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Effects of Caffeine, Halothane, and 4-Chloro-m-cresol on Skeletal Muscle Lactate and Pyruvate in Malignant Hyperthermia–susceptible and Normal Swine as Assessed by Microdialysis

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Background: **Skeletal muscle fibers from malignant hyperthermia (MH)–susceptible humans and swine are markedly more sensitive to ryanodine receptor (RyR1) agonists than those from normal individuals. Reproducible shifts in the dose–response of skeletal muscle to caffeine and halothane are the basis of the current *in vitro* diagnostic caffeine–halothane contracture test. In an attempt to develop a less invasive MH diagnostic test, the authors determined the effects of RyR1 agonists (caffeine, 4-chloro-*m*-cresol [4CmC], and halothane) on the adductor muscle with respect to the lactate–pyruvate (L/P) system that was percutaneously dialyzed using a microdialysis technique in homozygous MH-susceptible compared with normal swine.**

Methods: **Animals were anesthetized (ketamine–propofol) and artificially ventilated. Sets of six CMA/20 microdialysis catheters were implanted; each catheter was perfused with different RyR1 agonist concentrations. After a 30-min equilibration after implantation, one of the catheters was perfused (2 μl/min) with vehicle (0.9% saline or lipid emulsion), and the other five were perfused with caffeine (1–64 mM), 4CmC (0.1–8 mM), or halothane (prepared in lipid emulsion; 10–500 mM). Outflow dialysate fractions collected at 10-min intervals and L/P parameters were measured enzymatically.**

Results: **Only in the MH-susceptible group did all RyR1 agonists increase dialysate L/P in a dose-dependent manner. The dose–effect relations were most prominent with 4CmC. With the halothane lipid emulsion, data scatter was high compared with that of the caffeine group and especially the 4CmC group. There were no signs of global muscle rigidity, systemic hypermetabolism, or a clinical MH episode during microdialysis RyR1 perfusion.**

Conclusions: **The authors data demonstrate that the *in vivo* muscle microdialysis of the porcine L/P system reveals distinct differences between MH-susceptible and MH-normal muscle, especially in response to highly specific RyR1 agonists such as 4CmC. The microdialysis L/P technique seems to have an MH diagnostic potential in the clinical setting.**

MALIGNANT *hyperthermia* (MH) is a genetic disorder of skeletal muscle that predisposes to a life-threatening reaction, a clinical MH episode, in response to commonly used inhalational anesthetics and depolarizing muscle relaxants.[1] In MH-susceptible (MHS) individuals, the exposure triggers uncontrolled Ca^{2+} release from the sarcoplasmic reticulum *via* the ryanodine receptor (RyR1),[2,3] the main calcium release channel of the sarcoplasmic reticulum. Excess myoplasmic Ca^{2+} leads to hypermetabolism, muscle rigidity, massive rhabdomyolysis, local and systemic acidosis, and eventual hyperthermia. According to previous ^{31}P-nuclear magnetic resonance data, even unchallenged MHS patients typically have a decreased concentration ratio of phosphocreatine (PCr) and inorganic phosphate (P_i), suggesting a subnormal skeletal muscle cellular energy state, in forearm flexor muscle; in addition, their postexercise energetic recovery is delayed.[4,5] However, even prolonged exposure to halothane does not increase skeletal muscle levels of P_i or [lactate] in normal rats.[6,7]

Because MH is usually inherited as an autosomal dominant trait in humans, family history is helpful in the diagnosis, but it is not definitive and reliable. Reproducible shifts in the dose–response of skeletal muscle force development due to caffeine and halothane are the basis of the current MH diagnostic *in vitro* caffeine–halothane contracture test (CHCT).[8,9] However, there are a number of limitations associated with this *in vitro* bioassay procedure. The test requires an invasive surgical excision of a 3 × 1-cm muscle bundle; the test has a sensitivity of 97% (accurately detecting MH) and a specificity of 78% (22% false positive). In addition, these tests can only be performed at MH diagnostic centers, and there are only six such centers in the United States of America. The CHCT test has not been evaluated in children. The test is expensive ($6,000–9,000 per test), and declining viability of the excised muscle after surgical excision requires that the CHCT to be completed within 5 h after the biopsy.

The objective of this study was to develop a simple, minimally invasive, relatively inexpensive, and readily available clinical MH diagnostic test in a swine model. The hypothesis was that by exposing only a very small portion of skeletal muscle *in vivo* to RyR1 agonists using the microdialysis technique,[10] it would be possible to monitor the diffusible indices of cellular redox and energy states, the lactate–pyruvate (L/P) system, and thus distinguish normal (MHN) skeletal muscle from MHS muscle, taking advantage of *in vivo*–based L/P metabolic reaction to RyR1 agonists. The advantage of such

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an approach would be that the local microdialysis application of RyR1 agonists is unlikely to trigger a systemic MH reaction but powerful enough to trigger a strictly localized skeletal MH episode. We established dose–response relations for the three major RyR1 agonists (caffeine, 4-chloro-*m*-cresol [4CmC], and halothane) with respect to the dialysate L/P system and compared the responses of MHS skeletal muscle with those from MHN muscle. This approach was considered reasonable because the L/P redox ratio can inversely couple to the cytosolic energy state, and the latter decreases in response to increased cellular energy requirements,[11,12] including calcium overload in an MH crisis.[4,5] Anetseder *et al.*[13,14] first described an application of percutaneous partial pressure of carbon dioxide (P_{CO_2}) probes for the diagnosis of MH susceptibility measuring the caffeine-induced increase in P_{CO_2} in dialysates from skeletal muscle. Our study focuses on the local, *i.e.*, percutaneously assessed, L/P system as it responds to microdialysis perfusion with various RyR1 agonists over a wide pharmacologic range.

Materials and Methods

In Vitro *Studies with the Dialysis System*

Characteristics of the CMA-20 Microdialysis Membrane in Terms of Permeability to RyR1 Agonists. The permeation of the RyR1 agonists across the tip of the microdialysis catheter membrane (CMA-20; CMA/Microdialysis AB, Stockholm, Sweden; membrane: polycarbonate probe length 10 mm, shaft plus membrane length 24 mm, OD 0.5 mm, molecular cutoff 20,000 Da; tubing: inlet/outlet length 200 mm, dead volume 1.8 μl/100 mm) was measured by inserting the tip into microvials containing 0.1 ml normal saline (0.9% NaCl); this volume fully covered the catheter tip membrane. Perfusions were performed for up to 4 h at a perfusion rate of 2 μl/min (microdialysis pump, model CMA/102) at room temperature. Caffeine (2–8 mM; Sigma Chemical Co., St. Louis, MO) or 4CmC (0.1–0.8 mM; Calbiochem, La Jolla, CA) was prepared in normal saline. Halothane (Halocarbon Laboratories, River Edge, NJ), which is poorly soluble in aqueous solutions, was added in different amounts to an intravenous 20% lipid emulsion (Intralipid; Fresenius Kabi Clayton, Clayton, NC; pH = 8, 20% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerin); the mixtures were shaken for 24 h, yielding lipid emulsions containing 0.1–1 M halothane (HLE).[15] In the studies with HLE, microdialysis catheters were immersed in microvials containing 1.4 ml saline and perfused at flow rate of 2 μl/min. The larger saline volume in the microvials allowed the catheter membrane to remain in the aqueous phase media during the protocol. Outflow dialysate fractions were collected over 30 min in microvials containing 0.1 ml saline sealed with 0.1 ml *n*-heptane (Sigma Chemical Co.) to prevent halothane evaporation.

***In Vitro* Recovery of Lactate, Pyruvate, and Glucose.** To determine the permeation of lactate, pyruvate, or glucose from a microvial sample into the mobile phase of the microdialysis system, catheters were immersed in microvials containing 0.1 ml calibrated standard solutions of lactate (2.5 mM), pyruvate (0.25 mM), and glucose (5.5 mM) in normal saline; microdialysis catheters were perfused with normal saline with and without caffeine (32 mM), 4CmC (8 mM), and HLE (200 mM); dialysis perfusion rates were 0.5, 1.0, and 2.0 μl/min. The outflow dialysates were collected for 30 min and analyzed for lactate, pyruvate, and glucose using a CMA600 clinical analyzer.

Special procedures were required for the HLE studies. Here, the outflow dialysates containing HLE were centrifuged to separate the lipid phase (upper layer) from the aqueous phases (lower layer). Concentrations of lactate, pyruvate, and glucose were measured in the aqueous phases and compared with the concentrations of the standard solutions of the microvials. Recoveries were calculated as the ratio of metabolite concentration in outflow dialysate divided by the standard concentration in the microtube.

In Vivo *Studies Using the Percutaneous Microdialysis Implantation Technique*

Instrumented Porcine Methodology and Animal Groups. With approval of the institutional animal care committee, nine MHN pigs (not MH sensitive; Archer Farm, Belcamp, MD) and nine homozygous MHS swine (University of Minnesota, Experimental Farm, Becker, MN) weighing 20–40 kg were used. The animals were maintained on a normal diet but fasted overnight before the experimental procedure. The animals were sedated with ketamine (8–10 mg/kg intramuscular); an ear vein was cannulated for inducing and maintaining anesthesia with propofol ($0.2–0.4\ mg \cdot kg^{-1} \cdot min^{-1}$). An endotracheal tube was inserted, and ventilation was maintained with an end-tidal carbon dioxide of 40 ± 5 mmHg. A rectal thermometer probe was placed to monitor and maintain core body temperature at 38.5°C.

A 20-gauge cannula was inserted percutaneously into the superficial femoral artery. Arterial blood samples (7 ml) were collected before, during (every 10 min), and at the end of the microdialysis protocol. Blood samples were analyzed for pH, arterial oxygen tension (Pa_{O_2}), arterial carbon dioxide tension (Pa_{CO_2}), and serum potassium and creatine kinase. Mean arterial blood pressure, heart rate, respiratory rate, and body temperature were monitored continuously.

Experiments were performed in two groups. The groups were paired so that one group of MHS swine was compared with one group of MHN swine in each session with caffeine, 4CmC, or HLE. Each swine underwent

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tests four times, with 1-week intervals between tests: three treatments (caffeine, 4CmC, HLE) and time control. Animal test groups were matched in terms of sex, weight, and number of experiments performed per animal. At the conclusion of each experimental session, animals were allowed to wake up and recover for at least 7 days before they were subjected to the next session, albeit with a different RyR1 agonist.

***In Situ* Microdialysis Procedure.** After establishing surgical anesthesia, the skin above the adductor muscles was prepared using antiseptic (10% povidone-iodine solution); six CMA-20 microdialysis catheters were inserted percutaneously into the adductor muscles bilaterally using a catheter insertion guide to obtain uniform percutaneous depth of implantation. Catheters were advanced to a depth of 20–24 mm and were secured in place using adhesive tape. Catheter implantation sites were spaced 4–5 cm apart to minimize interference between catheter sites.

***In Situ* Time Course Protocol.** To determine the effects of the insertion trauma itself on the L/P parameters, the time course of the outflow dialysate [lactate] and [lactate]/[pyruvate] ratios were measured over 40 min using a perfusion rate of 2 μl/min of normal saline. Six microdialysis catheters were implanted percutaneously into adductor muscles of MHS (n = 9) and MHN (n = 9) swine as described in the *In Situ* Microdialysis Procedure section. Catheter perfusion with saline started either immediately after insertion (elapsed time zero) or with a delay of 5, 10, 20, 30, and 40 min (elapsed time to perfusion) after implantation. Outflow dialysate fractions were collected sequentially over 10 min and analyzed with a CMA-600 clinical analyzer for lactate and pyruvate.

At the conclusion of all microdialysis protocols, muscle biopsies were taken (musculus vastus lateralis) to perform the classic CHCT, which verified animal MH status.[8]

Dialysis Perfusion with Different RyR1 Agonists on Outflow Dialysate Lactate and Pyruvate. After microdialysis catheter implantation as described above, a 30-min "equilibration" period was used before starting the dose–response studies with the various RyR1 agonists. This period reduced the acute insertion-induced trauma as indicated by the fact that the L/P parameters reached near control at the end of this period. One of the six catheters was then perfused at 2 μl/min with saline or lipid emulsion (controls), and the remaining five catheters were perfused at 2 μl/min with different concentrations of caffeine (1–64 mM), 4CmC (0.1–8 mM), or HLE (10–500 mM). Six samples were collected over 10-min intervals for caffeine and 4CmC perfusions. In the HLE studies, samples were collected over 20-min intervals; this produced 40-μl aliquots, which were needed to reproducibly centrifuge and transfer the aqueous phase without contamination from the lipid phase to the CMA analyzer. The HLE dialysate samples were centrifuged at 10,000 rpm for 30 min (Eppendorf, model 5417R; Brinkmann Instrument Inc., Westbury, NY), which separated the aqueous and lipid phases. Outflow dialysate lactate, pyruvate, and glucose were measured using a CMA-600 clinical analyzer. Systemic arterial blood gases, hemodynamic parameters, and serum creatine kinase were measured before, every 10 min during, and after completion of each microdialysis session.

Chemical Analyses. Caffeine and 4CmC concentrations in microvials and outflow dialysates were assayed using ultraviolet spectrophotometry (model DU 640; Beckman, Fullerton, CA) at $\lambda = 273$ and 279 nm, respectively. Halothane concentrations were determined by *n*-heptane extraction (0.1 ml) and gas chromatography (model GC-14A; Shimadsu Co., Kyoto, Japan) using a Supelco-column (0.1% SP-1000, 80/100 Carbopack-C, 6' 1/8") operating at oven, detector, and injector temperatures of 140°, 300°, and 200°C, respectively, with a ^{63}Ni electron-capture detector.[16] Briefly, a halothane standard curve (1–20 μM/l) was constructed using the average peak area of three injections (10 μl) for each halothane concentration. Halothane concentrations of samples were determined in triplicate using the average peak area from 10-μl injections compared with the standard curve. In the studies with caffeine and 4CmC, lactate, pyruvate, and glucose were measured in the outflow dialysate samples using the CMA-600 clinical analyzer. The analyzer was calibrated once a week according to the manufacturer's instructions; in addition, we used two known concentrations of lactate, pyruvate, and glucose in the range of interest to test the CMA analyzer output on each experimental day. In the experiments with HLE, separate calibration curves were established for L-lactate (2.5 mM), pyruvate (0.25 mM), and glucose (5.5 mM) in 500 mM HLE (1:1, 1:2, and 1:4, vol/vol). The HLE emulsion was opaque and therefore did not allow direct measurement of the metabolites in the outflow HLE dialysate. However, after centrifugation of the HLE samples (30 min; 10,000 rpm) the aqueous layer separated from the lipid phase; lactate, pyruvate, and glucose were measured in the aqueous supernatants.

Statistics and Fitting Nonlinear Saturation Kinetics. Data are presented as mean ± SEM. Two-way analysis of variance with the Bonferroni correction was applied to test for differences between means between and within groups. $P < 0.05$ was considered to indicate statistical significance. The observed nonlinear RyR1 dose–response relations with respect to dialysate [lactate] levels or [lactate]/[pyruvate] ratios were fitted iteratively using the maximum-likelihood routines of Gauss 5.0/Gaussx 6.0 software (Aptech Systems, Kent, WA) essentially as described previously.[17] Regression coefficients were estimated assuming rectangular hyperbolic saturation kinetics in the following form:

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[Lactate] or [Lactate]/[Pyruvate] = [RyR1 agonist]/(a0 * [RYR1 agonist] + a1),

where 1/a0 = maximum effect, a1 = rate constant (equivalent of slope in double reciprocal Lineweaver-Burk plot), and $S_{0.5}$ = a1/a0 (half-maximum effective RyR1 agonist concentration). This nonlinear model overcomes one of the problems of the linearized double-reciprocal Lineweaver-Burk analysis, which is not defined for zero agonist concentrations, *i.e.*, the control level/response before application of the agonist (agonist concentration = 0). Our hyperbolic model, however, did allow the inclusion of the zero agonist controls (saline controls), which markedly improved precision of the fit and the behavior of the statistical residuals as well. Further, the maximum-likelihood method, unlike the least squares routines, is not based on the assumption of a normal distribution of observations and is therefore more generally applicable to obtain good estimates of coefficients and SEs in statistical models.[18] To enhance confidence in the fitted coefficients and their estimated SEMs, residual analyses were performed comprising the Durban-Watson statistic (testing for serial errors and model misspecification) and heteroscedasticity routines (testing for constancy of error variance).[19] In most cases of this study, the Durban-Watson statistics were near the ideal value of 2.0; however, error variance was significant, and this problem was accounted for by using the White algorithm of Gaussx 6.0 to obtain the heteroscedasticity-adjusted SEMs of the equation coefficients.

Results

In Vitro *Permeability Characteristics of the Microdialysis Catheter*

Recovery of RyR1 Agonists from Dialysis Medium in Microvials. The dose–response relations between RyR1 agonist concentrations (caffeine, 4CmC, and HLE) in the perfusion mobile phase and their concentrations in the microvials are shown in figure 1. These studies examined the transfer of the RyR1 agonists from the dialysis perfusion medium into the fixed volume in the microvials (see Materials and Methods section). In the case of caffeine, the agonist accumulated to approximately 2 mM over a period of 90 min when the dialysis perfusion medium concentration was 4 mM. This reflects approximately 50% permeability to caffeine under the test conditions. For 4CmC, 80 μM accumulated when the mobile phase contained 0.8 mM 4CmC over a period of 120 min, which amounted to only 10% permeability to 4CmC. For halothane, 2.5 mM accumulated in the microvial when 1 M HLE was in the mobile phase for 240 min, which reflected an even lower permeability of approximately 0.25% over a very extended sampling period of 4 h. Higher concentrations of HLE (≥ 2 M) could not be applied because it damaged the microdialysis membranes.

Recovery of Lactate, Pyruvate, and Glucose in Outflow Dialysates from Calibrated Solutions in the Microvials. The effect of different dialysis flow rates (0.5, 1.0, and 2.0 μl/min) on the transfer of lactate, pyruvate, and glucose from calibrated microvial solutions into the dialysate outflow are shown in table 1. Data are presented for the different mobile phases (dialysis perfusion media) used in the *in vivo* studies (normal saline, caffeine, 4CmC, and HLE solutions) when dialyzed against the calibrated solutions in the microvials. Recovery was judged by measuring the lactate and pyruvate concentrations in the outflow dialysate. At a perfusion rate of 0.5 μl/min with HLE, there was an insufficient amount of outflow dialysate (15 μl) to reproducibly centrifuge and transfer the aqueous phase without contamination from the lipid phase. The results show that the recoveries of lactate, pyruvate, or glucose were largely independent of the composition of the mobile dialysis medium (table 1).

Conversely but as expected, increasing the flow rate from 0.5 to 1.0 and 2 μl/min decreased recoveries progressively (table 1). We also observed that the recoveries of lactate and pyruvate were quantitatively similar except in the HLE experiments, where the pyruvate recovery was approximately half of that of lactate at a dialysis perfusion rate of 2 μl/min.

In Vivo *Effects of RyR1 Agonists on Outflow Dialysis L/P*

Effects of Percutaneous Catheter Insertion on Outflow Dialysate Lactate and Pyruvate. Immediately after percutaneous catheter insertion, the L/P parameters sharply increased in the MHS group but not in the MHN group (fig. 2). A period of approximately 30 min was required for the parameters to return to the baseline levels of the MHN. This is illustrated for [lactate] (fig. 2A) and [lactate]/[pyruvate] ratios (fig. 2B).

In the MHN group, dialysate [lactate] levels (1–1.5 mM) and the [lactate]/[pyruvate] ratios[15–25] remained virtually constant after implantation. However, in the MHS group, the parameters increased up to fourfold relative to MHN pigs during the initial 0–20 min after catheter insertion. At 30–40 min after implantation, the L/P parameters in the MHS samples had returned to time-matched controls, indicating catheter "equilibration" within the skeletal muscle microenvironment around the inserted catheter. The data show that artificial changes in the dialysate L/P parameters due to the insertion trauma became negligible when catheter perfusion began after a waiting period of 30 min or more after implantation. Such delay guaranteed that there were no measurable differences between MHN and MHS swine in terms of the dialysate L/P parameters under our conditions.

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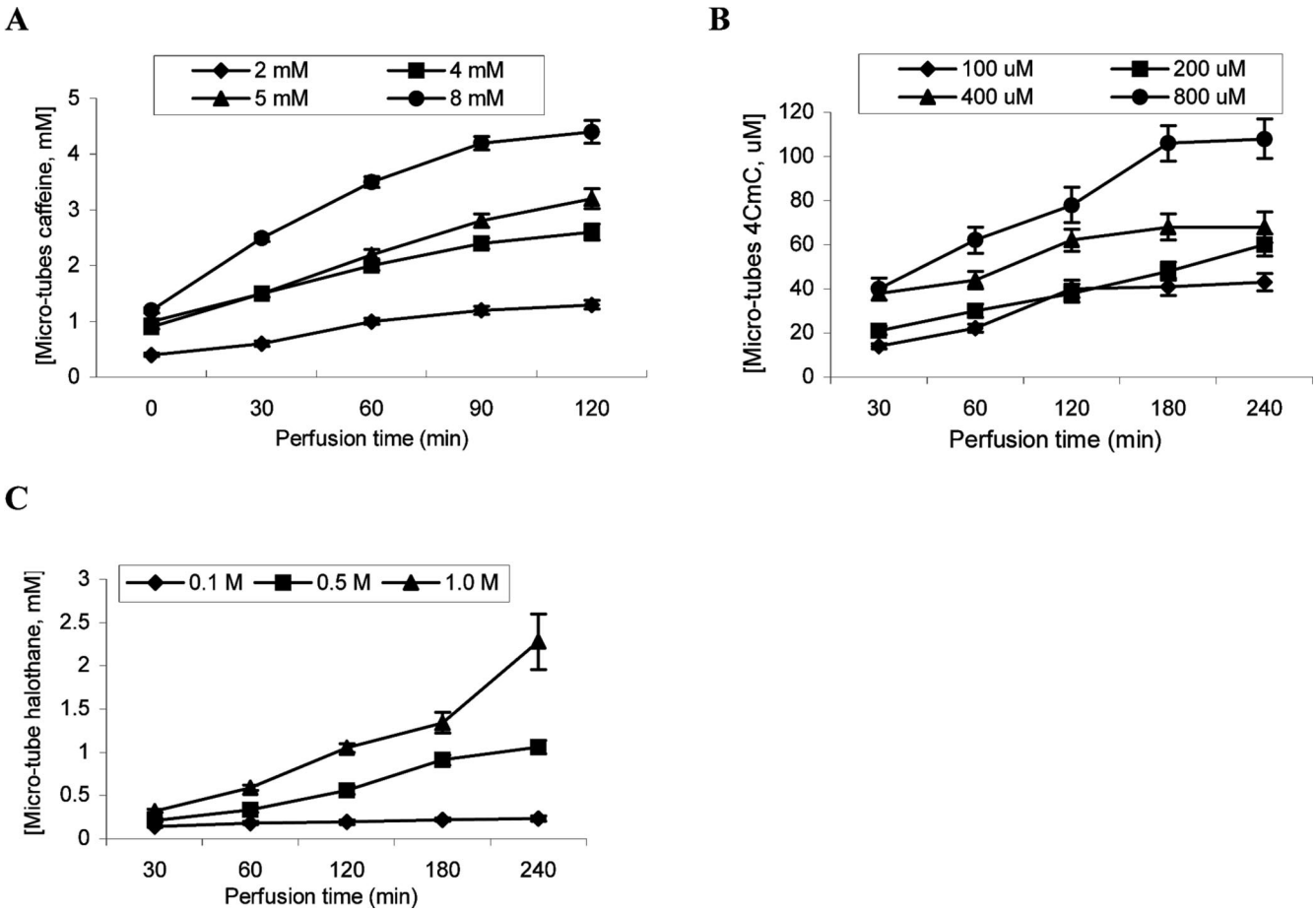

Fig. 1. ***In vitro*** **permeability of the microdialysis catheter membrane to caffeine, 4-chloro-*m*-cresol (4CmC), and halothane. Microdialysis catheters (n = 4) were placed in microtubes containing 0.1 ml normal saline and then perfused (2 μl/min) with varying caffeine or 4CmC concentrations prepared in normal saline solution (*A* and *B*, respectively). For halothane perfusion (2 μl/min), catheters (n = 4) were placed in microtubes containing 1.4 ml normal saline solution and then perfused with varying halothane concentrations prepared in lipid emulsion. Dialysate fractions were collected in microtubes containing 0.1 ml saline toped with 0.1 ml *n*-heptane to trap/extract halothane (*C*). The caffeine and 4CmC concentrations were analyzed using an ultraviolet spectrophotometer. Halothane was extracted in *n*-heptane and quantified using gas chromatography as detailed in the Materials and Methods section.**

Effects of RyR1 Microdialysis Perfusion on Outflow Dialysate Lactate and Pyruvate with Respect to the Dialysate Fractions. After implantation of catheters and the 30-min equilibration period, [lactate] levels and [lactate]/[pyruvate] ratios induced by microdialysis perfusion of RyR1 agonists showed characteristic time courses for all three RyR1 agonists (caffeine, 4CmC, HLE) tested. The parameters were increased severalfold immediately after perfusion, *i.e.*, in the first two 10-min outflow dialysate fractions, but only in the MHS pigs. This contrasted with the results from the MHN pigs where the [lactate] levels did not change and the [lactate]/[pyruvate] ratio only minimally increased immediately after the start of perfusion. After approximately 30 min of constant RyR1 perfusion, the increased values from MHS pigs typically declined toward the time-matched MHN baseline values within approximately the next 30–60 min of perfusion.

Figure 3 depicts a representative time course constructed from L/P data in response to saline with and without caffeine (16 mM) perfusion. Six 10-min dialysate fractions were sequentially collected according to protocol during a 60-min microdialysis perfusion with saline with and without 16 mM caffeine that was started at 30 min after implantation. The first two 10-min fractions collected from caffeine perfusion that covered a period of 20 min showed more than threefold increases in the [lactate] levels and an approximately twofold to threefold increase in the [lactate]/[pyruvate] ratios in the MHS pigs compared with time-matched MHN controls. In addition, in the MHN pigs, 16 mM caffeine did not significantly change the dialysate [lactate] levels or the [lactate]/[pyruvate] ratios (compare figs. 2A and B, lower data points, with figs. 3A and B, lower data points).

Effects of RyR1 Agonist Perfusion on Dialysate [Lactate] and [Lactate]/[Pyruvate] Ratio.

Caffeine. In dose–response studies, all caffeine concentrations tested (1–64 mM) induced significantly higher levels (twofold to threefold) of measured outflow

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Table 1. *In Vitro* Microdialysis Relative Recovery of Lactate, Pyruvate, and Glucose Respective to the Perfusion Solutions and to the Perfusion Flow Rates

Perfusion Flow Rate, μl/min	Lactate, %	Pyruvate, %	Glucose, %
Perfusion of normal saline			
0.5	77.7 ± 6.5	72.9 ± 2.2	63.1 ± 3.1
1.0	49.5 ± 3.1*	55.0 ± 4.9*	43.1 ± 3.2*
2.0	27.3 ± 3.1*	28.7 ± 3.3*	24.4 ± 1.6*
Perfusion of HLE			
0.5	—	—	—
1.0	45.3 ± 1.5	35.0 ± 2.3	34.5 ± 1.2
2.0	27.1 ± 1.2*	14.7 ± 1.0*	14.6 ± 1.1*
Perfusion of caffeine			
0.5	63.9 ± 3.9	69.2 ± 3.0	56.1 ± 3.0
1.0	39.1 ± 3.5*	46.7 ± 3.0*	36.5 ± 2.6*
2.0	21.3 ±1.9*	24.9 ± 2.4*	18.8 ± 1.6*
Perfusion of 4CmC			
0.5	72.9 ± 5.6	85.1 ± 7.1	54.3 ± 5.8
1.0	41.2 ± 3.7*	67.5 ± 5.5*	40.6 ± 2.2*
2.0	24.3 ± 1.7*	32.0 ± 2.3*	24.1 ± 1.2*

The microdialysis catheters (n = 8) were immersed in microtubes containing calibration standard solutions (2.5 mM lactate, 0.25 mM pyruvate, and 5.5 mM glucose prepared in normal saline) and perfused with normal saline with and without caffeine (32 mM), 4-chloro-*m*-cresol (4CmC; 8 mM), and halothane lipid emulsion (HLE; 200 mM) solutions at flow rates of 0.5, 1.0, and 2.0 μl/min. The dialysates were collected for 30 min. Data are expressed as mean ± SEM.

* Values are significantly lower than corresponding values at perfusion flow rates of 0.5 and 1.0 μl/min.

dialysate [lactate] in MHS compared with MHN control pigs (table 2). The [lactate]/[pyruvate] ratios were also significantly increased at caffeine concentrations of 4 mM or greater in the MHS compared with the MHN swine.

The single measurements from the first 10-min fractions of figure 3 (n = 45 in MHS, n = 52 in MHN) were modeled applying the rectangular saturation kinetics (see Materials and Methods) with the following modifications: An exponent a2 for the RyR1 agonist concentration (the dependent variable) was introduced to account for possible cooperativity. In addition, a constant or offset variable a3 was introduced to account for the physiologically preexisting baseline levels of [lactate] or the [lactate]/[pyruvate] ratio. This composite hyperbolic model proved to provide the best residual pattern: The Durban-Watson statistic was 2.2, *i.e.*, very close to the ideal value of 2.0, implying the absence of serial errors and model misspecification. This increased confidence in the SEs of the estimated coefficients a0, a1, a2, and a3. The following rectangular hyperbolic saturation model was used:

$$y = x^{a2}/(a0 * x^{a2} + a1) + a3,$$

where y is the dependent response variable ([lactate] or the [lactate]/[pyruvate] ratio), x is independent variable (caffeine concentration in the inflow dialysate), a0 and a1 are saturation kinetic parameters, a2 is an exponent to account for possible cooperativity, and a3 is the independent constant to account for RyR1-independent preexisting baseline levels of y (offset variable).

Using this model, the estimated baseline [lactate] level was 1.44 ± 0.47 mM at a [lactate]/[pyruvate] ratio of 27.6 ± 4.3 in the MHS pigs; the corresponding estimates in the MHN pigs were significantly lower, 0.94 ± 0.07 mM ($P \approx 0.05$) and 16.4 ± 4.2 ($P < 0.05$), respectively. Similarly, the estimated maximum [lactate]/[pyruvate] ratio was 2.5-fold higher in the MHS pigs than in MHN pigs, 71.9 ± 14.6 *versus* 28.3 ± 2.6 ($P < 0.01$). These results indicated that both the baseline and the caffeine-induced L/P redox states were more reduced in MHS muscle than in MHN muscle. However, because of data overlap between the MHS and MHN groups, the half-maximal effective caffeine concentration (EC_{50}) for increasing the dialysate [lactate]/[pyruvate] ratios could not be estimated with confidence (EC_{50} = 11.1 ± 11.4

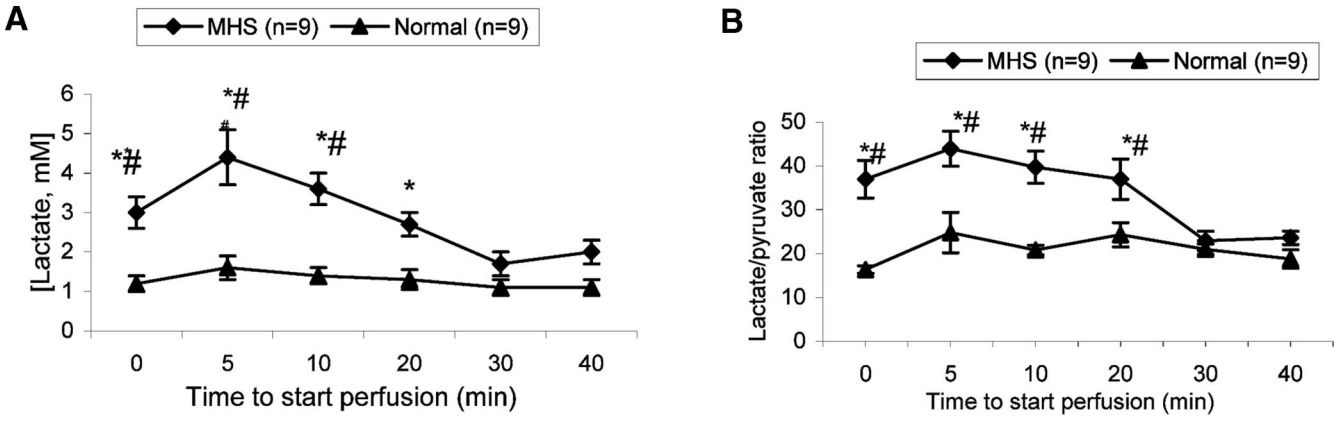

Fig. 2. This protocol was performed to assess effects of catheter implantation (cell injury) alone with respect to time on local intramuscular lactate and lactate/pyruvate ratios, assuming normal saline effect is negligible. Briefly, six microdialysis catheters were implanted percutaneously into adductor muscles of the right and left thighs (three catheters in each thigh) of malignant hyperthermia–susceptible (MHS) or normal swine. Perfusion of the catheters with normal saline (without ryanodine receptor agonist) were started immediately 0, 5, 10, 20, 30, and 40 min after implantation of each catheter in the muscle. Dialysate fraction was collected for 10 min. *A* and *B* show lactate and lactate/pyruvate molar ratios, respectively. These data suggest that at least 30 min *in situ* catheter equilibration is essential before the start of ryanodine receptor agonist perfusion. * Significantly higher than corresponding values in normal swine. # Significantly higher than 30- to 40-min data point in MHS group.

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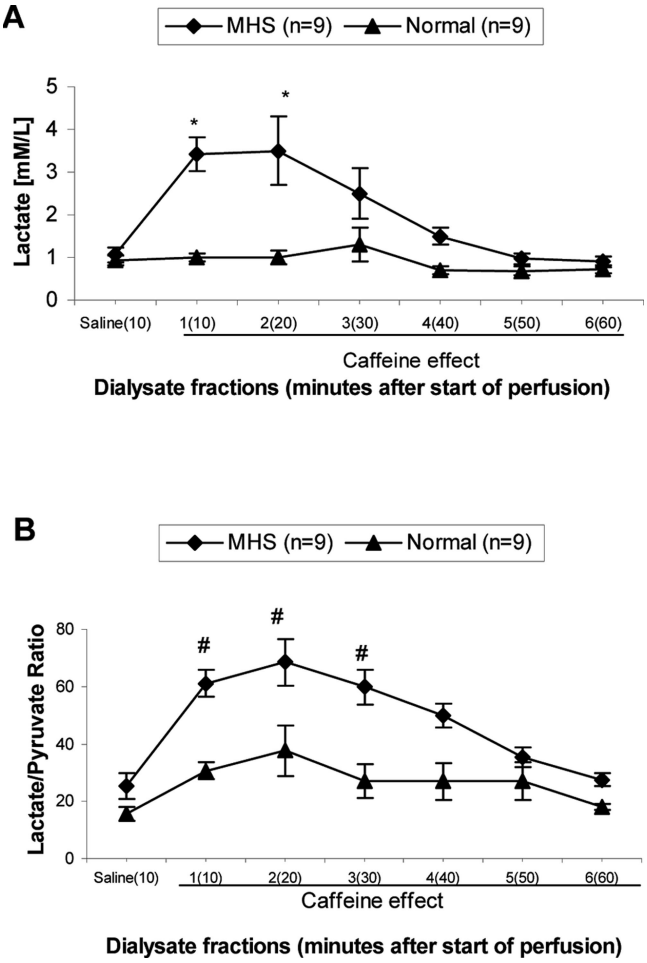

Fig. 3. Representative lactate and lactate/pyruvate ratio levels in dialysate fractions in response to saline and 16 mM caffeine. Microdialysis catheters were implanted percutaneously into the adductor muscle of either the right or the left inner thigh of malignant hyperthermia–susceptible (MHS) and normal swine. After a 30-min initial *in situ* equilibration period, the catheters were perfused with saline with and without caffeine (16 mM) solution for 60 min. Six fractions in 10-min intervals were collected. Numbers in parentheses refer to elapsed time. *A* and *B* show lactate levels and lactate/pyruvate molar ratios in dialysate fractions 1–6 respectively, indicating that collection of two dialysate fractions would be sufficient in a human trial. * Significantly higher than dialysate fraction 4–6 values in MHS group. # Significantly higher than dialysate fraction 5–6 values in MHS group.

mM [MHS] *vs.* 0.26 ± 1.1 mM [MHN]; not significant), because the SE equaled the estimated mean value.

4CmC. Complete dose–response curves for 4CmC-induced dialysate [lactate] concentrations and [lactate]/[pyruvate] ratios are presented in figure 4. Data depicted in semilogarithmic plots and solid lines represent the fitted curves using the modified rectangular hyperbolic model (see Statistics and Fitting Nonlinear Saturation Kinetics section).

In the MHS group, the estimated exponents a2 were similar: 2.09 ± 0.93 for [lactate] and 1.65 ± 0.89 for the [lactate]/[pyruvate] ratio ($P > 0.05$). When fitting the curves for the MHN group, the exponents were held at these previously estimated MHS values, which allowed better direct comparisons between the fitted MHS and MHN curves as shown in figures 4A and B.

The model estimations for baseline L/P values were again higher in the MHS than the MHN group: [lactate] = 1.23 ± 0.19 mM (MHS, n = 46) *versus* 0.87 ± 0.09 mM (MHN, n = 53) ($P < 0.05$); [lactate]/[pyruvate] ratio = 22.4 ± 2.2 (MHS) *versus* 17.9 ± 2.5 (MHN) ($P \approx 0.05$). Similarly, also the maximum [lactate] estimates were approximately 3.4-fold higher in the MHS than the MHN group: 3.89 ± 0.51 *versus* 1.14 ± 0.2 mM ($P < 0.01$). The estimated maximum [lactate]/[pyruvate] ratios in the MHS group were 2.8-fold above those of the MHN controls: 70.1 ± 9.2 *versus* 24.7 ± 2.4 ($P < 0.01$). The EC_{50}s were 0.65–0.70 ± 0.24 mM in the MHS group. The data showed that 4CmC significantly increased dialysate [lactate] and [lactate]/[pyruvate] ratios already below the EC_{50}, at 0.4 mM in MHS (fig. 4). The correlation coefficients R^2 for the two fitted curves in the MHS animals were, however, only moderate: $R^2 = 0.38$ for y = [lactate] and $R^2 = 0.48$ for y = [lactate]/[pyruvate], indicating moderate precision. In contrast but as expected, the correlation coefficients for the MHN group were insignificant (R^2 = 0.02–0.05), demonstrating that there was no relation between the dialysis 4CmC level and the outflow L/P parameters in the MHN muscle (fig. 4).

Hle. Data for HLE-induced changes in dialysate [lactate] and [lactate]/[pyruvate] ratios are compiled in table 3. Perfusion with the lipid emulsion in the absence of halothane (lipid emulsion, control) or with 10 mM halothane did not significantly increase dialysate L/P parameters in MHS or MHN swine. However, at HLE concentrations of 20 mM or greater, we consistently found higher levels of [lactate] (twofold to fourfold) in MHS than in MHN swine ($P < 0.02$). However, because of marked data overlap in the responses to HLE microdialysis infusion between MHS and MHN groups, model estimations of the maximum changes were highly uncertain. Despite these problems with the kinetic estimates, the estimated baseline values of [lactate] (1.77 ± 0.35 *vs.* 0.95 ± 0.06; $P < 0.01$) or of the [lactate]/[pyruvate] ratios (26.4 ± 3.9 *vs.* 18.5 ± 2.8; $P < 0.01$) were again acceptable and confirmed a small but significant increase in the baseline L/P values in the MHS group relative to the MHN animals.

Systemic Creatine Kinase, Blood Gases, and Electrolytes. Data from RyR1-induced changes in physiologic and blood gases are compiled in table 4. Baseline arterial serum creatine kinase levels of MHS swine before microdialysis were approximately twofold higher than those in MHN swine ($P < 0.003$). As expected, the creatine kinase levels did not significantly change during or 1 h after RyR1 agonist microdialysis perfusions. Therefore, the local infusion of RyR1 agonists using microdialysis did not cause systemic muscle damage typical of an acute clinical (*i.e.*, system-wide) MH episode. In addition, body temperature, arterial blood pressures, heart rate, and respiratory rate before, during, and after saline

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Table 2. Effect of Caffeine on Dialysate Lactate, Pyruvate, and Lactate/Pyruvate Ratios in MHS and Normal Swine

	Lactate Level, μM		Pyruvate Level, μM		Lactate/Pyruvate Ratio	
Perfusate Solution	MHS Swine	Normal Swine	MHS Swine	Normal Swine	MHS Swine	Normal Swine
Saline	1,061 ± 180 (350–1,960)	933 ± 107 (300–1,280)	42 ± 6.3 (14–63)	60.2 ± 9.2 (33–91)	25.3 ± 4.5 (14–54)	15.6 ± 2.4 (9–27)
1 mM	2,012 ± 664* (480−4,680)	861 ± 161 (450–1,470)	54.8 ± 10.9 (22–101)	42.2 ± 12.2 (16–96)	36.6 ± 5.1 (19–53)	20.4 ± 7.1 (14–68)
4 mM	2,183 ± 244* (1,270–2,880)	1,011 ± 200 (660–2,050)	56.9 ± 3.1 (44–71)	38 ± 3.8 (31–59)	38.5 ± 4.1* (24–52)	26.6 ± 2.6 (20–35)
16 mM	3753 ± 415* (2,640–6,310)	1,148 ± 184 (570–2,100)	62.4 ± 4.8 (41–83)	43.7 ± 7.3 (18–73)	60.3 ± 5.6* (37–93)	26.5 ± 4.0 (15–49)
32 mM	3,780 ± 736* (1,490–5,710)	1,033 ± 205 (490–2,130)	63.7 ± 10.5 (32–117)	38.3 ± 7.8 (11–68)	59.6 ± 7.2* (33–90)	27.1 ± 3.5 (17–43)
64 mM	5,250 ± 1,329* (2,730–10,440)	1,260 ± 393 (540–3,930)	75.5 ± 9.6 (45–101)	39.9 ± 5.7 (12–74)	69.5 ± 11.3* (38–103)	31.7 ± 6.5 (17–64)

Microdialysis catheters were implanted percutaneously into adductor muscles of the right and left inner thighs of malignant hyperthermia–susceptible (MHS; n = 9) and normal (n = 9) swine and then perfused with normal saline with or without different caffeine concentrations. Data are expressed as mean ± SEM (range). Values refer to the first dialysate fraction collected over 10-min periods.

* Values are significantly higher than corresponding values in normal swine.

or RyR1 agonist perfusion did not significantly change during RyR1 microdialysis perfusion. Also, arterial blood pH, $Paco_2$, Pao_2, Ca^{2+}, and K^+ remained within physiologic MHN values during and after saline or RyR1 agonist microdialysis infusion.

Cbct Results. Halothane (3%)–induced contractures in muscle strips from MHS and MHN swine were 22.5 ± 3.9 mN (range, 10.8–36.3 mN) and 1.0 ± 1.0 mN (range, 0–2 mN), respectively. Caffeine (2 mM)–induced contractures in muscle strips from MHS and MHN swine were 10.8 ± 2 mN (range, 4.9–17.4 mN) and 0 mN (range, 0–1 mN), respectively.

Discussion

The major findings in this study may be summarized as follows: (1) A simple *in vivo* percutaneous skeletal mus-

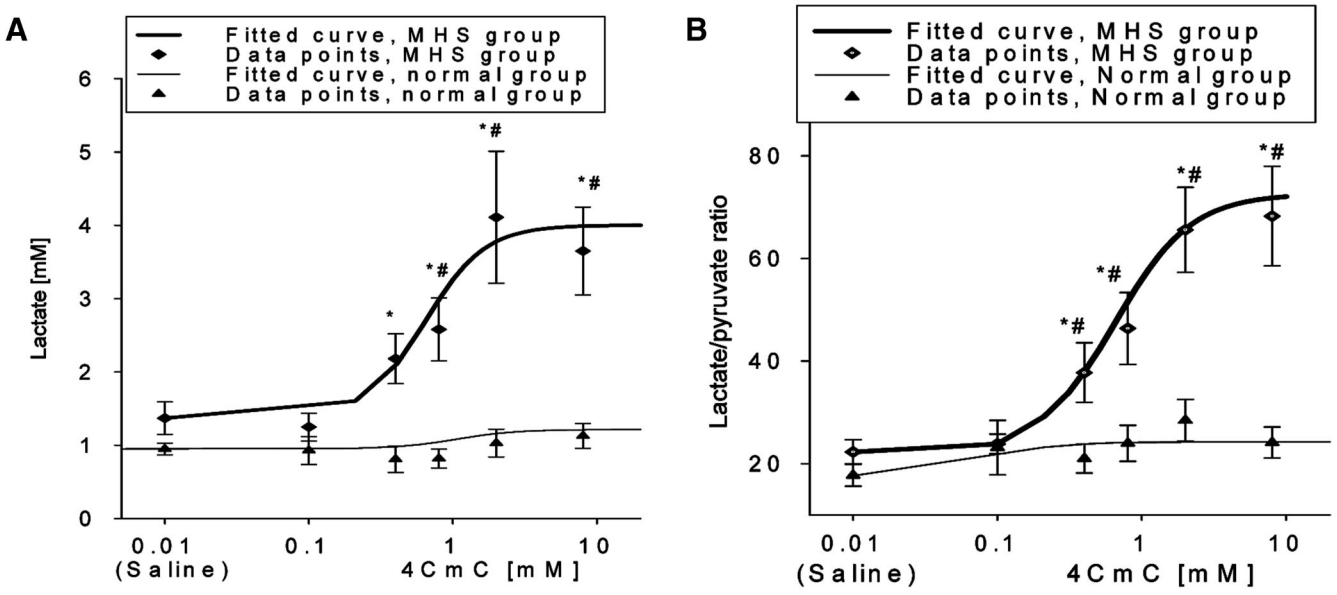

Fig. 4. Effect of 4-chloro-*m*-cresol (4CmC) on dialysate lactate and lactate/pyruvate ratios in malignant hyperthermia–susceptible (MHS) and normal swine. Microdialysis catheters were implanted percutaneously into adductor muscles of the right and left inner thigh of MHS (n = 9) and normal (n = 9) swine. After an initial *in situ* equilibration period, microdialysis catheters were perfused with normal saline with or without different 4CmC concentrations. Data are expressed as mean ± SEM. Values refer to the first dialysate fraction according to the Materials and Methods section. Measured lactate and lactate/pyruvate ratios in the absence of 4CmC (saline controls) are plotted against X = 0.01 mM to allow logarithmic display of the entire dose–response curves. Formulas used to illustrate the fitted curve in *A* for the MHS and normal groups were $Y = X^{2.09}/(0.38 * X^{2.09} + 0.15) + 1.26$ and $Y = X^{2.091}/(3.78 * X^{2.091} + 4.205) + 0.871$, respectively, and formulas used in *B* for the MHS and normal groups were $Y = X^{1.65}/(0.02 * X^{1.65} + 0.01) + 22.43$ and $Y = X^{1.65}/(0.146 * X^{1.65} + 0.002) + 17.86$, respectively. * Values are significantly higher than corresponding values in normal swine. # Significantly higher than saline data point in MHS group.

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Table 3. Effect of HLE on Dialysate Lactate, Pyruvate, and Lactate/Pyruvate Molar Ratios in MHS and Normal Swine

	Lactate Levels, μM		Pyruvate Levels, μM		Lactate/Pyruvate Ratios	
Perfusate Solution	MHS Swine	Normal Swine	MHS Swine	Normal Swine	MHS Swine	Normal Swine
Lipid	1,320 ± 190 (870–3,340)	1,050 ± 100 (790–1,280)	78 ± 8 (47–117)	71 ± 7.3 (24–97)	17 ± 3.8 (12–67)	15 ± 3.4 (10–33)
10 mM HLE	1,680 ± 585 (190–4,100)	871 ± 80 (490–1,230)	74 ± 22 (12–186)	55 ± 11 (24–133)	23 ± 4.5 (8–45)	16 ± 3.6 (7–40)
20 mM HLE	2,330 ± 320* (1,650–3,560)	1,006 ± 68 (650–1,310)	76 ± 9 (60–152)	53 ± 4.2 (34–77)	31 ± 3.4* (15–42)	19 ± 2.8 (8–30)
100 mM HLE	3,350 ± 570* (1,740–7,180)	1,197 ± 95 (440–1,410)	89 ± 15 (45–217)	55 ± 7.6 (17–79)	38 ± 5.3* (21–63)	22 ± 4.4 (14–48)
200 mM HLE	3,440 ± 640* (1,060–7,620)	1,210 ± 150 (670–2,000)	86 ± 13 (36–268)	53 ± 11 (22–96)	40 ± 7.7* (17–80)	23 ± 5 (15–68)
500 mM HLE	6,130 ± 820*	1,490 ± 257	87 ± 16	52 ± 3.8	70 ± 11*	29 ± 4.9

Microdialysis catheters were implanted percutaneously into adductor muscles of the right and left inner thighs of malignant hyperthermia–susceptible (MHS; n = 7) and normal (n = 7) swine and then perfused with lipid emulsion with or without halothane. Data are expressed as mean ± SEM (range). Values refer to the first dialysate fraction collected over 20-min periods.

* Values are significantly higher than corresponding values in normal swine.

HLE = halothane lipid emulsion.

cle microdialysis technique (iPMT) for physiologic monocarboxylates (lactate, pyruvate) is described; it allows reliable detection of changes of the local L/P system dialyzed from the interstitium of skeletal muscle in MHN and MHS pigs. (2) Percutaneous microdialysis catheter implantation trauma caused a rapid (within 5 min), up to threefold increase in dialysate L/P parameters in MHS pigs only without affecting systemic cardiovascular metabolic, electrolyte, or acid–base parameters. In MHN pigs, the dialysis L/P parameters were virtually stable, suggesting only minimal artificial change due to probe implantation trauma. (3) The postimplantation local injury in MHS muscle was transitory as the dialysate L/P parameters returned to baseline within 30 min after implantation. (4) Baseline muscle dialysate [lactate] and [lactate]/[pyruvate] ratios were increased in MHS compared with MHN pigs. (5) The RyR1 agonists caffeine, 4CmC, or halothane, when applied locally *via* microdialysis, caused severalfold accumulations of dialysate [lactate] or [lactate]/[pyruvate] ratios in MHS pigs with only minimal effects in the MHN pigs. (6) The data suggest that the skeletal muscle L/P system is more reduced in MHS than in MHN pigs, especially during challenges by RyR1 agonists such as caffeine or 4CmC. (7) The iPMT for muscle L/P parameters is an efficient and minimally invasive test for differentiating between porcine MHS and MHN skeletal muscle *in vivo*. Therefore, the test could have potential clinical utility for patients considered to be at risk for MH.

Table 4. Systemic, Blood Gas, and Electrolyte Levels before, during, and after Perfusion of RyR1 Agonists

	MHS Swine			Normal Swine		
Parameters	Before Perfusion of RyR1	During Perfusion of RyR1	After Perfusion of RyR1	Before Perfusion of RyR1	During Perfusion of RyR1	After Perfusion of RyR1
CK, U/l	3,390 ± 490 (1,759–5,272)	3,907 ± 492 (1,858–5,478)	4,050 ± 510 (1,958–5,649)	1,660 ± 100 (1,175–2,000)	1,740 ± 91 (1,188–1,952)	1,990 ± 210 (1,145–3,178)
K^+, mEq/l	3.8 ± 0.3 (2.9–4.2)	3.6 ± 0.2 (2.9–4.1)	3.5 ± 0.2 (2.9–4.1)	4.2 ± 0.1 (3.8–4.6)	4.3 ± 0.1 (3.9–4.8)	4.0 ± 0.1 (3.8–4.2)
pH	7.51 ± 0.04 (7.43–7.61)	7.50 ± 0.04 (7.44–7.63)	7.52 ± .04 (7.43–7.62)	7.45 ± 0.05 (7.39–7.59)	7.45 ± 0.04 (7.40–7.58)	7.42 ± 0.04 (7.39–7.56)
MAP, mmHg	90.8 ± 7.1 (61–130)	97.1 ± 5.9 (73–134)	100.8 ± 5.2 (75–127)	101.9 ± 8.2 (72–150)	98.5 ± 7.3 (70–130)	101.8 ± 6.9 (73–129)
HR, beats/min	117.2 ± 3.1 (93–136)	111.5 ± 5.6 (85–167)	112.8 ± 5.1 (84–157)	127 ± 8.2 (79–140)	114.9 ± 8.5 (74–166)	112.2 ± 7.7 (65–140)
Ca^{2+}, mM/l	1.42 ± 0.02 (1.36–1.43)	1.40 ± 0.01 (1.38–1.42)	1.37 ± 0.02 (1.32–1.41)	1.40 ± 0.02 (1.38–1.45)	1.38 ± 0.02 (1.36–1.42)	1.35 ± 0.02 (1.34–1.42)
Body temp, °C	38.1 ± 0.2 (36.6–38.6)	38.0 ± 0.2 (36.2–38.9)	37.8 ± 0.2 (36–38.7)	37.9 ± 0.19 (36.3–39.2)	38.3 ± 0.15 (36.3–39.2)	38.1 ± 0.14 (36.3–39.3)

Data (n = 25) in each group of malignant hyperthermia–susceptible (MHS) and normal swine are presented as mean ± SEM (range). Data are pooled from all experimental stages: before, during, and after perfusion of the ryanodine receptor (RyR1) agonists. Serum creatine kinase (CK) and K^+, blood pH, mean arterial blood pressure (MAP), heart rate (HR), blood Ca^{2+}, and body temperature did not significantly change during or 1 h after RyR1 agonist perfusions.

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Muscle Dialysate L/P as Indicator of Mh Status

The rationale for examining the skeletal muscle dialysate L/P system as a potential index of the MH status is indirect. Cellular energy state (indexed by the cytosolic phosphorylation potential, [adenosine triphosphate (ATP)]/([adenosine diphosphate (ADP)] · [P_i])[20,21] and the free cytosolic nicotinamide-adenine dinucleotide reduced form (NADH)/nicotinamide-adenine dinucleotide oxidized form (NAD^+) redox system have been found to be reciprocally related in the myocardium under near-equilibrium conditions.[20–23] Reciprocity requires that the net metabolic fluxes through glycolysis and lactate dehydrogenase (LDH) are small relative to their respective maximum capacities, including that of the abundant glyceraldehyde dehydrogenase (GAPDH)/phosphoglycerate kinase (PGK)/LDH system.[22–25] In early ^{31}P-nuclear magnetic resonance–based MH studies, local muscle MH episodes typically caused a decrease in the [PCr]/[P_i] ratio, indicating cellular deenergization, most likely due to the uncontrolled release of sarcoplasmic reticulum calcium *via* a defective or mutated RyR1 channel.[4,5] Such cytoplasmic Ca^{2+} overload triggers not only muscle contracture, but also, due to maximum activation of calcium pumps (SERCA1, sarcolemmal Ca^{2+} ATPase) and possible direct Ca^{2+} toxicity to mitochondrial energetics, substantial cellular deenergization. Cellular deenergization expresses itself in a decrease in [ATP]/([ADP] · [P_i]), the cytosolic phosphorylation potential.[20,22,26] The ability of the calcium pumps to maintain normal subcellular calcium gradients is related to the cytosolic phosphorylation potential, not to ATP content alone, which means that cellular calcium homeostasis is a function of [ATP]/([ADP] · [P_i]).[12,26–28] The level of [ATP]/([ADP] · [P_i]) determines the available free energy of ATP hydrolysis, which is known to depend on intracellular pH, free Mg^{2+}, and temperature.[29,30]

The free cytosolic [NADH] · [H^+]/[NAD^+] ratio is directly correlated with the intracellular [lactate]/[pyruvate] ratio *via* the abundant LDH.[31] Because cytosolic lactate and pyruvate are readily transported across the sarcolemma *via* proton-compensated monocarboxylate transporters,[32–34] the interstitial L/P system is likely directly reflecting the cytosolic L/P system, especially when the system is in a steady state and net transport across the sarcolemmal membrane (lactate or pyruvate release or uptake) is small compared with total capacity of the transporter.[35]

In the current iPMT study, the pyruvate and lactate net fluxes across the sarcolemmal membrane were not measured but were likely quite small, because global muscle metabolism and blood flow and hence interstitial washout were only marginally, if at all, enhanced after implantation and during RyR1 agonist dialysis perfusion as well. We consistently measured markedly increased dialysis [lactate]/[pyruvate] ratios in MHS muscle after implantation (fig. 2) and further increases during RyR1 perfusion (fig. 4 and tables 2 and 3). Assuming a close association between the interstitial [lactate]/[pyruvate] ratio and the intracellular [lactate]/[pyruvate] ratio, we reasoned that the steady state increases in the dialysate [lactate]/[pyruvate] ratio after implantation and those in responses to RyR1 agonists as well reflected significant increases in the free cytosolic [NADH] · [H^+]/[NAD^+] ratio. Such increased cytosolic NADH reduction could well indicate a decrease in [ATP]/([ADP] · [P_i]) mediated by the GAPDH/PGK/LDH system.

We assumed that the microdialysis-applied RyR1 agonists triggered an MH episode locally resulting in cellular calcium overload near the dialysis catheter. As a consequence of the calcium overload, the muscle cells likely decreased their [PCr]/[P_i] ratio, indicating a decrease in [ATP]/([ADP] · [P_i]). Because of the reported reciprocal relation between cytosolic energy state and redox state, it was conceivable that such deenergization was associated with an increase in the free cytoplasmic [NADH]/[NAD^+] ratio, which would increase the cytosolic [lactate]/[pyruvate] ratio. Subsequently, cellular lactate and pyruvate were equilibrating with interstitial lactate and pyruvate, which was recorded in the muscle dialysate as a steady state increase in the outflow [lactate]/[pyruvate] ratio (tables 2 and 3 and fig. 4).

As for the clinical implications of the current findings, it is tempting to suggest that the current iPMT provides a minimally invasive and relatively rapid test of the local cellular muscle energetics in response to selective and local application of RyR1 agonists, especially 4CmC (fig. 4). Obviously, this technique may have diagnostic and prognostic value. To validate these ideas, the predictor power of the new iPMT must be compared with the classic *in vitro* contracture test or CHCT tests as well as modern genetic RyR1 mutation analyses. The proposed minimally invasive iPMT could be an attractive alternative to the existing invasive CHCT/*in vitro* contracture test or the extremely expensive ^{31}P-nuclear magnetic resonance technologies. The main advantage of the current iPMT is its straightforward, relatively economical, and reproducible technology, at least in the homozygous MHS porcine model.

The ipmt Compared with the Classic Chct or Ivct

In vitro caffeine–halothane contracture tests that are the current standards for MH diagnostics have specific disadvantages that prevent their broad clinical application. The tests are invasive, expensive, not readily available, and time-consuming, with sensitivity of 97% (accurately detects those with MH) and specificity of 78% (the ability of the test to rule out disease when disease is absent [false positive]).[8] In cases requiring emergency surgery, these tests cannot be performed quickly enough to be of use for the MH-suspect patient. In the North American protocol (CHCT), the test has not been approved for children. In addition, patients with myopa-

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thies whose basic defect involves abnormally high levels of cellular calcium, such as muscular dystrophy, may display abnormal contracture to halothane and caffeine. Because of the invasive nature of the test, only approximately 10% of MH-suspect patients undergo testing. However, the proposed microdialysis technique is much less invasive, and the protocol is simple enough to be performed in outpatient offices, because it does not require significant surgery, only percutaneous implantation of dialysis catheters. It is also less expensive than the CHCT and may well be approved for use in children.

Nevertheless, the proposed iPMT has specific weaknesses. The percutaneous implantation trauma causes a rapid increase of outflow [lactate] and [lactate]/[pyruvate] ratio in the dialysates collected for the first 30 min after implantation. In our swine experiments, a period of 20–30 min after implantation was required before the L/P parameters returned to baseline in the MHS pigs. It is convenient that the dialysate fractions from caffeine or 4CmC perfusions can be used in the CMA analyzer without additional sample preparations. However, fractions collected during HLE perfusion required centrifugation and sample cleanup before analysis. This makes the HLE application time-consuming and complex. An alternative technique that could be used to overcome the opaque lipid contamination in the dialysate fractions during HLE perfusion would be to implant two microdialysis catheters immediately adjacent to each other, with one catheter used for perfusion with HLE and the other for sample collection for L/P analyses. However, implantation of two side-by-side catheters will increase the implantation trauma and thus likely decrease the reproducibility of the data, degrading its value for clinical application. Based on our findings, HLE would probably not be the drug of choice for microdialysis application in clinical MH diagnostics. We obtained the most reproducible and consistent data using 4CmC, likely because this is a more selective RyR1 agonist compared with HLE and caffeine. The iPMT also must be evaluated in patients with non-MH myopathies whose basic defect involves increased intracellular calcium, such as muscular dystrophy. In addition, the iPMT requires more definitive studies to define its sensitivity, specificity, and limitations in humans.

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