SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES – ADAPTED FOR BASIC SCIENCES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
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]; [1]Center for Functionally Integrative Neuroscience, Dept. of Clinical Medicine, Aarhus University, Denmark. [2]SYRCLE, Radboud University, The Netherlands [3]AUGUST, Dept. of Clinical Medicine, Aarhus University, Denmark. TR Hollyer (TRH) – study concept, study design, search design, search, study selection, data extraction, data analysis, manuscript preparation, manuscript editing. J van Luijk (JVL) - search design, search, study selection, process oversight, analytical support manuscript editing. 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	
7.	Date and location of protocol registration	October 2017	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration		

	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 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 animal/in vivo brain/ ex vivo brain derived vascular tissue or primary vascular cell in vitro /in vitro vascular cell lines. 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 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[15] and animal search filters[20, 21])	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, gender, disease model)	Inclusion criteria: Physiology based hypothesis including in vitro, ex vivo, in vivo, and human studies. Genetic modified models acceptable if it does not induce a disease state, fx: GFP-labelling or specific receptor knockout with no stated deleterious effect. Negative control data in studies investigating a disease model, Exclusion criteria: Used of a disease model in vitro, ex vivo, in vivo, and human studies	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Direct observation 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/naïve data	
	Study characteristics to be extracted (for assessment of external validity, reporting quality) ***To be presented in a table***		
31.	Study ID (*e.g.* authors, year)	Authors, Year, Title, Journal.	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Methods of assessment: biochemistry/molecular biology/cell physiology/vascular diameter/flow response/signal change in imaging paradigm.	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	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 ● A change in signal/ratio/quotient ● A change in uptake or release of labelled tracer.	
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[4] ☐By use of SYRCLE’s Risk of Bias tool, adapted as follows: ☐By use of CAMARADES' study quality checklist, e.g [22] ☐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	

	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.* random or fixed effects model)	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