Lack of functional effects of neuromuscular electrical stimulation on skeletal muscle oxidative metabolism in healthy humans

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Porcelli S, Marzorati M, Pugliese L, Adamo S, Gondin J, Bottinelli R, Grassi B. Lack of functional effects of neuromuscular electrical stimulation on skeletal muscle oxidative metabolism in healthy humans. J Appl Physiol 113: 1101–1109, 2012. First published August 16, 2012; doi:10.1152/japplphysiol.01627.2011.—A recent study has demonstrated that neuromuscular electrical stimulation (NMES) determines, in vitro, a fast-to-slow shift in the metabolic profile of muscle fibers. The aim of the present study was to evaluate if, in the same subjects, these changes would translate, in vivo, into an enhanced skeletal muscle oxidative metabolism. Seven young men were tested (cycle ergometer) during incremental exercises up to voluntary exhaustion and moderate and heavy constant-load exercises (CLE). Measurements were carried out before and after an 8-wk training program by isometric bilateral NMES (quadriceps muscles), which induced an ~25% increase in maximal isometric force. Breath-by-breath pulmonary O2 uptake (VO2) and vastus lateralis oxygenation indexes (by near-infrared spectroscopy) were determined. Skeletal muscle fractional O2 extraction was estimated by near-infrared spectroscopy on the basis of changes in concentration of deoxygenated hemoglobin + myoglobin. Values obtained at exhaustion were considered “peak” values. The following functional evaluation variables were unaffected by NMES: peak VO2; gas exchange threshold; the VO2 vs. work rate relationship (O2 cost of cycling); changes in concentration of deoxygenated hemoglobin + myoglobin vs. work rate relationship (related to the matching between O2 delivery and VO2); peak fractional O2 extraction; VO2 kinetics (during moderate and heavy CLE) and the amplitude of its slow component (during heavy CLE). Thus NMES did not affect several variables of functional evaluation of skeletal muscle oxidative metabolism. Muscle hypertrophy induced by NMES could impair peripheral O2 diffusion, possibly counterbalancing, in vivo, the fast-to-slow phenotypic changes that were observed in vitro, in a previous work, in the same subjects of the present study.

strength training; muscle hypertrophy; near-infrared spectroscopy; gas exchange kinetics

NEUROMUSCULAR ELECTRICAL STIMULATION (NMES) (26, 28) is widely utilized as a complement to voluntary exercise in athletes (27) and in patients, such as those with heart failure (46), chronic obstructive pulmonary disease (34), or cancer (6). NMES represents an obligatory choice for training in patients who cannot perform voluntary contractions, such as those with spinal cord lesions (10), or in patients immobilized for fractures or following ligament reconstruction surgery (42). It is well established that NMES leads to significant increases in muscle mass and maximal voluntary strength (13, 26, 39).

The effects of NMES on oxidative metabolism appear rather controversial. At molecular level, Perez et al. (36) reported a higher succinic dehydrogenase activity after NMES training, whereas both citrate synthase and phosphofructokinase activities were unchanged. Kim et al. (21) reported, after a NMES training program, no changes in citrate synthase activity and number of capillaries. Nuhu et al. (35) showed an increase in the activity of citrate synthase associated with a decrease in the activity of glyceroldehyde phosphate dehydrogenase. Interestingly, these molecular adaptations were not associated with clear effects in vivo. Perez et al. (36) observed an increase of the mean number of capillaries of fast-twitch (type II) fibers, but only minimal muscle hypertrophy, and no significant changes in aerobic performance. On the other hand, Nuhu et al. (35) reported an improved work capacity and O2 uptake (VO2) at the anaerobic threshold following NMES. Compared with voluntary contractions of the same intensity, it has been reported that contractions obtained by NMES are associated with a greater (30, 32) or similar (33) metabolic demand, and with an increased blood flow during recovery (32, 45).

In the light of these controversial results, Gondin et al. (12) performed a comprehensive in vitro evaluation of the skeletal muscle phenotypic adaptations following a training program by NMES. These authors observed the following (12): increases in maximum voluntary force and neural activation; muscle fibers hypertrophy; a fast-to-slow transition of muscle fiber types, as determined on the basis of myosin heavy chain (MHC) isoforms; a fast-to-slow phenotype shift in the expression of myofibrillar proteins; a glycolytic-to-oxidative shift in metabolic profile; and an enhancement of intracellular defenses against reactive oxygen species. As discussed by the authors (12), these muscular adaptations could be attributed to the peculiar motor unit recruitment pattern associated with NMES (12, 28) and appear of interest since they are typical of both resistance (strength gains and hypertrophy) and endurance (fast-to-slow shift in MHC and metabolic profile) training.

In the present study, which was carried out on seven of the eight subjects evaluated by Gondin et al. (12), we hypothesized that the phenotypic adaptations described in vitro by these authors would translate, in vivo, into improvements in some variables of functional evaluation of oxidative metabolism,
such as peak pulmonary VO$_2$ (VO$_{2\text{peak}}$), gas exchange threshold (GET), cycling efficiency, skeletal muscle fractional O$_2$ extraction [as estimated by near-infrared spectroscopy (NIRS)], matching of O$_2$ delivery and O$_2$ utilization in skeletal muscles (as estimated by NIRS), rate of adjustment of pulmonary VO$_2$ during metabolic transitions (VO$_2$ “kinetics”), and presence and amplitude of the slow component of the VO$_2$ kinetics. Several of these variables are specifically aimed at the skeletal muscle level (see DISCUSSION). An enhanced oxidative function in vivo would be of interest for a better comprehension of the overall effects of NMES. Moreover, if confirmed also in other populations, it would make this procedure particularly suited, alone or in combination with voluntary exercise, to treat conditions of disuse/immobilization.

MATERIALS AND METHODS

Subjects. Seven young healthy subjects (age: 25.9 ± 1.2 yr; height: 175.7 ± 3.1 cm) (mean value ± SD) volunteered for the study. As mentioned above, the subjects were from the group of eight evaluated in the study by Gondin et al. (12); one subject from that study was unavailable for our measurements. After considering the wealth of data, we decided to split them (as well as the authors) into two studies: the in vitro data in the study by Gondin et al. (12), and the in vivo data in the present study. The subjects were evaluated before (Before) and after (After) a NMES training program (see below). Body mass and body mass index were, respectively, 73.7 ± 14.5 kg and 23.7 ± 2.8 kg/m$^2$ in Before, and 72.9 ± 13.2 kg and 23.4 ± 2.4 kg/m$^2$ in After (no significant differences for both variables). None of the subjects had engaged in systematic strength training or NMES in the 12 mo before the beginning of the experiments. Five subjects were involved in various sport activities (average weekly volume of physical activity ~4–6 h/wk), whereas two subjects had no history of regular participation in physical activities (~2 h/wk) during the NMES training period, the subjects were strongly encouraged to keep their level of physical activity (apart from NMES) at the same level as before NMES. This was checked by analysis of daily diaries of physical activity, which the subjects kept during NMES training and the preceding weeks.

The subjects were highly motivated to participate in the research, and their collaboration was excellent. The subjects were informed about risks and discomfort associated with the experimental procedure before they gave their written consent to participate in the research program, which was approved by the ethical committee of the University of Pavia and conformed with standards set by the Declaration of Helsinki (last modified in 2000). All tests were conducted in the laboratories of the Department of Physiology of the University of Pavia under close medical supervision, and subjects were continuously monitored by 12-lead electrocardiography. After a detailed explanation of all experimental procedures, subjects were allowed enough time to familiarize themselves with the researchers and with the setup environment, as well as with the experimental protocol by short preliminary practice runs.

NMES training and MVC measurements. The training program consisted of twenty-five 18-min sessions of isometric NMES of both quadriceps, carried out over 8 wk, with three sessions/wk. Details on the NMES protocol can be found in Gondin et al. (12). The protocol had been successfully used in previous studies to increase knee extensor muscle strength (13).

Bilateral maximal voluntary contraction (MVC) during isometric knee extension was determined for both legs in Before and After by means of a strain gauge (DG-ST 250 kg, Dinamica Generale, Poggio Rusco, Italy) mounted on a leg extension machine (Oenmebi, AM311/DS2, Moglia, Italy). Details on the MVC measurements can be found in Gondin et al. (12).

Cycloergometric exercise protocols. A mechanical flywheel cycloergometer (Monark 818E, Stockholm, Sweden) was utilized. The following exercises were performed. 1) First was an incremental exercise (IE) up to voluntary exhaustion (15 W/min increments after an initial 75–90-W work rate carried out for 6 min). Voluntary exhaustion was defined as the inability to maintain the pedaling frequency, despite vigorous encouragement by the operators, as well as by maximal levels of self-perceived exertion using the validated Borg scale (3). Values of cardiovascular, ventilatory, gas exchange, and muscle oxygenation variables (see below) determined during the last 20 s of the exhausting work rate were considered “peak” values. 2) Second was three repetitions of 6-min constant-load exercise (CLE) of moderate intensity, at 60% of the GET (see below); each repetition was separated by a 15–30-min recovery period. On-transitions were from unloaded pedaling to the imposed work rate, which was attained in about 3 s. 3) Third was one repetition of a 6-min CLE of heavy intensity, that is, at a work rate corresponding to ~50% of the difference between GET and VO$_{2\text{peak}}$.

Measurements. Pulmonary ventilation [VE] expressed in BTPS, VO$_2$, and CO$_2$ output (VCO$_2$), both expressed in STPD, with determined breath by breath by a computerized metabolic cart (SensorMedics Vmax229e, Bihlthoven, The Netherlands). For details on this standard method, see Porcelli et al. (37). Gas exchange ratio was calculated as VCO$_2$/VO$_2$. The GET was determined by standard methods (1). Arterial blood O$_2$ saturation was continuously monitored by pulse oximetry (MasimoRAD-9, Masimo, Milan, Italy) at the ear lobe. Heart rate (HR) was determined by electrocardiography. Blood pressure was measured using a standard cuff sphygmomanometer. At rest, at the end of exercise, and at 1, 3, 5, and 7 min during the recovery period, blood lactate concentration was determined by an enzymatic method (Biosen 5030, EKF diagnostic, Eppendorf Italia, Milano, Italy) on 20 μl of capillary blood obtained at the earlobe.

Oxygenation changes in the vastus lateralis muscle were evaluated by NIRS. A portable near infrared single-distance, continuous-wave photometer (HEO-100, Omron, Japan), which adopts an algorithm based on diffusion theory (41), was utilized. The instrument provides separate measurements of changes in deoxygenated hemoglobin (Hb) and myoglobin (Mb) concentrations {Δ[Deoxy(Hb+Mb)]}, expressed in arbitrary units. Specific details on these measurements can be found in recent papers by our group ([14–16, 37]). The reliability of muscle oxygenation indexes obtained by NIRS, evaluated by the intraclass correlation coefficient for repeated measurements on the same subject during different days, was recently found to be very high (43). NIRS measurements in muscle tissue (22) have been shown to be well correlated with local venous O$_2$ saturation. Advantages and limitations of NIRS measurements in skeletal muscle are discussed in the reviews by Boushel et al. (4) and Ferrari et al. (8).

Average values of Δ[Deoxy(Hb+Mb)] were calculated during the last 10 s of each workload. The resulting Δ[deoxy(Hb+Mb)] data were averaged as a function of work rate and were fitted by a sigmoid function of the type:
Table 1. Peak values obtained during exhausting exercise of cardiovascular, ventilatory, pulmonary gas exchange, and muscle oxygenation variables

<table>
<thead>
<tr>
<th>Workload, W</th>
<th>HR</th>
<th>Vo2</th>
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<tbody>
<tr>
<td>Beats/min</td>
<td>%Predicted</td>
<td>l/min</td>
</tr>
<tr>
<td>Before</td>
<td>272.2 ± 52.3</td>
<td>176.0 ± 10.8</td>
</tr>
<tr>
<td>After</td>
<td>272.2 ± 37.1</td>
<td>166.8 ± 10.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR, heart rate; Vo2, pulmonary ventilation; VCO2, O2 uptake; R, gas exchange ratio; [La], blood lactate concentration; Δ[deoxy(Hb+Mb)], changes in concentration of deoxygenated hemoglobin and myoglobin, expressed as a ratio of the maximal values obtained during transient limb ischemia.

\[ f(x) = f_0 + A [1 + e^{-(c+d/x)}] \]  \tag{1}

In this function \( f_0 \) represents the baseline, \( A \) the amplitude, and \( c \) is a constant dependent on \( d \), the slope of the sigmoid (9). In this function \( cd \) gives the \( x \) value corresponding to \( (f_0 + A)/2 \), which is the work rate corresponding to half-amplitude \( (W_{so}) \).

**Kinetics analysis.** Vo2, HR, and Δ[deoxy(Hb+Mb)] kinetics were evaluated during the transition from unloaded pedaling to CLE. Breath-by-breath Vo2 values obtained in the various repetitions of CLE at the same workload were time aligned and then superimposed for each subject. Average Vo2 values every 10 s were calculated and utilized for kinetics analysis. Vo2 data obtained during the first 20 s of the transition (corresponding to the “cardiodynamic” phase (48)) were excluded from the analysis. Thus Vo2 kinetics analysis dealt with the “phase 2” (or “fundamental”) component of the response, which should more closely reflect gas exchange kinetics occurring at the skeletal muscle level (17, 48), as well as (for CLE) the “slow component” of the kinetics (48). As for HR and muscle oxygenation data \{Δ[deoxy(Hb+Mb)]\}, values obtained in the various repetitions of CLE were time aligned and superimposed, and average values were calculated, respectively, every 10 s and every 1 s.

To mathematically evaluate the Vo2, HR, and Δ[deoxy(Hb+Mb)] kinetics, data were fitted by a function of the type:

\[ y(t) = y_{bas} + A_1 [1 - e^{-(x-TD)/\tau_1}] + A_2 [1 - e^{-(x-TD)/\tau_2}] \]  \tag{2}

Parameter values \( (TD, \tau) \) were determined that yielded the lowest sum of squared residuals. In Eq. 2, \( y_{bas} \) indicates the baseline value; \( A_1 \), the amplitude between \( y_{bas} \) and the steady-state value during the fundamental component of the kinetics; \( TD_1 \), the time delay; and \( \tau_1 \), the time constant of the function for the fundamental component. The mean response time (MRT) was calculated as \( \tau_1 + TD_1 \).

To check the presence of a "slow component" of the kinetics (20), data were also fitted by a function of the type:

\[ y(t) = y_{bas} + A_1 [1 - e^{-(x-TD)/\tau_1}] + S(\tau_s - TD_s) \]  \tag{3}

In Eq. 3, \( A_1 \), TD, and \( \tau_s \) indicate, respectively, the amplitude, the time delay, and the time constant of the slow component of the kinetics.

Sometimes, after the first exponential rise, Vo2 increased linearly without reaching a steady-state value. In this case, Eq. 3 did not provide a good fit of data. Thus another equation was also utilized, with an exponential function for the fundamental component and a linear function for the slow component (exponential + linear fitting):

\[ y(t) = y_{bas} + A_1 [1 - e^{-(x-TD)/\tau_1}] + S(\tau_s - TD_s) \]  \tag{4}

where \( S \) (slope) is the angular coefficient of the linear regression of Vo2 vs. time (40).

The equation that best fit the experimental data was determined by the F-test. That is to say, when Eq. 3 or Eq. 4 provided a better fit of the data, a slow component of Vo2 kinetics was present, superimposed on the fundamental component. The actual amplitude of the slow component \( (A_2) \) was estimated as the difference between the average Vo2 value obtained during the last 20 s of CLE and the asymptotic value of the fundamental component \( (25) \). The percent contribution of the slow component to the total amplitude \( (A_{tot}) \) of the response \( (A_2/A_{tot}) \) was also calculated (37).

**Statistical analysis.** Results were expressed as means ± SD. The statistical significance of differences between two means was checked.

Fig. 1. Typical example of the time course of the near-infrared spectroscopy (NIRS)-derived variable \{changes in concentration of deoxygenated hemoglobin and myoglobin, Δ[deoxy(Hb+Mb)]\} utilized to estimate skeletal muscle O2 extraction during the incremental exercise (IE). The vertical dashed lines indicate the sequence of phases of the experiment: rest, unloaded pedaling (UL), constant load exercise (CLE), the various 1-min steps during IE, followed by recovery and transient limb ischemia (see MATERIALS AND METHODS). Data obtained during exercise were then expressed as a ratio of the maximal values obtained during the ischemia at the end of the test. In the example shown in the figure, peak values of Δ[deoxy(Hb+Mb)] \( \Delta[\text{deoxy(Hb+Mb)}] \) were ~0.8 of the values during ischemia \( \Delta[\text{deoxy(Hb+Mb) ischemia}] \). AU, arbitrary units.
RESULTS

Force. MVC values of the knee extensor muscles were significantly (by ~25%) higher in After (807.5 ± 185.6 N) than in Before (663.1 ± 161.7 N). This increase is in agreement with those reported in the literature after similar training programs (2, 11, 29).

Incremental exercise. “Peak” cardiovascular, ventilatory, pulmonary gas exchange, and muscle oxygenation variables obtained at exhaustion during IE are shown in Table 1. For all variables, the values obtained in After were not significantly different from those obtained in Before.

GET occurred at 66.6 ± 7.4% of \( \dot{V}O_2\text{peak} \) in Before vs. 67.6 ± 9.2% in After (no significant difference).

Mean \( \dot{V}O_2 \) values were calculated for each subject during the last 20 s of each work rate. Individual \( \dot{V}O_2 \) vs. work rate plots were drawn, and linear regressions were calculated. Mean (± SD) values of the slopes of the individual \( \dot{V}O_2 \) vs. work rate relationships were 10.2 ± 0.8 ml·min\(^{-1}\)·W\(^{-1}\) in Before, and 10.8 ± 0.9 ml·min\(^{-1}\)·W\(^{-1}\) in After (no significant difference).

The mechanical efficiency of oxidative metabolism was estimated by calculating the ratio between the difference in external mechanical power output (Δwork rate, expressed in Watts) and the corresponding change in oxidative energy output (Δ\( \dot{V}O_2 \)). Also this variable was expressed in Watts by assuming an energy equivalent of 20.9 kJ/liter of consumed \( O_2 \), and the equivalence 1 W = 1 J/s. Mechanical efficiency was 0.28 ± 0.02 in Before and 0.26 ± 0.01 in After. Thus NMES did not affect the \( O_2 \) cost of cycling during IE.

A typical example of a \( \Delta[\text{deoxy} (\text{Hb} + \text{Mb})] \) vs. time tracing during IE and the subsequent transient ischemia is shown in Fig. 1. Figure 2, left, shows a typical example of a \( \Delta[\text{deoxy} (\text{Hb} + \text{Mb})] \) vs. work rate relationship during IE, with the sigmoid function utilized to fit the data (see Eq. 1). Mean values (± SD) of \( c/d \) (\( W_50 \)) (see above) obtained by solving the function are shown in Fig. 2, right. No significant differences were observed between Before and After. Since peak work rate was not significantly different, \( W_50 \) allows the comparison of \( \Delta[\text{deoxy} (\text{Hb} + \text{Mb})] \) values for the same submaximal work rate in the two conditions. For the same submaximal work rate (and for the same \( \dot{V}O_2 \)), \( \Delta[\text{deoxy} (\text{Hb} + \text{Mb})] \) was unaffected by NMES.

Table 2. Steady-state (or end-exercise, when a slow component was detected) values obtained during CLE < GET and CLE > GET of cardiovascular, ventilatory, pulmonary gas exchange, and muscle oxygenation variables

<table>
<thead>
<tr>
<th>HR</th>
<th>Workload, W</th>
<th>Beats/min</th>
<th>%Predicted</th>
<th>( \dot{V}c ), l/min</th>
<th>( l/min )</th>
<th>ml·kg(^{-1})·min(^{-1} )</th>
<th>( \dot{V}CO_2, l/min )</th>
<th>( R )</th>
<th>[La], mM</th>
<th>( \Delta[\text{deoxy} (\text{Hb} + \text{Mb})] )</th>
</tr>
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<tbody>
<tr>
<td>&lt; GET</td>
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</tr>
<tr>
<td>Before</td>
<td>87.9 ± 5.7</td>
<td>115.2 ± 18.2</td>
<td>59.3 ± 9.6</td>
<td>32.9 ± 4.4</td>
<td>1.412 ± 0.144</td>
<td>19.5 ± 2.6</td>
<td>1.229 ± 0.114</td>
<td>0.87 ± 0.05</td>
<td>2.8 ± 0.4</td>
<td>0.18 ± 0.11</td>
</tr>
<tr>
<td>After</td>
<td>87.9 ± 5.7</td>
<td>114.9 ± 15.6</td>
<td>59.2 ± 8.5</td>
<td>32.7 ± 5.4</td>
<td>1.438 ± 0.124</td>
<td>20.1 ± 2.5</td>
<td>1.304 ± 0.176</td>
<td>0.90 ± 0.07</td>
<td>2.1 ± 0.2</td>
<td>0.19 ± 0.28</td>
</tr>
<tr>
<td>&gt; GET</td>
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<tr>
<td>Before</td>
<td>192.9 ± 35.1</td>
<td>163.9 ± 12.1</td>
<td>84.5 ± 6.8</td>
<td>76.0 ± 22.1</td>
<td>2.843 ± 0.581</td>
<td>38.9 ± 6.6</td>
<td>2.913 ± 0.650</td>
<td>1.02 ± 0.03</td>
<td>5.1 ± 0.8</td>
<td>0.77 ± 0.35</td>
</tr>
<tr>
<td>After</td>
<td>192.9 ± 34.1</td>
<td>165.1 ± 16.1</td>
<td>85.2 ± 7.3</td>
<td>73.9 ± 20.0</td>
<td>2.902 ± 0.444</td>
<td>40.3 ± 6.5</td>
<td>3.052 ± 0.599</td>
<td>1.04 ± 0.06</td>
<td>5.5 ± 1.1</td>
<td>0.84 ± 0.30</td>
</tr>
</tbody>
</table>

Values are means ± SD. CLE, constant load exercise; GET, gas exchange threshold.
Also $\Delta[\text{deoxy(Hb+Mb)}]$ peak values (last points of Fig. 2, left) were unaffected by NMES.

$\text{CLE}$. Steady-state values (or end-exercise values, when a slow component was detected) of the investigated variables obtained during CLE < GET and > GET are shown in Table 2. No significant differences were observed for any variable between Before and After. During CLE < GET and CLE > GET $\dot{V}O_2$ corresponded, respectively, to $\sim 45$ and $85\%$ of $\dot{V}O_2$peak. Gas exchange ratio and lactate concentration values confirm that CLE < GET corresponded to moderate-intensity exercise, whereas CLE > GET corresponded to heavy-intensity exercise.

The kinetics of adjustment during CLE of $\dot{V}O_2$, HR, and $\Delta[\text{deoxy(Hb+Mb)}]$ are shown for a typical subject in Fig. 3 (<GET) and Fig. 4 (>GET). During CLE < GET, a slow component was not detected in any subject for any of the variables, whereas a slow component was detected in all subjects and for all variables (with the exception of $\dot{V}O_2$ in one subject) during CLE > GET. The parameters calculated to evaluate the kinetics are given in Table 3. For both CLE <

![Fig. 3. Typical examples of O2 uptake ($\dot{V}O_2$), heart rate (HR), and muscle oxygenation ($\Delta[\text{deoxy(Hb+Mb)}]$) kinetics during constant-load exercise < gas exchange threshold (GET). Data obtained Before are shown on the left, whereas data obtained After are shown on the right. The vertical lines indicate the transition from UL to the imposed load. The monoexponential functions fitting the data and the values of time constants ($\tau$) for $\dot{V}O_2$ kinetics and for HR kinetics are also shown. As for $\Delta[\text{deoxy(Hb+Mb)}]$, the mean response time (MRT) is shown. (See text for further details).](image)
GET and CLE > GET, no significant differences were observed, between Before and After, for the $\tau_f$ of V$\dot{O}_2$ and HR kinetics, as well as for the MRT$_f$ of $\Delta$[deoxy(Hb+Mb)] kinetics. The $\tau_f$ values of V$\dot{O}_2$ kinetics were not significantly different during CLE < GET and CLE > GET, both in Before and in After. During CLE > GET, $A'_s/A_{tot}$ for V$\dot{O}_2$ kinetics was $17.8 \pm 8.8\%$ in Before vs. $11.7 \pm 4.4\%$ in After (no significant difference). Also for HR kinetics, no significant differences in $A'_s/A_{tot}$ were observed between Before and After. As for $\Delta$[deoxy(Hb+Mb)] kinetics, its time course confirms previous studies by our group (16), as well as by others (8). In CLE < GET, at the transition the variable kept constant for $\sim 10$ s (TD$_f$).
The O2 cost of cycling related to the efficiency of oxidative metabolism, and the fraction of that power that can respectively, the maximal power that can be sustained by oxidative metabolism during cycle ergometer exercise, by evaluating a comprehensive functional evaluation of skeletal muscle hypertrophy (fast-to-slow shift in MHC and endurance (fast-to-slow shift in MHC and metabolic profile. As deriving from the changes described in vitro by Gondin et al. (12), the increased CSA could impair peripheral O2 diffusion and O2 utilization, and then rapidly increased (suggesting an increased fractional O2 extraction), according to a monoeXponential function characterized by a τ of 5.4 ± 1.6 s in Before and 6.3 ± 0.8 s in After (no significant difference), to reach a steady-state value in ~20 s. For CLE > GET, a slow component ensued after ~2–3 min of exercise, whose amplitude, expressed as a percentage of the total amplitude of the response (A/A0 tot), was 11.5 ± 6.0% in Before and 12.2 ± 7.5% in After (no significant difference).

DISCUSSION

We hypothesized that the enhanced oxidative function observed in vitro in skeletal muscle fibers of subjects undergoing a NMES training program (12) would be associated, in the same subjects, with an enhanced oxidative function evaluated in vivo during exercise. Together with the increased muscle force (12), an enhanced oxidative function in vivo could significantly increase the exercise tolerance of the treated subjects, and, if confirmed also in other populations, could make NMES well suited to treat conditions of disuse/immobilization, or other pathological conditions characterized by an impaired muscle function. Rather surprisingly, however, the hypothesis was not confirmed by the obtained results, which demonstrated no significant changes of the investigated variables.

More specifically, following NMES training, Gondin et al. (12) described, in a group of untrained or moderately trained, healthy, young human male subjects, the following: increases in maximum voluntary force and muscle fibers hypertrophy, a fast-to-slow transition of muscle fiber types, a fast-to-slow phenotype shift in the expression of myofibrillar proteins, and a glycolytic-to-oxidative shift in the metabolic profile. As pointed out by the authors (12), these effects appear of interest, since they are typical of both resistance (strength gains and hypertrophy) and endurance (fast-to-slow shift in MHC and metabolic profile) training. In the present study, we performed a comprehensive functional evaluation of skeletal muscle oxidative metabolism during cycle ergometer exercise, by evaluating the following variables: 1) \( V'\text{O}_2\text{peak and GET} \) represent, respectively, the maximal power that can be sustained by oxidative metabolism, and the fraction of that power that can be sustained for relatively long periods of time (see, e.g., 47). 2) The O2 cost of cycling related to the efficiency of oxidative metabolism, during both IE to voluntary exhaustion and moderate-intensity and heavy-intensity constant work rate exercises. 3) The kinetics of adjustment of pulmonary \( \text{VO}_2 \) during moderate-intensity constant work rate exercise considered a functional evaluation tool specifically aimed at skeletal muscle oxidative metabolism (see e.g., Ref. 48). 4) For the “slow component” of pulmonary \( \text{VO}_2 \) kinetics during heavy-intensity constant work rate exercise, the presence and the amplitude of the slow component are considered to reflect a loss of “metabolic stability” in the working muscles and appear to be directly associated with muscle fatigue (see e.g., Refs. 20, 50). 5) The maximal capacity of fractional O2 extraction by skeletal muscle during exercise, as evaluated by NIRS (see e.g., Refs. 14, 24, 37). 6) The matching of O2 delivery and O2 utilization in skeletal muscles during incremental and constant work rate exercises, as evaluated by NIRS (see e.g., Refs. 24, 37). All variables were substantially unaffected by NMES, which, therefore, contrary to our hypothesis, did not enhance skeletal muscle oxidative metabolism.

In the light of the results reported by Gondin et al. (12), the absence of improvements for the above-mentioned functional evaluation variables appears puzzling. Some hypotheses can be forwarded to explain the apparent discrepancy. As a first possibility, the fast-to-slow skeletal muscle changes observed in vitro by Gondin et al. could be too small to induce a significant enhancement of skeletal muscle oxidative function in vivo. Alternatively, any enhancement of oxidative function deriving from the changes described in vitro by Gondin et al. could be counterbalanced, in vivo, by an impairment of peripheral O2 diffusion, attributable to the skeletal muscle fiber hypertrophy induced by NMES. Gondin et al. performed, in four of the subjects, a morphometric analysis of tissue obtained by muscle biopsy and measured the cross-sectional area (CSA) of individual fibers, observing 12% (for type 1 fibers) to 23% (for type 2A fibers) increases. If not associated with a proportionate increase in capillarization (this variable was not determined by Gondin et al., and this represents a limitation of our study), the increased CSA could impair peripheral O2 diffusion (23, 49), thereby possibly counterbalancing, in vivo, the fast-to-slow phenotypic changes observed in vitro.

Whereas some studies have investigated the capillarization of skeletal muscle fibers after resistance training (see e.g., Refs. 5, 18, 44), to the best of our knowledge only one study (36) has reported data on muscle fiber capillarization following NMES. These authors reported increases of oxidative capacity and mean number of capillaries for fast-twitch fibers, but in the presence of minimal muscle fiber hypertrophy. Thus a direct

<table>
<thead>
<tr>
<th>( \Delta[\text{Deoxy(Hb+Mb)}] ) Kinetics</th>
<th>HR Kinetics</th>
<th>( \Delta[\text{Vo2peak and GET}] ) Kinetics</th>
<th>V\text{O}2 Kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRTI, s</td>
<td>TD1, s</td>
<td>( \tau ), s</td>
<td>A/A0 tot, %</td>
</tr>
<tr>
<td>&lt; GET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>47.4 ± 17.9</td>
<td>12.7 ± 5.1</td>
<td>34.7 ± 17.9</td>
</tr>
<tr>
<td>After</td>
<td>51.9 ± 11.3</td>
<td>19.6 ± 3.9</td>
<td>32.3 ± 11.4</td>
</tr>
<tr>
<td>&gt; GET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>54.1 ± 8.9</td>
<td>16.7 ± 5.7</td>
<td>37.4 ± 10.2</td>
</tr>
<tr>
<td>After</td>
<td>52.8 ± 5.9</td>
<td>19.9 ± 5.5</td>
<td>32.9 ± 9.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. MRT, mean response time; TD, time delay; \( \tau \), time constant (f indicates that these parameters relate to the fundamental component of the kinetics). \( A_t \), total amplitude; \( A_r \), actual amplitude of the slow component of the kinetics. See text for further details.

\[ A/A0 _{tot,\%} = \frac{A/A0 _{tot}}{A/A0 _{tot,\%}} \]
comparison of the results by Pérez et al. (36) with those of the present study, in which a significant increase in fiber CSA was observed, is not possible. As for voluntary resistance exercise, contradictory results are found in the literature. Tesch (44) described, in weight and power lifters, similar number of capillaries per fiber compared with controls; considering the marked muscle fiber hypertrophy observed in the weight and power lifters, this translated into a significantly lower number of capillaries per unit of CSA (“capillary density”). The concept that resistance training often decreases capillary density, as a result of a fiber hypertrophy not associated with proportional angiogenesis, was put forward also in the recent review by Egginton (7). On the other hand, according to other studies (see e.g., Refs. 4, 29), fiber hypertrophy obtained by resistance exercise is associated with a proportional increase in the number of capillaries per fiber, with the net result being a substantially unchanged capillary density. As mentioned above, no similar data are available for muscle hypertrophy deriving from NMES training. Another limitation of the present study is that no (invasive) measurements of peripheral O2 diffusion (see e.g., Ref. 38) were performed.

The glass could be also be seen as being “half full” instead of “half empty”: despite the impairment of peripheral O2 diffusion deriving from muscle fibers hypertrophy, the fast-to-slow transition in the metabolic profile of fibers could allow oxidative function in vivo to be functionally unaffected. This concept would be in agreement with the unchanged skeletal oxidative function in vivo to be functionally unaffected. This slow transition in the metabolic profile of fibers could allow diffusion deriving from muscle fibers hypertrophy, the fast-to-slow transition of the metabolic profile of the fibers, did not affect, in vivo, several variables of functional evaluation of oxidative metabolism. Fiber hypertrophy could impair peripheral O2 diffusion, thereby possibly counterbalancing, in vivo, the fast-to-slow phenotypic changes observed in vitro. NMES can increase muscle strength without negatively affecting oxidative metabolism.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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