dipyridamol	$n = 1$	$n = 2$	None
Beta-blocker (*n*)	1/7	3/22	0/9
Metoprolol (mg/kg/d)	0.27 ± 0.0 ($n = 1$)	0.86 ± 0.6 ($n = 2$)	
Carvedilol (mg/kg/d)		0.42 ± 0.0 ($n = 1$)	

Adjunctive medication is expressed as mean ± SD in mg/kg/d. Diuretic therapy was classified as quadruple, triple, double, or single diuretic therapy
PLE protein-losing enteropathy, *d-TGA* dextro-transposition of the great arteries

Table 3. Patient characteristics and laboratory analysis

	Fontan with PLE	Fontan without PLE	d-TGA	*p*-value
n	7	22	9	
Sex	f = 4/m = 3	f = 10/m = 12	f = 2/m = 7	0.331
Age (years)	17.3 ± 6.7	16.4 ± 5.7	16.2 ± 5.3	0.921
Weight (kg)	40.6 ± 9.3	57.7 ± 21.1	57.1 ± 16.1	0.104
Height (cm)	147.6 ± 15.7	166.4 ± 19.4	164.1 ± 19.2	0.079
BMI	18.4 ± 0.9	19.9 ± 3.3	20.9 ± 2.4	0.227
n	5	13	6	
Weight percentiles (%)	5.8 ± 9.2	52.5±27.0	55.0 ± 28.9	**0.004**
				1–2: <**0.001**
				1–3: **0.017**
				2–3: 0.979
Height percentiles (%)	4.6 ± 6.8	54.2 ± 30.4	46.2 ± 36.5	**0.004**
				1–2: <**0.001**
				1–3: **0.083**
				2–3: 0.693
Hemoglobin (g/dl)	14.8 ± 2.4	14.9 ± 1.8	14.2 ± 1.9	0.643
MCV (fl)	94.7 ± 15.0	86.5 ± 4.1	84.1 ± 7.6	**0.0312**
				1–2: 0.133
				1–3: 0.048
				2–3: 0.525
MCH (pg)	30.3 ± 1.1	29.7 ± 1.8	28.6 ± 3.0	0.219
MCHC (g/dl)	32.6 ± 4.3	34.4 ± 0.9	34.1 ± 1.5	0.128
RDW (%)	14.3 ± 1.1	14.3 ± 2.0	13.3 ± 1.2	0.324
Hematocrit (%)	45.3 ± 0.8	43.4 ± 5.4	41.6 ± 4.7	0.348
Creatinine (mg/dl)	0.56 ± 0.2	0.73 ± 0.2	0.7 ± 0.2	0.278
Cystatine C (mg/l)	0.9 ± 0.3	1.0 ± 0.2	0.9 ± 0.1	0.433
Albumin (g/l)	27.6 ± 7.2	43.1 ± 2.9	44.2 ± 1.9	<**0.001**
				1–2: **0.003**
				1–3: **0.002**
				2–3: 0.485
Osmolality (mOsm/kg)	279.3 ± 2.4	282.5 ± 3.6	284.4 ± 2.1	**0.009**
				1–2: **0.042**
				1–3: **0.002**
				2–3: 0.164
Total protein (g/l)	46.3 ± 10.8	67.0 ± 3.9	67.0 ± 4.2	<**0.001**
				1–2: **0.005**
				1–3: **0.004**
				2–3: 0.99
IgG (g/l)	3.47 ± 4.2	9.7 ± 2.5	9.3 ± 2.6	<**0.001**
				1–2: **0.016**
				1–3: **0.023**
				2–3: 0.929
Serum sodium (mmol/l)	134.3 ± 1.6	136.4 ± 1.7	137.6 ± 1.0	<**0.001**
				1–2: **0.03**
				1–3: **0.002**
				2–3: 0.071
Fecal alpha-1 antitrypsin (µg/g)	1566.9 ± 616.8	255.7 ± 369.1	238.3 ± 150.5	<**0.001**
				1–2: **0.002**
				1–3: **0.003**
				2–3: 0.982

Percentiles were calculated for patients up to the age of 18 years, only. Parametric ANOVA was used to investigate group differences. A significant ANOVA was followed by pairwise post hoc tests (Games–Howell) comparing PLE, Fontan without PLE, and d-TGA patients. Results from the post hoc test between the groups are expressed as 1–2; 2–3; 1–3 (where 1 = PLE, 2 = Fontan without PLE, and 3 = d-TGA). Values are expressed as mean ± SD. Bold values represent significant results

f female, *m* male, *PLE* protein-losing enteropathy, *d-TGA* dextro-transposition of the great arteries, *MCH* mean cell hemoglobin, *MCV*mean corpuscular volume, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red cell distribution width

Study protocol

Patients underwent cardiac exercise testing by incremental ramp cycle exercise (Ergoselect 200P, Ergoline, Bitz, Germany) starting at 10 W (independently of bodyweight) with a continuous increase of resistance (15 W/min) by a standard protocol. Patients were required to maintain a pedal frequency of 60 rpm during exercise. Monitoring included continuous 12-lead electrocardiogram (ECG; Cardiodirect 12S Del Mar Reynolds Medical, Hertfort, UK), automated oscillometric blood pressure measurement on the right arm (Ergoline, Bitz, Germany), oxygen saturation measurement on the left index finger (Masimo SET Rad 5, Irvine, CA), and four NIRS electrodes, as shown in Fig. 1a.

The protocol included a 5-min pre-exercise rest period to determine rSO2 at baseline, resting heart rate, and blood pressure values. Subsequently, the patients were physically stressed until reaching voluntary or symptom-limited exhaustion. Thereafter, a 5-min recovery period started beginning with an initial cool-down phase of 1 min at 15 W followed by 4 min at 0-W resistance on the ergometer (Fig. 1b). Pulmonary exercise testing (spirometry) was not included in the study protocol. After cycling exercise, a 1-h period of physiological recovery, including normal daily activities (e.g., walking in the park) was recorded by portable NIRS for each patient (Fig. 1b).

NIRS measurement

Four-site rSO2 was measured using Nonin Sensmart X-100 (Nonin Medical, Inc., Plymouth, Minnesota, USA) near-infrared spectroscope with Nonin EQUANOX Advance Model 8004 CA NIRS sensors (Nonin Medical, Inc., Plymouth, Minnesota, USA) detecting rSO2 with spatially resolved spectroscopy. EQUANOX sensors feature two emitters and two detectors covering four wavelengths (775, 810, 847, and 919 nm). Nonin Sensmart X-100 was selected for this study, because it is a portable device with a weight of only 900 g and battery runtime of 3 h.[22] Measurement of rSO2 included four NIRS electrodes placed in the area of the frontal cortex, left triceps brachii muscle, right kidney (placement confirmed by a dorsal sagittal sonogram), and right quadriceps femoris muscle (Fig. 1a).

Critical threshold values for renal and cerebral rSO2 were selected based on current literature.[18,23–25] The duration of rSO2 measurements below these threshold values was analyzed for every patient group and during all time periods. For renal rSO2 values: <65% are described as critical, <55% as hazardous for kidney injury, and <45% as an acute risk for kidney injury in infants during surgery on cardiopulmonary bypass. For cerebral NIRS, critical rSO2 values were described as values below 45%.[25,26]

Ethics

The study was approved by the local ethics committee of the University of Erlangen-Nürnberg (Re.-No. 145_13B). Written informed consent was obtained from all participating individuals or their parents or legal guardians. All procedures were based on standard of care, and established clinical guidelines were followed. The study was conducted in accordance with the Declaration of Helsinki.

Data analysis and statistics

Nonin Sensmart X-100 measured rSO2 values every 4 s (=15/min). To minimize measurement variability, we used the arithmetical mean of 15 values per minute. Baseline and peak exercise values were determined. Absolute and relative values from baseline were calculated and compared.

Due to incomplete measurements over time (some patients interrupted the exercise early), the time axis was divided into the following binned categories: exercise: minute 1, minute 2, minute 3, and the last measured minute; recovery: minute 1, minute 2, and the last measured minute; daily activities: minutes 1–10, minutes 11–20, minutes 21–30, and minutes 31–40. Mean values within an interval were used for further analyses of the activities section.

All data are displayed as mean ± standard deviation (SD) or as minimum (min), maximum (max), and range, unless stated otherwise. For data presentation, the arithmetical mean was chosen above the median, as the presence of outliers had been excluded. Normality was analyzed by Shapiro–Wilk test and QQ plots.

Patient characteristics and ergometry measurements were normally distributed within the groups, and parametric ANOVA was used to investigate group differences. A significant ANOVA was followed by pairwise post hoc tests (Games–Howell).

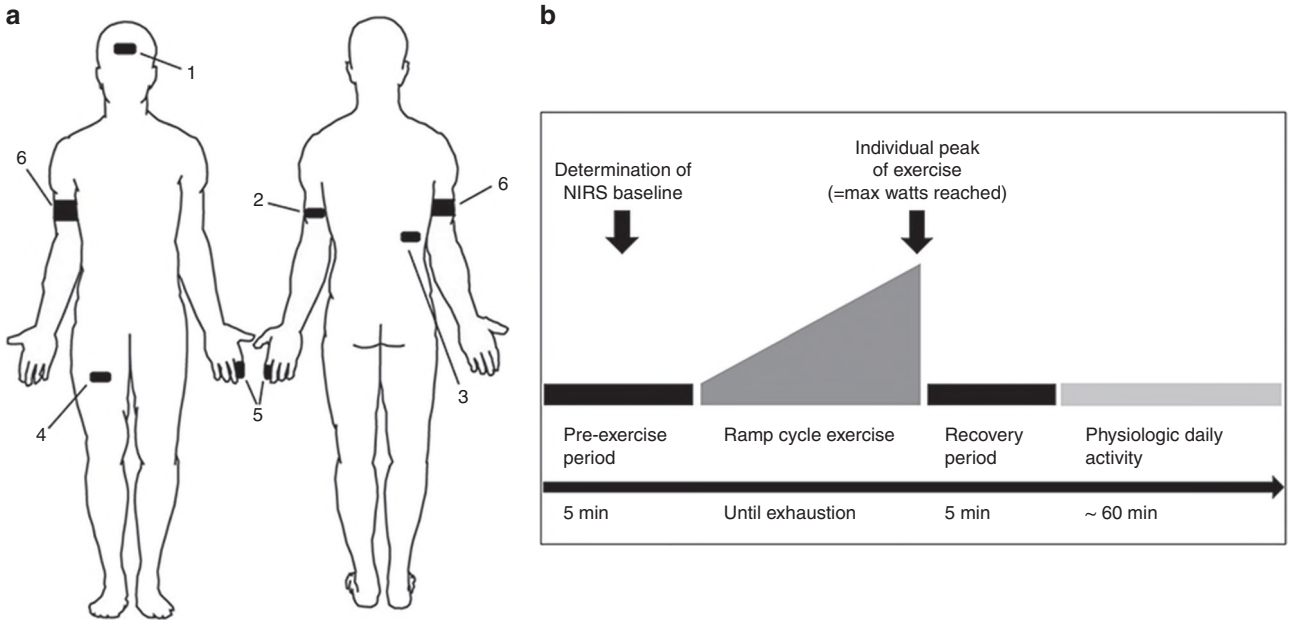

Fig. 1 Study protocol and NIRS body map. **a** NIRS body map (black marks indicate NIRS electrodes: 1 frontal cortex (cerebral); 2 triceps brachii muscle; 3 kidney (renal); 4 quadriceps femoris muscle; 5 pulse oximetry; 6 blood pressure cuff). **b** Study protocol included pre-exercise period, ramp cycle exercise, recovery period, and physiologic daily activities

Measurements over time did not show a normal distribution in general and the sample size was small. Thus, nonparametric analyses were used. The change over time was investigated by using difference values (Δ last − first minute, for each section) and differences between the three groups were analyzed. This analysis was performed for each section. For this comparison, nonparametric Mann–Whitney *U* test was calculated and the effect size measure Cohens *d* was reported.

The correlation between the control parameters and the difference values was checked by a nonparametric Spearman correlation. A level of significance of 5% was used for all analyses. All statistical analyses were performed with the software R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). Based on the effect size measurement, the required sample size was calculated by a post hoc sample size analysis with a power of 80% via G*Power software.[27,28]

For all measurements, a threshold analysis was performed comparing the duration (percentage in minutes) that the values were below the characterized threshold for (thresholds: renal 65%, 55%, and 45%, respectively, cerebral 45%) in each group. These percentage values were then investigated regarding the differences between the groups.

RESULTS

Patient cohort and laboratory analysis

Our single-center cohort included $n = 38$ individuals ($n = 22$ Fontan patients without PLE, $n = 7$ Fontan patients with PLE, and $n = 9$ d-TGA patients). Demographic and laboratory data are shown in Table 3.

There were no significant differences between all groups regarding height, weight, or age at the time of the study (Table 3). Subgroup analysis of percentiles in patients younger than 18 years showed that PLE patients had significantly lower weight ($p < 0.001$) and height ($p = 0.001$) than Fontan patients without PLE, and showed a significantly lower bodyweight than d-TGA patients ($p = 0.017$). No differences were seen between d-TGA and Fontan patients without PLE (Table 3).

Laboratory parameters in PLE patients showed significantly lower albumin, osmolarity, total serum protein, IgG, and serum sodium levels compared with Fontan patients without PLE and d-TGA patients. Fecal alpha-1 AT values were significantly higher in PLE patients compared with Fontan patients without PLE ($p = 0.002$) and d-TGA ($p = 0.003$); no differences were observed between d-TGA and Fontan patients without PLE for the above parameters (Table 3). No differences were observed for hemoglobin, hematocrit, creatinine, and cystatin c levels between all groups. Indirect markers of iron deficiency, i.e., mean cell hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were within their respective reference ranges. MCV was significantly elevated in Fontan patients with PLE when compared with d-TGA patients (Table 3). The underlying cardiac anatomy, cardiac magnetic resonance imaging (MRI) measurements, and adjunctive medication are summarized in Tables 1 and 2. One Fontan patient with PLE was listed for heart transplantation (HTX) 1 year after the study and died 2 days after HTX.

Exercise testing and cardiac monitoring

PLE patients showed a significantly shorter duration of exercise than Fontan patients without PLE ($p = 0.006$) and d-TGA patients ($p = 0.002$), reaching lower maximum watts ($p = 0.003$ and $p = 0.001$, respectively) and watt/kg ($p = 0.037$ and $p < 0.01$, respectively) (Table 4). Fontan patients without PLE reached lower watt/kg than d-TGA patients ($p = 0.018$), which was the only significant difference between these two groups in ergometry testing (Table 4).

Table 4. Ergometry characteristics, vital parameters, and NIRS baselines

	Fontan with PLE	Fontan without PLE	d-TGA	*p*-value
Exercise time (min)	5.0 ± 1.7	8.5 ± 3.4	10.9 ± 3.6	**0.003** 1–2:**0.006** 1–3:**0.002** 2–3:0.203
Maximum watts	80.6 ± 27.0	137.2 ± 51.0	174.3 ± 51.5	**0.002** 1–2:**0.003** 1–3:**0.001** 2–3:0.195
W/kg	2.0 ± 0.4	2.5 ± 0.5	3.1 ± 0.5	<**0.001** 1–2:**0.037** 1–3:<**0.001** 2–3:**0.018**
Resting heart rate (bpm)	91 ± 17	75 ± 14	70 ± 13	**0.020** 1–2:0.149 1–3:**0.046** 2–3:0.459
Peak heart rate (bpm)	164 ± 42	176 ± 25	182 ± 11	0.413
Sys BP baseline (mmHg)	104 ± 8	118 ± 10	125 ± 18	0.236
Dia BP baseline (mmHg)	62 ± 5	63±8	60 ± 9	0.457
Sys BP peak (mmHg)	118 ± 19	151 ± 36	164 ± 30	**0.026** 1–2:**0.017** 1–3:**0.007** 2–3:0.584
Dia BP peak (mmHg)	76 ± 15	72 ± 15	77 ± 13	0.636
Pulse oximetry baseline (SpO2%)	90.0 ± 4.7	89.6 ± 6.3	97.3 ± 1.1	**0.003** 1–2:0.986 1–3:**0.027** 2–3:<**0.001**
Pulse oximetry peak exercise (SpO2%)	79.6 ± 8.4	85.2 ± 6.4	93.4 ± 3.4	<**0.001** 1–2:0.356 1–3:**0.020** 2–3:<**0.001**
Baseline frontal cortex (rSO2%)	70.1 ± 13.2	70.4 ± 8.4	76.6 ± 7.3	0.216
Baseline triceps brachii (rSO2%)	64.4 ± 7.3	68.7 ± 8.5	67.6 ± 4.5	0.432
Baseline renal (rSO2%)	56.0 ± 9.9	74.5 ± 13.0	81.0 ± 9.6	<**0.001** 1–2:**0.010** 1–3:**0.002** 2–3:0.250
Baseline quadriceps femoris (rSO2%)	58.1 ± 7.7	57.1 ± 10.1	72.3 ± 7.3	<**0.001** 1–2:0.870 1–3:**0.003** 2–3:<**0.001**

Parametric ANOVA was used to investigate group differences. A significant ANOVA was followed by pairwise post hoc tests (Games–Howell) comparing PLE, Fontan without PLE, and d-TGA. Results from the post hoc test between the groups are expressed as 1–2; 2–3; 1–3 (where 1 = PLE, 2 = Fontan without PLE, and 3 = d-TGA). Values are expressed as mean ± SD. Bold values represent significant results

min minutes, *W* watts, *bpm* beats per minute, *Sys* systolic, *Dia* diastolic, *BP* blood pressure

PLE patients had a significantly higher mean resting heart rate of 91 ± 17/min before exercise. The maximum systolic blood pressure was significantly lower in PLE patients than in Fontan patients without PLE ($p = 0.017$) and d-TGA patients ($p = 0.007$) (Table 4).

Mean peripheral oxygen saturation (SpO2) was lower at rest/baseline in Fontan patients ($p < 0.01$) and PLE patients ($p = 0.027$) compared with patients with d-TGA, but there was no difference between Fontan patients with or without PLE. The individual peak exercise SpO2 was significantly lower in Fontan patients without PLE ($p < 0.001$) and with PLE ($p = 0.02$) compared with d-TGA patients. No significant difference in SpO2 during exercise was detected between Fontan patients with or without PLE (Table 4).

NIRS measurements

NIRS measurement did not show any significant differences in cerebral and triceps brachii rSO2 at baseline, during exercise, recovery, or daily activity phase between all groups (Supplemental Figure S1A/B). Correlations between SpO2 from pulse oximetry and rSO2 from NIRS were calculated to evaluate whether a priori lower SpO2 might have led to an impaired rSO2 in every group. No significant correlation could be identified during exercise (renal: $p = 0.860$; cerebral: $p = 0.451$; quadriceps femoris: $p = 0.777$; triceps brachii: $p = 0.771$) and recovery period (renal: $p = 0.387$; cerebral: $p = 0.311$; quadriceps femoris: $p = 0.436$; triceps brachii: $p = 0.258$). A correlation of SpO2 to renal rSO2 at peak exercise (where major changes in SpO2 and rSO2 were observed) was not significant ($p = 0.3208$; Supplemental Figure S2A).

No significant correlation between EF and SpO2 at rest ($p = 0.650$) and SpO2 at peak exercise ($p = 0.109$) could be identified. Correlations between EF and NIRS baselines (cerebral: $p = 0.755$; triceps: $p = 0.0218$; renal: $p = 0.100$; quadriceps: $p = 0.864$) are presented in the novel Supplemental Figure S2B. Further, no correlation between EF and renal rSO2 at peak exercise ($p = 0.332$) was found.

Renal tissue

Absolute baseline renal rSO2 was lower in Fontan patients with PLE compared with patients without PLE ($p = 0.01$) and d-TGA patients ($p = 0.002$) (Table 4). In all time sections (exercise time, recovery, and daily activity), renal rSO2 remained lower during the entire exercise time ($p = 0.0062$), recovery period ($p = 0.029$), and during daily activity ($p = 0.03$) in patients with PLE compared with d-TGA patients (Fig. 2a). In addition to the differences in absolute baseline, rSO2 levels of PLE patients were lower than those in Fontan patients without PLE during all time sections without reaching statistical significance. No significant difference in rSO2 was found between Fontan patients without PLE and d-TGA patients for all investigated renal rSO2 values (baseline, exercise time, recovery period, and daily activity) (Fig. 2a).

The decline of rSO2 levels from baseline to peak exercise was similar in every group over the entire exercise time for all NIRS electrodes.

Skeletal muscle

No differences were identified between Fontan patients with or without PLE in rSO2 values of quadriceps femoris at baseline ($p = 0.87$) (Table 4) and other time sections (exercise: $p = 0.57$; recovery: $p = 0.81$; daily activity: $p = 0.31$) (Fig. 2b). Baseline rSO2 of quadriceps femoris was significantly lower in Fontan patients with PLE ($p = 0.003$) and Fontan patients without PLE ($p < 0.001$) compared with d-TGA patients (Table 4). rSO2 of quadriceps femoris was significantly lower during exercise time ($p = 0.041$), recovery period ($p = 0.016$), and daily activity ($p = 0.008$) in Fontan patients with PLE compared with d-TGA patients. Fontan patients without PLE showed significant lower rSO2 values during exercise time ($p = 0.007$), recovery period ($p < 0.001$), and daily activity ($p = 0.02$) compared with d-TGA patients (Fig. 2b).

Threshold analysis

For all participating patients, renal rSO2 threshold analysis was performed, including their total exercise, recovery period, and physiologic daily activity time. We analyzed the duration of measurements below renal rSO2 thresholds (<65, <55, and <45%) in percentage (Table 5). PLE patients remained 95% of their monitored exercise time below the threshold of <65%, 48% of their monitored exercise time below <55%, and 33.3% of their exercise time below the threshold of <45%, respectively. PLE patients fell below the hazardous threshold of 55% already during

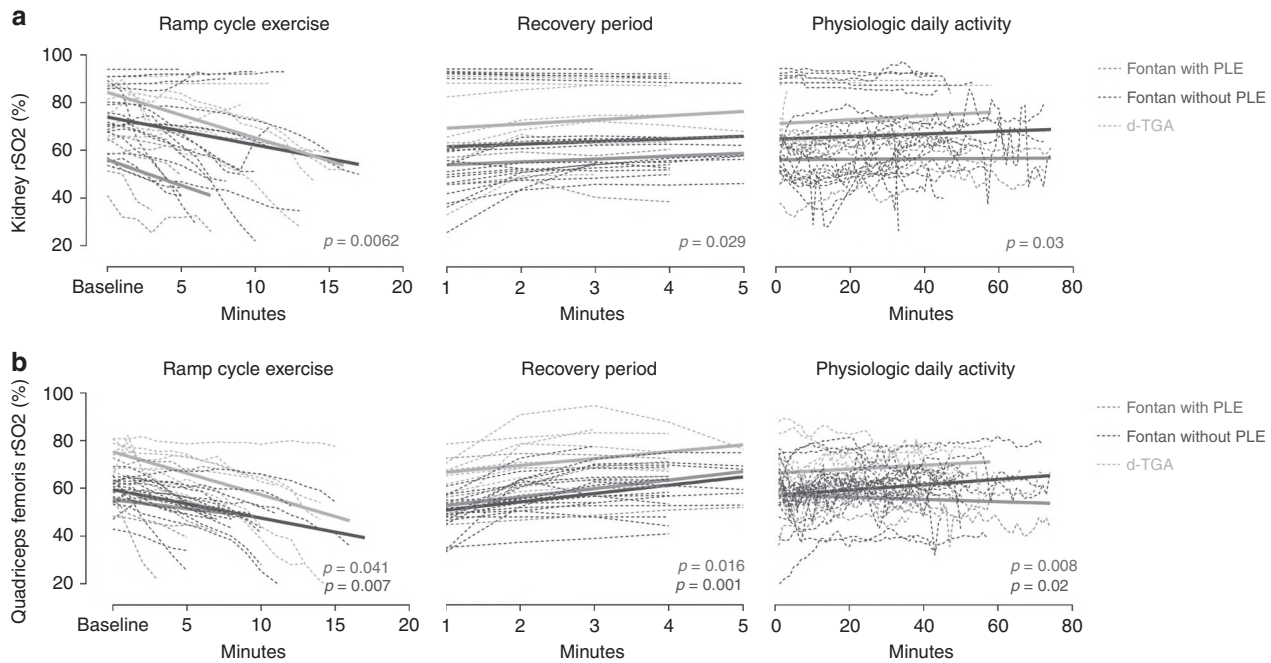

Fig. 2 Individual NIRS measurement of rSO2 in renal tissue and quadriceps femoris muscle. Graphs representing individual measurements of rSO2 in renal tissue (**a**) and quadriceps femoris (**b**) in Fontan patients with PLE (red), without PLE (blue), and d-TGA patients (green) during ramp cycle exercise, recovery period, and physiologic activities (left to right). A red *p* value (Fontan patients with PLE) and blue *p* value (Fontan patients without PLE) indicate significant differences compared with d-TGA, respectively

Table 5. Threshold analysis

	Fontan with PLE	Fontan without PLE	TGA
Renal rSO2			
<65% (critical)			
Exercise	95.0 ± 11.1	48.4 ± 39.1	26.1 ± 28.7
Daily activity	83.7 ± 34.8	53.0 ± 42.8	24.3 ± 27.1
<55% (hazardous)			
Exercise	48.0 ± 50.2	30.4 ± 33.4	9.1 ± 16
Daily activity	40.3 ± 52.6	30.6 ± 36.1	15.6 ± 35.2
<45% (acute risk)			
Exercise	33.3 ± 47.1	11.4 ± 18.3	2.9 ± 8.2
Daily activity	19.7 ± 41.8	4.7 ± 8.1	—
Cerebral rSO2			
<45% (critical)			
Exercise	14.3 ± 28.3	6.2 ± 22.9	—
Daily activity	4.1 ± 10.7	2.5 ± 10.9	—

Comparison of the relative duration (% of monitored total exercise time, time of daily activity) at each threshold of renal (65%, 55%, and 45%) and cerebral (45%) rSO2 values. Values are expressed as mean ± SD

40% of their monitored daily activity time. Fontan patients without PLE mostly remained above these rSO2 threshold limits and d-TGA patients never dropped below the critical threshold of 45% during daily activity, except during high exercise (Table 5). Analogously, cerebral threshold analysis was performed for the critical rSO2 threshold <45%. Fontan patients with PLE underwent this threshold during 14% of their exercise time, while Fontan patients without PLE underwent this threshold only during 6% of their exercise time. D-TGA patients did not decline below the critical cerebral rSO2 threshold (Table 5). The lowest renal rSO2 values (91.7% of the observational time period below the renal rSO2 threshold of 45%) were obtained from a Fontan patient with PLE who was listed for HTX and died shortly after transplantation.

DISCUSSION

This study presents results from multisite NIRS measurements in Fontan patients with and without PLE and patients with regular biventricular circulation after d-TGA repair at rest, intense exercise, and daily activities. We demonstrate significant differences in rSO2 between patients with univentricular and biventricular circulation in the skeletal muscle of the lower limbs and between Fontan patients with and without PLE in renal tissue.

The effect of desaturation in the activated muscle during exercise has been described in several studies involving NIRS measurements.[29–31] We found similar desaturation of these tissues in patients with univentricular and biventricular physiology. Our data demonstrate significantly lower pre-existing rSO2 values in the quadriceps femoris muscle of univentricular compared with biventricular patients. Fontan patients with and without PLE, did not show normal rSO2 values at rest, with rSO2 dropping significantly during physical exercise.

Patients with univentricular circulation presented a reduced exercise capacity. This is discussed to be due to circulatory impairment of the single ventricle, i.e., increased pulmonary vascular resistance, passive pulmonary perfusion, consecutive low cardiac filling, impaired chronotropic response, or reduced myocardial or valvular function, all resulting in a varying degree of low cardiac output.[7,17,32–34] However, cardiac MRI revealed no differences in single-ventricle function between Fontan patients with or without PLE. Blood pressure was lower in Fontan patients with PLE compared with Fontan patients without PLE and d-TGA patients. However, this finding might have been biased by diuretic treatment (Table 2). Further, peripheral SpO2 was significantly lower in all Fontan patients compared with biventricular patients, yet no correlation with rSO2 was found. In Fontan patients with PLE, desaturation as determined by renal rSO2, seemed to be disproportionally lower to concomitant SpO2 measurement (Supplemental Figure S2B). This might suggest that renal rSO2 could possibly present a more sensitive measurement for oxygen supply independently from ventricular function and peripheral SpO2.

The observed lower muscle rSO2 at rest and considerable desaturation even during daily activity (with low exercise) in Fontan patients correlates well with the common assumption that these patients suffer from mild-to-moderate heart failure with reduced cardiac output in the absence of clinical symptoms.[7,8,35] Regarding the exercise capacity in our cohort, Fontan patients and particularly those with PLE showed early exhaustion, lower maximum watt levels, and watts/kg compared with d-TGA patients after biventricular repair.[32,36,37]

Interestingly, rSO2 of the brain and upper limbs did not show any significant alteration in all univentricular patients when compared with the biventricular patient group. This may be a consequence of venous congestion of the abdominal and lower limb perfusion in Fontan patients, which might have been aggravated by the sitting posture while cycling.

Our analysis revealed low rSO2 in renal tissue at all investigated time points in Fontan patients with PLE only. Renal tissue oxygenation has been evaluated by NIRS in several studies[18,20,24,25] to predict the outcome and organ injury in critically ill children and adolescents. As demonstrated in the studies of Ruf et al.[18] and Choi et al.,[25] prolonged desaturation of renal tissue with rSO2 values below critical threshold values of 55% and 45%, respectively, during cardiopulmonary bypass, is associated with an increased risk for acute kidney injury and postoperative kidney failure. The authors concluded that renal NIRS might be a promising tool for monitoring kidney function and preventing ischemic kidney injury during surgery.[18] Examining adult patients with Fontan circulation without PLE, Navaratnam et al.[20] found a significant correlation between exercise and SVP augmentation. When compared with healthy controls, high-intensity exercise in these stable Fontan patients without PLE was associated with renal and cerebral deoxygenation, potentially contributing to end-organ dysfunction, such as hepatic fibrosis, a characteristic risk in the Fontan circulation.[20]

In line with these observations, our Fontan patients suffering from PLE repeatedly presented with rSO2 levels hazardous or critical for kidney injury. Thus, this finding might be associated with a potential risk for the development of chronic kidney disease or even failure in Fontan patients with PLE. In general, there is a large body of evidence, indicating that chronic kidney disease is driven by renal tissue hypoxemia.[38] End-organ dysfunction, including renal failure, is a late complication in Fontan patients with PLE and has been associated with poor outcome.[10] In line with this finding, we observed the highest mortality in our study in a highly morbid Fontan patient with PLE that presented with extremely low renal rSO2 values. A possible correlation between renal rSO2 and outcome parameters needs to be further analyzed in larger patient cohorts. Based on our data, we conclude that renal disease in Fontan patients with PLE could be caused by chronic and intermittent ischemic injury secondary to reduced tissue oxygenation. Further studies have to evaluate the pathophysiologic and circulatory mechanisms in patients with univentricular circulation, leading to this phenomenon. Our cohort of PLE patients did not present with reduced kidney function based on creatinine and cystatine C levels, which could be due to their young age and reduced muscle mass in the investigated cohort.[8,39] Measuring rSO2 via NIRS might be a promising tool to identify Fontan patients at risk for kidney failure during routine outpatient visits in long-term follow-up. Furthermore, our data

suggest that renal NIRS monitoring might even have a significant advantage over pulse oximetry when screening for PLE in Fontan patients.

Renal tissue rSO2 in Fontan patients with PLE may reflect tissue oxygenation of the splanchnic region, including the intestines. Decreased rSO2 in the splanchnic region in the setting of high systemic venous and intestinal lymphatic pressure due to the univentricular circulation may be due to microcirculatory disturbances, leading to impairment of the endothelial architecture. We hypothesize that similar mechanisms of decreased rSO2 may play a role in the development of PLE.

LIMITATIONS

This study has several limitations. The investigated sample size was small, and we did not employ a healthy control group, as biventricular-corrected d-TGA patients share important clinical features with Fontan patients: both present with neonatal hypoxemia/cyanosis and undergo neonatal cardiac surgery with sternotomy.

We did not include full cardiopulmonary exercise testing; thus, the influence of respiratory effort on NIRS remains undetermined in our patients, but has already been described in earlier studies.[13,16] The influence of other potential confounders, such as oxygen-carrying capacity (no Fe/ferritin/transferrin or SaO2 determined), adjunctive medication, cardiac anatomy, hemodynamics (no current cardiac catheterization data available), and cardiac rhythm, cannot be completely ruled out, partly due to a limited number of control (d-TGA) patients.

Our study was based on findings, indicating that chronic kidney disease is driven by renal tissue hypoxemia. However, we did not further examine potential negative influences of acute hypoxemia on renal function. Thus, our data are not suited to derive the final recommendations for exercise in Fontan patients with PLE. Also, while we used the term "renal NIRS" throughout this paper, it has to be noted that this merely refers to the placement of the probe, while the detected tissue oxygenation reading is composed of signals deriving from various neighboring regions, such as the splanchnic bed. No additional blood flow measurements were undertaken to further distinguish between these NIRS signal compounds.

CONCLUSION

Univentricular circulation in Fontan patients is associated with decreased rSO2 of skeletal muscle, particularly of the lower limbs, and with hypoxemia of renal tissue in Fontan patients with PLE, which was evident even at low exercise activities of daily life. Reduced oxygenation in tissues with high oxygen demand already during activities of daily life might play a role in the development of end-organ dysfunction, i.e., renal failure or PLE itself in this patient population. The role of acute and chronic tissue hypoxemia in Fontan patients associated with PLE and kidney failure needs to be clarified in further studies. Furthermore, multisite NIRS might be a promising tool for monitoring of Fontan patients at risk for kidney failure or PLE during routine outpatient visits.

ACKNOWLEDGEMENTS

This work was supported by the German Heart Foundation and German Society of Pediatric Cardiology (DGPK) through the Gerd-Killian grant. The presented work was performed in fulfillment of the requirements for obtaining the degree "Dr. med" at "Friedrich-Alexander University of Erlangen-Nürnberg (FAU)" of Simon Schröer. This work was performed in close cooperation with the German competence network for congenital heart disease. We thank our biostatistician D. Keller (Statistics and Consultation, Kürnach, Germany) for compiling the statistical analysis of the data and V. Laternser and J. Halbfass for English language editing. The paper was presented as a poster at the Annual Meeting of the Association for European Pediatric and Congenital Cardiology AEPC in Rome 2016.

AUTHOR CONTRIBUTIONS

J.M. and O.T. designed the study and contributed to the paper. S.D., R.C., H.R.T. and F.B.F. contributed to the design of the study. F.M. and A.R. offered the technical expertise of NIRS measurements. J.M., M.A., S.S. and F.M. collected the data. S.S., F.M. and J.M. analyzed and interpreted the data and drafted the paper. M.R. carried out the laboratory analysis of the data. All participating authors critically revised the paper before submission.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-019-0346-3) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Fontan, F. & Baudet, E. Surgical repair of tricuspid atresia. *Thorax* **26**, 240–248 (1971).
2. Khairy, P. et al. Long-term survival, modes of death, and predictors of mortality in patients with Fontan surgery. *Circulation* **117**, 85–92 (2008).
3. Wilson, T. G. et al. Twenty-five year outcomes of the lateral tunnel Fontan procedure. *Semin. Thorac. Cardiovasc. Surg.* **29**, 347–353 (2017).
4. Downing, T. E. et al. Long-term survival after the Fontan operation: twenty years of experience at a single center. *J. Thorac. Cardiovasc. Surg.* **154**, 243–253.e2 (2017).
5. Pundi, K. N. et al. 40-Year follow-up after the fontan operation: long-term outcomes of 1,052 patients. *J. Am. Coll. Cardiol.* **66**, 1700–1710 (2015).
6. Gewillig, M. & Brown, S. C. The Fontan circulation after 45 years: update in physiology. *Heart* **102**, 1081–1086 (2016).
7. Gewillig, M. et al. The Fontan circulation: who controls cardiac output? *Interact. Cardiovasc. Thorac. Surg.* **10**, 428–433 (2010).
8. Rychik, J. The relentless effects of the Fontan paradox. *Semin. Thorac. Cardiovasc. Surg. Pediatr. Card. Surg. Annu.* **19**, 37–43 (2016).
9. Alsaied, T. et al. Factors associated with long-term mortality after Fontan procedures: a systematic review. *Heart* **103**, 104–110 (2017).
10. Mori, M. et al. Beyond a broken heart: circulatory dysfunction in the failing Fontan. *Pediatr. Cardiol.* **35**, 569–579 (2014).
11. Mizuno, M. et al. Diverse multi-organ histopathologic changes in a failed Fontan patient. *Pediatr. Int.* **58**, 1061–1065 (2016).
12. Fleck, T. et al. Propofol effect on cerebral oxygenation in children with congenital heart disease. *Pediatr. Cardiol.* **36**, 543–549 (2015).
13. Loomba, R. S. et al. Effect of Fontan fenestration on regional venous oxygen saturation during exercise: further insights into Fontan fenestration closure. *Pediatr. Cardiol.* **35**, 514–520 (2014).
14. Hoffman, G. M. et al. Perioperative cerebral oxygen saturation in neonates with hypoplastic left heart syndrome and childhood neurodevelopmental outcome. *J. Thorac. Cardiovasc. Surg.* **146**, 1153–1164 (2013).
15. Rao, R. P. et al. Measurement of regional tissue bed venous weighted oximetric trends during exercise by near infrared spectroscopy. *Pediatr. Cardiol.* **30**, 465–471 (2009).
16. Rao, R. P. et al. Cerebral hemodynamics in the presence of decreased systemic venous compliance in patients with Fontan physiology may limit anaerobic exercise capacity. *Pediatr. Cardiol.* **31**, 208–214 (2010).
17. Forman, E. et al. Noninvasive continuous cardiac output and cerebral perfusion monitoring in term infants with neonatal encephalopathy: assessment of feasibility and reliability. *Pediatr. Res.* **82**, 789–795 (2017).
18. Ruf, B. et al. Intraoperative renal near-infrared spectroscopy indicates developing acute kidney injury in infants undergoing cardiac surgery with cardiopulmonary bypass: a case-control study. *Crit. Care* **19**, 27 (2015).
19. Su, X. W. et al. Improved cerebral oxygen saturation and blood flow pulsatility with pulsatile perfusion during pediatric cardiopulmonary bypass. *Pediatr. Res.* **70**, 181–185 (2011).
20. Navaratnam, D. et al. Exercise-induced systemic venous hypertension in the Fontan circulation. *Am. J. Cardiol.* **117**, 1667–1671 (2016).
21. Kromeyer-Hauschild, K. et al. Perzentile für den body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Mon. Kinderheilkd.* **149**, 807–818 (2001).
22. Horibata, Y., Murakami, T. & Niwa, K. Effect of the oral vasopressin receptor antagonist tolvaptan on congestive cardiac failure in a child with restrictive cardiomyopathy. *Cardiol. Young-*. **24**, 155–157 (2014).

23. Bernal, N. P. et al. Cerebral and somatic near-infrared spectroscopy in normal newborns. *J. Pediatr. Surg.* **45**, 1306–1310 (2010).
24. Colasacco, C. et al. Near-infrared spectroscopy monitoring to predict post-operative renal insufficiency following repair of congenital heart disease. *World J. Pediatr. Congenit. Heart Surg.* **2**, 536–540 (2011).
25. Choi, D. K. et al. Intraoperative renal regional oxygen desaturation can be a predictor for acute kidney injury after cardiac surgery. *J. Cardiothorac. Vasc. Anesth.* **28**, 564–571 (2014).
26. Dent, C. L. et al. Brain magnetic resonance imaging abnormalities after the Norwood procedure using regional cerebral perfusion. *J. Thorac. Cardiovasc. Surg.* **130**, 1523–1530 (2005).
27. Faul, F. et al. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav. Res. Methods* **41**, 1149–1160 (2009).
28. Faul, F. et al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **39**, 175–191 (2007).
29. Callewaert, M. et al. Quadriceps muscle fatigue in trained and untrained boys. *Int. J. Sports Med.* **34**, 14–20 (2013).
30. Bowen, T. S. et al. The spatial distribution of absolute skeletal muscle deoxygenation during ramp-incremental exercise is not influenced by hypoxia. *Adv. Exp. Med. Biol.* **876**, 19–26 (2016).
31. Okushima, D. et al. Muscle deoxygenation in the quadriceps during ramp incremental cycling: deep vs. superficial heterogeneity. *J. Appl. Physiol.* **2015**, 1313–1319 (1985).
32. Hager, A. et al. Predictors of sildenafil effects on exercise capacity in adolescents and adults with Fontan circulation. *Clin. Res. Cardiol.* **103**, 641–646 (2014).
33. Fredriksen, P. M. et al. Aerobic capacity in adults with various congenital heart diseases. *Am. J. Cardiol.* **87**, 310–314 (2001).
34. Bradley, E. A., Berman, D. & Daniels, C. J. First implantable hemodynamic monitoring device placement in single ventricle fontan anatomy. *Catheter. Cardiovasc. Interv.* **88**, 248–252 (2016).
35. Talwar, S. et al. Outcomes of patients undergoing primary fontan operation beyond first decade of life. *World J. Pediatr. Congenit. Heart Surg.* **8**, 487–494 (2017).
36. Ohuchi, H. Cardiopulmonary response to exercise in patients with the Fontan circulation. *Cardiol. Young-*. **15**(Suppl 3), 39–44 (2005).
37. Khiabani, R. H. et al. Exercise capacity in single-ventricle patients after Fontan correlates with haemodynamic energy loss in TCPC. *Heart* **101**, 139–143 (2015).
38. Fu, Q., Colgan, S. P. & Shelley, C. S. Hypoxia: the force that drives chronic kidney disease. *Clin. Med. Res.* **14**, 15–39 (2016).
39. Opotowsky, A. R. et al. Estimated glomerular filtration rate and urine biomarkers in patients with single-ventricle Fontan circulation. *Heart* **103**, 434–442 (2016).