Interstitial K⁺ in human skeletal muscle during and after dynamic graded exercise determined by microdialysis

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Juel, C., H. Pilegaard, J. J. Nielsen, and J. Bangsbo. Interstitial K⁺ in human skeletal muscle during and after dynamic graded exercise determined by microdialysis. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R400–R406, 2000.—Interstitial K⁺ concentrations were measured during one-legged knee-extensor exercise by use of microdialysis with probes inserted in the vastus lateralis muscle of the subjects. K⁺ in the dialysate was measured either by flame photometry or a K⁺-sensitive electrode placed in the perfusion outlet. The correction for fractional K⁺ recovery was based on the assumption of identical fractional thallium loss. The interstitial K⁺ was 4.19 ± 0.09 mM at rest and increased to 6.17 ± 0.19, 7.48 ± 1.18, and 9.04 ± 0.74 mM at 10, 30, and 50 W exercise, respectively. The individual probes demonstrated large variations in interstitial K⁺, and values >10 mM were obtained. The observed interstitial K⁺ was markedly higher than previously found for venous K⁺ concentrations at similar work intensities. The present data support a potential role for interstitial K⁺ in regulation of blood flow and development of fatigue.

Methods

DURING MUSCLE ACTIVITY, K⁺ is lost to the interstitium via voltage-dependent K⁺ channels activated during action potentials, resulting in interstitial K⁺ accumulation. The interstitial K⁺ concentration is important for cell excitability and, thereby, for development of fatigue. It is also expected to play a role in regulation of muscle perfusion as well as cardiac and ventilatory adjustments via muscle metaboreflexes (21, 32).

A number of human exercise studies have measured K⁺ concentrations in a vein draining the active muscle during contraction. During high-intensity dynamic knee-extensor exercise, femoral venous K⁺ has been shown to reach 5.9–6.8 mM (5, 8, 18, 30, 31). Higher values (up to 8.2 mM) have been reported after static exercise with restricted blood flow (27) and during bicycle exercise (33). However, venous values may not reflect the concentration in the interstitium, which is the site where K⁺ may have its effects. The changes in interstitial K⁺ concentrations during muscle activity have been difficult to quantify in humans for technical reasons. Vyskocil et al. (34) used inflexible glass electrodes and reported interstitial K⁺ values as high as 9.5 mM during muscle activity and, in a few experiments, up to 15 mM. These results may be questioned due to the dimension of the electrodes, which created a large artificial space in the muscle. Measurements with smaller (25 µm) electrodes in isolated mouse muscle stimulated to fatigue confirmed that interstitial K⁺ concentration can increase from 5 mM at rest to almost 10 mM at fatigue (17). However, in that model, the muscle was not perfused.

The microdialysis technique, developed to be used in human skeletal muscle, can be used to determine interstitial compounds. The determination of the interstitial concentrations of a compound of interest is, however, dependent on accurate estimates of the recovery (the fractional uptake), which can be determined using a labeled analog of the compound in the perfusate. Labeled potassium cannot be used due to the short half-life and high radiotoxicity. Instead, labeled thallium may be used as an internal reference (28), as this compound has been shown to diffuse in tissues at a rate similar to K⁺ (22). The fractional loss of thallium from the perfusate may, therefore, be used to correct for the partial K⁺ equilibration between interstitium and perfusate. This method has been used in a study of interstitial K⁺ during static contractions (12).

Thus the aim of the present study was to examine changes in interstitial K⁺ in human skeletal muscle during and after exercise at different intensities using the microdialysis technique.

Methods

In Vitro Experiments

In vitro experiments were performed to test the use of labeled thallium (²⁰¹TI) as an internal reference. A microdialysis probe was placed in a beaker with magnetic stirring and connected to a microdialysis pump. The perfusate contained 154 mM NaCl and ²⁰¹TI (no K⁺), whereas the medium consisted of 154 mM NaCl and either 4, 8, or 10 mM K⁺ and no thallium. The perfusion rate ranged from 1 to 10 µl/min. Dialysate samples were collected and analyzed for K⁺ by flame photometry and for ²⁰¹TI using a Packard autogamma counter. The thallium loss was used to calculate (see Calculations) the outer K⁺ concentration. In addition, experiments were performed to investigate the effect of the stirring speed.

In Vivo Experiments

Subjects. Six male subjects ranging in age from 24 to 27 yr and with an average weight and height of 85.5 (range 82–105) kg and 185.4 (180–192) cm, respectively, participated in the study. The subjects were informed of any risks associated
with the experiments before giving their consent to participate, and the study was approved by the local ethics committee. After local anesthesia (lidocaine, 1 ml of 20 mg/ml) of skin and subcutaneous tissue, six microdialysis probes (length of microdialysis membrane 30 mm, outer diameter 0.6 mm, CMA60; CMA Microdialysis, Sweden) were inserted parallel to the muscle fibers in the vastus lateralis muscle of one leg. To allow for analysis of the time course of changes in interstitial K⁺, two of the probes were used for continuous measurements of K⁺ concentration in the outlet by means of small electrodes (Diamond General, Ann Arbor, MI) placed in a small adapter. The perfusate was Ringer acetate containing (in mM) 130 Na⁺, 2 Ca²⁺, 3.5–4 K⁺, 1 Mg²⁺, and 30 Ac⁻. The exact perfusate K⁺ concentration was determined in each experiment. ²⁰¹Tl was added (activity <7,000 Bq/ml) to the perfusate of the four probes in which dialysate was collected.

Procedure. After insertion, the probes were flushed and connected to a pump (CMA 102). Then they were perfused at a rate of 2 µl/min for 1 h, and samples were collected during three 10-min periods. Thereafter, sampling was carried out at perfusion rates of 0.5, 2, and 5 µl/min with collection times of 40, 10, and 4 min, respectively, to evaluate in vivo how well thallium loss represents K⁺ recovery.

For the remaining part of the experiment, the perfusion rate was 5 µl/min. The subjects performed one-legged knee-extension exercise (kicking frequency 60/min) in a modified Krogh ergometer permitting the exercise to be confined to the quadriceps muscle (1). Passive movements (with the same range of movements as active leg movements and performed by moving the leg of the subject attached to the ergometer arm) and exercise at 10, 30, and 50 W were performed for 9 min in random order. Finally, the subjects who were able to sustain the load for 9 min carried out 70 and 90 W. All exercise periods were followed by a 12-min rest period. Dialysate samples were collected from four probes 2–5 and 5.25–8.25 min after onset as well as during 0–3 and 7.25–10.25 min after end of exercise, taking into account the delay (45 s) due to volume of the outlet tubes. The rate of perfusion of each probe was validated throughout the experiment by weighing the sample tube before and after collection. Samples were not analyzed when perfusion rate deviated ±5% or if there was any sign for hemoglobin in the dialysate. The K⁺ concentration of the samples was measured by a flame photometer (FLM3, Radiometer, Denmark) using lithium as internal standard, and 5 µl of each dialysate sample was counted in a Packard autogamma counter to determine thallium loss. Three subjects performed an additional experiment to test whether pressure influences thallium loss and concentration of interstitial K⁺. In these experiments, 2–5 microdialysis probes were inserted, and, after 1.5 h of recovery, two cuffs, placed around the leg above and below the probes, were rhythmically inflated and deflated every 2 s for 6 min.

Calculations. The relative loss (RL) of ²⁰¹Tl was calculated as RL(%) = (perfusate activity – dialysate activity)/perfusate activity. The interstitial K⁺ concentration was calculated from the dialysate samples, assuming that fractional ²⁰¹Tl loss from the perfusate was equal to the fractional K⁺ gain in the perfusate: K⁺ interstitial = K⁺ perfusate + [(K⁺ dialysate – K⁺ perfusate )/RL(%)], where K⁺ interstitial is the interstitial K⁺ concentration. From the K⁺ electrode measurements, the peak K⁺ concentration (peak K⁺) observed in the dialysate was calculated from the modified Nernst equation: log (peak K⁺) = E/55.8 + log A, where E is the change in electrode response (in mV), 55.8 is the actual electrode K⁺ sensitivity (increase in mV for 10 times increase in concentration) in the presence of 130 mM Na⁺, A is the K⁺ concentration in the dialysate collected at rest before the exercise period. The mean fractional loss, which was determined in the four other probes in the same experiment, was used to convert the peak dialysate K⁺ into the peak interstitial K⁺ concentration.

RESULTS

In Vivo Experiments

Dialysate K⁺ concentration was reduced with increasing perfusion rates, indicating a less-efficient exchange between perfusate and interstitium at higher perfusion rates. This was seen whether the initial K⁺ gradient was 4, 8, or 10 mM (Fig. 1). The linear regression between the perfusion flow and corrected K⁺ concentration, using the loss of thallium, displayed a slope not different from zero (Fig. 1), showing that the estimation resulted in a complete compensation for the partial equilibration of K⁺ across the probe membrane. The fractional loss of thallium without stirring (0.66) was lower than with fast stirring (0.82); in both cases, thallium loss represented K⁺ recovery.

In Vivo Experiments

Fractional loss. The mean fractional loss of ²⁰¹Tl at rest was reduced (P < 0.05) by increasing perfusion rates. Thus, with the use of pump rates of 0.5, 2, and 5 µl/min, the fractional thallium loss was 0.95 ± 0.01, 0.73 ± 0.01, and 0.47 ± 0.01, respectively (Fig. 2). At a perfusion rate of 5 µl/min, the fractional loss at rest of 0.47 ± 0.01 was increased to 0.64 ± 0.03 by passive movements and to 0.68 ± 0.02 during exercise at 10 W with no further increase at higher work rates. An
The present experiments demonstrated that the interstitial K⁺ concentration is increased with increasing work rates in a fashion similar to muscle blood flow (4). These findings support a role for K⁺ in regulation of blood flow, as suggested based on a number of in vitro studies (21, 32). It was also shown that the interstitial K⁺ concentrations obtained for the various probes varied markedly (Figs. 4 and 6). This inhomogeneous K⁺ distribution may reflect different activity of the muscle fibers around the probes, resulting in different K⁺ release, but it may also be the result of different fiber type composition and different capacity of Na-K pumps of the fibers near the probes. It has been reported that blood flow differs in various regions of the muscle (15, 20, 23), and it has been suggested that the active parts of the quadriceps muscle during knee-

![Fractional loss of thallium at different perfusate flow rates.](http://ajpregu.physiology.org/)

**Fig. 2.** Fractional loss of thallium at different perfusate flow rates. Fractional ²⁰¹Tl loss at rest was measured at flow rates of 0.5, 2, and 5 µl/min and at a flow rate of 5 µl/min during passive movements and at 10-, 30-, 50-, and 70-W exercise. Dashed line connects values obtained at 5 µl/min. Values are means ± SE.

The individual peak K⁺ concentrations obtained with the electrodes during passive and active exercise are depicted in Fig. 6, which demonstrates large variations between probes. In these probes, passive movements increased interstitial K⁺ from 4.32 ± 0.05 to 5.08 ± 0.35 mM (n = 9 probes). A significant increase in interstitial K⁺ to 6.09 ± 0.31 mM (n = 10 probes) occurred at 10 W, and a further increase to 6.92 ± 0.27 and 8.01 ± 0.36 mM was obtained at 30 and 50 W.

**DISCUSSION**

The main findings of the present study are that the interstitial K⁺ concentration was elevated with increasing work intensity and that there was a large spatial variation in interstitial K⁺ in the contracting muscle. Furthermore, the interstitial K⁺ concentration was considerably higher than previously reported exercise-induced concentrations in venous plasma K⁺ concentrations.

**Fig. 3.** Mean interstitial K⁺ at passive movements, preexercise, and at power outputs of 10, 30, and 50 W. Interstitial K⁺ was determined as a mean for each subject and then means ± SE of all subjects.

*Significantly different from preexercise.

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The K⁺ concentration rose quickly (one-half peak concentration after ~1 min) and reached the peak value ~4 min after the onset of exercise, after which it remained constant in the last part of exercise. After exercise, the K⁺ concentration decreased toward the resting value with a one-half time of 2–3 min.

Electrode measurements. A typical reading from a K⁺ electrode placed in the probe outlet is depicted in Fig. 5. The K⁺ concentration rose quickly (one-half peak concentration after ~1 min) and reached the peak value ~4 min after the onset of exercise, after which it increased interstitial K⁺ concentration was 5.98 ± 0.22, 4.45 ± 0.14, and 4.19 ± 0.06 mM at 10, 30, and 120 min, respectively, after the probes had been inserted.

At rest, with the use of pump rates of 0.5, 2, and 5 µl/min, the dialysate K⁺ concentration was 4.39 ± 0.09, 4.24 ± 0.06, and 3.95 ± 0.05 mM, respectively, with no difference in the calculated interstitial K⁺ concentration (4.53 ± 0.08, 4.40 ± 0.09, and 4.45 ± 0.14 mM, respectively).

During passive movements, dialysate K⁺ increased (P < 0.05) to 4.75 ± 0.29 mM, resulting in an interstitial K⁺ concentration of 5.78 ± 0.53 and 5.47 ± 0.50 mM in the first and second part of the period, respectively. Interstitial K⁺ declined to 4.32 ± 0.11 and 3.81 ± 0.12 mM 0–3 and 7.25–10.25 min after the passive movements.

The mean dialysate K⁺ concentration was 5.47 ± 0.16, 5.79 ± 0.38, and 7.00 ± 0.48 mM at 10, 30, and 50 W exercise, respectively, with no difference between first and second part of the exercise bouts. The mean interstitial K⁺ concentration increased (P < 0.05) during exercise at all intensities used (Fig. 3). Interstitial K⁺ was lower (P < 0.05) after exercise than during exercise, but it was still elevated (P < 0.05) 0–3 min after exercise compared with the preexercise value. In the second part of recovery (7.25–10.25 min), the K⁺ concentration was not different from the preexercise level, except for exercise at 30 W (Fig. 3). There was a large variation in the interstitial K⁺ concentrations obtained with different probes in the same subject (Fig. 4).

Elevated fractional loss (0.53–0.57) of thallium was also seen in the first sampling period after exercise.

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Extensor exercise are maximally perfused even at low exercise intensities (25). Thus it may be that local blood flow is regulated by the accumulation of $K^+$ in the region.

To accurately determine interstitial $K^+$ by microdialysis, the recovery of $K^+$ needs to be known. It was, therefore, investigated whether thallium loss could represent $K^+$ uptake. The in vitro experiments demonstrated that recovery of $K^+$ is only partial and that a full compensation can be obtained if the fractional thallium loss is used to calculate the $K^+$ concentration in the medium outside the probe. Thus the in vitro experiments justify the use of thallium loss to represent recovery of $K^+$. This is also supported by the in vivo measurements, which demonstrated that the calculated interstitial $K^+$ concentration at rest was the same at three different perfusion rates, which resulted in differences in the relative thallium loss and the dialysate $K^+$ concentrations. As observed for other compounds (3), the fractional loss of thallium was higher in vitro than in vivo, which may be due to a restricted diffusion in the interstitial space and/or that binding of proteins may affect the permeability of the probe.

Fig. 4.Interstitial $K^+$ measured in individual probes in 6 subjects at passive movements and at different work loads. Each symbol represents a probe. Lines connect values obtained with same probe.

Fig. 5. Continuous measurement of interstitial $K^+$ obtained with an electrode placed in outlet of microdialysis probe. $y$-Axis is in mV scale; values were later converted to mM $K^+$ as described in METHODS.
During one-legged knee-extensor exercise, the femoral vein does not only drain the active part of the quadriceps muscle, because a fraction of the venous blood comes from inactive tissues and inactive parts of the quadriceps muscle. This means that the venous concentration is likely to be less than the concentration in the interstitium of the contracting muscle. The difference can be evaluated from the following examples. At low-intensity, one-legged, knee-extensor exercise (10 W), femoral venous blood flow will be 2.0–2.5 l/min (4, 24). The exact distribution of blood flow between active and passive muscles is not known. It has been reported that knee-extensor exercise is restricted to the four muscles of the quadriceps muscle (1, 26), which may have a mass of 2.5–3 kg. Furthermore, the active parts of the quadriceps muscle have been suggested to be maximally perfused (−2.5 l·min⁻¹·kg⁻¹ of active muscle) (24). If it is assumed that 10-W exercise activates 10% of the knee extensors, then blood flow to active parts of quadriceps muscle will only be 0.6–0.8 l/min. A similar value is assumed if it is also assumed that the blood flow of −1.2 l/min (24) seen during passive movements also occurs in the muscle parts that are moved passively during low-intensity exercise. Using an average concentration of interstitial K⁺ of 6 mM for the active parts and assuming K⁺ equilibration as well as no exchange of K⁺ occurring for the inactive parts leads to a calculated mean femoral venous K⁺ concentration of 4.7–5.0 mM, which is only slightly higher than the observed concentration (Fig. 7). At an exercise intensity of 50 W, blood flow may be 4.3 l/min (4, 18), of which 1.2 l/min (24) may be perfusing inactive tissue. With the use of an average interstitial K⁺ concentration of 9.2 mM, the calculated mean femoral venous concentration is 7.6 mM, which is closer to but still higher than observed (Fig. 7).

It can be concluded that the dilution of femoral venous blood caused by flow from inactive tissues can explain part of the discrepancy between interstitial concentrations measured with microdialysis and femoral venous concentrations and that the relative deviation can be expected to be more pronounced at low-intensity exercise. Another possible explanation for the difference between interstitial and venous concentrations is that K⁺ may be taken up in inactive muscle (7, 29–31).

**Fig. 6.** Individual values of peak interstitial K⁺ during exercise measured with an electrode placed in outlet of microdialysis probe. Lines connect values obtained for a probe. Data from all 6 subjects.

**Fig. 7.** A comparison between femoral venous plasma K⁺ values obtained during one-legged knee-extensor exercise and interstitial K⁺ measured with microdialysis. Microdialysis data (present study) are means of electrode determinations (peak values; □, top) and mean of flame photometric measurements (○, bottom). Venous concentrations from literature represent highest value if more than 1 value is given. Venous K⁺ data from Refs. 18 (▼), 19 (▲), 31 (●), 30 (■), and 3 and 6 (●).
because arterial K⁺ concentration is increased. Such an uptake will lower the increase in femoral venous K⁺ concentration caused by release from active fibers.

The difference between the interstitial K⁺ and the venous samples might also be due to the presence of a concentration gradient between interstitium and blood plasma. During intense knee-extensor exercise, leg blood flow is increased more than 10-fold (1, 4, 6, 18). Thus the mean transit time of the blood passing the capillaries in the active part of the muscle may be reduced, which may result in only partial equilibration of K⁺ between interstitium and blood plasma. This proposal is at first contradicted by the observation that the venous K⁺ concentration, measured by electrodes, shows a steep drop immediately at the end of exercise and preexercise values are reached within 60 s after exercise (13, 14, 33). However, the "invisible" accumulated K⁺ may only amount to 0.16 mmol/kg, assuming that the interstitial K⁺ concentration at the end of exercise is 1 mM higher than the venous value and that the interstitial space is 16% of the muscle (31). At the end of exercise, the K⁺ release due to action potentials will immediately stop and the Na-K pump will mediate a net K⁺ uptake. If the maximal pump activity of 70–80 µmol·s⁻¹·kg⁻¹ (11) is obtained, any "extra" accumulated K⁺ is expected to be removed from the interstitium within a few seconds after termination of exercise. The accumulated K⁺ would, therefore, barely be detected even with venous K⁺ electrodes with low response time, and the analysis of venous K⁺ cannot exclude the presence of an interstitial-to-venous K⁺ gradient during intense exercise.

As discussed, "dilution" of the blood from active muscle, K⁺ uptake in inactive tissues, and incomplete K⁺ equilibration between interstitium and blood may explain the difference between interstitial and femoral venous K⁺ concentration. Nevertheless, the possibility that microdialysis, in combination with dynamic exercise, creates an artificial high interstitial K⁺ concentration should be considered. Passive movements of the leg lead to a marked increase in interstitial K⁺ (Fig. 4), which may reflect that some K⁺ was released due to fiber damage or other mechanisms creating large sarcolemmal K⁺ permeability. However, the facts that interstitial K⁺ is not different in the first and second sample during passive exercise and that interstitial K⁺ returned to preexercise levels in the recovery period after both exercise and passive movements indicate that such a condition, if present, is not permanent.

Effects of high interstitial K⁺ concentration. Increased extracellular K⁺ concentration has been shown to reduce the force development in isolated rat and mouse muscle. A minor reduction (10–20%) in force occurs with K⁺ concentrations up to 8 mM, whereas 10 and 12.5 mM K⁺ decreased force by 25–75% and 60–100%, respectively (2, 9, 10, 16). On the basis of the Nernst equation, the doubling of interstitial K⁺ (from 4 to 8 mM), which was observed at 50 W, is consistent with an 18-mV less-negative K⁺ equilibrium potential. The effect on K⁺ equilibrium potential is probably even larger as intracellular K⁺ decreases during exercise (29). The effect of a less-negative K⁺ equilibrium potential on resting membrane potential in human muscle is not known. On the basis of experiments with isolated rat muscle (9), a depolarization of approximately two-thirds of the change in K⁺ equilibrium potential is likely to occur during exercise. This is probably due to activation of the Na-K pump, which will counteract the depolarization due to high interstitial K⁺ (2). The depolarization during exercise probably causes a combined effect of a reduced Ca²⁺ release due to action potential depression and a complete loss of excitability in some fibers (10). Thus the finding of interstitial K⁺ concentrations >10 mM suggests that potassium affects force development during high-intensity exercise in humans.

Perspectives

This is the first study to determine K⁺ in muscle interstitium during dynamic exercise using microdialysis probes. It was demonstrated that muscle interstitial K⁺ was related to the exercise intensity. These findings support a role of K⁺ in muscle blood flow regulation either mediated by a direct effect on blood vessels or indirectly via the nervous system. Furthermore, an interstitial K⁺ concentration >10 mM was frequently observed, which likely has a major impact on the force development of the surrounding fibers. The finding of large variability in interstitial K⁺ within a contracting muscle and that interstitial K⁺ was considerably higher than venous K⁺ concentration illustrates the importance of measuring interstitial K⁺ to examine the effect of K⁺ on muscle metabolism and function.

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