Importance of pH regulation and lactate/H⁺ transport capacity for work production during supramaximal exercise in humans

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Submitted 20 June 2006; accepted in final form 5 February 2007


Many studies have shown that the rapid decline in force development during intense exercise (referred to as fatigue) coincides with metabolic changes occurring in the active muscles. The decline of cellular pH is among the potential candidates for muscle fatigue (11, 16, 17). The lactate anion per se has also been suggested to be involved in muscle function disturbances. Both H⁺ and lactate impair excitation-contraction coupling (10, 36), depress Ca²⁺-activated force (2, 7), and reduce tension development (9, 18, 30, 45). Changes in intracellular pH may also modify protein conformation, alter channel properties (40), and depress the activity of key enzymes in glycolysis (6, 28, 50), which reduce the rate of ATP resynthesis (16, 32, 42). Consequently, it may be crucial for exercising muscle cells to delay pH decline and lactate accumulation in the cytosol.

During periods of high muscle activity, protons and lactate are generated in the cells. The H⁺ and lactate produced can either be buffered and removed intracellularly or released to the interstitium. The cellular mechanisms of proton buffering and lactate clearance represent the first line of defense against acidosis and lactate accumulation. Elevated muscle buffering and lactate clearance mechanisms would enable the muscle to produce more lactate and protons before reaching lactate accumulation and pH limits. This would enhance the ability for energy production and improve work capacity (34, 43).

The presence of monocarboxylate transporter (MCT) 1 in the mitochondrial membrane (8, 14, 15) allows lactate and H⁺ ions to enter the mitochondria. Offering a dilution space for lactate and protons, mitochondria may also contribute to delay cytosolic lactate accumulation and pH decrease. From that point of view, one may consider that mitochondria also participate in the cellular buffering mechanisms. Furthermore, the existence of a mitochondrial lactate accumulation complex (14) supports the hypothesis that mitochondria may also take part in the intracellular lactate clearance (5).

The release of lactate and H⁺ ions to the outside of the muscle cell constitutes another protective mechanism against intracellular pH decrease and lactate accumulation. During high-intensity exercise, the release of these ions is mediated mainly by lactate-H⁺ cotransport via the MCT1 and MCT4 (21, 22). This mechanism predominates during intense exercise. It accounts for ~70–75% of the proton efflux (12) and 70–80% that of lactate (22). The proton efflux is completed by the Na⁺/H⁺ exchanger 1 (NHE1) system, which is less important during exercise than at rest (23). Thus it can be hypothesized that the muscle content of MCT1, MCT4, and NHE1 influence work capacity during high-intensity exercise.

The high concentration of HCO₃⁻ in the interstitial space provides the major buffering mechanism in the extracellular fluid for H⁺ leaving the muscle cells. Carbonic anhydrase (CA), which accelerates the hydration/dehydration reaction between CO₂, HCO₃⁻, and H⁺, is probably mandatory for making the process sufficiently fast to function in vivo. Two isoforms of CA (CAIV and CAXIV) have been found on the muscle membrane with their active centers oriented toward the extracellular space. By accelerating the hydration/dehydra-

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tion reaction in the interstitial space, CA associated with the sarcolemma plays a major role in maintaining a normal interstitial pH but also a sufficient release of protons and lactate from the muscle cells. Indeed, by consuming the H⁺ leaving the cell, a high CAIV and CAXIV activity may favor a large intra- to extracellular proton gradient and thus lactate and H⁺ transport across the sarcolemma (1, 25, 33, 41). In accordance with this idea, previous experiments have shown that inhibition of extracellular muscle CA by acetazolamide decreases lactate transport (51) and delays its accumulation in the blood (27, 44). Furthermore, the role played by sarcolemmal CA for H⁺ and lactate release is expected to become more important under non-steady-state conditions (12). All of this makes sarcolemmal CA a potential major factor in pH regulation and lactate transport during high-intensity exercise.

Despite the mechanisms mentioned above, maximal exercise generates protons and lactate molecules at a much higher rate than can be buffered or metabolized inside the muscle cell or released and subsequently eliminated outside it. As a result, pH generates protons and lactate molecules at a much higher rate during high-intensity exercise.

Furthermore, CAIV and CAXIV activity may favor a large intra- to extracellular proton gradient and thus lactate and H⁺ transport across the sarcolemma (1, 25, 33, 41). In accordance with this idea, previous experiments have shown that inhibition of extracellular muscle CA by acetazolamide decreases lactate transport (51) and delays its accumulation in the blood (27, 44). Furthermore, the role played by sarcolemmal CA for H⁺ and lactate release is expected to become more important under non-steady-state conditions (12). All of this makes sarcolemmal CA a potential major factor in pH regulation and lactate transport during high-intensity exercise.

Previous experiments have shown that metabolic alkalosis (Alk) (induced by bicarbonate or citrate ingestion) enhances work capacity for short-duration, high-intensity exercises (4, 35, 47). A large variability has been detected in performance improvement in response to Alk (29, 46). Higher net lactate release rates from the muscle have been obtained with metabolic Alk than with controls (19, 20). This faster lactate output was associated with a greater muscle lactate accumulation attributed by the authors to a higher lactate production (19).

However, the enhanced lactate efflux observed during Alk by Hollidge-Horvat et al. (19) took place without any change in the muscle-to-blood lactate gradient. This result suggests that metabolic Alk accelerates lactate transport. The most plausible explanation for this higher effectiveness for lactate transport is the higher muscle-to-blood proton gradient observed with Alk (19, 46). By increasing the extracellular buffer capacity, metabolic Alk maintains an elevated muscle-to-blood proton gradient and in turn promotes a higher efflux of lactate and protons from the muscle cells (41). If the efficiency of the transporters is increased by metabolic Alk, one can further hypothesize that subjects with a high muscle content of MCTs and CAs would take a greater benefit of Alk by increasing their lactate and proton transport to a larger extent and, consequently, their work capacity.

The main purpose of the present study is to investigate the influence of the muscle content of proteins involved in pH regulation and lactate transport on work capacity during supramaximal exercise. A secondary aim is to test the hypothesis that the muscle content of MCTs, NHE1, and CAs influences the improvement of work capacity induced by metabolic Alk.

MATERIALS AND METHODS

Subjects

Eight healthy, sedentary volunteers (three women and five men) participated in the study. Age, height, and weight were 20.5 ± 0.3 yr, 172 ± 2 cm, and 66.6 ± 2.9 kg (means ± SE), respectively. The study was approved by the ethics committee of the University of Saint-Etienne. Before giving their written consent, the subjects were fully informed of the objectives, all risks, possible discomforts, and potential benefits of the experiments.

Experimental Design

At least 8 days before the start of the experiments, the subjects were submitted to an inclusion protocol. This consisted of a physical examination, anthropometric measurements, and an incremental exercise to exhaustion to allow the subjects to become familiar with the experimental equipment and testing procedures of the incremental test. The experiment took place during the following 5 wk.

Preliminary measurements (week 1): incremental exercise to exhaustion. After the subjects had rested for 10 min on the cycle ergometer, the graded exercise started at 40 W for the women and 60 W for the men. Following 2 min of exercise at this load, the work rate was increased by 20 W for the women and 30 W for the men every 2 min thereafter. The exercise was stopped when the subjects were no longer able to sustain the work rate at the required pedaling frequency. Expired gas was collected in Douglas bags during the last 30 s of each work load and subsequently analyzed for oxygen uptake (VO₂). A capillary blood sample (micropuncture of 20 μl) was taken from the fingertip at the 3rd min of the recovery. This exercise session was performed for determination of the maximal heart rate (beats/min), maximal VO₂ (VO₂ max; l/min and ml·min⁻¹·kg⁻¹), work rate corresponding to VO₂ max (Wmax; W/kg), and capillary [Lac]b 3 min after exercise cessation.

Familiarization period (weeks 1 and 2). The subjects performed three familiarization sessions separated by at least 3 days to become accustomed to the performance events. Each session included successively a 15-min warm-up period at 50% Wmax, 15 min of rest, and 15 min of exercise at 60, 70, and 80% of Wmax for the first, second, and third familiarization session, respectively. After complete recovery, each session ended with an exercise to exhaustion at 120% Wmax.

Work capacity event (week 3): time to exhaustion at 120% Wmax. After oral ingestion of placebo (Con; lactose 0.5 g/kg body mass) or sodium citrate (Alk; 0.5 g/kg body mass), the subjects were asked to perform exercise to exhaustion at 120% Wmax. The Con and Alk events were performed in random order 3 days apart. These sessions were done to estimate the work capacity during supramaximal exercise and the effect of Alk on work capacity. Subjects arrived in the laboratory 1 h after having a light standard breakfast. Placebo or sodium citrate were ingested within 15 min after arrival. After 65 min of rest, the subjects performed a 15-min warm-up exercise at 50% Wmax. Then after another 10 min of rest, the subjects performed exercise at 120% Wmax until exhaustion. A blood sample was collected from the fingertip 3 min after the end of the exercise to determine the [Lac]b. Time to exhaustion at 120% Wmax was recorded (Tex). The work rate was measured throughout the period of exercise at 120% Wmax and the average was calculated (Wavg). The total work (Wtot, i.e., Wtot × Tex) and the supramaximal work performed [Wsup, i.e., (Wtot - Wmax) × Tex] during the period of exercise at 120% Wmax were calculated in Con (Wtot and Wsup) and Alk (Wtot-Alk and Wsup-Alk) for each subject and were used as work capacity criteria.

Standardized event (weeks 4 and 5): fixed time exercise at 120% Wmax. To compare muscle metabolic response for the same energy demand, subjects were asked to perform a standardized exercise in each condition (Alk and Con) in random order 1 wk apart. Subjects arrived in the laboratory 1 h after their light standard breakfast and ingested placebo or sodium citrate within 15 min of arrival. A preheparinized catheter (Quick-Cath, N1116, Travenol, 20 gauge, 0.85 mm) was inserted in the right radial artery under local anesthesia once Allen’s test had been performed (to make sure of the supply to the manually closed radial artery by the cubital artery). After 65 min of rest, the subjects performed a 15-min warm-up exercise at
50% $W_{\mathrm{max}}$. After another 10 min of rest, the first muscle biopsy of the vastus lateralis and an arterial blood sample were taken to determine resting muscle and blood pH and lactate concentrations. Then the subjects performed exercise at 120% $W_{\mathrm{max}}$ for an individually determined duration, which was the same in Con and Alk and equaled $T_{\mathrm{ex}}$ obtained in Con minus 20 s. In such conditions, exercise work rate and duration were individually the same in Con and Alk. Immediately after exercise, a second muscle biopsy was performed to determine muscle pH ($pH_m$) and [Lac]$_m$. An arterial blood sample was also taken to determine pH and [Lac]$_b$ at the end of exercise. Total and supramaximal work performed during the standardized exercise ($W_{\mathrm{tot-se}}$ and $W_{\mathrm{sup-se}}$, respectively) were calculated as described above for both conditions. Muscle biopsies were also used for Western blotting (see below) and determination of fiber-type distribution. The $pH_m$ decreases ($\Delta pH_m = \text{postexercise } pH_m - \text{resting } pH_m$) and lactate increases ($\Delta [\text{Lac}]_m = \text{postexercise } [\text{Lac}]_m - \text{resting } [\text{Lac}]_m$) during exercise were calculated. $\Delta pH_m$ and $\Delta [\text{Lac}]_m$ were also expressed per joule of $W_{\mathrm{tot-se}}$ or $W_{\mathrm{sup-se}}$. The muscle-to-blood lactate gradient at the end of exercise was estimated. Dynamic buffer capacities were calculated in Con and Alk (dynamic buffer capacity and dynamic buffer capacity,$\text{Alk}$, respectively), as proposed by Sahlin and Henriksson (43): (postexercise $[\text{Lac}]_m$ – resting $[\text{Lac}]_m$)/(resting pH$_m$ – postexercise pH$_m$).

**Ergometry**

All of the exercises were performed on cycle ergometers (Monark 818E, Stockholm, Sweden for the incremental exercise and MEV5000 Ergoméca, Le Pradet, France for the work capacity and standardized events). The instantaneous power output and the pedaling frequency (set at 75 rpm) were delivered online by a computer device. For all of the exercise tests, the subjects were in the seated position.

**Standard Breakfast**

The standard breakfast included 20 cl of apple juice, two slices of bread, and 10 g of strawberry jam that corresponded to a dietary energy intake of 66 g carbohydrates, 35 g proteins, and no fat (1170 kJ).

**Measurements**

Heart rate was displayed throughout the incremental exercise sessions by a continuous electrocardiogram as a safety and check procedure. $V\dot{O}_2$ was measured with an open gas-exchange apparatus. The subjects breathed through a two-way mouthpiece (Hans Rudolph 2700, Kansas City, MO). Expired gas was collected in Douglas bags for the flow measurements and analysis for $O_2$ and $CO_2$ by means of a D-Fend Datex (Helsinki, Finland) and an S3A/I Ametek (Pittsburgh, PA) analyzer, respectively. These devices were calibrated before the experiment using precision-analyzed gas mixtures. When $V\dot{O}_2$ reached a plateau while the work rate was still increasing, the plateau value was considered as $V\dot{O}_2\text{max}$. To establish that $V\dot{O}_2\text{max}$ was reached during the last step when a plateau was not observed, the following criteria were used: a respiratory exchange ratio greater than 1.1, an end-exercise lactate concentration higher than 9 mmol/l, the theoretical maximal cardiac frequency approximate reached ($\geq 210$ beats/min), and observation of a rapid increase in the ventilation rate during the last steps before exhaustion.

$W_{\text{max}}$ was determined by linear interpolation from the $V\dot{O}_2$ vs. work rate curve.

[Lac]$_w$ was determined enzymatically in hemolyzed blood for the fingertip samples or in plasma after centrifugation of the blood drawn from the radial artery. Analyses were performed using an LA 640 Kontron lactate analyzer (Roche Bio-electronics, Hoffman-La Roche, Basel, Switzerland).

**Muscle Analysis**

A small incision was made in the skin and fascia under local anesthesia. Biopsies of the vastus lateralis muscle were taken with a Weil Blakesley forceps, at rest and at the end of the standardized exercise. Part of the preexercise biopsy sample was mounted in Tissue-Tek II OCT compound for histochemical analysis. Cryostat serial transverse sections (~20μm, 10 μm) were stained for ATPase activity to determine the muscle fiber composition. The remainder was frozen and stored in liquid nitrogen until analysis. Postexercise muscle biopsies were immediately frozen in liquid nitrogen and stored until analysis. The delay before freezing averaged 15 s. The frozen samples were freeze-dried and then dissected free of connective tissue and blood. One portion was extracted with HClO$_4$ (650 mmol/l), neutralized, and assayed enzymatically for lactate concentration by fluorometric analysis. [Lac]$_b$ are expressed in millimoles per kilogram tissue dry mass. A second portion was weighed and homogenized at 4°C in a solution containing KCl (145 mmol/l), NaCl (10 mmol/l), and NaF (10 mmol/l) with 30 mg dry muscle/ml homogenizing solution.

$\text{pH}_b$ was determined with a microelectrode (MI 410, Microelectodes, Bedford, NH) at 37°C. The remaining portion was used for the Western blots.

**Western Blots**

Approximately 30 mg of muscle were homogenized (Polytron 2100, Kinematica, Newark, NJ) in a sucrose buffer (250 mM sucrose, 30 mM HEPES, 2 mM EGTA, 40 mM NaCl, 2 mM PMSF, pH 7.4) and centrifuged at 1,000 g for 5 min. This procedure removed heavy material, including a fraction of the mitochondria. The supernatant was spun at 190,000 g for 90 min at 4°C. The new supernatant (cytosolic fraction) was stored at ~80°C, while the new pellet (total muscle membrane fraction, including sarcolemmal and mitochondrial membrane fractions) was resuspended in Tris-SDS (10 mM Tris, 4% SDS, 1 mM EDTA, 2 mM PMSF, pH 7.4). Protein content was determined with a BSA standard (DC protein assay, Bio-Rad, Herlev, Denmark). Ten micrograms of protein from each sample were subjected to SDS-PAGE (excel 8–18% gradient gel; Amersham Biosciences, Uppsala, Sweden) and electroblotted to an Immobilon-P transfer membrane (Millipore, Copenhagen, Denmark). The membrane was blocked with a buffer containing 1% BSA, 0.1% Tween 20, and 0.5% low fat milk and further incubated with the primary antibody diluted in the same blocking buffer. After treatment with the secondary antibody and repeated washing, the membrane was incubated with enhanced chemiluminescence reagent (Amersham Biosciences, Arlington Heights, IL) and visualized on a hyper film (Amersham Biosciences). Quantification of the selected protein was performed by scanning the film and analyzing band densities with the SigmaGel software (SPSS, Chicago, IL). The NHE1 isoform (100 kDa), the lactate/H$^+$ cotransporter isoforms MCT1 and MCT4 (both 43 kDa), and the membrane-bound CA isoforms CAIV (39 kDa) and CAXIV (51 kDa) were measured on the total muscle membrane fraction, whereas the CA isoforms CAII and CAIII (both 31 kDa) were measured on the cytosolic fraction. The antibodies for NHE1 (no. MAB3140), MCT1 (no. AB3540P), MCT4 (no. AB3316P), and CAII (no. AB1828) were purchased from Chemicon (Chandlers Ford, UK), and from Spectral Diagnostics (Toronto, Canada) for CAIII (no. 4020). The antibodies for CAIV and CAXIV were provided by Professor W. Sly, St. Louis, MO. Some membranes were reused after treatment with a stripping solution (Re-Blot Plus, Chemicon).

**Statistical Analysis**

Descriptive statistics are expressed as means ± SE. Relationships between two variables were studied by means of linear regressions (confirmed by Pearson tests). In Figs. 1–6, solid and shaded symbols are used for men and women, respectively. Analysis by multiple regressions has also been used. Differences between values and
relationships among variables were considered to be significant for $P \leq 0.05$ and to represent a tendency for $0.05 < P \leq 0.10$.

**RESULTS**

**Muscle Characteristics and Incremental Exercise**

The distributions of muscle fiber types were 48.3 ± 3.9, 40.8 ± 3.7, and 10.9 ± 1.2% for types I, IIa, and IIx, respectively.

Maximal heart rate, $\dot{V}O_2\text{max}$, and $W_{\text{max}}$ were 198 ± 5 beats/min, 3.11 ± 0.22 l/min (47.3 ± 2.2 ml·min$^{-1}$·kg$^{-1}$), and 3.00 ± 0.16 W/kg, respectively. Three minutes after exercise, mean capillary $[\text{Lac}]_b$ reached 12.6 ± 4.0 mmol/l.

**Work Capacity Events**

The all-out supramaximal exercise was performed at 121.0 ± 3.0 and 119.9 ± 2.0% of $W_{\text{max}}$ in Con and Alk, respectively (not significant (NS)). This work rate was maintained during 263.8 ± 8.1 and 296.5 ± 16.0 s in Con and Alk, respectively ($P = 0.012$). The $W_{\text{tot}}$ and $W_{\text{tot}-\text{Alk}}$ were 63.5 ± 3.8 and 70.1 ± 3.8 kJ, respectively ($P = 0.015$). The $W_{\text{sup}}$ and $W_{\text{sup}-\text{Alk}}$ were 10.8 ± 0.7 and 11.1 ± 0.5 kJ, respectively (NS). Capillary hemolized $[\text{Lac}]_b$ reached 13.6 ± 0.5 and 17.1 ± 0.5 mmol/l in Con and Alk, respectively ($P = 0.018$). At exhaustion, mean heart rate was 190 ± 2 beats/min in Con and 193 ± 3 beats/min in Alk (NS).

**Standardized Events**

At rest (90 min postingestion), blood pH was lower in Con than in Alk (7.42 ± 0.03 vs. 7.46 ± 0.06, $P < 0.01$). On the other hand, $pH_m$ was not different (NS) in Con and Alk (7.16 ± 0.02 vs. 7.17 ± 0.02, respectively). $[\text{Lac}]_m$ (3.0 ± 1.1 vs. 3.1 ± 1.1 mmol/l) and $[\text{Lac}]_m$ (4.5 ± 0.3 vs. 4.0 ± 0.5 mmol/kg dry wt) were not different between the two conditions (NS).

At the end of the exercise, blood pH was lower in Con than in Alk (7.21 ± 0.02 vs. 7.29 ± 0.03, $P < 0.01$), while $pH_m$ was not different (NS) (6.49 ± 0.04 vs. 6.51 ± 0.06). $[\text{Lac}]_m$ was similar in both conditions (101.7 ± 6.0 vs. 103.1 ± 6.1 mmol/kg dry wt in Con and Alk, respectively). In other words, $pH_m$ decreased by 0.67 ± 0.05 and 0.66 ± 0.05 units and $[\text{Lac}]_m$ increased by 97.2 ± 5.9 and 99.2 ± 6.3 mmol/kg dry muscle in Con and Alk, respectively (NS). Plasma $[\text{Lac}]_b$ reached 17.6 ± 1.2 vs. 19.3 ± 0.9 mmol/l at the end of exercise in Con and Alk, respectively ($P = 0.046$).

**Linear and Multiple Correlations Between Variables in Con**

The decreases in $pH_m$ and muscle lactate accumulation per joule of supramaximal work in Con ($\Delta pH_m/W_{\text{sup-se}}$ and $\Delta [\text{Lac}]_m/W_{\text{sup-se}}$, respectively) were correlated with $W_{\text{sup}}$ (Fig. 1). The CAII content was negatively correlated with $\Delta [\text{Lac}]_m/W_{\text{sup-se}}$, while CAIII was positively correlated with $\Delta pH_m/W_{\text{sup-se}}$ (Table 1). Postexercise $[\text{Lac}]_b$ was positively correlated with MCT1 (Fig. 2A) and tended to be correlated with MCT4 (Fig. 2B), while no correlations were found between postexercise $[\text{Lac}]_m$ and MCT1 or MCT4 (Fig. 2, C and D, respectively). $W_{\text{sup}}$ was positively correlated with the muscle content of MCT1 (Fig. 3A) and tended to be correlated with MCT4 and NHE1 (Fig. 3, B and C, respectively). MCT1 was correlated with MCT4 ($r = 0.845$, $P = 0.008$). The membrane-bound CAIV isoform content was positively correlated with postexercise $[\text{Lac}]_b$ (Fig. 4A) and $W_{\text{tot}}$ (Fig. 4C).

The cytosolic CAII + CAIII contents were positively correlated with $\Delta pH_m/W_{\text{sup-se}}$ (Table 1) and negatively correlated with $\Delta [\text{Lac}]_m/W_{\text{sup-se}}$ (Table 1). The estimated muscle-to-blood lactate gradient in Con was negatively correlated with the MCT1 + MCT4 + NHE1 contents ($r = -0.955$, $P = 0.012$).

**Table 1. Correlations (simple and multiple) between some parameters**

<table>
<thead>
<tr>
<th></th>
<th>$\Delta pH_m/W_{\text{sup-se}}$</th>
<th>$\Delta [\text{Lac}]<em>m/W</em>{\text{sup-se}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAII</td>
<td>$r = 0.703$, $P = 0.052$</td>
<td>$r = -0.822$, $P = 0.012$</td>
</tr>
<tr>
<td>CAIII</td>
<td>$r = 0.759$, $P = 0.029$</td>
<td>$r = -0.564$, $P = 0.145$</td>
</tr>
<tr>
<td>CAII + CAIII</td>
<td>$r = 0.840$, $P = 0.047$</td>
<td>$r = -0.837$, $P = 0.048$</td>
</tr>
</tbody>
</table>

Values are correlation coefficients ($r$) and probabilities ($P$). CAII and CAIII (arbitrary units), isoforms II and III of carbonic anhydrase, respectively; $pH_m$, muscle pH; $W_{\text{sup-se}}$, supramaximal work performed after standardized exercise; $[\text{Lac}]_m$, muscle lactate concentration; $\Delta pH_m/W_{\text{sup-se}}$ (pH units/kJ) and $\Delta [\text{Lac}]_m/W_{\text{sup-se}}$ (μmol·kg$^{-1}$·kJ$^{-1}$), pH decrease and lactate accumulation per joule of supramaximal work performed in control condition, respectively.
Effects of Alk: Linear and Multiple Correlations Between Variables

Relationships between the intraindividual changes in response to Alk on the dynamic buffer capacity (dynamic buffer capacity_{AB}/dynamic buffer capacity) were closely related to those on the supramaximal work (W_{sup-Alk}/W_{sup}) completed during the work capacity event (Fig. 5). If the outlier (Fig. 6, A and B) is not taken into account, the present study shows close negative correlations between the changes induced by metabolic Alk on the W_{tot} in the work capacity event at 120% W_{\dot{max}} (W_{tot-Alk}/W_{tot}) and the muscle content of MCT1 and MCT4 (Fig. 6, C and D).

Fig. 2. Relationships between blood ([Lac]_{b}; A and B) or muscle ([Lac]_{m}; C and D) lactate concentrations (mmol/l and mmol/kg dry wt, respectively) at the end of the standardized exercise and muscle content of monocarboxylate transporter (MCT) 1 (A and C) and MCT4 (B and D). MCT1 and MCT4 values are in arbitrary units. Shaded circles are for women; solid circles for men. n, No. of subjects.

Fig. 3. Relationships between W_{sup} during the work capacity event (kJ) and the muscle content of MCT1 (A), MCT4 (B), or Na^{+}/H^{+} exchanger (NHE) 1 (arbitrary units) (C).
The intraindividual changes of dynamic buffer capacity Alk/dynamic was negatively related to MCT1/H11001/CAIII/H11001/CAIV/H11001/CAXIV (r = 0.972, P = 0.032), and those of Wsup (Wsup-Alk/Wsup) were negatively related to MCT1/H11001/NHE1/H11001/CAIII (r = 0.915, P = 0.050).

**DISCUSSION**

We have investigated the influence of muscle content of proteins involved in pH regulation (CAII, CAIII, CAIV, and CAXIV) and lactate and proton transport (MCT1, MCT4, and NHE1) on work capacity during supramaximal exercise in humans. The main results of this study are that the supramaximal work capacity $1$ is negatively correlated with the pHm decrease and lactate accumulation rates, and $2$ is or tended to be positively associated with the muscle contents of MCT1, MCT4, and NHE1. The present study is the first to emphasize the potential role of the cytosolic CAs (CAII and CAIII) to delay pHm decrease and [Lac]m accumulation in vivo during supramaximal exercise. We also found a correlation between the extracellular membrane-bound CAIV and Wtot performed during the supramaximal exercise. Finally, Alk was more efficient in improving performance, if the work capacity in Con and the protein contents involved in pH regulation and lactate and proton transport were low.

Wsup was correlated with pHm decrease and [Lac]m accumulation per joule of Wsup-se (Fig. 1). The slower the decrease of pH and accumulation of lactate in the exercising muscles, the higher the Wsup. The present results suggest that pHm regulation and lactate removal may be crucial determinants of a subject’s ability to perform supramaximal exercise. The extent to which pH decreases and lactate accumulates in the exercising muscle depends (in addition to lactate and H+ production) on the intracellular buffering mechanisms and lactate removal and the amount of protons and lactate released from the exercising muscle cells.

The intracellular buffers consist of inorganic phosphates, protein-bound histidine residues, dipeptide carnosine, creatine phosphate, and bicarbonate (12, 13, 37, 40). An index of muscle buffer capacity is frequently determined by titrating a homogenate of wet or freeze-dried muscle with a fixed acid (31). However, because bicarbonate is removed by freeze-drying or because the CO2 most likely evaporates during sample preparation, the titration technique does not include the bicarbonate contribution to the buffering system. The present study demonstrates correlations between the cytosolic content of CAII and CAIII (two isoforms of CA present in the human skeletal muscle cell) and the pHm decrease and lactate accumulation during exercise (Table 1). These correlations seem to indicate that the bicarbonate buffer system may be an important buffering mechanism in the cell (3) and that its effectiveness may have important implications for work capacity during high-intensity exercise. Furthermore, the presence of MCT1 in the mitochondrial membrane (8, 14, 15) and the existence of a mitochondrial lactate oxidation complex (14) suggest that mitochondria may also take part in cellular buffering mechanisms and lactate clearance (5). The measurements performed in the present study do not allow for determining the precise

**Fig. 4.** Relationships between muscle content of isoform IV of carbonic anhydrase (CAIV; arbitrary units) and [Lac]c (mmol/l) (A) and [Lac]m (mmol/kg dry wt) (B) at the end of the standardized exercise and total work performed (Wtot; kJ) (C) during supramaximal exercise until exhaustion. Shaded circles are for women; solid circles for men. n, No. of subjects.

The intraindividual changes of dynamic buffer capacity Alk/dynamic buffer capacity were negatively related to MCT1 + CAII + CAIII + CAIV + CAXIV (r = -0.972, P = 0.032), and those of Wsup (Wsup-Alk/Wsup) were negatively related to MCT1 + NHE1 + CAII (r = -0.915, P = 0.050).
role of mitochondria in pHm regulation and lactate clearance. Further studies would be necessary. Nevertheless, this possibility exists and deserves to be mentioned.

The efflux of protons and lactate from the muscle cell is another way to prevent both intracellular pH decrease and lactate accumulation. A previous study reported a correlation between the sarcolemmal content of MCT1 and the net lactate release rate after 5 min of exercise at 65% \( V_{\text{O}_2 \text{ max}} \) (8). In our study, a positive correlation was observed between postexercise [Lac]b and MCT1 (Fig. 2A), and a positive tendency was found between postexercise [Lac]b and MCT4 (Fig. 2B). On the other hand, neither MCT1 (Fig. 2C) nor MCT4 (Fig. 2D) nor postexercise [Lac]b \((r = 0.460, P = 0.252)\) were related to postexercise [Lac]m. In addition, the estimated muscle-to-blood lactate gradient was negatively correlated with the MCT1, MCT4, and NHE1 contents \((r = -0.955, P = 0.012)\). Taken together, these results indicate that the muscle content of MCT1, MCT4, and NHE1 may play an important role for the efflux of lactate and protons from the muscle cell during supramaximal exercise.

Interestingly, \( W_{\text{sup}} \) was positively correlated with the muscle content of MCT1 (Fig. 3A), and strong tendencies were observed for \( W_{\text{sup}} \) with MCT4 (Fig. 3B) and NHE1 (Fig. 3C). These results suggest that the mechanisms for lactate and proton transport are important for human work capacity during supramaximal exercise. In accordance with the present finding and interpretation, Pilegaard et al. (39) reported a weak but significant negative relationship between muscle lactate transport determined from giant sarcolemmal vesicles and fatigue index during 50-s maximal knee extensor exercise. A recent study also reported a negative correlation between the muscle MCT1 content of the vastus lateralis and fatigue index during a 1-min all-out cycling event (49).

The positive correlation found between \( \Delta [\text{Lac}]_b \) and CAIV (Fig. 4A) in the absence of correlations between \( \Delta [\text{Lac}]_m \) and CAIV (Fig. 4B) or \( \Delta [\text{Lac}]_b \) \((r = 0.460, P = 0.252)\) suggests that the extracellular CA activity may be important in regulating the efflux of protons and lactate from the muscle cell during supramaximal exercise. This conclusion is in agreement with those obtained previously after inhibition of the sarcolemmal CA by acetazolamide (27, 44, 51). Also of note is the positive correlation between CAIV and \( W_{\text{tot}} \) (Fig. 4C).

The present results show a close, positive correlation between changes in the dynamic buffer capacity in response to Alk and those in the amount of supramaximal work completed during the work capacity event (Fig. 5). Since dynamic buffer capacity results from intracellular buffering and lactate removal mechanisms and the release of lactate and protons to outside the muscle cells, this close correlation reinforces our previous conclusion that the mechanisms of pH regulation and lactate and proton transport may influence work capacity during supramaximal exercise in humans.

Hollidge-Horvat et al. (19) measured a net lactate release rate from muscle approximately twofold higher with Alk \((\sim 10 \text{ mmol/min})\) than in the Con condition \((\sim 4.5 \text{ mmol/min})\), while the muscle-to-blood (arterial) lactate gradient was similar in
both conditions (≅5.1 mmol/l). Their study was the first in humans that demonstrated an improvement of lactate transport in response to metabolic Alk. In the present study, the net lactate release rate was not measured. However, the fact that higher [Lac]b were obtained at the end of exercise in Alk than in Con when their muscle counterparts were not different suggests that lactate transport was improved in Alk and corroborates the observation of Hollidge-Horvat et al. (19).

The unexpected result of the present study is that the subjects who gained the most in terms of work capacity from metabolic Alk had the lowest work capacity in the Con condition and a low muscle content of proteins involved in pH regulation and lactate/H+ transport (Fig. 6, C and D). Since metabolic Alk would act mainly by improving carrier-mediated lactate (and proton) transport, this result suggests that the work capacity of individuals with a low level of performance would be, in part, determined and limited by the muscle content of proteins involved in buffering mechanisms and lactate/H+ transport. Subjects with a higher level of performance would be less dependent on the muscle content of these proteins. An elevated work capacity is often associated with higher muscle capillary supply and local blood flow, which, by increasing the efflux of lactate and thus protons to outside the muscle cell (38, 48), both favor washout of the muscle and maintenance of cellular homeostasis. Consequently, pHe regulation and lactate efflux would be much more efficient but less dependent on the muscle content of proteins involved in pH regulation and lactate/H+ transport in subjects with elevated work capacity. This might explain why the performance of subjects having elevated work capacity and content of proteins involved in pH regulation and lactate transport benefits less from metabolic Alk. This might also explain why the maximal effect of training on membrane transport proteins is often obtained within the first 8 wk of intensive training, while years of training do not seem to lead to further improvement (24). Although plausible, these interpretations still remain speculative, and further studies are necessary to elucidate the precise significance of these results.

In conclusion, the present study has shown the influence that intra- and extracellular mechanisms of pH regulation and muscle lactate/proton transport may have on human work capacity during supramaximal exercise. More specifically, we underline the role that the cytosolic CAs (CAII and CAIII) may play to delay pHm decrease and [Lac]m accumulation during exercise. Consequently, pHm regulation and lactate efflux of lactate and thus protons to outside the muscle cell (38, 48), both favor washout of the muscle and maintenance of cellular homeostasis. Consequently, pHm regulation and lactate efflux would be much more efficient but less dependent on the muscle content of proteins involved in pH regulation and lactate/H+ transport in subjects with elevated work capacity. This might explain why the performance of subjects having elevated work capacity and content of proteins involved in pH regulation and lactate transport benefits less from metabolic Alk. This might also explain why the maximal effect of training on membrane transport proteins is often obtained within the first 8 wk of intensive training, while years of training do not seem to lead to further improvement (24). Although plausible, these interpretations still remain speculative, and further studies are necessary to elucidate the precise significance of these results.

ACKNOWLEDGMENTS

The authors thank Helle Walas for technical assistance and Arthur Pape for helpful suggestions.

GRANTS

This study was supported by grants from l’Ambassade de France du Danemark in Copenhagen and from the Ministère Français de la Jeunesse et des Sports.

Part of the results have been published elsewhere for other purposes (29).

REFERENCES


J Appl Physiol • VOL 102 • MAY 2007 • www.jap.org


