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Nordström, Carl Henrik; Nielsen, Troels Halfeld; Schalén, Wilhelm; Reinstrup, Peter; Ungerstedt, Urban

Published in:
Acta Neurochirurgica

DOI:
10.1007/s00701-016-2835-z

2016

Document Version:
Peer reviewed version (aka post-print)

Biochemical indications of cerebral ischemia and mitochondrial dysfunction in severe brain trauma analyzed with regard to type of lesion

Carl-Henrik Nordström, Troels Halfeld Nielsen, Wilhelm Schalén, Peter Reinstrup, Urban Ungerstedt

From the Departments of Neurosurgery (CHN, THN) Odense University Hospital, Odense, Denmark, and the Department of Neurosurgery (WS, PR), Lund University Hospital, Lund, Sweden

Corresponding author: Carl-Henrik Nordström, Professor, Department of Neurosurgery, Odense University Hospital, Odense, Denmark.

Telephone: +4640263701

E-mail: carl-henrik.nordstrom@med.lu.se

Abstract

Background The study focuses on three questions related to the clinical usefulness of microdialysis in severe brain trauma: a) how frequently is disturbed cerebral energy metabolism observed in various types of lesions, b) how often does the biochemical pattern indicate cerebral Ischemia and Mitochondrial Dysfunction, c) how do these patterns relate to mortality.

Method The study includes 213 consecutive patients with severe brain trauma (342 intracerebral microdialysis catheters). The patients were classified in four groups according to the type of lesion: Extradural Hematoma (EDH), acute Subdural Hematoma (SDH), Cerebral Hemorrhagic Contusion (CHC) and No Mass lesion. Altogether about 500.000 biochemical analyses were performed during the initial 96 h after trauma.

Results Compromised aerobic metabolism occurred during 38 % of the study period. The biochemical pattern indicating Mitochondrial Dysfunction was more common than that of Ischemia. In EDH and No Mass lesion aerobic metabolism was generally close to normal. In SDH or CHC it was often severely compromised. Mortality was increased in SDH with impaired aerobic metabolism while CHC did not exhibit a similar relation.

Conclusions Compromised energy metabolism is most frequent in patients with SDH and CHC (32% and 49% of the study period, respectively). The biochemical pattern of Mitochondrial Dysfunction is more common than that of Ischemia (32% and 6% of the study period, respectively). A correlation between mortality and biochemical data is obtained provided the microdialysis catheter is placed in an area where energy metabolism reflects tissue outcome in a large part of the brain.

Keywords: Traumatic brain injury, microdialysis, ischemia, mitochondrial dysfunction, lactate, pyruvate, cytoplasmatic redox

Introduction

The technique of microdialysis was originally developed to monitor neurotransmitters in the animal brain during experimental conditions [32]. A microdialysis catheter intended for the human brain, an infusion pump, an analyzer for bedside analysis of the dialysate and display of biochemical variables related to cerebral energy metabolism was presented in 1995. This year the microdialysis technique was also introduced as a clinical routine at the Department of Neurosurgery, Lund University Hospital. The study presented here includes all patients with severe traumatic brain injuries (TBI) monitored with microdialysis during the period 1995 – 2009.

More than 300 articles with data obtained from intracerebral microdialysis have been published in patients with severe TBI. However, comprehensive information regarding cerebral energy metabolism has previously been presented in only two large series of TBI patients. One of these showed that variables obtained during microdialysis exhibited a poor correlation to global variables like intracranial pressure (ICP) and cerebral perfusion pressure (CPP) as well as clinical outcome while autocorrelation was high for all biochemical markers [15]. The second large study had a conflicting conclusion claiming that increase in interstitial lactate/pyruvate (LP) ratio was an independent marker of poor clinical outcome [30]. As intracerebal microdialysis gives biochemical information from a very small volume of tissue a general correlation between the biochemical patterns obtained and clinical outcome would not be expected. In both large studies there is a lack of information regarding the conditions necessary for obtaining such correlations. Further, there is a need of deeper understanding of the information obtained from the LP ratio.

The LP ratio obtained from microdialysis reflects cerebral cytoplasmatic redox state [2,3,25]. The latter is determined by the oxidative metabolism which in turn is primarily

dependent on adequate cerebral perfusion /oxygen delivery and mitochondrial function. Under clinical conditions an increase in LP ratio is mainly observed during ischemia and post-ischemic mitochondrial dysfunction. In experimental and clinical studies we have recently shown that the pattern of the biochemical variables obtained during routine microdialysis may discriminate between these two conditions [8,16,17].

In the present study we describe a) how frequently a perturbation of cerebral energy metabolism is observed in patients with severe TBI in four different types of lesions, b) how often the pattern of the biochemical variables indicates cerebral ischemia and mitochondrial dysfunction respectively and how often transitions between these two patterns occur, and c) how these biochemical patterns relate to mortality in the various diagnostic groups.

Materials and Methods

In 1991 the Ethical Committee of Lund University Medical Faculty approved the use of microdialysis with multiple intracerebral catheters as a standard clinical technique in neurocritical care. In 1995 microdialysis was introduced as part of clinical routine in patients with severe TBI. From the beginning bedside analysis and display of chemical variables related to cerebral energy metabolism was included. The present study is a retrospective analysis of data collected prospectively in all patients with severe TBI monitored with microdialysis during the period November 1995 – August 2009 at the Department of Neurosurgery, Lund University Hospital, Sweden.

The study includes 213 consecutive patients with altogether 342 intracerebral microdialysis catheters. The mean age ± S.D. of the patients was 39 ± 19 years. All patients

had an estimated post-resuscitation Glasgow Coma Scale (GCS) sum score <8 (GCS motor score <5) [28]. Basic data regarding the patients, the diagnostic groups and the number of microdialysis catheters in the different groups are given in Table 1. The patients were classified in four groups according to the type of lesion. Patients defined as extradural hematoma (EDH), acute subdural hematoma (SDH) or focal cerebral hemorrhagic contusion (CHC) were all acutely treated surgically with evacuation of the mentioned type of lesion. In patients with No Mass lesion evacuation of a focal mass was not necessary. In all patients ICP was continuously monitored from an intraventricular catheter [11]. Mean arterial pressure (MAP) was continuously monitored from an intra-arterial line and CPP was calculated as MAP - ICP. All patients were kept sedated and intubated with controlled normoventilation during the monitored period. Neurocritical care was conducted according to the principles developed and evaluated at the Department of Neurosurgery, Lund University Hospital ("Lund concept") [5,20]. Clinical outcome is in the present study described as mortality within six months after trauma. As transitions between the two categories of Ischemia and Mitochondrial Dysfunction were observed patients exhibiting a change from Ischemia to Mitochondrial Dysfunction were classified as mitochondrial dysfunction and *vice versa*.

Intracerebral microdialysis

Microdialysis was performed utilizing CMA 70 or 71 catheters (cut-off 20 and 100 kDa, respectively; CMA Micodialysis, Stockholm, Sweden) as described previously [20,22]. The microdialysis catheters were perfused (Perfusion Fluid, CMA Microdialysis) at a rate of 0.3 µl/min and the perfusates were collected in capped microvials at one-hour intervals. The samples were immediately analyzed for glucose, pyruvate, lactate, glutamate, and glycerol utilizing conventional enzymatic techniques (CMA 600 or ISCUS Microdialysis Analyzer)

and the results were displayed on a bedside monitor. The calculated LP ratio was used for evaluation of the cytoplasmatic redox state. All data obtained from cerebral microdialysis were integrated bedside with global biochemical and physiological data utilizing a specially developed computer program (ICU-pilot, CMA Microdialysis, Stockholm, Sweden). In the present study we report the biochemical data obtained during the initial 96 hours after trauma. During this time period altogether about 150.000 bedside biochemical analyses were conducted and displayed bedside.

In patients treated with open surgical evacuation (ECH, SDH, CHC) one microdialysis catheter was usually inserted into the vicinity of the focal lesion – "biochemical penumbra" [4]. In 105 of these patients one, two or three additional catheters were inserted into the ipsi- or contralateral hemisphere (Table 1). In patients with No Mass lesions one microdialysis catheter was introduced through a separate burr hole a few cm distant from the intraventricular catheter. In 4 of these patients a second microdialysis catheter was also inserted into the contralateral hemisphere.

Five patients who on admission were classified as GCS 3 with dilated non-reacting pupils (1 EDH, 4 SDH) died due to an intractable increase in ICP within 24 h of trauma. Data from these and two other patients who died within 4 days after trauma are excluded from the figures showing the time course of the biochemical changes during the studied period of 96 h but are included in the tables comparing the biochemical patterns in cerebral ischemia /mitochondrial dysfunction and mortality.

Cerebral biochemical variables

Normal reference levels for the studied biochemical variables were defined from data obtained in un-anesthetized normal human brain utilizing identical microdialysis and

analytical techniques [22]. In accordance with conventional principles in clinical chemistry normal range was defined as normal mean level ± 2 S.D. As the LP ratio in normal human brain is 23 ± 4 [22] the upper normal level for cerebral LP ratio was in the present study set at 30. For cerebral interstitial pyruvate concentration 70 μmol/L was defined as the lower normal level. In accordance with recent experimental and clinical studies LP ratio >30 at a pyruvate concentration ≥70 μmol/L was interpreted as a biochemical pattern indicating mitochondrial dysfunction while LP ratio >30 simultaneously with pyruvate <70 μmol/L was interpreted as a biochemical pattern indicating ischemia [8,16,17]. An increase in interstitial glutamate concentration above normal was interpreted as a sign of perturbation of cerebral energy metabolism resulting in impaired astrocytic re-uptake of glutamate [14,24]. Increase in glycerol above normal range was interpreted as an indication of degradation of cellular membranes with liberation of free fatty acids and glycerol [7,33].

Patients were classified as neither ischemia nor mitochondrial dysfunction if they during the whole study period of 96 h had LP ratio ≤30. Biochemical data for patients classified as ischemia or mitochondrial dysfunction are given selectively for the time periods the definitions given above were fulfilled. For the diagnosis of these two conditions the biochemical pattern defined above should have been obtained during three consecutive hours or more. Approximately 77.000 of the total number of analyses are included in the description of these three groups.

Statistics

Data for groups of patients are given as mean ± S.D. in figures 3-5 and as median and interquartile range (q1- q3) in Table 1 and 2. Statistical comparisons were performed utilizing unpaired t-test.

Results

Age distribution of the patients and the number of functioning microdialysis catheters in the four diagnostic groups are given in Table 1. Patients in the diagnostic groups of SDH and CHC were significantly older than those in the groups of EDH ($p<0.05$) and No Mass lesion ($p<0.001$).

Table 2 gives the levels of the biochemical variables obtained from all microdialysis catheters for the three diagnostic groups of Ischemia, Mitochondrial Dysfunction and LP ratio ≤ 30 (normal aerobic metabolism). In the table median level and interquartile range represent data from the total number of samples (N_{samp}) and analyses of each variable from all inserted microdialysis catheters. The levels of these variables obtained in normal human brain are shown as reference [22].

The statistical comparisons were performed from the median levels of the variables obtained in each catheter during each episode of ischemia ($N_{epis} = 41$), mitochondrial dysfunction ($N_{epis} = 112$) and LP ratio ≤ 30 ($N_{epis} = 130$), respectively. Due to the definition of the inclusion criteria the levels of pyruvate and the LP ratio differed between these two groups as well as between these groups and patients with LP ratio ≤ 30. Accordingly, the LP ratio and pyruvate level were not included in the statistical comparisons. The levels of glutamate and glycerol were during Ischemia significantly higher than during Mitochondrial Dysfunction while glucose was higher in the latter group. In the group of Ischemia as well as in the group of Mitochondrial Dysfunction lactate, glutamate and glycerol were significantly higher ($p<0.001$) and glucose was significantly lower ($p<0.001$) when compared with the group of LP ratio ≤ 30 (statistical comparisons not shown in the table).

Transition between the two categories of Ischemia and Mitochondrial Dysfunction was observed in altogether 40 catheters. In 22 of these cases the transition

occurred from Ischemia to Mitochondrial Dysfunction. In catheters with an observed transition from Ischemia to Mitochondrial Dysfunction mean pyruvate level increased from 46±21 (N=468) to 148±43 µmol/L (N=908). In catheters with transition from Mitochondrial Dysfunction to Ischemia mean pyruvate level decreased from 166±115 (N=327) to 40±25 µmol/L (N=349). The number of observed transitions and the distribution between the four diagnostic groups is shown in Table 3. The transitions were usually clearly delineated as illustrated in figures 1 and 2. In figure 1 (Transition from Mitochondrial Dysfunction to Ischemia) a rapid decrease in pyruvate from about 200 µmol/L to below 20 µmol/L simultaneously with a slow increase in the concentration of lactate resulted in a very pronounced increase in LP ratio from an initially moderately increased level (50-60). The simultaneously occurring change in glucose level is also shown. A late increase in glucose occurred although CPP remained below 20 mmHg. In figure 2 (Transition from Ischemia to Mitochondrial Dysfunction) the increase in pyruvate from a very low level (40-60 µmol/L) to a high normal level at an unchanged high level of lactate (9-12 mmol/L) is reflected in a decrease in LP ratio which, however, remained above normal at a level of about 50-60. The simultaneous increase in glucose concentration is also shown. These biochemical changes coincided with an increase in CPP from approximately 50 to 75 mmHg.

The changes over time for all biochemical variables measured bedside during the studied period of 96 h are shown (mean and standard deviation – S.D.) in figures 3-5. In these figures data obtained in seven patients who died during this time period (1 EDH; 6 SDH) are excluded. The S.D. is for each variable only shown in one direction.

In the EDH group LP ratio remained close to normal during the study period (fig. 3A). Also in the No Mass group LP ratio was relatively close to normal (fig. 3B). In the CHC group LP ratio was initially very high and exhibited a slow decline towards normality during the study (fig. 3B). In the SDH group the LP ratio was initially moderately elevated

and exhibited a transient pronounced increase during the following 48 hours (fig. 3A). During this period there was also a distinct increase in S.D. indicating that a very marked secondary increase in LP ratio occurred in some of these patients.

The changes in glutamate exhibited a pattern similar to the pattern in the LP ratio (fig. 4A and B). In the groups of EDH and No Mass lesion glutamate remained relatively close to normal. In the diagnostic groups of SDH and CHC glutamate concentration was initially markedly increased and in the SDH group a secondary increase was observed about 20 to 60 h after trauma (fig. 4A). Intracerebral glycerol was also considerably elevated in the groups of SDH and CHC (fig. 5A and B) and a secondary increase in glycerol was observed in the SDH group (fig. 5A).

Table 4 gives the total number of hours the biochemical patterns indicated ischemia, mitochondrial dysfunction and a normal LP ratio, respectively, in the four diagnostic groups. In the total material the biochemical pattern of ischemia was observed during 6 % of the study period while the pattern of mitochondrial dysfunction was obtained during 32 % of the time. Table 4 also shows mortality within 6 months of trauma. In the total group of 213 patients mortality was 17 per cent. Mortality was highest in the SDH group (29 %) and lowest in the group of No Mass lesion (9 %). In the total material mortality was higher in patients with a biochemical pattern of ischemia (38%) than in those with signs of mitochondrial dysfunction or a normal LP ratio (9 and 13 %, respectively). In the diagnostic groups of SDH and No Mass lesion mortality was markedly elevated for patients with a biochemical pattern of ischemia (73 and 33 %, respectively). The biochemical pattern of mitochondrial dysfunction appeared to be associated with increased mortality only in patients with SDH (40 %). In patients classified as CHC ischemia and mitochondrial dysfunction was not related to increased mortalit

Discussion

Multimodal monitoring aims at early detection of secondary physiological and biochemical deterioration that may affect clinical outcome. If the processes underlying the deterioration are identified and understood appropriate therapeutic interventions may be instituted. Correct clinical application and interpretation of intracerebral microdialysis necessitates substantial knowledge of the advantages and the limitations of the technique. First, the metabolic pathways related to cerebral energy metabolism are complex and the interpretation of the biochemical patterns observed must be based on known biochemical patterns obtained under defined experimental conditions. Normal limits for the variables studied must be based on our present knowledge of the variations occurring in normal human brain under physiological conditions. Second, changes over time for the observed chemical patterns are often more important than the absolute levels of individual variables. Information regarding such changes is difficult to convey in scientific publications but is often necessary for the clinical interpretation. Third, as the biochemical information originates from a very small volume of tissue it is usually not known whether this information is representative of global cerebral energy state. When microdialysis is intended to give an early warning of threatening deterioration the catheter is positioned in the especially vulnerable "penumbra zone" surrounding focal lesions [4]. In this situation a correlation to global variables and clinical outcome would be expected only if the "penumbra zone" includes a major part of a hemisphere.

Interpretation of biochemical data

Cerebral energy state is entirely dependent on oxidative metabolism. The cerebral cytoplasmatic redox state is determined by mitochondrial oxidative metabolism which

conventionally is described by the lactate dehydrogenase equilibrium as reflected in the LP ratio [2,3,25].

Lactate and pyruvate are water soluble. However, due to monocarboxylate transporters (MCTs) they equilibrate rapidly across cellular membranes. Out of the total family of 14 members three isoforms (MCT1, MCT2, MCT4) have been described in the brain [6]. The driving force for the transport of the monocarboxylates is obtained from the differences in concentration on both sides of the cellular membranes. As lactate and pyruvate rapidly equilibrate over the cell membrane the LP ratio obtained during cerebral microdialysis may be used as a measure of cytoplasmatic redox state [25]. Available data indicate that LP ratio obtained from microdialysis of un-anesthetized normal human brain is 23 ± 4 [22]. Based on conventional principles accepted in clinical chemistry we defined a LP ratio of 30 as the upper limit for normal aerobic metabolism. An increase in LP ratio above normal indicates compromised oxidative metabolism which in TBI patients is generally due to ischemia (insufficient blood supply) or mitochondrial dysfunction [37]. In cerebral ischemia the increase in LP ratio is associated with a very low pyruvate level while in mitochondrial dysfunction pyruvate level is increased or remains within normal limits [16]. Accordingly, these two conditions can be diagnosed and separated bedside by utilizing routine cerebral microdialysis. The patterns of ischemia and mitochondrial dysfunction were defined in accordance with recent experimental and clinical studies [8,16,17].

As mentioned previously normal ranges for the biochemical variables were defined in accordance with conventional principles in clinical chemistry. An LP ratio >30 at a pyruvate concentration ≥ 70 μmol/L was interpreted as mitochondrial dysfunction while LP ratio >30 simultaneously with pyruvate <70 μmol/L was interpreted as ischemia. Like in all clinical situations these limits should not be regarded as definite thresholds and the

information obtained by cerebral microdialysis should always be related to other biochemical, physiological and clinical information.

Table 2 shows that in TBI patients classified according to these principles and a LP ratio ≤ 30 (normal aerobic metabolism) all biochemical variables were close to the levels obtained in normal human brain [22]. This pattern of normal aerobic metabolism during the whole study period was obtained in approximately 62% of all analyzed samples. In the group of Ischemia (6% of the study period) as well as in the group of Mitochondrial Dysfunction (32% of the study period) glutamate and glycerol were significantly elevated indicating insufficient energy metabolism leading to cell membrane degradation. These indications of compromised cerebral energy metabolism and signs of degradation of membranes were significantly more pronounced in the group of Ischemia than in the group of Mitochondrial Dysfunction.

The possibility to identify and separate Ischemia and Mitochondrial Dysfunction bedside by microdialysis has clinical implications. When ischemia is diagnosed an increase in cerebral perfusion pressure (*c.f.* fig. 2) or therapy improving microcirculation has been shown to normalize the biochemical pattern [23]. In patients with Mitochondrial Dysfunction drugs supposed to improve mitochondrial function can be tested and microdialysis can be used to evaluate their biochemical effects (*e.g.* Cyclosporin A, ethyl-pyruvate) [1,13,31].

Transitions between Ischemia and Mitochondrial Dysfunction

During experimentally induced mitochondrial dysfunction the biochemical pattern is identical to the pattern in the group denoted Mitochondrial Dysfunction in the present study [8,16,17]. In experimental studies it has been shown that transient ischemia causes mitochondrial dysfunction [18,19,21]. However, although many acute and chronic neurologic conditions

seem to be associated with mitochondrial dysfunction the consequences of this disorder are not well-known in brain trauma or other acute and chronic cerebral diseases [26]. In addition to other possible patho-physiological consequences mitochondrial dysfunction might increase the risk of compromised energy metabolism at a reduction of cerebral perfusion.

As illustrated in figures 1 and 2 transitions between ischemia and mitochondrial dysfunction are characterized by rapid changes in pyruvate concentration resulting in dramatic changes in the LP ratio. As shown in the figures glucose concentration may in both conditions vary considerably. During mitochondrial dysfunction glucose concentration has been shown to vary from normal to very low levels [8,12,16,17]. During the period of ischemia illustrated in fig. 1 glucose increased from a very low to a normal level although CPP was extremely low (20 mmHg). The observation is explained by the fact that during a decrease in blood flow delivery of oxygen is critically compromised at a higher blood flow than glucose [25]. During the period of ischemia shown in fig. 1 glutamate and glycerol increased to extremely high levels (> 500 µmol/L and > 2000 µmo/L, respectively).

Biochemical pattern related to type of lesion

The differences between the time courses of the biochemical patterns obtained in different types of traumatic brain lesions have not been described previously. In patients with EDH and No Mass lesion the LP ratio was generally normal or close to normal with a relatively narrow S.D. (fig. 3). The higher mean LP ratio in the groups of SDH and CHC indicates that energy metabolism is generally more severely compromised in these patients. The high S.D.in both groups reflects a pronounced variability between patients. In particular in the SDH group the increase in LP ratio after 24-48 hours signals secondary adverse events (fig. 3A). In all groups the redox pattern is reflected in parallel changes in glutamate and glycerol. The observation

indicates that the degree of compromised energy metabolism translates to the degree of tissue damage. The biochemical patterns observed is in agreement with the fact that patients with focal intracerebral mass lesions in general have worse outcome than patients with No Mass lesion or EDH.

Intracerebral microdialysis and clinical outcome

As the microdialysis technique gives biochemical information from a very narrow zone surrounding the catheter a correlation between microdialysis data and clinical outcome should not be expected. By inserting multiple intracerebral microdialysis probes it has been shown that energy metabolism is severely compromised in the "penumbra zone" surrounding focal lesions while biochemistry in other parts of the brain remained close to normal [4]. Accordingly, intracerebral microdialysis would be expected to reflect local tissue outcome but not necessarily correlate to clinical outcome. A correlation between microdialysis/tissue outcome and clinical outcome would be expected only if the disturbance of cerebral energy metabolism is global or if the biochemical "penumbra zone" includes a large part of a hemisphere.

In the present study mortality within 6 months was very high (73%) in the SDH patients within the subgroup defined as Ischemia. In the corresponding subgroup in the CHC patients mortality was remarkably low (13%). In patients with normal aerobic metabolism (LP ratio ≤ 30) mortality was in these groups 14 % and 20 %, respectively. The high mortality in the group of Ischemia in SDH patients is probably explained by the fact that the hematoma has affected energy metabolism in a large part of the hemisphere. In patients with CHC a limited biochemical "penumbra zone" surrounds the focal lesion which is not representative of the hemisphere [4]. However, by monitoring deterioration of energy metabolism in this

particularly vulnerable zone an early warning may be obtained. A change in therapy might under these conditions prevent the deterioration to spread to adjacent areas of the brain. Further, a normal LP ratio also reflects energy state in a small volume of tissue which explains why in patients with CHC mortality was remarkably high in the group with LP ≤30 (Table 4).

Under clinical conditions it may be difficult or impossible to decide whether the biochemical information obtained from microdialysis is focal or representative. Accordingly, it would be of value to develop a bedside technique that reveals global cerebral energy state as a complement to the conventional microdialysis method. One possibility might be to measure biochemical variables obtained from intraventricular microdialysis [29]. A second possibility which avoids the necessity to insert an intracerebral catheter was recently presented in an experimental study: evaluation of global cerebral energy state by placing a microdialysis catheter in the cerebral venous drainage (superior sagittal sinus) and measure the LP ratio [9]. This technique might also be used in patients where a global deterioration of cerebral energy metabolism is expected but it is for technical and ethical perspectives impossible to insert intracerebral catheters (e.g. extracorporeal circulation during open heart surgery, resuscitation after cardiac standstill).

"Ischemia – Mitochondrial Dysfunction" and the concept of "Metabolic Crisis"

It has repeatedly been observed that in patients with TBI increased LP ratio is often not related to reduced cerebral blood flow and ischemia [35,36]. The concept of "Metabolic Crisis" has been defined as a low intracerebral glucose level and/or elevation in the LP ratio [36]. However, this concept is associated with certain problems. Firstly, it does not explain the biochemical or physiological background and can accordingly not lead to any specific

therapeutic considerations. Secondly, the biochemical limits used to define Metabolic Crisis varies in different publications [27,35,36].

The introduction of the two diagnostic groups of Ischemia and Mitochondrial Dysfunction circumvents these problems. In experimental studies it is well documented that ischemia causes mitochondrial dysfunction [18,19,21], it has been shown to occur in patients with brain trauma [10], and the limits used for the separation of the two conditions is based on experimental and clinical studies [8,16,17]. Both conditions may be associated with a low intracerebral glucose level [12,16]. Finally, the bedside diagnosis and separation of the two conditions is directly related to different therapeutic strategies [1,13, 23,31].

Conclusions

In patients with severe traumatic brain injuries compromised cerebral aerobic metabolism (LP ratio >30) was documented during 38 % of the study period. A biochemical pattern interpreted as Mitochondrial Dysfunction was more common than the pattern of Ischemia (32 % and 6 % of the study period, respectively). In patients with EDH and No Mass lesion aerobic metabolism was generally normal or close to normal while in patients with SDH or CHC it was often severely compromised. Compromised aerobic metabolism was translated to variables reflecting tissue damage (glutamate, glycerol). In SDH patients microdialysis frequently revealed secondary adverse events occurring 24-48 hours after trauma. In the total group of patients mortality within 6 months was increased for patients exhibiting a biochemical pattern of ischemia. In patients with impaired aerobic metabolism increased mortality was observed in SDH while patients with CHC did not exhibit a similar relation. This difference is explained by the fact that perturbation of energy metabolism usually occurs in a large part of the hemisphere in SDH but is more localized to a “penumbra zone” in CHC

patients. Ideally the conventional intracerebral microdialysis technique should be supplemented by a technique revealing global cerebral energy state.

Funding: No funding was received for this research

Conflict of Interest: Carl-Henrik Nordström is consulting for MDialysis Stockholm, Sweden. All other authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval: All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

Acknowledgements: We thank Katarina Nielsen, Department of Neurosurgery, Lund University Hospital, for help with the microdialysis equipment and biochemical analyses.

References:

1. Alessandri B, Rice AC, Levasseur J, DeFord M, Hamm RJ, Bullock MR (2002) Cyclosporin A improves brain tissue oxygen consumption and learning/memory performance after lateral fluid percussion injury in rats. J Neurotrauma 19:829-841
2. Dienel GA (2012) Brain lactate metabolism: the discoveries and the controversies. J Cereb Blood Flow Metab 32:1107-1138
3. Dienel GA (2014) Lactate shuttling and lactate use as fuel after traumatic brain injury: metabolic considerations. J Cereb Blood Flow Metab 34:1736-1748
4. Engström M, Polito A, Reinstrup P, Romner B, Ryding E, Ungerstedt U, Nordström CH (2005) Intracerebral microdialysis in clinical routine – the importance of catheter location. J Neurosurg 102:460-469
5. Grände PO, Asgeirsson B, Nordström CH (2002) Volume targeted therapy of increased intracranial pressure: the Lund concept unifies surgical and non-surgical treatments. Acta Anaesthesiol Scand 46:929-941
6. Halestrap AP (2012) The monocarboxylate transporter family--Structure and functional characterization. IUBMB Life 64:1-9
7. Hillered L, Valtysson J, Enblad P, Persson L (1998) Interstitial glycerol as a marker for membrane phospholipid degradation in the acutely injured human brain. J Neurol Neurosurg Psychiatry 64:486-491
8. Jacobsen A, Nielsen TH, Nilsson O, Schalén W, Nordström CH (2014) Bedside diagnosis of mitochondrial dysfunction in aneurysmal subarachnoid hemorrhage. Acta Neurol Scand 130:156-163
9. Jacobsen R, Nielsen TH, Granfeldt A, Toft P, Nordström CH (2016) A technique for continuous bedside monitoring of global cerebral energy state. Intensive Care Med Experimental 4:3

10. Lakshmanan R, Loo JA, Drake T, Leblanc J, Ytterberg AJ, McArthur DL, Etchepare M, Vespa PM (2010) Metabolic crisis after traumatic brain injury is associated with a novel microdialysis proteome. Neurocrit Care 12:324–336
11. Lundberg N (1960) Continuous recording and control of ventricular fluid pressure in neurosurgical practice. Acta Psychiatr Scand Suppl 36(149):1-193
12. MacMillan VH (1989) Cerebral energy metabolism in cyanide encephalopathy. J Cereb Blood Flow Metab 9:156-162
13. Moroa N, Suttona RL (2010) Beneficial Effects of Sodium or Ethyl Pyruvate after Traumatic Brain Injury in the Rat. Exp Neurol 225:391–401
14. Nedergaard M, Takano T, Hansen AJ (2002) Beyond the role of glutamate as a neurotransmitter. Nat Rev Neurosci 3:748-755
15. Nelson DW, Thornquist B, MacCallum RM, Nyström H, Holst A, Rudehill A, Wanecek M, Bellander BM, Weitzberg E (2011) Analyses of cerebral microdialysis in patients with traumatic brain injury: relations to intracranial pressure, cerebral perfusion pressure and catheter placement. BMC Medicine 9:21
16. Nielsen TH, Olsen NV, Toft P, Nordström CH (2013) Cerebral energy metabolism during mitochondrial dysfunction induced by cyanide in piglets. Acta Anaesthesiol Scand 57:793-801
17. Nielsen TH, Schalén W, Ståhl N, Toft P, Reinstrup P, Nordström CH (2014) Bedside diagnosis of mitochondrial dysfunction after malignant middle cerebral artery infarction. Neurocrit Care 21:35-42
18. Nordström CH, Rehncrona S, Siesjö BK (1978) Restitution of cerebral energy state, as well as of glycolytic metabolites, citric acid cycle intermediates and associated amino acids after 30 minutes of complete ischemia in rats anaesthetized with nitrous oxide or phenobarbital. J Neurochem 30:479-486

19. Nordström CH, Rehncrona S, Siesjö BK (1978) Effects of phenobarbital in cerebral ischemia. Part II: restitution of cerebral energy state, as well as of glycolytic metabolites, citric acid cycle intermediates and associated amino acids after pronounced incomplete ischemia. Stroke 9:335-343
20. Nordström CH, Reinstrup P, Xu W, Gärdenfors A, Ungerstedt U (2003) Assessment of the lower limit for cerebral perfusion pressure in severe head injuries by bedside monitoring of regional energy metabolism. Anesthesiology 98:809-814
21. Rehncrona S, Mela L, Siesjo BK (1979) Recovery of brain mitochondrial function in the rat after complete and incomplete cerebral ischemia. Stroke 10:437-446
22. Reinstrup P, Stahl N, Mellergard P, Uski T, Ungerstedt U, Nordström CH (2000) Intracerebral microdialysis in clinical practice: baseline values for chemical markers during wakefulness, anesthesia, and neurosurgery. Neurosurgery 47:701-709
23. Reinstrup P, Nordström CH (2011) Prostacyclin infusion may prevent secondary damage in pericontusional brain tissue. Neurocrit Care 14:441-446
24. Samuelsson C, Hillered L, Zetterling M, Enblad P, Hesselager G, Ryttlefors M, Kumlien E, Lewén A, Marklund N, Nilsson P, Salci K, Ronne-Engström1 E (2007) Cerebral glutamine and glutamate levels in relation to compromised energy metabolism: a microdialysis study in subarachnoid hemorrhage patients. J Cereb Blood Flow Metab 27:1309-1317
25. Siesjö BK (1978) Brain Energy Metabolism. John Wiley & Sons, Chichester, New York Brisbane Toronto 192-194
26. Soane L, Kahraman S, Kristian T, Fiskum G (2007) Mechanisms of impaired mitochondrial energy metabolism in acute and chronic neurodegenerative disorders. J Neurosci Res 85:3407-3415

27. Stein NR, McArthur DL, Etchepare M, Vespa PM (2012) Early cerebral metabolic crisis after TBI influences outcome despite adequate hemodynamic resuscitation. Neurocrit Care 17:49-57
28. Teasdale G, Jennett B (1974) Assessment of coma and impaired consciousness. A practical scale. Lancet 7872:81-84
29. Thelin EP, Nelson DW, Ghatan PH, Bellander BM (2014) Microdialysis Monitoring of CSF Parameters in Severe Traumatic Brain Injury Patients: A Novel Approach. Front Neurol 5:159
30. Timofeev I, Carpenter KL, Nortje J, Al-Rawi PG, O'Connell MT, Czosnyka M, Smielewski P, Pickard JD, Menon DK, KirkpatrickPJ, Gupta AK, Hutchinson PJ (2011) Cerebral extracellular chemistry and outcome following traumatic brain injury: a microdialysis study of 223 patients. Brain 134:484-494
31. Uchino H, Elmer E, Uchino K, Lindvall O, Siesjö BK (1998) Amelioration by cyclosporin A of brain damage in transient forebrain ischemia in the rat. Brain Res 812:216-226
32. Ungerstedt U, Pycock CH (1974) Functional correlates of dopamine neurotransmission. Bull der Schweiz Akad Med Wiss 30:44-55
33. Ungerstedt U, Bäckström T, Hallström Å, Grände PO, Mellergård P, Nordström CH. (1997) Microdialysis in normal and injured human brain. In: Kinney JM, Tucker HN, eds. Physiology, Stress, and Malnutrition: Functional Correlates, Nutritional Intervention . Philadelphia, New-York: Lippincott-Raven 361-374
34. Verweij BH, Muizelaar JP, Vinas FC, Peterson PL, Xiong Y, Lee CP (2000) Impaired cerebral mitochondrial function after traumatic brain injury in humans. J Neurosurg 93:815-820

35. Vespa P, Bergsneider M, Hattori N, Wu HM, Huang SC, Martin NA, Glenn TC, McArthur DL, Hovda DA (2005) Metabolic crisis without brain ischemia is common after traumatic brain injury: a combined microdialysis and positron emission tomography study. J Cereb Blood Flow Metab 25:763-774
36. Vespa PM, O'Phelan K, McArthur D, Miller C, Eliseo M, Hirt D, Glenn T, Hovda DA (2007) Pericontusional brain tissue exhibits persistent elevation of lactate/pyruvate ratio independent of cerebral perfusion pressure. Crit Care Med 35:1153-1160
37. Xiong Y, Gu Q, Peterson PL, Muizelaar JP, Lee CP (1997) Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. J Neurotrauma 14:23-34

Legends

Figure 1. Biochemical pattern during transition from a biochemical pattern of Mitochondrial Dysfunction to that of Ischemia. The change in lactate/pyruvate (LP) ratio is shown in relation to the simultaneously occurring changes in pyruvate, lactate and glucose. Due to the very high LP ratio this scale is logarithmic.

Figure 2. Biochemical pattern during transition from a biochemical pattern of Ischemia to that of Mitochondrial Dysfunction. The change in lactate/pyruvate (LP) ratio is shown in relation to the simultaneously occurring changes in pyruvate, lactate and glucose.

Figure 3. Variations in intracerebral lactate/pyruvate (LP) ratio in patients with extradural hematoma (EDH) and acute Subdural Hematoma (SDH) (A) and in patients with No Mass lesion and Cerebral Hemorrhagic Contusion (CHC) (B) during the 96 h study period. Data are given as mean values. The grey area indicates S.D.

Figure 4. Variations in intracerebral glutamate level in patients with extradural hematoma (EDH) and acute Subdural Hematoma (SDH) (A) and in patients with No Mass lesion and Cerebral Hemorrhagic Contusion (CHC) (B) during the 96 h study period. Data are given as mean values. The grey area indicates S.D.

Figure 5. Variations in intracerebral glycerol level in patients with extradural hematoma (EDH) and acute Subdural Hematoma (SDH) (A) and in patients with No Mass lesion and Cerebral Hemorrhagic Contusion (CHC) (B) during the 96 h study period. Data are given as mean values. The grey area indicates S.D.

TABLE 2 Pattern of biochemical variables in groups defined as Ischemia, Mitochondrial Dysfunction and normal LP-ratio (≤30)

		LP ratio	Lactate mmol/L	Pyruvate µmol/L	Glucose mmol/L	Glutamate µmol/L	Glycerol µmol/L
Ischemia							
N_{samp}= 985	Median	204	7.0	37	0.2	204	217
	q1 - q3	64 - 537	3.0 -9.9	16 - 56	0.1 - 0.6	37 - 361	99 - 474
Mitoch. Dysf.							
N_{samp}=4804	Median	42	7.4$^{n.s.}$	162	1.2***	48***	56**
	q1 - q3	35 - 59	5.4 – 9.7	122 - 216	0.6 - 2.0	8 - 98	28 - 137
LP ratio ≤ 30							
N_{samp}=9410	Median	18	2.4	135	1.7	5	33
	q1 - q3	15 - 22	1.8 - 3.4	103 - 187	1.1 - 2.3	3 - 15	17 - 62
Reference levels							
	Mean	23	2.9	166	1.7	16	82
	SD	4	0.9	47	0.9	16	44

Ischemia defined as LP-ratio >30 at Pyruvate < 70 µmol/L. Mitochondrial dysfunction (Mitoch. Dysf.) defined as LP-ratio > 30 at Pyruvate ≥ 70 µmol/L. Reference levels for the biochemical variables are based on data obtained from normal human brain [22]. In the table N_{samp} refers to the number of microdialysis samples in each group. Statistical comparison between the median levels of Lactate, Glucose, Glutamate and Glycerol during the episodes of Ischemia (N_{epis}=41) and Mitochondria dysfunction (N_{epis}=112) are shown ($p<0.001$ = ***; $p<p.002$=**).

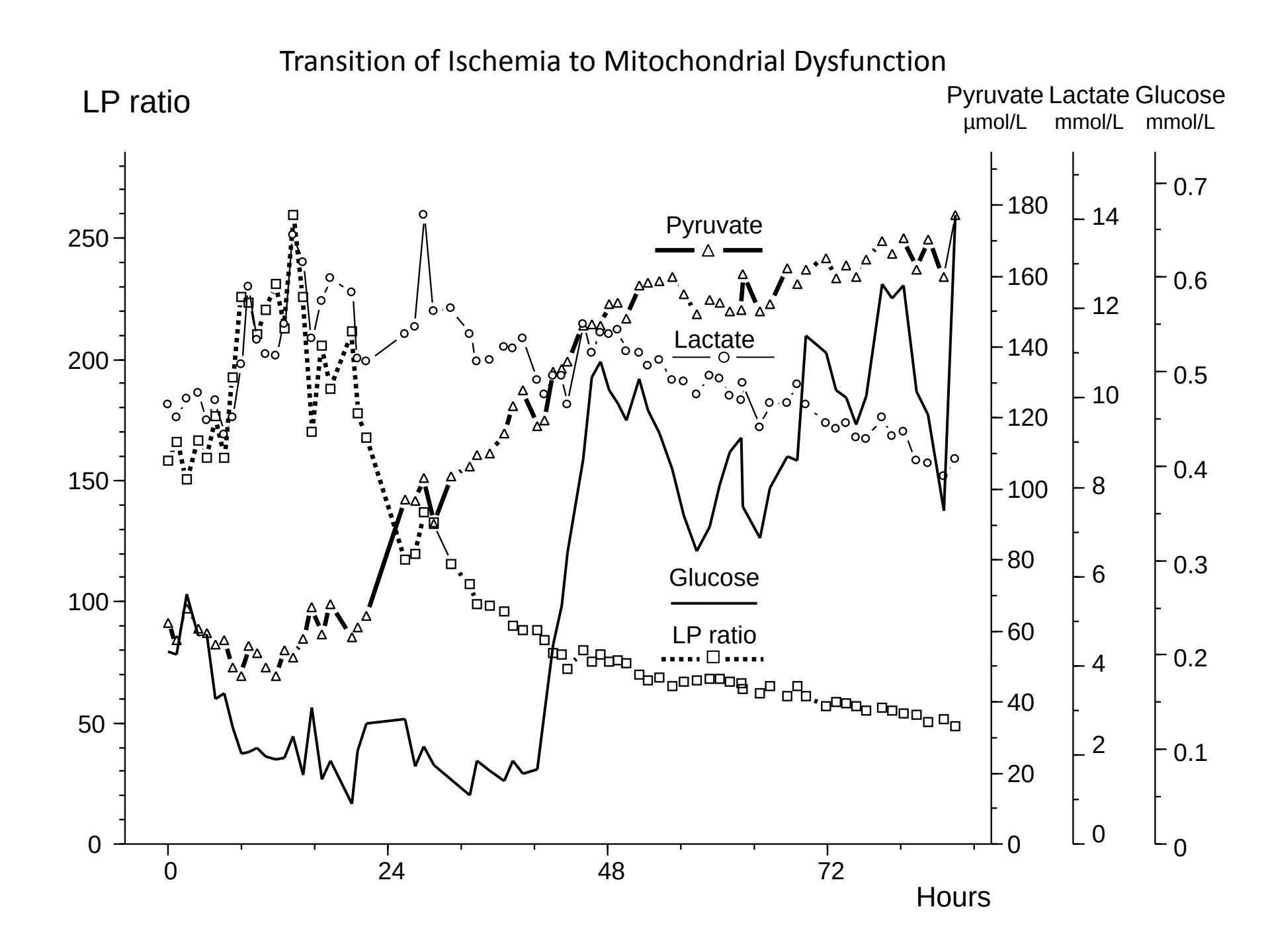

Transition of Ischemia to Mitochondrial Dysfunction
LP ratio
Pyruvate Lactate Glucose
µmol/L mmol/L mmol/L
Pyruvate
Lactate
Glucose
LP ratio
250
200
150
100
50
0
180
160
140
120
100
80
60
40
20
0
14
12
10
8
6
4
2
0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
24
48
72
Hours

TABLE 1 Basic data in four subgroups of TBI

	Patients	Median age	q1 - q3	Number of intracerebral MD catheters per patient			
	N	years	years	N=1	N=2	N=3	N=4
EDH	16	35	22 - 48	10	5	1	0
No Mass	52	27	19 - 42	48	4	0	0
SDH	62	49	28 - 63	26	32	3	1
CHC	83	45	26 - 58	24	43	14	2
Total	213	40	22 - 55	108	84	18	3

Diagnostic groups: Extradural Hematoma (EDH); acute Subdural Hematoma (SDH); Cerebral Hemorrhagic Contusion (CHC); No Mass denotes patients not treated with surgical evacuation. MD denotes microdialysis. Age is given as median and interquartile range (q1-q3).

TABLE 3 Number of observed transitions between Ischemia and Mitochondrial Dysfunction (Mit.Dysf.)

	EDH	No Mass	SDH	CHC
	N	N	N	N
Ischemia to Mit. Dysf.	0	2	6	14
Mit. Dysf. to Ischemia	0	0	8	10

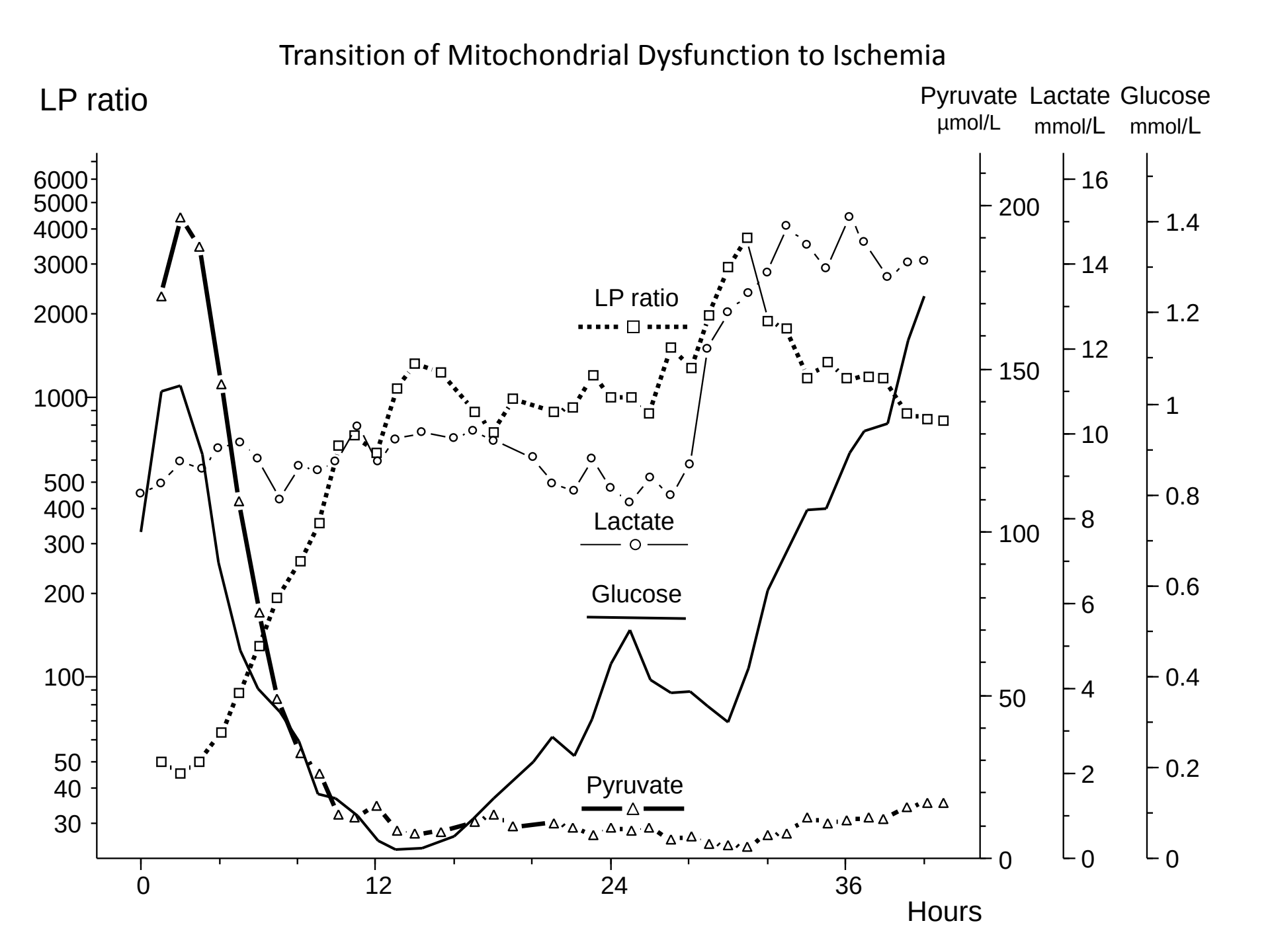
N represents the number of microdialysis catheters with observed biochemical transition from ischemia to mitochondrial dysfunction or reverse. Data regarding the underlying changes in pyruvate level are given in the text.

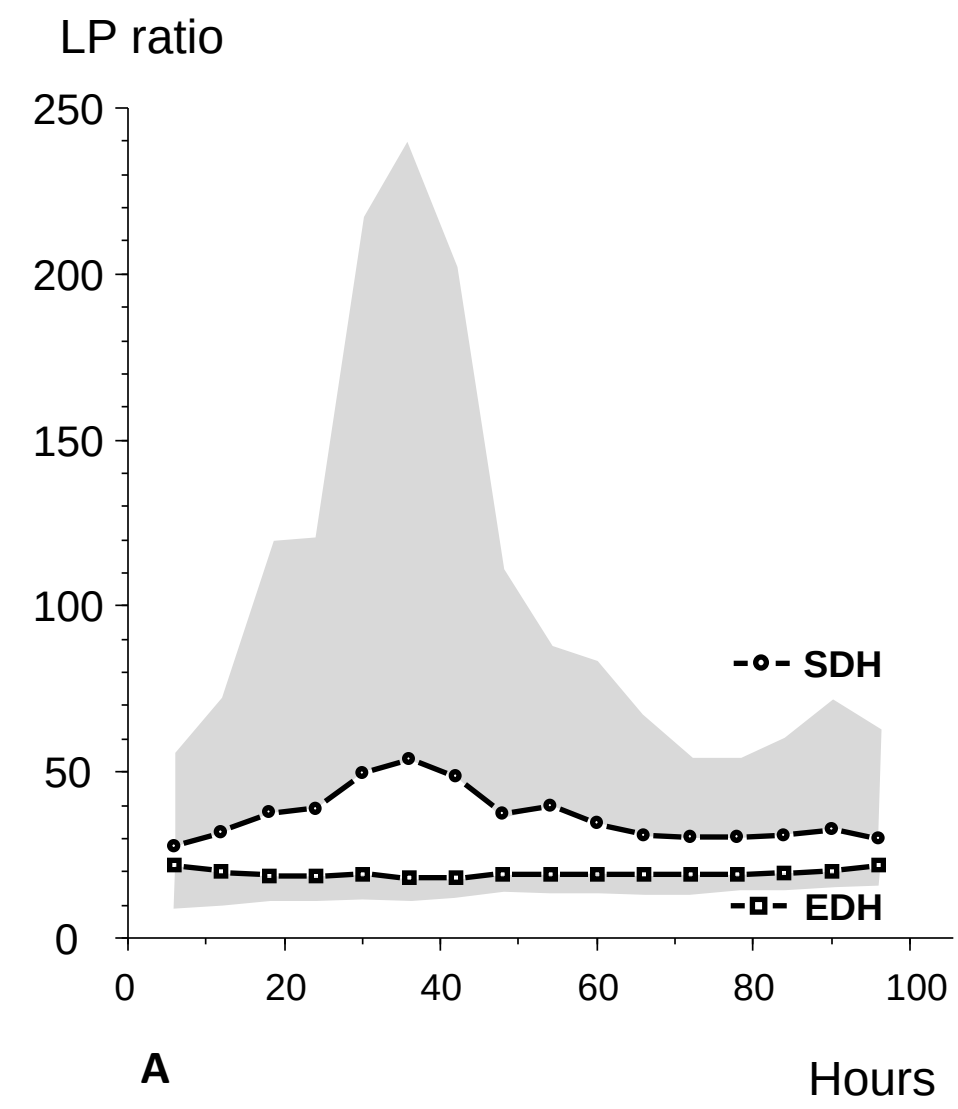
Diagnostic groups: Extradual Hematoma (EDH); acute Subdural Hematoma (SDH); Cerebral Hemorrhagic Contusion (CHC); No Mass denotes patients not treated with surgical evacuation.

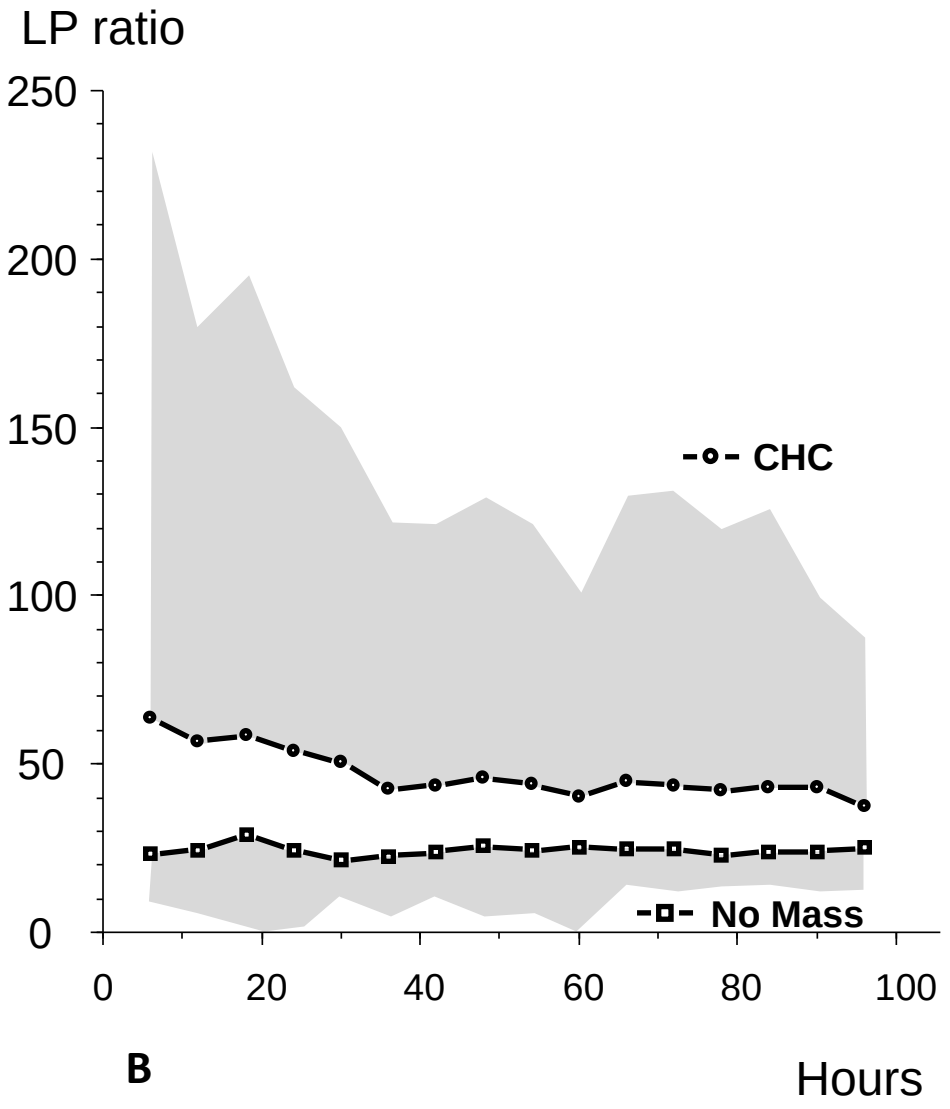
TABLE 4 Mortality in four diagnostic groups of TBI and its relation to the biochemical patterns of Ischemia and Mitochondrial Dysfunction

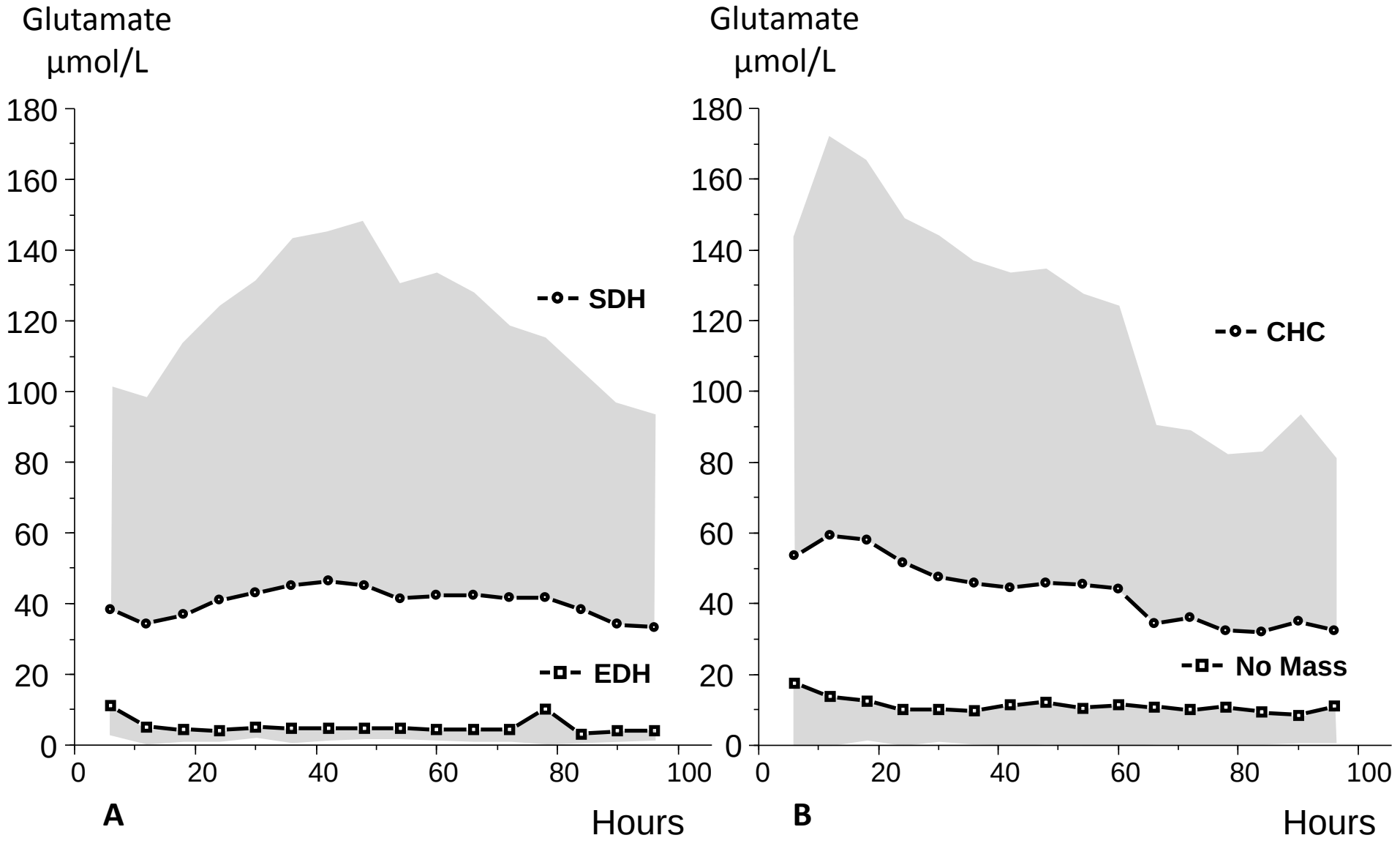
	EDH (N=16)			No Mass (N=52)			SDH (N=62)			CHC (N=83)			Total (N=213)		
Dead	2			5			18			12			37		
per cent	*13*			*9*			*29*			*14*			*17*		
	Isch	**Mit Dysf**	**LP≤30**	**Isch**	**Mit Dysf**	**LP≤30**	**Isch**	**Mit Dysf**	**LP≤30**	**Isch**	**Mit Dysf**	**LP≤30**	**Isch**	**Mit Dysf**	**LP≤30**
Hours	8	54	886	59	511	1468	403	1101	3196	515	3138	3860	985	4804	9410
per cent	*1*	*6*	*93*	*3*	*25*	*72*	*9*	*23*	*68*	*7*	*42*	*51*	*6*	*32*	*62*
Patients	1	3	12	6	15	31	11	10	41	23	19	41	41	47	125
Dead	1	0	1	2	1	2	8	4	6	3	1	8	14	6	17
per cent	*100*	*0*	*8*	*33*	*7*	*6*	*73*	*40*	*14*	*13*	*5*	*20*	*34*	*13*	*14*

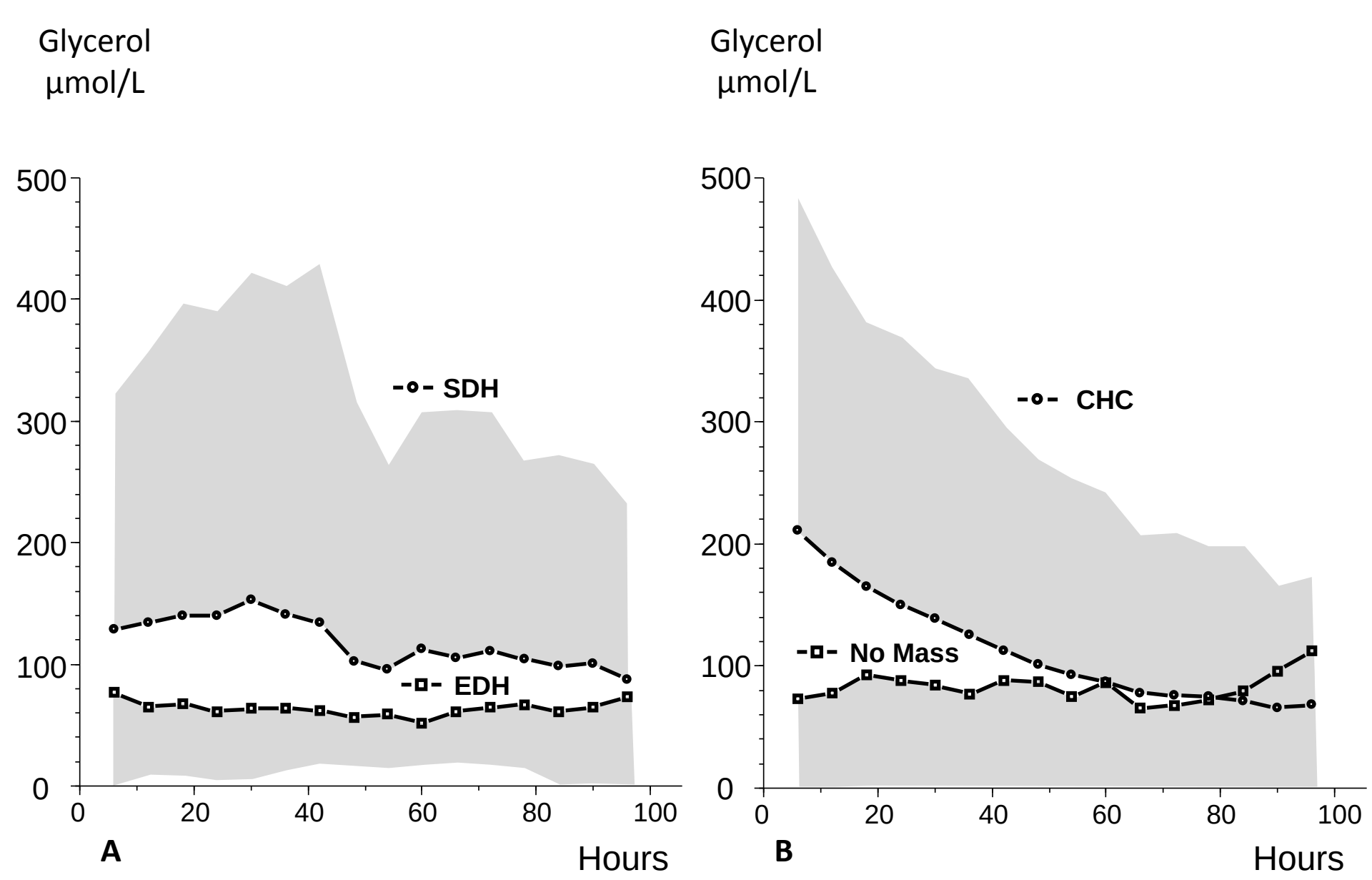
Mortality in four diagnostic groups: Extradural Hematoma (EDH); acute Subdural Hematoma (SDH); Cerebral Hemorrhagic Contusion (CHC); No Mass denotes patients not treated with surgical evacuation. Mortality is also given separately for the biochemical subgroups defined as Ischemia (Isch), Mitochondrial Dysfunction (Mit Dysf) and normal aerobic metabolism (LP-ratio ≤30) within each diagnostic group.


Transition of Mitochondrial Dysfunction to Ischemia
LP ratio
Pyruvate µmol/L
Lactate mmol/L
Glucose mmol/L
6000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
200
150
100
50
0
16
14
12
10
8
6
4
2
0
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LP ratio
Lactate
Glucose
Pyruvate
0
12
24
36
Hours


LP ratio
250
200
150
100
50
0
SDH
EDH
0
20
40
60
80
100
A
Hours


LP ratio
250
200
150
100
50
0
CHC
No Mass
0
20
40
60
80
100
B
Hours


Glutamate
µmol/L
180
160
140
120
100
80
60
40
20
0
SDH
EDH
0
20
40
60
80
100
A
Hours
Glutamate
µmol/L
180
160
140
120
100
80
60
40
20
0
CHC
No Mass
0
20
40
60
80
100
B
Hours


Glycerol
µmol/L
500
400
300
200
100
0
SDH
EDH
0
20
40
60
80
100
A
Hours
Glycerol
µmol/L
500
400
300
200
100
0
CHC
No Mass
0
20
40
60
80
100
B
Hours