Influence of oxygen therapy on glucose–lactate metabolism after diffuse brain injury

MICHAEL REINERT, M.D., BENOIT SCHALLER, M.D., HANS RUDOLF WIDMER, PH.D., ROLF SEILER, M.D., AND ROSS BULLOCK, M.D., PH.D.

Department of Neurosurgery, University of Bern, and Inselspital Bern, Switzerland; and Department of Neurosurgery, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

Object. Severe traumatic brain injury (TBI) imposes a huge metabolic load on brain tissue, which can be summarized initially as a state of hypermetabolism and hyperglycolysis. In experiments O2 consumption has been shown to increase early after trauma, especially in the presence of high lactate levels and forced O2 availability. In recent clinical studies the effect of increasing O2 availability on brain metabolism has been analyzed. By their nature, however, clinical trauma models suffer from a heterogeneous injury distribution. The aim of this study was to analyze, in a standardized diffuse brain injury model, the effect of increasing the fraction of inspired O2 on brain glucose and lactate levels, and to compare this effect with the metabolism of the noninjured sham-operated brain.

Methods. A diffuse severe TBI model developed by Foda and Maramarou, et al., in which a 420-g weight is dropped from a height of 2 m was used in this study. Forty-one male Wistar rats each weighing approximately 300 g were included. Anesthetized rats were monitored by placing a femoral arterial line for blood pressure and blood was drawn for a blood gas analysis. Two time periods were defined: Period A was defined as preinjury and Period B as postinjury. During Period B two levels of fraction of inspired oxygen (FiO2) were studied: air (FiO2 0.21) and oxygen (FiO2 1).

Four groups were studied including sham-operated animals: air-air-sham (AAS); air-O2-sham (AOS); air-air-trauma (AAT); and air-O2-trauma (AOT). In six rats the effect of increasing the FiO2 on serum glucose and lactate was analyzed.

During Period B lactate values in the brain determined using microdialysis were significantly lower (p < 0.05) in the AOT group than in the AAT group and glucose values in the brain determined using microdialysis were significantly higher (p < 0.04). No differences were demonstrated in the other groups. Increasing the FiO2 had no significant effect on the serum levels of glucose and lactate.

Conclusions. Increasing the FiO2 influences dialysate glucose and lactate levels in injured brain tissue. Using an FiO2 of 1 influences brain metabolism in such a way that lactate is significantly reduced and glucose significantly increased. No changes in dialysate glucose and lactate values were found in the noninjured brain.

Key Words • severe head injury • oxygen • glucose • lactate • rat

Early after trauma, brain tissue is exposed to a cascade of events resulting in hypermetabolism.1,2,3,4,5,6,7 Experimental and clinical research has unveiled a number of pathophysiologic mechanisms, some of them described by an exaggerated, uncontrolled physiological phenomenon such as glutamate excitotoxicity,8 increase in extracellular K,9 and increases in intracelluar as well as intramitochondrial Ca,10,11 These phenomena have been associated with posttraumatic hyperglycolysis, leading to an increase in lactate as a result of a mismatch between mitochondrial and glycolytic ATP production. Pellerin and Magistretti12,13,14,15,16,17 have shown that this pathway may be independent of an anerobic metabolic state.1 Yet O2 demand after trauma has been found to be increased, especially during the early phase.2 Secondary posttraumatic cerebral ischemia is very common after severe head injury and, as a result of a mismatch between demand and availability of O2, is associated with an unfavorable outcome.8 Hyperventilation resulting in cerebral vasodilatation has, therefore, been correlated with reduced brain-tissue O2 tensions18 and a worsening neurological outcome. This approach has thus been abandoned as a long-term therapeutic prophylaxis for the treatment of increased intracranial pressure.9 Low brain-tissue O2 tensions and reduced jugular bulb O2 saturation have been directly linked to bad outcomes in patients with severe head injury; monitoring of these parameters is routinely performed at present.10 In several clinical studies an improvement in brain tissue O2 has been demonstrated by increasing the FiO2.19-27 Lactate levels in brain dialysate lactate levels were lowered using this maneuver, whereas glucose levels in the brain showed a trend toward an increase. The impact of increasing the FiO2 as a therapeutic procedure is still controversial, especially as it relates to the negative effects of prolonged increased O2 therapy. Conclusion results are difficult to obtain in patients suffering from severe human head injury because of large differences in the morphological characteristics of the injury, its pro-
injury or sham injury during which all groups received ventilation using the same parameters (FiO2 0.21 [air]). Period B was the monitoring period after injury or sham injury during which different ventilation parameters were used according to the study group (FiO2 settings were analyzed (air level in which the O2 was 21% [air] and O2 level in which the O2 was 100% [O2]). Because FiO2 settings were analyzed (air level in which the O2 was 21% [air] and O2 level in which the O2 was 100% [O2]). Because

Since there was a trauma group and a sham-operated group, the study consisted of a total of four groups: AAT, AOT, AAS, and AOS (Fig. 1). Six sham-operated animals were used to assess the effect of increased O2 delivery (FiO2 from 0.21 to 1.0) on serum lactate and glucose levels.

**Trauma Model**

A standardized weight-drop injury model (420-g weight dropped from a height of 2 m) was used to produce a DBI with a level of injury described as “severe.” The trauma model–related mortality rate was 14.2%, which was described by Foda and Marmarou, et al.

The location of the hit and the location and depth of brain penetration made by the microdialysis probe are shown in Fig. 2. A total of 40 animals underwent surgery. Nine animals comprised Group AOT, seven Group AAT, seven Group AAS, and five Group AOS. Six animals were studied to assess their serum lactate and glucose levels.

**Induction of Anesthesia**

The rats were sedated by administering an isoflurane/air mixture. An intraperitoneal injection of 5 mg pentobarbital (Nembutal) was then given and the animals were intubated with a tracheal (Ohmeda Medical, Laurel, MD) catheter for intubation and anesthesia. Anesthesia was maintained at a level of 2 MAC by using a Harvard ventilator (Harvard Apparatus, Inc., Holliston, MA). Thereafter, the depth of anesthesia was steadily reduced during further animal preparation.

**Physiological Parameters**

An arterial line for continuous measurement of MABP was inserted for 30 seconds from the ventilator and isoflurane-induced anesthesia was stopped for 2 minutes and then resumed to standard levels. For trauma to be delivered, the rats were disconnected from the ventilator and isoflurane-induced anesthesia was stopped. An intraperitoneal injection of 5 mg pentobarbital (Nembutal) was then given and the animals were returned to the isoflurane chamber.

**Oxygen Therapy**

Oxygen therapy in different levels was delivered to the animals during the monitoring period. Period A was defined as the period before trauma or a sham operation; and Period B, which occurred after trauma or sham trauma (sham operation). In Period B two levels of ventilation FiO2 settings were analyzed (air level in which the O2 was 21% [air] and O2 level in which the O2 was 100% [O2]). Because there was a trauma group and a sham-operated group, the study consisted of a total of four groups: AAT, AOT, AAS, and AOS (Fig. 1). Six sham-operated animals were used to assess the effect of increased O2 delivery (FiO2 from 0.21 to 1.0) on serum lactate and glucose levels.

**Study Structure**

The study consisted of two phases: Period A, which took place before trauma or a sham operation; and Period B, which occurred after the trauma or sham trauma (sham operation). Period B two levels of ventilation FiO2 settings were analyzed (air level in which the O2 was 21% [air] and O2 level in which the O2 was 100% [O2]). Because

Therefore, it is mandatory to investigate pathophysiological events by using a standardized animal trauma model to clarify and compare the effects of enhanced O2 delivery in the traumatized and nontraumatized brain. For that reason, we studied brain glucose and lactate metabolism in a rat model of DBI.

**Material and Methods**

All experiments were performed with the approval of the Institutional Animal Care and Use Committee of the Kanton of Bern, Switzerland. All surgeries were performed by the same person throughout the study.

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### Table 1

<table>
<thead>
<tr>
<th>Factor &amp; Timing</th>
<th>AOS</th>
<th>AAS</th>
<th>AOT</th>
<th>AAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABG O₂ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period A</td>
<td>93.3 ± 19.7</td>
<td>79.8 ± 16.9</td>
<td>77.8 ± 10.2</td>
<td>81.4 ± 8.5</td>
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<tr>
<td>Period B</td>
<td>485.7 ± 121.6</td>
<td>93.5 ± 10.2</td>
<td>426.6 ± 72.4</td>
<td>85.9 ± 12.5</td>
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<tr>
<td>ABG CO₂ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period A</td>
<td>35.7 ± 7.3</td>
<td>34.3 ± 4</td>
<td>36.2 ± 8.5</td>
<td>37.5 ± 3.9</td>
</tr>
<tr>
<td>Period B</td>
<td>38.3 ± 6.8</td>
<td>37.3 ± 4.8</td>
<td>41.0 ± 9.1</td>
<td>40.0 ± 8.7</td>
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<tr>
<td>Temperature (ºC)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Period A</td>
<td>36.4 ± 0.6</td>
<td>36.7 ± 0.3</td>
<td>36.2 ± 0.5</td>
<td>36.3 ± 0.7</td>
</tr>
<tr>
<td>Period B</td>
<td>36.5 ± 0.4</td>
<td>36.4 ± 0.3</td>
<td>36.3 ± 0.5</td>
<td>36.3 ± 0.4</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period A</td>
<td>104.2 ± 10.8</td>
<td>100.4 ± 14.9</td>
<td>92.0 ± 11.1</td>
<td>100.5 ± 13.3</td>
</tr>
<tr>
<td>Period B</td>
<td>101.1 ± 8.4</td>
<td>95.3 ± 11.3</td>
<td>94.5 ± 13.2</td>
<td>94.2 ± 10.2</td>
</tr>
</tbody>
</table>

* Values are given as means ± SDs. Period A = before trauma or sham trauma; Period B = after trauma or sham trauma.
† Significantly different from all other groups (p < 0.001).

### Microdialysis Measurements

Probes (CMA/20; CMA Microdialysis AB, Solna, Sweden) with a collecting membrane of 4 mm and a molecular weight cutoff level of 20 kD were used. The probes were inserted into traumatized brain tissue at a depth of 5 mm (Fig. 2), according to the description of the trauma model. 

### Statistical Analysis

Statistical analyses were performed with the aid of Statistica (StatSoft, GmbH, Hamburg, Germany) and GraphPad (GraphPad Software, Inc., San Diego, CA) software by using the repeated-measures ANOVA test for microdialysis data and the standard ANOVA for physiological data. Significance was determined at a probability value lower than 0.05. Data are shown as means ± SDs.

### Results

#### Core Temperature and Mean Arterial Blood Pressure

There were no differences in the rats’ core temperatures throughout the study in any group (Table 1). In addition, there were no significant differences in MABP in any group. An overall tendency for a lower MABP was observed in Period B compared with Period A; however, this did not reach significance (Table 1).

#### Arterial Blood Gas O₂, and CO₂ Tensions

During Period B blood gas O₂ tensions did not differ between the AOS and AOT groups or between the AAS and AAT groups; however, they were significantly differ-
ent between the AAT (85.87 ± 12.4 mm Hg) and AOT (426.65 ± 72.4 mm Hg) groups (p < 0.001) and between the AAS (93.5 ± 10.2 mm Hg) and AOS (458.73 ± 121.6 mm Hg) groups (p < 0.0001), indicating that the delivered O2 (FiO2) resulted in increased tissue O2 levels (Table 1). There were no differences in blood gas CO2 tensions in any group.

**Serum Levels of Glucose and Lactate**

There was no difference in serum levels of glucose and serum lactate between the two FiO2 settings for sham-operated animals. For glucose we measured 5.58 ± 0.51 mmol/L and 5.54 ± 1 mmol/L for FiO2 1 and FiO2 0.21, respectively, and for lactate we measured 0.9 ± 0.15 mmol/L and 1.07 ± 0.35 mmol/L for FiO2 1 and FiO2 0.21, respectively (Fig. 3).

**Brain Dialysate Glucose**

Microdialysis showed that glucose levels were significantly higher in the AOT group than in the AAT group (p < 0.04) (Fig. 4 left). Moreover, glucose levels were observed to be higher in the AOT group than in all other groups (by 41, 53, and 50%, compared with the AAT, AOS, and AAS groups, respectively). No significant differences were noted for the sham-operated groups (Fig. 4 right).

**Brain Dialysate Lactate**

Microdialysis showed that lactate levels were significantly lower in the AOT group than in the AAT group (p < 0.05) (Fig. 5 left). Moreover, lactate levels were observed to be lower in the AOT group compared with all other groups (by 53, 34, and 21%, compared with the AAT, AOS, and AAS groups, respectively). No significant differences were noted for the sham-operated groups (Fig. 5 right).

**Discussion**

Severe head injury seen in a clinical situation can have multiple origins and thus is difficult to study systematically. Despite these difficulties in clinical research, energy metabolism in the brain has been shown to be influenced by changes in FiO2, in which levels of lactate in brain dialysate were significantly reduced by increasing the FiO2. The response of glucose in the dialysate has been less clear.

The present study was designed to validate the effect of changes in FiO2 on glucose and lactate levels in the dialysate within the controlled environment of a reproducible animal DBI model and to compare the response to these changes with the metabolism of the noninjured sham-operated brain.

In activated brain tissue, such as that observed during the early phase after trauma, lactate has been shown to be the preferred energy substrate over glucose.42–44 These results are supported by the finding that neurons can survive in a milieu depleted of glucose in which lactate is the sole energy substrate.45 Monocarboxylate transporters for lactate are supported by the finding that neurons can survive in a milieu depleted of glucose in which lactate is the sole energy substrate.45 Monocarboxylate transporters for lactate have been demonstrated to be selectively expressed between astrocytes and neurons,7,37,38,48 a finding that has led to the theory of compartmentalization of energy utilization in the brain, of which functional coupling between astrocytes and neurons is a primary tenet.35 Yet lactate is also a marker of anaerobic metabolism in cases in which intramitochondrial O2 tensions are low (decreasing to < 1.5 mm Hg),17 the redox potential of the brain tissue, especially in activated brain tissue, is analyzed using cytochrome oxidase staining and A

**How Can We Explain These Findings?**

A systemic effect of FiO2 on serum levels of glucose and lactate has been observed. The present results support the proposition that FiO2 can have a significant effect on the metabolism of the brain. The impact of increases in FiO2 on brain glucose and lactate concentration is well documented.

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**Conclusion**

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Left: Graph showing lactate in the brain dialysate in the posttrauma groups. Significantly lower lactate levels (p < 0.05) were found in the AOT group than in the AAT group in 15 samples. Right: Graph showing lactate in the brain dialysate in sham-operated groups (AAS and AOS). No significant differences between these groups were observed.

How Can We Explain These Findings?

A systemic effect of FiO2 can be excluded by its lack of influence on serum levels of glucose and lactate. Using the posttraumatic hyperglycolysis theory and the finding proposed by Pellerin and Magistretti that lactate is preferentially used over glucose in times of activation, it can be hypothesized that, by increasing the availability of O2, the reduction of lactate in the dialysate in the injured brain may be related to the final aerobic use of lactate in mitochondria. Lactate may be reduced due to the action of specific monocarboxylate transporters. In this situation the increase in glucose would thus reflect diminished usage to the disadvantage of lactate.

Thus, the simple maneuver of increasing FiO2 would favor aerobic lactate consumption. The finding of Daugherty, et al.,12 that mitochondrial O2 consumption is increased early after trauma and can be further enhanced by the coadministration of lactate and increased FiO2 would complement our findings. To enhance our findings further, an experimental design is required in which radiolabeled lactate and glucose are administered and mitochondrial activity is analyzed using cytochrome oxidase staining and ATP measurements.

Effect of Anesthesia

Concerning the effects of the anesthetic agent isoflurane, it has been reported that increasing the MAC from 0.5 to 1.5 does not influence transcranial Doppler velocities or cerebral blood flow.21 The cerebral metabolic rate of glucose, however, is reduced by increasing the MAC. Burst suppression for isoflurane has been described when MAC values are greater than 1.5.26 As all our groups were treated identically, we did not expect any intergroup differences regarding the brain metabolism of glucose and lactate. Nevertheless, in absolute terms, the overall brain metabolism is influenced by interfering with MAC values.

Free Radical Production

The enhanced production of free radicals is a major concern of prolonged O2 therapy and has been demonstrated in ischemia–reperfusion or fluid percussion models. This observation, however, remains equivocal because in many studies no elevation of free radical production has been observed following O2 enhancement. Free radical scavengers such as polyethylene glycol–superoxide dismutase and tirilazad mesylate have neither proved nor disproved a role for free radical scavengers in the treatment of head injury. Nevertheless, prolonged enhanced O2 therapy may potentially result in irreversible pulmonary injury especially in patients treated for longer than 24 hours with a FiO2 of 1 and a history of bleomycin administration. Therefore clinical protocols should not exceed the use of increased FiO2 for more than 24 hours and the risks may further be reduced by using the PaO2 upper limit of 250 mm Hg.

Future outcome analyses in severe head injury studies are needed to investigate whether short-term (≤ 24 hours) O2 therapy will have a positive long-term effect both on survival and the neurological performance score.

Conclusions

Increasing the FiO2 affects the levels of glucose and lactate in the brain dialysate of rats with injured brains. Lactate is significantly reduced and glucose increased compared with healthy air-ventilated rats. The noninjured brain demonstrated no changes during FiO2 alterations. Serum glucose and lactate levels were not influenced by variations in FiO2. We hypothesize that, by increasing the FiO2, aerobic lactate utilization may thus be enforced. For further inves-
tigations ATP measurements and histological staining with cytochrome oxidase in combination with electron microscopy of mitochondria will be helpful.

Acknowledgment

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7. Cater HL, Benham CD, Sundstrom LE: Neuroprotective role of cytochrome oxidase in combination with electron microscopy of mitochondria will be helpful.


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Manuscript received November 19, 2003. Accepted in final form April 14, 2004. Address reprint requests to: Michael Reinert M.D., Department of Neurosurgery, Inselspital Bern, University of Bern, 3010 Bern, Switzerland. email: michael.reinert@neurochirurgie-bern.ch.