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News & Views

The blood-brain barrier – a metabolic ecosystem

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Summary: A functional blood-brain barrier relies on a tightly controlled interplay between endothelial cells, pericytes and astrocytes, which together form the neurovascular unit. Recent work by Lee at al (2022) discovers endothelial cell-derived lactate as a crucial metabolic fuel for brain pericytes, revealing a new way of CNS vascular communication that links nutrient metabolism to blood-brain barrier function.

See also: **Lee HW *et al* (2022)**

eToC: Recent work reports a key role for lactate exchange between endothelial cells and neurovascular pericytes in support of brain vasculature integrity.

Main text: The brain is our most energy-consuming organ: Despite contributing to just 2% of body weight, it accounts for 20% of total energy expenditure. Surprisingly, its capacity to store energy is low, necessitating a continuous supply of nutrients and oxygen. This vital task is carried out by the brain vasculature, which is laid out with unique three-dimensional topology to optimally meet the neurons' metabolic demands. In humans, the estimated total length of this network is more than 400 miles, highlighting the intimate linkage between neuronal and vascular function (Kaplan *et al*, 2020).

However, before nutrients can reach the central nervous system (CNS), they must pass the blood-brain barrier (BBB) – a specialized cellular interface that separates the CNS from the circulation. The BBB is formed by an alliance of endothelial cells (ECs), pericytes (PCs) and astrocytes, which controls the selective trafficking of (metabolic) substances in and out of the CNS (Figure 1A) (Potente & Makinen, 2017; Zhao *et al*, 2015). Studies of the past years have examined these trafficking routes in detail and identified mechanisms that ensure transport selectivity. Examples include the shuttling of metabolites via dedicated solute carriers (SLCs) as well as the passaging of macromolecules via transcytosis (Kaplan *et al.*, 2020; Zhao *et al.*, 2015). Through this finely tuned machinery the BBB establishes a metabolic milieu that supports and maintains neuronal activity. Yet, how BBB ensures its own metabolic

homeostasis is unknown. In the journal's current issue, Lee et al. (2022) shed first light on this question by demonstrating that the metabolism of ECs and PCs is tightly coupled (Lee *et al*, 2022). They report that lactate, produced and secreted by ECs at high levels, is a crucial metabolic resource for brain PCs, which maintains PC homeostasis and BBB integrity (Figure 1B).

Lactate was long thought to be solely a waste product of glycolytic metabolism that is disposed of in the extracellular environment. However, recent studies revealed that lactate can be shuttled between adjacent cells or get into the circulation to participate in intercellular communication (Brooks, 2018). Because ECs are highly glycolytic cells, Lee and colleagues (2022) asked whether endothelial-derived lactate might have similar roles and assessed its impact on cells in their vicinity. To this end, the researchers genetically inactivated the endothelial *Glut1* gene (*Slc2a1*) of mice – the primary glucose transporter of ECs highly enriched at the BBB. In line with a previous report (Veys *et al*, 2020), the work by Lee and colleagues (2022) showed that endothelial *Glut1* inactivation had dramatic consequences for the mutant mice, particularly their brain vasculature: ECs lacking this transporter were unable to import glucose and, as a result, had reduced glycolytic activity, lactate levels and angiogenic capacity. The cerebral vasculature also showed a striking loss of PCs, caused by increased apoptosis, which compromised BBB integrity and led to profound vascular leakage. Noting the PC drop off, the authors posited that lactate, generated by ECs and released into the extracellular space, is required for healthy PC coverage and proper BBB function.

To further examine this model, the authors asked how EC-derived lactate might impact PC biology and traced its metabolic fate. Using a fluorescence-based lactate sensor combined with an *in vitro* co-culture system, they observed that PCs that are in close proximity to ECs take up lactate generated by endothelial glycolysis. Interestingly, even in the presence of competing nutrients such as glucose, pyruvate or glutamine, PCs consumed substantial amounts of lactate, suggesting that they are predestined to utilise this remnant of glycolytic metabolism. Lee and colleagues also performed metabolomic analyses to gain insight into PC lactate usage. These metabolic labelling studies indicated that lactate is a readily available carbon source for PCs, fuelling tricarboxylic acid cycle (TCA) activity, ATP production, and amino acid biosynthesis. Together, these data suggested an exchange of lactate molecules between ECs and PCs, with glycolytic ECs acting as *"producers"* and oxidative PCs as *"consumers".*

To further corroborate this model, Lee and team (2022) studied carriers that transport lactate in and out of cells. They found that MCT1 (SLC16A1) and MCT5 (SLC16A4) export lactate from ECs, while MCT12 (SLC16A12) imports it into PCs. Consistent with these data, pharmacological MCT1 inhibition compromised EC-PC lactate shuttling and mimicked phenotypes of GLUT1-deficiency, including a reduction in PCs and compromised barrier function. To finally test the specific requirement of lactate for BBB function, the authors performed lactate supplementation experiments in Glut1-deficient mice. They found that PC coverage and barrier integrity were ameliorated in lactate-treated mutants, suggesting that BBB defects are, at least in part, due to a lack of endothelium-derived lactate.

Lee and colleagues' (2022) findings are exciting for several reasons. First, they provide important insights into the metabolism of PCs, the role and regulation of which is still poorly understood. Second, they shed light on an intercellular communication strategy that is based on metabolites and reveal its physiological relevance in the brain vasculature.

The role of lactate as a messenger and fuel for PCs is surprising because previous *in vitro* studies reported that PCs rely on glucose and its metabolization via glycolysis (Cantelmo *et al*, 2016). In contrast, the current study suggests that PCs avidly consume lactate – even in the presence of ample glucose – implying substantial mitochondrial metabolism. One possible

explanation for these seemingly contradictory findings is that lactate consumption of PCs represents a peculiarity of the BBB, which is specialized for efficient nutrient transport and delivery (Kaplan *et al.*, 2020; Zhao *et al.*, 2015). By recycling the leftovers of endothelial glucose metabolism, brain PCs might spare glucose for use in other cells of the CNS, thereby making delivery more effective. According to this model, the BBB could be considered as a *"metabolic ecosystem"* that optimizes nutrient handling to maximise supply of neuronal tissue.

However, several caveats have to be taken into consideration. The inactivation of *Glut1 in vivo as a means to* block lactate production, also deprives ECs and the surrounding BBB milieu of glucose, which bears pervasive physiological implications, and might confound the interpretation of PC-specific responses to lactate deprivation. Further *in vivo* studies aimed at abrogating lactate influx into PCs should allow to determine direct causality. The lack of tools to precisely inspect metabolic fluxes across cells *in vivo* also hampers a more refined understanding of how lactate impacts the BBB. Yet, more readily answerable questions remain. For instance, whether a GLUT1-deficient state interferes with the paracrine ability of ECs to recruit PCs (e.g., via PDGFB/PDGFRB signalling); and whether PC attachment is altered by changes in pH in the basement membrane space (as lactate is co-transporter with H^+), were left unaddressed.

Notwithstanding these questions, this work illustrates the importance of lactate metabolism in the vascular wall and provides an exciting framework in which new questions can be explored. One obvious direction is whether lactate has direct signalling functions. In macrophages, for instance, lactate has recently been shown to direct epigenetic gene regulation via histone lactylation (Zhang *et al*, 2019) – a mechanism that might be relevant for organ-specific differentiation of PCs. At a more general level, Lee and colleagues' study also raises the question of whether other secreted products of metabolism are exchanged between vascular cells to influence each other's state. Examples of such metabolic messengers could be the Krebs cycle intermediate succinate, which signals via G-protein-coupled receptors (Reddy *et al*, 2020), or the α-ketoglutarate derivative *S*-2-hydroxyglutarate, which promotes endothelial quiescence (Andrade *et al*, 2021). Understanding the scope of this type of metabolic communication will provide exciting new avenues for vascular biology with wide-reaching implications for tissue development, homeostasis and regeneration.

Disclosure Statement & Competing Interests

The authors declare no conflict of interest.

Figure Legend

Figure 1. A new way of neurovascular communication via glycolysis-derived lactate. A) Schematic illustration of a cross section through a brain capillary illustrating the cellular constituents of the blood-brain barrier (BBB): endothelial cells (ECs), pericytes (PCs) and astrocytes (ACs). The scheme also shows the basement membrane (BM) in which both ECs and PCs are embedded. **B)** Lee and colleagues describe a new way of communication between ECs and PCs, which is based on the end product of glycolytic metabolism, lactate. ECs import glucose from the circulation via the glucose transporter GLUT1 and metabolize it through glycolysis to generate substantial amounts of lactate. The transporters MCT1 and MCT5 transport lactate out of ECs to dispose it in the extracellular space. PCs, which are close proximity to ECs, take up this lactate and break it down in mitochondria to generate ATP

and amino acids. Disruption of this mechanisms, for instance by depletion of GLUT1, causes PC demise and BBB breakdown.

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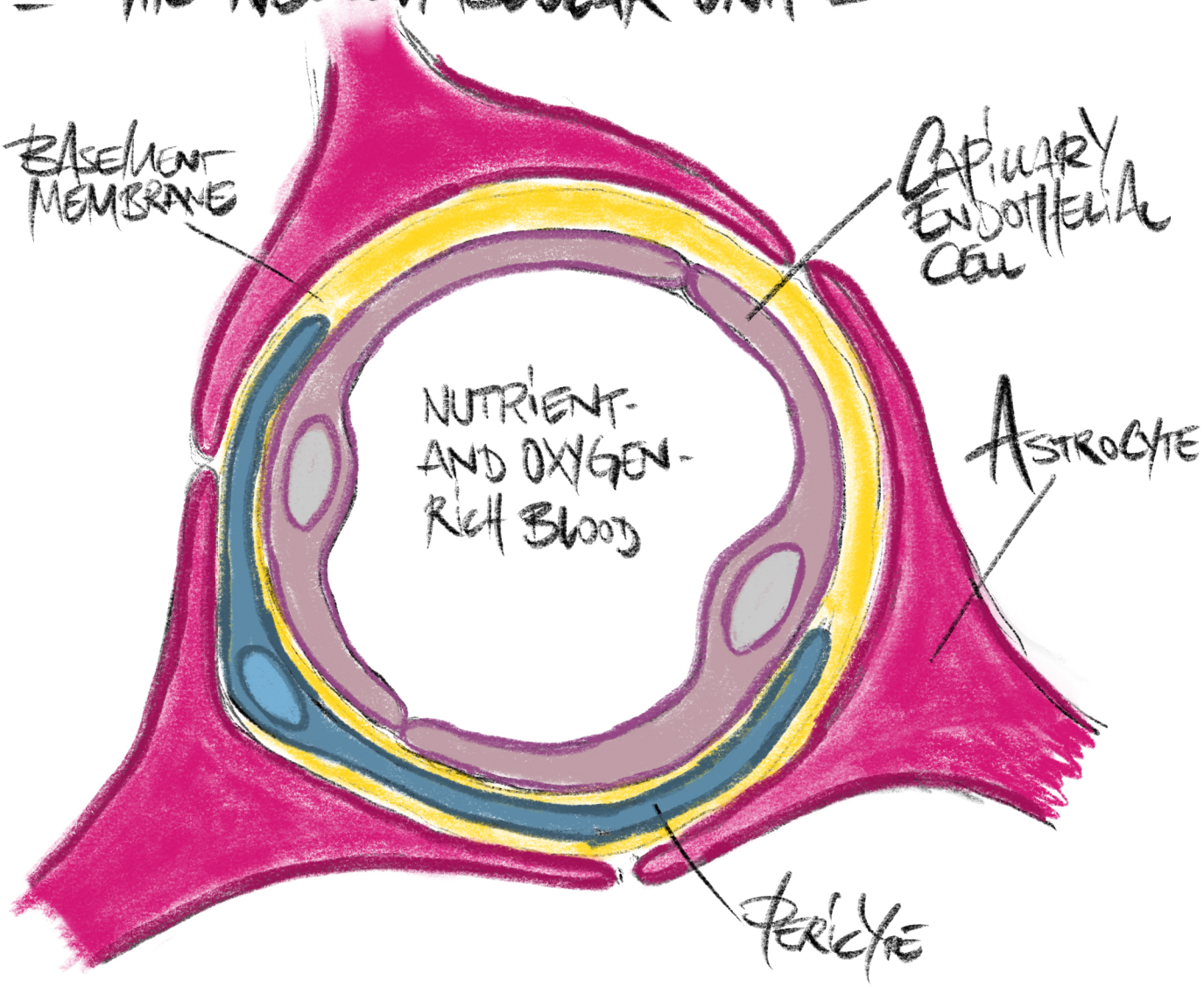
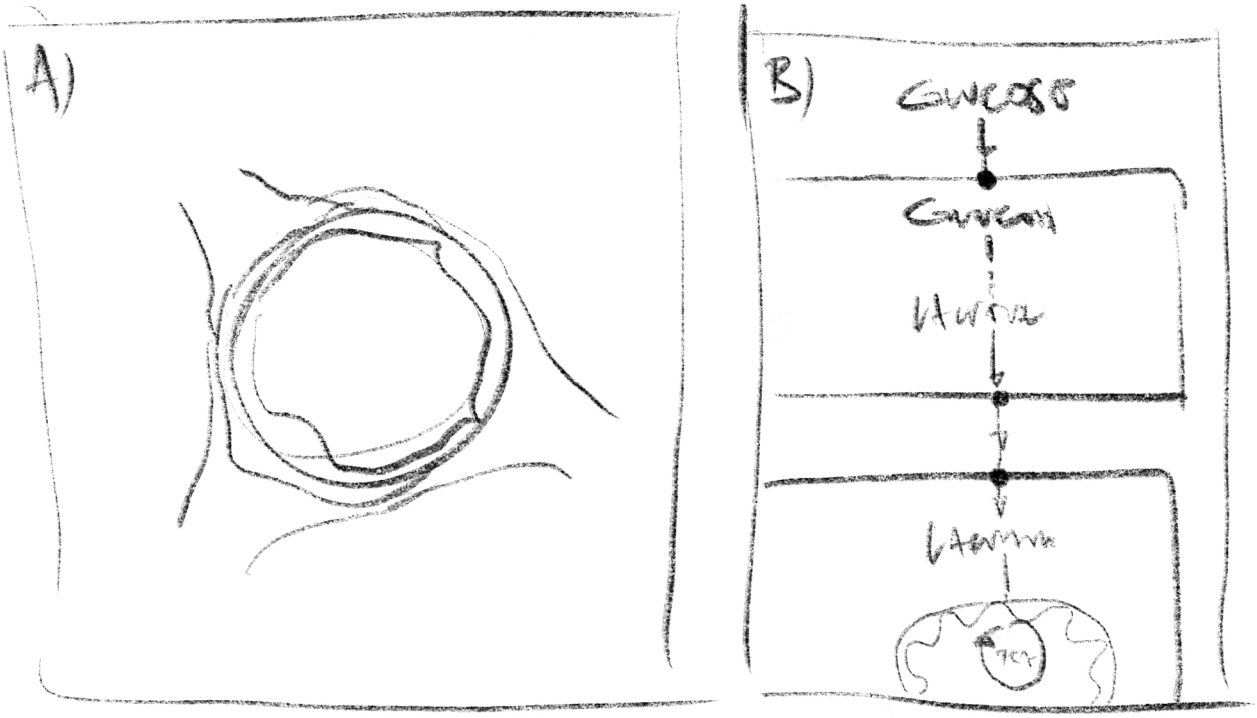
A) – THE NEUROVASCULAR UNIT –





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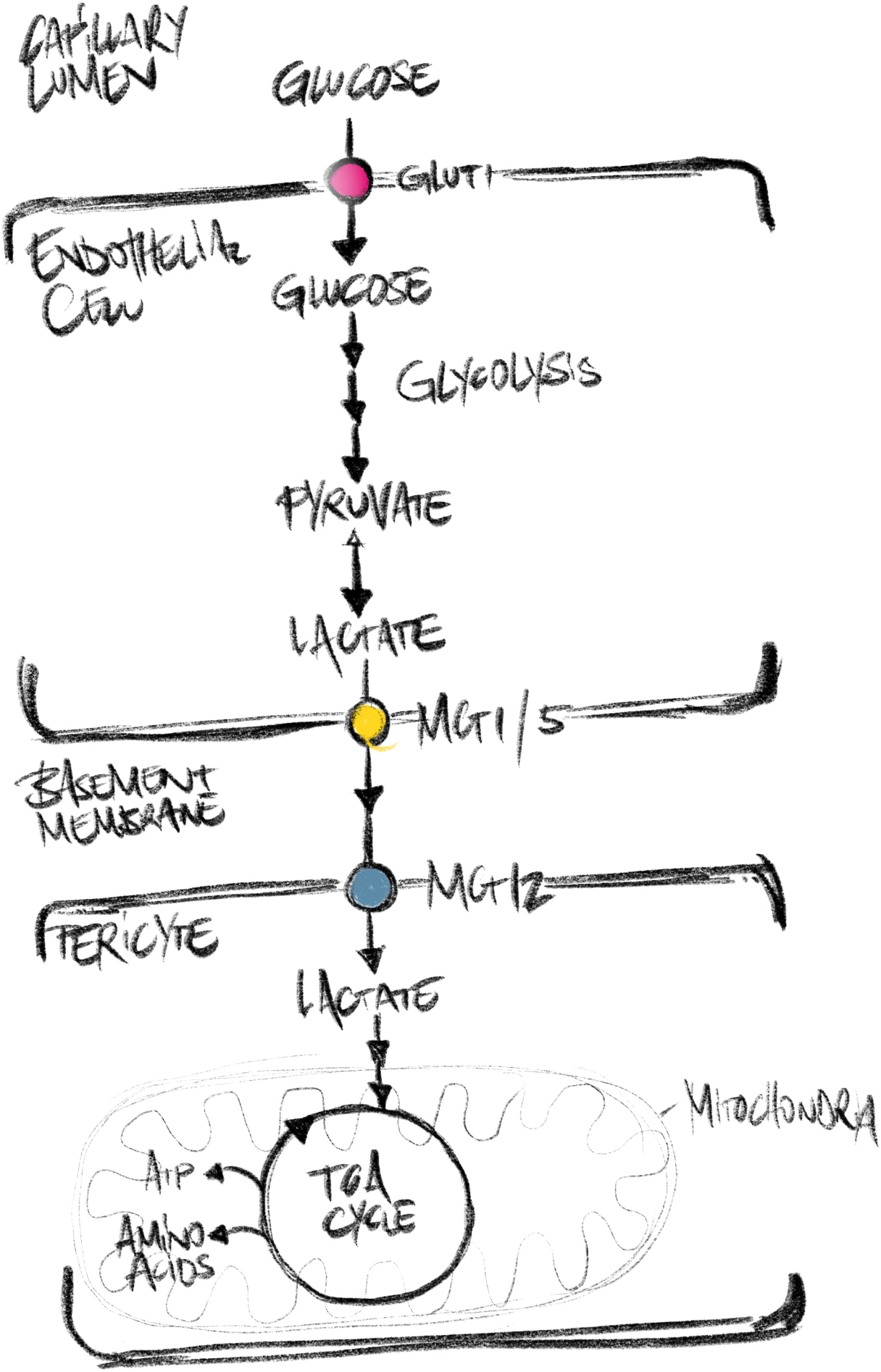
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GLUCOSE
GLUT1
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GLUCOSE
GLYCOLYSIS
PYRUVATE
LACTATE
MCT1/5
BASEMENT MEMBRANE
MCT1/2
PERICYTE
LACTATE
MITOCHONDRIA
ATP
AMINO ACIDS
TCA CYCLE