

Tourniquet-induced Changes of Energy Metabolism in Human Skeletal Muscle Monitored by Microdialysis

Ulrike Korth, M.D., Günter Merkel, M.D.,† Francisco F. Fernandez, M.D.,‡ Oliver Jandewerth, M.D.,§ Günes Dogan, Cand.Med.,‖ Thea Koch, M.D.,# Klaus van Ackern, M.D.,** Oksana Weichel, M.S.,†† Jochen Klein, Ph.D.‡‡*

Background: **Tourniquets are often used as part of orthopedic surgery but may cause local and remote organ injury. The authors hypothesized that the procedures used to induce ischemia (circulatory occlusion or exsanguination) may have differential effects on the metabolic state of the muscle that should be reflected in the interstitial levels of metabolites.**

Methods: **Microdialysis probes were implanted in both quadriceps femoris muscles of 18 patients. Interstitial fluid was obtained during tourniquet-induced ischemia and reperfusion and was analyzed for glucose, lactate, choline, and purines by high-performance liquid chromatography.**

Results: **At a flow rate of 2 μl/min, the average baseline concentrations in the dialysate were 2.5 mM for glucose, 1.7 mM for lactate, 5.2 μM for choline, and 14.3 μM for hypoxanthine. Circulatory occlusion by tourniquet caused a 40% decrease of the extracellular glucose concentration within 30 min. Concomitantly, the interstitial levels of lactate and hypoxanthine increased in a linear fashion to 206% (lactate) and 241% (hypoxanthine) of basal values. The extracellular concentration of choline was also significantly elevated. After exsanguination, the glucose levels were significantly more reduced (by 65%), and the levels of lactate (to 268%) and hypoxanthine (to 286%) were more increased than after circulatory occlusion alone.**

Conclusion: **Our microdialysis results demonstrate that the interstitial concentrations of glucose, lactate, and hypoxanthine, which are indicators of tissue ischemia, change more prominently after exsanguination than after circulatory occlusion alone. (Key words: Choline; glucose; hypoxanthine; lactate.)**

TO avoid intraoperative bleeding, tourniquets are often used in orthopedic surgery. Circulatory occlusion is achieved by the use of a pneumatic tourniquet, whereas traditional Esmarch ischemia additionally involves a previous exsanguination of the limb. These procedures induce muscle ischemia that is accompanied by anaerobic glycolysis, formation of lactate, and depletion of high-energy phosphates, resulting in the production of adenosine, inosine, and its oxidation product, hypoxanthine.[1,2] Prolonged ischemia results in loss of cellular homeostasis, disruption of ion gradients, and breakdown of membrane phospholipids, which is reflected by release of choline. After reperfusion, activation of neutrophils, formation of oxygen radicals, and release of vasoactive factors may cause damage to local and peripheral tissues.[1,2] Prominent secondary complications of prolonged lower-limb ischemia include acute compartment syndrome[3,4] and adult respiratory distress syndrome.[5–7]

In the present study, we used microdialysis in an operative setting to quantify the extent of ischemia in skeletal muscle. In the microdialysis procedure, a probe is inserted into the tissue of interest; perfusion of the probe allows the continuous sampling of interstitial fluid. The procedure has previously been used in experimental studies with volunteers to monitor changes of energy metabolism, *e.g.*, during exercise.[8,9] We measured the levels of glucose, lactate, purines, and choline as indicators of muscle energy metabolism and membrane breakdown. Our hypothesis was that the metabolic state of the muscle may be differentially affected by circulatory occlusion alone or additional previous exsanguination.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Patients and Methods

Eighteen patients undergoing elective surgery of the lower limb with an expected operation time of 60–75 min were included in the study. None of the patients had preexisting systemic diseases or was currently taking any medications. The patients were randomized so that nine received only the tourniquet, whereas in the nine others the operated leg was exsanguinated before the tourniquet was inflated (Esmarch ischemia). Both groups contained six men and three women. The primary diagnoses and surgical procedures were mainly internal fixations or metal removals from fractures of the distal tibia, fibula or ankle and did not differ between groups.

The study design was approved by the local Committee on Human Experimentation II (Faculty of Clinical Medicine Mannheim, University of Heidelberg, Germany). All patients received a detailed description of the study program, and written consent was obtained from each patient.

Study Protocol

All patients were fasted overnight and received a premedication of oral midazolam (7.5 mg) before leaving

* Resident, § Fellow, ‖ Medical Student, # Associate Professor, ** Professor, Department of Anesthesiology, † Attendant, ‡ Fellow, Department of Surgery, Faculty of Clinical Medicine Mannheim, University of Heidelberg. †† Technician, ‡‡ Associate Professor, Department of Pharmacology, University of Mainz.

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Address reprint requests to Dr. Klein: Department of Pharmacology, University of Mainz, Obere Zahlbacher Str. 67, D-55101 Mainz, Germany. Address electronic mail to: jklein@mail.uni-mainz.de.

the ward. All patients underwent spinal anesthesia (L3–L4) with isobaric bupivacaine (0.5%; 12.5–17.5 mg) and were monitored by electrocardiogram, automated noninvasive blood pressure measurement, and pulse oxymetry. Skin temperature was continuously measured with a thermistor probe (Dräger, Lübeck, Germany) 5 cm lateral of the dialysis probe insertion site on both legs. After induction of spinal anesthesia, the pneumatic tourniquet was applied but not inflated. We used I-shaped microdialysis probes (CMA/60, Carnegie Medicine, Stockholm, Sweden) with an exchange length of 30 mm (membrane, polyamide; OD, 0.6 mm; molecular cutoff, 20 kDa). Using guide cannulae supplied by the manufacturer, these probes were inserted at an angle of 45° through the subcutaneous tissue and, after passing the muscle fascia, at an angle of 20° into the vastus medialis of both quadriceps femoris muscles under aseptic conditions. The correct location of the probe in the muscle tissue was controlled by ultrasonography or the "fascial click." The probe on the operated side was located midline 2 cm below the tourniquet; an additional probe was inserted at the equivalent site of the other leg and served as control. During the period of observation, no clinical signs of inflammation were noted at the insertion site. The microdialysis probes were perfused at a rate of 2 μl/min with sterile Ringer's solution by means of a precision infusion pump (CMA/102, Carnegie Medicine); for flow analysis (fig. 1), different flow rates (0.3, 2, and 10 μl/min) were applied in nonoperated legs. Samples were collected on ice in 15-min intervals and stored at −20°C until analysis. In addition, blood samples were obtained from the femoral vein before ischemia and again 2, 60, and 120 min after deflation of the tourniquet. Laboratory parameters were inconspicuous; in particular, no signs of inflammation or infection were present.

The patients were separated in two groups. One group of patients (group I, "circ. occl." in figs. 2–4) underwent surgery with circulatory occlusion (leg elevation for a few minutes, then tourniquet inflation to a pressure of 380 mmHg to surpass arterial blood pressure). The second group of patients (group II, "exsang." in figs. 2–4) underwent surgery with Esmarch ischemia (leg elevation and exsanguination by a circumferential elastic bandage wrapped around the ankle and rolled toward the body, with subsequent tourniquet inflation to a pressure of 380 mmHg and removal of the elastic bandage).

Analytical Methods

The analysis of glucose, lactate, choline, and purines was conducted using a high-performance liquid chromatography system (Gynkotek 300C pump, Biometra EP-30 electrochemical detector; Gynkotek, Germering, Germany) coupled to a separation column and an enzyme reactor. The enzyme reactors contained specific immobilized enzymes (glucose oxidase, lactate oxidase, Biometra EP-12; choline oxidase, Biometra EP-13; xanthine oxidase, Biometra EP-11); these enzymes catalyzed the oxidation of the analytes with the formation of H_2O_2, which could be detected with high sensitivity (picomole range) at a platinum electrode operating at 0.5 V. Glucose and lactate were separated by high-performance liquid chromatography using 0.1 M sodium phosphate, pH 7.6, as eluent; the retention times were 2.0 min (glucose) and 8.8 min (lactate). Choline was measured using a nucleosil 5 SA column and 0.1 M sodium phosphate buffer, pH 7.4, containing 10 mM tetramethylammonium chloride as eluent; retention time was 3.5 min. Purines were separated on a nucleosil C18 column (250 × 4.6 mm) using 20 mM ammonium dihydrogenphosphate, pH 6.3, as eluent (flow rate: 0.8 ml/min); the retention times were 4.9 min (uric acid), 17.5 min (hypoxanthine), and 20.7 min (xanthine). The microdialysis samples were injected directly into the high-performance liquid chromatograph.

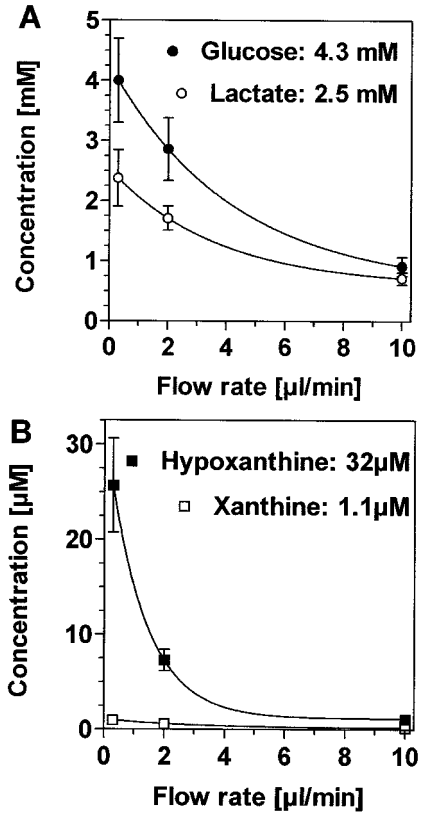

Fig. 1. Metabolite recovery during microdialysis at different flow rates. (*A*) Glucose and lactate. (*B*) Hypoxanthine and xanthine. Microdialysis probes were inserted into quadriceps muscle and perfused with Ringer's solution at the flow rates indicated (0.3, 2, and 10 μl/min). Absolute concentrations of the metabolites in the interstitial fluid were calculated by extrapolation of the curves to zero flow using the mathematical function for monoexponential decay (n = 4 for each curve).

Statistical Analysis

For data evaluation, the basal efflux of the analytes (glucose, lactate, choline, and hypoxanthine) was calculated as the average of three to four consecutive samples

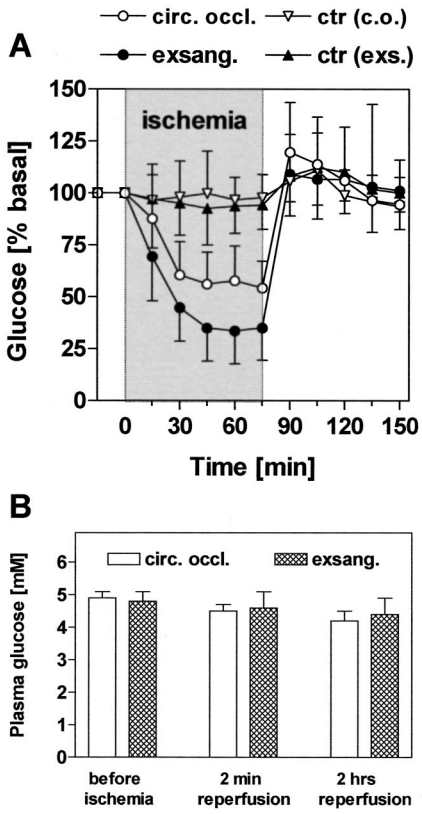

Fig. 2. Changes of glucose concentrations in muscle and venous plasma during ischemia and reperfusion. (*A*) Interstitial glucose concentration in quadriceps muscle: ischemia (shaded area) induced by circulatory occlusion (circ. occl.) or exsanguination (exsang.) in surgically treated and untreated (ctr) legs (n = 9 each). Data are mean ± SD and are given as percentage of baseline glucose concentrations (table 1). (*B*) Plasma glucose levels before ischemia, 2 min after ischemia, and after 2 h of reperfusion (venous blood from treated leg). Statistics in (*A*): Comparison between the curves for circulatory occlusion and exsanguination, two-way analysis of variance for repeated measurements, data points from 15–75 min: $F_{1,71} = 27.9$; $P < 0.001$.

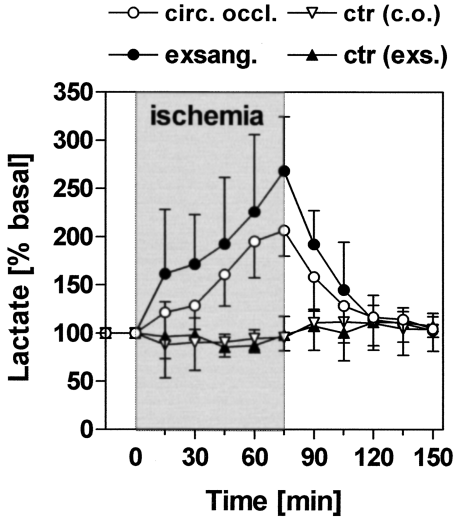

Fig. 3. Changes of interstitial lactate concentration in the quadriceps muscle during ischemia (shaded area) and reperfusion. Ischemia was induced by circulatory occlusion (circ. occl.) or by exsanguination (exsang.; n = 9 each). Controls are from nontreated legs. Data are mean ± SD and are given as percentage of baseline lactate concentrations (table 1). Statistics: Comparison between the curves for circulatory occlusion and exsanguination, two-way analysis of variance for repeated measurements, data points from 15–75 min; $F_{1,71} = 12.2$; $P < 0.001$. ctr (c.o.) = circulatory occlusion controls; ctr (exs.) = exsanguination controls.

taken before ischemia and defined as 100% for each curve. The average basal dialysate concentrations are given in table 1. All subsequent data points (figs. 2–4) were expressed as percentage of the basal efflux. Data in figures 2–4 are given as mean ± SD of nine experiments. To compare the data obtained from the two groups of patients, the two data curves ("circ. occl." *vs.* "exsang.") were statistically compared by two-way analysis of variance for repeated measurements using the Excel program package (Microsoft, Seattle, WA). In table 2, paired *t* test was used for statistical comparison.

Results

The two groups of patients were not significantly different in age, body height, or body mass (circulatory occlusion: age 42 ± 17 yr, height 171 ± 9 cm, and mass 81 ± 14 kg; Esmarch ischemia: age 41 ± 11 yr, height 175 ± 9 cm, and mass 75 ± 14 kg). No significant changes in heart rate, blood pressure, or oxygen saturation were recorded between the two groups either during operative care or after removal of the tourniquet. Furthermore, the time course of skin temperatures did not reveal any significant differences between the study groups.

When the dialysis fluid was analyzed for metabolite concentrations (glucose, lactate, choline, and purines), baseline levels were reached 15 min after probe insertion and remained constant for the time of observation (180 min) in control legs. In four patients, we determined the *in vivo* recoveries of our probes at different flow rates (0.3, 2, and 10 μl/min) in legs not undergoing surgery. Assuming a monoexponential relation between flow rate and recovery,[10,11] we calculated interstitial concentrations of the metabolites by extrapolation of the recovery curve to zero flow. By this method, the interstitial levels of glucose and lactate were determined as 4.3 and 2.5 mM, respectively (fig. 1A). Likewise, the extracellular concentrations of hypoxanthine and xanthine were calculated as 32 and 1.1 μM, respectively (fig. 1B), and for choline, 11.8 μM (not shown).

For investigations in ischemic muscle, we used a constant flow rate of 2 μl/min. At this flow rate, we measured the concentrations of glucose, lactate, choline, and the purines given in table 1. The effects of ischemia by circulatory occlusion or exsanguination on the dialysate concentrations of glucose, lactate, and hypoxanthine are shown in figures 2–4. We found that the levels of glucose decreased immediately after induction of ischemia and stabilized after 30 min; the decrease of glucose was significantly more pronounced after exsanguination

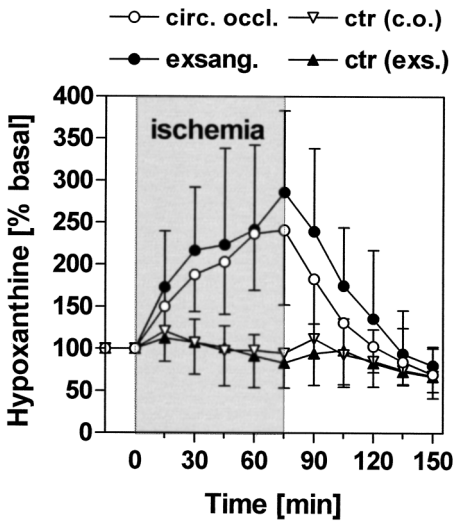

Fig. 4. Changes of interstitial hypoxanthine concentration in the quadriceps muscle during ischemia (shaded area) and reperfusion. Ischemia was induced by circulatory occlusion (circ. occl.) or by exsanguination (exsang.; n = 9). Controls are from nontreated legs, respectively. Data are mean ± SD and are given as percentage of baseline hypoxanthine concentrations (table 1). Statistics: Comparison between the curves for circulatory occlusion and exsanguination, two-way analysis of variance for repeated measurements, data points from 0–150 min: $F_{1,161} = 5.77$; $P = 0.018$. ctr (c.o.) = circulatory occlusion controls; ctr (exs.) = exsanguination controls.

($P < 0.001$; fig. 2A). It should be noted that the changes of extracellular glucose monitored by microdialysis were not reflected in the plasma glucose concentrations of venous blood taken from the ischemic leg (fig. 2B).

Parallel to the decrease in interstitial glucose, induction of ischemia caused an increase in the levels of lactate (fig. 3) and hypoxanthine (fig. 4). Again, the increases in lactate and hypoxanthine were more pronounced after exsanguination than during circulatory occlusion alone. The levels of xanthine were low in skeletal muscle (fig. 1) and did not significantly change during ischemia (data not shown); however, during reperfusion, xanthine levels increased to a maximum of 141 ± 21% ($P < 0.05$ *vs.* basal) at 45 min after deflation of the tourniquet. In some experiments, changes of uric acid levels were also monitored; similar to xanthine, the levels of uric acid did not change during ischemia but tended to increase during reperfusion. This phenomenon was observed in both legs and likely reflected systemic formation of xanthine and uric acid from hypoxanthine, probably by plasma or hepatic xanthine dehydrogenase–oxidase.[12,13]

Average levels of free choline in dialysates are given in table 2. During ischemia, the extracellular choline concentration increased to a maximum of 175% of basal levels and returned to baseline during reperfusion. In venous blood plasma, basal choline levels (4.8 ± 2.0 μM, n = 6) were significantly increased to 6.3 ± 1.8 μM 2 min after reperfusion and remained slightly elevated at 5.5 μM for 60–120 min (not illustrated).

Discussion

The present study demonstrates that interstitial levels of metabolites could be monitored by microdialysis in a routine operative setting. After induction of spinal anesthesia, the implantation of the dialysis probe required only a few minutes of handling, and the sampling of fluid was continued during anesthesiologic care. The procedure is associated with negligible stress for the patient and carries no more risk than intramuscular injections. Compared with noninvasive techniques such as nuclear magnetic resonance, microdialysis also allows the analysis of metabolites that are present in low (micromolar) concentrations, *e.g.*, hypoxanthine or choline. Online analysis of metabolites is possible with appropriate equipment.

While previous studies about the consequences of ischemia in skeletal muscle usually involved venous blood sampling or tissue biopsies,[13,14] the microdialysis procedure has the advantage that metabolite levels can be monitored directly in the interstitial fluid of the tissue even when blood flow is restricted. In our study, changes of the energy status in muscle were immediately visible after induction of ischemia when glucose levels decreased and the extracellular concentrations of lactate and hypoxanthine increased. Importantly, the microdialysis procedure revealed pronounced changes of the glucose levels *in situ* that were not reflected in pooled venous blood from the ischemic leg (figs. 2A and 2B).

There is a limited amount of information in the literature on metabolite levels in muscle interstitial fluid. The true interstitial levels for glucose (4.3 mM) and lactate (2.5 mM) calculated from the present data (fig. 1) are compatible with earlier data that reported glucose levels

Table 1. Concentrations of Metabolites in Dialysates from Muscle under Basal Conditions

	Circulatory Occlusion		Exsanguination	
	Control Leg	Ischemic Leg	Control Leg	Ischemic Leg
Glucose (mM)	2.5 ± 1.2	2.4 ± 1.2	2.5 ± 0.9	2.4 ± 0.3
Lactate (mM)	1.7 ± 0.9	1.8 ± 0.3	1.6 ± 0.3	1.5 ± 0.3
Hypoxanthine (μM)	15.7 ± 7.2	15.5 ± 9.2	13.7 ± 7.5	13.1 ± 6.0
Xanthine (μM)	0.9 ± 0.2	1.0 ± 0.5	0.6 ± 0.2	0.5 ± 0.3

Data represent the concentrations of metabolites in the dialysate (flow rate: 2 μl/min) and are given as mean ± SD (N = 9).

Table 2. Choline Concentrations in Muscle Dialysate during Ischemia and Reperfusion

	Control Leg	Circulatory Occlusion
Basal level	5.0 ± 2.6	5.4 ± 1.8
Ischemia	5.1 ± 3.2	9.5 ± 2.9*
Reperfusion	5.0 ± 2.6	6.3 ± 2.6

Data represent concentrations in the dialysate (in μM) and are given as mean ± SD (N = 6).

* $P < 0.01$ *versus* control leg (paired *t* test).

of 3.3–3.6 mM and lactate levels of 1.9–2.2 mM, respectively, in volunteers.[9,15] Therefore, interstitial glucose levels are slightly lower, and lactate levels are two times higher, than the corresponding plasma levels.[16,17] To our knowledge, the extracellular concentrations of choline (11.8 μM), hypoxanthine (32 μM), and xanthine (1.1 μM) have been quantified for the first time in the present study. The data in figure 1 also demonstrate that glucose and lactate could be efficiently dialyzed from the extracellular space when the flow rate was high. This indicates that the dialysis-induced depletion of the metabolites in the extracellular space is rapidly compensated for, probably by release of glucose and lactate from muscle tissue.[8] In contrast, no such compensation was observed in the case of hypoxanthine.

This study was conducted in human skeletal muscle during operative care. Two recent microdialysis studies had tested the effects of ischemia or exercise on muscle metabolites in volunteers.[8,9] In agreement with these studies, we found that the extracellular glucose concentration decreased immediately after induction of ischemia (fig. 2), whereas extracellular lactate increased (fig. 3). This was obviously caused by the disruption of blood flow and the subsequent shift from aerobic to anaerobic metabolism in ischemic tissue, which caused the muscle cells to consume large amounts of glucose to sustain the energetically inefficient anaerobic glycolysis. Although the glucose levels stabilized after 45 min, the levels of lactate increased linearly for 75 min; this lactate formation was probably caused by the well-known consumption of muscle glycogen during prolonged ischemia. It should be noted that these metabolic changes were strictly confined to the ischemic legs; when we monitored extracellular metabolites in the absence of tourniquet to assess the effect of surgery alone, no significant changes of glucose or lactate were observed (unpublished observations).

To follow the breakdown of adenosine triphosphate, we also monitored the interstitial levels of hypoxanthine, a product of adenosine oxidation. Increases of hypoxanthine after ischemia in muscle had previously been found in plasma samples taken after surgical interventions.[18,19] We found that the extracellular levels of hypoxanthine also rapidly increased, indicating that adenosine triphosphate breakdown was an immediate consequence of ischemia. It should be noted that hypoxanthine seemed to be the major purine metabolite in human muscle; the low levels of xanthine observed during basal conditions were not increased during ischemia, but xanthine and uric acid were formed during reperfusion, and increases were found in both legs. These observations are compatible with reports describing a low activity of xanthine dehydrogenase–oxidase in human skeletal muscle.[20,21]

In the present study, the continuous sampling of extracellular fluid by microdialysis allowed the sensitive detection of muscle metabolites during the ischemic period and, consequently, the quantitative comparison of two different techniques of bloodless lower-limb surgery. Our results give the first clear-cut evidence that exsanguination affects muscle metabolism more strongly than circulatory occlusion. As shown in figures 2–4, exsanguination caused a much stronger decrease of extracellular glucose, and a significantly stronger increase of lactate and hypoxanthine, than observed during circulatory occlusion alone. Moreover, basal levels of lactate and hypoxanthine were reached somewhat later when the exsanguination method was used. The reciprocal changes of metabolite levels indicate that these changes are not simply caused by a reduction of the extravasal volume induced by exsanguination. Rather, the concomitant changes of glucose, lactate, and hypoxanthine demonstrate that muscle cells have a higher rate of anaerobic glycolysis after exsanguination compared with circulatory occlusion. Although muscle tissue is believed to be relatively resistant to ischemia, short (and drastic) ischemic periods may already cause calcium overload in muscle and promote secondary complications in vulnerable patients such as compartment and adult respiratory distress syndrome. To address possible secondary complications, we provide preliminary data that short-term ischemia induces an immediate breakdown of cellular membranes. Hypoxic conditions are known to cause the activation of phospholipase A_2, leading to release of free choline from choline-containing phospholipids.[22] In the present work, the levels of free choline were enhanced after circulatory occlusion in plasma and, more importantly, in interstitial fluid of muscle (table 1). This finding is compatible with the formation of phospholipase A_2–derived lipid mediators such as thromboxane A_2 observed previously,[9] and may be indicative of cellular degeneration; this hypothesis is the focus of our present work.

In conclusion, using microdialysis to gain access to the interstitial fluid of skeletal muscle, we demonstrated that circulatory occlusion is a less demanding technique than exsanguination when the levels of glucose, lactate, and hypoxanthine are taken as indicators of tissue ischemia.

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