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# **Evolution of muscular oxygenation during a walking test in pre-term children.**

Owen-Jones Z. (M.Sc)<sup>1-2</sup>, Perrochon A. (Ph.D)<sup>1-2</sup>, Hermand E. (Ph.D)<sup>2-3</sup>, Ponthier L. (M.D)<sup>4</sup>, L. Fourcade (Pr./M.D)<sup>5</sup>, Borel B (Ph.D)<sup>1-2</sup>.

<sup>1</sup> ILFOMER, Institut Limousin de Formation aux Métiers de la Réadaptation, F-87000 Limoges, France

<sup>2</sup> Laboratoire HAVAE, EA6310, Université de Limoges, Limoges, France,

<sup>3</sup> Laboratoire UMR INSERM U1272 'Hypoxie & Poumon', University Paris 13, Bobigny, France

<sup>4</sup> Service de Réanimation néonatal et pédiatrique, CHU de Limoges, Limoges, France

<sup>5</sup> Département de Chirurgie pédiatrique, Hopital Mère Enfant, Université de Limoges, Limoges, France

## **Corresponding Author**

BOREL Benoit  
Laboratoire HAVAE (EA6310)  
Faculté des Sciences et Techniques – Université de LIMOGES  
123 Avenue Albert Thomas 87060 LIMOGES Cedex  
Phone: +33 (0)5 55 45 76 32

E-mail: benoit.borel@unilim.fr

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## **ABSTRACT**

**OBJECTIVE:** The aim of the study was to explore measures of peripheral muscular oxygenation, coupled to gait characteristics, between premature (PT) and full-term (FT) children during a 6-minute walking test (6MWT).

**STUDY DESIGN:** Prepubescent children performed a 6MWT. During the test, changes in muscular oxyhaemoglobin ( $\Delta\text{HbO}_2$ ), deoxyhaemoglobin ( $\Delta\text{HHb}$ ) and total haemoglobin ( $\Delta\text{tHb}$ ) were measured with NIRS technology, positioned on subjects' calves. Gait variables were monitored with an OPTOGAIT system.

**RESULTS:** 45 children (33 FT children and 12 PT children) participated in this study (mean age $\pm$ SD: 4.9 $\pm$ 0.7 and 4.6 $\pm$ 0.9 years, respectively). Statistical analysis highlighted a decreased walking performance for PT children, with significantly lower walking distance ( $p<0.05$ ) than children born FT (405.1 $\pm$ 91.8m vs. 461.0 $\pm$ 73.3m respectively, -9%). A concomitant increase of oxygen extraction (over  $\Delta\text{HHb}$  time course) was observed from the third minute of the test ( $p<0.05$ ). No statistically significant difference was found for other NIRS measures. Lastly, the analysis of gait variables highlighted a group effect for walking speed ( $p<0.05$ ) and stride length ( $p<0.01$ ).

**CONCLUSION:** Premature children showed decreased walking performance and greater change in peripheral muscular oxygen extraction, associated with lower walking speed and stride length. This may point to a muscular maladjustment and reduced functional capacities for children born PT. These phenomena could be responsible for greater muscular fatigue.

## **List of abbreviations**

6MWT: 6-Minute Walking Test

$\Delta\text{HbO}_2$ : Variation of Oxyhemoglobin

$\Delta\text{HbTot}$ : Variation of Total Hemoglobin

$\Delta\text{HHb}$ : Variation of Desoxyhemoglobin

BPD: Bronchopulmonary Dysplasia

COPD: Chronic Obstructive Pulmonary Disease

FT: Full-term children

NIRS: Near Infra-Red Spectroscopy

PT: Pre-term children

# **Evolution of muscular oxygenation during a walking test in pre-term children.**

**Keywords:** Preterm - Walking test - NIRS – Muscular oxidative capacity – functional capacity

## **INTRODUCTION**

Prematurity leads to multiple consequences and, in particular, causes an interruption of lung development, impacting the normal pulmonary vascular and alveolar development (1). The major respiratory pathology developed in prematurity is bronchopulmonary dysplasia (BPD), affecting up to 40 % of preterm (PT) children with birthweight below 1000g (2). During exercise, children with BPD show ventilatory limitations and a higher oxygen uptake for the same power output than full term (FT) children (3). Moreover, long term consequences seem to be important as adult survivors of preterm birth, with or without BPD, globally exhibit mechanical constraint to increase tidal volume during near-maximal exercise, associated with severe dyspnea and leg discomfort (4). Still, little information is available to explain how impaired lung function in PT children alters exercise capacity (5).

One way to investigate for muscle function is non-invasive techniques like Near Infra-Red Spectroscopy (NIRS) (6). NIRS technology has been previously used in neonates and premature babies to measure tissue oxygenation and perfusion in regions of interest, e.g. the brain, kidneys or peripheral muscles, in early states of shock (7). It has also been used in children with muscular pathologies and chronic heart disease, in whom different profiles of muscular oxygenation were evidenced, compared to healthy subjects (8,9). This method reveals a decrease in oxidative capacities in lower leg muscle that contribute to exercise intolerance in

these patients (10). Similarities between BPD and COPD suggest that children born PT may present a high risk of exhibiting similar muscular disorders, also responsible for the reduced functional capacities reported in this population (11).

The objective of this study was to compare measures of peripheral muscular oxygenation using NIRS between PT and FT children during a 6-minute walking test (6MWT). We hypothesized that children born PT have impaired muscular oxygenation, compared to children born FT.

## Methods

This cross-sectional study was conducted on two populations: i) an experimental group born prior to 32 weeks of gestation; and ii) a control population of children born after 37 weeks of gestation. PT children were recruited at the ‘Hopital Mère Enfant’ (HME) in Limoges (France), followed by the ‘Centre d’Action Médico-Sociale Précoce’ (CAMSP) until their sixth birthday. The control population was composed of FT children recruited in several recreational centres in Limoges. Patients aged from 3 to 6 years old were eligible. Exclusion criteria were moderate PT children, major neurological disabilities (cerebral palsy) or cardiac pathologies that would interfere with protocol completion and any refusal of participation by the child or parents. Parents signed a consent form and authorization before the participation of their children to this protocol. The study was carried out according to the standards set by the Declaration of Helsinki.

The study took place over a 3-month period. At their arrival children were asked to sit and the NIRS probe was placed on the right medial gastrocnemius (Figure 1) (12). They were asked to walk as fast as possible once the start had been given and this for 6 minutes (6MWT). The starting line and the turning area were marked by a line and a cone. The baseline NIRS measurements began before the walking test once children were asked to stand up. At the end of the test, children were asked to sit and the NIRS records were stopped.

\*\*\* Insert Figure 1 near here \*\*\*

Changes in muscle oxygenation and blood volume during the 6MWT was measured with a NIRS device (PortaMon, Artinis, Netherlands) (13). The NIRS probe was placed on the right medial gastrocnemius as illustrated in Figure 1.

NIRS acquisition was made through the Oxysoft software (version 3.0.97.1), with a 2 Hz sample frequency. Differential Pathlength Factor (DFP) was set on 4 (14). A 0.1 Hz low-pass filter was applied to the signal to remove physiological and instrumental noise. The relative concentrations in oxy- and deoxyhemoglobin ( $\Delta\text{HbO}_2$  and  $\Delta\text{HHb}$ , respectively,  $\mu\text{mol.L}^{-1}$ ) during the last 10 seconds of each minute of the 6MWT were then normalized by subtracting the mean value of the last 15 s of baseline, immediately before the beginning of the task, i.e., standing up. From these data the total haemoglobin ( $\Delta\text{HbTot} = \Delta\text{HbO}_2 + \Delta\text{HHb}$ ) was determined.

A 6MWT was conducted in accordance with American Thoracic Society recommendations (15,16). Children were asked to walk back and forth along a 30-meter flat corridor as quickly as possible for 6 minutes. The covered distance (6MWT, in meters) was then recorded upon the completion of the test. During the 6MWT, gait was measured using an OPTOGAIT system (Microgate, Bolzano, Italy) (17), placed at the beginning of the walking path 2 meters after the starting line in order to eliminate the acceleration and deceleration phase. Speed ( $\text{m.s}^{-1}$ ), cadence ( $\text{st.min}^{-1}$ ), stride step (cm) and stride time (s) were collected at each passage.

Unless otherwise stated, data are expressed as mean  $\pm$  SD.

Data analysis was performed, for all the tests described below, using Prism software (software version 6.0c, GraphPad, San Diego, CA, USA).

For each statistical test performed, a significance level was set at  $p < 0.05$ . The normality of data distribution was verified with the D'Agostino & Pearson normality test. Depending of the normality of data distribution, parametric and non-parametric tests were applied for further statistical analysis.

Mean walking performance was compared between the groups using an unpaired t-test.



All the muscular oxygenation measures ( $\Delta\text{HbO}_2$ ,  $\Delta\text{HHb}$  and  $\Delta\text{HbTot}$ ) and gait variables were analyzed by including the entire 6-minute walking test. NIRS data were analyzed by repeated-measures two-way analysis of variance (ANOVA) protocol, with “Group” and “Time (minute)” factors. In the case of a significant interaction, a multiple comparison procedure, the Bonferroni’s multiple comparisons test, was used.

## Results

Fifty-three children (38 FT and 15 PT) were recruited for this study and 33 FT children and 12 PT completed the entire protocol and were included for data analysis. The study flow chart is presented in Figure 2.

\*\*\* Insert Figure 2 near here \*\*\*

Population characteristics are presented in Table 1. All the PT children had a BPD diagnosis. As expected, PT children had lower birthweight, weight and height at the same age than FT children ( $p < 0.001$ ,  $p < 0.05$  and  $p < 0.05$ , respectively). The gait acquisition was also earlier for FT children than for PT ones ( $p < 0.001$ ), as well as independent walking ( $p < 0.001$ ). The two populations were similar in terms of average age ( $p > 0,05$ ).

\*\*\* Insert Table 1 near here \*\*\*

At the end of the 6MWT, FT children walked a greater distance than PT ones ( $461.0 \pm 73.3$  vs.  $405.1 \pm 91.8$  m,  $p < 0,05$ ).

The time-course variations of NIRS measures (i.e.  $\Delta\text{HbO}_2$ ,  $\Delta\text{HHb}$  and  $\Delta\text{HbTot}$ ) are illustrated in Figure 3.  $\Delta\text{HbO}_2$  decreases during the 6MWT, indicating that peripheral muscle shows a greater deoxygenation (defined as a fall of  $\text{HbO}_2$ ) at the onset of the 6MWT. This was followed by a plateau phase at the third minute, with no difference between the two populations ( $p > 0,05$ ). We observed an increase of  $\Delta\text{HHb}$ , then reaching also a plateau at the third minute (cf. Figure 3). The peripheral oxygen extraction is enhanced at the beginning of exercise and will then stabilized after 3 minutes of moderate intensity activity. There is a significative difference

between the two populations starting at the third minute of the 6MWT ( $p < 0.05$ ), with greater oxygen extraction (i.e. increase of  $\Delta\text{HHb}$ ) for PT group in comparison with FT group. This variation is maintained until the end of the test.

Lastly, there is no difference for  $\Delta\text{HbTot}$  between the two populations during the 6MWT.  $\Delta\text{HbTot}$  starts by slightly decreasing and then increases after 3 minutes of exercise.

\*\*\* Insert Figure 3 near here \*\*\*

Time-course evolution of gait variables are shown in Figure 4. The statistical analysis shows similar results for walking speed and stride length, with a significant “Group” effect ( $p < 0.05$  and  $p < 0.01$ , respectively), but with no significant effect of “Time” factor and without factors interaction. Stride cycle time presents no significant differences and no significant interaction.

\*\*\* Insert Figure 4 near here \*\*\*

## Discussion

In this work, our main finding highlights a decreased walking performance in the PT population. Thus, prematures exhibit a reduced functional capacity, associated with a greater variation in peripheral muscular oxygen extraction ( $\Delta\text{HHb}$ ) and a specific gait adaptation, compared to FT, during a standardized walking evaluation. Other results show no difference in terms of oxygen supply ( $\Delta\text{HbO}_2$ ) or blood volume (perfusion  $\Delta\text{HbTot}$ ).

The lower performance reported for the PT population has been previously observed in PT children with very low birthweight (11,18). Several explanations are possible for the decreased performance in the PT group. FT children are significantly taller, associated with higher stride length normalized to height. Therefore, with the same exercise time, measured height could help FT children to reach higher walking distance. Secondly, lower functional capacity could therefore be the expression of lung impairment and a global poor adaptation to exercise in the PT population. This could also be explained by decreased muscle mass. In order to investigate the potential impairment of muscle function in the PT population, we used a non-invasive monitoring method (NIRS), that uses near-infrared light for exploring muscular oxygenation and its adaptation to exercise.

The differences of muscular oxygen variables observed in the two populations are consistent with the NIRS data measured in adults and children (9,12–14). In both groups, a walking task first leads to a simultaneous reduction of  $\Delta\text{HbO}_2$  and increase of  $\Delta\text{HHb}$ , reflecting a temporary inadequacy between  $\text{O}_2$  consumption and delivery (19–21). After 3 minutes of walking, a plateau phase is then observed. As noted before:  $\text{O}_2$  delivery is then sufficient to compensate for metabolic requirements (22). However,  $\Delta\text{HHb}$  increases more in the PT population after 3

minutes of walking and until the end of the 6MWT. In previous studies, a greater O<sub>2</sub> consumption was observed in BPD children during exercise (5).

The skeletal muscular oxygenation data show a higher oxygen extraction during exercise in PT children compared to FT, reflecting the metabolic state of the activated muscle. Prematurity has numerous consequences for child development. A previous study by Lewandowski et al. reports specific left side cardiac adaptations due to prematurity (23). This could lead to reduced cardiac volume and reduced O<sub>2</sub> delivery, which could explain the greater O<sub>2</sub> extraction for PT population. However, in this study, the kinetics of oxyhaemoglobin (HbO<sub>2</sub>) do not differ between PT and FT children. This could indicate that the lower performance (distance) observed for PT children for the walking test is mainly associated with a muscle dysfunction, explaining the greater O<sub>2</sub> extraction.

In healthy subjects, muscular performance partially depends on the proportion of the different types of muscular fibers in the activated muscle. Prematurity has a negative impact on the respiratory function similar to COPD patients in which muscle morphologic adaptations such as fat infiltration, fibrosis, inflammation, increased subcutaneous adipose, loss of type I fibers and mitochondrial density may hamper muscle oxidative capacity (6,10). Muscular physiological characteristics of PT children with BPD could be similar to patients with COPD. Wenchich et al. (2002) showed a reduction of the number of mitochondrial proteins and activity of the PDH and COX enzymes in very PT neonates (24). The muscular oxygen metabolism is then impaired, and the subsequent ATP production is reduced. Skeletal muscle dysfunction and peripheral factors are major determinants of reduced exercise capacity and exercise intolerance, and could contribute to their reduced involvement in physical activities (9). Very PT children would be more prone to fatigue, during moderate intensity physical activity, requiring a greater O<sub>2</sub> extraction in activated muscle in order to respond to the metabolic needs.

Another potential explanation for the lower performance observed for PT population, related to the muscle function, could be the difference in gait observed in this study. Despite the absence of significant interaction for both factors (Time and Group), gait seemed to be influenced by group condition (PT vs. FT). Walking speed and stride length are both lower for the PT group, in comparison with FT group, throughout the 6-minute walking test. Walking speed could be directly linked to muscle function and contractile capacity but also to the efficiency of the walk for each child, PT children having a later age for independent walk. Lastly, the length of lower limbs could influence the length of the stride, as confirmed by a significant difference for normalized stride length to height between the two groups. However, this hypothesis requires a more precise assessment of sitting height, for the determination of the length of lower limbs.

They are limitations to report for this study. First, children from 3 to 6 years old may present various degree of psychomotor maturity (Cf. Table 1). Nevertheless, the mean age between the two populations was not significantly different, which allowed comparisons between the populations of PT and FT children.

The NIRS technique is a technology that is sensitive to methodological considerations and evaluates oxygenation variables in a limited area of a targeted muscle group. Also, muscle microvascular oxygenation is heterogeneous within the studied muscle (25); in order to avoid this issue, the probe placement was standardized (Cf. Figure 1). In order to have a global approach, it would have been interesting to couple the NIRS measures with  $VO_2$  measurements and cardiorespiratory adaptations. Furthermore, NIRS only offers a peripheral view and NIRS measurement could be influenced by central factors linked to cardio-vascular characteristics (like blood flow and blood volume variations, haemoglobin or haematocrit levels). It would be useful to couple NIRS assessment with other complementary control measurement in order to provide a more global point of view.

Children born PT with BPD showed reduced functional capacities during the 6MWT compared to FT children. This study supports the hypothesis that prematurity has pulmonary and muscular impact on childhood. Further studies are needed to clarify the strategy for respiratory monitoring of these children during childhood and adulthood.

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### **Authors contributions**

The different authors contributed at different levels to the different steps of the study conception, data acquisition, data analysis and manuscript writing and reviewing process.

Z.O-J: study conception, data acquisition, data analysis, manuscript writing and reviewing

A.P: study conception, manuscript writing and reviewing

E.H: data acquisition, data analysis and reviewing

L.P: reviewing

L.F: study conception and reviewing

B.B: study conception, data analysis, manuscript writing and reviewing

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Table 1 : Population characteristics

Values are expressed as mean  $\pm$  SD. Stride length was normalized to height of children, in order to consider significant difference for height between the two groups.

Figure 1 : NIRS setup on the lower limb of children

A/ Position of the probe on the calf during walking test realization ; B/ Near Infra-Red Spectroscopy (NIRS) probe (Portamon, Artinis) ; C/ Schematic representation of the evolution of NIRS parameters (HbO<sub>2</sub> : oxyhemoglobin, HHb : Deoxyhemoglobin, HbTot : total hemoglobin) during exercise.

First, a straight line (ST) between the middle of the popliteal fossa and calcaneus was traced. Larger calf circumference (LCC) was then measured and traced. At the intersection between the LCC and ST the probe was moved medially from LCC/8 cm and finally secured around the lower leg.

Figure 2 : Flow chart

Figure 3 : Evolution of muscular oxygenation measures during the 6MWT

Caption : PT : Pre-term children ; FT : Full-term children. Graph A (Top) : Oxyhemoglobin variation ; Graph B (Middle) : Deoxyhemoglobin variation ; Graph C (Bottom) : Total Hemoglobin variation.

\* :  $p < 0.05$  ; \*\* :  $p < 0.01$  ; \*\*\* :  $p < 0.001$

Figure 4 : Evolution of gait variables throughout the walking test

Caption : PT : Pre-term children ; FT : Full-term children. Graph A (Top) : walking speed (m.s-1) ; entre Graph B (Middle) : stride length (cm) ; Graph C (Bottom) : stride cycle time (seconds).