

## Skeletal muscle reoxygenation after high-intensity exercise in mitochondrial myopathy

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**Abstract** This study addressed whether O<sub>2</sub> delivery during recovery from high-intensity, supra-gas exchange threshold exercise would be matched to O<sub>2</sub> utilization at the microvascular level in patients with mitochondrial myopathy (MM). Off-exercise kinetics of (1) pulmonary O<sub>2</sub> uptake ( $\dot{V}O_{2p}$ ), (2) an index of fractional O<sub>2</sub> extraction by near-infrared spectroscopy ( $\Delta[\text{deoxy-Hb} + \text{Mb}]$ ) in the vastus lateralis and (3) cardiac output ( $Q'_T$ ) by impedance cardiography were assessed in 12 patients with biopsy-proven MM (chronic progressive external ophthalmoplegia) and 12 age- and gender-matched controls. Kinetics of  $\dot{V}O_{2p}$  were significantly slower in patients than controls ( $\tau = 53.8 \pm 16.5$  vs.  $38.8 \pm 7.6$  s, respectively;  $p < 0.05$ ).  $Q'_T$ , however, declined at similar rates ( $\tau = 64.7 \pm 18.8$  vs.  $73.0 \pm 21.6$  s;  $p > 0.05$ ) being typically slower than  $\dot{V}O_{2p}$  in both groups. Importantly,  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  dynamics (MRT) were equal to, or faster than,  $\tau\dot{V}O_{2p}$  in patients and controls, respectively. In fact, there were no between-group differences in  $\tau\dot{V}O_{2p}/\text{MRT}\Delta[\text{deoxy-Hb} + \text{Mb}]$  ( $1.1 \pm 0.4$  vs.  $1.0 \pm 0.2$ ,  $p > 0.05$ ) thereby indicating similar rates of microvascular O<sub>2</sub> delivery. These data indicate that the slower rate of recovery of muscle metabolism after high-intensity exercise is not related to impaired microvascular O<sub>2</sub> delivery in patients with MM. This phenomenon,

therefore, seems to reflect the intra-myocyte abnormalities that characterize this patient population.

**Keywords** Mitochondrial myopathy · Reoxygenation · Oxygen uptake · Kinetics · Exercise recovery

### Introduction

In similarity with the onset of exercise, sluggish pulmonary oxygen uptake ( $\dot{V}O_{2p}$ ) kinetics at the “off-transient” might be related to the impaired diffusive and/or convective O<sub>2</sub> transport to muscle mitochondria (Hughson et al. 2001; MacDonald et al. 2001) and/or abnormal metabolic activity of intra-myocyte machinery (Burnley et al. 2000; Grassi et al. 1996). The final consequence is a slower capability to replenish local phosphocreatine (PCr) stores, thereby retarding the restoration of muscle metabolism (Harris et al. 1976; McMahon and Jenkins 2002).

Near infrared spectroscopy (NIRS) is a non-invasive technique that has been widely used to investigate the (im)balance between microvascular O<sub>2</sub> delivery ( $Q'_O_{2mv}$ ) and utilization (Grassi et al. 2003; DeLorey et al. 2003; Koga et al. 2007). Among the variables provided by NIRS, deoxy-hemoglobin (Hb) + myoglobin (Mb) is particularly informative as it provides an index of muscle fractional O<sub>2</sub> extraction, i.e., the  $Q'_O_{2mv}$ -to- $\dot{V}O_{2p}$  relationship. In this context, previous studies have demonstrated that the off-transient kinetics of deoxy-Hb + Mb is faster than  $\dot{V}O_{2p}$  recovery in healthy subjects (Ferreira et al. 2005), thereby lending support to the notion that intra-myocyte metabolic inertia, not O<sub>2</sub> availability, is the key limiting step of  $\dot{V}O_{2p}$  kinetics after dynamic exercise (Harper et al. 2008; Ferreira et al. 2005).

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Mitochondrial myopathy (MM) is associated with slower  $\dot{V}O_{2p}$  kinetics at the onset of exercise, a finding thought to be related to impaired muscle  $O_2$  utilization (Grassi et al. 2009). It has been assumed that off-transient  $\dot{V}O_{2p}$  kinetics would also be slowed, as oxidative phosphorylation would take longer to replenish creatine (Cr), ADP, and inorganic phosphate (Pi) back to pre-exercise levels (Rossiter et al. 2002). If this is the case, deoxy-Hb + Mb would be expected to decrease at faster rates than  $\dot{V}O_{2p}$  thereby indicating that  $O_2$  availability is in excess to  $O_2$  needs.

There is, however, some intriguing evidence that convective  $O_2$  transport to contracting fibres could also be impaired in MM patients thereby predisposing  $\dot{V}O_{2p}$  kinetics to be delivery-limited. For instance, Jensen et al. (2002) found a decrease in forearm blood flow in MM patients and Trenell et al. (2007) described that  $O_2$  supplementation enhanced patients' ability to regenerate ATP. In fact, considering that  $O_2$  utilization per se might be important to spatially adjust muscle blood flow to  $O_2$  needs during exercise (Wolin 2009; Ellsworth et al. 2009; Sprague et al. 2010), it is possible that patients with impaired  $O_2$  utilization could not maintain  $Q'O_{2mv}$  well matched to demand. The usefulness of the deoxy-Hb + Mb analysis during exercise recovery in clinical populations has been demonstrated, for instance, by Kemps et al. (2009) who described slower rates of muscle reoxygenation relative to post-exercise PCr resynthesis in patients with decreased  $Q'O_{2mv}$  (chronic heart failure). Despite its potential to elucidate the mechanistic determinants of slower  $\dot{V}O_{2p}$  kinetics in disease states, this analytical approach has not been previously used in patients with MM.

Our objective, therefore, was to investigate whether  $Q'O_{2mv}$  would be commensurate to  $O_2$  utilization during recovery from high-intensity, supra-gas exchange threshold exercise in MM patients. Knowledge of the etiological mechanisms leading to the microcirculatory  $O_2$  exchange impairment in MM is crucial to understand the mechanisms of exercise intolerance and to plan efficacious therapeutic strategies for this patient population.

## Methods

### Subjects

The study population comprised of 12 patients (5 males) with biopsy-proven MM and 12 age-matched sedentary healthy controls (8 males). The patients had been followed at the Neuromuscular Division and volunteered for study participation. Consecutive patients who presented with ragged-red fibers pattern in all specimens and abnormally low mitochondrial electron transport chain activity

(cytochrome *c* oxidase) in muscle biopsy associated with symptoms and signs of chronic progressive external ophthalmoplegia (ptosis, exercise intolerance and chronic fatigue) were selected (Moraes et al. 1989). There was no evidence of cardiomyopathy or impaired cardiac conduction in any patient at rest. Subjects with mitochondrial encephalomyopathy, lactic acidosis and strokes episodes (MELAS), myoclonus epilepsy with ragged-red fibers (MERRF), and other MMs were excluded from the study. The patients have not been involved on any regular physical activity in the preceding year and the controls presented with very low daily activity scores according to the Baecke questionnaire (total score <6) (Baecke et al. 1982). Written informed consent to participate was obtained from each subject and the experimental protocol was approved by the Institutional Ethics Committee of the Federal University of Sao Paulo, Brazil.

### Study protocol

Subjects performed a ramp-incremental exercise test (5–10 W/min in patients and 15–20 W/min in controls) to determine parameters of aerobic function during exercise (Whipp et al. 1981). The tests were performed on an electronically braked cycle ergometer (Corival 400, Lode, The Netherlands) at 60 rpm and they were preceded by an unloaded baseline pedalling for 3 min. On a separate day, subjects performed a constant work rate exercise test at the same pedalling rate to the limit of tolerance ( $T_{lim}$ ) at an intensity of 70% of peak work rate.  $T_{lim}$  was defined as the time point in which the patients signaled to stop exercising or could not maintain the required pedalling rate for 10 s despite being encouraged by the investigators. Subjects also underwent an active 3-min recovery period of unloaded pedalling at the same rate.

### Measurements

#### *Cardiopulmonary exercise test (CPET)*

$\dot{V}O_{2p}$  (mL/min), pulmonary carbon dioxide output ( $\dot{V}CO_{2p}$ , mL/min) and minute ventilation ( $\dot{V}E$ , L/min) were measured breath-by-breath using a computer-based system (CardiO<sub>2</sub> System™, Medical Graphics, St. Paul, MN, USA). Gas exchange variables measured during the incremental test were averaged every 15 s, and  $\dot{V}O_{2peak}$  was defined as the highest value achieved during the test. Heart rate (HR, bpm) was determined using the R–R interval from a 12-lead electrocardiogram. Subjects were also asked to rate their 'shortness of breath' and 'leg effort' at exercise cessation using the 0–10 Borg's category-ratio scale. The  $\dot{V}O_{2pGET}$  was estimated by the gas-exchange

method, inspecting visually the inflection point of  $\dot{V}\text{CO}_2\text{p}$  with regard to  $\dot{V}\text{O}_2\text{p}$  (modified V-Slope) (Beaver et al. 1986) and by the ventilatory method when  $\dot{V}\text{E}$ -to- $\dot{V}\text{O}_2\text{p}$  ratio and end-tidal partial pressure of  $\text{O}_2$  increased, while  $\dot{V}\text{E}$ -to- $\dot{V}\text{CO}_2\text{p}$  ratio and end-tidal pressure of  $\text{CO}_2$  remained stable. The reading was performed independently by two experienced observers without knowledge of other results or subject identities.

### Skeletal muscle oxygenation

Skeletal muscle oxygenation profiles of the left vastus lateralis were determined by spatially resolved near-infrared spectroscopy (NIRS) (Hamamatsu NIRO 200™, Hamamatsu Photonics KK, Japan). This technique has been used in several studies to evaluate patients with MM (Bank and Chance 1997; Van Beekvelt et al. 1999a, b; Grassi et al. 2007, 2009). The theory of NIRS has been described in detail elsewhere (Ferrari et al. 2004). Briefly, one fiber optic bundle carried the NIR light produced by the laser diodes to the tissue of interest while a second fiber optic bundle returned the transmitted light from the tissue to a photon detector in the spectrometer. The intensity of incident and transmitted light were recorded continuously and, along with the relevant specific extinction coefficients, used to measure changes in the oxygenation status of Hb and Mb. A set of optodes was placed on the belly of the vastus lateralis muscle midway between the lateral epicondyle and greater trochanter of the femur. To ensure that the position of the optodes, relative to each other, was fixed and invariant, the optodes were housed in an optically dense plastic holder. The optode assembly was secured on the skin surface with tape, and then covered with an optically dense, black vinyl sheet, thus minimizing the intrusion of extraneous light and loss of NIR light.

Among the NIRS variables, several laboratories have adopted the deoxy-Hb + Mb concentration ([deoxy-Hb + Mb]) signal as the preferred indicator of changes in muscle microvascular oxygenation during exercise (Koga et al. 2007; DeLorey et al. 2005; Ferreira et al. 2005; Grassi et al. 2003). The [deoxy-Hb + Mb] response to exercise is then considered as a proxy of  $\text{O}_2$  extraction in the microcirculation when arterial oxygen content remains constant and has been referred as the balance between  $\text{O}_2$  delivery and utilization. Before exercise, care was taken that an arterial cuff occlusion (at 300 cmH<sub>2</sub>O) produced an increase in  $\Delta$ [deoxy-Hb + Mb] in all patients. The [deoxy-Hb + Mb] values were recorded as a change ( $\Delta$ ) from baseline in  $\mu\text{M}/\text{cm}$  and data were corrected by the maximal value obtained at peak of exercise and expressed in percentage. The NIRS system was ‘zeroed’ during the unloaded cycling portion of the tests.

### Central haemodynamics

Cardiac output ( $Q'_T$ , L/min) was measured non-invasively throughout the constant work rate test using impedance cardiography (PhysioFlow PF-05™, Manatec Biomedical, France) (Charloux et al. 2000). Before each exercise test, the system was auto-calibrated taking into consideration age, stature, body mass and blood pressure values. Signal quality was verified by visualizing the ECG tracing and its first derivative (dECG/dt) and the impedance waveform ( $\Delta Z$ ) with its first derivative (dZ/dt). In preliminary experiments, the coefficient of variation (CV) for changes in  $Q'_T$  during exercise was 3.3%. Although the method is not free from controversies, changes have been previously found to agree well with direct measurements in other disease populations (Borghi-Silva et al. 2008; Kemps et al. 2008; Tonelli et al. 2011).

### Kinetics analysis

The breath-by-breath  $\dot{V}\text{O}_2\text{p}$ ,  $\Delta$ [deoxy-Hb + Mb], and  $Q'_T$  data were interpolated second-by-second prior to kinetics analysis and expressed as % of total response (SigmaPlot 10.0, Systat Software Inc., San Jose, CA, USA). We opted to analyse data from the last 30 s of exercise to 180 s of recovery, i.e., we analyse only the kinetics of the primary component of response. Using this approach, we assured that the same amount of data was included in the kinetic analysis of  $\dot{V}\text{O}_2\text{p}$ ,  $\Delta$ [deoxy-Hb + Mb] and  $Q'_T$  for each subject (controls and patients), minimizing model-dependent effects on our results. The model used for fitting the kinetic response was:

$$[Y](t) = [Y]_{(ss)} - A_p \times (1 - e - (t - \text{TD}_p)/\tau_p)$$

where the subscripts “ss” and “p” refer to stable-state cycling and primary component, respectively; “A”, “TD”, and “ $\tau$ ” are the amplitude, time delay and time constant of the exponential response of interest ( $\sim$ time to reach 63% of the response following the end of exercise). The overall kinetics of  $\Delta$ [deoxy-Hb + Mb] were determined by the mean response time ( $\text{MRT} = \tau + \text{TD}$ ).

### Statistical analysis

The SPSS version 15.0 statistical software was used for data analysis (SPSS, Chicago, IL, USA). Results were summarized as mean  $\pm$  SD or median and interquartile ranges when applicable. Between-group differences were evaluated by means of independent-samples *t* test or Mann–Whitney *U* test. Wilcoxon test was used to evaluate within-group differences of  $\tau Q'_T$  and  $\tau \dot{V}\text{O}_2\text{p}$ . The level of statistical significance was set at  $p < 0.05$  for all tests.

## Results

### Sample characteristics

Patients presented with lower weight, height and body mass index (BMI) than controls. There were no significant between-group differences in pulmonary function variables with exception of lower MEP in patients (Table 1). As expected, they had reduced maximal work rate,  $\dot{V}O_{2\text{peak}}$ , HR,  $O_2$  pulse and  $\dot{V}O_{2\text{pGET}}$  as compared to controls ( $p < 0.05$ ; Table 2).  $\dot{V}O_{2\text{p}}$  at the constant work rate test corresponded to 86% and 75% of the  $\Delta\dot{V}O_{2\text{ppeak-GET}}$  difference in patients and controls, respectively.

### $\dot{V}O_{2\text{p}}$ and $\dot{Q}'_{\text{T}}$ kinetics

$\dot{V}O_{2\text{p}}$  dynamics were slower in patients than controls (see Fig. 1a for representative subjects, Fig. 2; Table 3).  $\dot{Q}'_{\text{T}}$ , however, declined at similar rates (Fig. 1b for representative subjects, Fig. 2; Table 3). Therefore,  $\dot{Q}'_{\text{T}}$  kinetics were equivalent to that of  $\dot{V}O_{2\text{p}}$  in patients and slower in controls (Fig. 2).

### Microvascular $O_2$ delivery and utilization

$\Delta[\text{deoxy-Hb} + \text{Mb}]$  amplitude was  $33.4 \pm 16.5\%$  of the “cuff” value with all patients showing a response larger than 20%. In similarity with  $\dot{V}O_{2\text{p}}$ ,  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  kinetics were slower in patients than controls (Fig. 1c for representative subjects, Fig. 2; Table 3); in fact,  $\text{MRT}\Delta[\text{deoxy-Hb} + \text{Mb}]$  values  $>40$  s were found in 8/12 MM patients but only in 3/12 controls. Importantly,  $\tau\dot{V}O_{2\text{p}}/\text{MRT}\Delta[\text{deoxy-Hb} + \text{Mb}]$ , an index of microvascular  $O_2$  delivery dynamics, did not differ between patients and controls ( $1.1 \pm 0.4$  vs.  $1.0 \pm 0.2$  s, respectively;  $p > 0.05$ ).

There was a weak, inverse relationship ( $r = -0.48$ ;  $p = 0.056$ ) between  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  kinetics and  $\dot{V}O_{2\text{peak}}$  in patients with MM.

## Discussion

This study investigated whether microvascular  $O_2$  delivery ( $\dot{Q}'O_{2\text{mv}}$ ) would be matched to  $O_2$  utilization during recovery from high-intensity exercise in patients with MM (chronic progressive external ophthalmoplegia) compared to healthy controls.  $\dot{Q}'_{\text{T}}$  declined at rates which were not faster than  $\dot{V}O_{2\text{p}}$  in both groups thereby indicating that “bulk”  $O_2$  delivery was sufficient to attend the peripheral demands for  $O_2$ . In addition,  $\dot{V}O_{2\text{p}}$  and  $\Delta[\text{deoxy-Hb} + \text{Mb}]$ , an index of muscle fractional  $O_2$  extraction at the microvascular level, recovered at similar rates. This finding indicates that muscle blood flow was precisely distributed to serve the  $O_2$  needs of individual fibers, i.e.,  $\dot{Q}'O_{2\text{mv}}$  was well matched to  $O_2$  utilization. These data, therefore, lend indirect support to the notion that the slower rate of recovery of muscle metabolism after high-intensity exercise is mechanistically linked to intra-myocyte disturbances in MM patients. This study sets the scene for future NIRS-based investigations aimed to investigate the consequences of interventions with a potential to improve post-exercise muscle metabolic abnormalities in this patient population.

Mitochondrial diseases are a heterogeneous group of disorders that shares the same basic pathophysiological feature, i.e., dysfunction of the mitochondrial respiratory chain. Impaired oxidative metabolism, therefore, is a hallmark of these diseases (Tarnopolski 2004; Tarnopolski and Raha 2005). During dynamic exercise, this has been traditionally assessed by the examination of some specific response which is assumed to reflect the adequacy of the

**Table 1** Resting characteristics of controls and patients with mitochondrial myopathy (MM)

|  | Controls ( $N = 12$ ) | MM ( $N = 12$ )   |
|--|-----------------------|-------------------|
| Demographics/anthropometrics               |                       |                   |
| Age (years)                                | $32.8 \pm 7.4$        | $35.1 \pm 11.8$   |
| Weight (Kg)                                | $71.3 \pm 13.7$       | $53.5 \pm 14.2^*$ |
| Height (cm)                                | $171.1 \pm 7.0$       | $160.7 \pm 9.1^*$ |
| Body mass index ( $\text{Kg}/\text{m}^2$ ) | $24.3 \pm 4.0$        | $20.6 \pm 4.5^*$  |
| Pulmonary function                         |                       |                   |
| FVC (% pred)                               | $97.4 \pm 9.0$        | $88.0 \pm 25.5$   |
| FEV <sub>1</sub> (% pred)                  | $94.2 \pm 15.5$       | $87.0 \pm 23.3$   |
| FEV <sub>1</sub> /FVC                      | $80.8 \pm 11.0$       | $83.7 \pm 10.2$   |
| MVV (% pred)                               | $108.2 \pm 16.6$      | $93.1 \pm 21.3$   |
| Respiratory muscle strength                |                       |                   |
| MIP (% pred)                               | $113.8 \pm 49.1$      | $79.7 \pm 45.8$   |
| MEP (% pred)                               | $125.4 \pm 46.1$      | $59.2 \pm 30.8^*$ |

Values are means  $\pm$  SD

FVC Forced vital capacity, FEV<sub>1</sub> forced expiratory volume in 1 s, MVV maximal voluntary ventilation, MIP maximal inspiratory pressure, MEP maximal expiratory pressure

\*  $p < 0.05$  for between-group comparisons

**Table 2** Metabolic, cardiovascular and subjective responses at peak incremental and constant work rate exercise tests in controls and patients with mitochondrial myopathy (MM)

|                                      | Controls ( <i>N</i> = 12) | MM ( <i>N</i> = 12)        |
|--------------------------------------|---------------------------|----------------------------|
| Incremental test                     |                           |                            |
| Power (W)                            | 170 ± 34                  | 87 ± 33*                   |
| $\dot{V}O_{2peak}$ (mL/min) (% pred) | 2,001 ± 628 (89.3 ± 12.5) | 1,072 ± 366* (62.3 ± 17.5) |
| $\dot{V}O_{2P_{GET}}$ (mL/min)       | 935 ± 190                 | 605 ± 178*                 |
| HR (bpm) (% pred)                    | 174 ± 13 (93.6 ± 4.4)     | 153 ± 21* (82.6 ± 9.9)*    |
| $\dot{V}O_{2p}/HR$ (mL/min/beat)     | 11.1 ± 3.1                | 7.1 ± 2.5*                 |
| Borg dyspnea scores                  | 5 (4–5.7)                 | 3 (1.5–7)                  |
| Borg leg effort scores               | 9 (5–9.7)                 | 6 (3–9.5)                  |
| Constant work rate exercise test     |                           |                            |
| Power (W)                            | 118 ± 25                  | 61 ± 23*                   |
| <i>Tlim</i> (s)                      | 557 ± 303                 | 421 ± 178                  |
| $\dot{V}O_{2p}$ (mL/min) (% pred)    | 1,742 ± 420 (79.0 ± 8.6)  | 1,016 ± 381* (58.9 ± 18.6) |
| $\dot{V}O_{2p}$ % $\dot{V}O_{2peak}$ | 89.5 ± 11.6               | 94.7 ± 12.9                |
| HR (bpm) (% max)                     | 167 ± 14 (88.7 ± 6.9)     | 154 ± 19 (83.6 ± 9.3)      |

Values are means ± SD with the exception of symptoms

$\dot{V}O_{2peak}$  Peak oxygen uptake, *GET* gas exchange threshold,  $\dot{V}E$  minute ventilation, *HR* heart rate, *Tlim* time to exercise intolerance

\* *p* < 0.05 for between-group comparisons

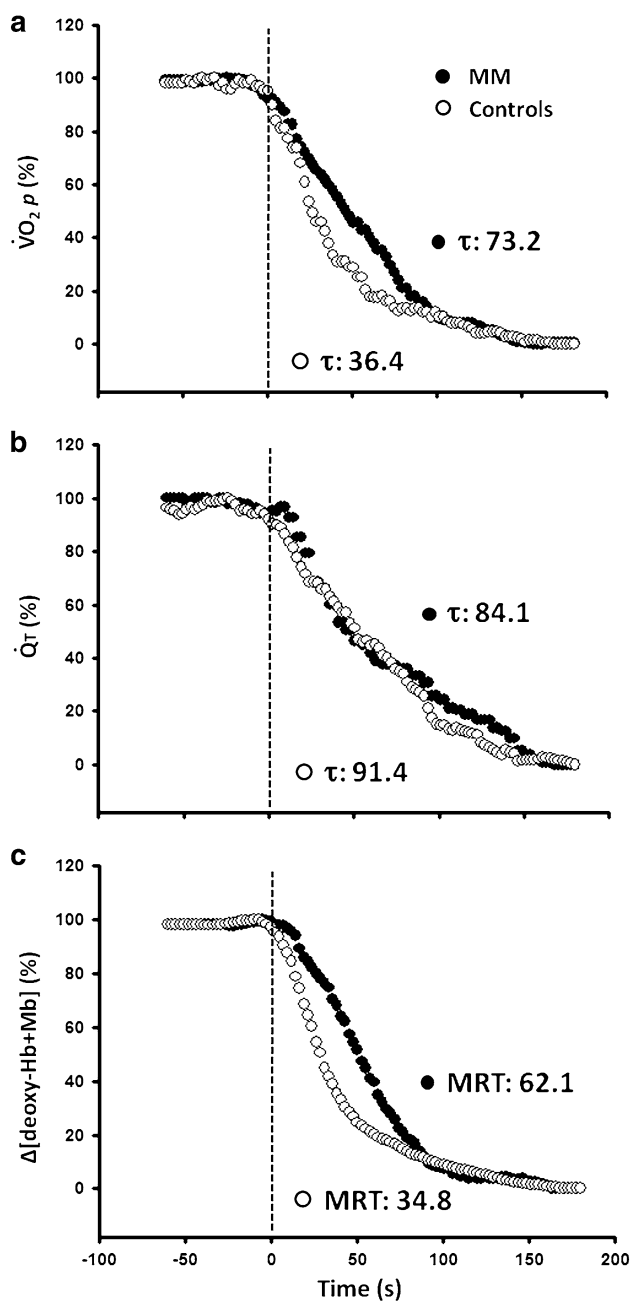
aerobic metabolism, such as  $\dot{V}O_2$  at peak exercise (Taivassalo et al. 2003) and lactate production (Jensen et al. 2002). In contrast, much less attention has been paid to the rate of change in  $\dot{V}O_{2p}$  at the transition to (“on-kinetics”) or from (“off-kinetics”) exercise. In fact, the study of  $\dot{V}O_{2p}$  kinetics is widely recognized as a fundamental tool to establish the “efficiency” (health) or appropriateness (disease) of the aerobic adjustments to exercise (Hughson et al. 2001; Grassi et al. 2009).

There is little doubt that under normal conditions muscle  $O_2$  delivery is unlikely to limit  $\dot{V}O_{2p}$  kinetics after the cessation of exercise (Ozyener et al. 2001; Perrey et al. 2002; Cleuziou et al. 2004; McDonough et al. 2004; Behnke et al. 2009). In patients with MM, however, we reasoned that this scenario could be different. For instance, Ellsworth et al. (2009) had put forward the hypothesis that the erythrocyte, when exposed to reduced  $O_2$  tensions, releases ATP in a controlled manner. This molecule, in turn, has been shown to interact with purinergic receptors on the endothelium producing both local and conducted vasodilation (Sprague et al. 2009) thereby enabling a precise matching of muscle  $O_2$  delivery to  $O_2$  needs (Sprague et al. 2010). In patients with MM, however, muscle capillary  $O_2$  tensions could not decrease to levels sufficiently low to elicit appreciable ATP release and, therefore, adequate vasodilation at the sites of (impaired)  $O_2$  utilization. In fact, intra-muscular perfusion heterogeneities might be more extensive in these patients (Sakuta and Nonaka 1989). Moreover, morphological and quantitative analysis of quadriceps muscle fibers in MM patients showed that mitochondrial changes affected predominantly type I fibers (Scelsi 1992). The recovery kinetics of estimated muscle blood flow in proportion to muscle  $\dot{V}O_2$  kinetics are faster in type II as compared to type I fibers (McDonough et al.

2004; Ferreira et al. 2005) thereby further increasing the likelihood of  $\dot{V}O_{2p}$  kinetics to be delivery-limited. Collectively, therefore, it seemed plausible that under conditions of high intra-muscular  $O_2$  needs, such as during recovery from high-intensity exercise, impaired  $Q'O_{2mv}$  could play a prominent role in slowing off-exercise  $\dot{V}O_{2p}$  kinetics in this patient population.

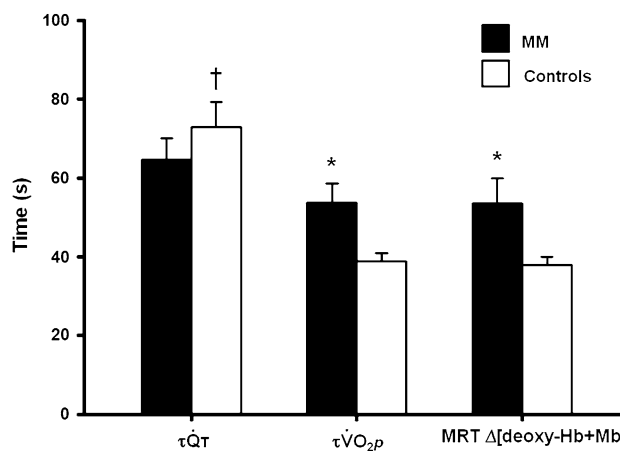
Despite these theoretical considerations, however, our main results did not indicate that impaired convective  $O_2$  delivery was etiologically linked to the sluggishness of  $\dot{V}O_{2p}$  kinetics in MM patients. Therefore, the findings of slower rates of decrease in  $Q'_T$  as compared to  $\dot{V}O_{2p}$  coupled with the temporal similarity between  $\dot{V}O_{2p}$  and  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  dynamics are consistent with the notion that  $Q'O_{2mv}$  was preserved in these patients. By exclusion, these data suggest that the defective intra-myocyte machinery set the limit of recovery of aerobic metabolism patients. In other words, assuming that exercise increased adenosine diphosphate (ADP), phosphate (Pi), and Cr in these patients (Kemp et al. 1993; Jeppesen et al. 2007; Trenell et al. 2007), this accumulated pool decreased slowly at exercise cessation, as compared to a normal, rapid recovery rate.

Some few previous studies used NIRS-derived parameters to investigate muscle metabolism in patients with MM (Van Beekvelt et al. 1999a, b, 2002; Grassi et al. 2007). In particular, Grassi et al. 2009 addressed the on-exercise  $\dot{V}O_{2p}$  kinetics in a heterogeneous group of patients including MM, myophosphorylase deficiency (McArdle’s disease) and patients with non-defined metabolic myopathy. These authors reported slower  $\dot{V}O_{2p}$  kinetics in patients as compared to controls; of note,  $\dot{V}O_{2p}$  kinetics correlated well with  $\Delta[\text{deoxy}(\text{Hb} + \text{Mb})]$  at peak



**Fig. 1** Representative examples of the pulmonary oxygen uptake ( $\dot{V}O_{2p}$ , **a**), cardiac output ( $Q_T$ , **b**) and change in deoxyhemoglobin + myoglobin concentration ( $\Delta[\text{deoxy-Hb} + \text{Mb}]$ , **c**) kinetic responses during recovery from a high-intensity constant work rate test in a patient with mitochondrial myopathy (closed circles) and a control subject (open circles). Data are expressed in percent of the maximal obtained value during exercise

exercise measured in a previous study (Grassi et al. 2007). The present results extend those from Grassi et al. by simultaneously assessing the behavior of  $\dot{V}O_{2p}$  and  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  after the cessation of exercise. The collective evidence brought about by these studies indicate that the analysis of  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  at peak exercise



**Fig. 2** Mean (SE) values of  $\tau Q_T$ ,  $\tau \dot{V}O_{2p}$  and MRT ( $\tau + \text{TD}$ )  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  at the recovery from a high-intensity constant work rate test in patients with MM (solid bars) and healthy controls (open bars). \* $p < 0.05$  for between-group comparisons; † $p < 0.05$  for within-group comparisons of  $Q_T$  versus  $\tau \dot{V}O_{2p}$

**Table 3** Kinetic parameters for controls and patients with mitochondrial myopathy

|                                       | Controls ( $N = 12$ ) | MM ( $N = 12$ ) |
|---------------------------------------|-----------------------|-----------------|
| $\dot{V}O_{2p}$                       |                       |                 |
| A (mL)                                | 1,154 ± 310           | 634 ± 293*      |
| TD (s)                                | 7.1 ± 5.5             | 10.1 ± 9.8      |
| $\tau$ (s)                            | 38.8 ± 7.6            | 53.8 ± 16.5*    |
| MRT (s)                               | 45.9 ± 9.0            | 63.8 ± 13.2*    |
| $Q_T$                                 |                       |                 |
| A (L)                                 | 5.5 ± 2.6             | 4.4 ± 1.5       |
| $\tau$ (s)                            | 73.0 ± 21.6           | 64.7 ± 18.8     |
| $\Delta[\text{deoxy-Hb} + \text{Mb}]$ |                       |                 |
| TD (s)                                | 10.3 ± 5.8            | 11.5 ± 5.2      |
| $\tau$ (s)                            | 27.5 ± 6.7            | 41.9 ± 19.6*    |
| MRT (s)                               | 37.8 ± 7.2            | 53.4 ± 22.3*    |

Values are means ± SD

$\dot{V}O_{2p}$  Pulmonary oxygen uptake, A amplitude of response, TD time delay,  $\tau$  constant time, MRT mean response time,  $Q_T$ , cardiac output,  $\Delta[\text{deoxy-Hb} + \text{Mb}]$ , variation of deoxyhemoglobin + myoglobin concentration by NIRS

\*  $p < 0.05$  for between-group comparisons

(Grassi et al. 2007) and during recovery from high-intensity exercise (present study) are likely to give valuable insights in the functioning of muscle metabolism in MM patients.

A fundamental critique to the present study is the assumption that  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  reflects fractional  $O_2$  extraction in a syndrome where impaired  $O_2$  extraction has been considered a key physiopathological marker (Taivassalo et al. 2002, 2003; Grassi et al. 2007). However, the amplitude of the  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  response

exceeded 20% of the maximum “cuff” value in all patients indicating that this variable can be used as a proxy of fractional  $O_2$  extraction in MM patients who are not severely affected by the disease process. In fact, the seminal studies that described a lack of directly determined reductions in  $PvO_2$  have valuated patients with more advanced disease performing small muscle mass exercise (Jensen et al. 2002; Taivassalo et al. 2003). Nevertheless, it should be recognized that our confidence in  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  as an index of fractional  $O_2$  extraction decreases as disease progresses which might limit the practical use of this approach in patients with end-stage disease.

Other potential points of concern might be related to the choice of supra-GET exercise (Ozyener et al. 2001) and the lack of analysis of on-exercise kinetics and the  $\dot{V}O_2$  response profile during exercise. As shown in Table 2, however, the GET occurred at very early exercise in these patients. Therefore, the amplitude of the  $\dot{V}O_{2p}$  response would have been too low for a reliable description of the on-exercise kinetic responses had we used a sub-GET work rate. In fact, pilot trials showed that the reproducibility of  $\Delta[\text{deoxy-Hb} + \text{Mb}]$  response was rather poor at the onset of exercise. In addition, thought visual inspection of the data did suggest the presence of the “slow” component of the  $\dot{V}O_{2p}$  response in all subjects, the excessive short duration of the tests in some MM had precluded a formal analysis of this important response profile. All of these aspects, therefore, await further investigation.

The adipose tissue thickness (ATT) has been described to influence in spreading the light of NIRS, leading to higher estimated muscle  $\dot{V}O_2$  in subjects with lower subcutaneous fat (van Beekvelt et al. 2002). However, Matsushita et al. (1998) concluded that NIR light penetrates shallow regions of muscle under the skin and subcutaneous fat even when the ATT was 1.5 cm. In the present study, patients with MM had lower weight and BMI than the controls and it is therefore unlikely that higher ATT in patients has influenced our results.

This study sets the scene for future NIRS-based investigations aimed to understand mechanisms of exercise intolerance and to determine the degree of muscle metabolic impairment in patients with MM. Furthermore, our results lend support to the assertion that NIRS might be useful to evaluate the effects of interventions with a potential to improve muscle metabolism in this patient population.

## Conclusion

The slower rate of recovery of muscle metabolism after high-intensity exercise was not related to a mismatching between  $\dot{Q}'O_{2mv}$  and  $O_2$  utilization in patients with MM. These data

lend indirect support to the notion that these disturbances are primarily linked to intra-myocyte disturbances being therefore amenable to be assessed by simultaneous analyses of  $\dot{V}O_{2p}$  and NIRS-derived indexes of muscle reoxygenation ( $\Delta[\text{deoxy-Hb} + \text{Mb}]$ ). Additional studies, however, are warranted to correlate the currently described abnormalities in  $O_2$  utilization during recovery with specific genotype and phenotype characteristics of MM patients.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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