Home-based aerobic exercise training improves skeletal muscle oxidative metabolism in patients with metabolic myopathies

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Porcelli S, Marzorati M, Morandi L, Grassi B. Home-based aerobic exercise training improves skeletal muscle oxidative metabolism in patients with metabolic myopathies (MM) and McArdle’s disease (McA). The aim of the study was to use noninvasive functional evaluation methods, specifically aimed at skeletal muscle oxidative metabolism, to evaluate the effects of an aerobic exercise training (cycle ergometer, 12 wk, 4 days/wk, ~65-70% of maximal heart rate) in 6 MM and 7 McA. Oxygen uptake and skeletal muscle vastus lateralis fractional O2 extraction by near-infrared spectroscopy were assessed during incremental and low-intensity constant work rate (CWR) exercises before (BEFORE) and at the end (AFTER) of training. Peak O2 uptake increased significantly with training both in MM [14.7 ± 1.2 vs. 17.6 ± 1.4 ml·kg⁻¹·min⁻¹ (mean ± SD)] and in McA [18.5 ± 1.8 ml·kg⁻¹·min⁻¹ vs. 21.6 ± 1.9]. Peak skeletal muscle fractional O2 extraction increased with training both in MM [22.0 ± 6.7 vs. 32.6 ± 5.9%] and in McA [18.5 ± 6.2 vs. 37.2 ± 7.2%]. During low-intensity CWR in both MM and McA: V̇O2 kinetics became faster in AFTER, but only in the patients with slow V̇O2 kinetics in BEFORE; the transient overshoot in fractional O2 extraction kinetics disappeared. The level of habitual physical activity was not higher 3 mo after training (FOLLOW-UP vs. PRE). In MM and McA patients a home-based aerobic training program significantly attenuated the impairment of skeletal muscle oxidative metabolism and improved variables associated with exercise tolerance. Our findings indicate that in MM and McA patients near-infrared spectroscopy and V̇O2 kinetics can effectively detect the functional improvements obtained by training.

near-infrared spectroscopy; V̇O2 kinetics; mitochondrial myopathies; McArdle’s disease

NEW & NOTEWORTHY

Twelve weeks of home-based moderate-intensity aerobic training in mitochondrial myopathies and McArdle’s disease patients attenuated the impairments of skeletal muscle oxidative metabolism and improved variables associated with exercise tolerance. Despite this, 3-mo after the termination of the exercise training program the level of habitual physical activity was not increased vs. the baseline.

METABOLIC MYOPATHIES are a heterogeneous group of disorders characterized by derangements of glycogen or lipid metabolism or mitochondrial function due to genetic mutations leading to defects of the main pathways of energy provision in skeletal muscle fibers. In some of these myopathies, such as myophosphorylase deficiency [McArdle’s disease (McA)] (23, 15) or mitochondrial myopathies (MM) (15, 46) the genetic defect significantly impairs oxidative metabolism. In MM the mutation(s) causes the impairment of the mitochondrial respiratory chain and of oxidative metabolism (9, 46). In McA the absence of myophosphorylase causes an incapacity to break down intramuscular glycogen. The reduced or absent flux of substrates along the glycolytic pathway impairs one of the two main routes of supply of substrates to the tricarboxylic acid cycle and disrupts the delicate interplay between carbohydrate and lipid metabolism.

In many MM and McA patients the impairment of oxidative metabolism leads to a phenotype characterized by a reduced exercise tolerance, often associated with progressive weakness and myalgia, negatively affecting the patients’ quality of life.

We have recently applied some noninvasive methods of functional evaluation of oxidative metabolism on MM and McA patients. Apart from confirming some observations by previous authors (15, 23, 25, 37), such as lower peak O2 uptake (V̇O2peak), lower peak power output, and an exaggerated cardiovascular response to exercise, we have observed, in these patients clear signs of impairment at the skeletal muscle level, such as an impaired capacity to increase skeletal muscle fractional O2 extraction during exercise, as evaluated by near-infrared spectroscopy (NIRS) (9); an “overshoot” of O2 extraction during constant work rate exercise (27), suggesting a transient imbalance between microvascular O2 delivery and O2 utilization (see, e.g., Ref. 6); a higher O2 cost of exercise, demonstrating an inefficiency of oxidative metabolism (12); and a slower pulmonary V̇O2 kinetics during transitions from rest to constant work rate exercise (12), a pronounced “slow component” of the kinetics (12, 27). Being noninvasive, the above-mentioned variables of functional evaluation are ideally suited to evaluate the effects of therapeutic/rehabilitation interventions, such as exercise training.

Recent studies have demonstrated that both in MM (18, 38, 39, 40, 41) and in McA (17, 24, 25) patients moderate-intensity aerobic exercise training is safe and effective in increasing exercise tolerance and in improving quality of life.

The main aim of the present study was to evaluate if the above-mentioned noninvasive methods of functional evaluation, specifically aimed at skeletal muscle oxidative metabolism, are capable to identify and quantify, in MM and McA patients, the improvements obtained by a program of exercise training. More specifically, we hypothesized that a program of home-based moderate-intensity aerobic exercise training would induce, in MM and McA patients: improved exercise tolerance (increases in V̇O2peak and peak work rate); enhanced capacity of fractional O2 extraction by skeletal muscle; impr-
proved matching between microvascular O$_2$ delivery and O$_2$ utilization; increased efficiency of skeletal muscle oxidative metabolism; faster pulmonary V˙O$_2$ kinetics; and less pro-

MATERIALS AND METHODS

Participants. Gender distribution, age, and body mass for MM and McA patients were as follows: MM, four males and two females, age (mean ± SD) = 51 ± 16 yr, body mass = 69.1 ± 18.1 kg, height = 171 ± 7 cm; body mass index (BMI) = 23.4 ± 4.9 kg/m$^2$; McA, three males and four females, age = 41 ± 13 yr, body mass = 71.2 ± 22.9 kg, height = 168 ± 12 cm; BMI = 24.6 ± 4.9 kg/m$^2$. Patients were from the Department of Neuromuscular Diseases, Neurological Institu-
tute “Carlo Besta,” Milan, Italy. The patients were recruited by a medical doctor in charge of their clinical assistance. Exclusion criteria were the presence of neoplastic and other major neurological/psychi-
atriac, orthopedic, rheumatologic, endocrine, pulmonary, or cardio-
vascular disorders. Patients of age <18 and >60 yr and patients not capable of performing exercises on a cycle ergometer were excluded.

The diagnosis of metabolic myopathy was based on clinical, morpho-
logical, biochemical, and molecular evaluations. The initial clinical evaluation was carried out in the occasion of the recruitment of the patients. The protocol included a detailed anamnestic evaluation and general physical and neurological examinations. Routine hematol-
egical examination [including creatine phosphokinase (CPK), lactate dehydrogenase (LDH), transaminase, aldolase], an electrocardiogra-
phy (ECG), and a cardiological evaluation, were performed. Clinical
details for the MM and McA patients were similar to those reported in our previous articles (9, 12). The functional impairment at the time of
enrollment was determined by the history (focused on exercise toler-
ance, habitual physical activity, etc.), physical examination, functional
evaluation scales, and muscle strength measurements. Their degree of
functional impairment varied from mild—virtually absent (no limita-
tions in activities of everyday life, no significant exercise intolerance) to severe (very limited exercise tolerance, impairment in activities of
daily living).

The patients were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate in the study, which was approved by the ethics committees of the Neurological Institute Carlo Besta. All procedures were in
accordance with the recommendations found in the Declaration of

Study design. This was an open interventional study in which each
patient acted as the control for herself/himself. Each patient was
involved in the study for 9 mo. During the first 3-mo period the
patients were invited to keep the same level of habitual physical activity they had before entering the study (PRE). This period was
used to record habitual physical activity and quality of life informa-
tion before intervention. During the subsequent 3-mo period the
subjects underwent the training protocol (see below for further de-
tails). The functional evaluation of oxidative metabolism (see below) was performed at the beginning (BEFORE) and immediately at the
end (AFTER) of the training period. During the last 3-mo period
(FOLLOW-UP) no specific recommendations were given, and the
patients were free to adjust at their will their level of physical activity.
At the end of this period, habitual physical activity and quality of life
data were again recorded.

Functional evaluation of oxidative metabolism. All tests were
carried out under close medical supervision, and patients were moni-
tored by 12-lead ECG. Patients were allowed time to gain familiarity
with the investigators and experimental protocol by means of short
preliminary practice runs. Measurements were carried out during 2
days: on day 1 the patients performed (see below) an incremental
exercise; on day 2 the patients performed two repetitions of 6 min
constant work rate exercises of low intensity. During each day, the
experimental session lasted about 1–1.5 h. A few minutes before the
incremental exercise McA patients ingested 330 ml of a caffeine—free
drink containing 37 g of sucrose to increase exercise tolerance and
peak exercise capacity and to abolish the “second-wind” phenomenon,
usually occurring after about 6–8 min of exercise (16).

An electromagnetically braked cycle ergometer (Corival, Lode, The Netherlands) was used. Pedalling frequency was digitally displayed
to the patients throughout the tests, and the patients were
encouraged to maintain relatively constant self-selected pedalling
frequency between 60 and 80 revolutions/min. During the incremental
exercise, after a few minutes of unloaded pedalling, exercise was
conducted at 25–50 W for 6 min, and thereafter the work rate was
increased by 10–25 W (according to the patient’s level of physical
fitness previously determined) every minute until voluntary exhaus-
tion was reached. The latter was defined when at least two of the
following criteria were met: 1) inability to maintain the pedalling
frequency (60–80 revolutions/min) despite encouragement by the
operators; 2) maximal levels (>15) of self—perceived exertion, using
the validated 6–20 Borg’s scale (3); and 3) heart rate (HR) values
>85% of the age—predicted maximum. On day 2, the patients
performed two repetitions of 6-min constant work rate low—inten-
sity exercise (CWR). Transitions from rest to the imposed work rate
were attained in ~3 s. The work rate was chosen to correspond to
~50% of peak work rate reached during the incremental exercise
before training. Repetitions were separated by at least 30-min recov-
er periods. Relatively long recovery periods were chosen to 1) be
sure that patients were in resting conditions before the next exercise
bout, 2) avoid the occurrence of a “second-wind phenomenon” in
McArdle patients (27), and 3) avoid a “priming effect” on the VO$_2$
kinetics (26).

Measurements. Pulmonary ventilation (V˙E), V˙O$_2$, and CO$_2$ output
(V˙CO$_2$) were determined breath—by—breath by a computerized meta-
bolic cart (Vmax229; SensorMedics). HR was determined from the
ECG signal. Stroke volume (SV) was estimated beat—by—beat by
impedance cardiography (Physio Flow; Manatec). The accuracy of
this device has been previously evaluated during incremental exercise
in healthy subjects against the direct Fick method (30). Cardiac output
(Q) was calculated as HR × SV. At rest and at various time points (1, 3, 5, and 7 min) during recovery after exercise, 20 µl of capillary
blood was obtained from a preheated earlobe for the determination of
blood lactate concentration ([Lacta]), by an enzymatic method (Biosen
5030; EKF Eppendorf). The highest [Lacta] value obtained during
recovery was retained for analysis. Gain values (G), variable estimat-
ing the O$_2$ cost of cycling, were calculated as ΔV˙O$_2$/V˙O$_2$ at the end of
CWR — resting V˙O$_2$ divided by work rate. Oxidative efficiency
during cycling was also evaluated as the ratio between the external
mechanical power output (work rate, expressed in W) and the oxida-
tive energy output. Also, this variable was expressed in watts by
assuming an energy equivalent of 20.9 kJ/l consumed O$_2$, and the
equivalence 1 W = 1 Jh.

Oxidation changes in the vastus lateralis muscle were evaluated by
near—infrared spectroscopy (NIRS) (4, 5). A portable NIR continu-
ous—wave photometer (PortaMon; Artinis) was used. Specific details
on the method and equipment can be found in a recent paper by our
research group (33). The instrument measures micromolar ([M]) changes
in oxygenated hemoglobin (Hb) + myoglobin (Mb) concentrations
[Δoxygen(Hb + Mb)], and in deoxygenated [Hb + Mb] [Δdeoxygen(Hb + Mb)], with respect to an initial value arbitrarily set equal to zero and
obtained during the resting condition preceding the test. Δ[deoxygen(Hb+Mb)] is relatively insensitive to changes in blood vol-
ume and has been considered an estimate of skeletal muscle fractional
O$_2$ extraction (ratio between O$_2$ consumption and O$_2$ delivery) (7, 8,
10, 21). Reliability of tissue oxygenation indexes obtained by NIRS, evaluated by the intraclass correlation coefficient, for repeated measurements on the same subject during different days, was found to be very high for skeletal muscle (36). A “physiological calibration” of $\Delta\text{[deoxy(Hb+Mb)]}$ values was performed by obtaining a transient ischemia of the limb after the exercise period (subject in the sitting position on the cycle ergometer). data obtained during exercise were expressed as a percentage of the values of maximal muscle deoxygenation obtained by pressure cuff inflation (at 300–350 mmHg), carried out at the inguinal crease of the thigh for a few minutes until $\Delta\text{[deoxy(Hb+Mb)]}$ increase reached a plateau (29).

Kinetics analysis. $V\dot{O}_2$ kinetics were evaluated during transitions from rest to low-intensity constant work rate exercise. Breath-by-breath $V\dot{O}_2$ values obtained in the two repetitions of the exercise were time aligned and then superimposed for each subject (22). Average $V\dot{O}_2$ values every 10 s were calculated. Data obtained during the first 20 s of the transition (“cardiodynamic” phase (45)) were excluded from analysis. Thus, $V\dot{O}_2$ kinetics analysis dealt mainly with the “phase 2” (or “fundamental”) component of the response, which more closely reflects gas exchange kinetics occurring in skeletal muscles (11). To evaluate mathematically the $V\dot{O}_2$ kinetics, data were fitted by the function:

$$y(t) = y_{\text{BAS}} + A_1 \left[ 1 - e^{-(t-TD_f)^\gamma_1} \right]$$ (1)

and parameter values $(TD_f, \gamma_1)$ were determined that yielded the lowest sum of squared residuals. In equation 1, $y_{\text{BAS}}$ indicates the baseline, $A_1$ is the amplitude between the $y_{\text{BAS}}$ and the steady state during the fundamental component, $TD_f$ is the time delay, and $\gamma_1$ is the time constant of the function for the fundamental component. To check the presence of a slow component (45) of the kinetics, data were also fitted by the function:

$$y(t) = y_{\text{BAS}} + A_1 \left[ 1 - e^{-(t-TD_u)^\gamma_2} \right] + A_s \left[ 1 - e^{-(t-TD_d)^\gamma_s} \right]$$ (2)

In equation 2, $A_s$, $TD_s$, and $\gamma_s$ indicate the amplitude, the time delay, and the time constant of the slow component, respectively. To confirm the presence/absence of an increase in $V\dot{O}_2$ as a function of time, average $V\dot{O}_2$ values were also calculated for each subject every 30 s, from the 3rd to the 6th min of exercise, and linear regression lines were drawn. The absence of a significantly positive slope would indicate that the variable has reached a steady state.

For the transitions from rest to CWR, $\Delta\text{[deoxy(Hb+Mb)]}$ data obtained in the various repetitions of CWR at the same work rate were time aligned and then superimposed for each subject. Average values every second were calculated and retained for analysis. To evaluate the $\Delta\text{[deoxy(Hb+Mb)]}$ kinetics and estimate the amplitude of its overshoot (7), data were fitted by a double exponential function of the type:

$$y(t) = y_{\text{BAS}} + A_1 \left[ 1 - e^{-(t-TD_s)^\gamma_2} \right] + A_s \left[ 1 - e^{-(t-TD_d)^\gamma_s} \right]$$ (3)

In equation 3, $y_{\text{BAS}}$ indicates the baseline, $A_s$ the amplitude of the upward component between $y_{\text{BAS}}$ and the transient steady-state value reached in the first seconds of the kinetics, $TD_s$ the time delay, and $\gamma_s$ the time constant of the function for the upward component. $A_1$, $TD_1$, and $\gamma_1$ indicate, respectively, the amplitude, time delay, and time constant of the downward component (32).

Exercise training. Training lasted 12 wk. The first one to two training sessions were conducted in the hospital, under the supervision of a researcher, who was in charge of giving adequate instructions to the patients about the training procedures. The patients conducted the remaining training sessions (4 days/wk) at home. Each session lasted about 1 h and began with $\sim$10–15 min of stretching exercises and exercises aimed at optimizing flexibility and balance. These activities were followed by 30 min (for the first 6 wk) or by 45 min (for the remaining 6 wk) of moderate-intensity aerobic training, conducted on a stationary cycle ergometer (Ergo bike CARDIO 3; Syloco srl). Patients were allowed to split at their will the 30- or 45-min total daily duration of the training session into combinations of 15- or 30-min training periods to make the compliance to the training regimen.

Exercise intensity was chosen as to correspond to $\sim$65–70% of maximal HR, determined for each subject during the incremental exercise described above. A software of the cycle ergometer regulated the work rate to keep the target HR. Work rate regulation as a function of HR was done every 30 s. HR was displayed and recorded during each exercise session using a Polar NV chest band and watch (s810; Polar Electro). The cycle ergometer recorded on a memory card the power output vs. time profile of each training session. These data were sent by e-mail to the researchers to check the adherence of the patients to the training regimen. The compliance to the training regimen was calculated as the relationship between minutes of work recorded by the memory card and minutes of exercise scheduled, and expressed as a percentage. Every week the patients (or someone close to them) were contacted by phone by the training supervisor to monitor progression, provide feedback and encouragement, and answer to any questions by the patients. The patients were instructed to maintain, during the training period, the same level of physical activity (apart from the training sessions) that characterized the 3 mo preceding training.

The patients were also instructed to keep a diary for each day of training. This diary was organized in a day-by-day table (one box for each hour) in which patients were invited to choose among sleep (B), sedentary (S), movement (M), and physical exercise (E). All forms of physical activity (type, duration, intensity) outside the training protocol were recorded. The hours spent by patients in the different domains were compared with the data obtained in PRE, and no significant differences were observed for M and E. The diary was kept also during the 3 mo after the completion of the training protocol.

Physical activity assessment. Physical activity was assessed using the Sensedwear Armband (SWA) (BodyMedia), a tri-axial accelerometer-based activity monitor coupled with several heat-related sensors (heat flux, body temperature, and galvanic skin response). The SWA has been shown to provide valid estimates of energy expenditure during exercise (19) and free-living physical activities (2) in previous studies. The SWA was placed on the patient’s right arm over the triceps muscle and was worn 24 h/day for three consecutive days, excluding Saturday and Sunday. The patients were instructed to continue their normal daily life activities while wearing SWA and to take the device only when doing water-related activities (i.e., swimming). Data from SWA were processed using a proprietary software package (version 8.0), and daily energy expenditure was expressed in kilocalories per kilogram per day and metabolic equivalents (METS). Steps per minute were summed to obtain the total number of steps per day. The physical activity assessment was not carried out during or immediately after training, but only during PRE and FOLLOW-UP phases.

Quality of life assessment. Quality of life and physical functioning were determined by a validated survey, the Short Form Health Survey Questionnaire (SF-36) (44). SF-36 assesses 8 health domains using 36 questions: the questions for physical function are 10, those for physical role limitation 4, for body pain 2, for general health 6, for mental health 5 (44). Answers are scored on a scale from 0 to 100, where 100 represents the highest level of functioning possible. A complete description of the scoring and measurement model is given elsewhere (44). The quality of life assessment was not carried out during or immediately after training, but only during PRE and FOLLOW-UP phases.

Statistical analysis. Results were expressed as mean values ± SD. Comparisons between patients before and after the training program were performed by two-sided Student’s $t$-test. Comparisons between more than two observations were performed by one-way ANOVA; a Tukey’s post hoc test was used when significant differences emerged at ANOVA. Data fitting by linear regression or by other functions was performed by the least-squares-residuals method. Comparisons between fittings with different function models were performed by
F-test. The level of significance was set at $P < 0.05$. Statistical analyses were performed by a software package (Prism 5.0; GraphPad).

RESULTS

Training. The compliance to the training regimen was evaluated as “high” (96% of all training sessions were completed as scheduled). Comparing the average work rate during the first three and the last three training sessions that patients carried at their homes a significant increase was observed in both MM (39.6 ± 8.2 and 49.8 ± 11.0 watt, respectively, $P = 0.04$) and McA (40.6 ± 8.7 and 49.1 ± 9.9 watt, respectively, $P < 0.01$).

Incremental exercise. Peak values of the main respiratory, cardiovascular, and metabolic variables are shown in Table 1. After training, an increase of ~20% in peak work rate and $V\dot{O}_{2}\text{peak}$ was observed both in MM and McA, indicating a significantly ($P = 0.02$) improved peak aerobic power, although the levels, in both groups, were substantially lower than those obtained in healthy controls (see Ref. 9). SV peak and $Q$ peak values were also significantly ($P = 0.04$) increased with training both in McA and MM. HR peak was significantly higher in McA vs. MM, corresponding, respectively, in the two groups to ~96% and ~83% of the age-predicted maximum. Training also improved ($P = 0.01$) peak skeletal muscle fractional $O_2$ extraction ($\Delta$(deoxy(Hb + Mb))peak), although the values, in both groups, were substantially lower than those obtained in healthy controls (see Ref. 9). As expected, in McA peak gas exchange ratio values were relatively low, and $[\text{La}]_b$ peak values were not different from those determined at rest (1.2 ± 0.1 mM). For the other variables, no differences were observed after training, either in McA or MM. The values of peak work rate, $V\dot{O}_{2}\text{peak}$, and peak skeletal muscle fractional $O_2$ extraction obtained by the two groups of patients in BEFORE and AFTER are summarized in Fig. 1; as a reference, values obtained by Grassi et al. (9) in healthy control subjects (CTRL) are also shown in Fig. 1. Although the values increased with training, both in MM and in McA, they were always substantially lower than the values observed in CTRL. The increase of $\Delta$(deoxy(Hb + Mb))peak with training was significantly and negatively correlated (monoeponential decreases; see Fig. 2) with $\Delta$(deoxy(Hb + Mb))peak before training. Thus, patients with greater impairment of skeletal muscle $O_2$ extraction before training benefited more, from training intervention, than patients with a relatively more preserved capacity to extract and utilize $O_2$.

Low-intensity constant work rate exercise. Mean ± SD values determined during the last ~30 s of CWR (carried out at the same absolute work rate in the two conditions) are presented in Table 2. $V\dot{O}_2$, $V_e$, HR, $Q$, and RPE values were significantly lower in AFTER vs. BEFORE, both in MM and McA. On the other hand, $\Delta$(deoxy(Hb + Mb)) was significantly ($P = 0.04$) higher in AFTER vs. BEFORE. In both groups of patients, the values of $O_2$ cost of cycling were significantly reduced by training (from 14.9 ± 1.6 to 12.8 ± 1.1 ml·min$^{-1}$·watt$^{-1}$ in MM and from 15.8 ± 1.3 to 13.6 ± 1.2 in McA, $P = 0.03$), even if they remained substantially higher than those usually observed in healthy subjects (~10 ml·min$^{-1}$·watt$^{-1}$; see, e.g., Ref. 12). Gross efficiency evaluated as the ratio between the external mechanical power output (work rate, expressed in W) and the oxidative energy output ($V\dot{O}_2$, expressed in W) increased with training in both MM and McA.

Table 1. Mean respiratory, cardiovascular, and metabolic variables determined at exhaustion (peak values) during the incremental exercise in MM and McA before (BEFORE) and after (AFTER) training. Values are means ± SD. MM, mitochondrial myopathy; McA, McArdle’s disease; $V\dot{O}_2$, oxygen uptake; $V\dot{CO}_2$, CO$_2$ output; $R$, gas exchange ratio; $V\dot{E}$, pulmonary ventilation; $V_t$, tidal volume; $fR$, breathing frequency; PETO$_2$, end-tidal O$_2$ partial pressure; PETCO$_2$, end-tidal CO$_2$ partial pressure; $[\text{La}]_b$, blood lactate concentration; RPE, rate of perceived exertion; HR, heart rate; SV, stroke volume; $Q\dot{\circ}$, cardiac output; $\Delta$(deoxy(Hb + Mb)), muscle oxygenation index obtained by near-infrared spectroscopy (NIRS); $\%$ of predicted, $%$ of expected value obtained BEFORE. See text for further details.
Fig. 1. The values of peak work rate, $V_{\text{O}_2}\text{peak}$, and peak skeletal muscle fractional $O_2$ extraction are summarized for the two groups of patients before (BEFORE) and after (AFTER) training. As a reference, values obtained by Grassi et al. (9) in healthy control subjects (CTRL) are also shown. $^\# P < 0.05$, significantly different from the corresponding value obtained in BEFORE.

Individual examples of $V_{\text{O}_2}$ kinetics in a representative MM patient (top) and in a representative McA patient (bottom) during CWR are shown in Fig. 3. In the MM patient, a slow component was not present either in BEFORE and in AFTER. As for the McA patient, in BEFORE a slow component was evident, whereas it disappeared in AFTER. The parameters of $V_{\text{O}_2}$ kinetics are shown in Table 3. In MM $T_\tau$ and $A_\tau$ values were significantly ($P = 0.03$) reduced in AFTER vs. BEFORE. In McA TD$_\tau$, $T_\tau$, and $A_\tau$ values were not significantly affected by training (although a clear trend toward lower $T_\tau$ values was observed). A significant linear relationship was observed (see Fig. 4) between the change in $T_\tau$ with training ($T_\tau$ in AFTER $- T_\tau$ in BEFORE) and the $T_\tau$ in BEFORE; in other words, the decrease in $T_\tau$ following training was more pronounced as a function of the “slowness” of the $V_{\text{O}_2}$ kinetics before training. A slow component, corresponding to $\sim 17\%$ of the total amplitude of the response, was present in all McA in BEFORE. After training, the slow component was reduced in amplitude in two patients (from 31.4 to 26.9$\%$ in patient 1 and from 26.7 to 17.3$\%$ in patient 2, in BEFORE and AFTER, respectively), and it disappeared in the remaining five. These results were confirmed by the linear regression analyses ($y = 0.766 + 0.01x$, $r^2 = 0.73$, $P < 0.0001$ in BEFORE and $y = 0.838x$, $r^2 = 0.92$, $P = 0.001$ in AFTER) of $V_{\text{O}_2}$ vs. time carried out on $V_{\text{O}_2}$ data averaged every 30 s from the 3rd to the 6th min of exercise (see MATERIALS AND METHODS for details).

Individual examples of $\Delta [\text{deoxy(Hb + Mb)}]$ kinetics of a typical MM patient (left) and of a typical McA patient (right) during CWR are shown in Fig. 5. The transient overshoot of the $\Delta [\text{deoxy(Hb + Mb)}]$ kinetics ($A_d$ in Eq. 3) was significantly ($P = 0.01$) more pronounced in BEFORE ($18.2 \pm 2.0$ and $28.2 \pm 4.3\%$ in MM and McA, respectively) than in AFTER ($1.8 \pm 2.4$ and $7.7 \pm 11.5\%$ in MM and McA, respectively). Following the overshoot, the $[\text{deoxy(Hb + Mb)}]$ time course in McA suggests $O_2$ extraction did not increase compared with the value determined at rest, confirming previous observations by our group (27).

Daily physical activity. Daily energy expenditure [total energy expenditure (TEE)] was not different in PRE ($36.6 \pm 9.2$ kcal·day$^{-1}$·kg$^{-1}$) compared with the “follow-up” 3 mo after the completion of the training period (FOLLOW-UP) ($35.7 \pm 13.4$ kcal·day$^{-1}$·kg$^{-1}$). TEE values corresponded, in both conditions, to a “low” level of habitual physical activity according to standard classifications (42). Similarly, METs ($1.55 \pm 0.3$ and $1.53 \pm 0.4$ kcal·kg$^{-1}$·h$^{-1}$ in PRE and FOLLOW-UP, respectively) and number of steps ($9,925 \pm 1,669$ and $8,337 \pm 2,046$ steps/day in PRE and FOLLOW-UP, respectively) were not different.
DISCUSSION

In the present study we investigated by noninvasive methods of functional evaluation the effects of 12 wk of home-based moderate-intensity aerobic training in MM and McA patients. Specific attention was given to variables related to skeletal muscle oxidative metabolism, which we had previously demonstrated to be impaired in these patients (9, 12, 27). In summary, we observed, after training: increased \( \dot{V}O_2 \)peak and peak work rate; increased skeletal muscle (vastus lateralis) fractional \( O_2 \) extraction, and signs of better matching between microvascular \( O_2 \) delivery and \( O_2 \) uptake during metabolic transitions, as estimated by NIRS; faster pulmonary \( V_o_2 \) kinetics during metabolic transitions (although only in the patients with markedly slow \( V_o_2 \) kinetics before training); and a decreased amplitude of the slow component of pulmonary \( V_o_2 \) kinetics and a decreased \( O_2 \) cost of exercise, suggesting an enhanced efficiency of oxidative metabolism. All of these improvements should directly increase exercise tolerance. In the present study, we indeed observed clear signs of increased exercise tolerance, such as an increased peak work rate, decreased heart rate, and decreased rates of perceived exertion during constant work rate submaximal exercise. However, the adopted exercise training program did not induce, 3 mo after the termination of the training program, an increased level of habitual physical activity. A limitation of the present study was that a supramaximal test, aimed at confirming \( V_o_2 \)peak values, could not be carried out.

MM and McA patients are affected by genetic disorders characterized by impairments of energy metabolism, which translate into reduced exercise tolerance and easy fatigability. At present, the therapeutic interventions available for these patients are very limited. Evidence from the literature suggests that moderate-intensity aerobic exercise training represents a safe intervention that may benefit MM and McA patients by increasing their exercise tolerance.

Taivassalo et al. (38) and Jeppesen et al. (18) demonstrated that 12–14 wk of moderate exercise training determined, in MM with single large-scale mitochondrial DNA (mtDNA) mutations, no changes in the level of mutated mtDNA, no changes in plasma CPK or alterations in muscle morphology, but increased \( V_{2max} \), work capacity, skeletal muscle \( O_2 \) extraction, and mitochondrial respiratory chain enzymatic activities. Thus, training affected positively the key pathophysiological mechanism responsible for the disease. Interestingly, in MM the main effect of aerobic training (increased capacity of \( O_2 \) extraction) was quite different from that (increased capacity of \( O_2 \) delivery by the cardiovascular system) usually described in control subjects. Similarly, Haller et al. (17) and Maté-Muñoz et al. (24) have shown that a low- to moderate-intensity aerobic exercise regimen increases peak power output, \( V_{2peak} \)-cardiac output, and mitochondrial enzymes in McA patients.

### Table 2. Main respiratory, cardiovascular, and metabolic variables determined in MM and McA at the end of the low-intensity constant work rate exercise before (BEFORE) and after (AFTER) training

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<thead>
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<th>Patient</th>
<th>( V_{O_2} ) peak</th>
<th>Work Rate, ( %W_{peak} )</th>
<th>RPE</th>
<th>( V_{O_2} ) kinetics</th>
<th>( O_2 ) extraction</th>
<th>Energy/Fatigue</th>
</tr>
</thead>
</table>
| MM BEFORE | 45 ± 5 | 11 ± # | # | 4.5 ± # | 0.95 ± 0.15 | 18.3% PRE | 51.1 ± 28.8% FOLLOW-UP in MM; 77.9 ± 13.5% PRE and 75.0 ± 18.3% FOLLOW-UP in McA and energy/fatigue (49.2 ± 28.9% PRE and 52.5 ± 25.9% FOLLOW-UP in MM; 60.0 ± 29.2% PRE and 59.8 ± 27.9% FOLLOW-UP in McA).

Values are means ± SD of the percentage of maximal work rate (%Wpeak). \#P < 0.05, significantly different from the corresponding value obtained in BEFORE. See text for further details.

### Quality of life assessment

Responses to questions in the SF-36 questionnaire did not differ significantly in PRE vs. FOLLOW-UP in either MM or McA. This applied to items related to physical function (50.8 ± 19.6% PRE and 51.1 ± 28.8% FOLLOW-UP in MM; 77.9 ± 13.5% PRE and 75.0 ± 18.3% FOLLOW-UP in McA) and energy/fatigue (49.2 ± 28.9% PRE and 52.5 ± 25.9% FOLLOW-UP in MM; 60.0 ± 29.2% PRE and 59.8 ± 27.9% FOLLOW-UP in McA).
suggesting that in these patients any increase in exercise capacity brought by exercise training is attributable not only to an improvement in cardiorespiratory function but also to an increased muscle oxidative capacity (24).

In the present study, we extended the observations of improvements, associated with exercise training, to other functional evaluation variables specifically related to skeletal muscle oxidative metabolism, which directly affect exercise tolerance. By doing so, our measurements also yielded pathophysiological insights (see below). For example, we observed a faster pulmonary VO2 kinetics, even though only in the patients showing markedly slow VO2 kinetics before training. As pointed out in a previous paper by our group (13), faster VO2 kinetics reflect a better performance of skeletal muscle oxidative metabolism and are associated with lower “O2 deficit” and higher exercise tolerance. We hypothesized that, if training induced an improvement of skeletal muscle oxidative metabolism, we would see faster VO2 kinetics in the patients after training. The fact that pulmonary VO2 kinetics got faster only in some patients (those with slow VO2 kinetics before training) may not be surprising. These data are in accordance with the results of a previous study by our group, carried out in MM and McA patients, in which we observed a hyperbolic relationship between the τ of the fundamental component of pulmonary VO2 kinetics, peak fractional O2 extraction, and peak VO2 [expressed as a fraction of the “normal” peak VO2, i.e., that of healthy subjects of the same age and sex (12)]. This indicated that only the patients with a markedly impaired oxidative metabolism had a slower VO2 kinetics, suggesting that analysis of this variable (which can be carried out during a constant work rate submaximal exercise, without the need to drive the patient to exhaustion during an incremental test) could represent a tool to identify the patients with the most pronounced metabolic impairment. The hyperbolic relationship between the τ of the VO2 kinetics and the other variables mentioned above is not surprising, since first-order kinetics predict linearity between VO2max and the velocity constant (k) of the kinetics, with k = 1/τ. In the present study, we observed a linear relationship, with a positive slope, between the changes of τ with training and the τ value before training.

The faster pulmonary VO2 kinetics was associated, in McA patients, with a significant decrease or with a disappearance of the slow component of the kinetics. The slow component represents a slowly developing increase in VO2, occurring in healthy subjects during constant work rate exercise above the

![VO2 kinetics graphs](https://example.com/vo2_kinetics.png)

**Table 3.** VO2 kinetics parameters determined during the low-intensity constant work rate exercise in MM and McA

<table>
<thead>
<tr>
<th></th>
<th>τf, s</th>
<th>Tδf, s</th>
<th>ybas, l/min</th>
<th>A1, l/min</th>
<th>A2, l/min</th>
<th>A1/A2, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEFORE</td>
<td>45.1 ± 6.6</td>
<td>1.9 ± 3.7</td>
<td>0.31 ± 0.03</td>
<td>0.55 ± 0.10</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>AFTER</td>
<td>35.9 ± 4.2#</td>
<td>2.3 ± 4.9</td>
<td>0.31 ± 0.05</td>
<td>0.48 ± 0.11#</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>McA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEFORE</td>
<td>46.7 ± 7.0</td>
<td>2.7 ± 2.5</td>
<td>0.31 ± 0.03</td>
<td>0.55 ± 0.06</td>
<td>0.09 ± 0.03</td>
<td>17.0 ± 4.4</td>
</tr>
<tr>
<td>AFTER</td>
<td>33.2 ± 4.9</td>
<td>6.4 ± 2.9</td>
<td>0.29 ± 0.03</td>
<td>0.53 ± 0.05</td>
<td>0.04 ± 0.03#</td>
<td>6.4 ± 4.3#</td>
</tr>
</tbody>
</table>

Values are means ± SD of baseline (ybas). Data obtained before (BEFORE) and after (AFTER) are shown. Tδf, time delay; τf, time constant; A1, amplitude of the fundamental component; A2, actual amplitude of the slow component; A1/A2, total amplitude of the response. #P < 0.05, significantly different from the corresponding value obtained in BEFORE. NA, not applicable. See text for further details.
lactate threshold” (the increase is progressive during constant work rate exercise above “critical power”) and is associated with a decreased efficiency of contraction and muscle fatigue (14). A decreased amplitude or the disappearance of the slow component, as observed in the present study in the McA patients, is intrinsically related to an improved exercise tolerance (14). The latter was directly demonstrated, in the present study, by the lower HR and RPE values observed during constant work rate exercise after training. In other words, in the present study, exercise training transformed the same mechanical power output from a “heavy-intensity exercise” (before training), characterized by the slow component, into a “moderate-intensity exercise” (after training), with no slow component (20). This effect, by definition, represents a sign of increased exercise tolerance.

A decreased amplitude or the disappearance of the slow components of the V˙O₂ kinetics have been recently described by our group (27), in McA patients, following a “warm-up” exercise, carried out a few minutes before the test; the effect was considered a sign of a second-wind phenomenon (see Ref. 15). This phenomenon, which is considered pathognomonic of the disease (43), is characterized by a sudden decrease in HR and improved exercise tolerance occurring after a few minutes of exercise and is attributable to an enhanced sympathoadrenal response and to an improved delivery of extramuscular energy substrates, free fatty acids, and glucose to working muscles, which would partially compensate for the impaired glycogen breakdown (15). It has been demonstrated that in McA patients a partial compensation of the impaired glycogen breakdown can be also obtained by the administration of oral glucose before the test; more specifically, glucose administration increases exercise tolerance and abolishes the second wind (16). In the present study, oral glucose was administered to the patients before the test, so that the observed improvements in skeletal muscle oxidative metabolism can be attributed to training by itself.

In the present study, training obtained, both in MM and in McA patients, a decreased O₂ cost during constant work rate exercise, suggesting an increased efficiency of skeletal muscle oxidative metabolism. In both patients’ populations, however, the O₂ cost of exercise, although significantly decreased following training, was still substantially higher (by ~30–40%) than the values usually described in healthy untrained controls (see, e.g., Ref. 12). In MM, the decreased O₂ cost of exercise was related to a decreased amplitude of the fundamental component of V˙O₂ kinetics, whereas in McA a decreased amplitude of the slow component was observed (see also above). Independently from the fitting details, however, the markedly increased O₂ cost of exercise observed in MM and McA patients, which is inevitably associated with an impaired exercise tolerance (see Ref. 14), was at least in part ameliorated by exercise training.

Another aspect, directly related to skeletal muscle oxidative metabolism, that was found to be positively affected by the training intervention was the overshoot of the NIRS-determined Δ[deoxy(Hb + Mb)] kinetics during the transition to the constant work rate exercise; this overshoot is considered to be caused by a transient mismatch between intramuscular O₂ delivery and O₂ utilization (7, 26). The mismatch could derive from a suboptimal nitric oxide (NO) signaling within the muscle, which would “uncouple” the heterogeneous microvascular blood flow increase from the presumably heterogeneous O₂ uptake (26). The overshoot would be associated with a decreased microvascular PO₂ and thereby with a decreased driving pressure for peripheral O₂ diffusion and with an impaired oxidative metabolism. An overshoot in Δ[deoxy(Hb + Mb)] during metabolic transitions has been described, among
others, in patients with chronic heart failure (35) and, by our group, in healthy young subjects undergoing bed rest deconditioning (28), as well as in McA and MM patients (27). In that study (27), in McA, but not in MM, the overshoot was eliminated by a warm-up exercise (second-wind phenomenon). The results of the present study demonstrate that both in McA and in MM patients the overshoot in Δ[deoxy(Hb + Mb)], and the mismatch between microvascular O₂ delivery and O₂ utilization presumably responsible for the overshoot, can be at least in part ameliorated by exercise training. Regarding skeletal muscle O₂ extraction, it is noteworthy that before training at the end of constant work rate exercise Δ[deoxy(Hb + Mb)] was very low, close to resting values (particularly in McA), confirming previous results by our group (27). The Δ[deoxy(Hb + Mb)] at the end of constant work rate exercise increased (>50%) with training both in MM and McA.

The habitual level of daily physical activity was evaluated by estimating TEE. Before and ~3 mo after the termination of the training period the subjects wore a dedicated device (SenseWear Armband) during three typical consecutive days. TEE was not different before vs. after training: TEE values were similar to other studies on patients with metabolic myopathies [~40 kcal·day⁻¹·kg⁻¹ (25)], and they corresponded to a low level of habitual physical activity according to standard classifications (1). In other words, the adopted exercise training program, which increased exercise tolerance of the patients, did not induce, a couple of months after the termination of the training program, an increased level of habitual physical activity. These results are in accordance with the observation that positive effects of exercise training in patients with metabolic myopathies substantially disappear when in MM patients training is interrupted (18). We have no specific data to explain this unexpected and rather disappointing finding. It may be hypothesized that to obtain an increase in the level of habitual physical activity specific interventions at a “motivational” level should be empowered (31). It should also be noted that exercise training was not associated with an improvement of the patients’ quality of life, as assessed by a SF-36 questionnaire. Although measurements carried out during or immediately after the training period could have been helpful to better clarify the reason for these unexpected findings, unfortunately they were not carried out and so no hypotheses can be forwarded.

In conclusion, in MM and McA patients a standardized home-based 12-wk training program with aerobic exercise of moderate intensity significantly improved several variables of noninvasive functional evaluation of skeletal muscle oxidative metabolism. More specifically, we observed an increased V̇O₂peak, faster pulmonary V̇O₂ kinetics, a decreased amplitude of the slow component, a decreased O₂ cost of cycling, an increased capacity to increase fractional O₂ extraction by skeletal muscles, and signs of improved matching between microvascular O₂ delivery and O₂ uptake within muscles. These improvements were associated with signs (e.g., increased peak work rate, decreased heart rate, and decreased rates of perceived exertion during constant work rate submaximal exercise) suggesting an enhanced exercise tolerance. The improvements in exercise tolerance obtained by the training program did not determine an increase in the habitual level of physical activity, as evaluated 3 mo after the termination of the training program. This study demonstrates the beneficial effects of relatively short-term aerobic exercise training on the exercise capacity in our patients, confirming the potential value of prescribed therapeutic exercise training for improving functional capacity and well-being even in patients with chronic neuromuscular disorders. Further studies should be carried out, in these patients, for the definition of methods and strategies aimed at increasing the level of habitual physical activity in the long period.

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DISCLOSURES

The authors declare they do not have conflict of interests.

AUTHOR CONTRIBUTIONS


REFERENCES


