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# Relationship between performance at different exercise intensities and skeletal muscle characteristics

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Iaia FM, Perez-Gomez J, Thomassen M, Nordsborg NB, Hellsten Y, Bangsbo J. Relationship between performance at different exercise intensities and skeletal muscle characteristics. J Appl Physiol 110: 1555-1563, 2011. First published March 24, 2011; doi:10.1152/japplphysiol.00420.2010.-The hypothesis investigated whether exercise performance over a broad range of intensities is determined by specific skeletal muscle characteristics. Seven subjects performed 8-10 exhaustive cycle trials at different workloads, ranging from 150 to 700 W (150 min to 20 s). No relationships between the performance times at high and low workloads were observed. A relationship (P < 0.05) was noticed between the percentage of fast-twitch x fibers and the exercise time at 579  $\pm$  21 W (~30 s;  $r^2 = 0.88$ ). Capillary-to-fiber-ratio ( $r^2$ : 0.58–0.85) was related (P <0.05) to exercise time at work intensities ranging from 395 to 270 W (2.5–21 min). Capillary density was correlated ( $r^2 = 0.68$ ; P < 0.05) with the net rate of plasma  $K^+$  accumulation during an  $\sim$ 3-min bout and was estimated to explain 50-80% (P < 0.05) of the total variance observed in exercise performances lasting  $\sim 30$  s to 3 min. The  $Na^+-K^+$  pump  $\beta_1$ -subunit expression was found to account for 13–34% (P < 0.05) during exhaustive exercise of  $\sim$ 1–4 min. In conclusion, exercise performance at different intensities is related to specific physiological variables. A large distribution of fast-twitch x fibers may play a role during very intense efforts, i.e.,  $\sim 30$  s. Muscle capillaries and the Na<sup>+</sup>-K<sup>+</sup> pump  $\beta_1$ -subunit seem to be important determinants for performance during exhaustive high-intensity exercises lasting between 30 s and 4 min.

high-intensity exercise; fatigue; capillaries; Na<sup>+</sup>-K<sup>+</sup> pump; pH

AN UNDERSTANDING OF THE PHYSIOLOGICAL variables that determine human exercise performance has intrigued physiologists for more than a century. It is well established that endurance performance is related to maximum oxygen uptake ( $\dot{V}o_{2max}$ ), work economy (mechanical efficiency), and the relative intensity (fractional utilization of  $\dot{V}o_{2max}$ ) that can be sustained throughout the exercise (8, 13, 15, 24, 33). There is, however, less consensus about the physiological determinants controlling short-term, high-intensity exercise performance.

The aerobic energy system has been reported to contribute up to 66% in a 800-m event (43), and recent findings have suggested that reduced oxygen delivery may limit performance in whole body supramaximal (110% of  $\dot{V}o_{2max}$ ; 2.1 ± 0.1 min) cycling exercise (35). On the other hand, a number of training studies have shown marked performance improvements during short-term exercise without changes in  $\dot{V}o_{2max}$  (6, 14, 20, 22). Capillarization has traditionally been associated with success in endurance performance (13, 40), but capillary density has also been linked with the recovery (46) and maintenance (45) of force, as well as improved time to fatigue in an  $\sim$ 8-min exhaustive incremental test (23). These findings suggest that capillaries may also be of importance for high-intensity efforts. However, no studies have examined the relationship between capillarization and performance during intense whole body exercise of different durations. Likewise, a high composition of fast-twitch (FT) muscle fibers has been shown to be typical in sprinters (12, 19), but its importance for intense exercise performance is not clear.

High-intensity exercise leads to a pronounced accumulation of  $H^+$  and lactate inside the working muscles (3, 26). The Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 1 (NHE1) and two isoforms of the monocarboxylate cotransporters (MCT1 and MCT4) operate as the predominant system in pH regulation, with the latter two accounting for 70-80% of the proton and lactate efflux during intense exercise (25). In human skeletal muscle, the lactate transport capacity has been reported higher in trained subjects compared with the untrained population (38). Furthermore, following a period of intense training, studies have found higher MCT1 protein expression in association with improved supramaximal exercise performance (6, 27, 34, 39). On the other hand, in two studies investigating endurance-trained runners (1, 22), an elevated short-term work capacity observed after a speed endurance training period was not associated with an augmented muscle MCT protein content, suggesting that other mechanisms may have been determinant for performance improvements. Thus it is unclear if the abundance of proteins involved in pH regulation is important for intense exercise performance.

Sarcolemmal inexcitability, especially due to extracellular K<sup>+</sup> accumulation, has also been suggested to limit highintensity exercise performance (41). Therefore, the  $Na^+-K^+$ pump may play a crucial role in preserving membrane excitability and ensuring skeletal muscle function, by maintaining the concentration gradients for Na<sup>+</sup> and K<sup>+</sup> across the muscle membrane (11). The suggestion that the  $Na^+$ -K<sup>+</sup> pump content is of importance for human fatigue development is supported by the findings that muscle interstitial  $K^+$  concentration ([K<sup>+</sup>]) during intense exercise is elevated to levels higher than 10 mM, which may cause inexcitability (36, 37). Moreover, exercise training leads to 15-40% increases in Na<sup>+</sup>-K<sup>+</sup> pump expression and reduced interstitial/venous K<sup>+</sup> accumulation, which may be part of the explanation of the associated improvements in high-intensity exercise performance (11, 22, 34, 36). Also, the  $Na^+$ - $K^+$ - $Cl^-$  cotransporter isoform 1 (NKCC1) may contribute to the maintenance of muscle function during

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# 1556

## PERFORMANCE AND MUSCLE CHARACTERISTICS

intense exercise, possibly by adding to the  $K^+$  reuptake (49). However, the extent by which the Na<sup>+</sup>-K<sup>+</sup> pump and NKCC1 density affect performance during high-intensity exercises of different duration is still unclear.

Thus the aim of the present study was to examine the hypothesis that the influence of  $\dot{V}o_{2max}$ , mechanical efficiency, capillary density, fiber-type distribution, and expression of selected muscle proteins on performance is related to the exercise intensity.

## METHODS

#### Subjects

Seven healthy men volunteered and gave written, informed consent to participate in this study, which was approved by the Ethics Committee of Copenhagen and Frederiksberg communities, according to code of Ethics of the World Medical Association (Declaration of Helsinki). Subjects' characteristics are reported in Table 1. The subjects were regularly physically active, but none of them were engaged in specific training for competition. Before testing, participants were fully informed of the protocol and the possible risks associated with the experimental procedures.

#### Experimental Protocol

The participants attended the laboratory between 17 and 19 times during a 2- to 3-mo period. The first four visits were used to familiarize subjects with the fatiguing performance trials. These consisted of several exhaustive cycle exercise bouts at different workloads, interspersed by 20–30 min of rest. During the following two visits, subjects underwent an incremental ramp protocol for  $\dot{V}o_{2max}$  assessment and a Wingate test for peak and mean power output determination. On the remaining occasions, subjects performed 8–10 exhaustive cycle trials at workloads ranging from 150 to 700 W (see Tables 4 and 5). In addition, three trials were repeated under invasive conditions to obtain physiological measurements.

 Table 1. Anthropometric, physiological, and muscle characteristics of the subjects

Age and anthropometric characteristics	
Age, yr	$24.7 \pm 1.4 (21 - 32)$
Height, cm	180.7 ± 3.3 (170–195)
Body mass, kg	$78.6 \pm 4.0  (63.5 - 91)$
Physiological characteristics	
$\dot{V}_{O_{2max}}, ml \cdot kg^{-1} \cdot min^{-1}$	$51.0 \pm 3.6 (38.8 - 68.2)$
Heart rate maximum, beats/min	$189 \pm 3 (178 - 203)$
Peak power output, W	$970 \pm 102 \ (683 - 1,500)$
Mean power output, W	$692 \pm 35 (587 - 829)$
Net cycling efficiency at 200 W, %	$22.5 \pm 0.8 (19.6 - 24.8)$
Net cycling efficiency at 270 W, %	$23.7 \pm 0.8 (20.8 - 25.4)$
Muscle characteristics	
Fiber type I, %	$62.3 \pm 6.3 (38.1 - 85.5)$
Fiber type IIa, %	$21.6 \pm 5.1 (7.8 - 46.8)$
Fiber type IIx, %	$16.1 \pm 4.8 \ (6.5 - 37.5)$
Capillary-to-fiber ratio	$3.36 \pm 0.49 \ (2.09 - 5.83)$
Capillary density, capillaries/mm <sup>2</sup>	561 ± 56 (439–813)
CK, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	$4,037 \pm 64 (3,715 - 4,224)$
PFK, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	$160 \pm 12 (107 - 187)$
CS, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	36 ± 3 (27–52)
HAD, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	35 ± 4 (24–52)
Buffering capacity, mmol H <sup>+</sup>	
$\cdot$ kg dry wt <sup>-1</sup> $\cdot$ pH unit <sup>-1</sup>	153 ± 7 (123–186)

Values are means  $\pm$  SE (with ranges in parentheses); n = 7 subjects.  $\dot{V}o_{2max}$ , maximum oxygen uptake; CK, creatine kinase; PFK, phosphofructokinase; CS, citrate synthase; HAD, 3-hydroxyacyl-CoA dehydrogenase.

## **Experimental Procedures**

Determination of  $\dot{V}o_{2max}$ .  $\dot{V}o_{2max}$  was measured using an incremental test to exhaustion, as previously described (21). Individual  $\dot{V}o_{2max}$  was determined as the highest value recorded in any 20-s period before the cessation of the test. A plateau in oxygen uptake  $(\dot{V}o_2)$  (increments lower than 2.1 ml·min<sup>-1</sup>·kg<sup>-1</sup>) (7), despite an increased power output and a respiratory exchange ratio > 1.15 were used as criteria for  $\dot{V}o_{2max}$  achievement.

*Wingate test.* The Wingate test was carried out on a mechanically braked cycle ergometer (model 824E, Monark, Stockholm, Sweden), as described (4), and data were subsequently analyzed for peak and mean power output determinations.

Exhaustive trials. The exhaustive trials were performed at least 3 days apart, in a cool room under the same standard environmental conditions (20°C; 40% relative humidity). All exercises were carried out on an electronically braked cycle ergometer (model 839E, Monark, Stockholm, Sweden), which was calibrated before each trial. Each trial was preceded by a 10-min warm-up at 75-100 W, followed by 5 min of rest. Subjects were informed to remain seated throughout the entire exercise bout. Subjects selected a cadence between 80 and 85 rpm and were instructed to maintain it ( $\pm 2$  rpm) for the entire test duration and throughout all of the exercise trials. Pedaling frequency and power output were recorded via a photoelectric sensor connected to a computer and subsequently analyzed by the use of the appropriate software (Monark 839E Analysis Software). During the trials, subjects were not given any temporal, verbal, or physiological feedback. Time to fatigue was defined as the point at which the pedaling frequency dropped <75 rpm for >5 s, despite strong verbal encouragement. The participants expressed maximum motivation and willingness in all of the trials. During the efforts lasting longer than 30 min, subjects were allowed to drink water.

To determine the reproducibility of exercise performances, the participants repeated exercise at several workloads, ranging from 270 to 600 W on a second occasion. The mean time to exhaustion between the first and second set of trials was not different (101.5  $\pm$  17.7 vs. 106.9  $\pm$  19.3 s; n = 33). The correlation coefficient was 0.99 (P < 0.05) for the test-retest, and the intraindividual difference between the tests averaged 5.4  $\pm$  2.6 s with a coefficient of variation value of 7.4%.

Invasive experiments. Three trials leading to exhaustion in  $\sim 30$  s (579 ± 21 W) (mean ± SE of 7 subjects),  $\sim 3 \min (354 \pm 25 \text{ W})$ , and  $\sim 2 \text{ h} (179 \pm 18 \text{ W})$  were performed with invasive measurements to obtain information on the interactions between exercise performance, metabolic response, and muscle characteristics. These experiments were executed in random order on separate days, at least 1 wk apart. The following procedure was common to all 3 days.

After subjects had been resting for 20 min in a supine position, a catheter (18 G, 32 mm) was inserted in an antecubital arm vein and covered by a wrist bandage. In preparation for collection of muscle biopsies, two small incisions (0.5–1 cm) through the skin and fascia over the medial part of vastus lateralis muscle were made under local anesthesia (1 ml; 20 mg/l lidocain without adrenalin) and covered by sterile band aid strips and a thigh bandage. The choice of leg was randomized. Blood samples were taken before, during, and within 10 s from the cessation of the exercise bout using 2-ml heparinized syringes. A muscle sample ( $\sim$ 100 mg) was taken at rest and immediately after the exhaustive exercise bout using the needle biopsy technique with suction (5). The muscle tissue was immediately frozen in liquid nitrogen.

On experimental days, subjects reported to the laboratory 3 h after having consumed a light meal at the same time of day and without having performed strenuous physical exercise in the 72 h preceding the trials. Subjects did not smoke and take caffeine on the day of experiment and abstained from alcohol consumption 24 h before the experiments. To minimize diet-induced changes on muscle metabolism and exercise performance, subjects were instructed to consume an adequate amount of carbohydrate on the day before testing and to replicate the same dietary pattern on the day before any experimental trial. During the experimental period, subjects maintained their habitual lifestyle, ordinary daily food intake, and physical activity practices with respect to the criteria above.

# Blood Analysis

Immediately after sampling, blood was rapidly centrifuged at 20,000 g for 30 s. Thereafter the plasma was collected in Eppendorf tubes and stored at  $-20^{\circ}$ C until analyzed. Plasma [K<sup>+</sup>] was determined by an ion selective electrode using a Hitachi 912 Automatic Analyzer (Roche Diagnostic). Another part of the blood sample (100 µl) was hemolysed in an ice-cold 100-µl Triton X-100 and was later analyzed for lactate concentration using an YSI 2300 lactate analyzer (Yellow Spring Instruments, Yellow Springs, OH). The rest of the blood sample was immediately placed in ice-cold water until measured for pH (ABL 700, Radiometer, Copenhagen, Denmark).

#### Muscle Analysis

The frozen muscle biopsies were weighed before and after freezedrying to determine the water content for later metabolite measurements. After freeze-drying, the muscle samples were dissected free of blood, fat, and connective tissue under a stereomicroscope in a room with a temperature of 18°C and a relative humidity <30%. Part of the muscle tissue obtained from the biopsies taken before the trials was utilized for determination of the muscle characteristics (i.e., ion transport proteins, enzymes, fiber-type distribution, and capillarization).

*Muscle ion transport proteins.* Expression of the ion transport proteins was determined by Western blotting, as previously described (22). In brief,  $\sim 4-5$  mg dry wt of muscle tissue taken at rest were homogenized on ice in a fresh batch of buffer for not more than 30 s. Samples were centrifuged for 30 min at 17,500 g at 4°C, and the lysate was collected as the supernatant. Protein concentrations were determined in the lysates using bovine serum albumin (BSA) standards (Pierce Reagents).

The lysates were diluted to appropriate protein concentrations in a  $6 \times$  sample buffer (0.5 M Tris base, DTT, SDS, glycerol, and bromphenol blue), and equal amounts of total protein were loaded for each sample in different wells. Afterwards, the gel electrophoresis proteins were transferred to a polyvinylidene difluoride membrane. The membrane was incubated with primary antibody overnight and then washed in Tris-buffered saline-Tween before incubation with horseradish peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark) for 1 h. The primary antibodies used were as follows: monoclonal Na<sup>+</sup>-K<sup>+</sup> pump  $\alpha_1$  ( $\alpha$ 6F, Iowa Hybridoma Bank), monoclonal  $\alpha_2$  (McB2 kindly donated by K. J. Sweadner to H. Bundgaard), monoclonal  $\beta_1$  (MA3-930, Affinity BioReagents), monoclonal NHE1, polyclonal NKCC1 (Sc-21545, Santa Cruz Biotechnology), and polyclonal MCT1 and MCT4 (MAB3140, AB3538P, and AB3316P; Chemicon). The membrane staining was visualized by incubation with a chemiluminescent horseradish peroxidase substrate (Millipore), gel images were digitalized (KODAK Image Station 2000MM), and the net band intensities quantified as the total minus the background intensity (Fig. 1).

*Muscle enzymes.* For the determination of enzymatic activity,  $\sim 2$  mg dry wt of muscle tissue were homogenized (1:400) in a 0.3 M phosphate buffer adjusted to pH 7.7 containing 0.5 mg/ml of BSA. Creatine kinase activity was determined flurometrically on whole muscle homogenized in a tetraethylammonium-BSA buffer (28). Homogenates for phosphofructokinase were prepared in 100 mM of potassium buffer phosphate (pH 8.2) containing 10 mM of glutathione, 0.5 mM of ATP, 5 mM of MgSO<sub>4</sub>, and 30 mM of NaF. Citrate synthase and 3-hydroxyacyl-CoA dehydrogenase maximal activities



Fig. 1. Representative Western blots for the muscle membrane transport proteins. MCT, monocarboxylate transporters; NHE1, Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 1; NKCC1, Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> 1 protein cotransporters; sub, subunit. Arrows indicate the molecular mass of the detected proteins determined by the Precision Plus Protein Standards All Blue and Dual Color (Bio Rad).

were determined by using fluorometric methods with NAD-NADH coupled reactions (28).

*Muscle fiber-type distribution.* Immediately after the extraction, a portion of fresh muscle tissue was mounted in an embedded medium (OCT Compound Tissue-Tek, Sakura Finetek, Zoeterwoude, the Netherlands), frozen in isopentane that was cooled to the freezing point in liquid nitrogen, and stored at  $-80^{\circ}$ C until analyzed for fiber-type distribution and capillarization. Serial 10-µm-thick sections were cut at  $-20^{\circ}$ C and incubated for myofibrillar ATPase reactions at pH 9.4, after preincubation at pH 4.3, 4.6, and 10.3 (9). Based on the myofibrillar ATP staining, three different fiber types were defined [slow twitch (ST), FTa, and FTx] under light microscopy. The number of fibers was determined using the software program Tema version 1.04 by CheckVision, Denmark.

Muscle capillarization. Staining of capillaries was performed on 8-µm transverse sections of frozen skeletal muscle samples. The sections were fixed in 2% formaldehyde for 2 min at room temperature and at  $-20^{\circ}$ C acetone for 30 s. The sections were rinsed with phosphate-buffered saline containing 1% BSA and thereafter blocked with 1% PBS containing BSA. The sections were then incubated for 1 h at room temperature with primary monoclonal antibody, mouse α-CD31 (50 µg/ml; Clone JC70A, DAKO A/S) for detection of endothelial cells. The sections were rinsed and thereafter incubated with a biotin-coupled rabbit  $\alpha$ -mouse antibody (E0354; DAKO A/S). Antibody binding was visualized with ABC complex with alkaline phosphatase (ABC/Complex/AP, DAKO A/S). Negative controls were achieved with staining without the primary antibody. Immunoreactive cells were examined in a Zeiss Axioplan Microscope. The number of capillaries was determined using the software program Tema version 1.04 (CheckVision).

*Muscle metabolites, pH, and buffer capacity.* About 2 mg dry wt tissue were extracted in a solution of 0.6 M perchloric acid and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M KHCO<sub>3</sub><sup>-</sup> and stored at  $-80^{\circ}$ C until analyzed for lactate and creatine phosphate contents by a flurometric assay (28). Another  $\sim$ 1 mg dry wt muscle tissue was

#### PERFORMANCE AND MUSCLE CHARACTERISTICS

extracted in 1 M HCl and hydrolysed at 100°C for 3 h, and the glycogen content was determined by the hexokinase method (28).

Muscle pH was measured by a small glass electrode (XC 161, Radiometer-analytical) after homogenization of  $\sim 1$  mg freeze-dried samples in a nonbuffered solution containing 145 mM KCl, 10 mM NaCl, and 5 mM NaF (29). The muscle buffer capacity was measured in samples collected at rest. After having adjusted pH of the sample to 7.1 with 0.01 M NaOH, the sample was titrated to pH 6.0 by serial additions of 0.01 M HCl, followed by titration back to pH 7.1 by serial additions of 0.01 M NaOH. The pH was measured after each addition. The non-HCO<sub>3</sub><sup>-</sup> physiochemical buffer capacity was determined from the number of moles of H<sup>+</sup> required to change pH from 7.1 to 6.5 and was expressed as millimoles H<sup>+</sup> per kilogram dry weight per unit of pH (29).

#### Calculations

The net cycling mechanical efficiency was estimated as the ratio between work rate (external power output) and the corresponding net energy expenditure. Net energy expenditure was calculated by multiplying  $\dot{V}_{02}$  measured during the last minute of each work intensity minus the resting  $\dot{V}_{02}$  assumed to be 3.6 ml·min<sup>-1</sup>·kg<sup>-1</sup> (30), by the caloric equivalent corrected for the corresponding respiratory exchange ratio (10).

#### **Statistics**

Before using parametric techniques, assumptions of normality were verified using the Kolmogorov-Smirnov test. Possible differences in muscle metabolites were evaluated using a two-way repeated-measures ANOVA, with one between-factor (type of trial: 30 s, 3 min, 2 h) and one within-factor (sampling time: before, after). In case of significant main effects or interactions, data were subsequently analyzed using a Student-Newman-Keuls post hoc test. Correlation coefficients were determined and tested for significance using the Pearson product-moment correlation. The variance in exercise performances was examined by applying multiple regression analyses (best subsets regression). The coefficient of variation was calculated as the standard deviation of the repeated measures divided by the mean and multiplied by 100. A significance level of 0.05 was chosen. Data are presented as means  $\pm$  SE to provide an estimate of the population mean.

#### RESULTS

# Performance

The exercise time at power outputs of 600, 550, and 500 W was 28.6  $\pm$  4.2 (mean  $\pm$  SE) (range: 21–53), 44.2  $\pm$  5.9 (24–68), and 61.6  $\pm$  8.2 (41–106) s, respectively, and, at 395, 340, 315, and 270 W, it was 2.5  $\pm$  0.4 (1.2–4.1), 4.2  $\pm$  0.9 (1.5–7.1), 6.8  $\pm$  1.6 (1.9–13.3), and 21.1  $\pm$  7.7 (3.3–59) min, respectively, with large individual differences, as illustrated in Fig. 2. No relationship between the performance time at high (600 W) and low (270 W) workloads was observed.

#### Metabolic Response to Exercise

The metabolic response to the various exercise bouts is shown in Tables 2 and 3. At the end of the ~2-h experiment, plasma [K<sup>+</sup>] was lower (P < 0.05) than after the ~3 min and ~30 s experiment, being  $4.9 \pm 0.1$ ,  $5.4 \pm 0.3$ , and  $5.7 \pm 0.3$  mM, respectively. After ~2 h of cycling, muscle creatine phosphate content was higher (P < 0.05) than following ~30 s and ~3 min ( $64.9 \pm 2.9$  vs.  $48.8 \pm 6.1$  and  $23.6 \pm 6.3$  mmol/kg dry wt, respectively), and it was also higher (P < 0.05) after ~30 s compared with ~3 min. At the end of the



Fig. 2. Individual relationships (n = 7) between time to fatigue and power output during exhaustive cycle exercise. Each subject is represented by a symbol. The subjects maintained a pedaling frequency of 80–85 rpm, and a test was terminated when the frequency fell <75 rpm.

exercise, muscle lactate levels were higher (P < 0.05) after  $\sim 3$ min compared with after  $\sim 2$  h and  $\sim 30$  s ( $82.8 \pm 8.4$  vs.  $9.7 \pm 2.3$  and  $59.7 \pm 10.3$  mmol/kg dry wt, respectively) and also higher (P < 0.05) following  $\sim 30$  s compared with  $\sim 2$  h. At the end of exercise, muscle pH was lower (P < 0.05) in the  $\sim 3$ -min and  $\sim 30$ -s experiments compared with  $\sim 2$  h ( $6.76 \pm 0.08$  and  $6.90 \pm 0.07$  vs.  $7.18 \pm 0.04$ , respectively). Muscle glycogen content at the end of the  $\sim 2$ -h experiment was lower (P < 0.05) than after the  $\sim 3$ -min and  $\sim 30$ -s experiments ( $164 \pm 31$  vs.  $311 \pm 16$  and  $467 \pm 27$  mmol/kg dry wt, respectively), with glycogen level following  $\sim 30$  s being higher than after  $\sim 3$  min.

# Vo<sub>2max</sub>, Cycling Efficiency, and Performance

Vo<sub>2max</sub> [51.0 ± 3.6 (38.8−68.2) ml·kg<sup>-1</sup>·min<sup>-1</sup>] was related (P < 0.05) to exercise time at 395 W ( $r^2 = 0.74$ ), 340 W ( $r^2 = 0.62$ ), 315 W ( $r^2 = 0.83$ ), and 270 W ( $r^2 = 0.92$ ). Net mechanical efficiency was 22.5 ± 0.8 (19.6−24.8)% at 200 W and 23.7 ± 0.8 (20.8−25.5)% at 270 W. Mechanical efficiency at 200 and 270 W was related ( $r^2 = 0.65$ ; P < 0.05) to the performance time during an exercise bout, leading to exhaustion in ~2 h (~65% Vo<sub>2max</sub>).

# Relationship Between Muscle Characteristics, Metabolic Response to Exercise, and Performance

The capillary density [561  $\pm$  56 (439–813) capillaries/ mm<sup>2</sup>] was related (P < 0.05) to exercise time at 550 ( $r^2 = 0.60$ ), 500 ( $r^2 = 0.78$ ), 395 ( $r^2 = 0.80$ ) (Fig. 3A), 340 ( $r^2 = 0.60$ )

#### PERFORMANCE AND MUSCLE CHARACTERISTICS

	Exercise Type	Before	After
CP, mmol/kg dry wt	~30 s	93.2 ± 4.8 (73.0–106.9)	$48.8 \pm 6.1 (21.4 - 72.0)$ †
	$\sim$ 3 min	$90.1 \pm 3.7 (77.6 - 106.6)$	$23.6 \pm 6.3 (8.2 - 46.5)^{*\dagger}$
	$\sim 2$ h	$96.6 \pm 2.6 \ (85.7 - 105.0)$	$64.9 \pm 2.9 (49.0 - 75.2)$
Lactate, mmol/kg dry wt	~30 s	$4.6 \pm 0.4 (3.1 - 6.1)$	$59.7 \pm 10.3 (39.0 - 102.0)^{\dagger}$
	$\sim$ 3 min	$6.5 \pm 0.3 (5.3 - 7.8)$	82.8 ± 8.4 (55.7–104.6)*†
	$\sim 2$ h	$3.9 \pm 0.3 (3.1 - 4.9)$	9.7 ± 2.3 (4.5–19.2)
pH, $-\log [H^+]$	~30 s	$7.14 \pm 0.04$ (7.01–7.26)	$6.90 \pm 0.07 (6.59 - 7.18)^{+}$
	$\sim$ 3 min	$7.18 \pm 0.02 (7.08 - 7.23)$	$6.76 \pm 0.08 (6.56 - 7.07) * \dagger$
	$\sim 2$ h	$7.23 \pm 0.02 (7.15 - 7.30)$	$7.18 \pm 0.04$ (6.98–7.25)
Glycogen, mmol/kg dry wt	~30 s	$529 \pm 22$ (463–626)	$467 \pm 27 (381 - 595)$ †
	$\sim$ 3 min	$514 \pm 5 (499 - 533)$	$311 \pm 16 (289 - 378) * \dagger$
	$\sim 2$ h	503 ± 4 (470–565)	$164 \pm 31 (33 - 265)$

Table 2. Muscle metabolite content and pH in vastus lateralis before and immediately after an  $\sim$ 30-s,  $\sim$ 3-min, and  $\sim$ 2-h exhaustive cycling exercise

Values are means  $\pm$  SE (with ranges in parentheses); n = 7 subjects. CP, creatine phosphate; [H<sup>+</sup>], H<sup>+</sup> concentration. \*Significant difference (P < 0.05) from  $\sim$ 30-s exercise. †Significant difference (P < 0.05) from  $\sim$ 2-h exercise.

0.58), 315 ( $r^2 = 0.63$ ), and 270 W ( $r^2 = 0.73$ ). Relationships (P < 0.05) were also observed between the capillary-to-fiberratio [3.36 ± 0.49 (2.09–5.83)] and exercise time at 395 ( $r^2 = 0.58$ ), 315 ( $r^2 = 0.79$ ), and 270 W ( $r^2 = 0.85$ ). The capillary density was related (P < 0.05) to the mean net rate of plasma K<sup>+</sup> accumulation ( $r^2 = 0.68$ ) and muscle pH ( $r^2 = 0.80$ ) during and at the end of the ~3-min exercise (354 ± 25 W), respectively.

A relationship (P < 0.05) was also observed between the percentage of FTx fibers (16.1 ± 4.8; 6.5–37.5%) and the exercise time at 579 ± 21 W, leading to exhaustion in ~30 s ( $r^2 = 0.88$ ). Furthermore, the percentage of FTx fibers was related (P < 0.05) to muscle lactate levels ( $r^2 = 0.85$ ) and H<sup>+</sup> concentration ( $r^2 = 0.85$ ) at the end of the ~30-s exhaustive exercise, as well as to the rate of muscle H<sup>+</sup> accumulation during the exercise ( $r^2 = 0.75$ ).

Performance during the exercise bout leading to exhaustion in ~30 s was significantly (P < 0.05) related to the muscle lactate content at the end of the exercise ( $r^2 = 0.56$ ) and to the mean net rate of muscle H<sup>+</sup> accumulation during the exercise ( $r^2 = 0.60$ ), as well as inversely related to the muscle pH at the end of the exercise ( $r^2 = 0.77$ , Fig. 3B). The expression of the Na<sup>+</sup>-K<sup>+</sup> pump  $\alpha_1$ -subunit was inversely related (P < 0.05) to the venous [K<sup>+</sup>] at the end of the ~30-s exercise ( $r^2 = 0.77$ ). Furthermore, performance during the exercise bout leading to exhaustion in ~30 s tended to be inversely related (P = 0.06) to the mean net rate of venous

Table 3. Blood metabolite concentrations and pH before and after an  $\sim$ 30-s,  $\sim$ 3-min, and  $\sim$ 2-h exhaustive cycling exercise

	Exercise Type	Before	After
Lactate, mM	~30 s	$0.8 \pm 0.1$	$2.7 \pm 0.4$
	$\sim 3 \min$	$0.7 \pm 0.1$	$8.1 \pm 0.5^{*}$
	$\sim 2 h$	$0.9 \pm 0.1$	$2.4 \pm 0.4$
pH	~30 s	$7.40 \pm 0.01$	$7.36 \pm 0.01$
-	$\sim 3 \min$	$7.38 \pm 0.01$	$7.19 \pm 0.02*$ †
	$\sim 2 h$	$7.38 \pm 0.02$	$7.42 \pm 0.02$
K <sup>+</sup> , mM	~30 s	$4.0 \pm 0.1$	$5.7 \pm 0.3 \ddagger$
	$\sim 3 \min$	$3.9 \pm 0.1$	$5.4 \pm 0.3*$
	$\sim 2 h$	$4.1 \pm 0.1$	$4.9 \pm 0.1$

Values are means  $\pm$  SE; n = 7 subjects. \*Significant difference (P < 0.05) from  $\sim$ 30-s exercise. †Significant difference (P < 0.05) from  $\sim$ 2-h exercise.

plasma K<sup>+</sup> accumulation during the exercise ( $r^2 = 0.52$ ). The citrate synthase activity was correlated (P < 0.05) to the performance time (21.1 ± 7.7 min) at 270 W ( $r^2 = 0.67$ ).



Fig. 3. A: individual relationship between capillary density and performance time during exhaustive cycling exercise at 395 W ( $\sim$ 136 ± 9.5% maximum oxygen uptake; 2:27 ± 0:25 min/s).  $r^2 = 0.80$ , P < 0.05; y = 0.0066x -1.2445. B: individual relationship between performance time during a very high-intensity exhaustive cycling exercise (579 ± 21 W;  $\sim$ 196 ± 8% maximum oxygen uptake; 0:29 ± 0:06 min/s) and muscle pH at the end of the exercise.  $r^2 = 0.77$ , P < 0.05; y = -67.746x + 496.07.

# 1560

# Multiple-regression Analysis

The capillary density was calculated to account for 50% (P < 0.05) of the variability during the 600-W performance (exercise time ~30 s). Muscle FTx fibers and creatine kinase activity contributed with an additional 30% (P = 0.07) and 12%, respectively (Table 4). The capillary density was estimated to explain 60–80% (P < 0.05) of the total variance observed in exercise performances lasting ~1–3 min, with the Na<sup>+</sup>-K<sup>+</sup> pump  $\beta_1$ -subunit expression accounting for an extra ~15% (P < 0.05) (Table 4). Over 80% (P < 0.05) of the variance in performances at 270 and 315 W (exercise time >4 min) was related to  $\dot{Vo}_{2max}$ , whereas the other 6–13% (P < 0.05) could be explained by cycling efficiency (Table 4).

# DISCUSSION

The subjects in the present study showed a broad range of performance abilities, as well as cardiorespiratory and skeletal muscle characteristics, which allowed for an evaluation of the importance of several variables for performance at different exercise intensities. The major findings of the present study were that significant relationships were observed between capillarization and the time to exhaustion during intense cycling bouts, ranging from 270 to 395 W (~93-135% Vo<sub>2max</sub>; 21-2.5 min). Furthermore, the capillary density was found to account for 50-80% of the variance observed in high-intensity exercise performances leading to exhaustion from 30 s to  $\sim$ 3 min, whereas the amount of the  $Na^+$ -K<sup>+</sup> pump  $\beta_1$ -subunit expressed in the muscle could explain an additional 13-34% for exhaustive exercise lasting  $\sim 1-4$  min. The percentage of FTx fibers also appears to be of importance during performance in an  $\sim$ 30-s exhaustive bout.

The number of muscle capillaries has often been positively associated with performance during low-intensity exercise (13, 40). Surprisingly, the present study also showed that the capillary density was the most dominant factor for exercise intensities leading to exhaustion within  $\sim$ 3 min, accounting for 80-50% of the total variability in whole body exercise performances at 395 and 600 W ( $\sim$ 135–205% Vo<sub>2max</sub>), respectively. In addition, the capillarization was significantly correlated to the time to exhaustion during intense cycling bouts lasting  $\sim$ 1–20 min. These findings are in accordance with those of Jensen et al. (23), who reported an increased muscle capillarization and enhanced performance during an ~3-min exercise bout in response to intense intermittent exercise training. A high capillary density is likely to lead to a shorter diffusion distance between capillaries and muscle fibers, as well as to a longer blood transit time and larger area available for diffusion. This may favor Vo<sub>2</sub> and release of compounds such as K<sup>+</sup> and H<sup>+</sup> from the extracellular space and, therefore, contribute to the elevated performance. In support, the capillary density was related to the rate of muscle H<sup>+</sup> decrease in recovery from the  $\sim$ 30 s exercise (data not shown) and to the mean net rate of plasma K<sup>+</sup> accumulation during the ~3-min exhaustive exercise bout. Together, these results indicate that angiogenesis observed with training is important for performance improvements, even during intense exercise.

Another novel finding of the present study was that 13–34% of the variance observed in exercise performances lasting  $\sim 1-4$  min was related to the expression of the Na<sup>+</sup>-K<sup>+</sup> pump  $\beta_1$ -subunit, which may reflect the number of functional skeletal

I auto 4.	Variance m	EXELLI	se perjur	ווומוורב												
									Physiological Ch	aracteristics	, %					
												Muscle n	nembrane transpo	ort proteins		
intensity, W	Duration, min/s	V <sub>O2max</sub>	Efficiency at 200 W	Efficienc at 270 V	y Fiber type V ST FTa F	Tx capillar	Capilla ies densit	ry Mus y CK Pl	scle enzymes FK CS HAD	Buffer capacity	$Na^+-K^+$ pump $\alpha_1$ -subunit	$Na^+-K^+$ pump $\alpha_2$ -subunit	$\frac{Na^{+}-K^{+}}{\beta_{1}-subunit}$	NKCC1 MCT1	MCT4 N	VHE1
600	$0:29 \pm 0:04$					30	50*	12								
550	$0:44 \pm 0:06$						*09						13*			24*
500	$1:02 \pm 0:08$				8*		78*						$14^{*}$			
395	$2:27 \pm 0:25$	5					80*						11			
340	$4:09 \pm 0.53$	62*				1							34*			
315	$6:50 \pm 1:36$	83*	$13^{*}$		ю											
270	$21:04 \pm 7:42$	92*		6*					1							
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Values are the percentage of the three most dominant factors that explain the total variance in performance at each workload. ST, slow twitch; FT, fast twitch; NKCC1, Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> 1 protein cotransporters; < 0.05MCT, monocarboxylate transporters; NHE1, Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 1. \*Statistical significance, P muscle Na<sup>+</sup>-K<sup>+</sup> pumps during exercise. This is in line with our laboratory's previous finding where the expression of the  $Na^+-K^+$  pump  $\beta_1$ -subunit after a period of speed endurance training was related to the capacity to perform short-term exhaustive and repeated intense exercise (22). Similarly, Mohr et al. (34) observed elevated levels of  $\beta_1$ -subunit in association with a reduced time to perform repeated sprints following a speed training program. A high Na<sup>+</sup>-K<sup>+</sup> pump activity protects against the depressive effect that contraction-induced disturbances in ion homeostasis exert on cell excitability and thereby may be of importance for preserving muscle force production during intense exercise. This effect could be mediated by either a higher  $K^+$  reuptake rate, leading to reduced extracellular  $K^+$ accumulation-induced depolarization, or by the increased  $Na^+-K^+$  pump activity per se, which would hyperpolarize the membrane potential due the electrogenity of the Na<sup>+</sup>-K<sup>+</sup> pump (41). Accordingly, in the present study, it was observed that the expression of the Na<sup>+</sup>-K<sup>+</sup> pump  $\alpha_1$ -subunit was inversely related to the venous  $[K^+]$  at the end of the ~30-s exhaustive bout, and performance tended to be related to a reduced rate of venous K<sup>+</sup> accumulation during the exercise. In agreement, a number of training studies have shown marked performance improvements during intense exercise, in association with elevated Na<sup>+</sup>-K<sup>+</sup> pump levels and reduced muscle interstitial or venous K<sup>+</sup> accumulation (17, 18, 22, 31, 34, 36). Taken together, these findings do support a role of the  $Na^+-K^+$  pump in delaying fatigue during intense exhaustive exercise lasting less than  $\sim$ 4 min. In addition, 30% of the variance in the  $\sim$ 30-s exhaustive cycling bout was related to the number of FTx fibers, indicating that a high composition of FTx fibers may play a role in maximal exercise efforts as also supported by other studies (12, 19).

The capacity of human skeletal muscles to delay the decline in pH has been suggested to be important for performance during high-intensity exercise (2). The muscle content of the lactate transporter MCT1 has been observed to be positively correlated to the maximal work performed during an exhaustive trial at 120% of Vo<sub>2max</sub> (32) and inversely related to the fatigue index during a 1-min all-out cycling event (48). In the present study, no relationship was observed between MCT protein expression and performance at high exercise intensities. This observation is in agreement with our laboratory's previous findings that endurance-trained runners after 4 and 8 wk of speed endurance training enhanced their high-intensity work capacity without concomitant changes in MCT protein expression (1, 22). On the other hand, the expression of NHE1 accounted for  $\sim 24\%$  in exercise performance of  $\sim 45$  s (Table 4). Besides being involved in pH regulation, a greater amount of NHE1 may result in an elevated Na<sup>+</sup> uptake inside the muscle cell, which, possibly via greater activation of the  $Na^+-K^+$  pump (16), would hyperpolarize the membrane potential, allowing a longer time to exhaustion during intense exercise. Other pH-regulatory membrane proteins influence performance during high-intensity exercise. Recently, some cellular carbonic anhydrase isoforms were reported to be positively correlated to the total amount of work performed during supramaximal exercise (32) and were suggested to have a mitigating role in muscle fatigue development (42). However, these were not investigated in the present study.

Relationships were observed between  $Vo_{2max}$  and performance at exercise intensities between 315 and 395 W, indicat-

ing that the oxygen supply to the contracting muscles plays a role during intense fatiguing exercise lasting  $\sim 2-8$  min. This observation is in line with findings that the aerobic energy system contributes around two-thirds of the energy production during an  $\sim$ 2-min whole body exhaustive exercise (43), and that, in the last minute, Vo<sub>2max</sub> drops (47) in association with impaired cardiac function and enhanced local vasoconstriction (35). On the other hand, in the present investigation, multipleregression analysis revealed a limited importance of Vo<sub>2max</sub> on intense exercise performances lasting less than  $\sim 4$  min, which is in agreement with training studies showing unchanged Vo<sub>2max</sub> values, despite marked performance improvements during intense exercise (6, 14, 20, 22). For the exhaustive exercises lasting longer than  $\sim$ 4 min, time to fatigue was correlated with Vo<sub>2max</sub>, which accounted for 62–92% of the total variance in performance. These findings confirm that a high Vo<sub>2max</sub> is important for medium- to long-term performance (13, 43, 44).

Generally, performance at the various exercise intensities is determined by different mechanisms, as shown in Fig. 2, whereby subjects with superior performance at high work intensities are not performing as well as others at low workloads. Such differences are further illustrated in Table 5, which shows a representative comparison between three subjects. *Subjects 1* and 2 had the same performance during short-term exercise, whereas *subject 1* had a much better medium-longterm performance, which was associated with a significantly higher  $Vo_{2max}$  and oxidative enzyme activity, as well as more capillaries and ST fibers. *Subject 3* had a better performance during short-term (<3 min) exercise than *subjects 1* and 2,

 Table 5. Exercise performances and anthropometric,
 physiological, and muscle characteristics of three subjects

	Subject 1	Subject 2	Subject 3
Age and anthropometric characteristics			
Age, yr	32	21	26
Height, cm	188	170	179
Body mass, kg	83.5	63.5	91
Exercise performances			
600 W, s	26	26	53
550 W, s	54	34	68
500 W, s	65	44	106
395 W, min	3.5	1.2	4.1
340 W, min	5.8	1.5	7.1
315 W, min	13.3	1.9	10.0
270 W, min	59.2	3.3	35.9
Physiological characteristics			
$\dot{V}_{O_{2}max}$ , ml·kg <sup>-1</sup> ·min <sup>-1</sup>	68.2	50.6	53.9
Heart rate maximum, beats/min	178	203	193
Peak power output, W	911	810	1,500
Mean power output, W	617	587	779
Net cycling efficiency 200 W, %	21.7	22.1	19.6
Net cycling efficiency 270 W, %	20.8	25.4	21.8
Muscle characteristics			
Fiber type I, %	85.5	48.7	38.1
Fiber type IIa, %	7.8	21.5	24.4
Fiber type IIx, %	6.7	29.8	37.5
Capillary-to-fiber ratio	5.83	2.09	4.13
Capillary density, capillaries/mm <sup>2</sup>	730	458	813
CK, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	4,224	4,064	4,048
PFK, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	107	184	163
CS, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	52	30	37
HAD, $\mu$ mol $\cdot$ g <sup>-1</sup> $\cdot$ min dry wt <sup>-1</sup>	52	27	29
Buffering capacity, mmol H <sup>+</sup>			
·kg dry wt <sup>-1</sup> ·pH unit <sup>-1</sup>	157	142	151

which was related to a higher peak power output and more FT fibers. On the other hand, for exercise lasting >10 min, *subject* 3 performed poorer than *subject* 1, who had a higher  $\dot{V}o_{2max}$ , more ST fibers, and greater oxidative enzyme activity. Despite having less relative number of ST fibers, *subject* 3 had a better long-term performance than *subject* 2, which may be partially due to a higher  $\dot{V}o_{2max}$ . These findings suggest that exercise performance at different intensities is determined by a complex interplay between various key factors.

The number of subjects in the present investigation was limited to seven due to the invasive and time-consuming nature of the experiment, which needs to be taken into account when interpreting the results. On the other hand, the conclusions of the present study are only based on the significant correlations observed and, therefore, appear valid. It should also be emphasized that only men were included, and it is unclear whether similar relationships would be obtained for women. It may be argued that the subjects changed their training status over the course of the investigation, but no differences in performance were detected between the same exhaustive trials carried out at the beginning and at the end of the experimental period for any of the individuals.

In summary, the present study showed that muscle capillarization and the expression of the Na<sup>+</sup>-K<sup>+</sup> pump  $\beta_1$ -subunit are influential determinants for performance during high-intensity exhaustive exercise lasting from 30 s to 4 min. A large relative distribution of FTx fibers seems to be of importance during very intense efforts, i.e., ~30 s.

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#### DISCLOSURES

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

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