



HAL
open science

Relationships between maximal muscle oxidative capacity and blood lactate removal after supramaximal exercise and fatigue indexes in humans

Claire Thomas, Pascal Sirvent, Stéphane Perrey, E. Raynaud, Jacques Mercier

► **To cite this version:**

Claire Thomas, Pascal Sirvent, Stéphane Perrey, E. Raynaud, Jacques Mercier. Relationships between maximal muscle oxidative capacity and blood lactate removal after supramaximal exercise and fatigue indexes in humans. *Journal of Applied Physiology*, 2004, 97 (6), pp.2132 - 2138. 10.1152/japphysiol.00387.2004 . hal-01587415

HAL Id: hal-01587415

<https://insep.hal.science/hal-01587415v1>

Submitted on 14 Sep 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Relationships between maximal muscle oxidative capacity and blood lactate removal after supramaximal exercise and fatigue indexes in humans

C. Thomas,¹ P. Sirvent,¹ S. Perrey,² E. Raynaud,¹ and J. Mercier¹

¹Laboratoire de Physiologie des Interactions (EA 701), Département de Physiologie, Institut de Biologie, 34060 Montpellier; and ²Efficiencia et Déficiencia Motrice (EA 2991), 34090 Montpellier, France

The present study investigated whether blood lactate removal after supramaximal exercise and fatigue indexes measured during continuous and intermittent supramaximal exercises are related to the maximal muscle oxidative capacity in humans with different training status. Lactate recovery curves were obtained after a 1-min all-out exercise. A biexponential time function was then used to determine the velocity constant of the slow phase (γ_2), which denoted the blood lactate removal ability. Fatigue indexes were calculated during all-out (FI_{AO}) and repeated 10-s cycling sprints (FI_{Sprint}). Biopsies were taken from the vastus lateralis muscle, and maximal ADP-stimulated mitochondrial respiration (V_{max}) was evaluated in an oxygraph cell on saponin-permeabilized muscle fibers with pyruvate + malate and glutamate + malate as substrates. Significant relationships were found between γ_2 and pyruvate + malate V_{max} ($r = 0.60$, $P < 0.05$), γ_2 and glutamate + malate V_{max} ($r = 0.66$, $P < 0.01$), and γ_2 and citrate synthase activity ($r = 0.76$, $P < 0.01$). In addition, γ_2 , glutamate + malate V_{max} , and pyruvate + malate V_{max} were related to FI_{AO} ($\gamma_2 - FI_{AO}$: $r = 0.85$; $P < 0.01$; glutamate + malate $V_{max} - FI_{AO}$: $r = 0.70$, $P < 0.01$; and pyruvate + malate $V_{max} - FI_{AO}$: $r = 0.63$, $P < 0.01$) and FI_{Sprint} ($\gamma_2 - FI_{Sprint}$: $r = 0.74$, $P < 0.01$; glutamate + malate $V_{max} - FI_{Sprint}$: $r = 0.64$, $P < 0.01$; and pyruvate + malate $V_{max} - FI_{Sprint}$: $r = 0.46$, $P < 0.01$). In conclusion, these results suggested that the maximal muscle oxidative capacity was related to blood lactate removal ability after a 1-min all-out test. Moreover, maximal muscle oxidative capacity and blood lactate removal ability were associated with the delay in the fatigue observed during continuous and intermittent supramaximal exercises in well-trained subjects.

lactate kinetics; biexponential mathematical model; all-out exercise; saponin-permeabilized muscle fibers

THE TIME COURSE OF ARTERIAL blood lactate concentration during recovery from muscular exercise can be described by a sum of two exponential functions (12, 15, 16). The velocity constants of these functions are indicators of the blood lactate recovery kinetics, because they specifically reflect the abilities to exchange lactate (high-velocity constant γ_1) between the previously worked muscle and the blood, and to remove it (low-velocity constant γ_2) from the total lactate diffusion space (15, 16). The use of this mathematical model, in contrast to isotope tracers, has an advantage because it can be applied to non-steady-state, supramaximal conditions.

The low-velocity constant γ_2 has been reported to be higher in trained individuals than in the untrained, after both supramaximal exercise (14, 26) and graded exercise up to exhaustion (13). Isotope tracer studies on rats indicated that previous endurance training increases lactate clearance during steady-state exercise (8) and resting hyperlactatemia (9, 10). Subsequent studies on humans showed that training reduced blood lactate at given levels of submaximal exercise by improving the capacity for lactate clearance (22, 23, 36).

It has been established that oxidation is the major metabolic pathway for lactate disposal during rest, sustained and submaximal exercises, and recovery (3, 7, 33). As well, significant lactate is removed through gluconeogenesis in rats (4) and men (2). In addition, muscle oxidative capacity has been shown to be significantly improved by endurance training (8, 11). However, during supramaximal exercise, the major pathways for ATP resynthesis are the breakdown of creatine phosphate and the degradation of muscle glycogen to lactic acid (i.e., lactate anions and protons) (34).

Requested bouts of high-intensity exercises (20) and supramaximal exercise (38) result in profound metabolic acidosis. In light of these studies, as lactate anions and associated protons are transported and disposed of together, and because oxidation is the major pathway of lactate disposal, we hypothesized that the maximal oxidative capacity of skeletal muscle (i.e., mitochondria respiratory capacity) is involved in blood lactate removal after supramaximal exercise. We further posited that the removal of lactate and protons from skeletal muscle would be important to maintain force and prevent fatigue during different types of supramaximal exercise. To test these hypotheses, we determined the velocity constants of net blood lactate removal during recovery from a supramaximal exercise. We then related these constants to the maximal muscle oxidative capacity by using saponin-skinned muscle fibers from subjects with different training status. We also quantified the fatigue developed during a 1-min all-out test and during repeated 10-s cycling sprints.

MATERIALS AND METHODS

Subjects

Seventeen male volunteers [(mean \pm SE) age: 26.2 \pm 1.4 yr, height: 178.9 \pm 1.4 cm, body mass: 74.5 \pm 2.5 kg] participated in this study. Inclusion criteria were <25% body fat, nonsmoker status, stable diet and weight, and injury- and disease-free status as determined by physical examination. Informed consent was obtained from all subjects after the nature and risks involved in study participation

Address for reprint requests and other correspondence: C. Thomas, Laboratoire de Physiologie des Interactions (EA 701), Département de Physiologie, Institut de Biologie, Bvd Henri IV, 34060 Montpellier Cedex 2, France (E-mail: thomasclaire@wanadoo.fr).

were explained. The study was approved by the local Ethics Committee and conformed to the Declaration of Helsinki regarding the use of human subjects.

Subjects with different training status were deliberately targeted for this study. The group consisted of untrained subjects ($n = 5$), recreational athletes ($n = 4$), and middle-distance (MD, $n = 4$) and long-distance (LD, $n = 4$) runners. These subgroups were determined from the subjects' responses to a physical activity questionnaire and their maximal oxygen uptake ($\dot{V}O_{2\max}$) values. The untrained subjects had a normal level of physical activity without any athletic training, whereas the recreational athletes trained two to three times per week but did not compete. The MD runners competed in the 800- and 1,500-m events and trained five to six times per week. Their training included frequent high-intensity sessions alternating with endurance sessions. The LD runners competed in events of >5 km. They trained 8–10 times per week and ran an average of 110 km per week, including high-intensity workouts with 400- to 3,000-m intervals and continuous running (10–25 km).

Experimental Design

All subjects came to the laboratory for the three exercise sessions, as well as for the skeletal muscle biopsy. At least 48 h separated the exercise sessions, and the muscle biopsy was then performed 1 wk later. The subjects performed all exercise tests at a laboratory temperature of 22°C. An initial laboratory visit was scheduled to obtain data on physical characteristics and individual $\dot{V}O_{2\max}$. During the second visit, the subjects were familiarized with the testing procedure on the cycle ergometer. In the last session, they performed a 1-min all-out exercise followed by 60 min of recovery, and then performed 10 short cycling sprints separated by 30-s recovery intervals. Each subject was instructed to refrain from intense physical exercise for 48 h before this third visit.

Assessment of $\dot{V}O_{2\max}$

At the beginning of the study, all subjects underwent an incremental maximal exercise test on a treadmill (LE 200 CE Jaegger, Hoechberg, Germany). During the test, oxygen uptake, carbon dioxide production, and minute ventilation were measured breath by breath by means of an open-circuit metabolic cart (Oxycon Pro, Jaegger). The exercise test increments were designed to exhaust the subject within 10–15 min. Each stage consisted of a 2-min exercise period and increased by 1 km/h.

Supramaximal Exercises

The subjects performed all exercise tests on a standard friction-loaded cycle ergometer (Monark type 818 E, Stockholm, Sweden) specifically equipped with both a strain gauge (Interface MFG type, Scottsdale, AZ) and an optical encoder (Hengstler type RIS IP50, Aldingen, Germany) (for details, see Ref. 1). The strain gauge and the optical encoder measured the friction force applied to the belt and the flywheel displacement, respectively. The flywheel velocity was determined by using a first-order derivation of the flywheel displacement, and the power output was calculated from the product of total force and flywheel velocity. The values of velocity, force, and power output were collected at 50 Hz and sent to a personal computer for subsequent analysis. For all tests, subjects were in the seated position during exercise and in the supine position on a bed during rest and recovery. All tests started with the front pedal crank at $\sim 45^\circ$ to the horizontal to facilitate the best starting push. All tests were performed between 8 and 10 AM after an overnight fast.

Session I. The first test on the cycle ergometer, a force-velocity test, consisted of the repetition of short maximal sprints using different braking forces after a 5-min warm-up. The duration of each sprint was fixed at 8 s, the time it took for a vigorously motivated subject to attain his maximal velocity as rapidly as possible after the starting signal.

All of the subjects started the test randomly against friction loads corresponding to 0.4, 0.6, and 0.8 N/kg of body mass. Each sprint was separated by at least 5 min of rest. At the signal, the subjects were told to remain on the saddle and to pedal as fast as possible to reach maximal pedaling rate. Each subject was verbally encouraged throughout each sprint. These sprints allowed us to familiarize the subjects with the cycle ergometer and to calculate the optimal friction load against which they would perform during the second session. In fact, the velocity, force, and power values (averaged per half-pedal revolution) recorded during the acceleration phase of the three sprints were used to draw the individual force- and power-velocity relationships (1). The optimal values of friction force and velocity at which the highest power output was reached were determined from these relationships.

Session II. The all-out test consisted of 1 min of supramaximal all-out cycling against the optimal load determined during the force-velocity test. Before this test, subjects warmed up for 10 min on the cycle ergometer and then rested for 5 min. They were then instructed to pedal as fast as possible from the start of exercise and were verbally encouraged to maintain maximal pedaling speed throughout the test. We generally informed subjects every 15 s up to 45 s and then counted down to the end of the test every 5 s. All subjects recovered in supine position for 1 h. The mean, mean per kilogram, peak, and end power outputs were determined, and the fatigue index of the all-out test (FI_{AO}) for power output was calculated as the percentage of decline from peak to end values for each subject.

The subjects then performed a 6-min warm-up on the cycle ergometer at a moderate power output. After 5 min of rest, the subjects performed 10 consecutive 10-s sprints separated by 30-s recovery intervals against a friction load corresponding to 50% of the optimal value of the friction force for each subject (31). The fatigue index of the sprints (FI_{Sprint}) for peak power output was calculated as the percentage of decline from the first sprint to the tenth sprint (31).

Blood Samples

Blood samples were drawn from a 32-mm catheter placed into a superficial forearm vein. The venous blood samples, used for determination of plasma lactate, were collected in tubes containing heparin: at rest; just after the warm-up; and at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 30, 40, 50, and 60 min of recovery from the all-out test.

Blood lactate. Blood lactate content was analyzed enzymatically according to the spectrophotometric method of Gutmann and Wahlefeld (19). Briefly, a 200- μ l aliquot of blood sample was immediately mixed with 800 μ l of ice-cold 7% perchloric acid and centrifuged at 1,500 g for 10 min at 4°C. The supernatant was stored at -80°C. For the analysis, an aliquot of the resulting supernatant was mixed with 2 N NaOH. The reaction began with the addition of NAD and lactate dehydrogenase in glycine-hydrazine buffer. The reaction followed the formation of $NADH_2$ at 340 nm.

Lactate kinetics analysis. Venous blood lactate was collected during the recovery periods following the supramaximal exercise test, and each individual curve was fitted by using the following biexponential equation (12, 16): $[La(t)]_v = [La(0)] + A_1(1 - e^{-Y_1 t}) + A_2(1 - e^{-Y_2 t})$, where $[La(t)]_v$ and $[La(0)]$ are the measured lactate concentrations in venous blood at time t after the end of exercise and at the beginning of the recovery, respectively; A_1 and A_2 (in millimoles per liter) are the amplitudes of the two exponential components; and Y_1 and Y_2 (per minute) are their respective velocity constants.

The individual parameters of the biexponential function were fitted by means of an iterative nonlinear technique, using DataFit 6.0 software to determine the values of A_1 , A_2 , Y_1 , and Y_2 . The percentage of the variance explained by the use of the biexponential curve fit was determined by correlation of the observed and the predicted $[La(t)]_v$ at each time and by squaring of the Pearson product correlation coefficient.

Table 1. Values of mechanical power output and the fatigue index during the 1-min all-out test

$\dot{W}_{\text{Mean AO, W}}$	452.5+13.4
$\dot{W}_{\text{Mean AO/kg, W/kg}}$	6.1+0.3
$\dot{W}_{\text{max AO, W}}$	1,207.3+49.3
$\dot{W}_{\text{End AO, W}}$	350.9+21.8
FI _{AO, %}	69.0+2.5

Values are means + SE; $n = 16$ subjects. $\dot{W}_{\text{Mean AO}}$, $\dot{W}_{\text{Mean AO/kg}}$, $\dot{W}_{\text{max AO}}$, and $\dot{W}_{\text{End AO}}$ are mean, mean per kilogram, peak, and end power outputs, respectively. FI_{AO} is the fatigue index.

Skeletal Muscle Biopsy

Vastus lateralis muscle biopsies were taken by the percutaneous Bergström technique after local anesthesia (Xylocaine) 1 wk after all cycling exercise tests. No complications were noted following the biopsies. The muscle samples were divided into two portions. One portion was immediately frozen in liquid nitrogen and stored at -80°C until enzymatic determination. The other portion was used for the in situ respiration studies and was immediately placed in an ice-cold relaxing solution [at ionic strength 160 (potassium methanesulfonate), pH 7.1] containing (in mM) 10 EGTA-calcium buffer (free Ca²⁺ concentration: 100 nmol/l), 20 imidazole, 3 KH₂PO₄, 1 MgCl₂, 20 taurine, 0.5 DTT, 5 MgATP, and 15 phosphocreatine.

The fiber bundles were separated with sharp-ended needles, leaving only small areas of contact, and were incubated in 1 ml of the above solution (4°C) containing 50 µg/ml saponin for 30 min with continuous stirring. To completely remove the saponin, the fibers were washed with continuous stirring with relaxing solution for 10 min (4°C); to remove free ATP, they were then washed with oxygraph solution for 2 X 5 min (4°C). This was of the same composition as the relaxing solution, except that MgATP and phosphocreatine were replaced by 2 mM malate, 3 mM phosphate, and 2 mM fatty acid-free bovine serum albumin (pH 7.1). After washing, the fibers were stored on ice in oxygraph solution until determination of mitochondrial respiration activity.

Muscle Analysis

Mitochondrial respiration. The respiratory parameters of the total mitochondrial population were studied in situ, as previously described (25, 37), using a Clark electrode (Strathkelvin Instruments, Glasgow, UK). Measurements were carried out at 30°C with continuous stirring in 3 ml of the oxygraph solution with different respiratory substrates (in mM): either 5 glutamate + 2 malate or 10 pyruvate + 2 malate. ADP-stimulated respiration above basal oxygen consumption was measured by stepwise addition of ADP (2.5–2,000 µM). At the end of measurement, we used the cytochrome *c* test to investigate the state of the outer mitochondrial membrane (32). After the following respiratory measurements, the fiber bundles were removed, dried overnight, and weighed the next day. Respiration rates were expressed in micromoles of O₂ per minute per gram of dry weight.

Maximal ADP-stimulated respiration (V_{max}) for each substrate was calculated by using a nonlinear monoexponential fitting of the

Table 2. Values of peak power output for sprints 1 and 10 and the fatigue index during the repeated short sprints

$\dot{W}_{\text{max Sprint 1, W}}$	1,283.3+45.4
$\dot{W}_{\text{max Sprint 10, W}}$	1,034.2+32.2
FI _{Max Sprint, %}	18.9+2.3

Values are means + SE; $n = 17$ subjects. $\dot{W}_{\text{max Sprint 1}}$ and $\dot{W}_{\text{max Sprint 10}}$ are the maximal power output during the 1st and the 10th sprints. FI_{Max Sprint} is the fatigue index.

Table 3. Parameters of the biexponential curves fitted to venous blood lactate recovery curves obtained from 1-min supramaximal cycling exercise

	[La(0)], mmol/l	A ₁ , mmol/l	'Y ₁ , min ⁻¹	A ₂ , mmol/l	'Y ₂ , min ⁻¹
Mean + SE	5.2+0.6	19.0+6.5	0.26+0.03	-27.0+6.2	0.033+0.005

Values are means + SE. A₁, 'Y₁, A₂, and 'Y₂ refer to the definition of Eq. 1 in lactate kinetics analysis. [La(0)] is the venous blood lactate concentration at the end of exercise.

Michaelis-Menten equation with DataFit 6.0 software. The acceptor control ratio was calculated as $V_{\text{max}}/\text{basal oxygen consumption}$.

Citrate synthase analysis. Homogenates for citrate synthase (CS) were prepared in buffer (in mM), 210 sucrose, 2 EGTA, 40 NaCl, 30 HEPES, 5 EDTA, and 2 phenylmethylsulfonyl fluoride (pH 7.4), and stored at -80°C. CS activity was assayed by a spectrophotometric method according to Ref. 35. Changes in absorbance were recorded over 3 min at 412 nm at 25°C.

Statistics

Descriptive statistics are expressed as means + SE. Individual relationships between variables were studied by means of linear regressions using Statview software. The level of significance was set at $P < 0.05$.

RESULTS

Establishment of the Subgroups

$\dot{V}_{\text{O}_2\text{max}}$ was used in addition to the physical activity questionnaire to subdivide the subjects into four subgroups. $\dot{V}_{\text{O}_2\text{max}}$ were 53.6 + 4.3 ml·min⁻¹·kg⁻¹ for the untrained subjects, 55.6 + 3 ml·min⁻¹·kg⁻¹ for the recreational subjects, 64.5 + 1.1 ml·min⁻¹·kg⁻¹ for the MD runners, and 66.9 + 1.9 ml·min⁻¹·kg⁻¹ for the LD runners.

Mechanical Performance

The values for peak power output and mean power output during the 1-min all-out test and the 10 short repeated sprints are given in Tables 1 and 2. During the continuous and intermittent supramaximal exercises, maximal power output decreased significantly by 69.0 + 2.5% ($P < 0.01$) and 18.9 + 2.3% ($P < 0.01$), respectively.

Peak Blood Lactate Concentrations Measured During 1-min All-Out Exercise Recovery

Peak blood lactate concentrations measured during 1-min all-out exercise recovery were 12.9 + 0.8 mmol/l for the untrained subjects, 14.1 + 1.2 mmol/l for the recreational

Table 4. Maximal respiratory rate with pyruvate + malate and, glutamate + malate in saponin-permeabilized muscle fibers and citrate synthase activity

	V_{max} Pyruvate + Malate, µmol O ₂ ·min ⁻¹ ·g dry wt ⁻¹	V_{max} Glutamate + Malate, µmol O ₂ ·min ⁻¹ ·g dry wt ⁻¹	Citrate Synthase Activity, µmol O ₂ ·min ⁻¹ ·mg protein ⁻¹
Mean + SE	7.4+0.7	5.9+0.5	16.7+0.4

Values are means + SE. V_{max} , maximal ADP-stimulated respiration.

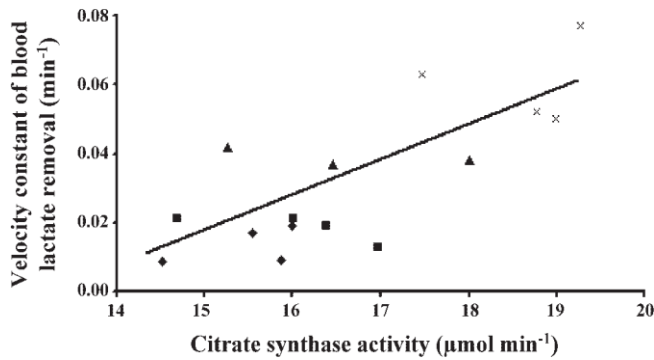


Fig. 1. Relationship between the velocity constant of blood lactate removal \dot{Y}_2 after the 1-min supramaximal all-out (AO) test and the citrate synthase activity ($r = 0.76$, $P < 0.01$, $n = 15$). Δ , Untrained subjects; \blacksquare , recreational athletes; \circ , middle-distance runners; \times , long-distance runners.

subjects, 13.8 ± 1.5 mmol/l for the MD runners, and 9.7 ± 0.7 mmol/l for the LD runners.

Blood Lactate Recovery Curves

The time course of blood lactate concentration during recovery showed the classic biphasic evolution pattern for all subjects. From the end of exercise, blood lactate concentration increased, reached a peak, and thereafter decreased progressively. The biexponential model accurately fitted the individual recovery curves obtained after the 1-min all-out exercise. The

mean values of the parameters of the fit of the biexponential equation to the lactate recovery curves are reported in Table 3. In most cases, the biexponential equation accounted for $>98\%$ of the variance in the lactate recovery curves. In addition, the fit accuracy for all subjects was comparable to that obtained previously (15, 26).

Mitochondrial Function

The mean basal respiration rates of the skeletal muscle mitochondria for each of the substrates, pyruvate + malate and glutamate + malate, were 1.8 ± 0.3 and 1.7 ± 0.2 $\mu\text{mol O}_2 \cdot \text{min}^{-1} \cdot \text{g dry weight}^{-1}$, respectively. Table 4 shows the mean maximal muscle oxidative capacity measured with pyruvate + malate and glutamate + malate. The acceptor control ratio, representing the degree of coupling between oxidation and phosphorylation, was, on average, 5.9 ± 1.5 for mitochondrial respiration with pyruvate + malate substrates and 7.5 ± 3.1 for mitochondrial respiration with glutamate + malate substrates. There was no change in maximal respiration after cytochrome *c* addition. CS activity is displayed in Table 4.

Relationships Between Variables

To distinguish the subjects with different training status, in the relationships, we identify the subject with different symbols in Figs. 1–5. As shown in Fig. 1, the velocity constant of net blood lactate removal (i.e., \dot{Y}_2) was positively and signifi-

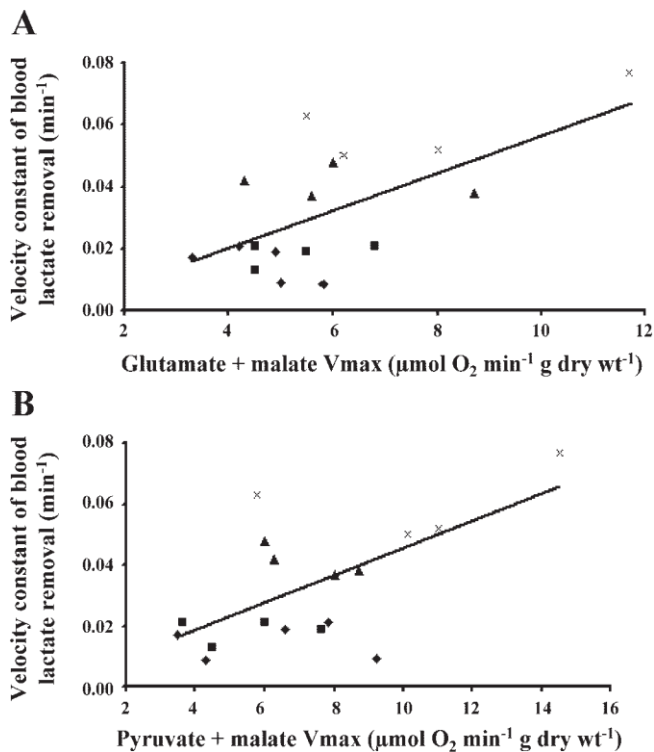


Fig. 2. Relationships between the velocity constant of blood lactate removal \dot{Y}_2 after the 1-min supramaximal AO test and the glutamate + malate maximal ADP-stimulated respiration (V_{max}) ($r = 0.66$, $P < 0.01$, $n = 17$; A) and the pyruvate + malate V_{max} ($r = 0.60$, $P < 0.05$, $n = 16$; B). Glutamate + malate V_{max} and pyruvate + malate V_{max} (expressed in $\mu\text{mol O}_2 \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$) are the V_{max} of the muscular fibers with glutamate + malate and pyruvate + malate as substrates. Symbols are as defined in Fig. 1 legend.

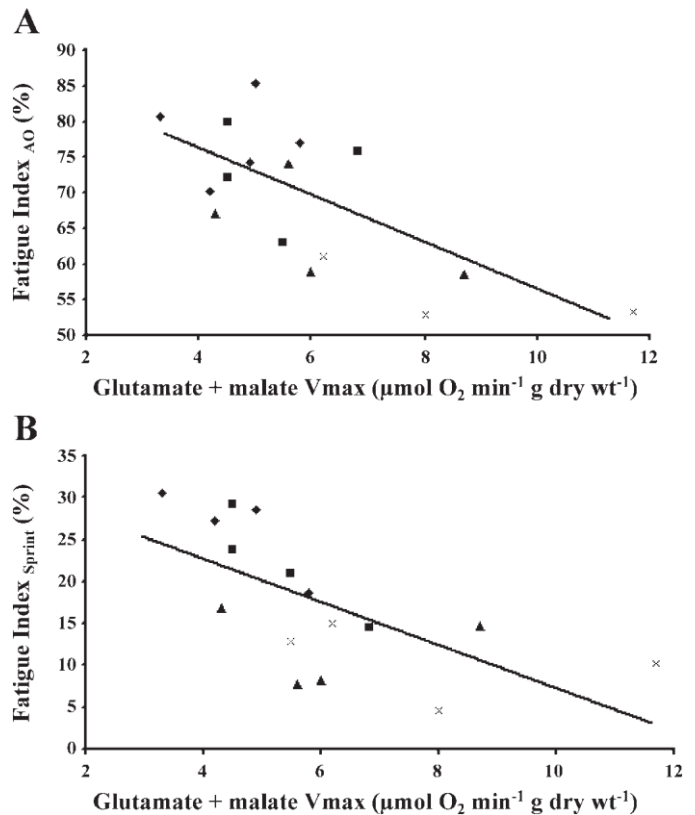


Fig. 3. Relationships between the fatigue index during the 1-min supramaximal AO exercise test and the glutamate + malate V_{max} ($r = 0.70$, $P < 0.01$, $n = 16$; A) and the fatigue index during the 10 short cycling sprints and the glutamate + malate V_{max} ($r = 0.64$, $P < 0.01$, $n = 16$; B). Symbols are as defined in Fig. 1 legend.

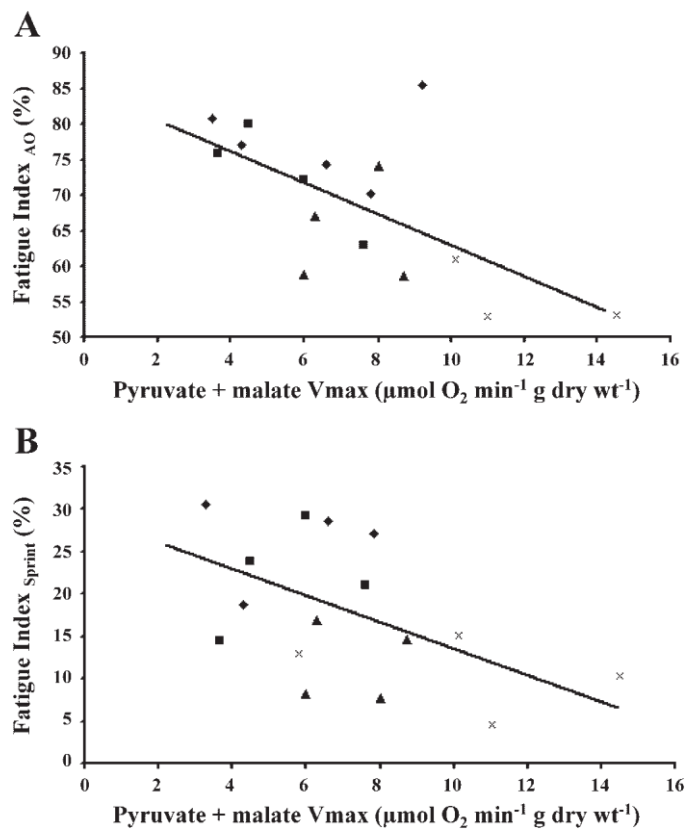


Fig. 4. Relationships between the fatigue index during the 1-min supramaximal AO exercise test and the pyruvate + malate V_{\max} ($r = 0.63$, $P < 0.01$, $n = 16$; A) and the fatigue index during the 10 short cycling sprints and the pyruvate + malate V_{\max} ($r = 0.46$, $P < 0.01$, $n = 16$; B). Symbols are as defined in Fig. 1.

cantly correlated with the CS activity ($r = 0.76$, $P < 0.01$, $n = 15$) and with the V_{\max} of the muscular fiber with glutamate + malate (Fig. 2A: $r = 0.66$, $P < 0.01$, $n = 17$) or pyruvate + malate (Fig. 2B: $r = 0.60$, $P < 0.05$, $n = 16$). Moreover, a significant correlation ($r = 0.68$, $P < 0.01$) was found between 1Y_2 and $\dot{V}O_{2\max}$.

The glutamate + malate V_{\max} was correlated with FI_{AO} (Fig. 3A: $r = 0.70$, $P < 0.01$) and FI_{Sprint} (Fig. 3B: $r = 0.64$, $P < 0.01$). The pyruvate + malate V_{\max} was also correlated with FI_{AO} (Fig. 4A: $r = 0.63$, $P < 0.01$) and FI_{Sprint} (Fig. 4B: $r = 1.46$, $P < 0.01$). In addition, as can be seen in Fig. 5, A and B, respectively, 1Y_2 was correlated to FI_{AO} ($r = 0.85$, $P < 0.01$, $n = 16$) and FI_{Sprint} ($r = 0.74$, $P < 0.01$, $n = 16$).

As shown in Figs. 3, 4, and 5, the LD and MD runners presented slower power output declines during both the 1-min all-out exercise and the 10 short cycling sprints compared with the untrained and recreational subjects.

DISCUSSION

The main finding of the present study was that the blood lactate removal ability (i.e., 1Y_2) after a 1-min all-out test was related to the maximal muscle oxidative capacity. To our knowledge, this is the first time that the biexponential mathematical model, proposed by Freund and Gendry (12) to describe lactate recovery kinetics, has been associated with a physiological state: the mitochondrial respiration measured in

situ in humans. In addition, the maximal muscle oxidative capacity and 1Y_2 were inversely related to the fatigue indexes measured during the 1-min all-out test and 10 successive cycling sprints.

Subjects with different training status were deliberately targeted for this study. To determine whether physiological responses are influenced by training status, we subdivided our population into four small groups (as shown in Figs. 1–5) based on their responses to a physical activity questionnaire and their $\dot{V}O_{2\max}$.

The lactate kinetics after supramaximal exercise were investigated by means of the biexponential model (12, 16, 28). Because of the limited information supplied by postexercise venous blood lactate kinetics (28), only the parameters of lactate disappearance were used for the analysis. As shown in Figs. 1, 2, and 5, we observed more efficient lactate removal in response to higher endurance training status in our human subjects. This result confirmed those of Refs. 14 and 27, which were also obtained after supramaximal exercise.

The most interesting finding was that training status influenced the relationship between 1Y_2 and the maximal muscle oxidative capacity (Fig. 2). One of the main adaptations of skeletal muscle in response to endurance training is improved oxidative capacity (8, 11). In this study, the maximal muscle oxidative capacity was measured in situ with different types of substrates and showed a difference in response to the subjects' training status. Because the authors of several studies used

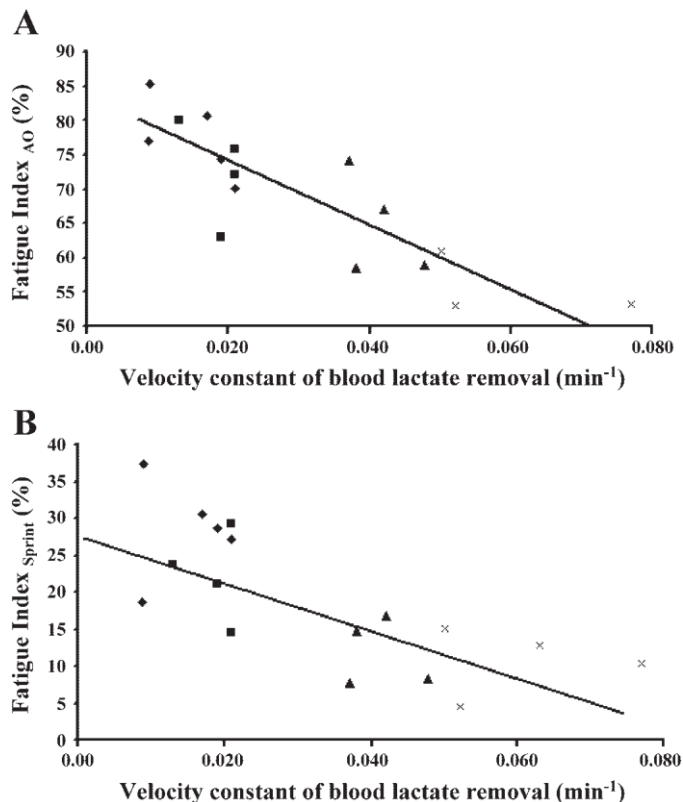


Fig. 5. Relationships between the fatigue index during the 1 min AO exercise test and the velocity constant of blood lactate removal 1Y_2 after the 1-min supramaximal AO test ($r = 0.85$, $P < 0.001$, $n = 16$; A) and the fatigue index during the 10 short cycling sprints and the rate of blood lactate removal 1Y_2 ($r = 0.74$, $P < 0.001$, $n = 17$; B). Symbols are as defined in Fig. 1.

glutamate + malate as the substrate to obtain the maximal oxidative capacity (25, 39), we also used it to permit comparison of our results with theirs. However, we also tested pyruvate + malate, because the first step in lactate disposal is oxidation to pyruvate, which is subsequently decarboxylated in the mitochondrial tricarboxylic acid cycle. As shown in Fig. 2, untrained and recreational subjects presented a trend for a lower maximal oxidative capacity determined with pyruvate + malate and glutamate + malate, compared with MD and LD runners. These results agree with those previously observed in humans with different levels of physical activity and with glutamate + malate as substrates (25, 39).

The biopsies were taken from the vastus lateralis because this muscle is a "prime mover" during leg cycling exercise. Stanley et al. (36) showed that the working skeletal muscle not only is a major site of blood lactate removal during exercise, but also appears to consume lactate during recovery (3, 4). Moreover, the faster removal during recovery in the well-trained subjects (MD and LD runners) compared with the untrained or little-trained (recreational) subjects could be explained by an increase in mitochondrial volume and density with training. This was indicated by the increase in the activity of muscle CS as a function of training status in the present study (Fig. 1) and several enzymes of oxidative metabolism (8, 11), as well as by the different mechanisms of respiratory control that vary in response to training status (39). Thus this result suggests the association between maximal muscle oxidative capacity and net blood lactate removal. However, we cannot rule out other potential factors, such as the level of lactate transporters in the sarcolemmal membrane (11) and capillary density (6), which increase with endurance training and may also be related to the blood lactate removal ability and the oxidative capacity. One might suggest the role of gluconeogenesis in lactate removal as well, but this represents only a minor fraction of lactate metabolism after exhausting exercise (4).

For the first time, the low-velocity constant of the biexponential model was found to be correlated with a cellular component. However, γ_2 was also related to the whole body $\dot{V}O_{2\max}$. This relationship, which confirmed results of McGrail et al. (24) and Oyono-Enguelle et al. (29), can be reasonably interpreted as being the consequence of concomitant respiratory, cardiovascular, and biochemical adaptations in the MD and LD runners induced by training, i.e., a high volume of endurance training, including exercise sessions close to $\dot{V}O_{2\max}$ intensity. In addition, Pilegaard et al. (30) have observed that the subjects exhibiting the highest lactate transport capacity were also those who displayed the highest $\dot{V}O_{2\max}$.

The fatigue indexes measured during the continuous and intermittent supramaximal exercise were related to the maximal oxidative capacity and γ_2 , as a function of endurance training status (Figs. 3, 4, and 5). The adaptations pointed out by Pilegaard et al. (30) no doubt allowed the highest trained subjects to obtain a higher energy fraction from oxidative metabolism during supramaximal exercises (5, 18), which may also have delayed fatigue. Indeed, some authors have shown that skeletal muscle acidosis impairs oxidative metabolism (17, 21), and reduced respiratory capacity may be one of the biggest factors contributing to the onset of fatigue during high-intensity exercise (17).

In conclusion, the present study demonstrated that the maximal muscle oxidative capacity investigated by the saponin-permeabilized muscle fiber technique was correlated with the velocity constant of net blood lactate removal after a 1-min all-out test and with the delayed fatigue measured during continuous and intermittent supramaximal all-out exercises.

ACKNOWLEDGMENTS

The authors thank Fabien Junius, Magali Bonnet, Marie-Chantal Granat, and Anne Levasseur for technical assistance.

GRANTS

The present study was supported by a Research Grant from Laboratoire Merck-MF Richelet (Paris, France).

REFERENCES

1. **Arsac LM, Belli A, and Lacour JR.** Muscle function during brief maximal exercise: accurate measurements on a friction-loaded cycle ergometer. *Eur J Appl Physiol Occup Physiol* 74: 100–106, 1996.
2. **Bergman BC, Horning MA, Casazza GA, Wolfel EE, Butterfield GE, and Brooks GA.** Endurance training increases gluconeogenesis during rest and exercise in men. *Am J Physiol Endocrinol Metab* 278: E244–E251, 2000.
3. **Brooks GA, Brauner KE, and Cassens RG.** Glycogen synthesis and metabolism of lactic acid after exercise. *Am J Physiol* 224: 1162–1166, 1973.
4. **Brooks GA and Gaesser GA.** End points of lactate and glucose metabolism after exhausting exercise. *J Appl Physiol* 49: 1057–1069, 1980.
5. **Calbet JA, De Paz JA, Garatachea N, Cabeza de Vaca S, and Chavarren J.** Anaerobic energy provision does not limit Wingate exercise performance in endurance-trained cyclists. *J Appl Physiol* 94: 668–676, 2003.
6. **Denis C, Chatard JC, Dormois D, Linossier MT, Geysant A, and Lacour JR.** Effects of endurance training on capillary supply of human skeletal muscle on two age groups (20 and 60 years). *J Physiol (Paris)* 81: 379–383, 1986.
7. **Depocas F, Minaire Y, and Chatonnet J.** Rates of formation and oxidation of lactic acid in dogs at rest and during moderate exercise. *Can J Physiol Pharmacol* 47: 603–610, 1969.
8. **Donovan CM and Brooks GA.** Endurance training affects lactate clearance, not lactate production. *Am J Physiol Endocrinol Metab* 244: E83–E92, 1983.
9. **Donovan CM and Pagliassotti MJ.** Endurance training enhances lactate clearance during hyperlactatemia. *Am J Physiol Endocrinol Metab* 257: E782–E789, 1989.
10. **Donovan CM and Pagliassotti MJ.** Enhanced efficiency of lactate removal after endurance training. *J Appl Physiol* 68: 1053–1058, 1990.
11. **Dubouchaud H, Butterfield GE, Wolfel EE, Bergman BC, and Brooks GA.** Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab* 278: E571–E579, 2000.
12. **Freund H and Gendry P.** Lactate kinetics after short strenuous exercise in man. *Eur J Appl Physiol Occup Physiol* 39: 123–135, 1978.
13. **Freund H, Lonsdorfer J, Oyono-Enguelle S, Lonsdorfer A, and Bogui P.** Lactate exchange and removal abilities in sickle cell patients and in untrained and trained healthy humans. *J Appl Physiol* 73: 2580–2587, 1992.
14. **Freund H and Oyono-Enguelle S.** [The effect of supramaximal exercise on the recovery kinetics of lactate.] *Schweiz Z Sportmed* 39: 65–76, 1991.
15. **Freund H, Oyono-Enguelle S, Heitz A, Marbach J, Ott C, Zouloumian P, and Lampert E.** Work rate-dependent lactate kinetics after exercise in humans. *J Appl Physiol* 61: 932–939, 1986.
16. **Freund H and Zouloumian P.** Lactate after exercise in man. I. Evolution kinetics in arterial blood. *Eur J Appl Physiol Occup Physiol* 46: 121–133, 1981.
17. **Gollnick PD, Bertocci LA, Kelso TB, Witt EH, and Hodgson DR.** The effect of high-intensity exercise on the respiratory capacity of skeletal muscle. *Pflügers Arch* 415: 407–413, 1990.

18. **Granier P, Mercier B, Mercier J, Anselme F, and Prefaut C.** Aerobic and anaerobic contribution to Wingate test performance in sprint and middle-distance runners. *Eur J Appl Physiol Occup Physiol* 70: 58–65, 1995.
19. **Gutmann I and Wahlefeld AM.** L-(+)-Lactate determination with lactate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis*. New York: Academic, 1974, p. 1464–1472.
20. **Hargreaves M, McKenna MJ, Jenkins DG, Warmington SA, Li JL, Snow RJ, and Febbraio MA.** Muscle metabolites and performance during high-intensity, intermittent exercise. *J Appl Physiol* 84: 1687–1691, 1998.
21. **Jubrias SA, Crowther GJ, Shankland EG, Gronka RK, and Conley KE.** Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. *J Physiol* 553: 589–599, 2003.
22. **MacRae HS, Dennis SC, Bosch AN, and Noakes TD.** Effects of training on lactate production and removal during progressive exercise in humans. *J Appl Physiol* 72: 1649–1656, 1992.
23. **Mazzeo RS, Brooks GA, Budinger TF, and Schoeller DA.** Pulse injection, ¹³C tracer studies of lactate metabolism in humans during rest and two levels of exercise. *Biomed Mass Spectrom* 9: 310–314, 1982.
24. **McGrail JC, Bonen A, and Belcastro AN.** Dependence of lactate removal on muscle metabolism in man. *Eur J Appl Physiol Occup Physiol* 39: 89–97, 1978.
25. **Mettauer B, Zoll J, Sanchez H, Lampert E, Ribera F, Veksler V, Bigard X, Mateo P, Epailly E, Lonsdorfer J, and Ventura-Clapier R.** Oxidative capacity of skeletal muscle in heart failure patients versus sedentary or active control subjects. *J Am Coll Cardiol* 38: 947–954, 2001.
26. **Oyono-Enguelle S and Freund H.** Ability to remove lactate in endurance-trained and -untrained humans. *J Appl Physiol* 72: 396–399, 1992.
27. **Oyono-Enguelle S, Freund H, Lonsdorfer J, and Pape A.** Impaired lactate exchange and removal abilities after supramaximal exercise in humans. *Med Sport Sci* 34: 140–161, 1992.
28. **Oyono-Enguelle S, Gartner M, Marbach J, Heitz A, Ott C, and Freund H.** Comparison of arterial and venous blood lactate kinetics after short exercise. *Int J Sports Med* 10: 16–24, 1989.
29. **Oyono-Enguelle S, Marbach J, Heitz A, Ott C, Gartner M, Pape A, Vollmer JC, and Freund H.** Lactate removal ability and graded exercise in humans. *J Appl Physiol* 68: 905–911, 1990.
30. **Pilegaard H, Bangsbo J, Richter EA, and Juel C.** Lactate transport studied in sarcolemmal giant vesicles from human muscle biopsies: relation to training status. *J Appl Physiol* 77: 1858–1862, 1994.
31. **Ratel S, Duche P, Hennegrave A, Van Praagh E, and Bedu M.** Acid-base balance during repeated cycling sprints in boys and men. *J Appl Physiol* 92: 479–485, 2002.
32. **Saks VA, Veksler VI, Kuznetsov AV, Kay L, Sikk P, Tiivel T, Tranqui L, Olivares J, Winkler K, Wiedemann F, and Kunz WS.** Permeabilized cell and skinned fiber techniques in studies of mitochondrial function in vivo. *Mol Cell Biochem* 184: 81–100, 1998.
33. **Searle GL and Cavalieri RR.** Determination of lactate kinetics in the human analysis of data from single injection vs. continuous infusion methods. *Proc Soc Exp Biol Med* 139: 1002–1006, 1972.
34. **Spriet LL, Lindinger MI, McKelvie RS, Heigenhauser GJ, and Jones NL.** Muscle glycogenolysis and H⁺ concentration during maximal intermittent cycling. *J Appl Physiol* 66: 8–13, 1989.
35. **Srere P.** Citrate synthase. *Methods Enzymol* 13: 3–5, 1969.
36. **Stanley WC, Gertz EW, Wisneski JA, Neese RA, Morris DL, and Brooks GA.** Lactate extraction during net lactate release in legs of humans during exercise. *J Appl Physiol* 60: 1116–1120, 1986.
37. **Veksler VI, Kuznetsov AV, Sharov VG, Kapelko VI, and Saks VA.** Mitochondrial respiratory parameters in cardiac tissue: a novel method of assessment by using saponin-skinned fibers. *Biochim Biophys Acta* 892: 191–196, 1987.
38. **Withers RT, Sherman WM, Clark DG, Esselbach PC, Nolan SR, Mackay MH, and Brinkman M.** Muscle metabolism during 30, 60 and 90 s of maximal cycling on an air-braked ergometer. *Eur J Appl Physiol Occup Physiol* 63: 354–362, 1991. [Corrigenda. *Eur J Appl Physiol Occup Physiol* 1992; 64(4): 387, and 1992; 64(5): 485.]
39. **Zoll J, Sanchez H, N'Guessan B, Ribera F, Lampert E, Bigard X, Serrurier B, Fortin D, Geny B, Veksler V, Ventura-Clapier R, and Mettauer B.** Physical activity changes the regulation of mitochondrial respiration in human skeletal muscle. *J Physiol* 543: 191–200, 2002.