Membrane leakage and increased content of Na\(^+\)-K\(^+\) pumps and Ca\(^{2+}\) in human muscle after a 100-km run

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membrane leakage supports the theory that Ca\(^{2+}\) plays a role in the initiation of degenerative processes in muscles after severe exercise.

Increasing support is that exercise leads to an increase in cytoplasmic Ca\(^{2+}\) and that the elevated resting Ca\(^{2+}\) level may activate proteolytic enzymes such as calpain to digest essential structural elements of the muscle fibers (4, 11, 17). This in turn leads to membrane damage, leakage of enzymes, and perhaps further accumulation of intracellular Ca\(^{2+}\), thus initiating a vicious cycle of cell degradation (3, 12). It was recently suggested that the events leading to loss of cellular integrity involve an early intracellular accumulation of Ca\(^{2+}\) as an important pathway. This was based on the finding that, in electrically stimulated rat muscles, the rate of Ca\(^{2+}\) uptake undergoes a rapid and pronounced increase (within 1 min) (10). This leads to a progressive accumulation of intracellular Ca\(^{2+}\), which is followed by a late loss of LDH from the muscle cells (11). In view of the implications of such events for performance and training, it is important to examine whether similar phenomena can be detected in human subjects.

Another possible performance-limiting factor is the pronounced decrease of the transmembrane Na\(^+\) and K\(^+\) gradients in skeletal muscle fibers during exercise (for reviews, see Refs. 36 and 37). This is evidenced by an increase in plasma K\(^+\) and intracellular Na\(^+\) and a decrease in intracellular K\(^+\). The increase in plasma K\(^+\) leads to an increase in ventilation and in blood flow to the working muscles (31, 39), and thus it can be seen as one of the signals in the regulatory adjustment to exercise. However, the possibility exists that if the transsarcolemmal K\(^+\) gradient (or Na\(^+\) gradient) is sufficiently reduced, a loss of muscle function due to reduced excitability may occur (16, 30, 37). It is important, therefore, that especially the K\(^+\) loss is kept at a relatively low rate during prolonged exercise. This is achieved by the acute activation of the Na\(^+\)-K\(^+\) pump (24). In addition, a number of studies have shown that various types of training lead to an upregulation of the Na\(^+\)-K\(^+\) pump content (9). This is some evidence that exercise in humans may also lead to an acute change in the proportion of Na\(^+\)-K\(^+\) pump content.

It is well known that prolonged exercise may lead to damage of muscle fibers, especially if there is a component of eccentric work involved in the exercise performed, as in running (3). Thus high levels of muscle-specific enzymes such as creatine kinase (CK) or lactate dehydrogenase (LDH) have repeatedly been observed in the blood of endurance runners after marathon and ultra-marathon events as reviewed in detail by Noakes (25). Extreme endurance sporting events are becoming increasingly popular, and the athletes who undertake such challenges experience an exercise-induced disruption of the muscle cell membranes that is generally accompanied by muscle soreness and pain and may ultimately lead to functional disability and limit performance. However, the mechanisms that underlie the development of cellular muscle damage after exercise are not yet clarified. One idea that has gained increasing support is that exercise leads to an increase in cytoplasmic Ca\(^{2+}\) and that the elevated resting Ca\(^{2+}\) level may activate proteolytic enzymes such as calpain to digest essential structural elements of the muscle fibers (4, 11, 17). This in turn leads to membrane damage, leakage of enzymes, and perhaps further accumulation of intracellular Ca\(^{2+}\), thus initiating a vicious cycle of cell degradation (3, 12). It was recently suggested that the events leading to loss of cellular integrity involve an early intracellular accumulation of Ca\(^{2+}\) as an important pathway. This was based on the finding that, in electrically stimulated rat muscles, the rate of Ca\(^{2+}\) uptake undergoes a rapid and pronounced increase (within 1 min) (10). This leads to a progressive accumulation of intracellular Ca\(^{2+}\), which is followed by a late loss of LDH from the muscle cells (11). In view of the implications of such events for performance and training, it is important to examine whether similar phenomena can be detected in human subjects.

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isoenzymes, and it was suggested that there is a translocation of Na\(^{+}\)-K\(^{+}\) pumps from an intracellular pool to the surface membranes during exercise (18). However, an acute upregulation of the total content of Na\(^{+}\)-K\(^{+}\) pumps was not found in rat muscles stimulated continuously for 24 h in vivo (8). The question remains whether exercise in humans can actually produce a significant increase in the total tissue Na\(^{+}\)-K\(^{+}\) pump content measured by the quantitative \(^{3}\text{H}\)ouabain binding technique. The extreme duration of the 100-km run provides a good experimental setting to test this possibility.

The present study was undertaken to test the following hypotheses that 1) prolonged running leads to an accumulation of Ca\(^{2+}\) in skeletal muscle that coincides or correlates with the occurrence of muscle cell damage assessed by measuring leakage of muscle-specific enzymes, 2) prolonged running leads to an upregulation of the content of Na\(^{+}\)-K\(^{+}\) pumps in skeletal muscle, and 3) running performance is related to the content of Na\(^{+}\)-K\(^{+}\) pumps in skeletal muscle.

METHODS

Subjects. Ten healthy men participated in this study. All were moderately to well-trained noncompetition runners, age 38 ± 2 yr (range = 25–51 yr), height 176 ± 2 cm (range = 169–185 cm), and weight 73 ± 2 kg (range = 61–83 kg). Subjects had been training regularly for at least 5–6 mo before the run, covering distances from 29 to 110 km/wk. The 100-km run was performed on a coastal route with no severe hills on a day with pleasant weather conditions for running (highest temperature ~20°C, modest wind, few showers). Subjects had given their informed consent of participation, and the study was approved by the local ethical committee.

Oxygen consumption rate and heart rate monitoring. Two to three weeks before the run, subjects performed a progressive maximal treadmill test in which running speed was increased every minute in steps of 1 km/h from 10 to 14 km/h, after which inclination was increased by 2% every minute until exhaustion. During treadmill testing, expired air was sampled continuously, and the rates of oxygen consumption and carbon dioxide production were determined every 10 s by an on-line respiratory gas exchange analyzer (model AMIS 2001, Innovision, Odense, Denmark). Maximal oxygen uptake (\(\text{VO}_2\max\)) of the subjects was calculated as the maximal rate achieved over any 60 s during the test. During this period, all subjects had a respiratory exchange ratio of at least 1.10. Heart rate was monitored continuously every 5 s during the test with a pulse monitor (Polar Accurex Plus, Polar Electro Oy, Kempele, Finland), and the highest heart rate measured during the period of \(\text{VO}_2\max\) for each subject was considered to be the maximal heart rate (HR\(\text{max}\)). During the 100-km run, subjects ran with pulse monitors, and the heart rate was saved to a file every minute. Also, the time to complete the run and split times for every 10 km were taken. Subjects were instructed to record their resting heart rate (HR\(\text{rest}\)) at home in the morning just after waking up. An intensity measure was calculated as a percentage of the heart rate reserve (HRR)

\[
\text{intensity} = \frac{\text{HR}_{\text{run}} - \text{HR}_{\text{rest}}}{\text{HR}_{\text{max}} - \text{HR}_{\text{rest}}}
\]

where HR\(\text{run}\) is the heart rate during running. Following this, oxygen consumption during running (\(\text{VO}_2\text{run}\)) could be estimated by assuming that there is a 1:1 linear relation between the %HRR and %\(\text{VO}_2\max\) reserve (\(\text{VO}_2\max R = \text{VO}_2\max - \text{VO}_2\text{rest}\)) (1)

\[
\text{VO}_2\text{run} = \text{intensity} \times \text{VO}_2\text{max R}
\]

Blood sampling and analysis. Blood samples were drawn from an antecubital vein by venipuncture. Samples were taken 4–6 wk before the run, immediately after the run (within 2 min of completion), and 1, 2, 3, 5, and 12 days after the run. Blood samples were divided in three and stored on ice in heparinized tubes until further analysis. Hemoglobin concentration and hematocrit were measured by using an automated hematology flow cytometry analyzer (Coulter SKTS, Beckman Coulter, Fullerton, CA). Plasma volume changes were calculated from hemoglobin and hematocrit values according to the method of Dill and Costill (7).

After centrifugation, plasma samples were analyzed for total content of Ca\(^{2+}\), Mg\(^{2+}\), Na\(^{+}\), K\(^{+}\), CK, LDH, and ionized Ca\(^{2+}\) by standard commercial kits applied in a multi-analyzer system (COBAS Integra 700, Hoffmann-La Roche, Basel, Switzerland).

Biopsies. Muscle biopsies (50–100 mg) were obtained 4–6 wk before the run and again about 30 min after the run. Biopsies were taken from the left vastus lateralis muscle with the use of a conchotome, according to the technique of Dietrichson et al. (6). The cutaneous, subcutaneous and fascial layers over the desired area of incision were anesthetized with xylocaine/adrenaline before an ~5-mm-long incision was performed. The conchotome was advanced into the muscle, opened, advanced a further short distance, and then closed and withdrawn. The biopsy material was immediately cooled with liquid nitrogen, and the samples were stored at ~80°C until further analysis.

Ouabain binding. The total concentration of \(^{3}\text{H}\)ouabain binding sites in the muscle biopsies was determined as previously described (28). Three to four small segments weighing 3–8 mg were cut from the biopsies and incubated for 2 × 10 min at 37°C in a buffer containing 10 mM Tris-HCl, 1 mM Tris-vanadate, and 250 mM sucrose (pH = 7.4). After this incubation, samples were then incubated for 120 min at 37°C in a similar buffer containing \(^{3}\text{H}\)ouabain (2 μCi/ml) and unlabeled ouabain to a final concentration of 10⁻⁶ M. This was followed by four 30-min washouts in ice-cold unlabeled buffer to remove \(^{3}\text{H}\)ouabain not bound to the receptors. At the end of washout, specimens were blotted, weighed, and soaked in 0.5 ml 0.3 M trichloroacetic acid (TCA) overnight before \(^{3}\text{H}\) activity was counted. On the basis of the specific activity of \(^{3}\text{H}\)ouabain in the incubation medium, the total amount of \(^{3}\text{H}\)ouabain retained in the samples was calculated and corrected for isotopic purity, incomplete saturation, unspecified uptake of \(^{3}\text{H}\)ouabain, and loss of specifically bound \(^{3}\text{H}\)ouabain occurring during washout (for details see Ref. 28).

Muscle Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), and water content. Biopsy samples weighing 16–32 mg were soaked overnight in 2.5 ml of 0.3 M TCA. In this TCA extract, Na\(^{+}\) and K\(^{+}\) contents were determined by using a flame photometer with lithium as internal standard (FLM3, Radiometer, Copenhagen, Denmark). Ca\(^{2+}\) content was determined by using the same TCA extract by atomic absorption spectrometry as described in detail by Gissel and Clausen (10). For each biopsy, this procedure was carried out twice. For the second procedure, water content was determined by weighing the samples before and after overnight drying at 60°C.

Statistics. All values are means ± SE, in some cases with the range of values in parentheses. For plasma concentrations, an ANOVA for repeated measures was first used to
identify possible differences within a time-group series. To test for differences between two specific groups, Student's t-test for paired observations was used, and only the P values obtained from this test are reported. To test for correlations between two parameters, linear regression analysis was performed. Significance level and a correlation coefficient (r) are reported for the performed correlations.

RESULTS

Physiological characteristics and performance of the subjects. The average VO₂max of the subjects was 4.3 ± 0.2 l O₂/min (3.1–5.1) or expressed relative to body weight 59 ± 2 ml O₂·min⁻¹·kg⁻¹ (51–67), indicating that subjects were moderately to well trained for an aerobically demanding event such as a 100-km run. The 100-km distance was completed by all subjects in an average time of 641 ± 23 min (496–719). Mean intensity of running calculated on the basis of heart rate was 68 ± 2% (58–77), and mean speed of running was 9.7 ± 0.4 km/h (8.6–12.2). There was a tendency for both the intensity and the speed of running to decrease during the course of the run. Thus mean speed from kilometers 0 to 50 was significantly higher compared with mean speed between kilometers 50 and 100 (10.9 ± 0.2 vs. 8.5 ± 0.2 km/h; n = 10, P < 0.001), and the mean intensity was also lowered when comparing kilometers 0–50 with kilometers 50–100 (70.5 ± 1.1 vs. 66.2 ± 1.0%, n = 9, P < 0.005).

Electrolytes in muscle and plasma. As shown in Fig. 1A (top), a muscle Ca²⁺ content of 0.84 ± 0.03 μmol/g wet weight (0.75–0.99) was found in the biopsies taken before the run. After the run, there was a significant increase in muscle Ca²⁺ content to 1.02 ± 0.04 μmol/g wet weight (0.86–1.24). This corresponds to a 22% increase in total muscle Ca²⁺ (P < 0.001). There was also an increase in plasma Ca²⁺ after the run from 2.42 ± 0.03 to 2.76 ± 0.06 mM (Fig. 1B, top; P < 0.001). We therefore examined whether this could have contributed to the increase in Ca²⁺ content of the biopsies. When an extracellular volume of 15% (38) and an extracellular Ca²⁺ concentration corresponding to 50% of the plasma concentration (due to lack of Ca²⁺ binding proteins in the interstitial space) is assumed and by correcting for the rise in extracellular Ca²⁺, it was calculated that the increase in cellular Ca²⁺ amounts to 24% (from 0.65 ± 0.03 to 0.81 ± 0.04 μmol/g total tissue wet weight, P < 0.005). Thus the main portion of the observed Ca²⁺ accumulation in muscle biopsies is confined to the muscle cells, and the increase in total tissue Ca²⁺ content could not be accounted for by a rise in extracellular Ca²⁺. It was found that the increase in muscle Ca²⁺ content (corrected for extracellular Ca²⁺) was negatively correlated to VO₂max (in ml O₂·min⁻¹·kg⁻¹) of the subjects (r = −0.68, P < 0.05), indicating that the training status of subjects affects the uptake of Ca²⁺ in muscle fibers (Fig. 2). Concentrations of both Na⁺ and K⁺ in plasma and in muscle biopsies were measured before and immediately after the run. There was no significant change in plasma Na⁺, muscle Na⁺, or muscle K⁺ (Fig. 1). There was, however, a significant increase of 37% in plasma K⁺ immediately after the run from 4.0 ± 0.1 to 5.5 ± 0.2 mM (P < 0.001; Fig. 1). As shown in Fig. 1, the plasma K⁺ level was normalized in the next plasma sample taken the next day.

Muscle enzymes in plasma. As shown in Fig. 3 there was a marked rise in plasma CK from a prerun value of 281 ± 104 (115–1,257) to 5,476 ± 1,130 U/l (1,014–11,566) just after the run, corresponding to an almost 20-fold rise (P < 0.005). In 7 of 10 subjects, maximal plasma values of CK were measured in the sample drawn immediately after the run, whereas the three remaining subjects had maximal elevation of CK 36 h after the run. Plasma CK values of all subjects were normalized at day 5 (Fig. 3). A similar but less pronounced elevation of plasma LDH from 412 ± 19 (295–506) to 1,252 ± 97 U/l (838–1,730) was found (P < 0.001). Maximal LDH values for all subjects was found immediately after the run, and the values of LDH were only normalized in the sample taken at day 12 after the run.

Despite the simultaneous occurrence of increased Ca²⁺ content and elevation of muscle-specific enzymes in plasma, individual values for the increase in Ca²⁺ content were only weakly and insignificantly correlated to the increases in plasma CK or LDH (r = 0.35 and 0.46, respectively, P > 0.05). This could, however, be because of the relatively large individual variation found in both plasma enzymes and in Ca²⁺ accumulation, which may be related to a number of other factors (see DISCUSSION). There was a significant positive correlation between LDH and CK values immediately after the run (r = 0.85, P < 0.005), in keeping with the assumption that these enzymes were both derived from the same source of damaged tissue.

Plasma volume changes. On the basis of the changes in hemoglobin and hematocrit, it could be calculated that there was a decrease in the plasma volume of 13 ± 7% from the prerun to postrun samples (P < 0.001). On days 1, 2, and 3, there was a slight elevation of plasma volume of 4 ± 6, 7 ± 7, and 5 ± 6%, respectively (P < 0.05). To test whether the electrolyte changes in plasma were caused by this hemoconcentration, plasma electrolyte values were individually corrected for plasma volume change. When this correction was done, the increase in plasma Ca²⁺ disappeared completely (ANOVA F test, P = 0.61; t-test prerun vs. postrun, P = 0.9), the increase in plasma K⁺ was reduced from 37 to 20% but was still significant (t-test prerun vs. postrun, P < 0.001), and finally there was a loss of Na⁺ from the plasma of 13 ± 7% from the prerun to the postrun sample (P < 0.001), indicating that plasma Na⁺ content changed in exact proportion to the plasma volume change, thereby keeping Na⁺ concentration at a constant level. On days 1, 2, and 3 after the run, there was also a tendency for plasma Na⁺ content (corrected for plasma volume) to be higher than in the prerun sample. This trend only reached the level of significance on day 2 after the run (P < 0.05), but this result indicates that plasma Na⁺ concentration is regulated very tightly during and after exercise (Fig. 1) even when plasma volume varies considerably.
Plasma volume changes had no significant influence on the pattern of changes in plasma enzyme content.

**Na⁺-K⁺ pump content.** The homeostasis of K⁺ is governed by the rates of uptake into and release from the muscle cells. Therefore, the rate of active Na⁺-K⁺ pumping is an important determinant for the total amount of K⁺ lost to the plasma. Although the content of Na⁺-K⁺ pumps cannot predict the actual amount of Na⁺ and K⁺ pumped through the membrane, it is a measure of the maximum capacity for Na⁺-K⁺ pumping. As shown in Fig. 4, there was a significant increase in the Na⁺-K⁺ pump content in muscle biopsies from 334 pmol/g wet wt before the run to 378 pmol/g wet wt after the run, corresponding to a 13% increase ($P < 0.05$). No significant correlations could be detected between the Na⁺-K⁺ pump content or increase in Na⁺-K⁺ pump content and either running time, V̇O₂ max, increase in total muscle Ca²⁺ content, or the increase seen in plasma K⁺.

**Muscle water content.** To test whether some of the observed changes in muscle Ca²⁺ or Na⁺-K⁺ pump content could be caused by a decrease in muscle water content, the dry and wet weights of the biopsies were determined. Total water content of the muscle biopsies was exactly the same before (78.3 ± 0.6%) and after (78.1 ± 0.3%) the 100-km run. Therefore, the observed increases in Ca²⁺ and Na⁺-K⁺ pump content could not be explained by a decrease in muscle water content.

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Fig. 1. Ca²⁺, K⁺, and Na⁺ in muscle and plasma before and after 100-km running. A: total Ca²⁺ (top), K⁺ (middle), and Na⁺ (bottom) contents in vastus lateralis biopsies taken 4–6 wk before (pre) or shortly after (post) completion of a 100-km run. B: plasma Ca²⁺ (top), K⁺ (middle), and Na⁺ (bottom) concentrations in samples taken 2 wk before (pre), immediately after (post), and 1, 2, 3, 5, or 12 days after (D1, D2, D3, D5, D12, respectively) completion of a 100-km run. Columns and symbols are means, with error bars showing SE ($n = 10$). *Significant difference from the pre-run sample ($P < 0.001$). NS, not significant.
Correlates of performance. Individual running times were not correlated to Na\(^+-\)K\(^+\) pump content before or after the run. Also there was no correlation between running time and plasma K\(^+\) values immediately after the run. Therefore, the present results do not support the idea that Na\(^+-\)K\(^+\) pump capacity or K\(^+\) accumulation in plasma present major limitations to the performance in endurance exercise.

Previous studies have suggested that \(\dot{V}_{O_2}\) max can be used as an indicator of performance in long distance running (27, 35). In our study, we found such a correlation between \(\dot{V}_{O_2}\) max and running time. However, although the correlation was significant, \(r\) was quite low compared with earlier studies (\(r = 0.65, P < 0.05\)), indicating that other factors were contributing to performance. The correlation between \(O_2\) consumption during running and running time was somewhat stronger (\(r = 0.78, P < 0.005\)), but even here a significant portion of the variation in running time was not accounted for, indicating that other factors including running economy are important.

**DISCUSSION**

It is well accepted that increases in plasma levels of muscle-specific enzymes such as CK and LDH after exercise are caused by leakage from damaged muscle fibers (3, 25). Values of CK in plasma after exercise depend largely on the duration and type of the exercise. Thus exercise of long duration and/or involving eccentric contractions leads to the largest elevations of plasma CK and other muscle enzymes (25). The values of plasma CK observed in this study were equal to (20) or higher than (25, 32) what has previously been reported for runners completing races of similar distances as the 100-km run. Interestingly, the peak value for plasma CK and LDH was found immediately after the run in our study and not 1–2 days after, as is often the case after marathon running (25). This could possibly be explained by the extreme duration of 8–12 h in this event. During such a long time period with a high blood flow through the muscles, there could be sufficient time for a washout of a major part of the enzymes released from the damaged fibers.

In a study by Schwane et al. (33), it was found that elevation of CK in plasma coincides with ultrastruc-
tural indications of muscle damage and muscle soreness and with a decrease in functional ability. Other studies, however, did not show any relationship between the levels of muscle enzymes in plasma after exercise and the amount of histologically observable damage (21) or the muscle mass involved in the exercise (29). This indicates that the mechanisms for release and removal of these enzymes to and from the plasma are not fully understood, but the discrepancies could also be because muscle damage after exercise is localized to only a small percentage of fibers and these fibers are not always present in the biopsy samples used to screen for histological changes. Furthermore, the mechanisms that underlie the actual muscle damage during exercise are also still widely debated. One idea that has gained increasing support is that exercise leads to an increase in cytoplasmic \(Ca^{2+}\) and that the elevated resting \(Ca^{2+}\) level may activate proteolytic enzymes such as calpain to digest essential structural elements of the muscle fibers (4,11,17). This, in turn, leads to membrane damage, leakage of enzymes, and perhaps further accumulation of intracellular \(Ca^{2+}\), thus initiating a vicious cycle of cell degradation (3,12).

We observed a negative correlation between \(\overline{V}_{O_2\text{max}}\) and increase in muscle \(Ca^{2+}\) content after 100-km running. Thus the most well-trained runners (with the highest \(\overline{V}_{O_2\text{max}}\)) are least likely to accumulate \(Ca^{2+}\) in their muscles. In line with this, other studies have shown that previous training protects against muscle damage after endurance events (26) or eccentric exercise (34). In addition to this, there could be an effect of fiber-type distribution because subjects with high \(\overline{V}_{O_2\text{max}}\) values (and low \(Ca^{2+}\) accumulation) are likely to have a relatively high percentage of slow twitch fibers. In keeping with this, in rats, the fast-twitch extensor digitorum longus muscles show a much greater \(Ca^{2+}\) accumulation after stimulation than the predominantly slow-twitch soleus muscles (11).

Our observation that large CK elevations are found simultaneously with increased \(Ca^{2+}\) content in muscle biopsies after 100-km running is consistent with the hypothesis that intracellular \(Ca^{2+}\) plays a role in the development of exercise-induced muscle damage. The relatively modest increase in total muscle \(Ca^{2+}\) might reflect a more pronounced uptake of \(Ca^{2+}\) confined to a few percent of the fibers. By assuming a plasma volume of 3.5 l, we find that the maximal amount of CK present in plasma after the 100-km run is 5,476 \(U/l \times 3.5 l\), which is \(\sim 19,200 U\). The content of CK in skeletal muscle of male marathon runners has been reported to be around 3,000 \(U/g\) wet wt (2). Thus, as a rough estimate of the extent of muscle damage, it can be calculated that the CK present in plasma after the 100-km run corresponds only to the total amount of CK present in 6.4 g of skeletal muscle, which amounts to around 0.02% of the total pool of muscle cells. This is likely to represent an underestimate but is in keeping with the idea that cellular damage is restricted to a very small fraction of the fibers.

In a number of previous studies, measurements of \(Na^+/K^+\) pumps in vastus lateralis muscle of humans using the \({^3H}\)ouabain binding technique ranged between 223 and 339 pmol/g wet wt (as reviewed in Ref. 5). \(Na^+/K^+\) pump content measured in the present study (334 pmol/g wet weight) is in the high end of this range. This is likely to be because our subjects had all been training regularly for a number of months. We observed a significant increase in the \(Na^+/K^+\) pump content of vastus lateralis after the 100-km run. This is in contrast to previous studies where \(Na^+/K^+\) pump content has been quantified by ouabain binding assays on electrically stimulated rat muscle, where no changes were seen after a variety of stimulation protocols of up to 24-h duration (8,22). In studies where rabbit muscle was stimulated for longer periods, it was found that there is a significant upregulation of \(Na^+/K^+\) pump content after 4–6 days of chronic low-frequency stimulation (14,15).

Another study on human muscle, in which the \(Na^+/K^+\) pump isoform distribution was determined, indicated that after exercise of only 5-min duration, previously compartmentalized pumps had been translocated to the surface membranes of the muscle fibers, thereby supposedly increasing the \(Na^+/K^+\) pump capacity (18).

Perhaps these discrepancies can be accounted for by methodological differences or by species differences in the regulation of \(Na^+/K^+\) pump content. Also, it is possible that, in humans, the duration of exercise of the current study is sufficient to allow more newly synthesized \(Na^+/K^+\) pumps to appear at the sarcolemma. Another possibility that could be considered is that the observed \(Na^+/K^+\) pump increase after running was in fact a training-induced response because the control biopsy was taken 4–6 wk before the run. However, subjects had at that point already reached their maximal training level and the training volume was in fact reduced during the last 4 wk before the run. Therefore, it is unlikely that subjects had any further training-induced effects on their muscles during the last 4 wk before the run.

Not surprisingly, the running performance was correlated to the \(\overline{V}_{O_2\text{max}}\) of the subjects. However, this correlation could only account for less than half of the observed variation in running time and therefore suggests that other factors may be limiting for running performance over 100 km. Nevertheless, we did not find any significant correlations between \(Na^+/K^+\) pump content and running time, indicating that in long distance events such as the 100-km run, performance is not limited by \(Na^+/K^+\) pump capacity. This is probably due to the low relative intensity of the performed work. However, the observed increase in plasma \(K^+\) shows that \(K^+\) balance is affected during 100-km running, and it is likely that plasma \(K^+\) concentration measured after the run approximates a steady-state level that was maintained for the entire duration of the run. Therefore, upregulation of \(Na^+/K^+\) pump content that was found after 100-km running may be a response to the prolonged increase in plasma \(K^+\) (8–12 h). It is interesting that...
even after such a long duration of exercise and hyperkalaemia, there seems to be no change in Na\(^+\) and K\(^+\) contents in vastus lateralis. Thus the hyperkalaemia is not associated with a net loss of muscle K\(^+\), and muscular Na\(^+\) and K\(^+\) homeostasis seems to be well maintained.

In conclusion, the highly increased plasma CK indicates that when running ultra-long distances, active muscles undergo a significant degree of sarcoplasmic damage already during the run. The simultaneous increase in muscle Ca\(^{2+}\) content suggests that an excessive intracellular Ca\(^{2+}\) accumulation may be involved in the development of cellular muscle damage.

Finally, Na\(^+\)-K\(^+\) pump capacity does not seem to be a crucial factor determining performance in ultra-distance running events such as the 100-km run. Nevertheless, the increase in plasma K\(^+\) seen after 100-km running indicates that, during this type of long-lasting exercise, Na\(^+\)-K\(^+\) pump activity in the skeletal muscles is inadequate to counterbalance the K\(^+\) efflux associated with the contractions. The upregulation of Na\(^+\)-K\(^+\) pumps could be an adaptation to cope with the ensuing disturbances of the Na\(^+\)/K\(^+\) gradients.

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