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1 **Neuroprotective effects of lactate and ketone bodies in acute brain injury**

2 Test

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46 Running head: Neuroprotective effects of lactate and ketones

47 Word count: 3709 words

48 **Unstructured abstract**

49 The goal of neurocritical care is to prevent and reverse the pathologic cascades of secondary
50 brain injury by optimizing cerebral blood flow, oxygen supply and substrate delivery. While
51 glucose is an essential energetic substrate for the brain, we frequently observe a strong decrease
52 in glucose delivery and/or a glucose metabolic dysregulation following acute brain injury. In
53 parallel, during the last decades, lactate and ketone bodies have been identified as potential
54 alternative fuels to provide energy to the brain, both under physiological conditions and in case of
55 glucose shortage. They are now viewed as integral parts of brain metabolism. In addition to their
56 energetic role, experimental evidence also supports their neuroprotective properties after acute
57 brain injury, regulating in particular intracranial pressure control, decreasing ischemic volume,
58 and even improving cognitive functions as well as survival. In this review, we present available
59 data supporting a future therapeutic use of lactate and ketone bodies in acute brain injury. We
60 also review preclinical and clinical evidence exploring the mechanisms underlying their
61 neuroprotective effects, and identify research priorities in the field of neuroenergetics.

62

63

64 Keywords: alternative brain fuels, brain metabolism, ketone bodies, lactate, neuroprotection,
65 stroke, TBI, hypoxia.

66

67 **Introduction**

68 Acute brain injury refers to brain damage that occurs suddenly, such as traumatic brain injury
69 (TBI), stroke, subarachnoid hemorrhage (SAH) or global ischemia, for example. The insult itself
70 leads to a primary injury, which is followed by secondary brain damage, a pathologic cascade that
71 arises hours to days after the initial injury. The main causes of this secondary brain injury are
72 cerebral blood flow disturbances, which lead to hypoxia and a strong decrease of glucose delivery
73 to the brain. To improve patient outcome, the goal of neurocritical care, after detection of the acute
74 brain injury, is to avoid or reverse the underlying cascades that occur during this secondary brain
75 injury period by optimizing cerebral blood flow, oxygen supply and substrate delivery to the brain.²
76 Glucose is an essential substrate to satisfy brain energy requirements. However, its availability is
77 frequently reduced following acute brain injury.^{3,4} In the last decades, lactate and ketone bodies
78 have been identified as alternative fuels to provide energy to the brain and preserve glucose for
79 other critical metabolic needs. Results are also accumulating concerning their potential
80 neuroprotective effects and clinical applications.

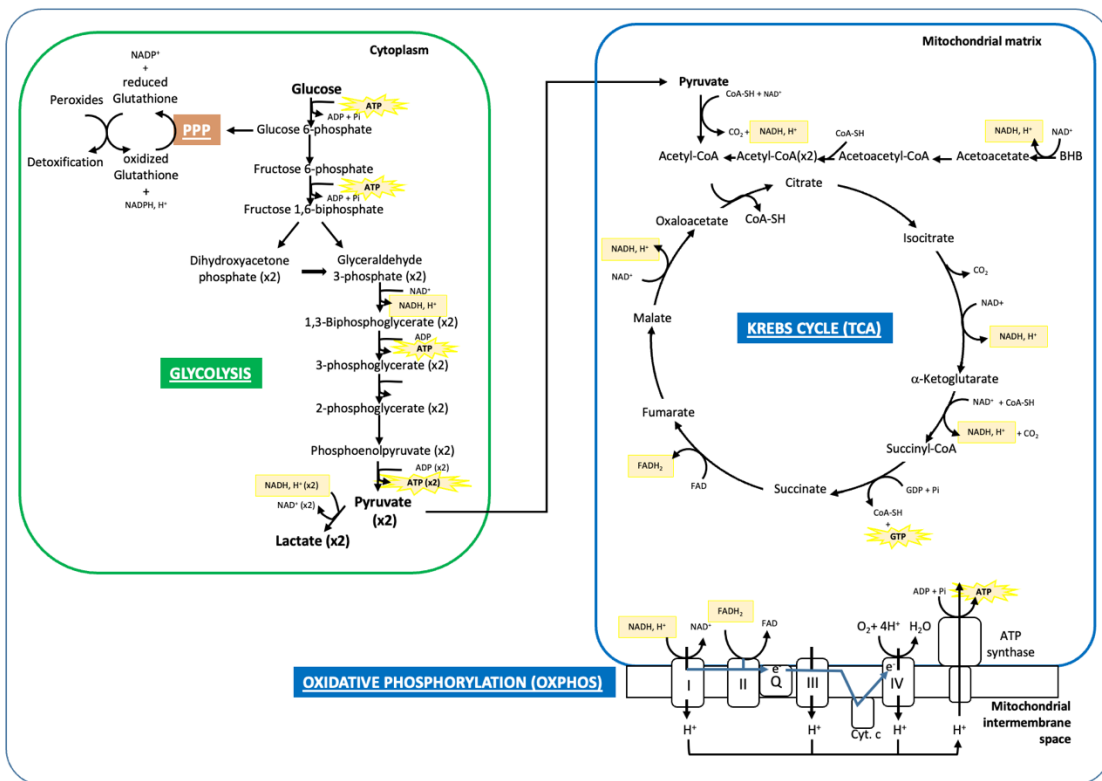
81 In this review, we present available data supporting a therapeutic use of lactate and ketone bodies
82 in acute brain injury. We also review preclinical and clinical evidence exploring the mechanisms
83 underlying their neuroprotective effects, and identify research priorities in the field of
84 neuroenergetics.

85

86 **Glucose for brain energy supply**

87 While the brain represents only 2% of the total body mass, its glucose consumption is around
88 20% of the systemic consumption, which represents more than 10 times the predicted value
89 relative to its mass. Glucose is an essential substrate to maintain normal brain metabolism and
90 function. It is required for glycolysis, which in turn provides 2 ATP, and 2 pyruvates that can enter
91 the tricarboxylic acid (TCA) cycle to ensure energy production, in majority through the generation
92 of reducing equivalents (NADH and FADH₂), which are then used in the electron transport chain

93 to generate around 30 more ATP molecules (the exact yield of ATP from one round of the TCA
 94 cycle can vary but it has been experimentally estimated that NADH can contribute about 2.5 to 3
 95 ATP, and each $FADH_2$ can contribute about 1.5 to 2 ATP) (**Figure 1**). In addition to provide
 96 energy to the brain, glucose is a precursor in the synthesis of neurotransmitters and participates
 97 in the pentose phosphate pathway (PPP), also known as the hexose monophosphate shunt,
 98 which is a metabolic pathway that runs parallel to glycolysis and is essential for NADPH and
 99 pentose sugar production, as well as for redox homeostasis. Indeed, the PPP plays a crucial role
 100 in maintaining reduced glutathione (GSH) levels in cells by supplying the necessary reducing
 101 equivalents in the form of NADPH. Reduced glutathione acts as a critical antioxidant and
 102 contributes to the cellular defense mechanisms against oxidative stress due to reactive oxygen
 103 species (ROS) production. The PPP is therefore a major pathway for neuronal protection after an
 104 acute brain insult during which high amount of ROS are produced.



105 **Figure 1: Glucose metabolism pathway.** In the cytoplasm, glucose is metabolized to pyruvate through
 106 glycolysis. This pyruvate can either provide lactate or be redirected to the mitochondria to provide ATP
 107 through (i) the Krebs cycle (TCA cycle) coupled to (ii) oxidative phosphorylation (OXPHOS).
 108

109

110 During the acute phase following brain injury, cerebral glucose availability may become
111 insufficient to meet the metabolic demands due to cerebral blood flow failure, blood-brain barrier
112 dysfunction or diffusion impairment. Indeed, glucose storage is very low in the brain. If glycogen
113 can be found in astrocytes, its consumption during ischemia can sustain brain functions for only
114 2 min (measurements made in rats (Swanson et al. 1989)). In human, a decrease in glycemia
115 linked with a decrease in cerebral glucose concentration was demonstrated to be deleterious in
116 the context of acute brain injury, leading to neuroglycopenia [Vespa P, McArthur DL, Stein N, et
117 al. Tight glyceic control increases metabolic distress in traumatic brain injury: a randomized
118 controlled withinsubjects trial. Crit Care Med 2012; 40:1923–1929 / Magnoni S, Tedesco C,
119 Carbonara M, et al. Relationship between systemic glucose and cerebral glucose is preserved in
120 patients with severe traumatic brain injury, but glucose delivery to the brain may become limited
121 when oxidative metabolism is impaired: implications for glyceic control. Crit Care Med 2012;
122 40:1785–1791].

123 Glucose infusion following brain injury has been tested decades ago on a rat model of brain
124 ischemia, aiming to reverse this substrate-deprived state and restore brain energy metabolism.
125 Surprisingly, it was associated with increased mortality and extension of injury.¹ Since then, these
126 results have been consistently replicated in various experimental models of ischemic brain
127 injury.^{1–3} In patients, spontaneous high plasma glucose levels have also been associated with
128 increased infarct size, worse clinical outcomes and increased mortality in ischemic stroke and
129 SAH, especially when it happens before or early after ischemia.^{1–3}

130 With the same idea in mind to control glucose level, insulin therapy was also tested, based on the
131 early promising results of intensive insulin therapy in critically ill surgical patients¹. Many trials
132 have investigated whether insulin-based glyceic control could improve the outcomes of brain-
133 injured patients. Among these, the large GIST-UK trial failed to show a benefit of intensive insulin
134 therapy in patients with acute stroke, although it was likely underpowered due to slow enrollment

135 which justified its early termination.¹ Overall, this trial and other studies pooled in two recent
136 systematic reviews suggest that tight glycemic control causes more symptomatic and
137 asymptomatic hypoglycemic episodes, both associated with poor outcomes.^{1,2}

138 **Ketone bodies and lactate as alternative fuels**

139 It is well established that the brain is metabolically flexible and can metabolize other substrates,
140 such as ketone bodies (KB) and lactate.⁵ KB are produced in the liver from fatty acids. They
141 represent a major source of energy for the brain in neonates, who rely on breastfeeding for their
142 diet which is rich in lipids. In adults, when glucose availability is limited in situations such as
143 fasting, a very low-carbohydrate diet (ketogenic diet), or prolonged exercise, KB are produced as
144 alternative fuels and used significantly by the brain. The three main KB synthesized by the liver
145 are acetoacetate (AcAc), beta-hydroxybutyrate (BHB; AcAc can be converted to BHB, which is
146 the predominant ketone body in circulation) and acetone, a minor KB that is produced as a
147 byproduct. KB enter the brain via monocarboxylate transporters (MCTs) and provide energy to
148 the brain in the form of acetyl-CoA entering the tricarboxylic acid cycle to be oxidized, hence
149 reducing reliance on glycolysis for energy production. This alternative energy production turns out
150 to be highly efficient: BHB, one of the main KB, is converted to acetyl-CoA through only three
151 enzymatic reactions, compared to the ten reactions with several rate-limiting enzymes required
152 to convert glucose to acetyl-CoA. It has been shown in non-fasted adult humans infused with [2,4-
153 ¹³C₂]-BHB and using ¹H-¹³C polarization transfer spectroscopy that KB can cross the blood-brain
154 barrier and are metabolized by the brain [PMID : 12142574]. These observations have been
155 confirmed in pre-clinical studies (Roy et al. 2015). In euglycemic conditions, ketone bodies are
156 predominantly metabolized by neurons and account for 6% of total coenzyme A oxidation in
157 normal conditions.¹ This phenomenon was found to be amplified in a model of trauma.¹ On a
158 juvenile rat model of TBI infused with [2,4-¹³C₂]-BHB, it has been shown using ¹³C magnetic
159 resonance spectroscopy (MRS) that KB were extensively metabolized, mainly in neurons [PMID
160 : 36005582].

161 Concerning lactate, experiments in the 1990s led by Pellerin and Magistretti suggest that lactate
162 is commonly used for basal brain metabolism under physiological conditions. They described a
163 metabolic cooperation between astrocytes and neurons, termed the astrocyte-neuron lactate
164 shuttle (ANLS) (Pellerin and Magistretti 1994). In this model, glutamate released as part of
165 synaptic activity is then taken up by astrocytes, one of their major function to avoid exitotoxicity.
166 Concomitantly with glutamate uptake, a Na^+ influx will occur and its intracellular concentration will
167 rise. As a consequence, Na^+/K^+ ATPase activity will increase, leading to enhanced glucose
168 utilization and lactate production by astrocytes. This astrocytic lactate is then transferred to
169 neurons, through the MCTs (isoforms MCT1 and 4 for astrocytes, MCT2 for neurons; same
170 transporters as the ones used for KB). Once in neurons, lactate is converted back to pyruvate
171 and used as an energetic substrate. Since its description, the ANLS has gained popularity and
172 has been validated in experimental models *in vitro*, *ex vivo* and *in vivo*, although remaining
173 controversial (Bak and Walls 2018, Barros and Weber 2018). Using infusion of $[3\text{-}^{13}\text{C}]\text{lactate}$ and
174 ^{13}C MRS it has been shown in rats that lactate efficiently cross the blood-brain-barrier and once
175 in the brain, it is preferentially a neuronal substrate (Bouzier et al. 2000). These observations
176 were confirmed in another study that showed that extracerebral lactate contributes at least two-
177 fold more to replenish the neuronal than the glial pyruvate pools [PMID : 25522255]. Authors
178 concluded that a primary utilization of exogenous lactate takes place in neurons rather than in
179 astrocytes (Bouzier et al. 2000). Moreover, lactate entry into neurons through its transporter
180 MCT2 was shown to be necessary to sustain brain function (Suzuki et al. 2011, Netzahualcoyotzi
181 and Pellerin 2020, Roumes et al. 2021). Under supraphysiological conditions, lactate can
182 represent up to 60% of brain energy supply. More recently, preferential lactate uptake by the brain
183 over glucose has been described in a healthy rat model¹ and in exercising man¹⁻³, likely reflecting
184 an adaptation of the cerebral metabolic ratio to transient hyperlactatemia and relative glucose
185 shortage. A similar observation has been made in patients with acute brain injury, for who an

186 increase in lactate production occurred during spontaneous glucose depletion despite the
187 absence of brain hypoxia.¹⁻³ Finally, blocking astrocyte lactate transport in a rat model of cerebral
188 ischemia altered functional recovery, suggesting an obligatory role of lactate in basal
189 neuroenergetics.¹ This obligatory role was further confirmed in another rat model of neonatal
190 hypoxia-ischemia, in which reductions in brain lesion volume and ROS production were observed
191 when lactate was injected, but not when it was co-administered with oxamate, a lactate
192 dehydrogenase inhibitor therefore avoiding its conversion into pyruvate and therefore its
193 metabolism (Roumes et al. 2021).

194 Thus both KB and lactate can serve as an alternative oxidative substrate for the brain and are
195 mainly metabolized by neurons. They both enter through MCTs and deliver energy after their
196 oxidation in the TCA cycle, entering either at the acetylCoA or at the pyruvate level, respectively
197 (**Figure 2**). In both cases, their metabolism also lead to the conversion of cytosolic NAD⁺ into
198 NADH and thus modify the redox potential of the cell (during the conversion of BHB into AcAc or
199 lactate into pyruvate).

200 Taken altogether, challenges in glucose availability after an acute brain injury, combined with the
201 possibility of the brain to metabolize efficiently KB and lactate, offer new perspectives. A direct
202 modulation of neuronal metabolism by these alternative energy substrates could be of major
203 interest in the search for therapeutic avenues in the context of acute brain injury.

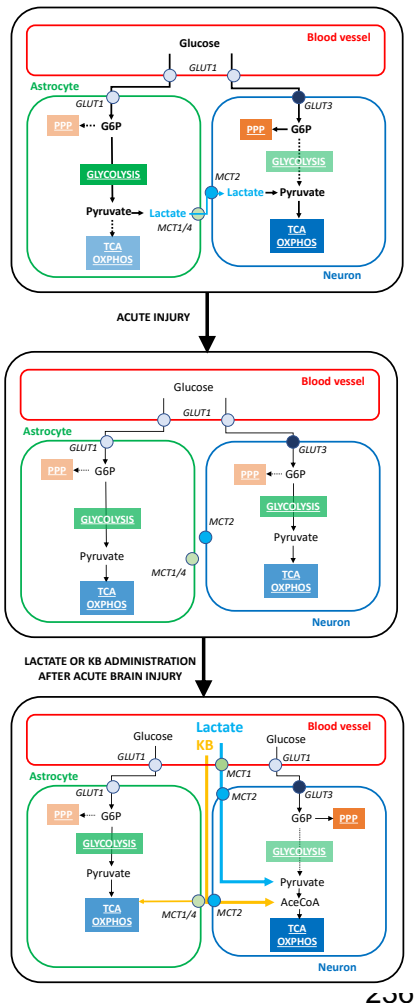


Figure 2: Compartmentalization of cerebral glucose metabolism and consequence in brain injury as well as for neuroprotection strategies. A- In the brain, glucose from the bloodstream enters both astrocytes and neurons. It is metabolized to pyruvate in the cytoplasm (glycolysis) then supplies ATP via pyruvate oxidation into the mitochondria (TCA cycle + OXPHOS). However, astrocytes have a greater glycolytic capacity compared to neurons, which are highly oxidative cells (Herrero-Mendez et al. 2009). Astrocytes are known to produce high level of lactate, which can be further transferred to neurons where it can be used as an energy substrate through the TCA and OXPHOS. Glucose can also enter directly into the neuron. Low glycolysis in neurons allows glucose conversion into the pentose phosphate pathway PPP to sustain antioxidant protection. B- In acute brain injury condition, the low level of circulating glucose can not meet cerebral energy needs and the metabolic cooperation between astrocyte and neurons can not be maintained. Glucose is mainly used for oxidative metabolism in both cell types to maintain ATP level. C- In order to overcome the energy deficit induced by acute brain injury, energy substitution strategies could be adopted. Lactate or exogenous ketone bodies enter directly into brain cells but predominantly in neurons (Pan et al. 2002, Duarte et al. 2015, Scafidi et al. 2022). Lactate is then converted into pyruvate, while ketone bodies are precursors of acetyl-CoA. Pyruvate and Acetyl-CoA are then oxidized through TCA cycle and OXPHOS to provide energy. Saved glucose can be redirected to the PPP pathway to reestablish the antioxidant defense. GLUT, glucose transporter; KB, ketone bodies; MCT, monocarboxylate transporter; OXPHOS, oxidative phosphorylation, PPP, pentose phosphate pathway; TCA, tricarboxylic acid cycles.

237 Alternative fuels and the pentose-phosphate pathway

238 Neuronal glycolysis is controlled by the enzymatic complex APC/C-Cdh1 that degrades the
 239 proglycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3). The
 240 enzyme PFKFB3 is less abundant in neurons than in astrocytes, resulting in a lower glycolytic
 241 rate and in the redirection of glucose-6-phosphate through the PPP.^{30,92} Astrocytes, on the other
 242 hand, exhibit a greater PFKFB3 activity, resulting in higher glycolytic rate and lactate production.
 243 Hence, this compartmentalization suggests that neurons use glucose preferentially for regulation
 244 of their redox state rather than for energy requirements (Bolanos 2016). In stressed states, such
 245 after an oxygen-glucose deprived condition, the reperfused neurons showed elevated PFKFB3
 246 expression (Li et al. 2019). Such changes directed neuronal glucose metabolism from PPP to

247 aerobic glycolysis compared to normal neurons, resulting in increased ROS production and
248 apoptosis during reperfusion. Interestingly, both KB and lactate are oxidative substrates and
249 provide acetylCoA or pyruvate, respectively, while contributing to the conversion of cytosolic
250 NAD⁺ into NADH. An increase in the NAD⁺/NADH ratio will inhibit glycolysis and redirect glucose
251 through the PPP, and therefore will increase the pool of reduced glutathione. Indeed, it has been
252 shown that both alternative fuels can decrease ROS damages réf 12 + Kim, do Y., et al. Ketone
253 bodies are protective against oxidative stress in neocortical neurons. J. Neurochem. 2007;
254 101:1316–1326. [PubMed: 17403035] Maalouf M, et al. Ketones inhibit mitochondrial production
255 of reactive oxygen species production following glutamate excitotoxicity by increasing NADH
256 oxidation. Neuroscience. 2007; 145:256– 264. [PubMed: 17240074] Jarrett S, et al. The ketogenic
257 diet increases mitochondrial glutathione levels. J. Neurochem. 2008; 106:1044–1051. [PubMed:
258 18466343] + [Joseph Lemire 1, Christopher Auger, Ryan Mailloux, Vasu D Appanna.
259 Mitochondrial lactate metabolism is involved in antioxidative defense in human astrocytoma cells.
260 J Neurosci Res . 2014 Apr;92(4):464-75. doi: 10.1002/jnr.23338. Epub 2014 Jan 22.] whereas
261 the activity of G6PD, the key enzyme of the first (and irreversible) step of the PPP, was
262 significantly increased with lactate in neuronal cultures Pöttsch A, Zocher S, Bernas SN, Leiter
263 O, Rünker AE, Kempermann G. L-lactate exerts a pro-proliferative effect on adult hippocampal
264 precursor cells *in vitro*. iScience. 2021 Feb 3;24(2):102126. doi: 10.1016/j.isci.2021.102126.
265 PMID: 33659884; PMCID: PMC7895751.

266

267 **Lactate and ketone bodies to control neuroinflammation**

268 Emerging data suggest that lactate and ketone bodies have anti-inflammatory properties, but the
269 underlying mechanisms remain unclear. Microglia are key regulators of brain homeostasis and
270 act as Janus-faced cells regarding neuroinflammation.⁹³ After an ischemic event, microglia are
271 activated and release pro-inflammatory factors, such as TNF- α , IL-1 β , IL-6 and IFN- γ , exhibiting

272 a pro-inflammatory or “M1” microglial phenotype.⁹⁴ On the other hand, microglia can also promote
273 tissue and vascular remodeling via expression of anti-inflammatory factors such as IL-10, VEGF,
274 TGF- β and BDNF, exhibiting an anti-inflammatory or “M2” phenotype. These microglial
275 phenotypes are tightly linked to their metabolism, and lactate was shown to switch microglial
276 profiles from a glycolytic M1 to an oxidative M2 phenotype, hence promoting microglial energy
277 production.⁹⁵ Lactate has been shown to inhibit microglia-mediated neuroinflammation through
278 the Akt-pathway leading to elongation of microglial processes [Hong, Hongxiang; Su, Jianbin;
279 Zhang, Yi; Xu, Guanhua; Huang, Chao; Bao, Guofeng; Cui, Zhiming. A novel role of lactate:
280 Promotion of Akt-dependent elongation of microglial process. International Immunopharmacology
281 June 2023 119 DOI: 10.1016/j.intimp.2023.110136]. BHB was shown to exert similar effects
282 through induction of microglial ramifications and of an anti-inflammatory response in a Akt-
283 dependant manner.⁹⁶ There is also evidence that a ketogenic diet reduces ROS generation and
284 suppresses inflammasome activation.⁹⁷ In another study, ketogenic diet and BHB post-treatment
285 reduced infarct volume and suppressed the over-activation of microglia in a mouse model of MCA
286 occlusion reference 85.

287 Together with glucose redirection through the PPP, these anti-inflammatory effects might
288 constitute an elegant hypothesis to explain some of the clinical benefits observed with alternative
289 fuel supplementation in acute brain injury.

290

291 **Increase in cerebral blood flow**

292 In an *in vitro* study on rat hippocampal and neocortical slices, cerebral vasomotor tone was
293 influenced by the metabolic state of the tissue. In hypoxic conditions, astrocyte glycolysis and
294 lactate production were increased, causing vasodilation via an attenuation of prostaglandin
295 uptake.^{6,68} In a cohort of patients with severe acute brain injury (SAH and TBI), a lactate infusion
296 increased extracellular glucose levels and improved cerebral blood flow estimated by transcranial
297 Doppler.⁵⁰ Explanation of this mechanism is not well understood. Lactate could act as a

298 transmitter of metabolic information by modulating prostaglandin action and cerebral
299 vasodilatation causing cerebral blood flow to increase, regulates the NAD⁺/NADH redox ratio by
300 conversion to pyruvate, and/or activates a G-protein coupled receptor in neurons, astrocytes,
301 and capillaries (Gordon 2008, Begersen 2012, Lauritzen 2014). Interestingly, KB can also increase
302 cerebral blood flow as shown in mice by MRI⁶⁹, and in human healthy subjects by PET scan⁶⁵ and
303 the Kety-Schmidt technique⁷⁰.

304

305 **Control of brain edema**

306 Intracranial hypertension is one of the major challenges faced by neurocritical care clinicians
307 following TBI, massive SAH or hypoxic-ischemic brain injury. To control intracranial pressure
308 (ICP), conventional treatment is based on a stepwise approach mostly described in TBI and
309 involves deep sedation and analgesia, correct positioning of the patient, and hypertonic sodium
310 chloride or mannitol infusions.⁷¹ If the efficacy of osmotic therapy to control ICP is well established,
311 it is also associated with side effects, among which are hyperchloremic acidosis and fluid
312 overload.⁷² Based on its favorable molecular profile, sodium lactate has been identified as a
313 potential agent to control ICP while avoiding these deleterious effects. It was first tested in a
314 randomized trial in humans against mannitol in the treatment of intracranial hypertension during
315 severe TBI. In this study, lactate was associated with a greater and more prolonged decrease in
316 ICP than mannitol.⁵¹ This result has since been replicated, and was also shown to prevent
317 intracranial hypertensive episodes and fluid overload.^{49,73,74} Moreover, half-molar sodium lactate
318 administered to patients with severe head trauma was associated with a mild metabolic alkalosis,
319 counteracting the hyperchloremic acidosis associated with “traditional” osmotic therapy.⁵² This
320 control of brain edema might explain why lactate has been associated with increased tissue
321 oxygenation. In rat models of TBI, exogenous sodium lactate increased cerebral tissue oxygen
322 pressure, possibly via improvement of oxygen diffusion due to reduced tissue edema.^{75,76} The
323 antiedematous effect of sodium lactate might be induced by an increased expression of VRAC,

324 KCC2 and AQP4, channels involved in cell volume regulation and free water movements across
325 membranes, rather than by an osmotic effect.⁷⁵ Concerning KB, their neuroprotective effects
326 were examined in rats with transient occlusion of middle cerebral artery. The administration of
327 BHB (30 mg.kg⁻¹.h⁻¹) significantly reduced cerebral infarct area and edema formation.
328 <https://doi.org/10.1254/jip.89.36>. Réf 81

329

330 **Reduction of ischemic lesion volume**

331 As described just before, KG have been associated with reductions in infarct volume after middle
332 cerebral artery occlusion^{81 85} but also after TBI.^{82,83} *In vitro* experiments on cultured cortical
333 neurons showed that a BHB-based treatment prevents neuronal death induced by glucose
334 deprivation or hypoxia by stimulation of the autophagic flux thus preventing autophagosome
335 accumulation and improving neuronal survival.⁸⁴ Moreover, both lactate and ketone bodies can
336 limit excitotoxicity, reducing neuronal death as well as lesion volume. In a glutamate excitotoxicity
337 context, pyruvate coming from lactate is metabolized to produce ATP. This ATP in turn activates
338 the PI3K pathway leading to KATP channels opening. This results in the hyperpolarization of the
339 neuron, decreasing neuronal excitability and leading to a neuroprotective effect [P. Jourdain^{1,2},
340 K. Rothenfusser^{1,2}, C. Ben-Adiba², I. Allaman¹, P. Marquet^{2,3,4,5} & P. J. Magistretti^{1,2,6}. Dual
341 action of L-Lactate on the activity of NR2B-containing NMDA receptors: from potentiation to
342 neuroprotection *Scientific Reports* Open Access Volume 6:19 February 2016 Article number
343 21250]. The neuroprotective effect of ketone bodies, targeting glutamate excitotoxicity, has also
344 been shown in rat neocortical neurons [Maalouf M, Sullivan PG, Davis L, Kim DY, Rho JM.
345 Ketones inhibit mitochondrial production of reactive oxygen species production following
346 glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience*. (2007) 145:256–64. doi:
347 10.1016/j.neuroscience.2006.11.065]. In this deleterious context, ketone bodies can compete
348 with the Cl⁻ anion and inactivate the vesicular glutamate transporters (VGLUTs), decreasing

349 presynaptic glutamate release [Juge N, Gray JA, Omote H, Miyaji T, Inoue T, Hara C, et al.
350 Metabolic control of vesicular glutamate transport and release. *Neuron*. (2010) 68:99–112. doi:
351 10.1016/j.neuron.2010.09.00]. A summary of *in vivo* studies showing a reduction of the infarct
352 volume after KB administration can be found in Table 2 of the following review
353 (<https://doi.org/10.1038/sj.jcbfm.9600>).

354 Lactate was also shown to decrease brain lesion volume in several rat models after acute brain
355 injuries such as neonatal hypoxia-ischemia réf 12 et 80, TBI and stroke,^{12,77–80} ajouter Castillo
356 2015 JCBFM.

357

358 **Improvement of recovery and survival**

359 Both substrates have been associated with improvement of cognitive function. In a rat model of
360 neonatal hypoxia-ischemia, lactate administration was associated with short and long term
361 improvement of motor and cognitive functions (réf 12). Improvement of long term cognitive
362 functions was also observed after lactate injections in rat models of TBI or stroke.^{87–89} Ketone
363 bodies might as well promote cognitive recovery after TBI since they act as histone deacetylase
364 inhibitors, an enzyme involved in memory and cognitive impairment in neurodegenerative
365 diseases.⁴⁴ A ketogenic diet or BHB administered post-treatment improved neurological function
366 in a mouse model of MCA occlusion.⁸⁵ Finally, in a recent meta-analysis of 49 studies on murine
367 models of TBI, ketones were associated with an improved survival, particularly in the adult
368 subgroup.⁹⁰ Interestingly, the plasma level of ketosis was the only predictor of the neuroprotective
369 effect. Another meta-analysis of studies on animal models of stroke described a protective effect
370 of ketosis on outcome⁹¹ while a recent review summarized the different studies showing the
371 neuroprotective effect of KB in acute brain injuries (different models, different species, different
372 ages) <https://doi.org/10.1038/sj.jcbfm.9600543>.

373

374 **Lactate and ketone bodies as pharmacological agents**

375 Lactate can be administered as an intravenous solution of sodium lactate.¹ All interventional
376 studies on TBI used solutions with an osmolality ranging from 500 mmol/L to 1000 mmol/L, the
377 latter being approximately iso-osmolar to 20% mannitol.^{48–54} In two studies, investigators injected
378 sodium lactate directly in the brain using a microdialysis technique using 4 and 8 mmol/L
379 microdialysis solutions.^{55,56} Both methods of lactate administration aimed to improve brain
380 function and reduce damage associated with head trauma. However, they differed since systemic
381 injection involves administering lactate intravenously and therefore distributing the compound
382 throughout the body. The objective of this method is to provide an additional energy substrate to
383 the entire body including brain cells. Lactate can be used by neurons to produce energy, even
384 when brain metabolism is disrupted by head trauma. In contrast, cerebral lactate injection involves
385 directly administering lactate into the brain, usually by microdialysis or intracerebral injection. This
386 method helps to deliver a high concentration of lactate directly to brain cells. Currently, there is
387 no clear consensus on the optimal method of administration of lactate.

388 Ketone bodies can be administered through various formulations. Ketogenic diets are based on
389 carbohydrate deprivation, either by fasting or by enteral formulas containing medium-chain
390 triglycerides and low amounts of carbohydrates. An exclusive ketogenic diet such as used to treat
391 epilepsy in children takes 3 to 5 days to reach therapeutic blood levels. These delays render the
392 ketogenic diet of limited interest in acute neurocritical care. Moreover, ketogenic diets are difficult
393 to prepare and compliance is a challenge.⁵⁷ Ketone bodies may also be infused as intravenous
394 sodium BHB or sodium acetoacetate solutions. Finally, enteral ketone bodies are now available
395 in the form of ketone esters (such as 1,3-butanediol monoester of beta-hydroxybutyrate or
396 glyceryl-tris-3-hydroxybutyrate, two precursors of ketone bodies). The compound (R)-3-
397 hydroxybutyl (R)-3-hydroxybutyrate is now considered a safe food supplement for humans by the
398 U.S. Food and Drug Administration. Ketone esters induce a rapid and significant ketosis after a
399 single oral intake and as such, represent an interesting therapeutic avenue in acute care.^{57,58}

400

401 **Clinical trials**

402 Based on such preclinical evidence, the idea of providing fuel support to brain-injured patients
403 progressively emerged and a few clinical trials started. Bouzat *et al.* were the first to infuse
404 hypertonic lactate in patients with severe TBI undergoing CMD monitoring. They observed a
405 significant increase in extracellular cerebral glucose.⁴⁹ Overall, these data suggest that both
406 endogenous and exogenous lactate are associated with an increased cerebral glucose
407 availability. This “excess” glucose may then be redirected for other metabolic purposes, such as
408 the neuronal PPP.⁵⁶

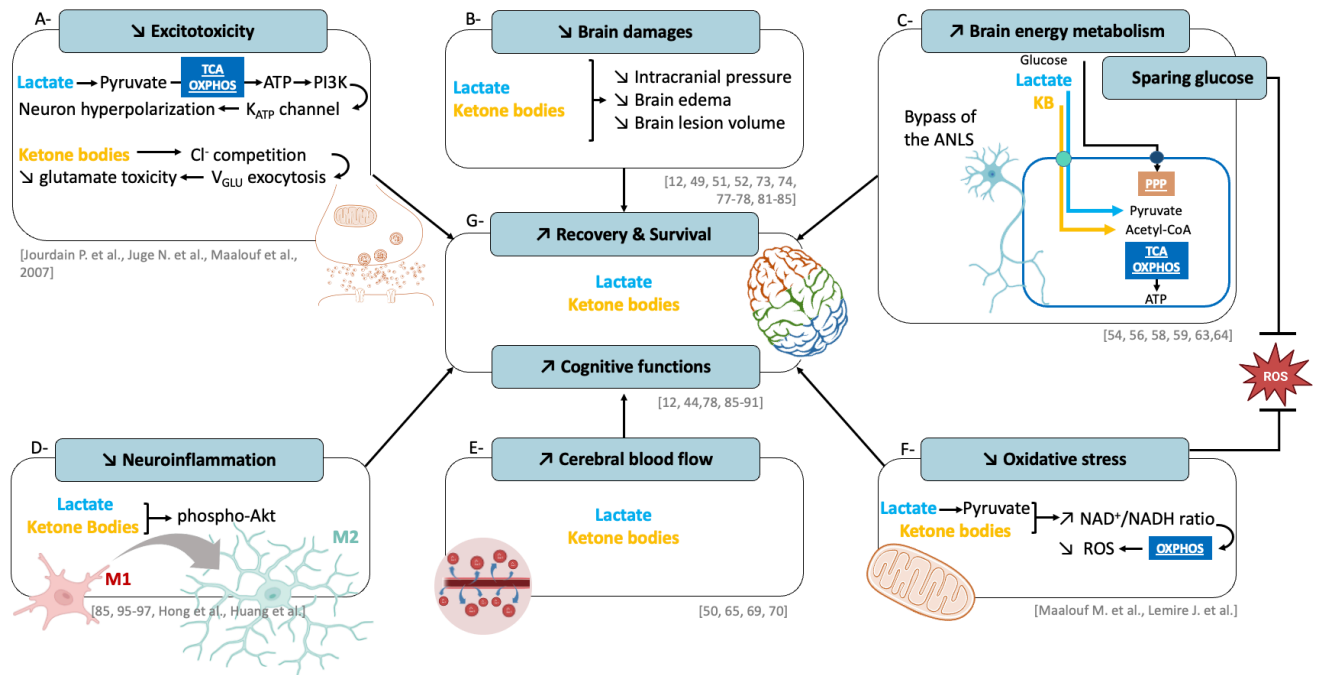
409 In human, clinical trials of lactate administration after TBI or SAH....Developper un peu plus les
410 essais cliniques TBI, SAH et lactate et what about KB ou ester de cetones ?

411

412 **Conclusion and Perspectives**

413 After being considered as unwanted metabolic wastes, lactate and KB are now widely viewed as
414 integral parts of brain metabolism. Data accumulating over the last thirty years support their
415 important role in energy production during rest states, as well as alternative substrates in case of
416 glucose shortage or unavailability. They have also been tested as therapeutics in animal models
417 and human cohorts with acute brain injury with beneficial results concerning excitotoxicity,
418 neuroinflammation, cerebral blood flow control, reduction of brain lesion volume, improvement of
419 cognitive function and survival (**Figure 3**). In addition, KB and lactate share a commun
420 mechanism: modulation of brain metabolism. Indeed, when administrered, lactate and ketone
421 bodies exert a glucose-sparing effect leading to neuronal glycolytic regulation and redirection of
422 glucose through the PPP which helps to fight against ROS produced during oxidative stress
423 (**Figure 3**). Overall, data in animal models and small human cohorts call for a confirmation of their
424 benefits in large therapeutic trials targeting acute neurocritical care populations. After decades of

425 preclinical research and encouraging new clinical data, we are at the dawn of a new period during
 426 which lactate and ketone bodies might become an integral part of neurocritical care therapeutics.



427
 428 **Figure 3: Neuroprotection by exogenous lactate and ketone bodies, as alternative energy substrates,**
 429 **in a context of brain injury.** A- Lactate conetracts glutamate excitotoxicity through the PI3K pathway
 430 leading to neuronal hyperpolarization. Ketone bodies also decrease excitotoxicity through a competition
 431 with Cl⁻, responsible of a decrease in vGLU exocytosis. B- Both exogenous lactate and ketone bodies limit
 432 brain damages by decreasing intracranial pressure, brain edema as well as brain lesion volume. C- Lactate
 433 and ketone bodies increase brain energy metabolism by providing respectively pyruvate and acetyl-CoA to
 434 feed the TCA cycle and OXPHOS. D- Both alternative substrates also switch microglia from an inflammatory
 435 M1 to a anti-inflammatory M2 phenotype, through a stimulation of the Akt pathway. E- Lactate and ketone
 436 bodies increase cerebral blood flow. F- Both lactate and ketone bodies regulate the oxidative stress by
 437 increasing the NAD⁺/NADH ratio. G- Altogether, beneficial effects of exogenous lactate and ketone bodies
 438 lead to increase recovery and survival, as well as improvement of cognitive functions.

439 ANLS, astrocyte-neurone lactate shuttle; OXPHOS, oxidative phosphorylation; PPP, pentose phosphate
 440 pathway; ROS, reactive oxygen species; TCA, tricarboxylic acid; vGLU, vesicular glutamate transporters.
 441

442 **Disclosure/conflict of interest**

443 All authors have no disclosures or conflict of interests.

444 **References**

445

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725 **Figure legend**

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