Effects of Glycemic Regulation on Chronic Postischemia Pain

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ABSTRACT

Background: Ischemia–reperfusion (I/R) injuries consist of enhanced oxidative and inflammatory responses along with microvascular dysfunction after prolonged ischemia and reperfusion. Because I/R injuries induce chronic postsurgical pain (CPIP) in laboratory animals, it is possible that surgical procedures using prolonged ischemia may result in chronic postoperative pain. Glycemic modulation during ischemia and reperfusion could affect pain after I/R injury because glucose triggers oxidative, inflammatory, and thrombotic reactions, whereas insulin has antioxidant, antiinflammatory, and vasodilatory properties.

Methods: One hundred ten rats underwent a 3-h period of ischemia followed by reperfusion to produce CPIP. Rats with CPIP had previously been divided into six groups with differing glycemic modulation paradigms: normal feeding; fasting; fasting with normal saline administration; fasting with dextrose administration; normal feeding with insulin administration; and normal feeding with insulin and dextrose administration. Blood glucose concentration was assessed during I/R injury and 3 h after I/R injury in these separate groups of rats, and these rats were tested for mechanical and cold allosthenia over the 21 days afterward (on days 2, 5, 7, 9, 12, and 21 after I/R injury).

Results: I/R injury in rats with normoglycemia or relative hyperglycemia (normal feeding and fasting with dextrose administration groups) led to significant mechanical and cold allosthenia; conversely, relative hypoglycemia associated with insulin treatment or fasting (fasting, fasting with normal saline administration, and normal feeding with insulin administration groups) reduced allosthenia induced by I/R injury. Importantly, insulin treatment did not reduce allosthenia when administered to fed rats given dextrose (normal feeding with dextrose and insulin administration group).

Conclusion: Study results suggest that glucose levels at the time of I/R injury significantly modulate postinjury pain thresholds in rats with CPIP. Strict glycemic control during I/R injury significantly reduces CPIP and, conversely, hyperglycemia significantly enhances it, which could have potential clinical applications especially in the surgical field.

What We Already Know about This Topic

- Ischemia and reperfusion of extremities in rats leads to hypersensitivity to touch and cold and represents a model of chronic pain after injury
- Whether glucose concentration or regulation affects this model is not known

What This Article Tells Us That Is New

- In rats with hind paw ischemia and reperfusion, hyperglycemia exacerbated the long-lasting hypersensitivity to touch and cold after reperfusion, suggesting glycemic control during surgeries involving prolonged ischemia may be important in the prevention of chronic pain after surgery

Numerous surgical procedures involve intentional ischemic states, to reduce blood loss and produce a “clean” surgical field, from orthopedic procedures using a tourniquet to any surgery involving organ resection, repair, or transplant requiring vascular clamps. Ischemia–reperfusion (I/R) injury, a complex condition consisting of intracellular injury and inflammatory phenomena,1–3 is a well-established posts ischemic consequence of a prolonged...
ischemic episode. Chronic pain disorders and various painful syndromes can result from procedures involving ischemia, and several factors that can affect the extent of I/R injury have been described, such as the duration of the ischemic period, the extent of the ischemic region, and the type of tourniquet used.

Various animal models of I/R injury have been created to study the underlying pathophysiological mechanisms of this phenomenon. Chronic postischemia pain (CPIP) is an animal model in which rats undergo hind paw ischemia for 3 h, induced by the application of a tight-fitting tourniquet around the ankle, followed by reperfusion. This I/R injury has been demonstrated to result in chronic mechanical and cold allodynia lasting for at least 4 weeks after the insulting event. In CPIP rats, well-described mechanisms of I/R injury have been demonstrated, such as increased oxidative and inflammatory responses and microvascular dysfunction. Indeed, in CPIP rats, free-radical induced lipid peroxidation, increased concentrations of proinflammatory cytokines tumor necrosis factor-α, interleukin-6, and interleukin-1β, increased concentrations of proinflammatory transcription factor nuclear factor κB and microvascular dysfunction (arterial vasospasms, capillary slow flow/no reflow) were demonstrated in the affected hind paw.

Factors that can decrease CPIP after I/R injury are not well known. Glycemia modulation, at the moment of the insult (i.e., during I/R injury), could potentially affect the chronic pain outcome. Glucose induces profound oxidative and inflammatory changes at the cellular and molecular levels, by activating several proinflammatory transcription factors and could potentially worsen CPIP. In contrast, insulin exerts vasodilatory and antiinflammatory effects by increasing the production of nitric oxide, and by suppressing proinflammatory cytokines synthesis, respectively, which could be potentially beneficial in alleviating CPIP symptomatology.

Glycemic modulation using insulin infusion has been demonstrated to be of benefit in reducing mortality and morbidity in the surgical intensive care unit, during cardiac surgery, and in patients with an acute myocardial infarction. Hyperglycemia has also been shown to worsen I/R injury in other organs such as the kidneys and brain. Numerous studies have observed and described neuropathic pain as a consequence of chronic hyperglycemia, using diabetic animal models, but nothing is known about the role of acute glycemic modulation during an I/R injury and its consequences on postischemia pain.

We hypothesize that hyperglycemia (relative hyperglycemia) at the time of the insult will be associated with an enhanced postischemia pain, whereas strict glycemic control (relative hypoglycemia) at the time of the insult will be associated with a reduced postischemia pain. The current study was aimed at testing this hypothesis in CPIP rats after modulating the glycemia of the animals during I/R.

### Materials and Methods

#### Animals

The current study was conducted on male Long Evans rats (300–500 g, Charles River, Senneville, Quebec, Canada). A total of 110 rats were used for the study. Animals were housed in groups of two to four, under controlled lighting (12-h light-dark cycle) and temperature conditions. Food and water were available ad libitum, except on the day before and on the day of the experimental procedures (see Glycemic Treatments section). The experiments performed in the current study were approved by the McGill Animal Care and Ethics committees, and were in conformance with the ethical guidelines of the Canadian Council on Animal Care and the International Association for the Study of Pain.

#### Hind Paw I/R

An I/R injury of the left hind paw was induced as previously described. Rats were anesthetized for a period of 3 h with an intraperitoneal bolus (55 mg/kg) of sodium pentobarbital, followed by an intraperitoneal infusion of the anesthetic for 2 h (27.5 mg/kg/h). After induction of anesthesia, a Nitrile 70 Durometer O-ring (O-rings, Seattle, WA) with a 5.5-mm internal diameter was placed around the rat’s left hind paw, proximal to the ankle and left in place for 3 h. After a 3-h period of ischemia, the O-ring was cut and normal blood flow to the injured limb was reestablished. We have previ-

### Table 1. Analysis of Variance Table for Glucose Measurements

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic condition</td>
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<td>5</td>
<td>157.70</td>
<td>54.737</td>
<td>0.000000</td>
</tr>
<tr>
<td>Injury</td>
<td>0.28</td>
<td>1</td>
<td>0.28</td>
<td>0.096</td>
<td>0.757888</td>
</tr>
<tr>
<td>Condition × injury</td>
<td>21.34</td>
<td>5</td>
<td>4.27</td>
<td>1.482</td>
<td>0.203222</td>
</tr>
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<td>Error</td>
<td>270.83</td>
<td>94</td>
<td>2.88</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Time</td>
<td>22.49</td>
<td>6</td>
<td>3.75</td>
<td>4.303</td>
<td>0.000296</td>
</tr>
<tr>
<td>Time × condition</td>
<td>558.03</td>
<td>30</td>
<td>18.60</td>
<td>21.352</td>
<td>0.000000</td>
</tr>
<tr>
<td>Time × injury</td>
<td>9.52</td>
<td>6</td>
<td>1.59</td>
<td>1.821</td>
<td>0.092723</td>
</tr>
<tr>
<td>Time × condition × injury</td>
<td>106.00</td>
<td>30</td>
<td>3.53</td>
<td>4.056</td>
<td>0.000000</td>
</tr>
<tr>
<td>Error</td>
<td>491.34</td>
<td>564</td>
<td>0.87</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Glycemic condition and injury are independent groups factors and time is a repeated factor. df = degrees of freedom; F = F statistic; MS = mean square; SS = sum of squares.
pentobarbital infusion, the rats had recovered a normal respiratory pattern and could be safely returned to their housing cages.

**Glycemic Treatments**

To study the effect of glycemic modulation on chronic postischemia pain, six groups of rats were submitted to different glycemic conditions. Glycemia was manipulated (increased, decreased, or unaltered) to make comparisons between groups of rats with differing mean glycemia. Fifteen to 21 rats were included in each group. In the regular diet (fed) group, animals had unlimited access to food and water before anesthesia. The second group of animals (fasted) had access to food withdrawn 18 h before anesthesia, but had unlimited access to water. The third group of animals (fasted + normal saline [NS]) had access to food withdrawn 18 h before anesthesia, but had unlimited access to water; these rats received three intraperitoneal injections of 1 ml NS at baseline, and at 60 and 120 min after the start of ischemia. The fourth group of rats (fasted + dextrose) had access to food withdrawn 18 h before anesthesia but had normal access to water; these rats received three intraperitoneal injections, each 1 ml, of dextrose dissolved in deionized water (final concentration: 40%, w/v, from now on referred to as DW40%): at baseline, and at 60 and 120 min after the start of ischemia. The fifth group of rats (fed + insulin) were fed normally but were administered subcutaneous injections of insulin R diluted in NS, according to a sliding scale we created for this experiment (glycemia at baseline 6 or less: 0.5 IU/kg; > 6 but < 7: 1 IU/kg; > 7 but < 8: 1.5 IU/kg; > 8 but < 9: 2 IU/kg; > 9 but < 10: 2.5 IU/kg; because none of the rats had a baseline glycemia more than 10, it was not necessary to further extend the insulin sliding scale). The total dose of insulin was administered in two half-doses, the first one immediately after the onset of ischemia and the second 60 min after induction of ischemia. The maximal volume of insulin administered was 50 μl. The last group of rats (fed + insulin + dextrose) were fed normally and received insulin according to a sliding scale (as group 5) at the same time points. However, they were also administered intraperitoneal DW40% (as group 4) to maintain their glycemia above the baseline value.

**Table 2.** Analysis of Variance Table for Ipsilateral Paw-Withdrawal Thresholds

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic condition</td>
<td>1,378.42</td>
<td>5</td>
<td>275.68</td>
<td>13.659</td>
<td>0.000000</td>
</tr>
<tr>
<td>Injury</td>
<td>4,296.72</td>
<td>1</td>
<td>4,296.72</td>
<td>212.882</td>
<td>0.000000</td>
</tr>
<tr>
<td>Condition × injury</td>
<td>548.22</td>
<td>5</td>
<td>109.64</td>
<td>2.788</td>
<td>0.016978</td>
</tr>
<tr>
<td>Error</td>
<td>2,058.72</td>
<td>102</td>
<td>20.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>158.75</td>
<td>5</td>
<td>31.75</td>
<td>3.645</td>
<td>0.003000</td>
</tr>
<tr>
<td>Time × condition</td>
<td>231.86</td>
<td>25</td>
<td>9.27</td>
<td>1.065</td>
<td>0.380037</td>
</tr>
<tr>
<td>Time × injury</td>
<td>121.44</td>
<td>5</td>
<td>24.29</td>
<td>2.788</td>
<td>0.016978</td>
</tr>
<tr>
<td>Time ×condition × injury</td>
<td>478.93</td>
<td>25</td>
<td>19.16</td>
<td>2.199</td>
<td>0.000801</td>
</tr>
<tr>
<td>Error</td>
<td>4,442.82</td>
<td>510</td>
<td>8.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glycemic condition and injury are independent groups factors and time is a repeated factor.

df = degrees of freedom; F = F statistic; MS = mean square; SS = sum of squares.
**Glucose Measurements**

Glucose levels were measured with a glucometer *FreeStyle mini* (Abbott Diabetes Care, Mississauga, Ontario, Canada). Blood was withdrawn from the extremity of the rat tail, from a submillimetric incision, by an observer blinded to treatment. Volume of blood withdrawn varied between 0.3 and 0.5 ml. The glycemia of the rats was regularly monitored during the ischemic period and the first hour of reperfusion. Blood samples were withdrawn at baseline, and then 30, 90, and 150 min after induction of ischemia, as well as 20, 40, and 60 min after reperfusion.

**Mechanical and Thermal Sensitivity**

**Hind Paw Mechanoallodynia.** Mechanical alldynia was assessed by measuring the 50% withdrawal response to von Frey filaments, according to a modification of a previously published method,32 by an observer blinded to treatment. The animals were placed in Plexiglas® cages with a wire grid bottom. von Frey filaments (Stoelting, Wood Dale, IL) were applied to the plantar surface of the hind paw for 10 s or less if the rat responded to the stimulus by withdrawing, flicking, stamping, or licking its paw. Filaments were applied in ascending or descending order of strength to determine the filament closest to the response threshold. The minimal stimulus intensity was 0.25 g, whereas the maximal intensity was 15 g.

**Cold Allodynia**

Cold alldynia was evaluated by exposing the rats’ paw to a drop of acetone, using a modification of a previously published method,33 by an observer blinded to treatment. A drop...
of acetone was applied on the plantar surface of the heel, and the rat’s response was observed for 20 s afterward. Normal rats either ignore the stimulus or respond with a rapid, brief withdrawal. The intensity and duration of the rats’ responses to the nociceptive stimulus were scored according to the following rating scale:

1. Rapid paw flicking, stamping, or shaking, less than 1 s;
2. Repeated paw stamping, shaking, or paw lift less than 3 s;
3. Above behaviors or hind paw licking for more than 3 s.

Acetone was applied 3 times over 3 min, and the 3 trials were then averaged.

Statistical Analysis
Glucose levels (mM), paw withdrawal thresholds (g), and acetone scores (nociceptive cold scores) were expressed as mean ± 95% confidence limits. Statistical differences between glycemic values at baseline before ischemia and at six subsequent points in time (i30, i90, i150, r20, r40, and r60; i = ischemia, r = reperfusion) were assessed with Tukey post hoc tests after a three-way ANOVA (glycemic condition by injury (CPIP or sham CPIP) by time as the repeated factor). Statistical differences between groups, collapsed over time on mechanical and cold allodynia of the ipsilateral and contralateral hind paws, were similarly assessed using a mixed model repeated measures ANOVA and Tukey post hoc tests.

Comparisons were considered significant if $P < 0.05$ (two-tailed tests in all cases). Correlations between glucose levels (averaged over the ischemia, reperfusion or ischemia and reperfusion periods) and von Frey or acetone cold scores (averaged for each subject over the entire behavioral testing period) were calculated using the Spearman rank correlation coefficient ($\rho$). The coefficients of determinations were obtained using the Pearson correlation. Differences between correlation coefficients were assessed using the Z score. Statistics were computed with Statistica (Version 6, Statsoft, Tulsa, OK), and graphs were prepared with GraphPad Prism 5.00 (GraphPad Software, Inc., 2007, La Jolla, CA).

Results
Glycemic Measurements
Three-way repeated measures ANOVA (see table 1) revealed nonsignificant main effects of injury (i.e., sham or CPIP) on glucose level, and main effect analysis indicated that there were no significant differences in the mean glucose levels between the CPIP groups and their sham control groups for the fed animals (6.75 ± 0.40 vs. 6.83 ± 0.57), the fasted + ns animals (4.62 ± 0.46 vs. 4.65 ± 0.57), the fed + insulin animals (5.62 ± 0.42 vs. 5.50 ± 0.44), the fasted + dextrose animals (7.25 ± 0.46 vs. 6.96 ± 0.44), the fed + insulin +

Table 3. Analysis of Variance Table for Contralateral Paw-Withdrawal Thresholds

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic condition</td>
<td>585.16</td>
<td>5</td>
<td>117.03</td>
<td>4.734</td>
<td>0.000626</td>
</tr>
<tr>
<td>Injury</td>
<td>1,417.38</td>
<td>1</td>
<td>1,417.38</td>
<td>57.334</td>
<td>0.000000</td>
</tr>
<tr>
<td>Condition × injury</td>
<td>446.40</td>
<td>5</td>
<td>89.28</td>
<td>3.611</td>
<td>0.004763</td>
</tr>
<tr>
<td>Error</td>
<td>2,521.59</td>
<td>102</td>
<td>24.72</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Time</td>
<td>155.27</td>
<td>5</td>
<td>31.05</td>
<td>3.314</td>
<td>0.005904</td>
</tr>
<tr>
<td>Time × condition</td>
<td>211.74</td>
<td>25</td>
<td>8.47</td>
<td>0.904</td>
<td>0.600716</td>
</tr>
<tr>
<td>Time × injury</td>
<td>49.52</td>
<td>5</td>
<td>9.90</td>
<td>1.057</td>
<td>0.383477</td>
</tr>
<tr>
<td>Time × condition × injury</td>
<td>243.28</td>
<td>25</td>
<td>9.73</td>
<td>1.038</td>
<td>0.413632</td>
</tr>
<tr>
<td>Error</td>
<td>4,779.32</td>
<td>510</td>
<td>9.37</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Glycemic condition and injury are independent groups factors and time is a repeated factor.

df = degrees of freedom; F = F statistic; MS = mean square; SS = sum of squares.

Fig. 4. (A–F) Time course of contralateral paw withdrawal thresholds (PWTs) (mean ± 95% confidence limits) for the fed, fasted, fasted + normal saline (NS), fasted + dextrose (dex), fed + insulin (ins) and fed + ins + dex groups. Contralateral PWTs of rats with chronic postischemia pain were not significantly lower than those of rats in the sham group on any testing day for rats in all glycemic conditions.
Mechanical and Thermal Hypersensitivity

Ipsilateral Paw Withdrawal Thresholds. Generally, CPIP animals had significantly lower ipsilateral paw withdrawal thresholds (PWTs) than animals in the sham group. Three-way repeated measures ANOVA (see table 2) revealed a significant main effect of injury (i.e., sham group or CPIP group). Time course analysis with post hoc pairwise comparisons, depicted in figure 2, showed that the ipsilateral PWTs of CPIP rats were lower than that of rats in the sham group on each testing day for rats in the fed and fasted + dextrose glycemic conditions, and for four of the six test days for the fed + insulin + dextrose glycemic condition (\( P < 0.01; \) Tukey post hoc test).

In order to examine the highly significant glycemic condition \( \times \) injury interaction (see table 2) we plotted PWTs grouped by glycemic condition and injury averaged over time for the ipsilateral hind paw (fig. 3A) and contralateral hind paw (fig. 3B). Ipsilateral PWTs were significantly lower for CPIP rats compared with rats in the sham group in five of the six glycemic conditions (i.e., fed, fasted, fasted + dex, fed + insulin, fed + insulin + dextrose, but not in the fasted + NS condition (fig. 3A) \( * = P < 0.05; ** = P < 0.01; \) Tukey tests). Comparing CPIP rats across conditions, the fed rats exhibited significantly lower PWTs compared with the fasted group (\( \$ = P < 0.01 \)), and the fasted + dextrose group of rats exhibited significantly lower PWTs than fasted + ns rats (\( \# = P < 0.01 \)). The fed CPIP rats also showed significantly lower PWTs than did the fed + insulin rats (\( \& = P < 0.01 \)). The PWTs of the fed + insulin + dextrose group of CPIP rats were significantly lower than those of the fed + insulin group (\( \% = P < 0.01 \)) (all Tukey tests).

Contralateral PWTs. CPIP rats generally appeared to exhibit lower contralateral PWTs than rats in the sham group, with greater effects for the fasted + dextrose and fed + insulin + dextrose conditions. Three-way repeated measures ANOVA (see table 3) revealed a significant main effect of injury (i.e., sham or CPIP). However, time course analysis with post hoc pairwise comparisons, depicted in figure 4, showed that the contralateral PWTs of CPIP rats were not significantly lower than rats in the sham group on any testing day for rats in all glycemic conditions (Tukey post hoc test).

Once again to examine the highly significant glycemic condition \( \times \) injury interaction (see table 3) we plotted PWTs grouped by glycemic condition and injury averaged over time for the contralateral hind paw (fig. 3B). PWTs were significantly lower for CPIP rats compared with rats in

dextrose animals (7.66 \( \pm \) 0.49 vs. 7.65 \( \pm \) 0.46), and the fasted group (4.75 \( \pm \) 0.42 vs. 5.42 \( \pm \) 0.44) (all \( P > 0.05 \), Tukey post hoc tests).

Time course analysis with post hoc pairwise comparisons, depicted in figure 1 showed that glucose levels were significantly increased over baseline in the fasted + dextrose and fed + insulin + dextrose groups, and significantly decreased in the fasted, fasted + ns, and fed + ins groups, at the times indicated by the asterisks in figure 1 (\( * = P < 0.05; ** = P < 0.01 \), Tukey post hoc test). There were also significant differences in glucose levels between the fed and the fasted groups (\( \dagger = P < 0.01 \), Tukey post hoc test), the fasted + NS and the fasted + dextrose groups (\( \$ = P < 0.01 \)), the fed and the fed + insulin groups (\( \& = P < 0.01 \)), and between the fed + insulin and the fed + insulin + dextrose groups (\( \% = P < 0.01 \)) at the times indicated by the symbols (Tukey post hoc test). Generally glucose levels varied between glycemic conditions to a greater degree during ischemia than during reperfusion, as documented by the significant glycemic condition by time interaction (see table 1).

Fig. 5. Scatterplot illustrating the relationship between ipsilateral paw withdrawal thresholds and glycemia in rats with chronic postischemia pain (CPIP) using glucose levels for the entire ischemia-reperfusion injury (A), for only the ischemic period (B) or only the reperfusion period (C). Specific groups in the scatterplot are identified in the symbol legend, with open symbols used for relatively hypoglycemic groups and closed symbols for relatively hyperglycemic groups. For each plot there was a significant negative correlation between the paw withdrawal thresholds and the mean glucose level (mM) during ischemia/reperfusion injury for CPIP rats \( (R^2 = \text{coefficient of determination}) \). dex = dextrose; ins = insulin; NS = normal saline; PWT = paw withdrawal threshold.
the sham group in three of the six glycemic conditions (i.e., fasted + dextrose, fed + insulin, fed + insulin + dextrose, but not in the fed, fasted, or fasted + NS conditions (* = $P < 0.05$; ** = $P < 0.01$; Tukey post hoc tests). Although the differences were not as extensive as for ipsilateral PWTs, contralateral PWTs of CPIP rats also differed significantly between glycemic conditions. Thus, the fed + insulin + dextrose group had significantly lower PWTs than the fed + ins group († = $P < 0.01$) and the fed group (§ = $P < 0.01$). The fasted + dextrose group also exhibited significantly lower contralateral PWTs than the fasted + NS group (# $P < 0.01$) (all Tukey post hoc test).

As displayed in the scatterplot of ipsilateral PWTs versus glucose levels in figure 5A, relatively hyperglycemic CPIP animals have lower PWTs than relatively hypoglycemic CPIP animals. Indeed, there is a negative correlation ($rs = -0.679, P = 0.000019$) between the mean PWTs and glucose levels measured during the entire I/R injury period across all groups. Thus, 46.1% of the observed variation in the ipsilateral PWTs depends on glycemia. This negative correlation is also significant when using glucose levels from only the ischemic period ($rs = -0.704, P = 0.000022$) or from only the reperfusion period ($rs = -0.588, P = 0.015$), with 49.4% (fig. 5B) and 34.6% (fig. 5C), of the observed variation in ipsilateral PWTs depending on the glucose levels measured during these periods, respectively. The correlation between PWTs and glucose levels during ischemia is not statistically different from the correlation between PWTs and glucose levels during reperfusion ($Z = 0.783, P > 0.05$).

**Cold Scores**

**Ipsilateral Cold Scores.** Generally, CPIP animals developed significantly higher ipsilateral cold scores than those in the sham group. Three-way repeated measures ANOVA (see table 4) revealed a significant main effect of injury (i.e., sham or CPIP). Time course analysis with post hoc pairwise comparisons, depicted in figure 6, showed that the ipsilateral cold scores of CPIP rats were higher than those of rats in the sham group on three of the testing day for rats in the fed glycemic condition, and for two of the testing days for the fasted + dextrose glycemic condition (Tukey post hoc test). To examine the highly significant glycemic condition × injury interaction (see table 2) we plotted cold scores grouped by glycemic condition and injury averaged over time for the ipsilateral hind paw (fig. 7A) and the contralateral hind paw (fig. 7B). Ipsilateral nociceptive cold scores generally increased with the number of postischemic days (fig. 6A). The fasted animals had significantly lower cold scores than the fed group on test days 5, 9, and 12 for rats in the fed glycemic condition, and on test days 7 and 12 for the fasted + dex glycemic condition (** $P < 0.01$, Tukey post hoc test). CPIP = chronic postischemia pain.

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**Table 4. Analysis of Variance Table for Ipsilateral Nociceptive Cold Scores**

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P Value</th>
</tr>
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<tr>
<td>Glycemic condition</td>
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<td>7.8719</td>
<td>9.408</td>
<td>0.000000</td>
</tr>
<tr>
<td>Injury</td>
<td>87.5721</td>
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<td>87.5721</td>
<td>104.656</td>
<td>0.000000</td>
</tr>
<tr>
<td>Condition × injury</td>
<td>13.8772</td>
<td>5</td>
<td>2.7754</td>
<td>3.317</td>
<td>0.008494</td>
</tr>
<tr>
<td>Error</td>
<td>76.1453</td>
<td>91</td>
<td>0.8368</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Time</td>
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<td>4.4357</td>
<td>14.770</td>
<td>0.000000</td>
</tr>
<tr>
<td>Time × condition</td>
<td>9.8359</td>
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<td>0.3934</td>
<td>1.310</td>
<td>0.146277</td>
</tr>
<tr>
<td>Time × injury</td>
<td>1.2454</td>
<td>5</td>
<td>0.2491</td>
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<td>0.529180</td>
</tr>
<tr>
<td>Time × condition × injury</td>
<td>13.4952</td>
<td>25</td>
<td>0.5398</td>
<td>1.797</td>
<td>0.011062</td>
</tr>
<tr>
<td>Error</td>
<td>136.6437</td>
<td>455</td>
<td>0.3003</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Glycemic condition and injury are independent groups factors and time is a repeated factor. df = degrees of freedom; F = F statistic; MS = mean square; SS = sum of squares.
Contralateral Cold Scores. CPIP rats generally appeared to exhibit higher contralateral cold scores than rats in the sham group, with greater effects for the fasted + ns and fed + insulin + dextrose conditions. Three-way repeated measures ANOVA (see table 5) revealed a significant main effect of injury (i.e., sham or CPIP). Time course analysis with post hoc pairwise comparisons, depicted in figure 8, showed that the contralateral PW Ts of CPIP rats were significantly higher than rats in the sham group on only one test day for rats in the fasted + ns and fed + insulin + dextrose glycemic conditions (* = P < 0.05, ** = P < 0.01, Tukey post hoc test).

Once again we plotted nociceptive cold scores grouped by glycemic condition and injury averaged over time for the contralateral hind paw (fig. 7B). Nociceptive cold scores were significantly higher for CPIP rats compared with rats in the sham group in only the fed + insulin + dextrose glycemic condition (fig. 3A) (** = P < 0.01; Tukey post hoc tests). Furthermore for CPIP rats, the fed + insulin + dextrose group exhibited significantly higher contralateral cold scores than the fed + insulin group († = P < 0.01) and the fed group (§ = P < 0.01). There was no significant difference between the contralateral cold scores of the fasted + dextrose group compared with the fasted + NS group (P > 0.05).

As displayed in the plot of ipsilateral cold scores versus glucose levels in figure 9A, relatively hyperglycemic CPIP animals have higher cold scores than relatively hypoglycemic CPIP animals. Thus, there is a positive correlation (rs = 0.490, P = 0.0055) between the mean cold scores and the glucose levels measured during the entire I/R period. Thus, 24% of the observed variation in the acetone scores depends on glycemia. This positive correlation is also significant when using glucose levels from only the ischemic period (rs = 0.528, P = 0.0203) or only the reperfusion period (rs = 0.419, P < 0.022), with 27.9% (fig. 9B) and 17.5% (fig. 9C) of the observed variation in ipsilateral cold responses depending on the glucose levels measured during these periods, respectively. The correlation between ipsilateral cold scores and glucose levels during ischemia is not statistically different from the correlation between ipsilateral cold responses and glucose levels during reperfusion (Z = 0.456, P > 0.05). Consequently, the glycemia present during these two periods similarly influence the ipsilateral cold alldynia exhibited after I/R injury.

Discussion

This study has demonstrated that manipulating glycemia during I/R injury significantly influences the degree of alldynia in CPIP rats. Thus, CPIP animals who have a higher glucose concentration, such as the normally fed animals and the animals receiving DW40%, exhibited significantly higher mechanical and cold alldynia than the animals with decreased glucose concentration, such as the fasted rats and fed rats in whom insulin was administered. These results

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strongly suggest a protective role for relative hypoglycemia during I/R injury.

The Effect of Glucose on Postischemia Pain

Hyperglycemia can increase oxidative stress by several mechanisms. Thus, in a hyperglycemic state, the production of reactive oxygen species such as the superoxide radical is increased. In addition, the concentration of endogenous antioxidants, such as \( \alpha \)-tocopherol, are decreased by hyperglycemia. Along with oxidative stress, hyperglycemia activates several proinflammatory transcription factors, such as nuclear factor \( \kappa B \), activator protein 1, and early growth response protein 1. The activation of these transcription factors leads to increased production of several proinflammatory mediators such as cytokines, chemokines, cell adhesion molecules, inflammatory enzymes, and some acute phase proteins, which amplify the inflammatory process.

Hyperglycemia has previously been shown to increase the levels of proinflammatory mediators in a rat model of cerebral I/R, exacerbating the ischemic insult. Thus, it is possible that hyperglycemic rats in our study suffered from an increased ischemic insult, the burden of which was added to the oxidative and inflammatory states. Although the relative contribution of these three pathologic phenomena is yet to be determined, their increases are likely to explain the enhanced cold and mechanical allodynia we observed.

Another potential contributory mechanism that could have led to an altered pain threshold in hyperglycemic rats could be related to thrombotic phenomena. Hence, glucose plays a role in the activation of a cascade ultimately leading to increased production of several proinflammatory mediators such as cytokines, chemokines, cell adhesion molecules, inflammatory enzymes, and some acute phase proteins, which amplify the inflammatory process.

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The Effect of Insulin on Postischemia Pain

Fed animals in whom insulin was administered have less allodynia than the fed animals that did not receive insulin. In addition, fed animals that received both DW40% and insulin...
lin exhibited more allodynia than the fed animals that received insulin without DW40%. The difference in pain thresholds for the latter two groups could be caused by two factors. By decreasing blood glucose, insulin could counteract the oxidative, inflammatory, and thrombotic cascades that occur during a hyperglycemic state, or alternatively insulin could itself exert intrinsic antiinflammatory, antioxidative, and antithrombotic effects. However, considering that insulin was administered to these two groups according to the same sliding scale, and that the only variable changed was the supplemental dextrose (which clearly increased blood glucose), the results support the hypothesis that insulin administration \textit{per se} is not as protective as decreasing glucose levels.

**Contralateral Effects**

Significant contralateral allodynia has been described for the normally fed CPIP animals and in various models of neuropathic and inflammatory pain. The accepted explanation for these contralateral effects is central sensitization caused by the peripheral activation of C-afferent nociceptive fibers, which modify the functional response of the spinal cord neurons to other inputs applied after the conditioning input. Interestingly, in our experiments, the fed + insulin + dextrose CPIP rat group exhibited significantly lower PWTs and higher cold scores on the contralateral side than the fed CPIP rat group, which suggests that central sensitization was more prominent in these rats. To explain this observation, we hypothesize that hyperglycemia associated with the administration of dextrose leads to more significant ischemic injury and increased central inputs, which in turn increase central sensitization. A second possibility (although unsupported by previous literature) is that dextrose has central effects that enhance pain facilitation.

**Potential Clinical Applications**

The observation that glycemic modulation affects postischemia pain has clinical implications, especially in the operating room. Postischemia pain and painful syndromes such as complex regional pain syndrome type I can theoretically follow any surgical procedure or trauma involving prolonged ischemia, such as open reduction and internal fixation of fractures or arthroscopic surgery. The occurrence and chronicity of postischemia pain could potentially be significantly reduced by intraoperative maintenance of strict glycemic control, potentially by an insulin infusion as patients usually are already fasting before a surgery, keeping in mind, of course, the deleterious effects of severe hypoglycemia. It is also tempting to extrapolate that dextrose infusions during a surgical procedure may contribute to postoperative pain, and should therefore be avoided if possible.

**Conclusions**

Our investigations point to a critical role of glycemia in CPIP, with significantly increased pain sensitivity when animals have higher glucose levels at the time of the injury, and significantly lower pain sensitivity when animals have lower glucose levels at the time of the injury, whether this is achieved by fasting or by insulin administration. As mentioned in the discussion, we believe that the prooxidative, inflammatory, and thrombotic properties of hyperglycemia play a critical role in leading to enhanced allodynic/hyperalgesic states. Of interest, our results demonstrate that higher glucose levels at the time of I/R injury also lead to enhanced pain sensitivity in the contralateral limb, suggesting that hypoglucemia might be beneficial in reducing postischemic pain.

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**Fig. 9.** Scatterplots illustrating the relationship between ipsilateral nociceptive score and glycemia in rats with chronic posts ischemia pain (CPIP) using glucose levels for the entire ischemia-reperfusion injury (A), for only the ischemic period (B) or only the reperfusion period (C). Specific groups in the scatterplot are identified in the symbol legend, with open symbols used for relatively hypoglycemic groups and closed symbols for relatively hyperglycemic groups. For each period (ischemia and reperfusion), there was a significant positive correlation between the nociceptive cold score and the mean glucose value (mM) during ischemia/reperfusion injury for CPIP rats ($R^2$ = coefficient of determination). dex = dextrose; ins = insulin; NS = normal saline.
perglycemia might play a role in the development of central sensitization. Our results might lead to several interesting clinical applications, most notably in the surgical field. Hence, it is tempting to extrapolate that maintaining strict glucose levels during a surgical procedure might lead to a reduction in postsurgical pain.

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