Potassium depletion increases potassium clearance capacity in skeletal muscles in vivo during acute repletion

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Bundgaard, Henning, and Keld Kjeldsen. Potassium depletion increases potassium clearance capacity in skeletal muscles in vivo during acute repletion. Am J Physiol Cell Physiol 283: C1163–C1170, 2002; 10.1152/ajpcell.00588.2001.—Muscular K uptake depends on skeletal muscle Na-K-ATPase concentration and activity. Reduced K uptake is observed in vitro in K-depleted rats. We evaluated skeletal muscle K clearance capacity in vivo in rats K depleted for 14 days. [3H]ouabain binding, α1 and α2 Na-K-ATPase isofrom abundance, and K, Na, and Mg content were measured in skeletal muscles. Skeletal muscle K, Na, and Mg and plasma K were measured in relation to intravenous KCl infusion that continued until animals died, i.e., maximum KCl dose was administered. In soleus, extensor digitorum longus (EDL), and gastrocnemius muscles K depletion significantly reduced K content by 18%, 15%, and 19%, [3H]ouabain binding by 36%, 41%, and 68%, and α2 isoform abundance by 34%, 44%, and 70%, respectively. No significant change was observed in α1 isoform abundance. In EDL and gastrocnemius muscles K depletion significantly increased Na (48% and 59%) and Mg (10% and 17%) content, but only tendencies to increase were observed in soleus muscle. K-depleted rats tolerated up to a fourfold higher KCl dose. This was associated with a reduced rate of increase in plasma K and increases in soleus, EDL, and gastrocnemius muscle K of 56%, 42%, and 41%, respectively, but only tendencies to increase in controls. However, whereas K uptake was highest in K-depleted animals, the K uptake rate was highest in controls. In vivo K depletion is associated with markedly increased K tolerance and K clearance despite significantly reduced skeletal muscle Na-K-ATPase concentration. The concern of an increased risk for K intoxication during K repletion seems unwarranted.

Important stimulators of activity of skeletal muscle Na-K-ATPase are high extracellular K concentration, high intracellular Na concentration, insulin, and catecholamines. Important factors increasing muscular Na-K-ATPase concentration are chronically high K intake, hyperthyroidism (2, 19), and increased physical activity, whereas decreases are seen in K depletion, hypothyroidism, immobilization, untreated diabetes mellitus, and starvation (for review see Refs. 5, 6).

Skeletal muscles normally contain ~75% of total body K. During K depletion this store serves an important buffer function, whereas myocardium and brain K contents are only reduced to a minor extent (23). In rats, 2 wk of K depletion caused a skeletal muscle K loss of ~20% and a reversible reduction in skeletal muscle Na-K-ATPase concentration of up to 78% (23). A downregulation is seen in white- as well as red-fiber muscles (23). In humans a diuretic-induced increase in renal K excretion was associated with a skeletal muscle K reduction of up to 24% and a skeletal muscle Na-K-ATPase reduction of ~18% (12).

Reduced skeletal muscle Na-K-ATPase concentration in K-depleted rats has been shown to reduce muscular K uptake capacity as evaluated in a study using oral K loading as well as in vitro K loading (23), in an in vitro study using intracellular Na loading (7), and in a study using oral KCl feeding (24). Recently, Choi et al. (3) developed a K clamp technique feasible for evaluation of K homeostasis in vivo. It was found that K depletion for only 2 days leads to almost complete insulin resistance of cellular K uptake even before any change in skeletal muscle Na-K-ATPase expression is present. However, the study was not designed to assess K uptake capacity. A clinical concern from the former findings is that in K-depleted patients K repletion may easily lead to excessive and possibly dangerous rises in plasma K (10). This may lead to inadequate management of potentially life-threatening profound hypokalemia (29). However, in the former studies of K repletion (23, 24) muscle K content was not measured until 8 h after K repletion, gastrointestinal K absorption and renal K excretion were not taken into account, and a maximum 42K uptake of only ~15% of the theoretical
maximum K uptake capacity of 16,000 K ions per Na-K pump per minute (7) of the remaining 22% of the Na-K pumps was reached. It can be calculated that an increase in the Na-K-ATPase activity to ~70% would have compensated for the downregulation of the Na-K-ATPase concentration. This consideration and the findings reported by Choi et al. (3) may challenge the point of view that skeletal muscle K uptake may be predicted from the size of the skeletal muscle Na-K-ATPase pool alone. Increased intracellular Na content, hyperpolarization, and perhaps an altered Na-K-ATPase affinity for K may, in K depletion in vivo, be factors of major importance for K uptake capacity. On this basis we evaluated in vivo K tolerance and K-stimulated skeletal muscle K uptake capacity in K-depleted rats using continuous intravenous KCl infusions with repeated plasma K measurements and measurements of skeletal muscle ion contents.

MATERIALS AND METHODS

Female Wistar rats bought at an age of 8 wk (~125 g) (Mellegaard Breeding Centre, Lille Skensved, Denmark) were used in the study. The study was conducted in agreement with the legislation for experimental animals given by the Danish Ministry of Justice. The animals were kept in animal housing facilities at 22°C with a 12:12-h light-dark cycle (8 AM to 8 PM). Animals allocated to K depletion were housed in cages without access to urine and feces, were fed a low-K-content diet containing (in mmol/100 g chow; \( n = 4 \)) 0.11 ± 0.01 K (mean ± SE), 11.52 ± 2.00 Na, and 3.30 ± 0.23 Mg, and had free access to distilled water. Control animals had free access to standard chow containing (in mmol/100 g chow; \( n = 4 \)) 22.87 ± 0.55 K, 9.09 ± 1.83 Na, and 7.37 ± 0.49 Mg to maintain body weights comparable between the two groups. Animals were taken for experiments after 2 wk of treatment.

Instrumentation. The instrumentation of the animals was previously described in detail (2). In brief, animals were anesthetized by intraperitoneal injection of 0.05 mg/g body wt of pentobarbital sodium (Mebumal, 50 mg/ml; Nycomed, Copenhagen, Denmark) before catheterization of the jugular vein for infusion and the carotid artery for blood sampling. Furthermore, under a microscope, a polyethylene catheter with a diameter of 1 mm was inserted through the urethra into the bladder. The exterior end of the catheter was attached to a syringe, and the bladder was emptied 15 min after completion of the surgical procedures (\( t = 0 \)). After infusions were finished (see below) the urine was collected for measurements of volume and K concentration by a K-sensitive electrode and for plasma K by a metabolic electrode with an ABL 605 (Radiometer). Skeletal muscle and chow K and Na contents were measured by flame photometry with an FLM3 (Radiometer) with lithium as an internal standard. A sample of ~25 mg wet weight was dissolved in 1 ml of 30% \( \text{H}_2\text{O}_2 \), and the suspension was maintained at 90°C for 12 h to allow complete evaporation. After addition of 2 ml of trichloroacetic acid (TCA; 5% wt/vol), 0.5 ml of the solution was used for flame photometry after final addition of a further 0.5 ml of 5% TCA and 1.5 ml of 5 mmol/l LiCl. Skeletal muscle and chow Mg contents were measured by atomic absorption (AAAnalyst 100; Perkin Elmer, Norwalk, CT) at a wavelength of 285.2 nm using a solution as used for flame photometry except that LiCl was substituted by 1.5 ml of redistilled water. Measurements were performed in duplicate.

\(^{3}\text{H}\)ouabain binding. Measurements of \(^{3}\text{H}\)ouabain binding were performed as previously described in detail for intact skeletal muscle samples (15). In brief, all procedures were performed by using freshly made vanadate (Merek, Darmstadt, Germany) buffer containing (in mmol/l) 10 Tris-HCl, 250 sucrose, 3 MgSO\(_4\), and 1 vanadate. pH was adjusted to 7.3 with Tris-HCl. Samples of ~4 mg wet weight were cut from the muscles and prewashed in unlabeled buffer at 0°C for 20 min (2 × 10 min). Incubations took place at 37°C in buffer containing \(^{3}\text{H}\)ouabain (2.1 µCi/ml) (Amersham International, Little Chalfont, UK) and ouabain (Sigma Chemical, St. Louis, MO) added to a final concentration of 1 × 10\(^{-4}\) mol/l for 2 h (2 × 1 h). Washout at 0°C in unlabeled buffer for 2 h (4 × 30 min) was then performed to reduce the amount of \(^{3}\text{H}\)ouabain in the extracellular space, thereby enhancing the precision of the method. After washout, samples were blotted, weighed, and soaked overnight in vials containing 0.5 ml of 5% (wt/vol) TCA. Thereafter, 2.5 ml of Opti-fluor scintillation mixture (Packard Instruments, Downers Grove, IL) was added and \(^{3}\text{H}\) activity in samples and incubation medium was assayed by liquid scintillation counting (Tri-Carb, 1600TR; Packard Instruments). On the basis of sample wet weight, \(^{3}\text{H}\) activity in the incubation medium, and \(^{3}\text{H}\) activity retained in the samples, the concentration of \(^{3}\text{H}\)ouabain binding sites in the samples was calculated and expressed in picomoles per gram of wet weight. Na-K pumps internalized to endosomal pools or the ouabain-resistant \( \alpha _{1} \) isoform of the rat skeletal muscle Na-K pump are not detected by \(^{3}\text{H}\)ouabain binding.

Muscle water and protein contents were determined as previously described in detail (2). Weights of soleus and EDL muscles were determined immediately after they were dissected out and tendons had been removed.
**Immunoblotting.** Immunoblotting was performed using crude homogenates (10 mg tissue/ml in His buffer; Ref. 21) as previously described in detail (1). Isoform-specific antibodies McK1 and Mcb2 against the α1 and α2 Na-K-ATPase subtypes, respectively, were kindly provided by K. Sweadner, Harvard University. Protein concentration was determined by the method of Lowry et al. (20). Equal amounts of protein were dissolved in Laemml buffer (Bio-Rad) and loaded on a 7.5% Tris-HCl gel together with molecular weight standards (Precision Protein Standards, Bio-Rad) and run on a Mini-Protean 3 cell electrophoresis system (Bio-Rad). Gels were blotted onto Immobilon 0.45-μm polyvinylidene difluoride (PVDF) membranes (Bio-Rad) with a Trans-Blot Semi-Dry transfer system (Bio-Rad) according to the manufacturer’s instructions. Membranes were blocked in PBS, 0.2% Tween 20, and 5% bovine albumin fraction V (AppliChem; Darmstadt, Germany) overnight at 4°C. Membranes were incubated with isotype-specific antibodies diluted 1:2,500 in blocking buffer overnight at 4°C, washed in PBS and 0.2% Tween 20, and incubated with anti-mouse IgG horseradish peroxidase-linked whole antibody (Amersham Life Science) at 4°C overnight at 4°C. Membranes were incubated with isotype-specific antibodies diluted 1:2,500 in blocking buffer overnight at 4°C, washed in PBS and 0.2% Tween 20, and incubated with anti-mouse IgG horseradish peroxidase-linked whole antibody (Amersham Life Science) at 4°C overnight at 4°C. Membranes were then washed in PBS and 0.2% Tween followed by a wash in PBS only. The signal was detected with an enhanced chemiluminescence (ECL) kit and Hybond ECL lum (Amersham Life Science). Multiple exposures were made to ensure that signals were within the linear range of the film. Immunoblots were quantified by scanning densitometry using Kodak Digital Science 1D Image Analysis Software (Rochester, NY). Changes in isoform abundances in K-depleted animals were expressed relative to values obtained in controls.

**Calculations and statistics.** Skeletal muscle net K uptake rate; e.g., soleus muscle K uptake rate in K-depleted rats during KCl infusion, was calculated as the difference in K content between each soleus muscle after infusion and the mean soleus muscle K content in the K-depleted group that had not undergone KCl infusion. Results are expressed relative to duration of KCl infusion (mol K ions·g wet wt⁻¹·min⁻¹). Skeletal muscle net K uptake rate per [³H]ouabain binding site, e.g., soleus muscle net K uptake rate per [³H]ouabain binding site, was calculated as the soleus muscle K uptake rate (mol K/min) divided by the mean soleus muscle [³H]ouabain binding site concentration. Results are expressed as moles of K per [³H]ouabain binding site per minute. All results are given as means ± SE. Statistical significance among groups was ascertained by Student’s two-tailed t-test for unpaired observations. Linear regression analysis was performed with the least-squares method. Bonferroni’s correction was applied to correct for multiple comparisons. Only corrected P values are given. Corrected P values <0.05 were considered significant.

**RESULTS**

K depletion was associated with reduced weight gain, but otherwise the dietary regimens were well tolerated and animals were clinically unaffected. No significant difference in body weight was observed between K-depleted and control rats, i.e., rats with limited access to standard chow (139 ± 2 vs. 138 ± 2 g; P = 0.9, n = 6). For rats at this age with free access to standard chow we previously reported (2) a mean body weight of 169 g.

**Plasma K and Na.** Plasma K was reduced to 2.0 ± 0.2 mmol/l in the K-depleted group compared with 3.8 ± 0.2 mmol/l in the control group, i.e., by 46% (P < 0.01, n = 6; Fig. 1). Plasma K changes in response to KCl infusions are described in K clearance capacity. At t = 0 there was no significant difference in plasma Na between K-depleted and control rats (140 ± 1 vs. 141 ± 1 mmol/l; P = 0.4, n = 6). It was of interest to assess whether the observed decrease in skeletal muscle Na during K infusions (see Skeletal muscle Na and Mg) was associated with changes in plasma Na. During infusions no significant plasma Na differences were observed within the first hour of infusion. However, in the K-depleted group a linear relationship (y = 0.04x + 138.82; r² = 0.96) between KCl dose and plasma Na was observed with plasma Na values of 147–149 mmol/l at t = 210–270 min (n = 1–4).

**Skeletal muscle K content.** In K-depleted rats soleus, EDL, and gastrocnemius muscle K content were reduced to 79 ± 3, 99 ± 2 and 87 ± 3 μmol/g wet wt, respectively, compared with 96 ± 5, 116 ± 5, and 108 ± 3 μmol/g wet wt, respectively, in controls (P < 0.01, n = 6; Fig. 2). These reductions are in agreement with previous findings (18, 23, 28). Skeletal muscle K content changes in response to KCl infusions are described in K clearance capacity.

**Skeletal muscle Na-K-ATPase.** In the K-depleted group soleus, EDL, and gastrocnemius muscle [³H]ouabain binding site concentrations were reduced to 118 ± 7, 168 ± 11, and 62 ± 6 pmol/g wet wt, respectively, compared with 184 ± 9, 285 ± 14, and 193 ± 9 pmol/g wet wt, respectively, in controls (P < 0.01, n = 6; Fig. 3). No significant changes were observed in K-depleted rats compared with controls in Na-K-ATPase α1 abundance in soleus (8%; n = 6, P > 0.4), EDL (11%; n = 5, P > 0.3), or gastrocnemius (5%; n = 5–6,
increased from 6.1 ± 0.4 to 8.6 ± 0.4 mmol/l (P < 0.01, n = 6) in controls, whereas no significant change was observed in K-depleted rats (from 5.7 ± 0.4 to 5.6 ± 0.3 mmol/l; P > 0.8, n = 6). During KCl-glucose infusion from t = 0 to t = 30 min plasma glucose increased from 5.5 ± 0.2 to 18.2 ± 1.6 mmol/l (P < 0.01, n = 6) in controls and from 5.7 ± 0.4 to 16.3 ± 2.0 mmol/l (P < 0.01, n = 6) in K-depleted rats. At t = 30 min plasma K had increased in control rats to 10.4 ± 0.7 mmol/l during KCl infusion and to 9.1 ± 0.4 mmol/l (P > 0.12, n = 6) during KCl-glucose infusion and in K-depleted animals to 6.0 ± 0.3 and 6.6 ± 0.6 mmol/l (P > 0.3, n = 6), respectively. Thus the pronounced hyperglycemia obtained by administration of KCl-glucose compared with KCl alone did not seem to affect the plasma K response significantly.

During the KCl infusion until the animals died, soleus, EDL, and gastrocnemius muscles K content in the K-depleted group had increased to 123 ± 4, 141 ± 8, and 123 ± 6 µmol/g wet wt, i.e., by 56%, 42%, and 41%, respectively (P < 0.05, n = 6), whereas only tendencies to increase were observed in muscles in the control group (Fig. 2). After KCl infusion, tendencies to higher K content were observed in each of the three muscles in the K-depleted group compared with controls.

Whereas total muscle K uptake during KCl infusions was highest in the K-depleted group, the skeletal muscle net K uptake rate showed a tendency to be higher in the control group in soleus and EDL muscles and reached a level of significance in the gastrocnemius muscle (0.30 ± 0.05 vs. 0.18 ± 0.02 µmol·g wet wt⁻¹·min⁻¹; P < 0.05, n = 6) and for all three muscles when combined, i.e., 0.27 ± 0.03 in controls and 0.19 ± 0.02 µmol·g wet wt⁻¹·min⁻¹ in the K-depleted rats (P < 0.05, n = 6). In the K-depleted group the observation of a lower plasma K increase despite the reduced muscle K uptake rate may at least to some extent be explained by increased renal K excretion (seeRenal K excretion) but could also represent repletion of K.

Plasma glucose was measured during KCl infusion alone and during KCl-glucose infusion. During KCl infusion from t = 0 to t = 30 min plasma glucose
K stores in other tissues, e.g., liver, myocardium, smooth muscles, blood cells, and neural tissue.

The calculated skeletal muscle net K uptake rate per [3H]ouabain binding site tended to be highest in the K-depleted group for soleus and EDL muscles and [3H]ouabain binding site tended to be highest in the smooth muscles, blood cells, and neural tissue. K stores in other tissues, e.g., liver, myocardium, smooth muscles, blood cells, and neural tissue.

Skeletal muscle Na and Mg. Before infusions Na and Mg contents were increased by 48% and 10%, respectively (P < 0.05, n = 6) in EDL and by 59 and 17%, respectively (P < 0.01, n = 6) in gastrocnemius muscles in the K-depleted rats compared with controls, whereas only tendencies to higher levels were observed in soleus muscle (Fig. 4). After KCl infusions Na content reductions were observed in both groups, but the Na content was still significantly higher in all three muscles in the K-depleted group. No major changes in Mg content were observed in response to KCl infusions. It should be noted that before (see Skeletal muscle Na-K-ATPase) as well as after infusions, no significant differences were observed between the K-depleted and control rats in water content in soleus (73.5 ± 0.6% vs. 73.9 ± 0.7%; P = 0.7, n = 6), EDL (73.1 ± 0.7% vs. 74.1 ± 0.7%; P = 0.3, n = 6), or gastrocnemius muscle (73.4 ± 0.7% vs. 75.0 ± 0.5%; P = 0.1, n = 6).

Renal K excretion. In anesthetized rats steady-state renal K excretion was measured during a 2-h period without KCl infusion. In K-depleted rats renal K excretion was 1.2 ± 0.3 μmol/h (urine volume 0.16 ± 0.03 ml/h, K concentration 8 ± 1 mmol/l) compared with 20.8 ± 3.7 μmol/h (urine volume 0.22 ± 0.05 ml/h, K concentration 100 ± 16 mmol/l) in controls (n = 5–6, P < 0.01). Thus at steady state the renal K excretion in

the K-depleted animals was reduced to 6% of the level in controls. During KCl infusions renal K excretion (from t = 0 until the animals died) in the K-depleted rats was 176 ± 31 μmol/h (urine volume 1.37 ± 0.15 ml/h, K concentration 125 ± 11 mmol/l) compared with 119 ± 49 μmol/h (urine volume 0.50 ± 0.22 ml/h, K concentration 272 ± 27 mmol/l) in controls (P = 0.4, n = 6). Figure 5 shows the amount of K retained and indicates renal K excretion during KCl infusion. Thus K depletion was associated with a profoundly reduced renal K excretion rate at steady state, but the rate increased 150-fold during KCl infusion compared with a 6-fold increase in controls, leading to a tendency to a KCl infusion-induced higher renal K excretion rate in the K-depleted group.

DISCUSSION

The present study demonstrates for the first time a marked increase in K clearance capacity in vivo in K-depleted rats exposed to intravenous K repletion. Thus, compared with controls, K-depleted rats tolerated up to a fourfold higher K dose associated with a significantly increased K uptake in skeletal muscles. The increased K tolerance was not the outcome of a higher skeletal muscle K uptake rate but reflects the total amount of K needed to normalize the skeletal muscle K stores, whereas in controls the K uptake represents a defense against K intoxication.

The observation of increased K tolerance is in accord with an observed normalization of gastrocnemius muscle K after K repletion for 1 day of rats K depleted for 3 wk without plasma K exceeding control level (23) and a 3.7-fold higher increase in gastrocnemius muscle K compared with controls after oral K loading for 1 day of rats K depleted for 4 wk (24). However, at variance with the present study, it was concluded in the former reports that K depletion reduces skeletal muscle K

Fig. 4. Na and Mg content in rat soleus, EDL, and gastrocnemius muscles after 2 wk of K depletion. K-depleted animals were given chow with low K content (0.11 mmol/100 g chow), and controls had limited access to standard chow to maintain body comparable weights between the 2 groups. Columns represent means and bars represent SE; n = 6.

Fig. 5. K retained in anesthetized rats during infusion of 0.5 mol/l KCl at a rate of 1.5 ml/100 g body wt h⁻¹. K-depleted animals were given chow with low K content (0.11 mmol/100 g chow), and controls had limited access to standard chow to maintain body comparable weights between the 2 groups. Line indicates the amount of K infused. For each animal, the amount of K retained was calculated as the difference between amounts of infused K and K excreted in the urine.

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uptake capacity. An obvious explanation for these different conclusions is that the term “uptake capacity” has been related to K uptake rates, i.e., relative to time, as well as to total K uptake. Thus, during K repletion of K-depleted rats, skeletal muscle K uptake rates are reduced and total K uptake increased. We confirmed that K depletion reduces plasma K and skeletal muscle K and Na-K-ATPase concentration and excluded the possibility that these changes were the outcome of differences in age or body weight or in muscle weight, water, or protein content (16, 24). Clinically, the results indicate that the concern that reduced skeletal muscle Na-K-ATPase concentration makes K-depleted patients more exposed to development of dangerous hyperkalemia during acute K repletion seems unwarranted.

It is important to assess whether the presently observed increases in plasma K during KCl infusion reflect K uptake into the intracellular volume (ICV) or only distribution of K in the extracellular volume (ECV). Assuming an ECV of 20% of body weight and immediately equal distribution of K in the ECV, the presently used KCl infusion rate would increase ECV K by \(-3 \text{ mmol/l}\) in 5 min [infused amount of K \(0.75 \text{ mmol KCl/100 g body wt/h \times body wt (140 g/100 g \times \text{infusion time (5 min/60 min)) \times ECV (140 g \times 0.2)}\). Plasma K increases were \(<3 \text{ mmol/l}\) after 5 min in both groups. This indicates that K uptake into ICV is initiated within the first minutes. Assuming a K activation of Na-K-ATPase of 50% at an ECV K of 0.8 mmol/l and of 85% at 4.5 mmol/l (25), a pronounced increase in Na-K-ATPase activity within the first minutes of infusion may have been induced in K-depleted rats compared with controls. It is well known that increased ICV Na stimulates Na-K-ATPase (4). Thus the pronounced increase in skeletal muscle Na level in the K-depleted group may also have added to increased Na-K-ATPase activity in the K-depleted group. This is in accord with the observation that the muscle with the highest Na content—the gastrocnemius muscle—also showed the highest K uptake per \[^{3}H\]ouabain binding site and that in all three muscles a tendency to a higher maximum K content after K infusion was seen in the K-depleted group. A similar tendency was previously observed in controls compared with K-supplemented rats with a reduced skeletal muscle Na content (2). During KCl infusions muscular Na-K-ATPase activity may also have been stimulated by increased insulin levels induced by high plasma K (8) and by hyperglycemia during KCl-glucose infusion as well as by increased catecholamine levels (9) induced by anesthesia. However, skeletal muscle net K uptake is a product not only of activity and concentration of Na-K-ATPase but also of the Na-K-2Cl and K-2Cl cotransporters and the efflux pathways, as well as the transmembrane K gradient. This indicates that in the present study calculations of Na-K-ATPase-mediated K uptake can only be made with a number of limitations. Within these limitations it was found that despite the various stimuli, the Na-K pump activity calculated on the basis of \[^{3}H\]ouabain binding sites and net muscle K uptake was only stimulated up to 18% of the theoretical maximum of 16,000 K ions per Na-K pump per minute (7). This indicates that, at variance with the activity achieved by in vitro Na loading or electrical stimulation (7, 13), at rest only stimulation of a minor fraction of the Na-K pumps or a minor activity of the pumps is achieved by the present levels of the stimulating factors or that the measured skeletal muscle net K uptake is the outcome of a higher K uptake but also a major K leakage. The latter may mainly be the case when K stores are full, leading to a higher ICV-to-ECV K ratio across the plasma membrane, i.e., first in controls. It should be noted that the calculated Na-K-ATPase activities relate to net skeletal muscle K uptake and are mean values, i.e., temporarily higher or lower activities may have been present. Compared with the present study it is of interest that \(^{42}K\) uptake measured in vitro in soleus muscle from K-depleted rats reached 13% of the theoretical maximum, although the measurements were carried out after in vitro K loading for 75 min to 96% of the control level (23). In that study control rats reached 9% compared with 10% in the present study. However, in that study (23) soleus muscle was assessed, whereas in the present study assessment of white- and mixed-fiber muscles revealed the highest calculated Na-K-ATPase activity in gastrocnemius muscle, i.e., mixed-fiber muscle. In the in vitro study as well as in the present study the activities were calculated relative to ouabain binding, i.e., for the \(\alpha_{2}\) isoform. However, \(\alpha_{1}\) isoform abundance was not affected by K depletion and inclusion of these pumps in the calculations would result in even lower activities. Our previous studies of K homeostasis in skeletal muscle (2) and the heart (1) in response to intravenous K infusion in rats chronically administered a high-K-content diet as well as the present study support the concept of a positive relationship between Na-K-ATPase concentration changes and active transmembrane K transport capacity. However, previous studies as well as the presently calculated Na-K pump activities indicate that changes in pump activity may compensate for considerable concentration reductions and that in vivo K uptake cannot be predicted from the size of the pool of skeletal muscle Na-K pumps alone. Skeletal muscle Na-K-ATPase was quantified by ouabain binding, i.e., the ouabain-sensitive \(\alpha_{2}\) isoform of the \(\alpha\beta\) heterodimer of Na-K-ATPase was determined. The \[^{3}H\]ouabain binding measurements were in good agreement with measurements of changes in \(\alpha_{2}\) isoform abundance by immunoblotting. The ouabain-insensitive \(\alpha_{1}\) isoform previously reported to compose \(\sim 50\%\) (27) has recently been reported to compose 15–25% of the total skeletal muscle Na-K-ATPase in rats (14). In accord with the present findings, rats K-depleted for 10 days showed no significant changes in skeletal muscle \(\alpha_{1}\) isoform abundance, whereas marked decreases were observed in the \(\alpha_{2}\) isoform (27, 28). In a study by Thompson et al. (27) no correlation could be established between level of K loss and \(\alpha_{2}\) downregulation in slow-twitch oxidative or fast-twitch glycolytic muscles. This was confirmed in the present
study. However, during KCl infusions almost similar amounts of K were accumulated in the three muscles, despite decreases in the α2 isoform varying from 35% to 70%. This observation, together with the unaltered α1 isoform abundances, may implicate that the α1 and α2 isoforms may have separate roles during K repletion. On the other hand, it is interesting that the present results indicate a positive correlation between magnitude of K depletion-induced Na-K-ATPase downregulation and increase in muscle Na and Mg. Thus the combined changes in skeletal muscle Na-K-ATPase and Na and Mg seem to be decisive for muscle K level as well as K uptake capacity. In K depletion, down-regulation of muscle Na-K-ATPase has been interpreted as a beneficial change that facilitates the large skeletal muscle K store to buffer the ECV K loss, and it has been speculated as to how the decline in ECV K is sensed by the muscles (27). The decline in ECV K leads to hyperpolarization of the cell membrane, and both of these changes tend to reduce Na-K-ATPase activity. Thus it is tempting to suggest that a decrease in muscle Na-K-ATPase activity during K depletion will—like inactivity of biological systems in general—secondarily reduce synthesis and/or increase degradation of Na-K-ATPase. During K depletion, the coincident increase in ICV Na and perhaps also the increase in Mg ensures a high activity of the remaining Na-K pumps when ECV K rises, i.e., during K repletion.

The comparable levels of hyperglycemia achieved by KCl-glucose infusion in K-depleted and control rats did not significantly affect the plasma K response compared with the response during KCl infusion alone. Thus no further increase in skeletal muscle Na-K-ATPase activity due to an expected hyperglycemia-induced increase in insulin levels seemed to be present. However, plasma insulin levels were not measured, but the results suggest that the observed K-stimulated K uptake resembles the more natural or physiological condition when K and glucose are coadministered. In the in vivo study of K depletion by Choi et al. (3) cellular K uptake in response to skeletal muscle Na-K-ATPase stimulation by insulin infusion was measured as the amount of K infused to clamp plasma K at the basal level, i.e., 3.8 mmol/l in rats K depleted for 2 days and 4.2 mmol/l in controls. At the same time, glucose was infused to clamp plasma glucose as well. Unexpectedly, 2 days of K depletion reduced the K uptake by 80% although no changes in skeletal muscle α2 isoform abundance were present. A 96% reduction in K uptake was observed after K depletion for 10 days, but at that time a ~40% reduction in the α2 isoform abundance was found. It was concluded that K depletion leads to insulin resistance of the K uptake. However, the putative skeletal muscle K content increases in response to K infusion were not measured, and in the K-depleted rats the amounts of K needed to clamp plasma K were not corrected for an almost 100% reduction in renal K excretion during K infusion. It may be speculated that because plasma K was clamped at a level at which plasma K per se (25) reduces skeletal muscle Na-K pump activity, the resistance of the simultaneous stimulation of the pump by insulin may be the balanced outcome of these two opposite forces. However, in accord with the present study, it was preliminarily found that during K clamping of the K-depleted rats at the plasma K level measured in the controls, substantially higher K infusion rates were needed.

In K depletion the K-conserving reduction in renal K excretion is explained by reduced K secretion—most likely due to reduced apical K channels—as well as increased reabsorption achieved by induction of apical H-K-ATPase (22). Accordingly, urinary K concentrations were lowest in the K-depleted group before as well as during infusions. However, during K infusions a 150-fold increase in the renal K excretion rate in K-depleted rats compared with a 6-fold increase in controls caused a tendency to a higher K excretion in the K-depleted group. After 30 min of KCl infusion a difference in plasma K increase of ~2.5 mmol/l was observed between the two groups. Assuming a plasma volume of 4% of the body weight (and ECV of 20%) this difference corresponds to 14 μmol of K in plasma or 70 μmol of K in ECV. Assuming a constant rate of renal K excretion during this infusion period (t = 0–30 min), there was a ~25-μmol higher renal K excretion (176 μmol K/h in K-depleted rats and 119 μmol K/h in controls) in the K-depleted group. Thus in the K-depleted group the tendency to an increased renal K excretion may have been of importance for the observed lower plasma K rise, although the skeletal muscle K uptake rate was reduced in K-depleted rats compared with controls.

It was of interest to make an overall account of the infused KCl vs. the measured tissue and renal K changes. Thus in the K-depleted group a total of ~3.6 mmol of KCl was infused (infusion rate × infusion time of 209 min). Assuming a skeletal muscle weight of 40% of body weight (~140 g) and a mean skeletal muscle K content of ~90 μmol/g wet wt before infusion (Fig. 2), a total skeletal muscle K content before infusion of ~5 mmol can be calculated (body wt × 0.4 × skeletal muscle K content). Thus the observed skeletal muscle K content increase after KCl infusion of ~50% corresponds to an increase of ~2.5 mmol. When also considering the renal K excretion of ~0.6 mmol (176 μmol/h for 209 min) and an increase in ECV K of ~0.3 mmol (ECV × plasma K increase during infusion from 2.0 to 10.6 mmol/l), a total of ~3.4 mmol of the infused ~3.6 mmol of KCl can be recovered. These calculations are hampered by a number of assumptions and approximations, but they may indicate that except for kidneys and skeletal muscles, other organs only accumulated a minute amount of K during KCl infusion.

K depletion significantly increased skeletal muscle Mg content. The increase was not due to decreased muscle water content or increased Mg content in the chow. Increased renal K and Mg excretion induced by diuretics leads to skeletal muscle K and Mg depletion in humans (12), and, generally, skeletal muscle K and Mg have been suggested to be positively correlated, which is supported by the observation that Mg depletion per se leads to K depletion (11, 17). However, the
present finding is in accord with the report (11) of nonsignificant increases of 12% and 19% in Mg content in soleus and EDL muscles, respectively, from K-depleted rats. Thus, opposite to development of K depletion in selective Mg depletion, selective K depletion increased skeletal muscle Mg.

In conclusion, low K intake reduces plasma K and skeletal muscle K and Na-K-ATPase and increases skeletal muscle Na and Mg content. In response to intravenous K repletion these changes reduced the skeletal muscle net K uptake rate but allowed the skeletal muscle K stores to be repleted during acute K repletion a lower rise in plasma K was related to the observation that the K depletion-induced reduction in skeletal muscle Na-K-ATPase concentration was in part compensated for by an increased activity of the remaining Na-K pumps and by an increased renal K excretion. In humans, K depletion is frequently seen as a result of diuretic therapy, reduced K intake, or increased gastrointestinal loss. In light of the present observations, the need for the strong precautions taken in clinical practice to avoid iatrogenic hyperkalemia during K repletion may be overestimated.

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REFERENCES


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