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Original Articles

Intraparenchymal Microdialysis after Acute Spinal Cord Injury Reveals Differential Metabolic Responses to Contusive versus Compressive Mechanisms of Injury

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Abstract

In animal models, spinal cord injury (SCI) is typically imparted by contusion alone (e.g., weight drop) or by compression alone (e.g., clip compression). In humans, however, the cord is typically injured by a combination of violent contusion followed by varying degrees of ongoing mechanical compression. Understanding how the combination of contusion and compression influences the early pathophysiology of SCI is important for the pre-clinical development of neuroprotective therapies that are applicable to the human condition. Disturbances in the metabolism of energy-related substrates such as lactate, pyruvate, and glucose are important aspects of secondary damage. In this study, we used a porcine model of traumatic SCI to determine the extent to which these metabolites were influenced by contusion followed by sustained compression, using the microdialysis technique. Following contusion injury, lactate and pyruvate levels near the epicenter both increased, while glucose remained quite stable. When the contusion injury was followed by sustained compression, we observed a transient rise in lactate, while pyruvate and glucose levels dropped rapidly, which may reflect decreased regional spinal cord blood flow. Furthermore, contusion with sustained compression produced a prolonged and dramatic increase in the lactate-pyruvate (L/P) ratio as a marker of tissue hypoxia, whereas after contusion injury alone, a transient and less significant elevation of the L/P ratio was observed. In this study, we demonstrate that disturbances in energy metabolism within the injured spinal cord vary greatly depending upon the biomechanical nature of the injury. Such differences are likely to be relevant to the applicability of novel therapies targeting specific aspects of the early secondary injury cascade after acute human SCI.

Key words: compression; glucose; lactate; pyruvate; SCI

Introduction

Spinal cord injury (SCI) has a devastating impact on an individual's quality of life, and at a societal level is associated with a heavy economic burden; recently estimated at $3.6 billion per year in Canada.[1] Considerable scientific efforts over the past three decades have garnered important insights into the neurobiological challenges in SCI, from which a growing number of novel neuroprotective and neuroregenerative strategies have emerged.[2–4] Unfortunately, none of the treatments to date that have been translated forward into clinical trials of acute human SCI have been found to be convincingly efficacious.[5,6]

The failure to recapitulate in human SCI the effectiveness of novel therapies that show promise in animal models of SCI has many potential explanations. The most widely used model organisms in mammalian SCI research are rodents, although increasingly, investigators are utilizing large animals such as cats, dogs, pigs, and nonhuman primates as translational models.[7–10] Aside from inevitable biological differences between the animal and human SCI condition, from a methodological perspective, the biomechanical precision with which injuries to the spinal cord are induced experimentally in animal models stands in stark contrast to the considerable variability with which such injuries occur in human patients. Traumatic SCIs are clinically heterogeneous in nature and can produce a mixture of contusion, compression, maceration, and laceration. Although it may not be feasible to establish animal models that perfectly reproduce the myriad of different mechanisms by which traumatic SCI occurs in human individuals, it is at the very least possible to consider the two that are most commonly employed in animal experimental models of SCI: contusion and compression.

Starting with the weight-drop SCI model described by Allen, many devices have been developed to deliver consistent contusive

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or compressive injuries to the spinal cord.[11–13] To produce a controlled, reproducible contusion injury to the spinal cord in rodents and mice, a number of commercially available ''impactor'' devices have been developed. These include the NYU/MASCIS impactor, the Infinite Horizon impactor, and the Ohio State University impactor, all of which employ the relatively rapid delivery of a blunt force to the spinal cord.[14–20] Compressive SCI in rodent models is usually induced by the extradural application of a modified aneurysmal clip around the cord, which allows precise monitoring of the force and duration of compression.[21–23] Although there is some debate about which is the more ''clinically relevant'' mechanism of injury for these experimental models, it is recognized that most human traumatic injuries are not caused by either contusion or compression alone, but rather, by varying degrees of these two mechanisms.[24] It could be validly argued that regardless of injury mechanism (contusion or compression), the subsequent behavioral deficits and the final histological results are similar, and the important experimental objective of consistency/reproducibility is comparably achieved with either injury model. However, the early pathophysiologic responses within the injured spinal cord to these different mechanisms of injury are likely to be different.[25–29] Such early differences have translational relevance, because experimental therapies for acute SCI are routinely tested during this early post-injury period, and, therefore, their efficacy is likely to be influenced by these acute pathophysiologic processes.

Although each injury model mimics certain aspects of how human injuries occur, there is limited understanding of what occurs in the clinically relevant scenario in which the sudden contusive injury from fractured bone fragments or spinal displacement is followed by a period of prolonged compression. Using a contusion model of SCI followed by a placement of an epidural spacer to simulate persistent compression, Kubota and colleagues showed that compression is an important factor influencing spinal cord blood flow and the subsequent recovery of the blood flow.[30] This finding highlights how differences in the nature of the mechanical insult to the cord influence specific early pathophysiologic responses within the cord. Reduced cord perfusion can at some point lead to hypoxia, and the downstream metabolic consequences of this are an important component of the secondary injury cascade that will strongly influence the tissue's survival and the eventual neurological outcome.[31–33] The jeopardized energy state of the hypoperfused tissue around the injury site will be manifested by altered extracellular concentrations of energy-related metabolites such as glucose, lactate, and pyruvate, as glycolytic pathways are activated to meet the energy demands.[31] Hence, by characterizing how glucose, lactate, and pyruvate levels are altered within the spinal cord after contusion and compression injuries, we may better understand how these injury mechanisms influence energy metabolism and secondary injury. Disturbances in energy metabolism have been demonstrated previously using microdialysis techniques in rodent models of spinal cord compression.[34,35] However, the combination of contusion with sustained compression has not previously been evaluated or directly compared with contusion alone.

In the present study, we utilized a novel porcine model of SCI to compare the early metabolic responses within the injured spinal cord after contusion or contusion plus sustained compression.[7] The model utilizes a 25–35 kg mini-pig, which possesses a spinal cord much more similar to that of humans in terms of size, structure, vascular supply, and subarachnoid space than a 250–300 g rodent. Additionally, the metabolic rates of pigs are much closer to those in humans than are those in rodents.[36] Serial analysis of lactate, pyruvate, and glucose from extracellular fluid from the spinal cord adjacent to the injury site was achieved with microdialysis, one of the few well-established techniques available for the *in vivo* assessment of the interstitial chemistry of neural tissue in acute trauma.[37,38] We measured dialysate levels of lactate, pyruvate and glucose and calculated the lactate-pyruvate (L/P) ratio (an indicator of ischemia) in order to temporally and spatially characterize the dysfunction in the energy-related metabolism in the injured spinal cord after either contusion or contusion plus sustained compression.

Methods

All animal protocols and procedures employed in this study were approved by the Animal Care Committee of the University of British Columbia and were compliant with the policies of the Canadian Council on Animal Care.

Porcine model of traumatic SCI

This study utilized a novel large animal model of SCI that we have recently developed.[7] Briefly, 25–35 kg female Yorkshire pigs were anesthetized with an intramuscular injection of ketamine (20 mg/kg) and maintained on isoflurane anesthesia (2–3%). Animals were intubated for pressure ventilation (10–12 breaths/min; tidal volume at 10–12 mL/kg) (Draeger Medical, Inc., Telford, PA). Their temperature was monitored with a rectal probe and maintained at 37.0°C with a heating pad. During the entire surgery, heart rate, respiratory rate, blood pressure, and oxygen saturation was monitored (pulse oximeter 8600V, Nonin Medical Inc., Markham, ON, Canada and Cardell® MAX-12HD Veterinary Monitor, Paragon Medical Supply Inc., Coral Springs, FL). Hydration was maintained with intravenous (IV) lactated Ringer's solution.

Prior to the skin incision, animals were given a single IV injection of hydromorphone (0.15 mg/kg) and the dorsal subcutaneous tissue around the incision site was infiltrated with bupivacaine (1–2 mg/kg). They also received a single IV injection of cefazolin (15 mg/kg). A dorsal midline incision was made from T4 to L3. Using electrocautery, the spine was exposed and a laminectomy was performed from T7 to T14. Three pedicle screws (3.5 mm Vertex screws, Medtronic, Memphis, TN) were inserted on the left at T9, T10, and T11, and a 3.2 mm titanium rod was affixed to the screws to rigidly stabilize the spinal column and to serve as the base for our custom-made weight drop impactor with a projected impact at T10 (Fig. 1).[7] For this study, the impactor weighing 50 g was dropped from a height of 50 cm onto the exposed spinal cord to induce a severe contusion injury. After 5 min, the impactor was removed from the cord. For the condition of ''sustained compression,'' following the weight drop injury, we added an additional weight of 100 g onto the 50 g impactor after injury, and left this on throughout the course of the experiment (4 h). This 100 g weight was not added in the ''contusion alone'' group. The force of the contusion impact was recorded from a load cell within the impactor tip, and this was analyzed with customized Labview software (National Instruments Corporation, Austin, TX).

Spinal cord microdialysis procedure

Four microdialysis probes (CMA #11 microdialysis probe with 2 mm membrane length; cutoff 6 kDa; M dialysis Inc., N. Chelmsford, MA) were inserted through the dura and into the spinal cord using a custom-made guide cannula. The probes were inserted at a 30 degree angle to the spinal cord, ~1 mm from midline (Fig. 2). The entry points for the probes into the spinal cord were at 20 mm and 40 mm cranial and caudal to the intended impact site, and by advancing the probe 7 mm into the cord, the tip of the probe was brought to ~15 and 35 mm from the impact site. The interface between the probe and dura was secured with acrylic glue to prevent further cerebrospinal fluid (CSF) leakage. The dialysis probes

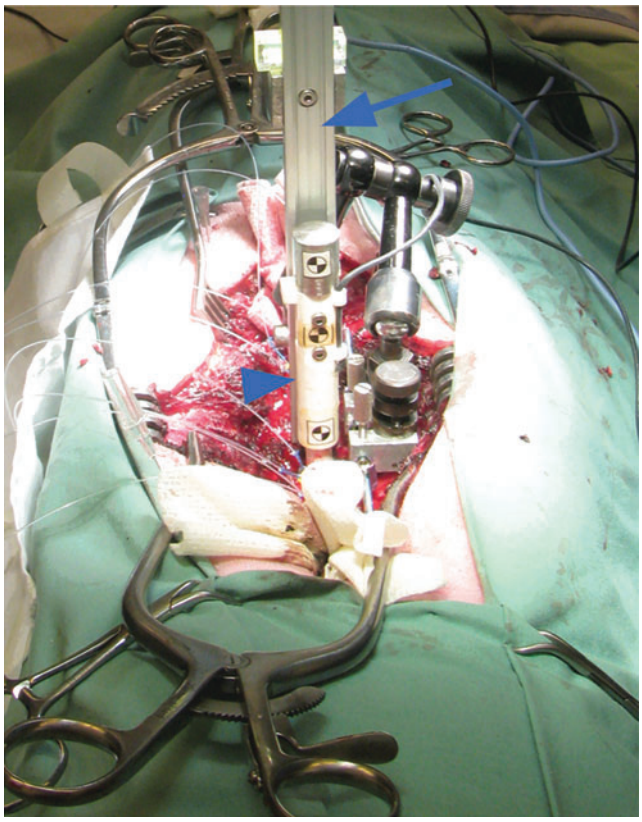

FIG. 1. Weight drop impactor on spinal cord. The 50 g impactor (arrowhead) falls along the guide rail (arrow) from a height of 50 cm and strikes the cord. Sustained compression on the cord is applied by leaving the impactor on the cord and adding a cylindrical 100 g weight to rest on top of it for the duration of the experiment (4 h). The 100 g weight is not shown in this figure. Color image is available online at www.liebertpub.com/neu

were perfused continuously at a rate of 0.5 μL/min with artificial CSF (in mM: 147 NaCl, 2.7 KCl, 1.2 $CaCl_2$, 0.85 $MgCl_2$; M dialysis Inc., N. Chelmsford, MA). The microdialysis probes remained in place within the spinal cord during the impact so as to avoid the additional local parenchymal disturbance that would occur by removing and replacing them before and after inducing the injury, as has been done in previous neurotrauma studies.[34,35,39]

Recognizing that probe insertion would invariably cause some acute tissue damage and metabolic changes, we allowed at least 1 h for ''equilibration'' within the spinal cord extracellular fluid. After this period of ''equilibration,'' dialysate samples were collected into small collection vials every 10 min for 1 h. The concentrations in these first six dialysate samples were averaged to represent the ''baseline'' level of each metabolite. After this 1 h of baseline sampling, the traumatic SCI was induced, and samples continued to be collected every 10 min for the next 4 h post-injury. Samples were immediately sealed after collection and stored on dry ice until assayed for lactate, pyruvate, and glucose. Dialysate measurements were made with the ISCUS^{flex} Microdialysis Analyzer (art. code 8003295; Microdialysis Analyzer for Research Use, M Dialysis, Stockholm, Sweden).[40] All reagents required for analysis were obtained from M Dialysis Inc., N. Chelmsford, MA (P000063 pyruvate reagent; P000024 lactate reagent; P000023 glucose reagent). The L/P ratio, a well-recognized indicator of hypoxia, was calculated from the measured values of lactate and pyruvate concentrations. For calculation of L/P ratio, observations that read a numeric value of 0 for pyruvate were excluded from the analysis (in total two samples). Lactate, pyruvate, L/P ratio, and glucose expression levels were calculated and expressed as percentage change versus baseline: change (%) = (expression after SCI/baseline levels) * 100%.

Experimental groups: Contusion only, contusion with compression, sham

Animals were divided into either: 1) contusion only group ($n = 6$), in which the impactor weighing 50 g was dropped onto the exposed cord from a height of 50 cm and left resting on the cord for 5 min before removal; 2) contusion with sustained compression

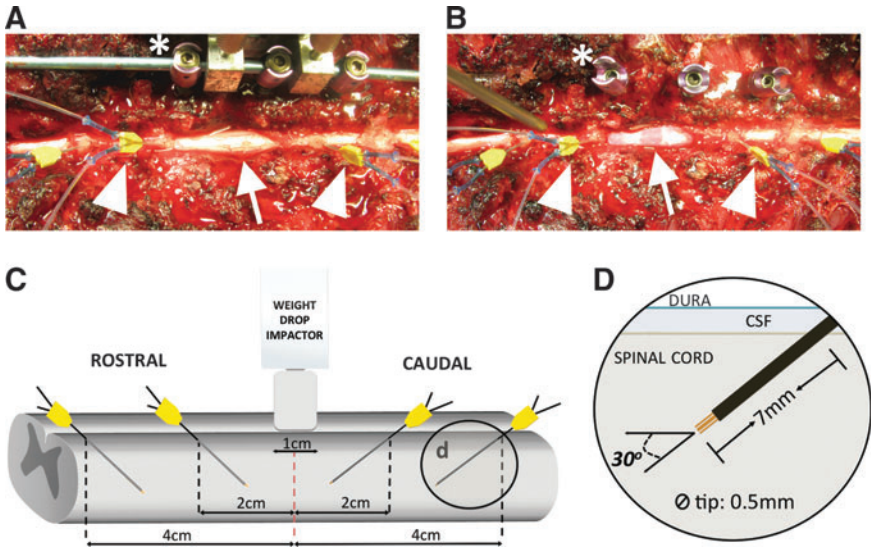

FIG. 2. Microdialysis probe insertion into spinal cord. After laminectomy, pedicle screws (asterisks) have been inserted at T9, 10, and 11 to hold the impactor guide rail. The microdialysis probes (arrowheads) are inserted on either side of the weight drop site at T10 (arrow). The cord is shown prior to the injury **(A)**, and after the weight has been dropped onto the cord **(B)**. Note in **B**, the significant hemorrhage at the injury site (arrow). **(C)** While the probe is inserted 2 cm from the site of injury, because of the angulation of the insertion path, the microdialysis membrane at the tip typically resides ~1.5 cm from the actual injury epicenter. **(D)** Magnified view of probe tip within the spinal cord. The probe is inserted to a depth of approximately 7 mm at a 30 degree angle to the longitudinal axis of the cord. Color image is available online at www.liebertpub.com/neu

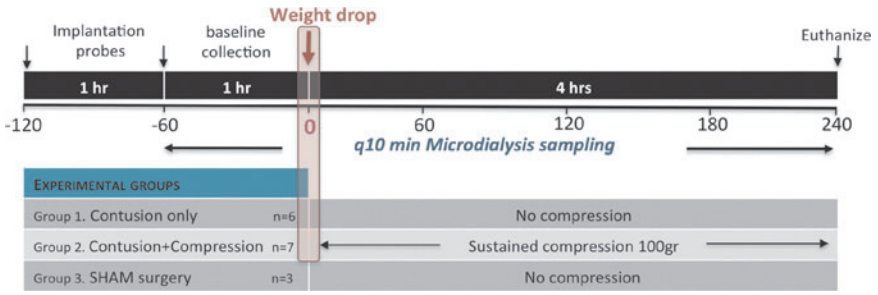

FIG. 3. The design for the microdialysis experiment. Animals are divided into: 1) contusion group, in which the impactor weighing 50 g was dropped onto the exposed cord and left resting on the cord for 5 min before removal; 2) compression group, in which the animals received the same weight drop injury followed by continued compression for the total duration of the experiment; or 3) sham animals, in which the animals underwent the entire surgical procedure, including laminectomy and microdialysis probe insertion, but no spinal cord injury (SCI). Color image is available online at www.liebertpub.com/neu

group ($n=7$), in which the animals received the same weight drop injury (50 g from a height of 50 cm), immediately followed by compression from the addition of a 100 g weight on top of the impactor, which remained in place for the total duration of the experiment (4 h); or 3) sham animals ($n=3$), in which the animals underwent the entire surgical procedure, including laminectomy and microdialysis probe insertion, but without SCI. A schematic overview of the experimental design is illustrated in Figure 3.

Histology

Four hours post-injury, animals were euthanized with an IV overdose of Euthanyl (Vetoquinal N.-A. Inc., Lavaltrie, QC, Canada), and then a 10 cm segment of spinal cord centered around the impact site was collected and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 3 days. After cryoprotection in sucrose, by gradually increasing the sucrose concentration from 12% to 30%, 1 cm spinal cord segments were cut and embedded in tissue freezing medium (Tissue-tek, Sakura, Japan). Each spinal cord section was then frozen on dry ice and cut (20 μm) on a cryostat. To histologically evaluate the position of the probe tip, sections through the probe sites were mounted on slides, dried, and stained with Eriochrome cyanine (EC) R as described previously.[7,41] Single images of EC-stained spinal cord sections were taken using a digital color camera (Leica DFC420, Leica Microsystems, Concord, ON, Canada) attached to a microscope (Leica DM5000B, Leica Microsystems, Concord, ON, Canada). For each section, single images were taken at 2.5 × objective and stitched together using Photoshop (Adobe Systems Inc., San Jose, CA).

Statistical analysis

Data are reported as average ± standard error of mean (SEM). SCI characteristics and physiologic parameters were analyzed with the Student's *t* test. To analyze changes in lactate, pyruvate, L/P ratio, and glucose between groups over time we used a generalized estimating equations (GEE) model. The GEE procedure allowed us to test the significance of the relationship between two major outcome variables (experimental group and microdialysis parameter) while simultaneously accounting for the temporally repeated observations for each animal. To capture differences in the very early metabolic responses among groups we compared the changes occurring within the first 60 min after injury (t0–60 min). We also compared the metabolite levels during the last 60 min of the 4 h post-injury observation period (t180–240 min), to determine if there were differences in how the levels ''plateaued'' over time. The Pearson correlations coefficients between the average glucose and pyruvate levels and L/P ratio during the first 60 min and the last 60 min of the experiment were determined. A value of $p \leq 0.05$ was considered statistically significant.

Results

SCI characteristics and physiologic parameters

The body weights of the animals in the three experimental groups were comparable. No significant differences in average maximal force of the impact were found; reaching 6543 ± 494 kdyn in the contusion only group and 6275 ± 164 kdyn in the group with sustained compression following contusion (Table 1). Mean arterial pressure (MAP) prior to SCI was lower in the contusion only group compared with the sustained compression group ($p=0.005$) and averaged respectively 49.1 ± 1.11, 54.6 ± 0.83 mm Hg. For both groups, MAP values were not different from those of the sham group (59.6 ± 9.62 mm Hg). Baseline heart rate averaged 96.0 ± 1.89 and 99.1 ± 2.27 bpm for contusion only or sustained compression group, respectively. SCI did not affect MAP or heart rate within the first 60 min (t0–60) after injury, and no differences were observed between groups (Table 1). During the last hour of the experiment (t180–t240), a slight decline in MAP was observed;

TABLE 1. PHYSIOLOGICAL PARAMETERS

Parameters measured	*Sham*	*Contusion only*	*Contusion + compression*
Number of animals (n)	3	6	7
Force (kdyn)	n.a.	6543 ± 494	6275 ± 164
Body weight (kg)	33.3 ± 1.0	31.1 ± 1.2	28.6 ± 1.1
Temperature (°C)	37.4 ± 0.05	37.3 ± 0.38	37.7 ± 0.35
SpO_2	96.2 ± 0.83	96.9 ± 0.32	97.3 ± 0.69
MAP (mm Hg)			
- Baseline	59.6 ± 9.62	49.1 ± 1.11	54.6 ± 0.83*
- t0–60 min	56.1 ± 7.30	47.1 ± 1.25	55.5 ± 1.79*
- t180–240 min	52.4 ± 6.28	46.5 ± 3.47	50.8 ± 1.64
Pulse (bpm)			
- Baseline	92.8 ± 4.91	96.0 ± 1.89	99.1 ± 2.27
- t0–60 min	93.2 ± 5.59	99.7 ± 2.12	101.3 ± 3.29
- t180–240	99.3 ± 3.90	106.7 ± 2.35	105.6 ± 3.94

Values are expressed as means ± SEM. For MAP and pulse there were no significant differences in these physiologic parameters between baseline and SCI values.

*Denotes significantly different from contusion only group, $p<0.05$.

SCI: spinal cord injury; MAP: mean arterial blood pressure.

however, this decreased to approximately the same extent for all three groups. Additionally, a slight increase in heart rate was observed during the last hour (t180–240) compared with baseline values, but again, this increase was similar among groups.

Histology

All probe tips were situated on the left side of the spinal cord at the white matter–gray matter (ventral funiculus–ventral horn) interface (Fig. 4). Modest disruption of tissue architecture within the ventral white and gray matter was evident close to the probe tip, as indicated by less tightly packed myelin, increased intercellular space, and less EC staining.

Baseline concentrations of lactate, pyruvate, and glucose

The average baseline concentrations of lactate, pyruvate, and glucose were derived from six dialysate samples obtained over 1 h, starting 1 h after microdialysis probe insertion. As shown in Table 2, there were no significant differences in the absolute baseline concentrations among the animal groups. All of the microdialysis data described below are presented as the percentage change from this baseline value.

Metabolic changes in sham animals (no SCI)

To provide perspective on the metabolic changes that occur after acute SCI, we first present the changes observed in the sham animals (summarized in Fig. 5). Three sham animals without the weight drop SCI were included to determine the changes in lactate, pyruvate, and glucose that resulted merely from the anaesthetic, surgical exposure, laminectomy, and microdialysis probe placement (i.e., all experimental conditions except the actual traumatic SCI). Like the animals receiving an SCI, a period of at least 1 h was allowed for ''equilibration'' after the four microdialysis probes were inserted, and then the ''baseline values'' were generated from the six samples obtained every 10 min over the following hour. ''Time zero'' was assigned at the conclusion of this 1 h of baseline sampling, when the SCI would have been induced if these were not sham animals.

Table 2. Baseline Levels of Lactate, Pyruvate, L/P Ratio, and Glucose

Microdialysis parameters	*Sham*	*Contusion only*	*Contusion + compression*
Lactate (mM)			
- 2cm	0.54 ± 0.06	0.57 ± 0.11	0.52 ± 0.05
- 4cm	0.51 ± 0.08	0.51 ± 0.05	0.53 ± 0.06
Pyruvate (M)			
- 2cm	34.69 ± 1.66	34.11 ± 5.73	29.55 ± 3.09
- 4cm	29.21 ± 2.55	29.56 ± 3.64	34.80 ± 5.78
L/P ratio			
- 2cm	15.85 ± 1.94	18.67 ± 1.25	18.61 ± 1.18
- 4cm	17.34 ± 1.41	18.84 ± 1.32	17.49 ± 1.41
Glucose (mM)			
- 2cm	0.22 ± 0.05	0.20 ± 0.02	0.18 ± 0.04
- 4cm	0.20 ± 0.03	0.23 ± 0.05	0.18 ± 0.04

Values are expressed as means ± SEM.

For the first 80 min after time zero, the lactate and pyruvate levels remained stable within a relatively small range. After 80 min, the lactate levels rose to ~150% of baseline. During this time, the pyruvate levels remained stable; therefore, there was a rise in the L/P ratio of ~150%. For glucose, we observed a transient increase to ~125% of baseline levels within the first hour after time zero, after which the glucose levels decreased steadily to ~50% of baseline levels by the end of the 4 h observation period. For lactate,

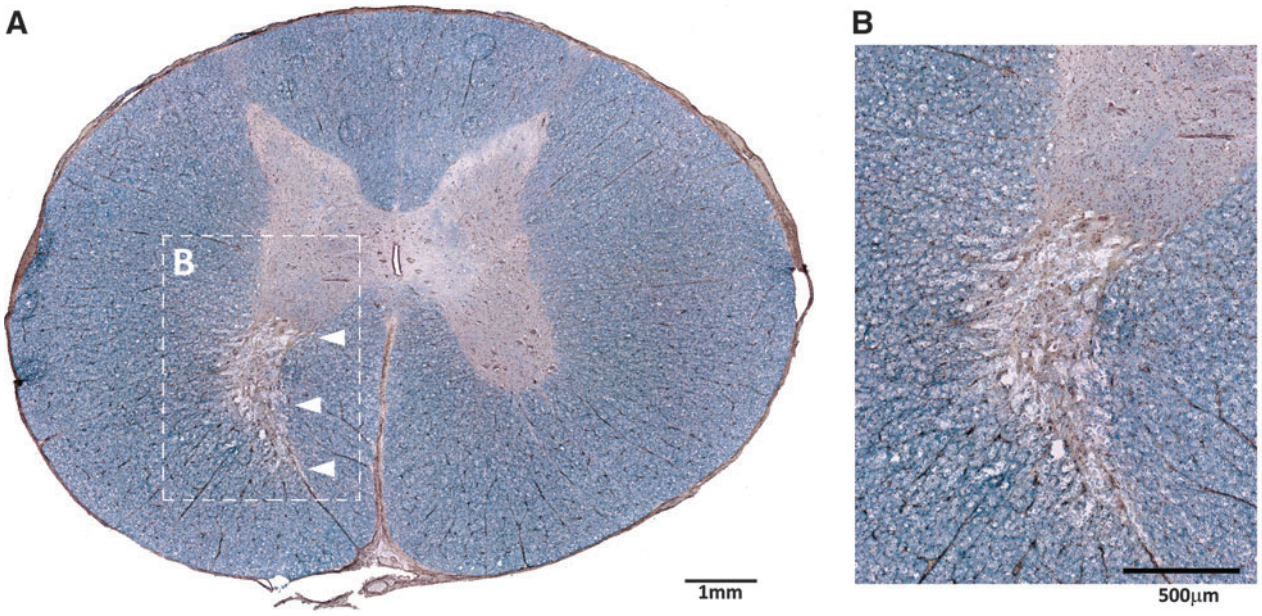

FIG. 4. Location of the microdialysis probe in the porcine spinal cord. The spinal cord was harvested and sectioned axially to determine the location of the probe tip. The microdialysis membrane occupies the final 2 mm at the tip of the probe, and measures 0.5 mm in diameter. **(A)** An example section of the spinal cord stained with Eriochrome cyanine R. All probe tips were situated on the left side of the spinal cord at the white matter–gray matter (ventral funiculus–ventral horn) interface. The white box shows the discrete region of damage within the gray and white matter where the probe tip resides. **(B)** is a higher magnification view of the boxed region in **(A)**. Color image is available online at www.liebertpub.com/neu

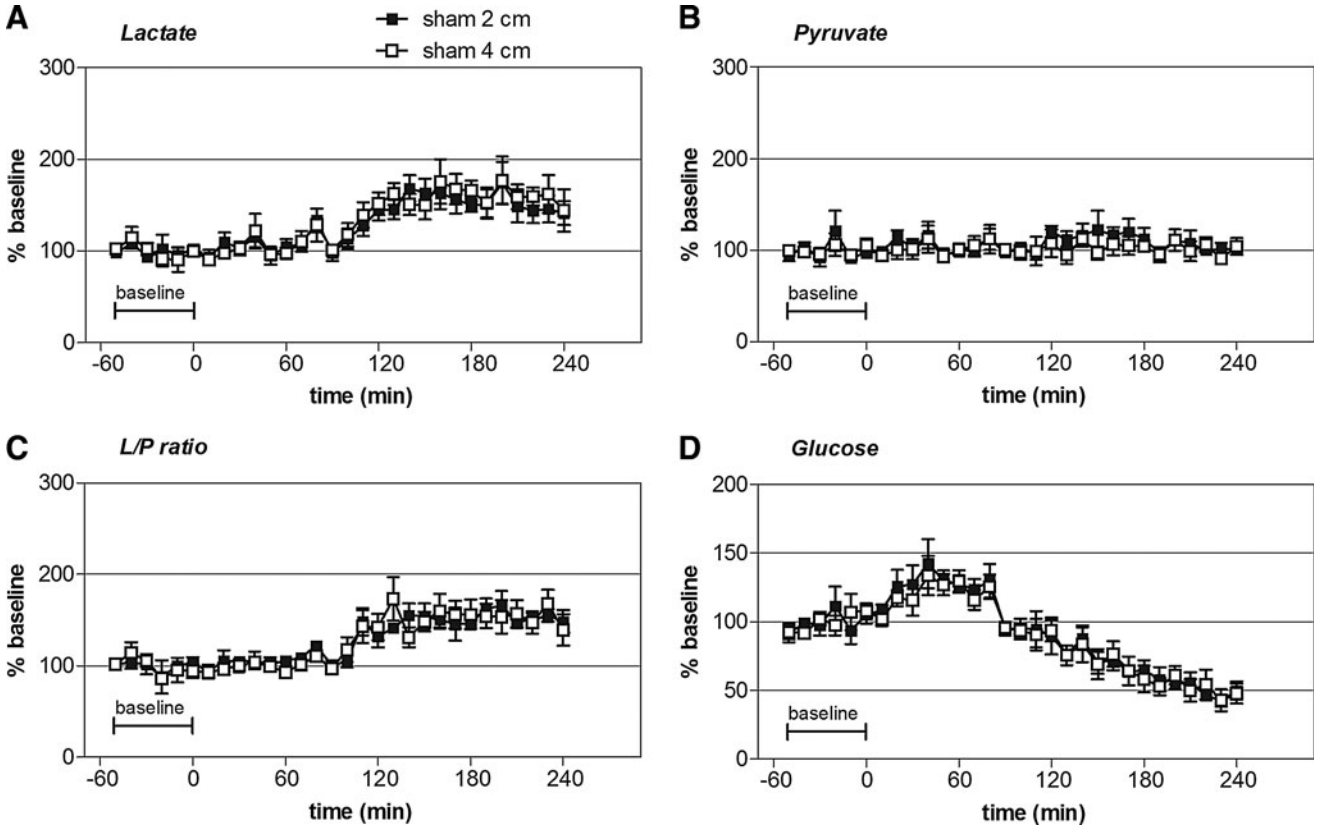

FIG. 5. Time course of changes in dialysate lactate, pyruvate, lactate/pyruvate (L/P), and glucose concentration in the spinal cord during sham surgery**.** Lactate **(A)**, pyruvate **(B)**, the L/P ratio **(C)**, glucose **(D)**. Open squares represent average values from the probes inserted at 2 cm from the epicenter; Black squares represent average values from the probes located 4 cm from the epicenter.

pyruvate, and glucose, the levels recorded at microdialysis probes placed 2 cm and 4 cm from the ''injury'' were very consistent over time, as would be expected in the absence of SCI.

Metabolic changes in SCI animals

Four microdialysis probes were inserted into the spinal cord: 4 cm rostral, 2 cm rostral, 2 cm caudal, and 4 cm caudal to the injury site. Because of the angle of insertion, the tips of the probes were actually closer to the injury site than 4 and 2 cm (Fig. 2); however, for simplicity, we refer to them here as the ''4 cm'' and ''2 cm'' rostral and caudal probes. There were no significant differences in the metabolite levels between the 4 cm rostral and 4 cm caudal probes, and no significant differences between the 2 cm rostral and 2 cm caudal probes (data not shown). Therefore, the values between the 2 cm rostral and caudal probes were averaged to provide a value at 2 cm ''from injury,'' and the values between the 4 cm rostral and caudal probes were averaged to provide a value at 4 cm ''from injury.''

Microdialysis measurements nearest to the injury site (2 cm probes)

The effects of contusion injury and the effects of contusion injury with sustained compression on lactate, pyruvate, the L/P ratio, and glucose levels at 2 cm from the SCI site are shown in Figure 6, and at 4 cm from the SCI site in Figure 7. Average values at the 2 cm probes during the first 60 min (t0–60 min), and last 60 min (t180–240 min) after SCI or sham surgery are shown in Table 3.

Metabolic changes after contusion (2 cm). The pattern of change for the various metabolites measured are displayed in Figure 6. The contusion injury resulted in an increase in lactate to a maximum of ~260% compared with baseline levels by 40 min post-injury. During the first 60 min post-injury, these lactate levels were significantly greater than those observed in the sham animals (contusion vs. sham [t0–60 min]: $p < 0.001$). After this initial peak, the lactate levels gradually decreased, but remained elevated compared with baseline. However, during the last hour of the experiment, these elevated lactate levels were not significantly different from those observed in the sham animals (contusion vs. sham [t180–240 min]: $p = 0.251$).

Approximately 10 min after contusion injury, pyruvate levels were modestly increased to ~150% of baseline. For the first hour post-injury, the pyruvate levels were significantly greater than those in the sham animals (contusion vs. sham [t0–60min]: $p = 0.002$). The pyruvate levels then decreased slightly and were not significantly different from sham during the last 60 min of the experiment (contusion vs. sham [t180–240 min]: $p = 0.102$). Similarly, the L/P ratio increased after contusion to a peak of ~220% of baseline within 40 min post-injury. During the first 60 min, this change in L/P ratio was significantly greater than sham (contusion vs. sham [t0–60 min]: $p = 0.001$). After the first 60 min post-injury, the L/P ratio began to return toward baseline, and during the last 60 min of the experiment, was not significantly different from sham (contusion vs. sham [t180–240 min]: $p = 0.723$).

Glucose levels in the contusion group remained relatively steady and hovered around baseline levels for the 4 h post-injury period, in contrast to the transient rise and then progressive decline of glucose

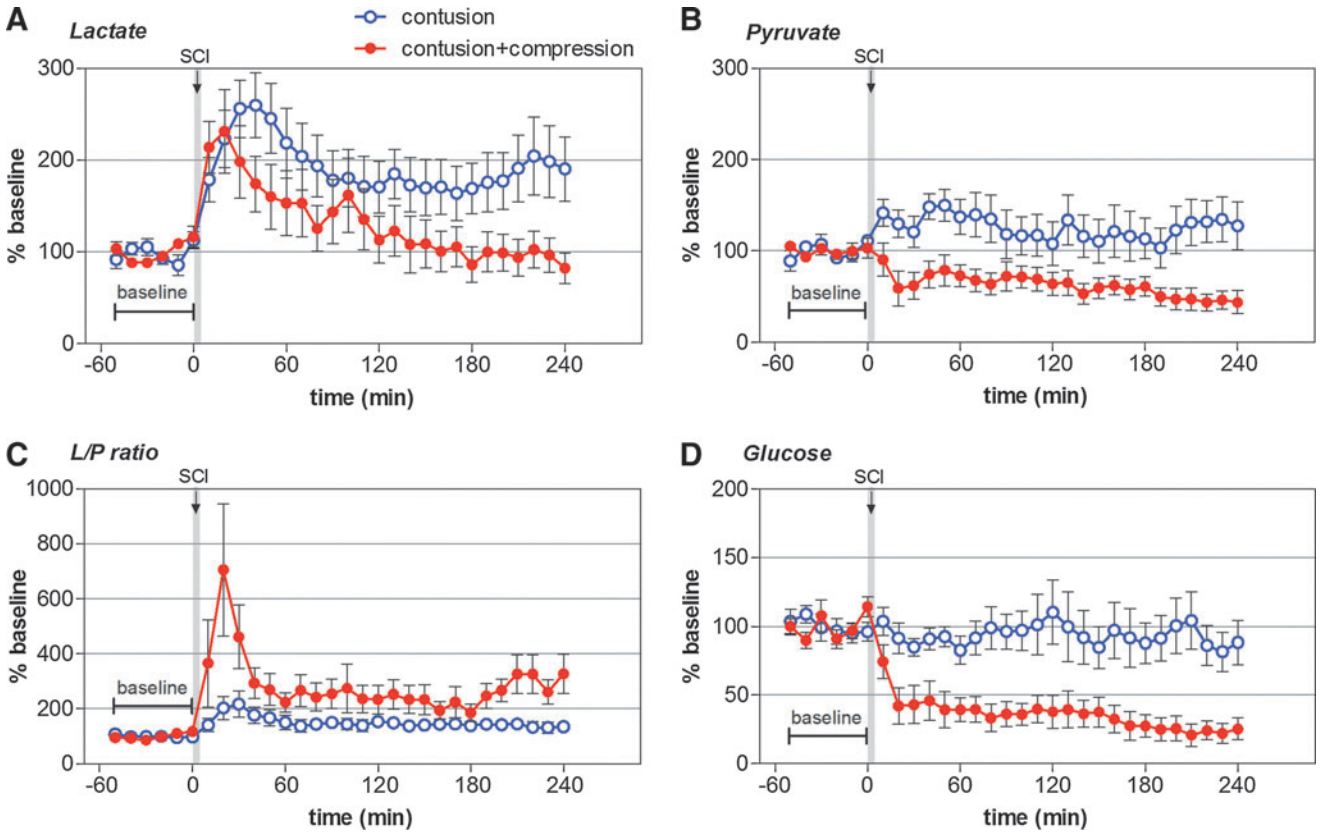

FIG. 6. Changes in spinal cord microdialysis parameters after spinal cord injury (SCI) with or without sustained compression, 2 cm from injury site. Spinal cord dynamics for **(A)** lactate, **(B)** pyruvate, **(C)** the lactate/pyruvate ratio, and **(D)** glucose measured from the dialysates obtained from the microdialsyis probe inserted at 2 cm from the epicenter. The blue line represents the average concentration profile of the contusion only group; the red line represents the average concentration profile of the contusion and sustained compression group. Color image is available online at www.liebertpub.com/neu

levels seen in sham animals. The glucose levels in the contusion group were, therefore, significantly lower than those in the sham animals during the first hour (contusion vs. sham [t0–60 min]: $p = 0.001$), but then significantly higher during the last hour of observation (contusion vs. sham, [t180–240 min]: $p = 0.018$).

Metabolic changes after contusion with sustained compression (2 cm). In the contusion injury with sustained compression (herein described as the ''compression group''), the lactate levels rose rapidly (contusion vs. sham [t0–60 min]: $p < 0.001$) to a peak of ~220% of baseline by 20 min post-injury. The increase in lactate over the course of this first hour, however, was less pronounced than that observed in the contusion group (compression vs. contusion [t0–60 min]: $p = 0.047$) After this initial peak, lactate levels progressively decreased to baseline levels, resulting in lower levels compared with those of the contusion group and even those of the sham group during the final hour of the experiment (compression vs. contusion [t180–240 min]: $p = 0.031$; compression vs. sham [t180–240 min]: $p = 0.002$).

Pyruvate levels in the compression group decreased rapidly after injury to ~60% of baseline, in contrast to the increase of ~150% observed in the contusion group and the unchanged levels in the sham group. These differences in pyruvate levels were statistically significant during both the first and last 60 min after injury (compression vs. contusion [t0–60 min]: $p < 0.001$, and [t180–240 min]: $p = 0.003$) and (compression vs. sham [t0–60 min]: $p = 0.014$, and [t180–240 min]: $p < 0.001$). The resultant L/P ratio increased rapidly to ~705% of baseline within the first 30–40 min post-injury, before quickly dropping down to ~250% of baseline. The L/P ratio during the first 60 min post-injury was significantly higher in the compression group than in the contusion and sham groups (compression vs. contusion [t0–60 min]: $p = 0.022$; compression vs. sham [t0–60 min]: $p < 0.001$). The L/P ratio remained elevated at 200–300% of baseline, and continued to be significantly higher in the compression group during the last 60 min of observation (compression vs. contusion [t180–240 min]: $p = 0.009$; compression vs. sham [t180–240 min]: $p = 0.006$).

Glucose levels in the compression group decreased rapidly after injury to < 50% of baseline levels, and continued to decline over time. This was markedly different from both the contusion and sham groups, in which glucose levels were either constant or even slightly elevated (compression vs. contusion [t0–60 min]: $p < 0.001$; compression vs. sham [t0–60 min]: $p < 0.001$). At the conclusion of the 4 h post-injury observation period, the glucose levels in the compression group were significantly lower than in the contusion and sham groups, dropping to ~25% of those in the contusion group and 50% of those in the sham group (compression vs. contusion [t180–240 min]: $p < 0.001$; compression vs. sham [t180–240 min]: $p = 0.014$).

In the animals subjected to contusion with sustained compression, correlations between glucose and pyruvate and the L/P ratio were established. During the first hour post-injury, there was a

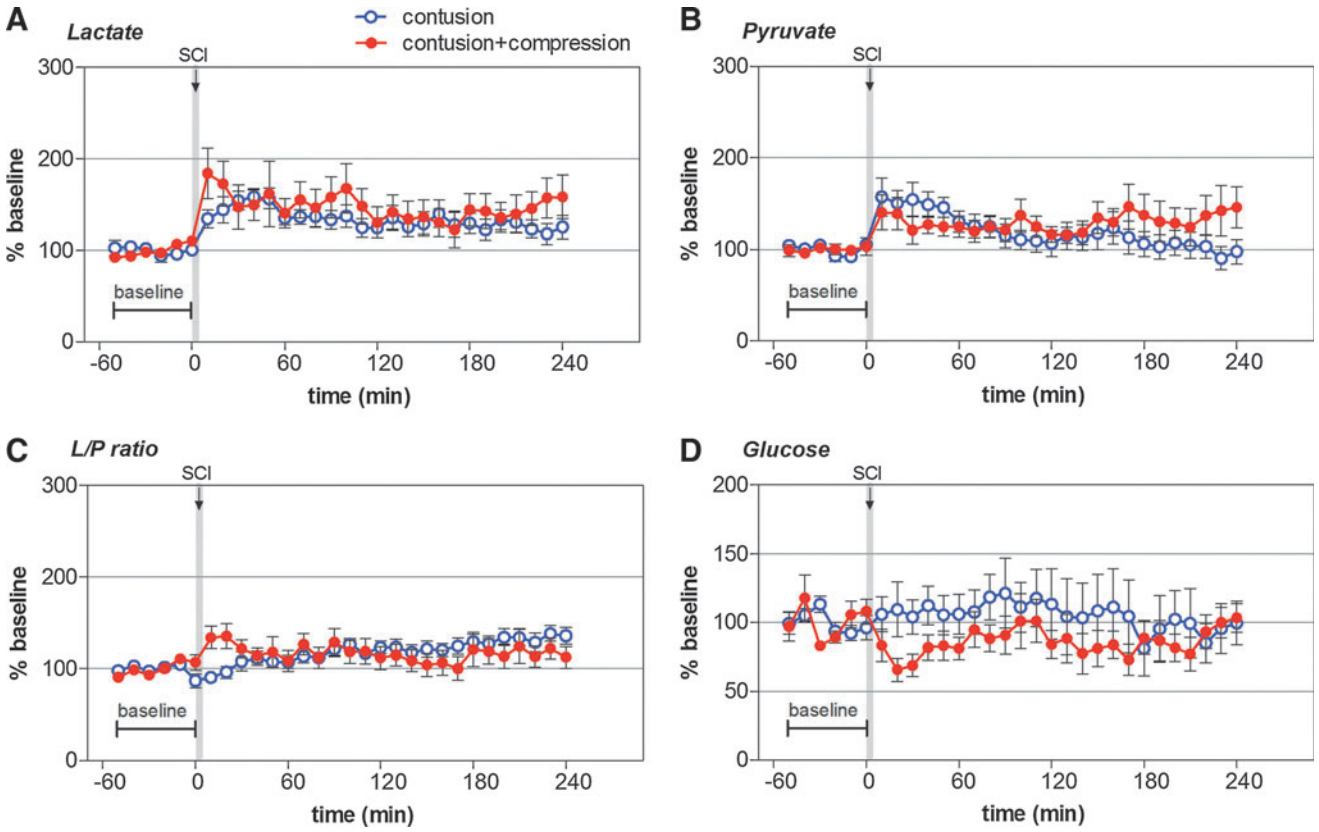

FIG. 7. Changes in spinal cord microdialysis parameters after spinal cord injury (SCI) with or without sustained compression, 4 cm from the injury site. Spinal cord dynamics for **(A)** lactate, **(B)** pyruvate, **(C)** the lactate/pyruvate ratio, and **(D)** glucose measured from the dialysates obtained from microdialsyis probe inserted at 4 cm from the epicenter. The blue line represents the average concentration profile of the contusion only group; the red line represents the average concentration profile of the contusion and sustained compression group. Color image is available online at www.liebertpub.com/neu

TABLE 3. TWO CM PROBE: EFFECT OF INJURY TYPE ON THE AVERAGE LACTATE, PYRUVATE, L/P RATIO, AND GLUCOSE LEVELS DURING THE FIRST AND LAST 60 MIN AFTER SCI OR SHAM SURGERY

Microdialysis parameters	*Sham*	*Contusion only*	*Contusion + compression*
Lactate (%)			
- Baseline	100	100	100
- t0–60 min	103.9 ± 03.4	↑224.0 ± 21.7†	↑188.4 ± 18.3†,*
- t180–240 min	151.6 ± 08.9	207.1 ± 57.5	↓093.6 ± 18.5†,*
Pyruvate (%)			
- Baseline	100	100	100
- t0–60 min	103.2 ± 07.5	↑126.3 ± 11.5†	↓73.0 ± 4.66†,*
- t180–240 min	99.8 ± 05.6	152.3 ± 41.1	↓44.9 ± 12.4†,*
L/P ratio (%)			
- Baseline	100	100	100
- t0–60 min	102.0 ± 08.0	↑176.3 ± 33.0†	↑386.7 ± 72.3†,*
- t180–240 min	152.6 ± 12.0	145.9 ± 15.8	↑300.0 ± 56.5†,*
Glucose (%)			
- Baseline	100	100	100
- t0–60 min	127.0 ± 06.6	↓88.6 ± 06.1†	↓43.0 ± 12.7†,*
- t180–240 min	051.6 ± 06.7	↑93.2 ± 16.2†	↓21.8 ± 07.6†,*

Values are the means ± SEM.

†Denotes significantly different from sham group, ↑ value increased versus sham; ↓ value decreased versus sham. *Denotes significantly different from contusion only group. Tested with regression analysis, $p < 0.05$.

L/P, lactate/pyruvate ratio; SCI, spinal cord injury.

statistically significant correlation between the glucose and pyruvate levels 2 cm from the injury site ([t0–60 min]: $r = 0.857$, $p = 0.01$). The correlation between the decrease in glucose and the increase in the L/P ratio at 2 cm was close to statistically significant during this period as well ([t0–60 min]: $r = -0.698$, $p = 0.085$). During the last hour of observation, the glucose levels 2 cm from injury were correlated positively to the pyruvate levels ([t180–240 min]: $r = 0.862$, $p = 0.009$) and inversely to the L/P ratio ([t180–240 min]: $r = -0.908$, $p = 0.002$). In contrast, such statistically significant correlations were not observed 2 cm from injury in the animals subjected to contusion without sustained compression. In neither group were correlations between the L/P ratio and glucose established at 4 cm from injury.

Microdialysis measurements distant to the injury site (4 cm probes)

The effects of contusion with or without compression on dialysate concentrations of lactate, pyruvate, glucose, and the L/P ratio at 4 cm from the center of the impact are shown in Figure 7. Average values at the 4 cm probes during for the first 60 min (t0–60 min), and last 60 min (t180–240 min) after SCI or sham surgery are shown in Table 4.

Metabolic changes after contusion injury (4 cm). Although the increases in both lactate and pyruvate at 4 cm were less than the changes observed at the 2 cm probe, the levels were

Table 4. Four cm Probe: Effect of Injury Type on Lactate, Pyruvate, L/P Ratio and Glucose Levels during the First and Last 60 Min after SCI or Sham Surgery

Microdialysis parameters (%)	*Sham*	*Contusion only*	*Contusion + compression*
Lactate			
- Baseline	100	100	100
- t0–60 min	100.9 ± 05.0	↑147.3 ± 08.5†	↑159.5 ± 25.1†
- t180–240 min	156.6 ± 23.0	129.2 ± 12.6	148.3 ± 24.0
Pyruvate			
- Baseline	100	100	100
- t0–60 min	100.3 ± 10.3	↑148.0 ± 13.7†	129.0 ± 13.2
- t180–240 min	101.3 ± 08.6	101.7 ± 15.0	133.0 ± 27.3
L/P ratio			
- Baseline	100	100	100
- t0–60 min	097.8 ± 04.8	103.6 ± 07.1	↑122.2 ± 10.2†
- t180–240 min	150.9 ± 17.9	134.7 ± 10.3	118.3 ± 16.7
Glucose			
- Baseline	100	100	100
- t0–60 min	121.0 ± 4.1	105.0 ± 10.9	↓76.8 ± 09.0†,*
- t180–240 min	051.6 ± 5.6	102.4 ± 27.8	↑89.6 ± 13.6† ,0

Values are the means ± SEM.

†Denotes Significantly different from sham group, ↑ value increased versus sham, ↓ value decreased versus Sham. *Denotes significantly different from contusion only group. Tested with regression analysis, $p < 0.05$.

L/P, lactate/pyruvate ratio; SCI, spinal cord injury.

still significantly greater than in the sham animals, suggesting that considerable metabolic alterations were occuring quite distant from the injury site (contusion vs. sham [t0–60 min]: $p < 0.001$ and $p = 0.002$, respectively). After this initial increase, both lactate and pyruvate levels decreased over time, such that by the end of the observation period they approximated the lactate and pyruvate levels of the sham group (contusion vs. sham [t180–240 min]: $p = 0.149$ and $p = 0.924$, respectively). At the 2 cm probe, an acute increase in the L/P ratio was observed, this effect, however, was not evident at the 4 cm probe. Instead, a slow and gradual increase was observed over the course of 4 h such that the L/P ratio was not significantly different than that observed in the sham group (contusion vs. sham [t0–60 min]: $p = 0.433$, and [t180–240 min]: $p = 0.307$). Similar to that observed at the 2 cm probe, glucose levels remained relatively stable, and did not differ significantly from the sham group during the first hour post-injury (contusion vs. sham, [t0–60 min]: $p = 0.105$). The glucose levels remained close to baseline at the conclusion of the 4 h observation period, which approached being significantly greater than the gradually decreasing glucose levels in the sham group (contusion vs. sham [t180–240 min]: $p = 0.056$).

Metabolic changes after contusion with sustained compression (4 cm). Compression injury resulted in a modest increase of ~160% of baseline lactate levels at the 4 cm probe in the first hour post-injury as compared with the increase of ~220% measured at the 2 cm probe. This increased lactate level at 4 cm was still greater than that of the sham animals during the first hour post-injury (compression vs. sham [t0–60 min]: $p = 0.013$). Unlike at 2 cm where the pyruvate levels dropped, pyruvate levels in the compression group trended toward being significantly greater than in sham animals (compression vs. sham [t0–60 min]: $p = 0.056$). These changes in lactate and pyruvate were not significantly different from the changes observed in the contusion group (compression vs. contusion [t0–60 min]: $p = 0.618$, and $p = 0.273$, respectively). In addition, compared with that observed at the 2 cm probe, a similar, albeit less pronounced, increase in the L/P ratio was observed (contusion vs. sham [t0–60 min]: $p = 0.006$). L/P level tended to be higher than that of contusion only injury immediately after SCI; however, it was not significantly different (compression vs. contusion [t0–60 min]: $p = 0.061$). In the last hour of the experiment, none of the changes in lactate, pyruvate, or L/P ratio in the compression group were significantly different from those in the sham group (compression vs. sham [t180–240 min]: $p = 0.686$; $p = 0.224$; $p = 0.131$, respectively) or those in the contusion group (compression vs. contusion [t180–240 min]: $p = 0.407$, $p = 0.246$, $p = 0.423$, respectively). This pattern, however, was in stark contrast to that observed at the 2 cm probe, where the L/P ratio levels remained increased, and pyruvate fell significantly compared with the sham group.

Similar to what was observed at 2 cm, the glucose levels at 4 cm also decreased rapidly after the compression injury, albeit not to the same extent. During the first hour post-injury, the glucose levels in the compression group were significantly lower than in the contusion or sham groups (compression vs. contusion [t0–60 min]: $p = 0.042$; compression vs. sham [t0–60 min]: $p < 0.001$). However, unlike at 2 cm where the glucose levels remained low and continued to decrease, the glucose levels at 4 cm slowly recovered over time and remained near baseline levels, similar to those of the contusion group at the conclusion of the experiment (compression vs. contusion [t180–240 min]: $p = 0.666$). These glucose levels in the compression group were significantly higher than those of the sham animals at the end the experiment (compression vs. sham [t180–240 min]: $p = 0.004$), which was related to the gradual decrease in glucose levels over time observed in the sham animals (Fig. 5).

Discussion

Traumatic spinal column and resultant SCI occur in humans with great biomechanical variability, which is then superimposed upon considerable variations in age, medical comorbidities, and genetic background. Clearly, this heterogeneity of human SCI is challenging to address in pre-clinical studies, where researchers typically strive to minimize variations in the most minute of experimental parameters. A better understanding of how the injured spinal cord responds to different mechanisms of injury is important for developing acute neuroprotective treatments that target these pathophysiologic responses. It could be validly argued that because we currently have no convincingly effective therapies for acute SCI patients, we do not have the luxury of attempting to develop treatments that are specific to one mechanism or another. However, from a translational perspective, if early pathophysiologic responses differ among distinct injury mechanisms, then the administration in a clinical trial of a neuroprotective agent to all acute SCI patients regardless of their injury mechanism, might be effective in some, but ineffective in others, making it impossible to discern a positive treatment effect in the trial. Here, we chose to study the two injury mechanisms – contusion and compression – that are commonly employed in pre-clinical studies. Importantly, the extent to which the spinal cord is being subjected to ongoing compression can be determined in human SCI patients with current imaging techniques (CT scan and MRI). The vast majority of pre-clinical rodent SCI studies employ either compression or contusion to generate the experimental injury. Given that human injury typically occurs after a contusion and compression, we sought to evaluate

how this combination influenced the metabolic responses in the cord.

The aim of this study was to characterize how the metabolic responses within the injured spinal cord differ after two clinically relevant mechanisms of SCI: contusion with and without sustained compression. In the present study, we used the microdialysis technique to demonstrate considerable differences in the intraparenchymal metabolic responses dependent upon the presence or absence of sustained compression after the contusion injury. With sustained compression, the glucose and pyruvate levels decreased significantly, and the L/P ratio increased dramatically (albeit transiently) in the first hour post-injury, suggesting marked ischemia caused by oxygen/glucose deprivation. Moreover, there were significant correlations between the L/P ratio and the glucose levels and between the glucose levels and the pyruvate levels among the individual animals in the compression group in the dialysates from the 2 cm probes. Such a drop in glucose and pyruvate was not observed in the contusion group, although there was a modest rise in the L/P ratio immediately after injury. In general, these metabolic parameters changed less dramatically at 4 cm than at 2 cm for both the contusion and compression groups, as one might expect, given that the former was 2 cm further away from the injury site.

Although we did not measure blood flow in the current study, previous rodent and large animal models of SCI have convincingly shown compromised local perfusion after compressive SCI lesions without contusion.[30,42–44] Frykholm and colleagues showed that in nonhuman primates, glucose in the brain (monitored by microdialysis), correlated significantly with cerebral blood flow and cerebral metabolic oxygen consumption.[45] Metabolic data also showed that during middle cerebral artery occlusion in primates, the L/P ratio significantly increased.[46] Similar to our study, Bäckström and colleagues reported significant decreases in glucose and pyruvate, with an increase in the L/P ratio after subjecting the porcine spinal cord to a severe global ischemic insult by cross-clamping the aorta.[47] Given the very strong correlation between decreased glucose and pyruvate levels 2 cm from the injury site in our animals subjected to sustained compression (with a concomitant increase in the L/P ratio), we assumed that decreased local spinal cord perfusion was similarly in effect during this experimental condition. Such correlations were not observed 2 cm from the SCI in the contusion injury group, or in either animal group at 4 cm from the injury, suggesting that hypoperfusion was not as prominent an issue.

Here, we sought to characterize the downstream metabolic consequences of presumptive hypoperfusion, acknowledging that whereas alterations in blood flow are important, it is the subsequent metabolic derangement that will ultimately dictate tissue survival. It has previously been shown that increases in the L/P ratio >40 with a concurrent fall in glucose, are reliable indicators of anaerobic metabolism and ischemia that can lead to irreversible cell damage in the brain.[46,48–51] Vespa and colleagues showed that under conditions of human brain ischemia, the L/P ratio increases to >40 and often plateaus in the range of 80–120.[50,52] Under conditions of impending brain death, the L/P ratio has been documented in the range of 500–1000. The increase in the ratio is comprised primarily of a reduction in the pyruvate concentration by 10– 100-fold, compared with increases in lactate by 2–5-fold.[52]

This supports the assumption that the metabolic changes we observed following contusion injury in the presence of sustained compression are indicative of tissue ischemia. In our study, in addition to a dramatic drop in glucose, we observed a rapid, significant, and persistent twofold decrease in pyruvate levels. Lactate levels also increased, but returned to baseline levels of the course of 4 h. Whereas the baseline L/P ratio was <20, following contusion with sustained compression we observed a rapid and distinct increase in the L/P ratio to $\sim$120 within 20 min, followed by a decrease after 60 min to reach a plateau of $\sim$45 for the remainder of the experiment. Of note, an L/P ratio >40 was reported by Vespa and colleagues to be indicative of irreversible ischemia.[50,52]

Similar to SCI with ongoing compression, the contusion injury also resulted in a transient increase in lactate and the lactate/pyruvate. This, however, was less pronounced and not associated with a drop in glucose, which might suggest an increased post-injury energy demand and the activation of glycolysis.[53] This potential contusion-related increase in glycolysis lasted $\sim$60 min after the injury, during which time cells were exposed to increased lactic acid. Other investigators have shown that neural injury and the acutely increased demand for energy, particularly to restore ionic homeostasis, quickly activates glycolysis and leads to the production of lactate.[53–55] In addition to hyperglycolysis, experimental contusive injury to the brain and spinal cord is also characterized by subsequent oxidative depression observed as early as 15–30 min post-injury.[56–59] These impaired mitochondrial bioenergetics can lead to reduced adenosine triphosphate (ATP) production, which provides an additional stimulus for increased glycolysis. Our observations suggest if this is a period of increased glycolysis, it is temporary. By 1 h, a decline in lactate and L/P ratio was observed, implying a recovery of oxidative metabolism.

It is noteworthy here that in the current study, the contusion group was tested with a drop height of 50 cm, but with subsequent period of compression of 5 min. Previously, we assessed the behavioral and histological consequences of such 5 min of compression following contusion injury.[7] This revealed quite dramatic differences in both the rate and extent of recovery, as well as histological outcome. Animals without compression achieved some level of walking, whereas the animals with 5 min of compression were at the earliest stages of stepping. Furthermore the longitudinal extent of the damage was less in the group without compression. At the lesion epicenter, white matter sparing was $\sim$25% in the animals without compression.[7] Therefore, the added 5 min of compression in our current study might have actually reduced the differences between our two injury conditions. Most likely, the metabolic derangements would have been even less pronounced following contusion injury in the absence of this brief 5 min period of compression afterwards.

The average MAP in the contusion only group was significantly lower than that of the contusion with compression group during the baseline period and the first hour post-injury (49.1 vs. 54.6 mm Hg during the baseline period, 47.1 vs. 55.5 mm Hg during the first hour). The reason for this difference in the baseline period was unclear. However, despite the contusion only group having the lower MAP during baseline and the first hour post-injury, this group did not have the metabolic changes consistent with ischemia that were observed in the group with sustained compression. Therefore, the fact that the contusion only group experienced a lower MAP does not significantly alter our interpretation of the different metabolic response after the addition of compression.

Methodological considerations

Although the microdialysate concentrations measured in our experimental model are likely related to metabolic activity within cells of the spinal cord extravasating into the extracellular fluid,

leakage caused by breakdown of the blood–spinal cord barrier contributing to these observations cannot be ruled out.[60–63] Probe insertion invariably causes a limited focal injury to the spinal cord, which can lead to a local compromise of blood–spinal cord barrier integrity and changes in oxygenation or blood flow in the immediate vicinity of the probe.[64–67] In our study, we observed in the sham animals a slight but steady increase in lactate and L/P ratio, with a late decrease in glucose levels. Possible explanations might be a result of the local damage induced by the probes or from the surgery and laminectomy themselves (or a combination thereof). By the end of the 4 h evaluation period, the animal had been under anaesthesia for close to 8 h, had undergone an extensive laminectomy, and had lost a considerable amount of blood. Various articles have demonstrated that probe insertion results in ''probe encapsulation'' and initiates an inflammatory response around the probe that can affect the recovery of small molecules such as those of glucose.[68,69] These changes, however, usually begin 24–36 h after probe implantation.[70] Because of the relative short duration of our experiments (total 6 h after probe insertion) most likely these substantial chronic processes have not yet occurred. We acknowledge that this complicates the interpretation of our results, but also highlights the importance of including sham groups in research studies. In the compression group, for example, lactate levels were elevated by twofold over pre-injury levels at the conclusion of the experiment; however, this was not statistically significant as compared with the sham animals, and would otherwise have been falsely interpreted as a persistent SCI-induced increase in lactate. Likewise, in the contusion group, the glucose levels were not significantly increased over pre-injury levels at the conclusion of the experiment; however, these glucose levels were almost twofold higher than in the sham animals.

Overall, the kinetics of the measured metabolites appeared to be highly reproducible when changes in metabolite levels in dialysates were compared among animals. Additionally, the low variability seen in the measurements of the sham animals suggests that microdialysis in this porcine model of SCI is an effective tool with which to study energy-related metabolic response. These results also reveal that these metabolic responses are still active and changing at 4 h post-injury and that we may not observe the full extent of the changes because of the time frame of the experiment. Whereas it would be very interesting to continue the experiment past 4 h, there are limitations for the animal (which undergoes almost 8 h of anaesthesia, with ongoing blood loss from the large posterior exposure and extensive laminectomy).

Conclusion and Significance

In the current study, we showed profound energy-related metabolic perturbations in the spinal cord upon injury, and these effects varied greatly depending upon the biomechanical nature of the injury. Our data on the disturbances in the energy metabolite levels are an important addition to the knowledge on the early pathogenesis of the SCI, particularly as it is influenced by the mechanisms of contusion and sustained compression. Clearly, past investigators have demonstrated the ability to monitor changes within the injured central nervous system (CNS) and CSF using microdialysis. However, in these studies, the ''insult'' has been either contusion alone or compression alone, or they are nontraumatic (ischemic) in nature. Using a contusion model of SCI followed by persistent compression, we confirmed that compression was an important factor that might jeopardize energy state of the hypoperfused tissue around the injury site manifested by altered extracellular concentrations of energy-related metabolites such as glucose, lactate, and pyruvate, as glycolytic pathways. The different mechanisms of energy disturbances in contusion with or without compression highlight the potential need for different treatment approaches to these injuries, particularly given that the degree of compression can be assessed clinically in patients with traumatic SCI.

Our model of contusion and of sustained compression was achieved through force applied to the dorsal surface of the spinal cord. Whereas this employed common methods of inducing injury to the spinal cord experimentally, it obviously differed from the clinical setting, in which contusion and compression are typically applied to the ventral surface of the cord. Our model of persistent dorsal compression also differed somewhat from the rodent clip compression model, in which compression is applied to both the ventral and dorsal surfaces of the cord. It is uncertain whether these differences in how sustained compression is applied would influence the metabolic responses of the surrounding tissue. We do feel, however, that the similarities in size of the pig spinal cord to the human spinal cord allow for a more comparable analysis of the spatial differences in metabolic response to the injury (e.g., at 2 and 4 cm from the injury site).

Our findings set the stage for further studies to discern how the spinal cord tissue adjacent to the injury site may respond to basic resuscitative measures, such as increased systemic blood pressure. The 4 h observation period bears some clinical relevance, in that patients with acute SCI are typically triaged in an emergency room setting within this time frame. Hemodynamic resuscitation measures are typically implemented within this time frame, and patients may also be considered for enrolment into trials of pharmacological treatments. Hence, the observation during this time period that the ''penumbra'' in the contusion only injury is responding very differently than that of the contusion plus compression injury is potentially important for the efficacy of these interventions. For example, in the case of the contusion injury without sustained compression, the metabolic changes that we observed were not that consistent with significant hypoperfusion and ischemia. This could then raise the question of whether aggressive hemodynamic interventions to drive up systemic blood pressure to maximize spinal cord blood flow are justified in such cases. Such a question can now be addressed in this model using these measurement techniques. Delineating how different injury mechanisms influence secondary pathophysiologic responses may help to inform the clinical community in its efforts to optimize the treatment of acutely injured SCI patients.

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Author Disclosure Statement

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