Human muscle net K^+ release during exercise is unaffected by elevated anaerobic metabolism, but reduced after prolonged acclimatization to 4,100 m

Nikolai B. Nordsborg, José A. L. Calbet, Mikael Sander, Gerrit van Hall, Carsten Juel, Bengt Saltin and Carsten Lundby

Am J Physiol Regul Integr Comp Physiol 299:R306-R313, 2010. First published 21 April 2010; doi:10.1152/ajpregu.00062.2010

Am J Physiol Regul Integr Comp Physiol 299: R306–R313, 2010.
First published April 21, 2010; doi:10.1152/ajpregu.00062.2010.

Human muscle net K^+ release during exercise is unaffected by elevated anaerobic metabolism, but reduced after prolonged acclimatization to 4,100 m

Nikolai B. Nordsborg,[2] José A. L. Calbet,[1,3] Mikael Sander,[1] Gerrit van Hall,[1] Carsten Juel,[4] Bengt Saltin,[1] and Carsten Lundby[1,5]

[1]*Copenhagen Muscle Research Center, Rigshospitalet Section, Copenhagen Ø, Denmark;* [2]*Department of Exercise and Sport Sciences, Faculty of Science, University of Copenhagen, Copenhagen, Denmark;* [3]*Department of Physical Education, University of Las Palmas de Gran Canaria, Palmas de Gran Canaria, Spain;* [4]*Department of Biology, University of Copenhagen, Copenhagen, Denmark; and* [5]*Center for Integrative Human Physiology, Institute of Physiology, University of Zurich, Zurich, Switzerland*

Submitted 26 January 2010; accepted in final form 20 April 2010

Nordsborg NB, Calbet JA, Sander M, van Hall G, Juel C, Saltin B, Lundby C. Human muscle net K^+ release during exercise is unaffected by elevated anaerobic metabolism, but reduced after prolonged acclimatization to 4,100 m. *Am J Physiol Regul Integr Comp Physiol* 299: R306–R313, 2010. First published April 21, 2010; doi:10.1152/ajpregu.00062.2010.—It was investigated whether skeletal muscle K^+ release is linked to the degree of anaerobic energy production. Six subjects performed an incremental bicycle exercise test in normoxic and hypoxic conditions prior to and after 2 and 8 wk of acclimatization to 4,100 m. The highest workload completed by all subjects in all trials was 260 W. With acute hypoxic exposure prior to acclimatization, venous plasma [K^+] was lower ($P < 0.05$) in normoxia (4.9 ± 0.1 mM) than hypoxia (5.2 ± 0.2 mM) at 260 W, but similar at exhaustion, which occurred at 400 ± 9 W and 307 ± 7 W ($P < 0.05$), respectively. At the same absolute exercise intensity, leg net K^+ release was unaffected by hypoxic exposure independent of acclimatization. After 8 wk of acclimatization, no difference existed in venous plasma [K^+] between the normoxic and hypoxic trial, either at submaximal intensities or at exhaustion (360 ± 14 W vs. 313 ± 8 W; $P < 0.05$). At the same absolute exercise intensity, leg net K^+ release was less ($P < 0.001$) than prior to acclimatization and reached negative values in both hypoxic and normoxic conditions after acclimatization. Moreover, the reduction in plasma volume during exercise relative to rest was less ($P < 0.01$) in normoxic than hypoxic conditions, irrespective of the degree of acclimatization (at 260 W prior to acclimatization: −4.9 ± 0.8% in normoxia and −10.0 ± 0.4% in hypoxia). It is concluded that leg net K^+ release is unrelated to anaerobic energy production and that acclimatization reduces leg net K^+ release during exercise.

acclimatization; plasma volume; fatigue; cycling

INTENSE EXERCISE IS ASSOCIATED with a high anaerobic energy production and a rapid development of muscular fatigue, which is of utmost importance to prevent detrimental intramuscular ATP depletion. One of many factors proposed to limit muscle function during intense exercise (1, 9, 28) is extracellular K^+ accumulation, which may decrease muscle excitability (6, 15, 31). It has been well described that the degree of muscle net K^+ release (11, 12), as well as extracellular K^+ buildup (13, 18) is related to exercise intensity. It is, however, unresolved as to whether a part of the intensity-dependent muscle net K^+ release is directly coupled to the degree of anaerobic metabolism.

Anaerobic energy production is associated with the development of metabolic acidosis, due to the high ATP hydrolysis rate (30). Because in vitro investigations have shown that intramuscular acidosis can increase muscular K_{ATP} channel opening probability (8), it is possible that anaerobic energy production is coupled to muscular K^+ release. However, previous studies have yielded conflicting results. Knee-extension exercise preceded by arm exercise, results in accelerated intramuscular acidosis, a higher quadriceps K^+ release (3), and accelerated interstitial K^+ accumulation (27) in musculus vastus lateralis. This indicates that accelerated intramuscular acidosis can increase muscular K^+ release. Moreover, ingestion of sodium citrate reduces both interstitial H^+ and K^+ accumulation during intense exercise (34). One reason for this finding could be that a reduced interstitial H^+ concentration facilitated the release of H^+ from the muscle cell via the Na^+, H^+ exchanger, and the monocarboxyltransporters (MCT1 and 4) (16), resulting in reduced intramuscular acidosis and thereby reduced K^+ release. Together, these studies support the suggestion of a link between anaerobic energy production and muscular K^+ release via the degree of intramuscular acidosis. However, an attempt to block the K_{ATP} channels during exercise failed to affect interstitial K^+ accumulation (26). This finding may be because K_{ATP} channels are not carrying a significant K^+ current during exercise or because the pharmacological intervention was not effective. In addition, systemic alkalosis has been shown to increase muscle K^+ efflux during exhaustive finger flexion exercise (33). Because systemic alkalosis should be expected to facilitate H^+ efflux from the muscle cell, it can be expected that muscular acidosis during exercise was unaffected or even reduced. Thus, these findings do not support a link between intramuscular acidosis and K^+ release. Taken together, it is still unclear whether muscle net K^+ release is related to the degree of anaerobic metabolism.

Hypoxic Exposure as a Model to Study the Impact of Increased Anaerobic Metabolism on K^+ Homeostasis

Ergometer cycling in acute normobaric hypoxia ($F_{I_{O_2}}$ 12%) increases systemic lactate levels (23) and leg lactate release compared with cycling at the same absolute intensity in normoxia (14). Thus, hypoxia induces a higher degree of anaerobic metabolism during exercise performed at the same absolute workload compared with in normoxia. Furthermore, the higher anaerobic energy production is likely to be associated with

Address for reprint requests and other correspondence: N. B. Nordsborg, Copenhagen Muscle Research Centre, Univ. of Copenhagen, Dept. of Exercise and Sport Sciences, Universitetsparken 13, 2100 Copenhagen, Denmark (e-mail: nnordsborg@ifi.ku.dk).

accelerated intramuscular acidosis. This is supported by the estimation of similar intracellular pH levels at exhaustion in normoxia and acute normobaric hypoxia despite a 22% reduction in peak workload during an exhaustive incremental workload test (14). Thus, acute hypoxic exposure in combination with intense exercise provides the possibility to investigate whether K^+ homeostasis is affected by accelerated anaerobic energy production. Previously, the peak venous K^+ concentration was found to be higher during cycling at 100% of $\dot{V}O_{2max}$ in normobaric hypoxia ($F_{I_{O_2}}$ 14.5%) compared with normoxia, despite a lower $\dot{V}O_{2max}$ and thus a lower absolute workload in the hypoxic condition (5). Because leg plasma flow and arterial K^+ were not determined in that study, it is unknown whether the higher peak K^+ was due to a faster K^+ release from the exercising muscles in the hypoxic condition.

After acclimatization to 4,300 m for 15 days, the anaerobic energy production at a given absolute workload appears to be similar to prior to acclimatization, as judged by similar net leg lactate release (36). However, muscle pH at exhaustion after 12 min of cycling was higher than after 30 min of sea-level exercise at the same absolute workload prior to acclimatization (37). Thus, it appears that acclimatization reduces intramuscular acidosis during intense exercise. In human skeletal muscle, acclimatization increases the capacity for H^+ transport via increased capacity of the muscular Na^+/H^+ exchanger and Na^+/HCO_3^- cotransporter (17). Furthermore, the erythrocyte expression of the Cl^-/HCO_3^- exchanger and lactate (La^-)/H^+ cotransporter MCT1 is elevated (17). Thus, prolonged hypoxic exposure increases the capacity for lactate, HCO_3^-, and H^+ removal from exercising muscle to blood and from blood to erythrocytes. In accordance with these observations is the acclimatization-induced increase in muscle buffer capacity (25) and the markedly increased in vivo buffer capacity determined as $\Delta La^-/\Delta pH$ in blood during exercise (4).

On the basis of the above considerations, acute hypoxic exposure can be used as a tool to investigate the effect of accelerated anaerobic metabolism on K^+ release from human skeletal muscle during cycling exercise. Moreover, a period of acclimatization to high altitude can be used to investigate whether an improved muscular capacity for H^+ handling can reverse possible hypoxia-induced effects on K^+ regulation during exercise.

Aims

The aims of the present study were twofold: *1*) to investigate whether accelerated anaerobic energy production, as imposed by acute hypoxic exposure during intense whole body exercise at the same absolute workload as in normoxia, results in increased K^+ efflux from the exercising muscles; and *2*) to investigate whether exercise induced skeletal muscle K^+ release is diminished at the same absolute workload after a period of acclimatization.

METHODS

Subjects

We studied six Danish male lowlanders, 26 yr (22–31), 187 cm (175–191), 82 kg (75–91 kg) (mean and range). The lowlanders were all physical education students participating regularly in a variety of club sports and outdoor recreational activities. Subjects were encouraged to remain active throughout the stay with activities such as cycling, soccer, basketball, hiking, and rock climbing. Subjects received written and oral information about the study and provided informed consent to the protocols. The protocol was approved by the Ethics Committee for Copenhagen and Frederiksberg (KF 11–050/01).

The subjects performed six incremental bicycle exercise tests on a cycle ergometer. At sea level (Copenhagen, Denmark), the subjects were tested twice on the same day, with 2 h between the trials. In the first tests, the subjects were breathing ambient air, and in the second test, they were breathing a hypoxic gas-mixture: 12.6% O_2 in N_2 (normobaric hypoxia). Approximately 1 mo after the test at sea level, subjects traveled by plane to La Paz, Bolivia. Initially, they spent 2 nights in La Paz (~3,700 m), and then moved to El Alto (4,100 m, barometric pressure: ~470 mmHg) for the remaining 8 wk of the study. During *weeks 3–7* of acclimatization, the subjects had short excursions from El Alto but were not allowed to descend below 3,700 m. The first testing period at altitude was between *day 11* and *day 17* after arriving to La Paz. The incremental bicycle exercise test was carried out first while breathing ambient air (hypobaric hypoxia), followed ~2 h later by the second exercise test, while breathing a high-oxygen gas mixture eliciting sea level conditions: 38% O_2 in N_2 (hypobaric normoxia). The second test period at altitude was between *day 52* and *day 60* after arriving at La Paz and was conducted exactly the same as the first tests.

Experimental Procedures

The subjects had a light breakfast and reported to the laboratory at 8 AM. Using local anesthesia (lidocaine, 20 mg/ml), we placed catheters in a femoral artery (20 G, 12 cm; Arrow, Copenhagen, Denmark) and vein (18 G, Radiopack TFE; Cook, Bjaerverskov, Denmark) for blood sampling. The venous catheter was used to measure leg blood flow by the constant infusion thermodilution technique (2). In brief, a thermistor (model 94–030-2.5F T.D. probe; Edslab, Baxter, Irvine, CA) was inserted to measure femoral blood temperature, while infusate temperature was determined with a flow-through chamber thermistor (model 93–505; Edslab, Baxter) connected to the venous catheter. The signal from both thermistors was conditioned and amplified by a custom-built interface (FBJ Industries, Copenhagen, Denmark). During catheterization and the remainder of the study, we continuously monitored the ECG (BIO amp, ADinstruments, Oxfordshire, UK) and arterial blood pressure. We used an analog-digital converter and data acquisition software (Powerlab/8SP and Chart 4; ADinstruments) to display and store the ECG, blood pressure, and temperature data on a portable computer (Dell, Round Rock, TX). After catheterization, the subjects remained supine for at least 30 min. Subjects were then seated on a bicycle ergometer (Monark 824E, Varberg, Sweden) and fitted with a mouthpiece and nose clip to enable measurements of ventilation, oxygen uptake ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) from expired gas (Oxygen Analyzer S-3A/I, Ametek, Paoli, PA; LB-2, Beckman Coulter, Pasadena, CA; VRDC/HC^{-1}; Parvo Medics, Sandy, UT). Blood was sampled anaerobically in heparinized syringes and immediately analyzed for hemoglobin (Hb) (OSM3 hemoxymeter, Radiometer, Brønshøj, Denmark), and blood pH, K^+ (ABL5, Radiometer, Den-

mark). Hematocrit (Hct) was determined by centrifuging capillary tubes. Results unrelated to the present study have been reported elsewhere (20–23, 36).

Protocol

Resting measurements started 10 min after placement of the mouthpiece, while the subjects were seated, and at this time point, the resting blood samples were obtained. Exercise started with 15-min warm-up at 100 W, and blood samples were obtained every 5 min. After warm-up, the workload was increased by 40 W every 2.5 min until exhaustion. During the last minute of each workload, blood flow was measured followed by blood sampling and an additional blood flow measurement. At altitude, 2 h after the first incremental exercise, the protocol was repeated, while breathing the oxygen-supplemented gas-mixture ($F_{I_{O_2}} = 0.38$). All subjects were familiar with maximal exercise testing on cycle ergometers from participation in previous experiments and were familiarized with the actual cycle ergometer used during pretrials. All studies were performed with vigorous verbal encouragement as the subjects approached exhaustion.

Calculations

To calculate leg net K^+ flux, the amount of K^+ entering the leg in the plasma compartment of arteria femoralis was subtracted from the amount leaving the leg in the plasma compartment of vena femoralis. This way, it was ensured that the calculation of net leg K^+ flux was unaffected by changes in the blood plasma volume. Venous leg plasma flow was determined by measuring total blood flow in v. femoralis (Q_v) and multiplying it by the determined plasma fraction (1 − venous hematocrit). On the basis of the assumption that equal amounts of hemoglobin enters and leaves the leg during exercise, arterial blood flow (Q_a) was calculated from the measurement of venous blood flow as $Q_a = Q_v \times [Hb_v]/[Hb_a]$, and arterial plasma flow was determined as $Q_a \times (1 -$ arterial hematocrit). Thus, the amount of K^+ entering the leg in the femoral artery was calculated as arterial plasma flow × arterial plasma $[K^+]$. Likewise, the amount of K^+ leaving the leg was calculated as venous plasma flow × venous plasma $[K^+]$. Net leg K^+ flux was then calculated as the amount of K^+ leaving the leg minus the amount entering the leg. In short, $K^+_{release} = (Q_v \times [K_v] \times (1-(Hct_v/100))) - ([K_a] \times ([Hb_v]/[Hb_a]) \times (1-(Hct_a/100)))$. Leg blood lactate release was calculated as $La^-_{release} = Q_v \times (La^-_v - La^-_a \times [Hb_v]/[Hb_a])$. By calculation (32) of arterial standard base excess, $SBE_a = (1 - [Hb_a] \times 0.023) \times \{[HCO_3^-{}_a]-24.4) + (2.3 \times [Hb_a]) + (7.7)\} \times ([pH_a] - 7.4)$ and venous standard base excess, $SBE_v = (1 - [Hb_v] \times 0.023) \times \{[HCO_3^-{}_v] - 24.4) + (2.3 \times [Hb_v]) + (7.7)\} \times ([pH_v] - 7.4)$ leg H^+ release was calculated as $H^+_{release} = SBE_v \times Q_v - SBE_a \times Q_a$. Plasma bicarbonate was calculated according to Siggaard-Andersen (32). All plasma flows have been calculated as $Q_{plasma} = Q_{blood} \times [1 - (Hct/100)]$.

Exercise-induced changes in plasma volume (dPV) can be calculated as $dPV = 100 \times (BV_{ex} \times (1 - Hct_{ex})/BV_{rest} \times (1-Hct_{rest}) - 1)$, where BV is blood volume, ex is exercise, and rest is measurements from the resting condition. Assuming that arterial hemoglobin represents average systemic hemoglobin concentration and that the total hemoglobin content of the blood compartment is constant then $[Hb_a]_{rest} \times BV_{rest} = [Hb_a]_{ex} \times BV_{ex}$. Thus, $dPV = 100 \{[Hb_a]_{rest}/[Hb_a]_{ex} \times (1 - Hct_{ex})/(1 - Hct_{rest}) - 1\}$.

Statistics

Results are presented as means ± SE. All statistical analyses have been performed using SPSS 17.0 software package (SPSS). To investigate whether the physiological response of any reported variable differed between the fixed effects "site" (arterial, venous), "condition" (hypoxia, normoxia), "week" (0, 2, or 8 wk) or "sample" (all samples included where $n = 6$) the SPSS mixed model was used (7). Site, condition, week, and sample were set as repeated effects for each subject with correction for autocorrelation (type AR1 in SPSS Mixed). When significant effects ($P < 0.05$) were detected for any fixed effect, post hoc Bonferroni adjusted multiple pairwise comparisons were performed. Possible difference in parameters determined at exhaustion was also analyzed by a mixed model where condition, week, and sample were set as a repeated effect for each subject with correction for autocorrelation (type AR1 in SPSS Mixed). Post hoc multiple pairwise comparisons were Bonferroni adjusted. The level of significance was set at $P < 0.05$.

RESULTS

Performance

Before acclimatization, exhaustion occurred at a 30% higher ($P < 0.001$) workload in normobaric normoxia (400 ± 9 W) than in normobaric hypoxia (307 ± 7 W). After 2 wk acclimatization to 4,100 m, exhaustion occurred at a 13% higher ($P < 0.001$) workload (340 ± 10 W) in hypobaric normoxia than in hypobaric hypoxia (300 ± 10 W). It may be noted that exhaustion in hypobaric hypoxia after 2 wk of acclimatization occurred at a workload 25% less ($P < 0.001$) than in normobaric normoxia prior to acclimatization. Subsequent to 8 wk of acclimatization, exhaustion in the hypobaric normoxia trial occurred at a 15% higher ($P < 0.001$) workload (360 ± 14 W) than in the hypobaric hypoxia trial (313 ± 8 W). The exhaustive workload in hypobaric hypoxia after 8 wk of acclimatization was 22% less ($P < 0.001$) than in the normobaric normoxia trial before acclimatization. Because 260 W was the highest workload completed by all subjects in all trials, exact values of measured parameters are given for this workload in the following section.

K^+ Homeostasis During Exercise: Effect of Acute Normobaric Hypoxic Exposure Prior to Acclimatization

Plasma K^+. Both arterial and venous plasma $[K^+]$ during low-intensity exercise was similar in normobaric normoxia and normobaric hypoxia (Fig. 1*A*). When the exercise intensity reached 260 W, venous plasma $[K^+]$ was lower ($P < 0.05$) in normobaric normoxia (4.9 ± 0.1 mM) than normobaric hypoxia (5.2 ± 0.2 mM), and at 300 W, both venous and arterial plasma $[K^+]$ was lower ($P < 0.001$) in normobaric normoxia (5.1 ± 0.2 mM and 5.0 ± 0.1 mM, respectively) than normobaric hypoxia (5.6 ± 0.2 mM and 5.5 ± 0.2 mM, respectively). At exhaustion, the venous plasma $[K^+]$ was similar in normobaric normoxia and normobaric hypoxia (5.9 ± 0.2 vs. 5.8 ± 0.3 mM).

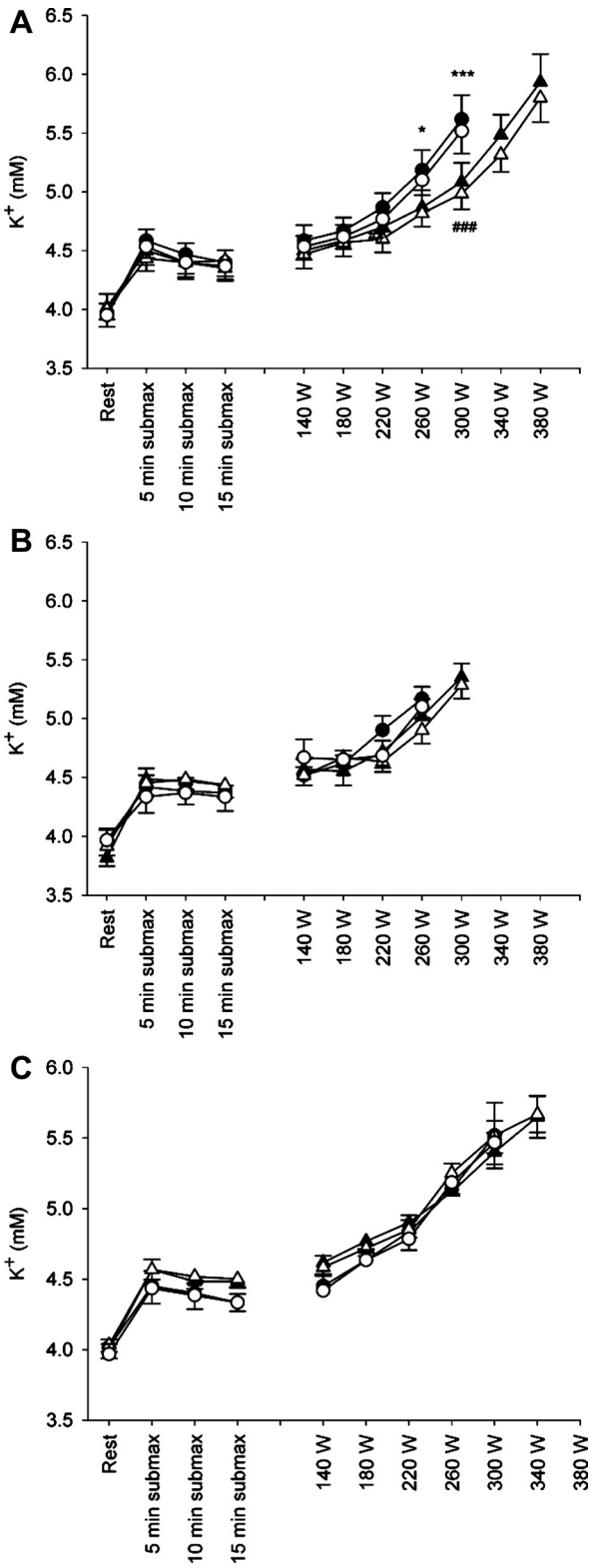

Fig. 1. Femoral venous (solid symbols) and arterial (open symbols) plasma [K^+] determined during cycling exercise ($n = 6$) in normoxia (triangles) and hypoxia (circles) after 0 wk (*A*), 2 wk (*B*), and 8 wk (*C*) of acclimatization to 4,100 m. Only data points where $n = 6$ are shown. See text for exhaustion values. Results are expressed as means ± SE. Significant differences between normoxic and hypoxic conditions are indicated for venous (*$P < 0.05$, ***$P < 0.001$) and arterial (###$P < 0.001$) samples.

K^+ release. In normobaric normoxia, K^+ release was increased ($P < 0.001$) from 0.0 ± 0.0 mmol/min at rest to 0.3 ± 0.2 mmol/min at 260 W (Fig. 2*A*). In normobaric hypoxic conditions, K^+ release was unchanged ($P = 0.07$) from 0.0 ± 0.0 mmol/min at rest to 0.5 ± 0.3 mmol/min at 260 W. No difference was apparent in K^+ release during exercise performed at the same absolute workload in normobaric normoxia and hypoxia (Fig. 2*A*). K^+ release was also similar at exhaustion (0.4 ± 0.3 in normobaric normoxia vs. 0.5 ± 0.2 mmol/min in normobaric hypoxia).

K^+ Homeostasis During Exercise: Effect of 2- and 8-wk Acclimatization to 4,100 m

Plasma K^+. No difference existed between arterial and venous plasma [K^+] changes during exercise performed in hypobaric normoxia or hypobaric hypoxia following 2 wk off acclimatization (Fig. 1*B*). Venous plasma [K^+] at 260 W was 5.0 ± 0.1 mM and 5.2 ± 0.1 mM in hypobaric normoxia and hypobaric hypoxia, respectively. At exhaustion, venous plasma [K^+] was similar in hypobaric normoxia (5.8 ± 0.2 mM) and hypobaric hypoxia (5.5 ± 0.2 mM) after 2 wk of acclimatization. Also, acclimatization for 2 wk did not affect the exercise-induced increase in venous plasma [K^+] under hypobaric normoxic or hypobaric hypoxic conditions compared with before acclimatization.

Subsequent to 8 wk of acclimatization, exercise in hypobaric normoxia compared with hypobaric hypoxia induced similar changes in venous and arterial plasma [K^+]. Venous plasma [K^+] reached 5.1 ± 0.1 mM and 5.1 ± 0.2 mM during exercise at 260 W in hypobaric normoxia and hypobaric hypoxia, respectively. Likewise, venous plasma [K^+] at exhaustion was similar in hypobaric normoxia (5.9 ± 0.2 mM) and hypobaric hypoxia (5.7 ± 0.2 mM). Like hypoxic exposure for 2 wk, acclimatization for 8 wk did also not affect the exercise-induced increase in venous plasma [K^+] under hypobaric normoxic or hypobaric hypoxic conditions compared with before acclimatization.

K^+ release. After 2 wk of acclimatization, K^+ release during exercise in hypobaric normoxic and hypobaric hypoxic conditions was similar. The K^+ release was −0.1 ± 0.0 and 0.0 ± 0.0 mmol/min at rest and 0.2 ± 0.2 vs. 0.2 ± 0.2 mmol/min at 260 W in hypobaric normoxia and hypobaric hypoxia, respectively. Likewise, no difference was apparent in K^+ release at exhaustion in hypobaric normoxia (0.2 ± 0.2 mmol/min) and hypobaric hypoxia (−0.1 ± 0.1 mmol/min). K^+ release at 260 W investigated prior to and after 2 wk of acclimatization was similar. Like after 2 wk of acclimatization, K^+ release during exercise in hypobaric normoxia and hypobaric hypoxia was also similar subsequent to 8 wk of acclimatization with K^+ release being 0.0 ± 0.0 mmol/min and 0.0 ± 0.0 mmol/min at rest and −0.4 ± 0.5 and −0.3 ± 0.4 mmol/min at 260 W in hypobaric normoxia and hypobaric hypoxia, respectively (Fig. 2*A*). Notably, K^+ release during intense exercise after 8 wk of acclimatization reached negative values and was reduced ($P < 0.001$) relative to before and after 2 wk of acclimatization (Fig. 2*A*). The K^+ release at 260 W amounted to −0.4 ± 0.5 mmol/min and −0.3 ± 0.4 mmol/min in hypobaric normoxia and hypobaric hypoxia, respectively. It should be noted that the leg K^+ flux was less negative ($P < 0.05$) in hypobaric nor-

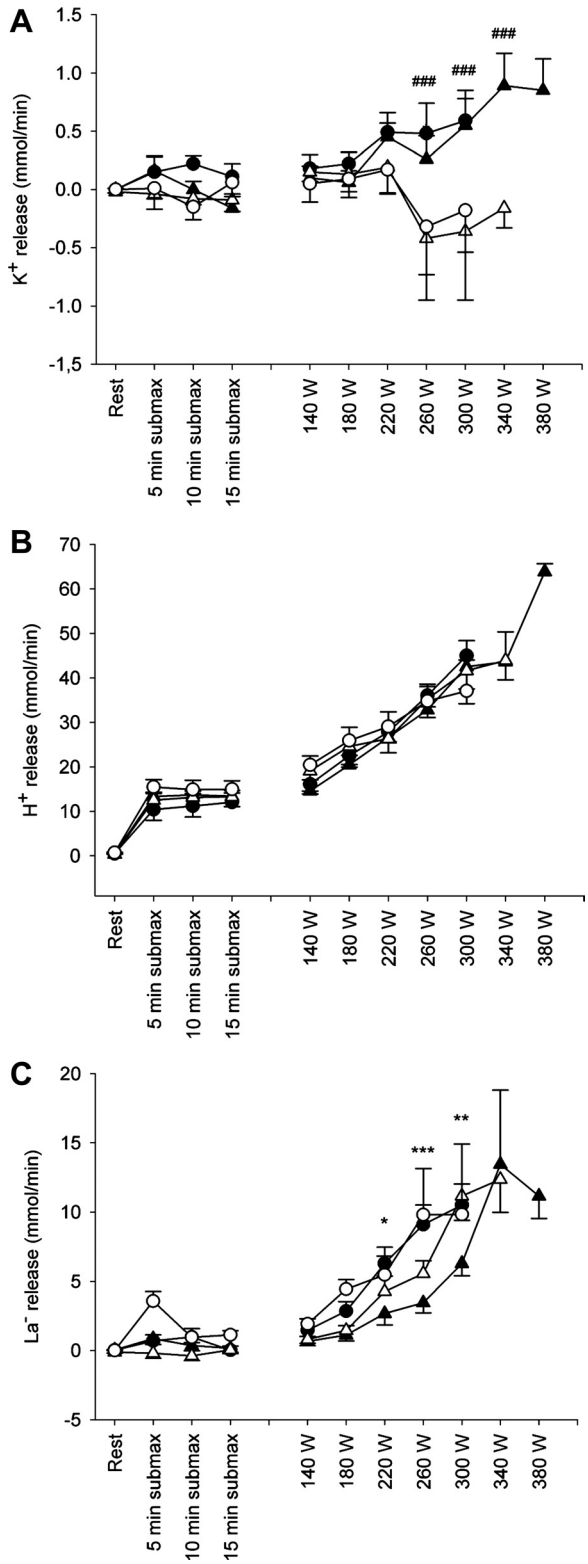

Fig. 2. Release of K^+ (*A*), H^+ (*B*), and lactate (La^-; *C*) from one leg during cycling ($n = 6$) in normoxia (triangles) and hypoxia (circles) after 0 (solid symbols) and 8 wk (open symbols) of acclimatization to 4,100 m. Results from the 2-wk trial have been omitted for clarity. Only data points where $n = 6$ are shown. See text for exhaustion values. Results are expressed as means ± SE. Time-specific difference (###$P < 0.001$) between 0 and 8 wk, when hypoxic or normoxic condition is not included in the analysis. Time-specific difference (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) between hypoxia and normoxia, when weeks of acclimatization are not taken into account. See RESULTS for details.

moxia (−0.4 ± 0.2 mmol/min) than in hypobaric hypoxia (−1.2 ± 0.4 mmol/min) at exhaustion in the 8-wk trial.

Lactate homeostasis. Detailed data on lactate homeostasis have been published elsewhere (36). Leg lactate release was generally lower ($P < 0.01$) in normoxia than hypoxia (Fig. 2*C*). Specifically, leg lactate release at 260 W was lower under normoxic than hypoxic conditions both before (3.4 ± 0.7 vs. 9.1 ± 1.4 mmol/min; $P < 0.05$) and after 2 wk of acclimatization (3.8 ± 0.5 vs. 11.8 ± 4.0 mmol/min; $P < 0.001$). After 8 wk of acclimatization, leg lactate release tended to be lower at 260 W (5.6 ± 0.9 vs. 9.8 ± 3.3 mmol/min; $P = 0.056$) in hypobaric normoxia than hypobaric hypoxia. At 300 W, leg lactate release in normoxic conditions vs. hypoxic conditions tended to be lower in the 0-wk trial (6.3 ± 0.9 vs. 10.5 ± 1.5 mM, $P = 0.056$), was lower in the 2-wk trial (6.5 ± 1.3 vs. 15.1 ± 4.1 mM, $P < 0.001$), whereas no difference was apparent after 8 wk of acclimatization (11.2 ± 1.8 vs. 9.8 ± 5.1 mM). The observed leg lactate release was not affected by acclimatization when analyzed independently of hypoxic or normoxic conditions or when including condition as an effect.

H^+ homeostasis. There was no difference in leg H^+ release either dependent on hypoxic or normoxic exposure or on the degree of acclimatization (Fig. 2*B*). At 260 W, leg H^+ release in normoxia vs. hypoxia was 32.8 ± 0.7 vs. 36.0 ± 2.0 mmol/min before acclimatization, 37.2 ± 3.6 vs. 36.1 ± 4.2 mmol/min after 2 wk, and 35.3 ± 4.2 vs. 34.8 vs. 3.8 mmol/min after 8 wk of acclimatization.

Plasma volume. The estimated change in total plasma volume during exercise was calculated to evaluate whether the increased plasma K^+ level in the hypoxic exercise trial before acclimatization could be explained by changes in K^+ dilution space. The reduction in plasma volume during exercise was generally less in normoxia than hypoxia before ($P < 0.01$) and after 2 wk of acclimatization ($P < 0.001$), whereas no statistically significant difference was apparent after 8 wk of acclimatization. Specifically, before acclimatization the change in plasma volume was apparent from 10 min of submaximal exercise and throughout the rest of the exercise period (Fig. 3). At 260 W, the change in plasma volume was less ($P < 0.01$) in normoxic (−4.9 ± 0.8%) than in hypoxic (−10.0 ± 0.4%) conditions. After 2 wk of acclimatization, the change in plasma volume during exercise was less in normoxia than hypoxia from 15 min and throughout the exercise period ($P < 0.01$ at all time points). At 260 W, the change in plasma volume was less ($P < 0.001$) in normoxia (−2.1 ± 2.1%) than hypoxia (−8.4 ± 1.2%). After 8 wk of acclimatization, the change in plasma volume at 260 W in normoxia (−5.0 ± 1.5%) did not differ from the change observed in hypoxia (−7.6 ± 2.0%). Despite the lack of difference between the normoxic and hypoxic trial after 8 wk of acclimatization and the observed differences at the 0- and 2-wk trials, no general effect of acclimatization was detected on the change in plasma volume during exercise.

DISCUSSION

A major finding in the present study was that leg K^+ release during ergometer cycling in normoxia was similar to the release in hypoxia, both before and after 8 wk of acclimatization to 4,100 m. Thus, muscular K^+ release is unaffected by increased muscular anaerobic energy production. Another

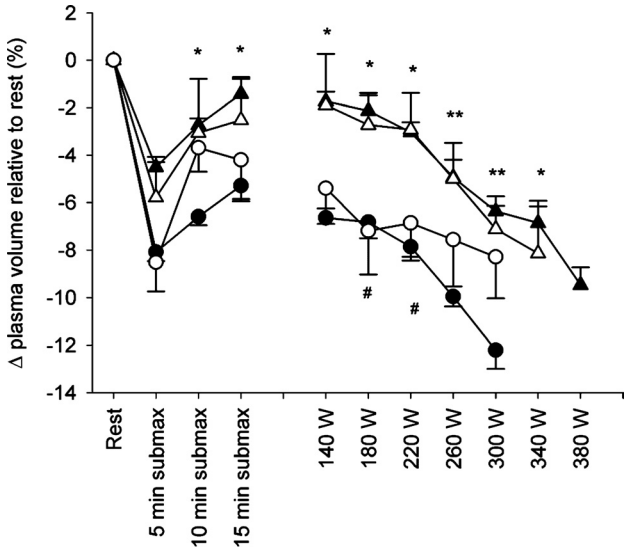

Fig. 3. Plasma volume change relative to rest before exercise. Cycling ($n = 6$) was performed in normoxia (triangles) and hypoxia (circles) after 0 (solid symbols) and 8 wk (open symbols) of acclimatization to 4,100 m. Only data points where $n = 6$ are shown. Results are expressed as means ± SE. Significant differences between the response to exercise in normoxia and hypoxia are indicated by ($*P < 0.05$, $**P < 0.01$, respectively) for 0 wk and ($\#P < 0.05$) for 8 wk.

novel finding was that acclimatization reduces leg K^+ release during intense exercise.

Effect of Acute Sea Level Hypoxic Exposure on Exercise K^+ Homeostasis Prior to Acclimatization

In contrast to the study hypothesis, leg K^+ release during exercise was found to be unaffected by acute hypoxic exposure at sea level. Because leg lactate release at 260 W in normoxia was only 1/3 of the release in hypoxia, it seems clear that hypoxic exposure markedly increased anaerobic energy production. Thus, no link appears to exist between anaerobic energy production and muscular net K^+ release in vivo. Moreover, it appears likely that the rate of intramuscular acidosis would have been higher during exercise in hypoxia. Thus, the present finding does not support the suggestion of a mechanism that couples K^+ release to intramuscluar acidosis (3, 27, 33, 34). In one series of studies the suggestion of a link between intramuscular acidosis and K^+ release was based on observed faster intramuscular acidosis, higher leg K^+ release (3), and faster interstitial K^+ accumulation (27) when leg exercise was preceded by intense arm exercise. However, in those studies, other factors may also have affected leg K^+ release. For example, the higher blood lactate concentration causes plasma osmolality to increase, which results in a fluid shift from the interstitium to the blood. Because a high diffusional barrier may exist for K^+ across endothelial cells (26, 29), interstitial K^+ may simply have been elevated by a reduced dilution space, and K^+ release may have been higher because the concentration gradient between the interstitial space and the blood has been increased. Another approach to studying the potential coupling of intramuscular acidosis to K^+ release has been by manipulation of systemic or local acid/base homeostasis by ingestion or infusion of buffers. After citrate ingestion, the exercise-induced increase in interstitial K^+ is reduced (34), possibly indicating an attenuated K^+ release. However, an increase of the intracellular to extracellular H^+ gradient may allow for a larger lactate efflux (16), as supported by reports of citrate-induced increases of blood lactate concentrations after intense exercise (24). In this scenario, extracellular [K^+] may be reduced due to increased interstitial volume caused by elevated osmolaility. In a study where $NaHCO_3$ was infused in an exercising human arm, the K^+ release was found to be elevated compared with infusion of $CaCO_3$ (33). This finding confirms that manipulation of extracellular pH with the potential to reduce intracellular acidification does not reduce K^+ release. The mechanism by which intramuscular acidosis could be coupled to K^+ release has been speculated to be metabolic acidosis-induced increased open probability of K_{ATP} channels (8, 27). However, local infusion of the K_{ATP} channel blocker glibenclamide did not affect K^+ release during intense exercise, which also indicates that muscle K^+ release is unrelated to intramuscular acidosis. Thus, the present results appear to support previous findings showing that muscle K^+ release is unrelated to intramuscular acidosis, at least during cycle exercise with stepwise increments of workload in humans. It should, however, be noted that the importance of acidosis per se was not investigated in the present study, but it is clearly demonstrated that the degree of anaerobic energy production is unrelated to leg net K^+ release.

Another novel observation in the present study was that intense exercise in hypoxia prior to acclimatization resulted in higher levels of arterial and venous plasma [K^+] compared with normoxia. Because leg K^+ release was unaffected by hypoxic exposure, it was investigated whether the K^+ dilution space was altered. During exercise in hypoxia, the relative change in plasma volume was more pronounced than in normoxia. Thus, the increase in plasma [K^+] is possibly related to a reduced dilution space. This suggestion is supported by the observation that no difference existed in plasma [K^+] changes between intense exercise in normoxic or hypoxic conditions when adjusting the measured plasma [K^+] for the difference in plasma volume change between normoxia and hypoxia (Fig. 4). It is unknown what caused the difference in plasma volume changes during exercise in normoxia and hypoxia. Possibly, a higher intracellular osmotic pressure existed in the active muscle during hypoxia due to accelerated lactate production, which may have caused a fluid shift from the bloodstream to interstitial/intracellular compartments. It may be noted that the normoxia sea-level trial in the present study was always completed before the hypoxia trial. Thus, an order effect cannot be excluded. However, it appears unlikely that repeating the exercise trial would induce a more than twofold increase in lactate production at 260 W.

Effect of Acclimatization on Exercise K^+ Homeostatsis in Hypobaric Normoxia and Hypoxia

After 8 wk of acclimatization, leg K^+ release was reduced in both normoxia and hypoxia. Prior to the study, it was hypothesized that acclimatization would result in unaltered anaerobic energy production but reduced muscular acidosis during intense exercise at the same absolute workload and that this would result in reduced leg K^+ release. Indeed, leg K^+ release was reduced, but the anaerobic energy production, as judged by net leg efflux of lactate, was unaffected by acclimatization. Thus, it appears unlikely that the reduced K^+ release after

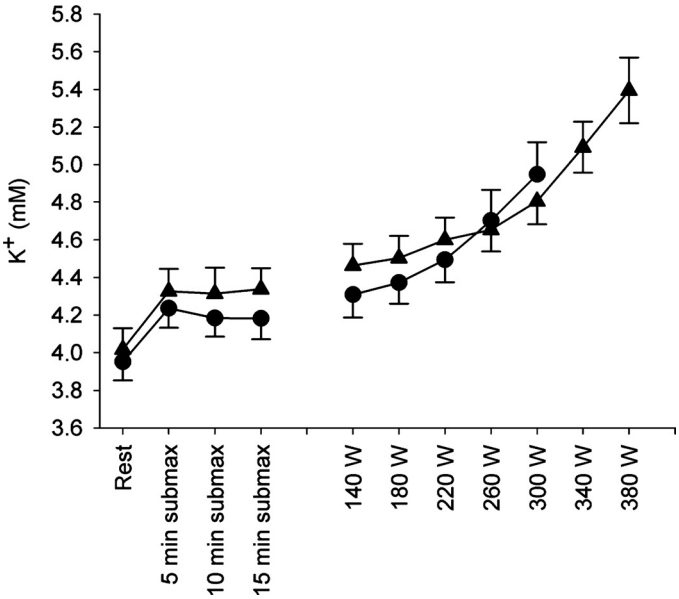

Fig. 4. Calculated venous plasma K^+ concentration, when correcting for exercise-induced changes in plasma volume. Cycling ($n = 6$) was performed in normobaric normoxia (triangles) and normobaric hypoxia (circles) prior to acclimatization. Only data points where $n = 6$ are shown. Results are expressed as means ± SE.

acclimatization is related to reduced anaerobic energy production. Because the net leg H^+ efflux was similar to before acclimatization, it also appears unlikely that intramuscular acidosis was reduced. Thus, the observed reduction in net leg K^+ release cannot be explained by either changes in anaerobic energy production or intramuscular acidosis. It may be noted that a higher lactate release during exercise in both hypoxic and normoxic conditions relative to before acclimatization was expected on the basis of results obtained at 5,260 m above sea level (35), possibly caused by an increased capacity for lactate transport in muscle and erythrocytes (17).

The reduction or even reversion of net leg K^+ release may have been caused by several factors. Net leg K^+ release is determined by muscular K^+ efflux, muscular K^+ uptake, K^+ flux from the interstitial space to the bloodstream, K^+ flux from the bloodstream to the interstitial space, and the associated changes in interstitial K^+ content. In previous studies of prolonged acclimatization, it has been found that muscular expression of Na^+, K^+ pumps is unaffected (17) or reduced 14% (10). Thus, an increased uptake of K^+ by increased Na^+, K^+ pump activity appears unlikely. From the present data, it is not possible to evaluate the flux across the endothelia or the interstitial accumulation. One striking effect of acclimatization was the reduction in maximal blood flow during exercise (19, 22) and the reduction in blood flow at a given submaximal exercise intensity. It can be speculated that K^+ release is related to capillary mean transit time, with increased capillary flow causing a K^+ washout and reduced capillary flow resulting in reduced K^+ release. However, the mechanism behind the acclimatization-induced uptake of K^+ at high intensities of exercise remains elusive. Also, it should be borne in mind that the change in leg K^+ balance is rather modest compared with the leg K^+ inflow-outflow of ~25 mmol/min prior to acclimatization and ~21 mmol/min after 8 wk of acclimatization.

After acclimatization, venous plasma [K^+] continued to increase during intense exercise in both normoxic and hypoxic conditions, despite the diminished or even reverted leg K^+ flux. The fact that K^+ was not released from the exercising leg after acclimatization at the investigated timepoints, can also be observed in Fig. 1, where differences between venous and arterial concentrations are evident during exercise at 0 wk but not at 8 wk. One possible explanation for this counterintuitive phenomenon is that the dilution space for K^+ is more severely reduced during exercise prior to acclimatization than after, which is in accordance with our observation of less pronounced changes in plasma volume after a period of acclimatization. K^+ concentration may be affected by an altered dilution space because fluid uptake by red blood cells is dissociated from K^+ uptake (5) and because a diffusion barrier may exist for K^+ from the interstitial space to the bloodstream, as previously mentioned. However, changes in K^+ dilution space do not explain all of the observed increase in venous K^+ during exercise. An additional explanation may be that after workload transitions, arterial-venous difference in plasma K^+ content are most pronounced during the first seconds on the new workload (13). Thus, sampling of blood during the last minute of the 2.5-min exercise period in the present study may not reveal the full picture of leg net K^+ release. Thus, it can be speculated that the rise in systemic K^+ levels with exercise after acclimatization was still caused by a net leg K^+ release but that the leg K^+ release was limited to a shorter time period after each workload transition as a consequence of the 8-wk acclimatization. However, it is a fact that leg K^+ release during the last minute of each exercise workload was reduced after acclimatization.

Notably, after acclimatization the hypoxia-induced right shift of the K^+ vs. workload curve (Fig. 1) was no longer apparent, and the reduction in plasma volume at high exercise intensities was no longer different between the normoxic and hypoxic exercise trial. Thus, acclimatization appears to diminish the exercise-induced reduction of total plasma volume during hypoxic exposure, but the mechanism is unclear.

Exhaustion and venous K^+. Exhaustion occurred at similar venous K^+ levels in all conditions despite a 20–25% difference in exhaustive workload. It has previously been suggested that development of muscular fatigue and exhaustion is caused by sarcolemmal inexcitability caused by extracellular K^+ accumulation (27, 31). The present data on venous K^+ values at exhaustion support this suggestion. However, the numerous adaptations to high altitude do not allow for a conclusion regarding what caused fatigue.

Conclusion. In conclusion, exercise-induced net loss of K^+ from exercising muscle is unrelated to anaerobic energy production. Moreover, acclimatization to 4,100 m for 8 wk results in reduced leg K^+ release during intense cycling exercise, which is possibly related to a reduced plasma flow at high-exercise intensities.

Perspectives and Significance

The present results show that mechanisms identified in vitro are not necessarily of importance in vivo. The results also call for further investigation into regulation of K^+ homeostasis with hypoxic exposure and acclimatization. An important question is how muscular net K^+ balance is affected at the

onset of exercise in hypoxic vs. normoxic conditions. Moreover, the study supports the suggestion of K^+ as a fatigue-inducing agent, but studies aiming at isolating the role of K^+ for fatigue during intense human exercise are warranted.

DISCLOSURES

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

REFERENCES

1. **Allen DG, Lamb GD, Westerblad H.** Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88: 287–332, 2008.
2. **Andersen P, Saltin B.** Maximal perfusion of skeletal muscle in man. *J Physiol* 366: 233–249, 1985.
3. **Bangsbo J, Madsen K, Kiens B, Richter EA.** Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J Physiol* 495: 587–596, 1996.
4. **Boning D, Maassen N, Thomas A, Steinacker JM.** Extracellular pH defense against lactic acid in normoxia and hypoxia before and after a Himalayan expedition. *Eur J Appl Physiol* 84: 78–86, 2001.
5. **Bouissou P, Peronnet F, Brisson G, Helie R, Ledoux M.** Fluid-electrolyte shift and renin-aldosterone responses to exercise under hypoxia. *Horm Metab Res* 19: 331–334, 1987.
6. **Cairns SP, Hing WA, Slack JR, Mills RG, Loiselle DS.** Different effects of raised $[K^+]_o$ on membrane potential and contraction in mouse fast- and slow-twitch muscle. *Am J Physiol Cell Physiol* 273: C598–C611, 1997.
7. **Cnaan A, Laird NM, Slasor P.** Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Stat Med* 16: 2349–2380, 1997.
8. **Davies NW.** Modulation of ATP-sensitive K^+ channels in skeletal muscle by intracellular protons. *Nature* 343: 375–377, 1990.
9. **Fitts RH.** Cellular mechanisms of muscle fatigue. *Physiol Rev* 74: 49–94, 1994.
10. **Green H, Roy B, Grant S, Burnett M, Tupling R, Otto C, Pipe A, McKenzie D.** Downregulation in muscle Na^+-K^+-ATPase following a 21-day expedition to 6,194 m. *J Appl Physiol* 88: 634–640, 2000.
11. **Greig C, Sargeant AJ, Vøllestad NK.** Muscle force and fibre recruitment during dynamic exercise in man. *J Physiol* 371: 176P, 1985.
12. **Hallen J.** K^+ balance in humans during exercise. *Acta Physiol Scand* 156: 279–286, 1996.
13. **Hallen J, Gullestad L, Sejersted OM.** K^+ shifts of skeletal muscle during stepwise bicycle exercise with and without beta-adrenoceptor blockade. *J Physiol* 477: 149–159, 1994.
14. **Jacobs RA, Donovan EL, Robinson MM.** Parallels of snipe hunting and ROS research: the challenges of studying ROS and redox signalling in response to exercise. *J Physiol* 587: 927–928, 2009.
15. **Juel C.** Muscle action potential propagation velocity changes during activity. *Muscle Nerve* 11: 714–719, 1988.
16. **Juel C.** Current aspects of lactate exchange: lactate/H^+ transport in human skeletal muscle. *Eur J Appl Physiol* 86: 12–16, 2001.
17. **Juel C, Lundby C, Sander M, Calbet JA, Hall G.** Human skeletal muscle and erythrocyte proteins involved in acid-base homeostasis: adaptations to chronic hypoxia. *J Physiol* 548: 639–648, 2003.
18. **Juel C, Pilegaard H, Nielsen JJ, Bangsbo J.** Interstitial K^+ in human skeletal muscle during and after dynamic graded exercise determined by microdialysis. *Am J Physiol Regul Integr Comp Physiol* 278: R400–R406, 2000.
19. **Lundby C, Boushel R, Robach P, Moller K, Saltin B, Calbet JA.** During hypoxic exercise some vasoconstriction is needed to match O_2 delivery with O_2 demand at the microcirculatory level. *J Physiol* 586: 123–130, 2008.
20. **Lundby C, Calbet JA, van HG, Saltin B, Sander M.** Pulmonary gas exchange at maximal exercise in Danish lowlanders during 8 wk of acclimatization to 4,100 m and in high-altitude Aymara natives. *Am J Physiol Regul Integr Comp Physiol* 287: R1202–R1208, 2004.
21. **Lundby C, Nielsen TK, Dela F, Damsgaard R.** The influence of intermittent altitude exposure to 4100 m on exercise capacity and blood variables. *Scand J Med Sci Sports* 15: 182–187, 2005.
22. **Lundby C, Sander M, van HG, Saltin B, Calbet JA.** Maximal exercise and muscle oxygen extraction in acclimatizing lowlanders and high altitude natives. *J Physiol* 573: 535–547, 2006.
23. **Lundby C, van Hall G.** Substrate utilization in sea level residents during exercise in acute hypoxia and after 4 weeks of acclimatization to 4100 m. *Acta Physiol Scand* 176: 195–201, 2002.
24. **McNaughton L, Cedaro R.** Sodium citrate ingestion and its effects on maximal anaerobic exercise of different durations. *Eur J Appl Physiol Occup Physiol* 64: 36–41, 1992.
25. **Mizuno M, Savard GK, Areskog NH, Lundby C, Saltin B.** Skeletal muscle adaptations to prolonged exposure to extreme altitude: a role of physical activity? *High Alt Med Biol* 9: 311–317, 2008.
26. **Nielsen JJ, Mohr M, Klarskov C, Kristensen M, Krustrup P, Juel C, Bangsbo J.** Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle. *J Physiol* 554: 857–870, 2004.
27. **Nordsborg N, Mohr M, Pedersen LD, Nielsen JJ, Langberg H, Bangsbo J.** Muscle interstitial potassium kinetics during intense exhaustive exercise: effect of previous arm exercise. *Am J Physiol Regul Integr Comp Physiol* 285: R143–R148, 2003.
28. **Reid MB.** Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. *Free Radic Biol Med* 44: 169–179, 2008.
29. **Renkin EM.** Exchangeability of tissue potassium in skeletal muscle. *Am J Physiol* 197: 1211–1215, 1959.
30. **Robergs RA, Ghiasvand F, Parker D.** Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* 287: R502–R516, 2004.
31. **Sejersted OM, Sjogaard G.** Dynamics and consequences of potassium shifts in skeletal muscle and heart during exercise. *Physiol Rev* 80: 1411–1481, 2000.
32. **Siggaard-Andersen O.** The van Slyke equation. *Scand J Clin Lab Invest Suppl* 37: 15–20, 1977.
33. **Sostaric SM, Skinner SL, Brown MJ, Sangkabutra T, Medved I, Medley T, Selig SE, Fairweather I, Rutar D, McKenna MJ.** Alkalosis increases muscle K^+ release, but lowers plasma $[K^+]$ and delays fatigue during dynamic forearm exercise. *J Physiol* 570: 185–205, 2006.
34. **Street D, Nielsen JJ, Bangsbo J, Juel C.** Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. *J Physiol* 566: 481–489, 2005.
35. **van Hall G, Calbet JA, Sondergaard H, Saltin B.** The re-establishment of the normal blood lactate response to exercise in humans after prolonged acclimatization to altitude. *J Physiol* 536: 963–975, 2001.
36. **van Hall G, Lundby C, Araoz M, Calbet JA, Sander M, Saltin B.** The lactate paradox revisited in lowlanders during acclimatization to 4100 m and in high-altitude natives. *J Physiol* 587: 1117–1129, 2009.
37. **Young AJ, Evans WJ, Fisher EC, Sharp RL, Costill DL, Maher JT.** Skeletal muscle metabolism of sea-level natives following short-term high-altitude residence. *Eur J Appl Physiol Occup Physiol* 52: 463–466, 1984.