

Endotoxemia stimulates skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ and raises blood lactate under aerobic conditions in humans

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Bundgaard, Henning, Keld Kjeldsen, Karen Suarez Krabbe, Gerrit van Hall, Lene Simonsen, Jesper Qvist, Christian Muff Hansen, Kirsten Møller, Lise Fonsmark, Per Lav Madsen, and Bente Klarlund Pedersen. Endotoxemia stimulates skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ and raises blood lactate under aerobic conditions in humans. *Am J Physiol Heart Circ Physiol* 284: H1028–H1034, 2003.—First published November 21, 2002; 10.1152/ajpheart.00639.2002.—We assessed the hypothesis that the epinephrine surge present during sepsis accelerates aerobic glycolysis and lactate production by increasing activity of skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$. Healthy volunteers received an intravenous bolus of endotoxin or placebo in a randomized order on two different days. Endotoxemia induced a response resembling sepsis. Endotoxemia increased plasma epinephrine to a maximum at $t = 2$ h of 0.7 ± 0.1 vs. 0.3 ± 0.1 nmol/l ($P < 0.05$, $n = 6-7$). Endotoxemia reduced plasma K^+ reaching a nadir at $t = 5$ h of 3.3 ± 0.1 vs. 3.8 ± 0.1 mmol/l ($P < 0.01$, $n = 6-7$), followed by an increase to placebo level at $t = 7-8$ h. During the declining plasma K^+ , a relative accumulation of K^+ was seen reaching a maximum at $t = 6$ h of 8.7 ± 3.8 mmol/leg ($P < 0.05$). Plasma lactate increased to a maximum at $t = 1$ h of 2.5 ± 0.5 vs. 0.9 ± 0.1 mmol/l ($P < 0.05$, $n = 8$) in association with increased release of lactate from the legs. These changes were not associated with hypoperfusion or hypoxia. During the first 24 h after endotoxin infusion, renal K^+ excretion was 27 ± 7 mmol, i.e., 58% higher than after placebo. Combination of the well-known stimulatory effect of catecholamines on skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, with the present confirmation of an expected $\text{Na}^+\text{-K}^+\text{-ATPase}$ -induced decline in plasma K^+ , suggests that the increased lactate release was due to increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, supporting our hypothesis. Thus increased lactate levels in acutely and severely ill patients should not be managed only from the point of view that it reflects hypoxia.

K^+ homeostasis; sepsis; glycolysis

PATIENTS WITH TRAUMA or sepsis hyperlactacidemia are usually managed from the point of view that it reflects anaerobic glycolysis caused by tissue hypoxia. However, it has been suggested that increased activity of

skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ caused by the epinephrine surge present in such patients causes aerobic glycolysis and lactate production (12, 13), i.e., the production of 2 mol ATP by glycolysis compared with 38 mol by complete oxidation of 1 mol of glucose may not be a consequence of anaerobic conditions, but an effect linked to the $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. This hypothesis is favored by several observations. First, a 46% increase in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity has been observed in extensor digitorum longus muscles from septic rats (22). Second, in well-oxygenated skeletal muscles, increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity induced by monensin (12), epinephrine, amylin (14), or insulin (21) leads to increased lactate production, and, on the other hand, inhibition of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ by ouabain or reduced extracellular K^+ concentration reduces lactate production (14). Third, increased skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity is induced by increased catecholamine levels that are present in trauma and sepsis. Fourth, a positive correlation between plasma lactate and plasma catecholamine concentrations has been observed and a reduction or blockage of hyperlactacidemia can be achieved by adrenergic blockage (for a review, see Ref. 13). If an increased catecholamine level is responsible for a part of the increase in blood lactate level in traumatized or septic patients, this has to be taken into account because it may be present in patients after compromised perfusion and tissue oxygen delivery have been normalized. It is well known that the catecholamine stimulation of the skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ is achieved via β_2 -adrenoceptors (2), and that increased activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$, caused by pumping Na^+ out and K^+ into the cell in a 3:2 relationship, leads to increased cellular K^+ uptake (2). This may lead to hypokalemia, which is actually a frequent finding at admission of severely ill patients (5, 19). Furthermore, a catecholamine-induced increase in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity may not only induce skeletal muscle K^+ uptake, but catecholamines

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may also via α - and β -adrenoceptors alter renal tubular $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity (11) and thus affect renal K^+ excretion.

Several observations favoring the hypothesis that catecholamine stimulation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ secondary leads to lactate production, comes from studies of isolated cells and animal models, whereas studies in humans have not been reported. Therefore, in the present study, we assessed the effect in humans of endotoxin on the K^+ homeostasis and lactate concentration changes to evaluate the hypothesis that the endotoxin-induced increase in epinephrine level leads to increased blood lactate level and hypokalemia under aerobic conditions.

MATERIALS AND METHODS

Volunteers

Eight healthy young volunteers (21–25 yr, 77 ± 3 kg body wt) were studied. None of the subjects had a history of medical problems, and physical examination revealed no abnormalities. Blood analyses showed normal hemoglobin, white blood cell count (WBC), WBC differential count, C-reactive protein (CRP), and blood glucose as well as normal kidney function (urea and creatinine), normal liver function (alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase), normal coagulation system [activated partial thromboplastin time, antithrombin III, prothrombin time, and international normalized ratio (INR)], and normal thyroid-stimulating hormone. All had a normal ECG. The volunteers did not use any medication, and they did not have any febrile illness in the 2 wk preceding the study.

Study Design

The presently used study design has previously been described in detail (18). In brief, the study was performed in an intensive care unit setting under the continuous supervision of an anesthesiologist, with emergency and resuscitation equipment immediately available. The volunteers were studied after endotoxin infusion and at least 14 days apart after placebo infusion. The sequence endotoxin/placebo was randomly chosen. Because of the obvious symptomatic (temperature and blood pressure) changes after infusion of endotoxin, neither the volunteers nor the staff was blinded to the study protocol.

The volunteers fasted overnight before endotoxin or placebo administration. Rectal temperature was recorded continuously. Furthermore, the subjects were monitored with respect to blood pressure (by a radial artery catheter) and heart rate. A femoral vein was catheterized for blood sampling. Isotonic saline solution was infused. From 8 AM until 8 PM, isotonic glucose ($50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was continuously infused. Subjects were given an intravenous bolus of endotoxin from *Escherichia coli* (lot EC-6, United States Pharmacopodia Convention; Rockville, MD) of 2 ng/kg body wt at $t = 0$, where t is time. The study was approved by the regional scientific ethical committee (no. 01-144/98) and written informed consent was obtained from each volunteer.

Blood and Urine Sampling

Blood was drawn at $t = 0$ and at 2, 4, 8, 10, 24, and 32 h after injection for differential WBC counts, CRP, hemoglobin, and plasma electrolytes. Blood was taken for chemical analyses of liver and kidney function at the same time points,

except at $t = 2$ h. Blood for measurements of cortisol, adrenocorticotropin (ACTH), insulin, orosomucoid, and fibrinogen was drawn at $t = 0, 4$, and 32 h and at $t = 8, 10$, and 24 h for orosomucoid and fibrinogen only. Blood for isolation of serum and plasma was drawn at $t = 0, 2, 4, 8, 24$, and 32 h. Blood samples were obtained simultaneously from the femoral vein and the radial artery at $t = 0, 1, 2, 3, 4, 5, 6, 7, 8$, and 10 h for measurements of plasma K^+ , Na^+ , lactate, pH, PO_2 , PCO_2 , glucose, as well as blood hemoglobin and oxygen saturation. These parameters were measured with the use of a blood gas analyzer (model ABL605, Radiometer), except for plasma lactate, which was automatically analyzed enzymatically with lactate dehydrogenase (Cobas Fara, Roche; Basel, Switzerland). The volunteers were encouraged to empty the bladder just before endotoxin was injected, and urine was collected for the period $t = 0$ –24 h.

Analyses

Catecholamines. Blood samples for measurements of epinephrine and norepinephrine were drawn into ice-cold glass tubes containing EGTA glutathione. Plasma was stored at -80°C until analyzed by high-performance liquid chromatography (Hewlett-Packard; Waldbronn, Germany) with electrochemical detection as previously described (10).

Tumor necrosis factor- α . Blood samples were drawn into ice-cold tubes containing EDTA and trasylol and then spun immediately at $2,200 g$ for 15 min at 4°C . Plasma was stored at -80°C until analyzed. Tumor necrosis factor- α (TNF- α) was determined with the use of an ELISA kit (detection limit 0.5 pg/ml , R&D Systems; Minneapolis, MN). According to the manufacturer, the TNF- α ELISA kits are insensitive to the addition of recombinant forms of the soluble receptor, and the measurements therefore correspond to both soluble and receptor-bound cytokine. All cytokine determinations were run as duplicates, and mean values were calculated.

Clinical chemistry tests. Standard laboratory procedures were employed. Urine and plasma K^+ were measured with a potentiometer (model 917, Hitachi). Only plasma K^+ measurements obtained from the Hitachi 917 at $t = 24$ and 32 h are given in RESULTS.

Leg blood flow. The femoral arterial leg blood flow (LBF) was measured at $t = 0$, and each hour until $t = 8$ h with the ultrasound Doppler technique, as previously validated (25). An ultrasound Doppler (model CFM 800; Vingmed Sound; Horton, Norway) equipped with an annular phased-array transducer probe 11.5-mm diameter (Vingmed Sound) operating at an imaging frequency of 7.5 MHz and the variable Doppler frequencies of 4.0–6.0 MHz (high-pulsed repetition frequency mode 4–36 kHz was used) (25).

Calculations and Statistics

K^+ uptake in one leg was calculated as the arteriovenous plasma K^+ difference multiplied by leg plasma flow [$\text{LBF} \times (1 - \text{hematocrit})$]. Lactate release from one leg was calculated as the venoarterial plasma lactate difference multiplied by LBF. Accumulated K^+ uptake was calculated as the sum of K^+ uptake each hour during the period of interest. Difference in accumulated K^+ uptake between the two groups was calculated as the accumulated K^+ uptake after placebo subtracted from the accumulated K^+ uptake after endotoxin.

Statistical significance between measurements obtained at two or more different time points after placebo and after endotoxin (paired design) was ascertained by two-way repeated-measures ANOVA with SigmaStat for Windows version 2.03 (SPSS). The two factors were treatment (placebo vs. endotoxin) and time. After a statistically significant treat-

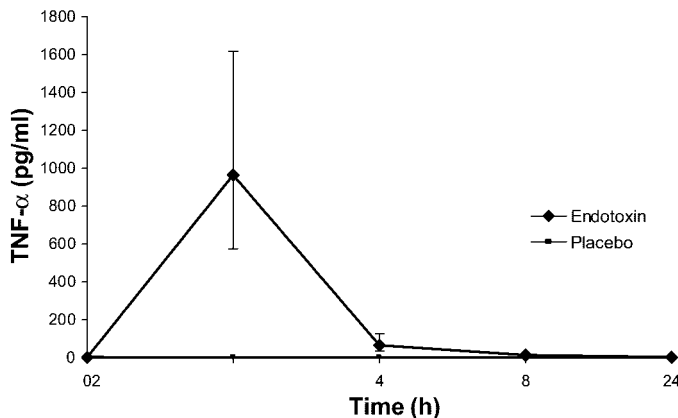


Fig. 1. Plasma tumor necrosis factor- α (TNF- α) concentrations measured in 8 healthy human subjects after infusion of endotoxin or placebo at time (t) = 0. Statistics: with the use of two-way repeated-measures (RM) ANOVA (see MATERIALS AND METHODS), the plasma TNF- α level was found to be statistically significantly increased after endotoxin compared with placebo. Geometric means and 95% confidence intervals are indicated.

ment effect (or treatment times time interaction) was detected, a multiple-comparison procedure (Tukey's test) was used to localize significant differences at each time point. Measurements that were not normally distributed were log transformed before statistical assessment. Parameters measured only at baseline and once after endotoxin infusion were compared with the use of Student's t -test for paired observations. Linear regression analysis was performed using the method of the least squares. The squared correlation coefficient (r^2) and the coefficient of inclination (α) are given. Results are given as means \pm SE or confidence interval as indicated. P values <0.05 were considered statistically significant.

RESULTS

Infusion of endotoxin significantly increased body temperature to a maximum at $t = 4$ h of 39.1 ± 0.2 vs. $36.9 \pm 0.2^\circ\text{C}$ after placebo ($P < 0.01$, $n = 8$). This was followed by a gradual decline to $37.7 \pm 0.1^\circ\text{C}$ at $t = 9$ h. During the rise in body temperature, the patients were shivering. At $t = 5$ h a maximum decline in mean arterial blood pressure from 95 ± 2 to 73 ± 2 mmHg ($P < 0.01$, $n = 8$) and at $t = 4$ h an increase in heart rate from 65 ± 4 to a maximum of 97 ± 5 beats/min ($P < 0.01$, $n = 8$) were observed. These hemodynamic changes were associated with a maximum increase in LBF at $t = 0$ from 450 ± 57 in the endotoxin group and 405 ± 38 ml/min ($P > 0.6$, $n = 7$) in the placebo group to 731 ± 79 versus 533 ± 77 ml/min at $t = 5$ h ($P < 0.05$, $n = 7$), respectively. The increased LBF level was maintained after endotoxin infusion for the next 3 h. Laboratory measurements on blood samples showed that maximum increases after endotoxin versus placebo were the following: TNF- α [964 vs. 3 pg/ml (geometric mean) at $t = 2$ h] (Fig. 1), CRP (481 ± 62 vs. <48 mg/l at $t = 24$ h, $P < 0.01$), orosomucoid (22 ± 2 vs. 16 ± 1 g/l at $t = 32$ h, $P < 0.01$), fibrinogen (8.0 ± 0.4 vs. 6.3 ± 0.5 $\mu\text{mol/l}$ at $t = 32$ h, $P < 0.05$), leucocyte count (13.0 ± 0.8 vs. 6.3 ± 0.4 $10^9/\text{l}$ at $t = 8$ h, $P < 0.01$), and neutrophil count (12.1 ± 0.7 vs. 3.9 ± 0.4

$10^9/\text{l}$ at $t = 8$ h, $P < 0.01$), plasma ACTH (79 ± 28 vs. 6 ± 1 pmol/l at $t = 4$ h, $P < 0.05$), and plasma cortisol (710 ± 101 vs. 295 ± 66 nmol/l at $t = 4$ h, $P < 0.01$). The maximum reductions were the following: lymphocyte count (0.5 ± 0.2 vs. 1.6 ± 0.1 $10^9/\text{l}$ at $t = 4$ h, $P < 0.01$) and monocyte count (0.05 ± 0.01 vs. 0.5 ± 0.1 $10^9/\text{l}$ at $t = 2$ h, $P < 0.01$). A significantly higher skeletal muscle glucose uptake was observed after endotoxin infusion compared with placebo (data not shown). No significant changes were observed in parameters analyzed for kidney and liver function, hemoglobin, plasma Na^+ , plasma myoglobin, or plasma insulin between the two groups. Taken together, the changes in temperature and in hemodynamic, immunologic, and hormonal parameters observed in response to infusion of endotoxin all resemble the acute response observed in patients with severe infections caused by bacteria that produces endotoxin.

Catecholamines

Infusion of endotoxin significantly increased the plasma epinephrine concentration to a maximum at $t = 2$ h of 0.7 ± 0.1 versus 0.3 ± 0.1 nmol/l ($P < 0.05$, $n = 6-7$) (Fig. 2) whereas only a tendency to an increase was seen in the plasma norepinephrine level (data not shown).

Plasma K^+

Infusion of endotoxin significantly reduced femoral venous plasma K^+ , reaching a nadir at $t = 5$ h of 3.3 ± 0.1 compared with 3.8 ± 0.1 mmol/l after placebo infusion ($P < 0.01$, $n = 6-7$) (Fig. 3A). After the nadir was reached, venous plasma K^+ increased to the level measured after placebo infusion after another 2-3 h. Similar changes were seen in arterial plasma K^+ . At $t = 8$ h the initial plasma K^+ level was reached and plasma K^+ remained stable for the next 24 h in both groups. At $t = 24$ and 32 h, venous plasma K^+ values

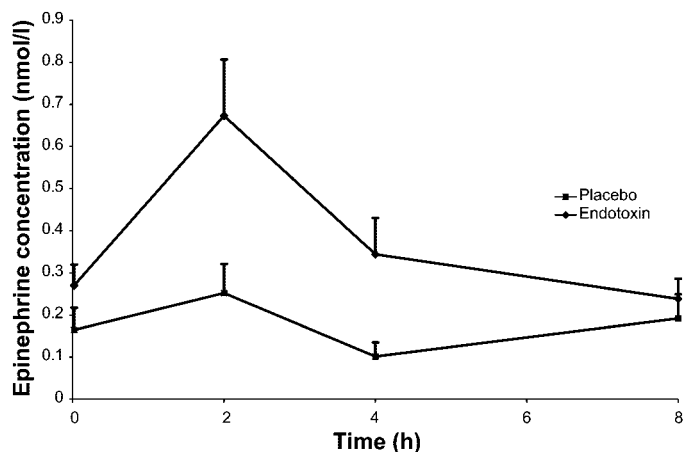


Fig. 2. Plasma epinephrine concentrations measured in 8 healthy human subjects after infusion of endotoxin or placebo at $t = 0$. Statistics: with the use of two-way RM ANOVA (see MATERIALS AND METHODS), the plasma epinephrine level was found to be statistically significantly increased after endotoxin compared with placebo. Values are means \pm SE.

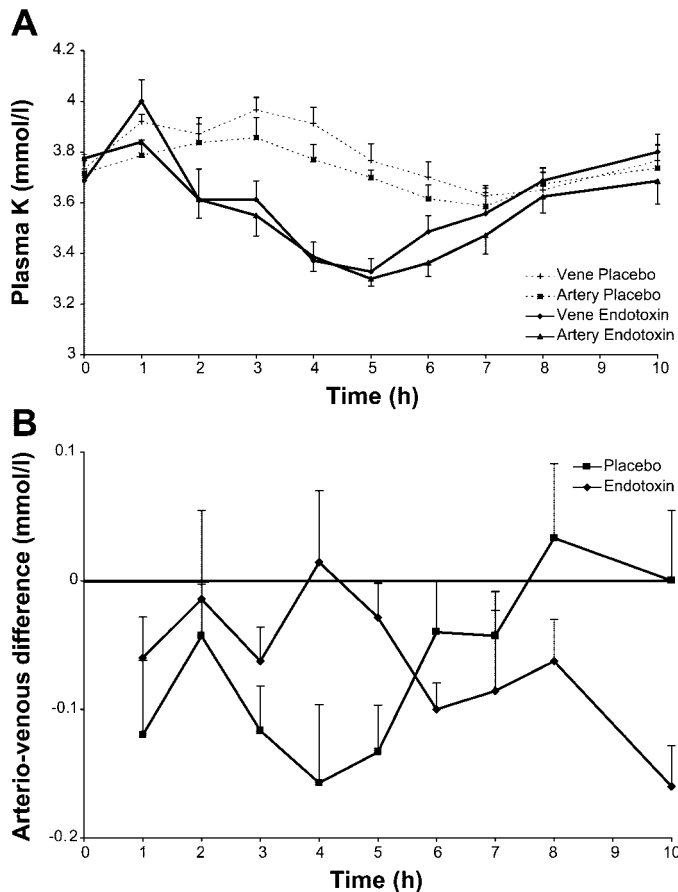


Fig. 3. A: arterial and venous plasma K^+ measured in 8 healthy human subjects after infusion of endotoxin or placebo at $t = 0$. Arterial plasma K^+ was measured in blood samples drawn from the arm and venous plasma K^+ in blood samples drawn from the femoral vein. Statistics: with the use of two-way RM ANOVA (see MATERIALS AND METHODS), both the arterial and venous plasma K^+ levels were found to be statistically significantly decreased after endotoxin compared with placebo. B: arteriovenous plasma K^+ differences in 8 healthy human subjects after infusion of endotoxin or placebo at $t = 0$. Statistics: with two-way RM ANOVA (see MATERIALS AND METHODS), the arteriovenous plasma K^+ difference level was found to be statistically significantly different after endotoxin compared with placebo. Values are means \pm SE.

after endotoxin and placebo were 3.8 ± 0.1 vs. 3.9 ± 0.1 mmol/l ($P = 0.8$, $n = 8$) and 3.7 ± 0.1 vs. 3.7 ± 0.1 mmol/l ($P < 0.6$, $n = 8$), respectively.

K^+ Exchange Across Leg

After placebo, venous plasma K^+ was slightly higher than arterial plasma K^+ , which reflects a small K^+ loss from the resting leg (Fig. 3, A and B). Until $t = 5$ h, the arteriovenous plasma K^+ difference was numerically higher after placebo compared with endotoxin, reaching a maximum at $t = 4$ h of -0.2 ± 0.1 versus 0.0 ± 0.1 mmol/l ($P < 0.05$, $n = 8$) (Fig. 3B). In combination with the increased LBF this indicates that until $t = 5$ – 6 h there was a reduced loss of K^+ from the leg after endotoxin infusion. On the basis of arteriovenous plasma K^+ differences and LBF measurements obtained each hour after endotoxin and placebo infusions,

the accumulated transport of K^+ to or from the leg could be calculated (Fig. 4). After placebo infusion, the accumulated K^+ exchange across the leg showed a constant loss of K^+ from the leg (linear regression analysis; $\alpha = -1.75 \pm 0.13$ mmol K^+ /h, $r^2 = 0.95$, $P < 0.01$). However, after endotoxin infusion, the loss of K^+ from the leg was reduced within the first hours. Thus the difference in accumulated K^+ (accumulated K^+ after endotoxin placebo) showed a gradual increase reaching a maximum after 6 h of 8.7 ± 3.8 mmol per leg ($P < 0.05$) (Fig. 4).

Renal K^+ Excretion

Urine volumes for the first 24 h after infusions were similar after endotoxin infusion ($2,869 \pm 253$) and placebo infusion ($2,788 \pm 252$ ml) ($P > 0.7$, $n = 8$). After endotoxin infusion urine K^+ concentration was 27 ± 3 compared with 18 ± 2 mmol/l after placebo infusion, i.e., an increase of 50% ($P < 0.05$, $n = 8$). Thus during the first 24 h after endotoxin infusion, the total renal K^+ excretion was 27 ± 7 mmol, i.e., 58% ($P < 0.01$) higher compared with placebo.

Plasma Lactate and Oxygen Saturation

After infusion of endotoxin significantly higher arterial plasma lactate concentrations were observed from $t = 1$ to $t = 10$ h, reaching a maximum after 1 h of 2.5 ± 0.5 compared with 0.9 ± 0.1 mmol/l after placebo infusion ($P < 0.05$, $n = 8$) (Fig. 5A). The lactate release (venoarterial plasma lactate difference multiplied by LBF) was stable after placebo, but increased after endotoxin (Fig. 5B). During the first 10 h, arterial oxygen saturation was for each subject at each time of

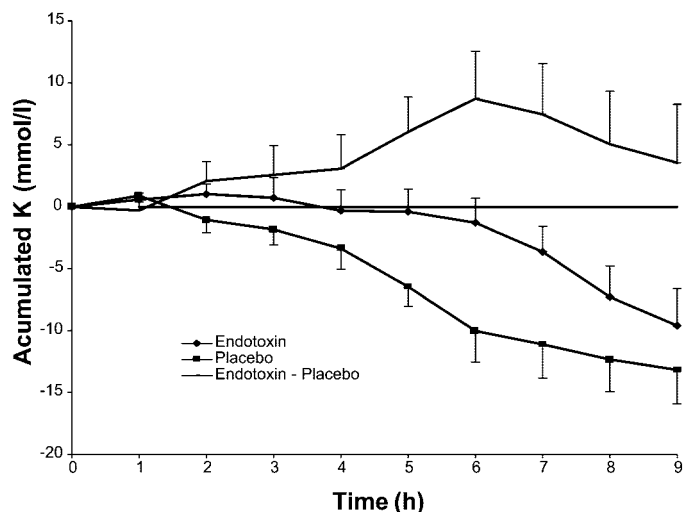


Fig. 4. Accumulated amount of K^+ per leg in 8 healthy human subjects after infusion of endotoxin or placebo at $t = 0$. Accumulated K^+ was calculated as the sum $\sum_{0-9 \text{ h}}$ of arteriovenous plasma K^+ difference times leg plasma flow. The difference between accumulated K^+ after infusion of endotoxin and accumulated K^+ after infusion of placebo is indicated (endotoxin placebo). Statistics: with the use of two-way RM ANOVA (see MATERIALS AND METHODS) the level of accumulated K^+ was found to be statistically significantly increased after endotoxin compared with placebo. Values are means \pm SE.

measuring $>97\%$ and no significant differences in venous oxygen saturation was observed. These observations, taken together with the observed increase in LBF, indicate that changes in blood lactate concentrations were not associated with reduced tissue oxygen supply. Moreover, the increase in plasma lactate was associated with a significant increase in plasma pH from 7.39 ± 0.01 to a maximum of 7.43 ± 0.01 ($P < 0.05$, $n = 8$) at $t = 5$ h, whereas no changes were seen after placebo infusion. The increase in pH may have been related in part to hyperventilation because a decrease in PCO_2 after endotoxin infusion was seen, reaching a nadir at $t = 5$ h of 4.7 ± 0.1 compared with 5.3 ± 0.1 kPa ($P < 0.05$, $n = 7-8$) after placebo.

DISCUSSION

The present study demonstrates that in healthy humans endotoxemia leads to hypokalemia and a relative accumulation of K^+ in the legs associated with increases in plasma catecholamines and plasma lactate concentrations without hypoxia, signs of hypoperfusion or reduced pH. In this model, endotoxin induced clinical,

hemodynamic, immunologic, and hormonal responses resembling the responses seen in patients with sepsis, which ensures clinical relevance of the obtained results.

In endotoxic rats, an increase in the activity of the pool of skeletal muscle $\text{Na}^+\text{-K}^+$ pumps has been shown without an associated increase in expression of messenger RNA for the α - or the β -subunits of the pump or increasing $\text{Na}^+\text{-K}^+$ pump number (22). Thus the present study supports the hypothesis that endotoxin exposure increases skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity (13) as assessed by a significant decrease in plasma K^+ and a relative increase in K^+ uptake in the leg induced by increased levels of catecholamines. The present observation of hypokalemia after endotoxin exposure has previously been observed in cows (23). We observed a constant loss of K^+ from the muscles in the placebo group. This is in accordance with previous studies in humans showing that at rest venous plasma K^+ is higher than arterial plasma K^+ (6, 9, 15, 17, 27). This may be explained by clearance of K^+ into the muscles for buffering after a meal, followed by a gradual loss of K^+ back to the extracellular fluid volume (ECV) and eventually excretion by the kidneys. After endotoxin infusion the plasma lactate level remained elevated after maximum changes in the K^+ homeostasis and in plasma epinephrine level were seen (Fig. 5A). This observation most likely reflects the variability in the measurements of plasma lactate as also seen in Fig. 5B, but may also reflect that plasma epinephrine level did not completely reach control level during this period (Fig. 2). However, a highly significant increase in plasma lactate of up to 177% was induced by endotoxin and it is of interest that this increase was of same order of magnitude as the insulin-induced ouabain-suppressible increase of 135% observed in humans during stimulation of skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ by an euglycemic-hyperinsulinemic clamp (21). However, it should be noted that in the present study neither the plasma lactate increase, nor the plasma K^+ decrease, was due to an insulin-induced $\text{Na}^+\text{-K}^+\text{-ATPase}$ stimulation because no significant differences in plasma insulin concentrations were observed after endotoxin infusion compared with placebo. The presently observed indications of increased skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity during endotoxemia are at variance with a report of no difference in cellular ^{86}Rb uptake in soleus muscle from rats treated with *Salmonella enteritidis* endotoxin (16). However, the soleus muscles were examined after excision and incubation in endotoxin-free buffers. Therefore, an ongoing effect of endotoxin could not be expected. This illustrates one of the possible pitfalls in in vitro assays compared with in vivo studies.

Assuming a total body skeletal muscle weight of $\sim 40\%$ of body weight, a skeletal muscle mass in one leg drained by the femoral vein of 10% of body weight (20) and a skeletal muscle K^+ content of $\sim 75\%$ of total body K^+ content of $\sim 3,500$ mmol (3) the accumulated difference in leg K^+ content in each leg 6 h after endotoxin and placebo infusions of ~ 9 mmol, corresponds to ~ 1

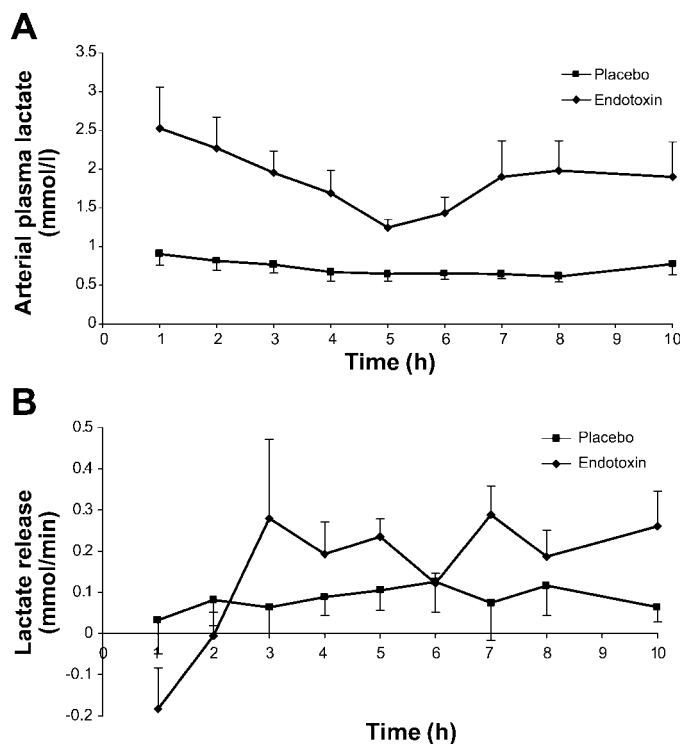


Fig. 5. A: arterial plasma lactate concentration measured in 8 healthy human subjects after infusion of endotoxin or placebo at $t = 0$. Statistics: with the use of two-way RM ANOVA (see MATERIALS AND METHODS) the arterial plasma lactate level was found to be statistically significantly increased after endotoxin compared with placebo. B: leg lactate release in 8 healthy human subjects after infusion of endotoxin or placebo at $t = 0$. Arterial plasma lactate was measured in blood samples drawn from the arm and venous plasma lactate in blood samples drawn from the femoral vein. Lactate release was calculated as venoarterial difference multiplied by leg blood flow. Statistics: with the use of two-way RM ANOVA (see MATERIALS AND METHODS), the level of lactate release from the leg to plasma was found to be statistically significantly increased after endotoxin compared with placebo. Values are means \pm SE.

mmol/kg K^+ skeletal muscle, i.e., 1.2%. Furthermore, assuming similar skeletal muscle K^+ accumulation throughout the body, a total body skeletal muscle K^+ accumulation of ~ 35 mmol at $t = 6$ h can be calculated after endotoxin infusion compared with after placebo infusion. The magnitude of this relative increase in skeletal muscle K^+ cannot fully be explained by the simultaneous decline in total ECV K^+ of 8 mmol [ECV (20% of body wt) \times plasma K^+ decrease (~ 0.5 mmol)]. This indicates that the K^+ changes were to a certain extent the result of K^+ shifts between ECV and skeletal muscles, but other organs, or regulatory mechanisms, e.g., temporal changes in renal K^+ excretion, may also have been involved. After the plasma K^+ nadir at $t = 5$ h, a gradual increase in plasma K^+ was observed with an increased venoarterial K^+ difference compared with before the nadir was reached (Fig. 3A). This indicates that the relative skeletal muscle K^+ accumulation ceased at $t = 5\text{--}6$ h and was followed by an increased loss of K^+ to the extracellular phase. Thus as presently as well as previously seen (18) the major effects of a single endotoxin bolus seems to vanish after ~ 5 h. Thus the study shows the initial effects of endotoxin on the extrarenal K^+ homeostasis, but the study design does not allow further assessment of the clinical situation with the septic patient with ongoing endotoxin production. It is important to notice that the shivering observed during the increase in temperature represents muscle work. During muscle work, the excitation-contraction sequence leads to a net loss of K^+ to the extracellular space (26). Thus shivering per se would have been expected to increase plasma K^+ . In contrast, we observed a decline in plasma K^+ , which can be explained by the catecholamine-induced stimulation of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. It was ensured that the plasma K^+ increase after the nadir at $t = 5$ h was not a result of rhabdomyolysis as indicated by unaltered plasma myoglobin concentrations.

To the best of our knowledge, the observed significant increase in renal K^+ excretion has not previously been reported. It should be noted that the higher K^+ excretion after endotoxin infusion was measured for the 24-h period after the infusions, whereas the skeletal muscle K^+ content changes were assessed after the initial 6 h. The combination of a reduction in skeletal muscle K^+ loss and an increase in renal K^+ excretion would be expected to reduce plasma K^+ or involve K^+ loss from other compartments or organs. However, it should be stressed that during the period of increased renal K^+ excretion ($t = 0\text{--}24$ h) skeletal muscle K^+ underwent a biphasic change: first, a relative accumulation, followed by an increased K^+ loss and at $t = 24$ h, skeletal muscle K^+ may actually have been reduced after endotoxin infusion compared with placebo. This suggestion is supported by the observation of a tendency to lower plasma K^+ concentrations at $t = 24\text{--}32$ h after endotoxin infusion.

Catecholamine stimulation of proximal tubule α -adrenoceptor increases $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, whereas a decrease is obtained by β -adrenoceptor stimulation and dual stimulation may counterbalance each other

(11). A micropuncture study showed that epinephrine reduces renal K^+ excretion (4) and a study using isolated kidneys showed that norepinephrine also reduces K^+ excretion (1). Thus it is most likely that the presently observed increase in renal K^+ excretion after endotoxin infusion may have been the combined outcome of a reduced renal K^+ excretion due to increased levels of catecholamines but at the same time an increased excretion induced by increased ACTH and cortisol concentration levels.

In vitro studies have shown that endotoxin via increased levels of IL-6 and TNF- α reduce hepatocyte (7, 8, 28) and thyroid cell (24) $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, respectively. Such changes would tend to increase plasma K^+ . Moreover, these results indicate that the numerous responses seen in sepsis, i.e., inflammatory, hormonal, metabolic, and hemodynamic changes may induce different or even opposite changes in different organs.

Taken together, the present study supports the hypothesis that in humans blood lactate level may increase under aerobic conditions. The combination of the well-known stimulatory effect on skeletal muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of catecholamines and the present confirmation of the correspondingly expected $\text{Na}^+\text{-K}^+\text{-ATPase}$ -induced decline in plasma K^+ suggests that the increase in skeletal muscle lactate release was due to increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. The findings also point to the importance of the K^+ homeostasis. It should be noted that the presently observed effect on the K^+ homeostasis is not supposed to be a specific response to endotoxemia, but may be present in general in acutely and severely ill patients with a stress response. The present study indicates that in such patients hypokalemia may not necessarily be interpreted as K^+ deficiency, as it may reflect (temporarily) increased muscle K^+ uptake, but on the other hand it is important to compensate for an increased renal K^+ excretion.

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