Evaluation of muscle oxygenation by near-infrared spectroscopy in patients with Becker muscular dystrophy

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Abstract

Several authors have reported alterations in vasodilation during effort in patients with dystrophinopathies, in which a lack of neuronal NO synthase is thought to lead to functional muscle ischemia. In order to determine changes in muscle oxygenation during effort in patients with Becker muscular dystrophy (BMD) and assess the parameters’ links with disease severity and functional status, 10 BMD patients and 10 age-matched controls performed isokinetic, constant-load knee extension exercises at (i) 20\% of their extensors’ peak torque (i.e. the same relative load) and (ii) the same absolute load (20 Nm). Muscle oxygenation was evaluated noninvasively using near-infrared spectroscopy (NIRS), with the time course of deoxygenation as the main criterion. As expected, BMD patients displayed a lower peak torque than controls (62\%). During both types of exercise, initial muscle deoxygenation was faster (by 27–41\%) in BMD patients than in controls. Greater disease severity (according to the Motor Function Measure) and functional impairment (walking endurance) were associated with a faster second deoxygenation phase (\tau). The validity and relevance of muscle deoxygenation parameters and the alteration of vasodilatation by nNOS deficiency in dystrophinopathies should be assessed by further studies.

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1. Introduction

Becker muscular dystrophy (BMD) is an X-linked, inherited muscle disease caused by mutation of the dystrophin gene. The main symptom is progressive, proximal muscle weakness. Clinically, BMD constitutes a milder variant of Duchenne muscular dystrophy (DMD, also a dystrophinopathy). Dystrophin is part of the dystrophin-associated glycoprotein complex, which spans the sarcolemma and acts as a link between the sarcolemma and the extracellular matrix on one hand and the contractile fibers on the other [1]. However, this complex also exerts a metabolic function by enabling the sarcolemmal localiza-

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infrared spectroscopy (NIRS) enables non-invasive, real-time monitoring of muscle oxygenation at rest and during exercise. It is based on the principle whereby the near-infrared light absorption characteristics of hemoglobin (Hb) and myoglobin (Mb) depend on their O₂ saturation. The NIRS signal reflects the balance between O₂ supply by the circulation and O₂ consumption by the muscle and thus enables the measurement of not only quantitative variations in muscle oxygenation but also the kinetics of deoxygenation and reoxygenation (for a review, see [6,7]). In the field of neuromuscular disease, NIRS has mainly been used to explore impaired O₂ uptake in metabolic myopathies [8]. To the best of our knowledge, studies in BMD patients have not been published and there are only two reports on muscle oxygenation in DMD patients during exercise [5,9]. The latter showed that patients and controls have the same level of deoxygenation during exercise at the same absolute load, whereas deoxygenation is lower in patients at the same relative load (because patients are weaker). However, the researchers did not study the initial deoxygenation kinetics.

The primary objective of the present study was to describe and compare muscle oxygenation patterns during constant-load exercise in BMD patients and healthy age-matched controls, with a focus on the initial muscle deoxygenation kinetics at the onset of exercise, but we also sought to compare the NIRS peak signals in the two groups. Our secondary objective was to identify potential links between local muscle oxygenation levels on one hand and a subject’s overall exercise tolerance and (for patients) motor function and functional gait parameters on the other.

2. Patients and methods

2.1. Subjects

Ten BMD patients and ten healthy, age-matched, male controls (mean ± SD age: 31.7 ± 12.4) took part in this prospective, single-center study. Patients were monitored in the neuromuscular diseases referral center at Lille University Medical Center (Lille, France). For the patients, the inclusion criteria included genetically confirmed BMD, the ability to walk (whether unaided or with a technical aid) and a quadriceps strength rating of at least 4 out of 5 on the Medical Research Council (MRC) scale. Eight patients had genetic abnormalities in exons 41–46. In terms of cardiac and respiratory status, two patients had a low left ventricle ejection fraction (41% and 53%, respectively). In BMD patients, the forced expired volume in 1 s and the forced vital capacity were respectively 97.7 ± 20% and 97.7 ± 18% of the theoretical values.

2.2. Exercise protocol

Subjects performed knee extension exercises on a CON-TREX isokinetic dynamometer (MEDIMEX®, Sainte Foy les Lyon, France). This type of instrument enables the assessment of muscle strength during a constant velocity displacement and records the moment, work and power during maximal or submaximal exercises. Subjects sat in the CON-TREX device with a back angle of 90° to the vertical and were strapped according to the manufacturer’s protocol. The device was then calibrated according to the manufacturer’s recommendations. The knee range of motion was set from 10° to 100° of flexion for all subjects and all assessments were made on the stronger leg. All exercises consisted in rhythmic, voluntary, isokinetic, concentric contractions of the quadriceps at 90°/s, whereas the return was performed passively at the same speed. A summary of the exercise protocol is presented in Fig. 1.

Subjects were first familiarized with the experimental protocol by means of two practice runs of five submaximal repetitions each, with a 30-s interval between repetitions. We then assessed the maximum muscle strength for five contractions (referred to as “MAX exercise”), during which the subjects were given verbal encouragement. The highest peak torque value of the five contractions was used thereafter as the maximal muscle strength parameter.

After a 15-min rest period, subjects performed a constant-load exercise according to the above-described parameters at a rate of one cycle every 2 s and at 20% of their peak torque (i.e. same relative workload for all subjects, referred to as “REL exercise”) for up to 4 min. Lastly, after a 30-min rest period, subjects performed another exercise session at the same absolute workload of 20 Nm (the “ABS exercise”) for up to 4 min. A visual feedback allowed the subjects to adjust their quadriceps contraction strength. The criteria for stopping the exercise before the end of the 4-min test period were exhaustion, insupportable pain and the occurrence of three successive contractions at below the target power.

2.3. Muscle oxygenation monitoring by NIRS

Muscle oxygenation in the vastus lateralis was monitored during the two constant-load exercises by a three-channel, portable continuous-wave NIRS device (PORT-AMON, Artinis Medical Systems®, Zetten, The Nether-
lands). Emitted light (peak wavelengths of 750 nm and 850 nm) is mainly absorbed by oxygenated and deoxygenated hemoglobin (HbO₂ and HHb, respectively) in small arterioles, capillaries and venules within the muscle [7]. The changes in optical density are converted into HbO₂ and HHb concentration changes by using a modified Lambert–Beer law in which a differential path length factor is used to correct photon scattering within the tissue [10]. The sum of the absorbances at the two wavelengths yields the change in local blood volume (attributed to a change in total hemoglobin (tHb)). The PORTAMON device measures also the tissue saturation index (TSI), which corresponds to the HbO₂ as a proportion of tHb. The TSI is derived from the relative absorption coefficients obtained from the slopes of light attenuation at three interoptode distances and by taking the diffusion scattering law into account [11,12]. The HHb signal has been preferred by some researchers because it is essentially blood-volume-insensitive during exercise (relative to HbO₂) and has been validated as a reliable estimator of changes in O₂ extraction in this field of investigation [13]. At the beginning of constant-load exercise, HHb rises rapidly after a time delay (TD) and then remains at a steady-state value (Fig. 2). This signal can be fitted by the monoexponential function \[ HHb(t) = HHb_{(ampl)} \times \left(1 - e^{-t/TD/s}\right), \] where \( HHb_{(ampl)} \) represents the steady-state HHb value, TD is the time delay and \( s \) is the time constant (i.e. the time needed for the HHb signal to reach 63% of its steady-state value) [14,15]. The

Fig. 1. Summary of the exercise protocol. MAX exercise: determination of the peak torque; REL exercise: at the same relative workload (20% of the peak torque); ABS exercise: at the same absolute workload (20 Nm). The text under the boxes describes the exercise and the text between the boxes indicates the rest period.

Fig. 2. Example of a muscle oxygenation recording during effort. (A) Change over time in HHb, HbO₂ and tHb. ½TR: recovery half time. (B) Change over time in TSI. (C) Focus on the initial deoxygenation parameters. TD: time delay; \( s \): time constant; MRT: mean response time. The vertical dotted lines show the onset and end of the exercise.
sum of TD and $\tau$ corresponds to the mean response time (MRT). The signal is arbitrarily set to zero at rest and so the equation does not include the baseline HHb level. At the end of the exercise, the HbO$_2$ signal rises and this reoxygenation can be studied by measuring the recovery half-time ($\frac{1}{2}$TR) (Fig. 2), which corresponds to the time required (after the termination of exercise) for the HbO$_2$ level to reach half of its resting value [16]. The skinfold thickness at the NIRS probe’s site of application was determined (at the end of the exercise protocol) with a Harpenden caliper. The two groups did not differ significantly in terms of the measured mean skin and subcutaneous tissue thicknesses ($6.5 \pm 2.4$ mm in patients; $6.9 \pm 2.9$ mm in controls); these values enabled efficient measurement of the muscle tissue because the probe’s 2.5–3.5 cm source-to-detector separation gives enables accurate measurements at depths of up to 17.5 mm [17,18].

Once the subjects were sitting comfortably on the dynamometer, the NIRS probe was placed on the belly of vastus lateralis midway between the lateral epicondyle and the great trochanter of the femur (in order to obtain the same relative position in each subject, since vastus lateralis oxygenation is known to be spatially heterogeneous [19]). The probe was secured with tape and its position was then marked with pen on the skin, to ensure that the probe did not slip during the exercise. To prevent signal interference by ambient light, an opaque, black, cloth sleeve was placed around the probe. The signal was sampled at 10 Hz using the manufacturer computer software and the data were filtered by a 10 s moving average window. Resting values were set to zero absorption units (AU).

We took into account the following NIRS measurements (Fig. 2): (i) the initial HHb deoxygenation kinetics, i.e. TD, $\tau$ and MRT, (ii) the HHb level at each minute of exercise (as an average of the 10 last seconds), (iii) $\frac{1}{2}$TR and (iv) TSI at baseline and at each minute of exercise.

2.4. Overall exercise tolerance in subjects

In order to assess each subject’s overall exercise tolerance, we measured the endurance time, the rate of perceived exertion (RPE, on the Borg scale from 6 to 20) and pain in the tested quadriceps (on a visual analog scale (VAS) from 0 to 100).

2.5. Assessment of motor function in patients

Motor function was assessed by the Motor Function Measure (MFM), a 32-item activity scale that has been validated in children and adults with neuromuscular disease [20]. Each MFM item is scored from 0 (“does not initiate movement or starting position cannot be maintained”) to 3 (“completes the exercise with a standard pattern”). Items can be grouped into three representative dimensions (D); D1, posture and transfers; D2, axial and proximal motor functions; D3, distal motor functions. The results are expressed as the score for each item, the score for each dimension (mfmD1, mfmD2 and mfmD3) and the total score (mfmT) expressed as a percentage of each possible maximal score. The MFM was assessed by a physical therapist with a validated qualification in the use of this tool.

2.6. Assessment of functional gait parameters in patients

Two-dimensional spatiotemporal gait parameters were measured using the GAITRITE electronic walkway (CIR Systems Inc., Havertown, PA, USA) which is 5 m long, allows repeat analyses and can be used easily even when the subject walks with a technical aid. We evaluated the average, comfortable (self-paced) walking speed and cadence (step/min) over three recording sessions. The maximum walking speed was determined in a fourth recording session.

Walking endurance was measured with a 6-min walking test on a circular walkway marked at 10-m intervals. Patients were not verbally encouraged or advised and only the 2-min and 4-min time points were announced.

2.7. Statistical analysis

Our results are expressed as the mean ± SD. Inter-group comparisons (i.e. BMD patients vs. healthy subjects) of NIRS parameters were tested with a linear mixed model, with group as the fixed effect and pairing block as the random effect. In view of the small sample size, the data were rank-transformed prior to a non-parametric analysis. Intra-group comparisons were performed with a Wilcoxon signed-rank test. The associations between NIRS parameters and the other parameters were studied by using Spearman’s rank correlation coefficient. All statistical analyses were performed with SPSS software (version 18, SPSS Inc., Chicago, IL, USA). The threshold for statistical significance was set to $p < 0.05$.

3. Results

3.1. Overall exercise tolerance (Table 1)

As expected, patients were much weaker than controls (peak torque: $65.1 \pm 40.2$ vs. $170.4 \pm 48.3$ Nm, respectively; $p < 0.0001$). Endurance did not differ when comparing patients and controls in terms of REL exercise but was significantly worse in patients for ABS exercise. The RPE was also identical for REL exercise but was significantly greater in patients for ABS exercise. Both exercise types were significantly more painful for the patients.

3.2. Muscle oxygenation

The changes over time in HHb, HbO$_2$ and TSI in a typical subject are shown in Fig. 2. The theoretical monoexponential model fitted the HHb signal well in all subjects. The parameters for the initial deoxygenation kinetics were faster in patients than in controls for both types of exercise.
(Fig. 3). The TD was significantly shorter (−41% for REL, −37% for ABS) in patients. The patients had also lower \( r \) values but the difference was only statistically significant for ABS exercise (−27%). Lastly, the MRT was significantly shorter in patients (−28% for REL; −31% for ABS). In both groups, the values of these parameters were independent of the exercise intensity.

We found no differences in HHb levels, \( \frac{1}{2} \text{TR} \) and TSI values when comparing patients and controls (for both REL and ABS exercises).

### 3.3. Motor function and gait parameters in patients

As expected, the MFM score’s D1 dimension was most impaired in patients (68.7 ± 26.1%). The D2 dimension was rated at 96.2 ± 7.3%, the D3 dimension was 95.2 ± 4.5% and the total score was 84.8 ± 13.2%. The patients’ mean comfortable walking speed was 1.03 ± 0.23 m/s, with a cadence of 97.4 ± 15 steps/min and a maximum walking speed of 1.44 ± 0.41 m/s. The patients walked a mean distance of 414.7 ± 107.2 m during the 6-min test.

### 3.4. Correlations (Table 2)

Since the initial deoxygenation kinetics enabled us to discriminate between patients and controls, we decided to study the correlations with clinical data. We found moderately to strongly positive correlations between \( r \) (in both exercise modes) on one hand and the MFM D1 dimension score, the comfortable walking speed and cadence and the 6-min walking distance on the other. The initial deoxygenation rate was faster in BMD patients than in controls. The speed of deoxygenation was positively correlated with the severity of the patients’ muscle and functional impairments.

We did not find any correlation between the deoxygenation parameters and the patient’s age, symptom duration or overall exercise tolerance.

### 4. Discussion

The present study’s main objectives were to describe and compare muscle oxygenation parameters during effort in BMD patients and age-matched healthy controls (with a focus on the initial deoxygenation kinetics) and to identify potential associations between local muscle oxygenation on the one hand and the subject’s overall exercise tolerance and (for patients) motor function and gait parameters on the other. The major findings of our study were that the initial muscle deoxygenation kinetics appears to be faster in BMD patients (whatever the exercise intensity) and that this phenomenon is (at least in part) related to the degree of muscle impairment and functional impairment. The other NIRS parameters did not differ significantly when comparing the two groups.

#### 4.1. The initial deoxygenation kinetics

The evolution of each NIRS parameter during exercise is quite similar to findings of duManoir et al. during a moderate intensity isotonic repetitive knee extension exercise, and DeLorey et al. for a constant-load cycling exercise [14,21]. We also showed that the kinetic parameters were independent of exercise intensity (at least at low intensities) in BMD patients as was already known for healthy subjects.

According to the theory of NIRS, a faster rise in the HHb signal may be due to lower \( O_2 \) supply or greater \( O_2 \) extraction (i.e. consumption). Several authors have shown a lack of vasodilation in dystrophinopathies during exercise and thus a decrease in the blood supply, which is related to nNOS dysfunction. Thomas et al. [4] observed this phenomenon in mdx mouse muscles by Doppler ultrasound measurements during electrically induced leg exercise after pharmacologic, adrenergic stimulation: the femoral blood flow (FBF) was significantly impaired in mdx mice, when compared with healthy mice. Similar results were obtained in nNOS gene knock-out mice. In humans, Sander et al. [5] reached the same conclusions by using NIRS to record the \( HbO_2 \) signal in 10 children with DMD and 13 healthy controls at rest and during a handgrip exercise. In this case, reflex adrenergic stimulation was induced by negative pressure around the legs. In contrast, \( O_2 \) extraction does not seem to be higher in patients suffering from dystrophinopathies, although data on this topic are scarce (especially in BMD), oxidative function is known to be altered in both mdx mice [22]
and DMD patients [23], although it does not seem to be true in BMD patients [24]. Thus, an alteration of vasodilation caused by nNOS deficiency could explain the faster deoxygenation in BMD patients.

However, some possible bias of measurement must be taken into account. Indeed, BMD patients have a muscular fatty tissue infiltration and interstitial fibrosis. This could firstly affect NIRS measurement validity by lowering muscle fiber mass in the measurement area and by modifying scattering of the emitted light and secondly it could cause a decrease in muscle blood flow caused by possible alteration of muscle blood supply. However, muscle histological modifications seem to be moderate in walking BMD patients [25,26], and both Sander et al. and Thomas et al.
Table 2
Correlations between the time constant (τ), muscle impairment and functional status in the BMD patient group. REL exercise: exercise at the same relative workload (20% of peak torque); ABS exercise: exercise at the same absolute workload (20 Nm).

<table>
<thead>
<tr>
<th></th>
<th>τ REL</th>
<th>τ ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFM D1 score (%)</td>
<td>0.794* (0.006)</td>
<td>0.636* (0.048)</td>
</tr>
<tr>
<td>6 min walking test (m)</td>
<td>0.782** (0.008)</td>
<td>0.673** (0.033)</td>
</tr>
<tr>
<td>Comfortable walking speed (m/s)</td>
<td>0.709* (0.033)</td>
<td>0.685* (0.020)</td>
</tr>
<tr>
<td>Walking cadence (step/min)</td>
<td>0.709* (0.022)</td>
<td>0.782** (0.008)</td>
</tr>
</tbody>
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p values are given in brackets.
* the correlation is significant at p < 0.05.
** the correlation is significant at p < 0.01.

found identical maximal deoxygenation (NIRS) in DMD patients and controls for an exercise at the same absolute workload, and a strong correlation with arterial blood flow as in healthy people [4,5]. Quaresima and Ferrari found similar results on maximal deoxygenation [27]. These experimental data suggest that NIRS remains valid in mild BMD patients, but further studies will be necessary to assess the links between muscle oxygenation trends (NIRS) and muscle dystrophy (fibrosis, fat infiltration), using imaging techniques (MRI, echography) or histological examination. It would be also interesting to measure the temporal and quantitative aspects of FBF in order to establish whether faster muscle deoxygenation in dystrophinopathies is indeed due to a poor O2 supply.

Moreover, the physiological significance of the TD period could be discussed. It represents the balance between Hb–Mb deoxygenation, O2 delivery, and the effect of muscle contraction on microvascular volume [21]. To minimize the effect of initial blood displacement, some authors begin their exercise protocol by passive movements of the muscles being studied. This was not done in our study, however, the workload during our two exercise sessions was probably low enough to limit this initial blood displacement.

Two other causes of impairment in the O2 supply must be taken in account. Firstly, patients may have developed cardiopulmonary complications as a result of BMD. However, none of the patients in our studied population displayed respiratory insufficiency and only two patients had an asymptomatic moderate heart failure. Secondly, sedentary, dystrophic muscles may have a low capillary density. However, this possibility has not been reported by the few specific studies in this area [28,29].

Finally, recent findings suggest that the nNOS dysfunction is not specific to dystrophinopathies [30]. Hence, the specificity of faster muscle deoxygenation patterns in BMD will have to be checked against other dystrophinopathies (e.g. DMD and limb girdle dystrophy) and non-dystrophic neuromuscular disorders (FSH dystrophy).

4.2. Correlations (Table 2)

We found strong correlations between τ and the MFM D1 dimension (computed from 12 items evaluating proximal muscles that are generally impaired in dystrophinopathies) but this should be confirmed in a larger patient population. We also found that τ is associated with comfortable walking speed and cadence and the distance covered in the 6-min walk test. The observed correlation between muscle oxygenation and motor and functional status is particularly noteworthy, since it could be a possible indicator of disease progression, even if the nNOS vasodilatation impairment is not proven by the initial deoxygenation kinetics parameters.

4.3. Other NIRS parameters

None of the other NIRS parameters enabled us to discriminate between patients and controls. This finding contradicts the results of Quaresima and Ferrari [30] and Sander [5] who found that deoxygenation was lower in BMD patients than controls during exercises at the same relative load but identical for exercises at the same absolute load. This might be explained by the small difference between workloads in the REL conditions (13 Nm for patients vs. 34 Nm for controls).

4.4. Advantages of the method

Our experimental protocol combines the benefits of the NIRS measurement of initial deoxygenation kinetics and those of low-intensity, constant-workload exercise. Near-infrared spectroscopy enables the continuous, non-invasive measurement of oxygenation in a muscle’s small blood vessels – exactly where NO acts – with a fairly good spatial resolution and during a short exercise.

A low-intensity, constant-workload exercise is easier, safer and more comfortable for patients. The use of an isokinetic dynamometer enables well-calibrated exercise that is limited to a single muscle or muscle group. It is also possible to control effective muscle activation (in contrast to cycling or walking protocols).

References


