Abstract

Lactate’s role in the brain is understood as a contributor to brain energy metabolism, but it may also regulate the cerebral microcirculation. The purpose of this systematic review was to evaluate evidence of lactate as a physiological effector within the normal cerebral microcirculation in reports ranging from in vitro experiments to in vivo studies in animals and humans. Following pre-registration of a review protocol, we systematically searched the PubMed, EMBASE, and Cochrane databases for literature covering themes of ‘lactate’, ‘the brain’, and ‘microcirculation’. Abstracts were screened, and data extracted independently by two individuals. We excluded studies evaluating lactate in disease models. Twenty-eight papers were identified, 18 of which were in vivo animal experiments (65%), four on human studies (14%), and six on in vitro or ex vivo experiments (21%). Approximately half of the papers identified lactate as an augmenter of the hyperemic response to functional activation by a visual stimulus or as an instigator of hyperemia in a dose-dependent manner, without external stimulation. The mechanisms are likely to be coupled to NAD\(^+\)/NADH redox state influencing the production of nitric oxide. Unfortunately, only 38% of these studies demonstrated any control for bias, which makes reliable generalizations of the conclusions insecure. This systematic review identifies that lactate may act as a dose-dependent regulator of cerebral microcirculation by augmenting the hyperemic response to functional activation below 5 mmol/kg, and by initiating a hyperemic response above 5 mmol/kg.

Keywords: brain, cerebral blood flow, lactate, microcirculation, systematic review.

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In brain tissue, proton-coupled lactate transport via monocarboxylate transporters (Bergersen 2015) and lactate exchange via ion channels (Sotelo-Hitschfeld et al. 2015; Karagiannis et al. 2016; Hadjihambi et al. 2017) suggest a broader role of lactate as a signaling molecule. Accordingly, lactate modulates neuronal excitability (Sotelo-Hitschfeld et al. 2015) and is also thought to act as a volume transmitter, coordinating energy metabolism and blood flow in the brain and other organs, possibly via mechanisms that involve NADH and hence cellular redox state (Bergersen and Gjedde 2012; Mosienko et al. 2015; Proia et al. 2016). Given the complexity of the mechanisms that control cerebral blood flow (CBF) at a microvascular level (Attwell et al. 2010; Hall et al. 2014) it is crucial to better understand the vascular effects of lactate.

Progress in biomedical research is impeded if studies are underpowered or experimental procedures incompletely reported, as this increases the risk of positive reporting bias (Macleod et al. 2015). The resulting poor reproducibility, in turn, is a source of wasted resources (Freedman et al. 2015). With greater attention focusing toward the translational efficacy and reporting quality of pre-clinical research, it is pertinent to consider evaluating existing literature prior to conducting in vivo experiments. This approach also sustains the 3R principles of Replacement, Reduction, and Refinement (Russell and Burch 1959). Moreover, it has been emphasized that systematic meta-research should be conducted to identify factors contributing to high translational ability of scientific findings, as well as to help demarcate the ideal ratio between basic and applied research to achieve these aims (Chalmers et al. 2014). Systematic reviews provide a means of identifying trends in the existing research pool, of improving the quality and translational efficacy of studies, and of identifying unaddressed aspects in experimental design, which otherwise create a risk of bias (Hooijmans and Ritskes-Hoitinga 2013; Ritskes-Hoitinga and Wever 2018). Systematic reviews therefore help drawing more reliable conclusions from the existing literature while identifying ways of improving the design of future works.

This systematic review investigates the current evidence for how the cerebral microvasculature responds to lactate in studies ranging from the cellular level to human experiments. Using this broad scoped approach, we aimed to expand the systematic review paradigm with a focus on intervention studies, to demonstrate that novel extractions of existing data help guide the formulation of new hypotheses.

Materials and methods

Review protocol & amendments
The protocol for this systematic review was pre-defined using the SYRCLE guidelines (de Vries et al. 2015) and published on www.radboudumc.nl/en/research/radboud-technology-centers/animal-research-facility/systematic-review-center-for-laboratory-animal-experimentation/protocols on 5th December 2017 prior to completion of primary screening. Post-publication modifications were made to the protocol as follows: (i) At the primary-screening phase, discrepancies on decision to include were resolved by Tristan R. Hollyer (TRH) and Birgitte S Kousholt (BSK). (ii) At the end of primary screening, Luca Bordoni (LB) was recruited to conduct the full text screening and data extraction as BSK and Judith van Luijk (JvL) were unable to contribute to these processes further. (iii) Discrepancies on decision at the full-text screening phase were resolved by TRH and LB. (iv) Leif Østergaard (LØ) was included as a contributing author. (v) A modified number of risk of bias measures were decided upon and then evaluated by TRH as described below.

During the data extraction process, we identified several studies which evaluated the effect of lactate on cerebral blood flow by different experimental measurements both in animal and human studies. We decided to extract these datasets in an aim to identify potential trends in findings. To further determine the methodological quality of these extracted studies, TRH assessed them for risk of bias (RoB) by determining if each study reported the use of bias limiting measures such as population randomization, blinding, or sample size calculation.

Study search, selection, screening, and extraction
The PubMed, EMBASE, and Cochrane databases were systematically searched electronically on 14th October 2017. The search strategy was comprised of three categories: ‘lactate (and related enzymes, transporters, and receptors)’, ‘microvasculature’, and ‘brain’. Within each category, medical subject headings terms were determined and relevant synonyms were sought within titles, abstracts, and keywords. The full search strategy is detailed in Table S1. No restrictions were applied to language or publication date. The search results were pooled, with duplicates removed, and indexed in EndNoteX8 Software (Clarivate Analytics, Philadelphia, PA, USA). Original articles and clinical trials were included, and review articles excluded. The library was then uploaded to the online systematic review management platform Covidence (http://covidence.org).

As stated in the study protocol, studies were included if they examined lactate’s role in cerebral circulation only in physiological conditions. Where disease/pathological models were used, we extracted data from appropriate controls whenever available. Selected populations ranged from endothelial cell lines, ex vivo tissue, in vivo animal, and human studies. Interventions were defined as any modification of lactate or its pharmacological effectors, for example, receptors, transporters, generating enzymes (LDH) and any non-harmful genetic modification, for example, receptor knockouts, or relevant control or baseline data. Defined outcomes were any stated measures related to the effects of the experimental treatments on population biochemistry or physiology; cerebral vascular cell/tissue behavior (such as diameter or flow behavior) evaluated directly or indirectly.

Titels and abstracts were screened for inclusion independently by TRH and BSK/JvL, the latter at a proportion of 80/20%. Disputes were resolved by TRH and BSK. The reference sections of all texts selected for full-text screening were checked for additional references of interest. Studies included for full-text screened were evaluated, and data extracted independently by TRH and LB.

Extracted studies are summarized in characteristics tables (Tables S2–S4). In each table, the author, publication data, species, strain,
the presence of LDH and lactate uptake by cortical vessels (Spatz et al. 1978; Rieke and Cannon 1985). Detailed anatomical investigations by Lauritzen et al. (2014) identified the presence of the HCA1 receptor on the luminal and abluminal membranes of the mouse endothelial cell at a density twice as that on astrocytic end-feet. Cellular responses to exogenous lactate were studied separately twice. Sub-physiological levels of lactate had no effect on cell survival (Pirchl et al. 2006), but 20 mmol/L lactate applied to human brain endothelial cells induced a marked response to cellular lactate uptake and cellular proliferation (Miranda-Gonçalves et al. 2017). Gordon et al. (2008) was the only ex vivo study that evaluated the pharmacological mechanisms behind lactate signaling in rat arterioles. It was found that lactate induces arteriolar dilation by reducing the reuptake of PGE2 at astrocyte end-feet, allowing for continued PGE2 binding to prostaglandin receptors on vascular smooth muscle cells.

**In vivo studies**

The majority of in vivo animal studies (Table S3) were conducted in mongrel dogs (Harper and Bell 1963; Iwabuchi et al. 1973; Hermansen et al. 1984; Young et al. 1991), or rats (Hallström et al. 1990; Ido et al. 2001, 2004; Provent et al. 2007). Almost half (10) of the in vivo studies evaluated the effect of systemic administration of lactate (either as an acid or its sodium salt) on CBF in animals. To elucidate any trends in these findings, results from these experiments were evaluated in combination with similar human studies (see below, Fig. 2 and Table S3).

Lactate only induced a hyperemic response (identified via radiolabeling techniques) if administered in doses of above 5 mmol/kg (clear symbols). Higher doses of lactate elicited a CBF response in the absence of stimulation (dark symbols). Animal studies (circle), human studies (triangle). Details of individual studies in Table 1.
5 mmol/kg (Bucciarelli and Eitzman 1979; Young et al. 1991), with resulting blood plasma concentrations of 30 mmol/L reported in Young et al. (1991). Studies infusing lactate at lower concentrations found no direct hyperemic response. However, in both rats and non-human primates, an increase in blood plasma lactate concentrations of 2.6 ± 0.0 and 3.5 ± 0.4 (Ido et al. 2004), and 2.5 ± 0.9 mmol/L (von Pöstl et al. 2012), led to an augmentation of the CBF response to visual stimuli.

The long-term effects of both exhaustive exercise and repeated administration of lactate were studied in two separate experimental groups of mice (Lezì et al. 2013; Morland et al. 2017). Both studies found that increased plasma lactate levels brought about by both exercise and systemic administration induced a comparable increase in brain vascular endothelial growth factor-A (VEGF-A) and their related signaling pathways. Morland et al. (2017) subsequently found that VEGF-A had a pro-angiogenic effect in both the hippocampus and sensorimotor cortex.

### Human studies

Selected human studies are characterized in Table S4. Two of these studies (Stewart et al. 1988; Reiman et al. 1989) evaluated how lactate infusion (a reported anxiolytic) influenced CBF in patients with anxiety disorders and control subjects. The latter showed a mean increase in CBF of 20% when administered 500 mmol/kg lactate (Stewart et al. 1988) but no CBF change when given a lower concentration (up to 133 mmol/kg) (Reiman et al. 1989). Mintun et al. (2004), infused 1 mmol/kg lactate, increasing blood plasma concentrations to 10.7 ± 2.8 mmol/L, and observed unaltered resting CBF, but augmented CBF response to stimulation, by up to 53%.

### Effects of lactate on CBF across species

We identified 10 in vivo animal and 3 human publications which evaluated the effect of direct systemic administration of lactate on CBF. To elucidate any trends in findings, results from these experiments were extracted and evaluated as shown in Fig. 2 (details are presented in Table 1). At concentrations of over 5 mmol/kg, lactate-induced cerebral hyperemia. At concentrations lower than 5 mmol/kg, lactate augmented CBF response to stimuli.

### Risk of bias assessment

We then conducted a modified assessment of reporting bias to evaluate the validity of these findings. Only one paper reported the use of blinding, randomization and sample size calculation methods to control for bias (Dostalova et al. 2018). Two reported the use of randomizing of subjects to treatment (Bucciarelli and Eitzman 1979; Ong et al. 1986) and two of blinding subjects to treatment (Reiman et al. 1989; Mintun et al. 2004). The remaining papers did not state as to whether they took bias controlling measures.

### Evidence on biochemical regulation of cerebral microvessels by lactate

Experiments conducted in vitro, or ex vivo comprised only 21% of all selected studies. The earliest studies showed age and vessel size-dependent responses to lactate uptake and production, respectively (Spatz et al. 1978; Rieke and Cannon 1985). The later anatomical study by Lauritzen et al. (2014) identified the presence of the HCA1 receptor in differing amounts within the neurovascular unit. In particular, they reported that twice as many receptors were found on endothelial cell membranes compared to astrocytic end-feet, suggesting a greater sensitivity of cerebral microvessels to lactate than astrocytes. Furthermore, Miranda-Gonçalves et al. (2017) showed that glucose uptake was down-regulated in favor of lactate uptake in response to high extracellular concentrations of lactate in immortalized cells derived from human brain endothelium. In addition, large increases in mitochondrial activity, cell migration, and formation of capillary-like structures and associated pro-angiogenic factors such as hypoxia inducible factor-1a (HIF-1a), a transcriptional regulator of VEGF-A occurred (Semenza 2010). At the arteriolar level, Gordon et al. (2008) showed that 1 mM lactate-induced arteriolar dilation via a cyclo-oxygenase (COX) dependent manner, further complementing earlier evidence (Kaidi et al. 2006; Benderro and LaManna 2014) that HIF-1a is a key regulator of COX-2. Alongside the findings of Morland et al. (2017), that repeated exposure to lactate, artificially or through exercise, promotes VEGF expression, it appears that lactate exerts both short- and long-term angiogenic effects on the cerebral microvascular in via a common mechanism. Such responses are perhaps unsurprising, considering the classical view of lactate being produced during anaerobic glycolysis as a consequence of exercise or hypoxia. Therefore, it demonstrates that the pathways, which are up-regulated in response to hypoxia and exercise, also have a role in cerebral vascular homeostasis and hemodynamics, ensuring that sufficient glucose and oxygen are delivered to the cells of the brain.

Intravenous administration of up to 2.0 mmol/L lactate has no effects on resting CBF, regardless of choice of anesthesia (Fig. 2 and Table S3), but enhances the CBF response to stimulation in both animals and humans (Ido et al. 2001,

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2004; Mintun et al. 2004). Indeed, CBF responses to functional activation seemingly correlate with arterial lactate/pyruvate ratios (Mintun et al. 2004), which is coupled to the NADH/NAD\(^+\) ratio.

Ido et al. (2001, 2004) hypothesized that the NADH/NAD\(^+\) ratio acts as sensor acting as a regulator of CBF when increased via activation of constitutive nitric oxide synthase. In their studies, the absence of any CBF response in the unstimulated regions of the brain is suggested to be due to exogenous lactate being oxidized to pyruvate (via LDH). This leads to a concurrent increase in NADH/NAD\(^+\) ratio which is balanced by transfer of NADH to the glycerol-phosphate and malate-aspartate shuttles (see Fig. 3) (Mintun et al. 2004). It is only when these pathways become saturated, due to the additive effect of > 5 mmol/kg lactate or functional activation (resulting in an accumulation of NADH), that a subsequent increase

| Authors                  | Species | Salt/ Acid | Dose (mmol/kg) | Delivery | Anesthesia (neuromuscular agents) | CBF assessment method | Reported response | Notes                                      |
|--------------------------|---------|------------|----------------|----------|----------------------------------|-----------------------|-------------------|-------------------------------------------|
| Bucciarelli and Eitzman (1979) | Goat    | Acid       | 5-10           | IV       | Chloralose                       | Radiolabeled microspheres | +46%              | Plasma lactate not reported               |
| Dostalova et al. (2018)   | Rabbit  | Salt       | 1.87           | IV       | Isoflurane and fentanyl (pipercuronium) | Side stream dark-field | No change         |                                           |
| Harper and Bell (1963)    | Dog     | Acid       | 0.22 mmol/L    | IA       | Thiopentone (suxamethonium)      | \(^{86}\)Kr washout | No change         |                                           |
| Hermansen et al. (1984)   | Dog     | Acid       | 2.20           | IV       | Pentobarbital (pancuronium)      | Radiolabeled microspheres | No change         |                                           |
| Ido et al. (2001)         | Rat     | Salt       | 1.00           | IV       | Urethane                         | \(^{125}\)I-desmethylimipramine | +100%             | Augmented stimulus response               |
| Ido et al. (2004)         | Rat     | n/a        | 1.00           | IV       | Urethane                         | \(^{125}\)I-desmethylimipramine | +11%              | Augmented stimulus response               |
| Ong et al. (1986)         | Sheep   | Acid       | 3.30           | IV       | d-Tubocurarine                    | \(^{113}\)Xe washout | No change         |                                           |
| Powell et al. (1985)      | Dog     | Acid       | 3.75           | IV       | Halothane (pancuronium)          | \(^{14}\)Ciodoantipyrine | No change         | Once corrected for pCO\(_2\)               |
| von Pfostl et al. (2012)  | Monkey  | Salt       | 0.04           | IV       | Remifentanil (mivacurium chloride) | MRI                  | No change         | Detection threshold/augment BOLD signal   |
| Young et al. (1991)       | Dog     | Acid       | 33.3 mol/L     | IV       | Halothane (pancuronium)          | \(^{14}\)Ciodoantipyrine | +36%              | Plasma lactate 30 mmol/L                  |
| Stewart et al. (1988)     | Human   | Salt       | 500            | IV       | n/a                              | Inhaled \(^{133}\)Xe CAT | +20%              | Plasma lactate not reported               |
| Reiman et al. (1989)      | Human   | Salt       | 89             | IV       | n/a                              | \(^{15}\)O water PET  | No change         |                                           |
| Mintun et al. (2004)      | Human   | n/a        | 1.00           | IV       | n/a                              | \(^{15}\)O water PET  | +38%              | Augmented stimulus response               |

**Table 1** Selected in vivo and human studies which reported cerebral blood flow measurements when administering lactate systemically.

BOLD, blood-oxygen-level dependent; CBF, cerebral blood flow; CAT, computer-assisted tomography; IV, intravenous; IA, intra-arterial; MRI, magnetic resonance imaging; PET, positron emission tomography.

The concentration of lactate (either as a sodium salt or acid), route of administration, anesthesia used, method of CBF evaluation, and reported effects are evaluated.
in reactive oxygen species results in elevated $\text{Ca}^{2+}$ before recruitment of nitric oxide synthase (NOS) pathways (Wolin 1996; Buccarelli and Eitzman 1979, Stewart et al. 1988, and Young et al. 1991) (Table S3), reported large increases in resting CBF following administration concentrations of lactate higher than 5 mmol/kg. It is therefore likely that the higher blood plasma concentrations of lactate in these experiments (as illustrated in Fig. 3), lead to much greater rises in the lactate/pyruvate ratio and NADH : NAD$^+$ ratios, causing a greater accumulation of NADH and reactive oxygen species, leading to a much larger increase in CBF. However, Reiman et al. contradicts this hypothesis showing no CBF response. Furthermore, what was not explored was how cellular redox potential is driven also by glyceraldehyde-3-phosphate dehydrogenase and, also the phosphorylation state of the cytosolic adenine nucleotide system (Veech et al. 1970).

Monocarboxylate transporter 4 is the predominant monocarboxylate transporter on astrocytes (Bergersen 2015) with a $K_m$ of 28 mmol/L (Manning-Fox et al. 2000). This is indicative of astrocytes having a far greater capacity for lactate uptake than neurons (Dienel 2012). Changes in astrocytic NADH : NAD$^+$ ratios has been linked to nitric oxide production by astrocytes (Buskila et al. 2005). However, transcriptome data (GOAD database, http://bioinf.nl:8080/GOAD2/databaseSelectServlet) suggest that astrocytes do not express NOS isoforms. Furthermore, calcium influx (also induced by oxidative stress) leads to activation of nNOS –derived NO in neurons and release of vasoconstrictors from astrocytes. Meanwhile, nNOS derived NO inhibits astrocytic COX-2 and thus the production of astrocyte-derived vasodilators (Attwell et al. 2010). Nitric oxide is also known to have positive reciprocal regulatory relationship with HIF-1$\alpha$ (Poyton and Hendrickson 2015), whose relationship with lactate is described above.

Tissue pH is a powerful regulator of arterial tone (Yoon et al. 2012), and the augmented CBF observed in response to elevated lactate levels might therefore be related to parallel acidification via co-transport of protons via monocarboxylate transporters, Experimental data suggest, however, that lactate-induced CBF changes are caused by the higher lactate concentrations rather than the parallel changes in pH (Laptook et al. 1988).

One of the aim of this systematic review was to collectively analyze evidence showing that lactate can serve as a regulator of cerebral microvasculature. Although we lack direct experimental proofs to confirm this specific hypothesis, the current evidence points toward a coordinated system of local control of the cerebral microvasculature in which lactate is a key regulator with concentration specific thresholds for the magnitude of the response. This complements our own modeling of microvessel flow patterns which has shown that during functional hyperemia, glucose uptake is facilitated more so than oxygen favoring non-oxidative glucose consumption suggesting that lactate may feedback into these control systems (Angleys et al. 2016).

**Considerations on RoB**

Only five of the 13 papers (38%), which evaluated CBF responses to lactate, reported using methods to control for bias. As interest in research reproducibility increases, it is important that research which is exploratory in nature (regardless of the model) takes a robust approach to study design and control for bias (Kimmelman et al. 2014; Dirnagl 2016). We believe that our modified RoB assessment underscores the need for implementation of the systematic review methodology in basic science.

**Assumptions**

During this review, we have made some assumptions about some of the data extracted. Several of the early studies administer lactic acid as a model of perinatal hypoxia under the hypothesis that lactic acidosis may have deleterious consequences to CBF or cerebral autoregulation (Harper and

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**Resting brain or < 5 mmol/kg administered lactate**

$$\text{lactate} + \text{NAD}^+ \rightleftharpoons \text{NADH} + \text{H}^+ + \text{pyruvate}$$

**Functional activation or > 5 mmol/kg administered lactate**

$$\text{lactate} + \text{NAD}^+ \rightleftharpoons \text{LDH} \rightarrow \text{NADH} + \text{H}^+ + \text{pyruvate}$$

**Fig. 3** Reaction scheme illustrating the thresholds for which excess production of NADH (from lactate) alters redox state, inducing hyperemia during functional activation or administration of over 5 mmol/kg lactate.

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Bell 1963; Bucciarelli and Eitzman 1979; Hermansen et al. 1984; Powell et al. 1985; Ong et al. 1986). In this review we considered the induction of hyperlactemia (using lactic acid) as a non-pathological or disease related intervention as long normoxia was maintained.

Contradictory literature reports may partly result from differences in the physiological mechanisms resulting in changes in lactate concentration due to the fact that multiple physiological situations may lead to alterations in lactate levels.

Additional relevant literature
We wish to direct to the reader to the fact that, due to the methodology of this review, we noted that several papers provide insights into the effects of lactate on microvessels that are located outside the brain parenchyma. An elegant set of studies by Yamanishi et al. (2005) evaluated lactate’s role in the function of retinal microvessels and pericytes and demonstrated that the contractile responses of pericytes to lactate were dependent on oxygenation of the preparation. Hein et al. (2006) also demonstrated this in porcine retinal arterioles dilated in response to lactate via nitric oxide synthase (NOS) pathways. However, it should be noted that the retina is a highly glycolytic environment (Winkler 1981) and as such, these studies may illustrate physiological responses unique to the retinal microvasculature. Cochlear capillaries have also been shown to dilate in response to lactate via NOS (Dai et al. 2011). It therefore may be likely that lactate acts (either directly or via NADH) on microvessels via a common nitric oxide-dependent mechanisms across multiple systems.

Works by Rasmussen et al. (2006, 2009) were excluded on the basis that blood flow velocities were recorded from the middle cerebral artery, which is not a microvessel. However, they do report that lactate/pyruvate ratios (a representation of redox state) may be a regulating factor in CBF during activation and during the onset of exercise which is further supported by Vlassenko et al. (2006). The mechanisms highlighted in this review would benefit from replication and further investigation, in particular mechanisms which control the threshold at which lactate switches from an augmentor of hyperemia with a separate stimulus (e.g. visual) to an initiator (without another stimulus).

This review has used systematic literature search techniques to comprehensively assess existing evidence on the role of lactate in the cerebral microcirculation. Using systematic review methodology to probe questions of a fundamental physiological nature in studies ranging from in vitro experiments to human studies, allows us to fully appreciate the field in the entire research chain. This approach has identified that exogenous administered lactate may act as a regulator of cerebral blood flow in a dose-dependent manner whereby at a threshold of 5 mmol/kg there is a switch from augmentation of the hyperemic response, to one of an initiator. We hope that this review provides a guide to the novel physiological properties of lactate in the brain, stimulates new interpretations of existing data, and highlights routes of exploration for further research.

Acknowledgments and conflict of interest disclosure
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This article has received a badge for *Pre-registration* because it made the data publicly available. The data can be accessed at www.radboudumc.nl/getmedia/53625326-d1df-432c-980f-27c7c80d1a90/THollyer_lactate_protocol.aspx. The complete Open Science Disclosure form for this article can be found at the end of the article. More information about the Open Practices badges can be found at https://cos.io/our-se rvices/open-science-badges/.

Supporting information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Search strategies of the PubMed, Embase, and Cochrane databases.
Table S2. Characteristics of in vitro and ex vivo studies.
Table S3. Characteristics of selected in vivo animal studies.
Table S4. Characteristics of selected human studies.

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Open Practices Disclosure

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# Systematic Review Protocol for Animal Intervention Studies – Adapted for Basic Sciences

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| Item # | Section/Subsection/Item | Description | Check for approval |
|--------|-------------------------|-------------|-------------------|
| 1.     | Title of the review     | The role of lactate on cerebral microvascular physiology: a systematic review. | |
| 2.     | Authors (names, affiliations, contributions) | Hollyer, T\(^1\); van Luijk, J\(^2\); Kousholt, BS\(^3\); Ritses, M\(^2\);  
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TR Hollyer (TRH) – study concept, study design, search design, search, study selection, data extraction, data analysis, manuscript preparation, manuscript editing.  
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BS Kousholt (BSK) – search, study selection, process oversight, manuscript editing.  
M Ritskes (MR) – study design, process oversight, manuscript editing. | |
| 3.     | Other contributors (names, affiliations, contributions) | Karen Tølbøl, Health Library (KT) – searches.  
Leif Østergaard offered to review manuscripts and will accept a mention in acknowledgments. | |
| 4.     | Contact person + e-mail address | Dr Tristan Hollyer, Tristan@cfin.au.dk | |
| 5.     | Funding sources/sponsors | CFIN/AUGUST | |
| 6.     | Conflicts of interest | n/a | |
|   | Date and location of protocol registration | October 2017 |
|---|------------------------------------------|-------------|
| 8. | Registration number (if applicable) | blank |
| 9. | Stage of review at time of registration | blank |

### B. Objectives

#### Background

10. What is already known about this disease/model/intervention? Why is it important to do this review?
The role of lactate in the brain as an energy source has been a widely studied, e.g. the astrocyte neuron lactate shuttle. However, lactate may have other roles such as a vasoactive substance in the brain. This systematic-review will evaluate the current literature on this concept and identify gaps in knowledge which may provide further insight into future experimental hypotheses and novel treatment avenues.

#### Research question

11. Specify the disease/health problem of interest
The effect of lactate on cerebral microvasculature in all in silico, in vitro, ex vivo, in vivo, and human studies in absence of a disease-state

12. Specify the population/species studied
Non-disease state brain imaging in human and animals/ex vivo brain/ ex vivo brain derived vascular tissue or primary vascular cell in vitro /in vitro vascular cell lines and computer models. In studies featuring disease models, negative control i.e. naïve data, shall be identified and used.

13. Specify the intervention/exposure
An assessment of the properties of lactate on the cerebral microvasculature by direct modification or measurement of lactate concentrations or manipulation/intervention of lactate pharmacology including lactate transporters, lactate dehydrogenase, and lactate receptors or any non-harmful/disease related genetic modification or negative control data.

14. Specify the control population
Populations where no manipulation/modification occurred; baseline data acquired prior to intervention; naïve (negative control) data in disease models.

15. Specify the outcome measures
Measures or models related to the effects of modification or manipulation stated above on cerebral vascular cell biochemistry/physiology or cerebral vessel vascular behaviour such as diameter/flow or changes in directly/indirectly acquired imaging indices
|   |   |   |
|---|---|---|
| 16. | State your research question (based on items 11-15) | What is the role of lactate in vitro, in vivo, ex vivo, and human models of cerebral microvascular behaviour? |
|   |   |   |
| **C. Methods** |   |   |
|   | Search and study identification |   |
| 17. | Identify literature databases to search (e.g. Pubmed, Embase, Web of science) | □ MEDLINE via PubMed □ Web of Science □ SCOPUS □ EMBASE □ Other, namely: Cochrane CENTRAL □ Specific journal(s), namely: |
| 18. | Define electronic search strategies (e.g. use the step by step search guide and animal search filters) | When available, please add a supplementary file containing your search strategy: [see last parts] |
| 19. | Identify other sources for study identification | □ Reference lists of included studies □ Books □ Reference lists of relevant reviews □ Conference proceedings, namely: □ Contacting authors/ organisations, namely: □ Other, namely: |
| 20. | Define search strategy for these other sources | Determine if references have been identified through search terms and include for evaluation if it meets the same criteria |
|   | Study selection |   |
| 21. | Define screening phases (e.g. pre-screening based on title/abstract, full text screening, both) | 1. Pool search results from databases in one reference management programme and remove duplicates. 2. Pre-screen based on title and abstract according to criteria stated below 3. Full-text screening on records which pass pre-screening |
| 22. | Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved | Two reviewers per phase. Discrepancies: Pre-screening – any paper which arises will be included for full-screening. Full-screening – inclusion criteria should, by design, prevent such occurrences. If it does occur, ask an independent researcher to evaluate according to the criteria. |
|   | Define all inclusion and exclusion criteria based on: |   |
| 23. | Type of study (design) | Inclusion criteria: Original article, clinical trial Exclusion criteria: review |
| 24. | Type of animals/population (e.g. age, | Inclusion criteria: Physiology based hypothesis including in |
| 25. | Type of intervention (e.g. dosage, timing, frequency) | Inclusion criteria: Direct observation/model of normal state in model, and/or a modification of lactate concentrations/behaviour/pharmacology through addition of lactate to model system/ manipulations of lactate transport, metabolism, receptor pharmacology.
Exclusion criteria: Stated use of disease model or induction of a disease like state by pharmacologic or genetically modifying means |
| 26. | Outcome measures | Inclusion criteria: a stated effect on the potential role of lactate on cerebral microvasculature as a result of experimental investigation at a cellular to whole-brain vasculature level.
Exclusion criteria: The stated effect of lactate in a disease model/state where the effects under pathological circumstances are under investigation |
| 27. | Language restrictions | Inclusion criteria: |
Exclusion criteria: none |
| 28. | Publication date restrictions | Inclusion criteria: |
Exclusion criteria: none |
| 29. | Other | Inclusion criteria: |
Exclusion criteria: |
| 30. | Sort and prioritize your exclusion criteria per selection phase | **Selection phase: Pre-screening**
1. Not primary literature or clinical trial.
2. Does not involve investigation of lactate in the brain

**Selection phase: Full-screening**
1. Use of a disease model or induction of disease state with no reported negative control/naive data |
| 31. | Study ID (e.g. authors, year) | Authors, Year, Title, Journal. |
| 32. | Study design characteristics (e.g. | Methods of assessment: mathematical model/ |
|   |   |
|---|---|
| **33.** | Animal model characteristics (*e.g.* species, gender, disease induction) |
|   | In silico: basis on existing models *e.g.* Kety-Schmidt.  
|   | In vitro: cell type/origin, cell line  
|   | In vivo: species, strain, sex and age.  
|   | Human: sex, age (weight if applicable) |
| **34.** | Intervention characteristics (*e.g.* intervention, timing, duration) |
|   | Investigation or use of lactate and or relevant substrate/treatment/intervention or (as defined in 25.) |
| **35.** | Outcome measures |
|   | Outcome measures in relation to the microcirculation (relevant cell types in vitro or in vivo and clinical measurements) behavior are classed as either direct or indirect.  
|   | Cell types refers to those identified in “microvessel” search category.  
|   | Primary outcome measures: DIRECT  
|   | ● Cell contractility (fiber length, thickness)  
|   | ● DNA/RNA/microRNA/Protein expression  
|   | ● Hormone/neurotransmitter/other signaling molecule release/uptake measured in concentration or volume.  
|   | ● Change in intracellular ion change – concentration or current changes/flux/potential difference  
|   | ● Vessel diameter  
|   | ● Plasma velocity or distribution  
|   | ● RBC/erythrocyte cell velocity  
|   | ● RBC/erythrocyte cell flux  
|   | ● Capillary heterogeneity (CTH)  
|   | ● Mean transit time (MTT)  
|   | ● Vessel density (direct count / number per unit volume)  
|   | Secondary outcome measures: INDIRECT *e.g.* imaging modalities such as PET / MRI or computer models  
|   | ● A change in signal/ratio/quotient  
|   | ● A change in uptake or release of labelled tracer.  
|   | ● Prediction of change in behaviour |
| **36.** | Other (*e.g.* drop-outs) |
### Assessment risk of bias (internal validity) or study quality

| 37. | Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved | 2 reviewers, Tristan Hollyer, and Judith van Luijk |
| --- | --- | --- |
| 38. | Define criteria to assess (a) the internal validity of included studies (e.g. selection, performance, detection and attrition bias) and/or (b) other study quality measures (e.g. reporting quality, power) | □ By use of SYRCLE’s Risk of Bias tool  
□ By use of SYRCLE’s Risk of Bias tool, adapted as follows:  
□ By use of CAMARADES’ study quality checklist, e.g.  
□ By use of CAMARADES’ study quality checklist, adapted as follows:  
□ Other criteria, namely: Cochrane RoB? Limited on in vitro work (OHAT currently refining) [https://ntp.niehs.nih.gov/pubhealth/hat/review/index-2.html#Systematic-Review-Methods](https://ntp.niehs.nih.gov/pubhealth/hat/review/index-2.html#Systematic-Review-Methods) |

### Collection of outcome data

| 39. | For each outcome measure, define the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement) | Data is likely to be a quantitative statement of the findings of the study. A responses or magnitude can also be found. Qualitative assessments may also be made and narrative assessments used to summarise findings. |
| 40. | Methods for data extraction/retrieval (e.g. first extraction from graphs using a digital screen ruler, then contacting authors) | Data will be extracted the following way:  
1. If results are presenting in text in a discrete format e.g. number/ % change. This shall be taken  
2. If 1. is not available the extract from graph using screen ruler or similar  
3. Contact authors if not available. |
| 41. | Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved | 2 reviewers, if discrepancies occur, ask an independent researcher to evaluate according to the criteria. |

### Data analysis/synthesis

| 42. | Specify (per outcome measure) how you are planning to combine/compare the data (e.g. descriptive summary, meta-analysis) | Table of findings with corresponding table with narrative synthesis in text |
| 43. | Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed | n/a |

*If a meta-analysis seems feasible/sensible, specify (for each outcome measure):*

| 44. | The effect measure to be used (e.g. mean difference, standardized mean difference, risk ratio, odds ratio) | n/a |
| 45. | The statistical model of analysis (e.g. | n/a |
|   |   |
|---|---|
| 46. | The statistical methods to assess heterogeneity (e.g. $I^2$, $Q$) | n/a |
| 47. | Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis) | n/a |
| 48. | Any sensitivity analyses you propose to perform | n/a |
| 49. | Other details meta-analysis (e.g. correction for multiple testing, correction for multiple use of control group) | n/a |
| 50. | The method for assessment of publication bias | n/a |

Final approval by (names, affiliations):  
Date: Oct. 2017