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Effect of high-intensity intermittent training on lactate and H^+ release from human skeletal muscle

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Juel, Carsten, Christina Klarskov, Jens Jung Nielsen, Peter Krustrup, Magni Mohr, and Jens Bangsbo. Effect of high-intensity intermittent training on lactate and H^+ release from human skeletal muscle. *Am J Physiol Endocrinol Metab* 286: E245–E251, 2004. First published October 14, 2003; 10.1152/ajpendo.00303.2003.—The study investigated the effect of training on lactate and H^+ release from human skeletal muscle during one-legged knee-extensor exercise. Six subjects were tested after 7–8 wk of training (fifteen 1-min bouts at ~150% of thigh maximal O_2 uptake per day). Blood samples, blood flow, and muscle biopsies were obtained during and after a 30-W exercise bout and an incremental test to exhaustion of both trained (T) and untrained (UT) legs. Blood flow was 16% higher in the T than in the UT leg. In the 30-W test, venous lactate and lactate release were lower in the T compared with the UT leg. In the incremental test, time to fatigue was 10.6 ± 0.7 and 8.2 ± 0.7 min, respectively, in the T and UT legs ($P < 0.05$). At exhaustion, venous blood lactate was 10.7 ± 0.4 and 8.0 ± 0.9 mmol/l in T and UT legs ($P < 0.05$), respectively, and lactate release was 19.4 ± 3.6 and 10.6 ± 2.0 mmol/min ($P < 0.05$). H^+ release at exhaustion was higher in the T than in the UT leg. Muscle lactate content was 59.0 ± 15.1 and 96.5 ± 14.5 mmol/kg dry wt in the T and UT legs, and muscle pH was 6.82 ± 0.05 and 6.69 ± 0.04 in the T and UT legs ($P = 0.06$). The membrane contents of the monocarboxylate transporters MCT1 and MCT4 and the Na^+/H^+ exchanger were 115 ± 5 ($P < 0.05$), 111 ± 11, and 116 ± 6% ($P < 0.05$), respectively, in the T compared with the UT leg. The reason for the training-induced increase in peak lactate and H^+ release during exercise is a combination of an increased density of the lactate and H^+ transporting systems, an improved blood flow and blood flow distribution, and an increased systemic lactate and H^+ clearance.

monocarboxylate transporter proteins 1 and 4; Na^+/H^+ exchange; Na^+/H^+ exchanger protein 1

DURING INTENSE EXERCISE, lactate and H^+ accumulate in the contracting muscle. The accumulated lactate is either removed by oxidation or gluconeogenesis in the muscle or is transported to the blood and removed by other cells (6, 14). The fluxes of lactate and H^+ across the muscle membrane are facilitated by two monocarboxylate transporter proteins, MCT1 and MCT4 (21). These transporters contribute to the pH regulation during intense muscle activity, whereas pH regulation at rest is mainly dependent on an efflux of H^+ mediated by Na^+/H^+ exchange or transport systems involving bicarbonate (11, 12). However, it has been demonstrated that the H^+ transport systems not involving lactate are also further activated during muscle activity and in the recovery phase after exercise (4).

Training induces numerous adaptive changes in skeletal muscle. A few reports have described some of these changes in lactate transporters at the protein level. The density of MCT1 and MCT4 in human skeletal muscle has been demonstrated to be elevated after a period of endurance training (7), high-intensity training (5, 16), and a single prolonged exercise bout (9). In the study by Pilegaard et al. (16), the training-induced elevation in MCT density was associated with a reduction in muscle lactate during exercise. The effect of training on the Na^+/H^+ exchanger in muscle is less investigated. One study in rats has demonstrated that the amount of the Na^+/H^+ exchanger proteins is elevated after a period of high-intensity treadmill training (13), but no studies have examined the effect of training on the Na^+/H^+ exchanger in human skeletal muscle.

It is hypothesized that intense training increases the release of H^+ and lactate from muscle, and that these changes are related to an increased expression of the MCT and Na^+/H^+ exchanger proteins.

MATERIALS AND METHODS

Subjects. Six healthy male subjects participated in the study. Age, height, weight, and maximal O_2 uptake ($\dot{V}O_{2\,max}$) before training were 25.3 ± 2.9 (±SD) yr, 185.0 ± 3.9 cm, 82.8 ± 11.8 kg, and 50.2 ± 1.2 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$, respectively. The subjects were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate. The study conforms to the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of the Copenhagen and Frederiksberg Communities, Denmark.

Training. Training was performed for 7–8 wk with one (T) leg, whereas the untrained (UT) leg served as control. Before training, the subjects were familiarized to the one-legged knee extensor model, and measurements were carried out to confirm that the work was only performed with m. quadriceps femoris (2). The leg to be trained was selected randomly. At least two incremental tests to fatigue were performed with each leg to determine the initial load in the training period. The subjects performed one-legged knee extensor exercise (60 kicks/min) on an ergometer in a supine position. Each training session consisted of a 5-min warm-up at a load of 10 W, 3 min of rest, and fifteen 1-min exercise bouts at ~150% of thigh $\dot{V}O_{2\,max}$ separated by 3 min of rest. The training was performed 3 times/wk in *weeks 1* and *2*, 4 times/wk in *weeks 3* and *4*, and 5 times/wk for the final weeks. The workload was adjusted to keep the relative load constant.

Main experiment. Muscle mass was determined before and after training from thigh length, three circumferences of the thigh, and skin fold thickness.

An identical protocol was carried out for T and UT legs on separate days. The subject rested in the supine position, and a catheter was placed in the femoral vein under local anesthesia. The tip of the catheter was positioned ~1–2 cm proximal to the inguinal ligament. This catheter was used for femoral venous blood sampling. Through the catheter a thermistor was placed and advanced 8–10 cm proximal to the tip of the catheter for measurement of femoral venous blood

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flow by the constant infusion-thermodilution technique. Briefly, ice-cold saline was infused at a constant rate for 10–15 s, and leg blood flow could be calculated from the temperature decrease. An occlusion cuff placed below the knee was inflated during exercise to avoid contribution of blood from the lower leg. A second catheter was inserted in the femoral artery of the resting leg.

Each protocol consisted of warm-up and 30 min of submaximal exercise at 30 W, which makes up ~28% of the final load (106 ± 9 W) obtained in the incremental test. After 60 min of rest, exercise was performed at 10 W, followed by 10 min of rest and an incremental test starting with 50 W for 4 min; subsequently, the load was increased every 2 min by 10 W until exhaustion. Time to fatigue was defined as the time when the kicking frequency reached below 55 rpm. Heparinized syringes were used to draw blood simultaneously from the femoral artery and the vein. The blood samples were placed on ice-cold water.

Blood analysis. Plasma pH, plasma HCO_3^-, whole blood hemoglobin, and hematocrit were measured in an ABL 615 blood analyzer (Radiometer Denmark). Actual base excess (ABE) for oxygenated blood was calculated from plasma HCO_3^-, blood pH, and hemoglobin according to the method of Siggaard-Andersen (19), and total H^+ release was calculated from blood flow and the venous-arterial (v-a) concentration difference for ABE, which was corrected for the changes in buffer capacity due to various levels of hemoglobin oxygenation. A 100-μl sample of each blood sample was treated with 20 g/l of Triton X-100 and used for determination of whole blood lactate on a YSI (Yellow Spring Instruments) lactate analyzer.

Muscle analysis. The needle biopsy technique was used to obtain muscle samples from the vastus lateralis. Muscle biopsies were obtained at rest before training, after 2 and 4 wk of training, as well as after the training period (7–8 wk). In the main experiment, biopsies were obtained before and immediately after exercise in the incremental test.

The samples were immediately frozen in liquid nitrogen and stored at −80°C. One part of each muscle sample was used with the Western blotting technique to measure membrane proteins and for fiber-type determination. The rest was freeze-dried, and water content was determined. One part was homogenized and used for pH determination with a small glass electrode; another part was extracted with 0.6 M perchloric acid, neutralized, and used for fluorometric lactate determination (in mmol/kg dry wt).

Western blot. Approximately 30 mg of each muscle sample were homogenized in a sucrose buffer (250 mM sucrose, 30 mM HEPES, 2 mM EGTA, 40 mM NaCl, and 2 mM PMSF, pH 7.4) with a Polytron 2100 and centrifuged at 1,000 *g* for 5 min; this procedure removes heavy material, including a fraction of the mitochondria. The pellet was used for determination of fiber types on the basis of the distribution of myosin heavy-chain (MHC) isoforms by SDS-PAGE (1, 19). The supernatant was spun at 190,000 *g* for 90 min at 4°C, the new pellet was resuspended in Tris-SDS (10 mM Tris, 4% SDS, 1 mM EDTA, and 2 mM PMSF, pH 7.4), and protein content was determined with a BSA standard (DC protein assay, Bio-Rad). Ten micrograms of protein from each sample were subjected to SDS-PAGE (Excell 8–18% gradient gel) and electroblotted to a Millipore Immobilon-P polyvinylidene difluoride membrane. The membrane was blocked by BSA, 0.5% Tween 20, and low-fat milk and was incubated with the primary antibody diluted in a BSA-containing buffer. After treatment with the horseradish peroxidase-coupled secondary antibody and repeated washing, the membrane was incubated with enhanced chemiluminescence reagent (Amersham) and visualized on film. The quantification of protein was performed by scanning the film and analyzing band intensities with SigmaGel software. Samples from the same subject were always run on the same gel and with identical amounts of protein per lane.

The Na^+/H^+ exchanger isoform NHE1 was detected by the antibody MAB3080 (Chemicon). The antibodies to the human lactate/H^+ cotransporter isoforms MCT1 and MCT4 were a gift from Professor A. Halestrap (Bristol, UK) (21).

Calculations. The concentration of lactate in cell water was calculated from muscle lactate (mmol/kg dry wt) and muscle water content (%) and was corrected for extracellular lactate, with the assumption of an interstitial space of 15%. The concentration gradient for lactate from muscle to blood at exhaustion from the incremental test was calculated as the difference between the intracellular muscle lactate concentration (mmol/l cell water) and the arterial plasma lactate concentration. The H^+ gradient was calculated from the H^+ concentration in muscle (pH in the homogenate was chosen to represent intracellular pH) and in the arterial blood. Release of lactate and H^+ was calculated from the v-a differences and blood flow.

Statistics. Two-way analysis of variance (ANOVA) was used for comparison between data sets from T and UT legs. Student's paired *t*-test was used to locate the differences if data passed a normality test; otherwise, a Wilcoxon rank test was used. One-way repeated-measures ANOVA was used to test the changes in protein content. The differences were located using the Tukey test (SigmaStat software). A significance level of $P = 0.05$ was chosen.

RESULTS

Muscle mass. The thigh muscle mass in the T leg increased ($P < 0.05$) by 4.5% (from 2.44 ± 0.13 to 2.55 ± 0.13 kg) during the training period, whereas the muscle mass of the UT leg remained unaltered.

Fiber-type distribution. The myosin heavy-chain fiber-type distribution after training in T and UT legs was 44/47/9 and 44/50/6% MHC-I/MHC-IIa/MHC-IIx, respectively.

Performance. The workload increased ($P < 0.05$) during the training period from 92 ± 8 to 106 ± 9 W. The time to fatigue in the incremental test was longer ($P < 0.05$) in the T leg than in the UT leg (10.6 ± 0.7 vs. 8.2 ± 0.70 min).

Leg blood flow. In the 30-W test, leg blood flow reached the peak value of ~3.5 l/min at ~3 min after onset of exercise, after which the flow remained constant in both T and UT legs.

In the incremental test, thigh blood flow increased with increasing workload with no differences between T and UT legs. However, the peak blood flow was higher ($P < 0.05$) in the T leg than in the UT leg (7.1 ± 0.5 vs. 5.7 ± 0.6 l/min). In the first sample in recovery (~5 s from exhaustion), leg blood flow was still higher ($P < 0.05$) in the T leg (6.7 ± 1.0 l/min) than in the UT leg (5.7 ± 0.3 l/min). Later in recovery, blood flow was gradually reduced toward the resting level without any difference between T and UT legs.

Blood lactate and lactate release. In the 30-W test, femoral venous blood lactate increased rapidly at onset of exercise and reached a lower ($P < 0.05$) peak value (1.5 ± 0.2 mmol/l) in the T leg than in the UT leg (2.0 ± 0.3 mmol/l) after 3 min, after which blood lactate declined toward resting level at the end of the exercise period (Fig. 1*A*).

The v-a concentration difference reached the peak value 3 min after onset of exercise and declined to zero at the end of the exercise period (Fig. 2*A*). In the first 5 min of exercise, the v-a lactate concentration difference was lower ($P < 0.05$) in the T leg than in the UT leg (Fig. 2*A*). The peak thigh lactate release was 2.1 ± 0.5 mmol/min in the T leg and 3.2 ± 0.5 mmol/min in the UT leg ($P < 0.05$) during the 30-W exercise test. Lactate release decreased toward the end of exercise and was only slightly higher at 30 min than at rest (Fig. 3*A*).

In the incremental test, blood lactate increased with increasing power output (Fig. 1*B*). At exhaustion, the femoral venous

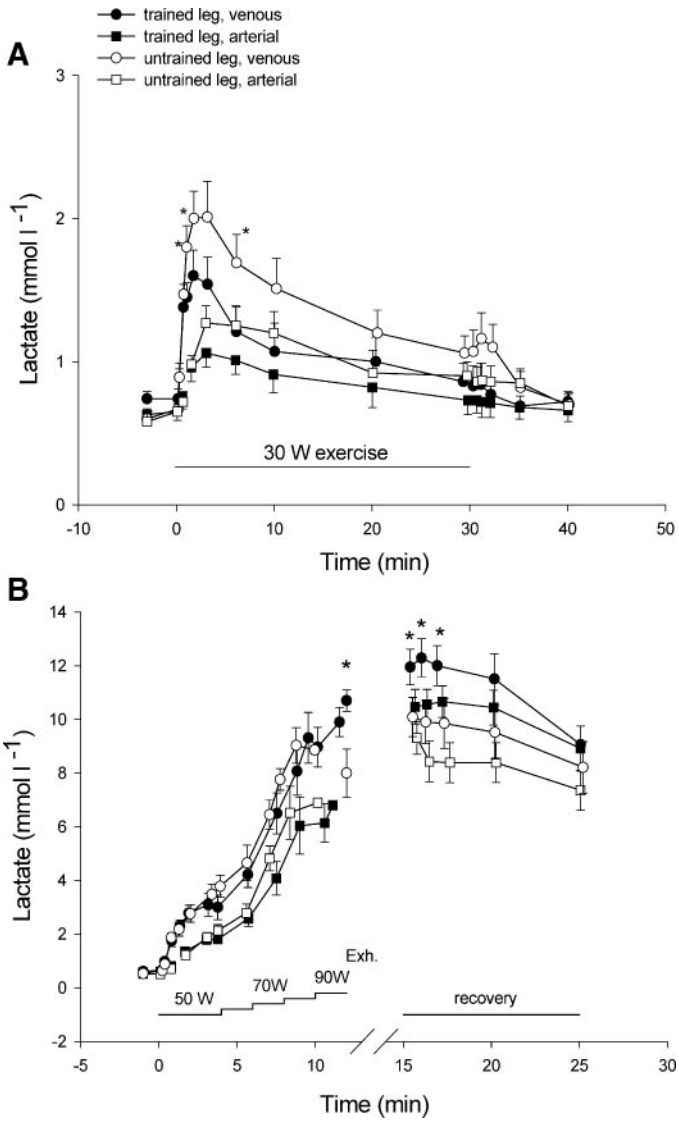

Fig. 1. Arterial and venous blood lactate in the 30-W test (*A*) and the incremental test (*B*). Because of the individual time to fatigue, not all subjects were represented in the last 3 mean values of the incremental test, whereas the mean value (Exh) is calculated as mean of all individual values at exhaustion ($n = 6$). Mean values from trained and untrained legs were significantly (*$P <$ 0.05) different.

blood lactate was higher ($P < 0.05$) in the T leg than in the UT leg (10.7 ± 0.4 vs. 8.0 ± 0.9 mmol/l). Also, in the first part of recovery, venous blood lactate was higher ($P < 0.05$) in the T compared with the UT leg (Fig. 1*B*). The v-a lactate concentration difference at exhaustion was 3.2 ± 0.5 and 1.9 ± 0.3 mmol/l in T and UT legs, respectively ($P < 0.05$), whereas no differences between T and UT legs were observed in recovery (Fig. 2*B*). Lactate release increased with increasing exercise intensity (Fig. 3*B*). At exhaustion, lactate release was greater ($P < 0.05$) in the T leg than in the UT leg (19.4 ± 3.6 vs. 10.6 ± 2.0 mmol/min), whereas no difference between T and UT legs was observed 5 s into recovery (9.3 ± 1.2 and 7.9 ± 1.1 mmol/min, respectively). In both T and UT legs, lactate release decreased toward zero during the 10-min recovery period.

Blood pH. At onset of the 30-W exercise, femoral venous blood pH declined rapidly and reached the lowest value after 3 min, when it then increased slowly during the rest of the exercise (Fig. 4*A*). However, venous blood pH was still reduced after 30 min. Arterial blood pH did not change during exercise.

In the incremental test, venous blood pH was gradually reduced during exercise (Fig. 4*B*). At exhaustion, venous blood pH was lower ($P < 0.05$) in the T leg than in the UT leg (7.07 ± 0.02 vs. 7.13 ± 0.02). Also, in the first part of recovery, venous blood pH was lower ($P < 0.05$) in the T leg compared with the UT leg (Fig. 4*B*).

H^+ release. The H^+ release in the 30-W exercise test increased rapidly at onset of exercise and reached a plateau after 3 min, which lasted for the rest of the 30-min exercise period. The peak H^+ release (10–11 mmol/min) was not different in T and UT legs (Fig. 5*A*).

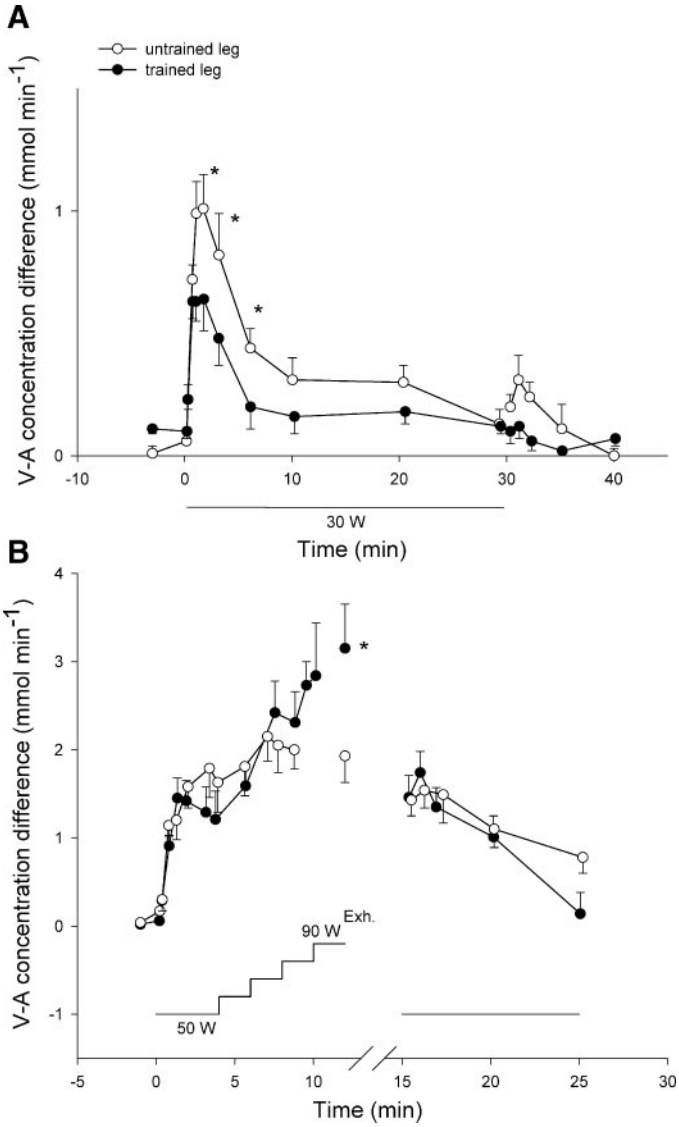

Fig. 2. Venous-arterial (v-a) lactate concentration difference in the 30-W test (*A*) and the incremental test (*B*). Because of individual times to fatigue, not all 6 subjects were represented in the last 3 mean values of the incremental test. Mean values from trained and untrained legs were significantly (*$P < 0.05$) different.

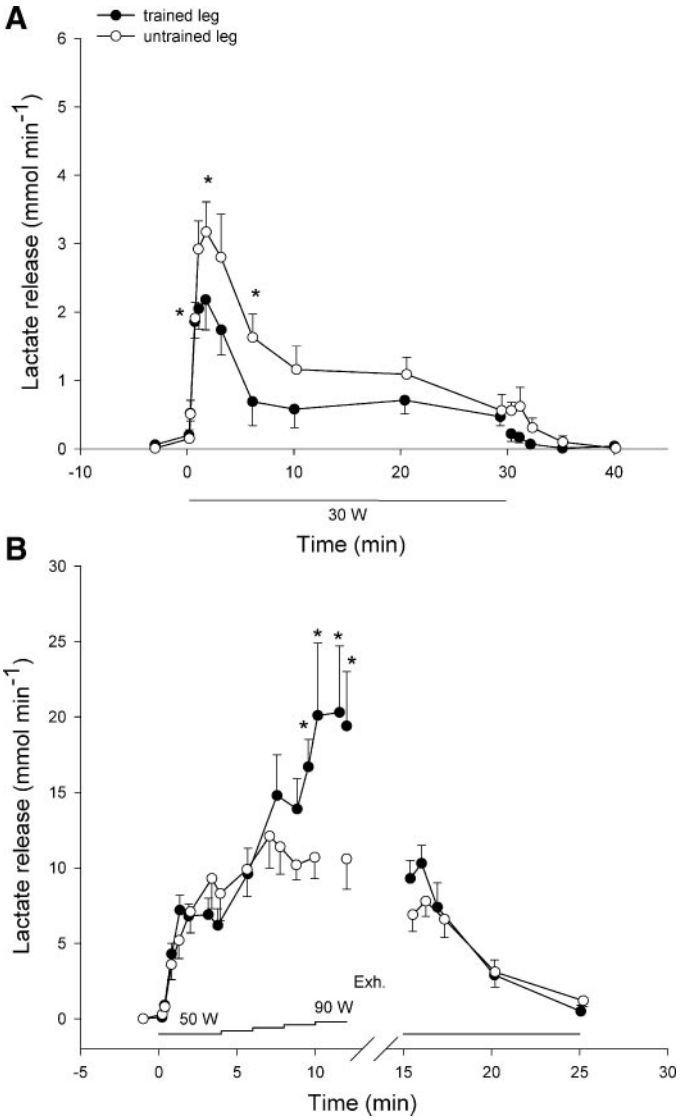

Fig. 3. Lactate release in the 30-W test (*A*) and the incremental test (*B*). Because of individual times to fatigue, not all 6 subjects were represented in the last 3 mean values of the incremental test. Mean values from trained and untrained legs were significantly (*$P < 0.05$) different.

In the incremental test, H^+ release (Fig. 5*B*) increased gradually with exercise intensity and reached 36.9 ± 3.1 and 24.2 ± 1.5 mmol/min at exhaustion in the T and UT legs, respectively ($P < 0.05$). H^+ release was also higher ($P < 0.05$) in the T leg compared with the UT leg in the first two measurements (5 and 75 s) in recovery.

Muscle lactate and muscle pH. Muscle lactate after the 30-W exercise bout was 8.4 ± 1.7 and 5.9 ± 1.0 mmol/kg dry wt in the T and UT legs, respectively. At exhaustion in the incremental test, muscle lactate in the T and UT legs was 59.0 ± 15.1 and 96.5 ± 14.5 mmol/kg dry wt, respectively, corresponding to 22.3 and 29.8 mmol/l of muscle water ($P = 0.06$).

Muscle pH at the end of the 30-W exercise test was 7.13 ± 0.04 in the T leg and 7.09 ± 0.03 in the UT leg. At exhaustion in the incremental test, muscle pH was 6.82 ± 0.05 in the T leg and 6.69 ± 0.04 in the UT leg.

Muscle-to-blood gradients for lactate and H^+. In the incremental test, the muscle-to-blood lactate gradient at exhaustion was 15.3 ± 4.4 mmol/l in the T leg and 23.5 ± 4.5 mmol/l in the UT leg ($P = 0.1$). Figure 6*A* shows the individual relationship between lactate release and the muscle-to-blood lactate gradient.

The H^+ gradient from muscle to arterial blood at exhaustion was lower ($P < 0.05$) in the T than in the UT leg [101 (range 54–132) vs. 158 (range 121–207) nM]. In Fig. 6*B*, individual H^+ release is plotted as a function of the muscle-to-blood H^+ gradient.

MCT1, MCT4, and NHE1 protein expression. After 2, 4, and 7–8 wk of training, the MCT1 content was 108 ± 10, 138 ± 19, and 115 ± 5% ($P < 0.05$), respectively, compared with the pretraining value (Fig. 7). The MCT4 content was 98 ± 9, 112 ± 7, and 111 ± 11% after 2, 4, and 7–8 weeks of training, respectively, compared with the pretraining values (Fig. 7).

The content of the Na^+/H^+ exchanger protein NHE1 was 131 ± 21, 131 ± 13 ($P < 0.05$), and 116 ± 6% ($P < 0.05$)

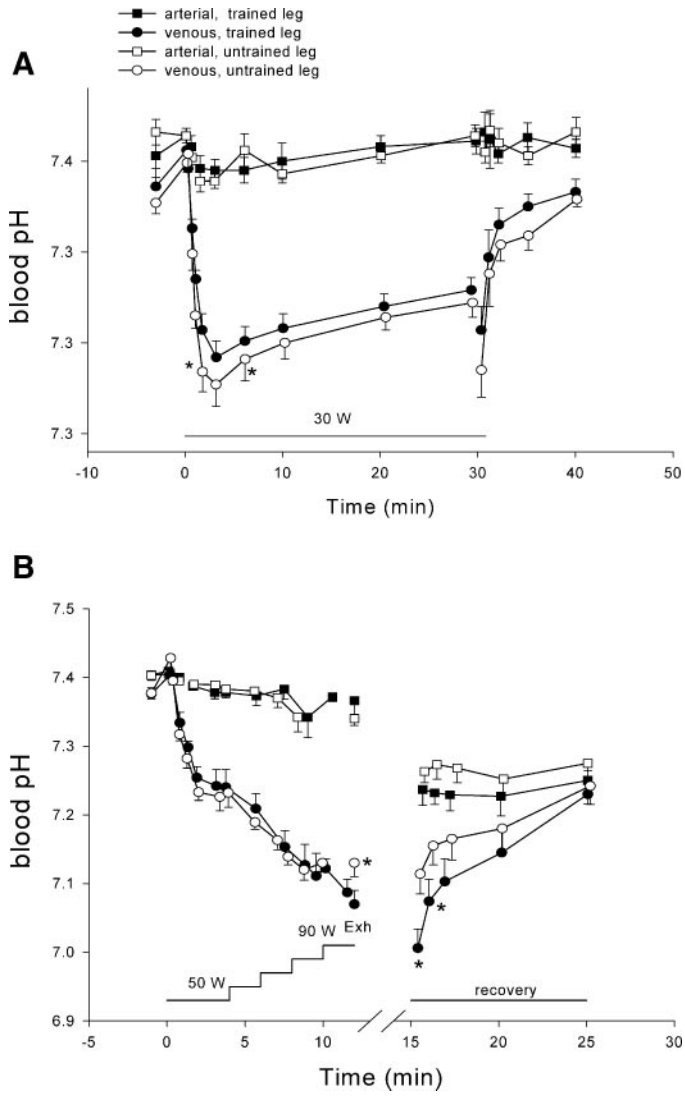

Fig. 4. Blood pH during exercise in the 30-W test (*A*) and the incremental test (*B*). Because of individual times to fatigue, not all 6 subjects were represented in the last 3 mean values of the incremental test. Mean values from trained and untrained legs were significantly (*$P < 0.05$) different.

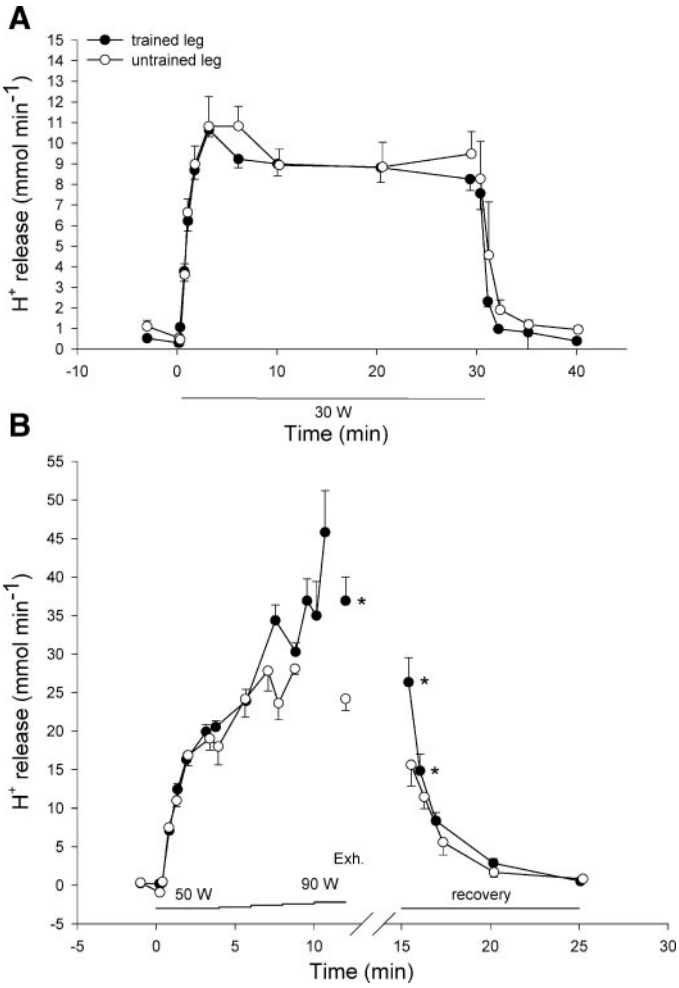

Fig. 5. H^+ release during the 30-W exercise test (*A*) and the incremental test (*B*). Because of individual times to fatigue, not all 6 subjects were represented in the last 3 mean values of the incremental test. Mean values from trained and untrained legs were significantly ($*P < 0.05$) different.

after 2, 4, and 7–8 wk, respectively, relative to the individual values before training (Fig. 7).

DISCUSSION

The main findings in the present study were that, at submaximal exercise, the femoral v-a lactate difference and lactate release were lower in the trained than in the untrained leg. On the other hand, in the incremental test, femoral v-a lactate difference at exhaustion was higher in the trained than the untrained leg, and peak lactate release was higher in the trained than in the untrained leg, although the lactate gradient from muscle to blood tended to be lower in the trained compared with the untrained leg. In addition, the H^+ release at exhaustion in the trained leg was higher than in untrained leg, although the H^+ gradient was lower. Training induced an increase in MCT1 and Na^+/H^+ exchanger proteins.

The lower total lactate production in trained compared with untrained legs is probably due to the lower relative exercise intensity. During the 30-W exercise bout, lactate release rose rapidly, peaked at 3 min, and approached zero (<1 mmol/min) at the termination of the period (Fig. 3*A*). This is probably due to a large lactate production early in the exercise period. In contrast, H^+ release (9–10 mmol/min) was nearly constant in the entire exercise period (Fig. 5*A*). Therefore, only a small fraction of the H^+ release seems to be coupled to lactate release mediated by lactate/H^+ cotransport and diffusion of undissociated lactic acid. The dominant part of the H^+ release at low-intensity exercise can therefore be ascribed to other mechanisms, i.e., HCO_3^--dependent transport, the Na^+/H^+ exchange system, and/or diffusion of CO_2. This is in accord with a study combining arm and leg exercise (4), in which it was observed that H^+ release can occur simultaneously with a net lactate uptake.

In the incremental test, both lactate release (Fig. 3*B*) and H^+ efflux (Fig. 5*B*) increased by increasing power output. If the nonlactate-coupled H^+ release at exhaustion is calculated from the total H^+ release (36.9 and 24.2 mmol/min in trained and untrained legs, respectively) and the lactate release (19.4 and 10.6 mmol/min in trained and untrained legs), it can be seen that the lactate-coupled H^+ release accounted for 53 and 44%

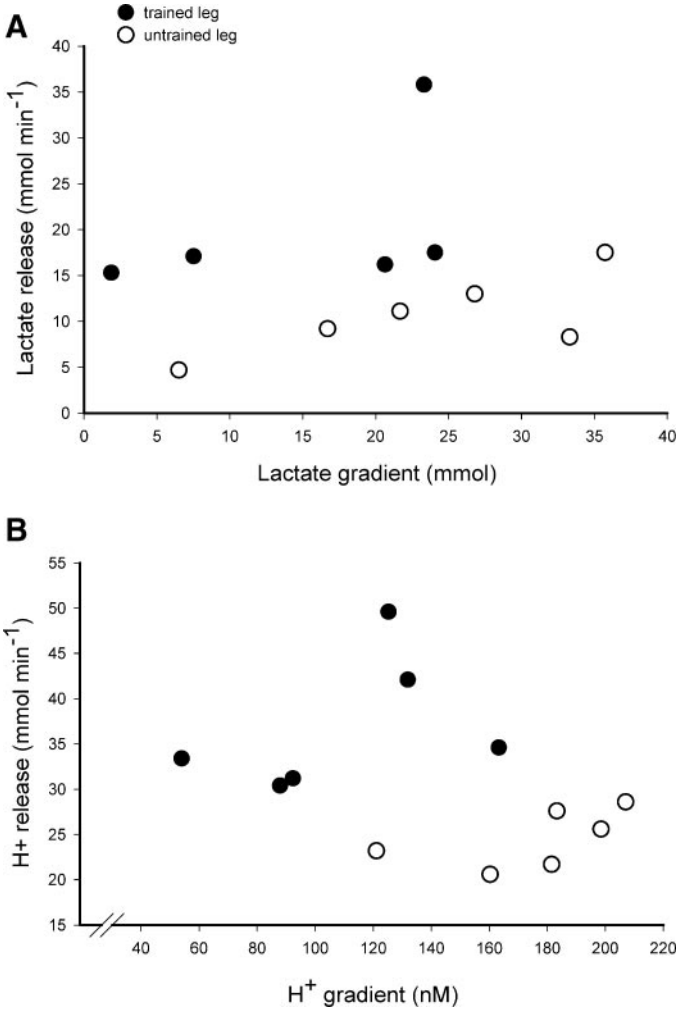

Fig. 6. Release of lactate and H^+ compared with muscle-to-arterial concentration gradients for lactate and H^+ at exhaustion from the incremental test. *A*: lactate release. The lactate gradient was calculated as the concentration difference between arterial blood and muscle lactate (mmol/l of cell water) calculated from water content and lactate concentration per kg dry wt ($n = 5$). *B*: H^+ release. Muscle H^+ concentration gradient (in nM, $n = 6$) was calculated as the concentration difference between the muscle H^+ concentration and arterial blood H^+ concentration at exhaustion.

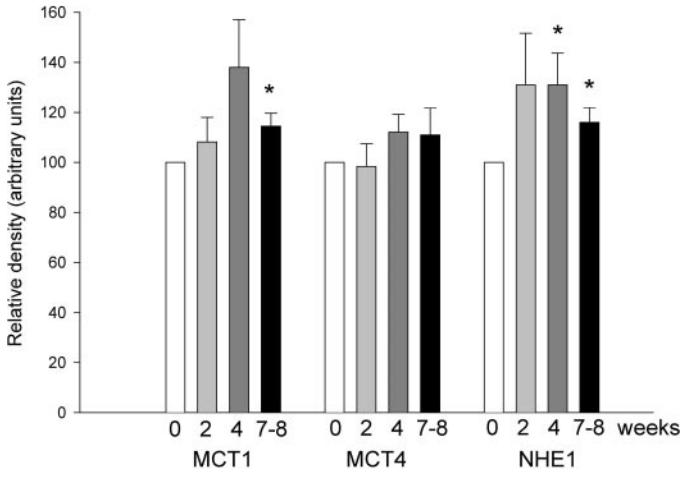

Fig. 7. Training-induced changes in the lactate/H^+ monocarboxylate cotransporter proteins MCT1, MCT4, and the Na^+/H^+ exchanger protein NHE1 determined by Western blotting. Protein contents were measured in biopsy material obtained before (open bar), after 2 (light gray bar) and 4 wk (dark gray bar) of training, and after the last training (7–8 wk; solid bar). *y*-Axis, arbitrary relative density units. Individual values were calculated relative to those before training. $*P < 0.05$, significantly different from mean value before training.

of the total H^+ release in trained and untrained legs, respectively. In addition, it appears that training affected both the lactate-coupled H^+ release (+52%) and the non-lactate-coupled H^+ release (+83%).

The present exercise model involved a small muscle mass, which produced only limited changes in arterial H^+ and lactate concentrations. It is therefore possible to study the effect of the muscle-to-blood gradient for H^+ and lactate release. The concentration gradients for lactate and H^+ are important factors for the transporter-mediated flux of lactate from muscle to blood. However, in the present study, a higher lactate release at exhaustion was observed in the trained leg despite a tendency toward a lower muscle-to-blood gradient for lactate (Fig. 6). Similarly, a large H^+ gradient is known to stimulate lactate/H^+ cotransport (11) and the Na^+/H^+ exchange system (12). In the present study, the H^+ gradient was lower in the trained than in the untrained leg, whereas the H^+ release was higher. Thus higher driving forces for lactate and H^+ cannot explain the higher release of lactate and H^+ in the trained leg. These findings are in accord with a previous study (16) showing that a given lactate release after training was obtained at smaller lactate and H^+ gradients.

In the present study, the MCT1 content was higher, and MCT4 content tended to be higher, after training than before it. These findings are in accord with observations in other studies. It has been demonstrated that a period of training can increase the density of the MCT1 proteins (1, 7, 9, 16), and MCT4 has also been shown to increase by high-intensity training (16), although a short-term (7-day) training study (5) and a long-term (5-mo) training study (8) did not observe any increase in MCT4. Thus MCT4 seems to be less sensitive to training than MCT1. In one study (20), plasma membrane MCT4, but not MCT1, was demonstrated to be acutely downregulated after one bout of exercise, whereas the membrane transport capacity for lactate was elevated. The moderate changes (+15 and +11%) in MCTs in the present study can probably explain only a fraction of the improved lactate release ability after training. The Na^+/H^+ exchanger protein NHE1, which in the present study for the first time was quantified in human muscle in association with training, was significantly elevated (+16%) after the training period. Thus the increase in the total H^+ transport capacity (estimated from the increase in NHE1 and MCTs) was ~15%. It is a possibility that training-induced changes in intrinsic activity of the transporter proteins are also involved, but this has not been studied. Apparently, the improved lactate and H^+ release can partly be explained by an increased density of membrane proteins, but other mechanisms are probably involved, because the training-induced improvement in H^+ release is larger than the increase in protein densities.

The higher lactate and H^+ release in the trained leg may also in part be due to a higher blood flow. At exhaustion, the thigh blood flow was ~16% higher in the trained leg, which by itself should increase lactate release, since elevated blood flow has been shown to increase lactate release (15). In the present study, an increased capillary-to-fiber ratio from 1.7 ± 0.1 to 2.5 ± 0.1 (+47%) after the training (10) may also in part explain the higher lactate release. The greater relative increase in capillarization than in leg blood flow in the trained leg may indicate that the mean transit time for blood was elevated, allowing a higher degree of equilibration. Thus the release of lactate to the bloodstream would be higher at a given concentration gradient.

Other factors might also be of importance. It has been demonstrated that passive leg movements increase thigh blood flow from ~0.4 to 1.2 l/min (17). Blood flow can therefore be expected to increase in the passive muscle of the thigh during the knee extensor exercise. Consequently, the increase in blood flow in the active muscle is >16% if it is assumed that the training-induced increase in blood flow is located to the active muscle.

The possibility also exists that training changes the ratio of flow between passive and active muscle. It is striking in connection with this possibility that the v-a concentration difference and lactate release in the trained leg are reduced at the transition from exercise to the first part of recovery (although leg blood flow is still high), whereas this is less marked in the untrained leg (Figs. 2*B* and 3*B*). This could indicate a different distribution of local flow during exercise in the trained compared with the untrained leg. In summary, higher leg blood flow and altered flow distribution are expected to account for a fraction of the higher lactate and H^+ release with training.

The total lactate release during exercise can be estimated from the area under the curves in Fig. 3*B*. Release in the trained leg was considerably higher (105 ± 24 mmol) than in the untrained leg (48 ± 4 mmol). With a blood volume of 5.5 liters, the theoretical arterial lactate concentrations at exhaustion are ~19 and 9 mM in the trained and untrained leg, respectively. Since the arterial lactate concentration increased by only 7.5 and 6.1 mM during exercise in the trained and untrained leg, respectively, it can be concluded that a considerably higher amount of lactate is removed from the circulation during exercise in the trained compared with the untrained leg (63 vs. 16 mmol). This large difference can only partly be explained by the increased (+29%) time to fatigue in the trained leg. Thus, although training was performed with only a

small muscle mass, there was a considerable effect on systemic lactate clearance.

Arterial and venous lactate concentrations stayed high in the first part of recovery, suggesting a balance between lactate release and clearance. Because release is reduced within the first 10 min of recovery, there must also be a parallel fall in clearance from the blood. This is probably due to the gradually reduced blood flow, since uptake in resting muscle is related to blood flow (3). Furthermore, lactate uptake in the heart is expected to be reduced during the recovery period. Together, these mechanisms are responsible for the small changes in arterial and venous lactate in the first part of recovery.

In summary, the reason for the higher peak lactate and H^+ release during exercise in the trained muscle is a combination of an increased density of the lactate- and H^+-transporting proteins MCT1 and NHE1, an improved blood flow and blood flow distribution, and an increased systemic lactate and H^+ clearance.

GRANTS

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