



## SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

FORMAT BY SYRCLE ([www.syrcle.nl](http://www.syrcle.nl))

VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
<b>A. General</b>			
1.	Title of the review	Impact of exercise on knee joint cartilage structure in animals – a systematic review.	
2.	Authors (names, affiliations, contributions)	<p>Alessio Bricca (First reviewer, supporting data analyses, manuscript preparation)</p> <p>Carsten Juhl (Second reviewer, methodological support, data analyses, reviewing manuscript)</p> <p>Martijn Steultjens (Reviewing manuscript)</p> <p>Ewa Roos (Methodological support, reviewing manuscript, clinical perspective)</p>	
3.	Other contributors (names, affiliations, contributions)	No other contributors	
4.	Contact person + e-mail address	Alessio Bricca, abricca@health.sdu.dk	
5.	Funding sources/sponsors	European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 607510.	
6.	Conflicts of interest	None of the authors have any conflicts of interests	
7.	Date and location of protocol registration	22-10-2015 Odense, Denmark	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Preliminary search strategy has been performed	
<b>B. Objectives</b>			
Background			
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	No cure for knee osteoarthritis exists but exercise therapy is considered a central non-pharmacological treatment. In animal models individual studies have examined the effect of exercise on knee cartilage structure, showing a possible relationship between the dose of exercise and cartilage structural changes. Therefore, we will conduct a systematic review of these studies to generate additional knowledge on the mechanism on the impact of exercise on the cartilage, identifying knowledge gaps for future studies.	
Research question			
11.	Specify the disease/health problem of interest	Knee Osteoarthritis/ the effectiveness of exercise therapy or physical activity in knee cartilage health.	
12.	Specify the population/species	<ul style="list-style-type: none"> <li>• Healthy animals</li> </ul>	



		group not offering “Exercise Therapy” OR “Physical Activity” Exclusion criteria: Study without a control group	
24.	Type of animals/population (e.g. age, gender, disease model)	Inclusion criteria: All type animal models Exclusion criteria: In vitro studies, animal with comorbidities and genetically modified animals	
25.	Type of intervention (e.g. dosage, timing, frequency)	Inclusion criteria: Interventions defined as “Exercise Therapy” OR “Physical Activity” Exclusion criteria: Passive motion studies	
26.	Outcome measures	Inclusion criteria: Quantifiable outcome measure on articular cartilage. Exclusion criteria: No quantifiable outcome measure on articular cartilage.	
27.	Language restrictions	Inclusion criteria: English. Exclusion criteria: Other languages. The results presented in the English abstracts of articles in non-English language will be compared with the result of the included studies	
28.	Publication date restrictions	Inclusion criteria: None Exclusion criteria: None	
29.	Other	Inclusion criteria: None Exclusion criteria: None	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: First screening based on title/abstract 1. NOT Exercise Therapy OR Physical Activity 2. NOT Osteoarthritis in the knee OR cartilage structure 3. NOT in vivo animal model  Selection phase: Second screening based on full text 1. NOT Exercise Therapy OR Physical Activity 2. NOT Osteoarthritis in the knee OR cartilage structure 3. NOT in vivo animal model 4. NOT a full publication containing original data 5. NOT a full text retrievable 6. NOT genetically modified animal model 7. NOT comorbidities non connected with the osteoarthritis model	
<b>Study characteristics to be extracted (for assessment of external validity, reporting quality)</b>			
31.	Study ID (e.g. authors, year)	Authors, title, year, language, contact author e-mail	
32.	Study design characteristics (e.g. experimental groups, number of animals)	Study design: Randomized Control Trial or Clinical Controlled Trial.  Number of animals in experimental and control group.	
33.	Animal model characteristics (e.g. species, gender, disease induction)	Animal species, gender, age, weight, osteoarthritis model	
34.	Intervention characteristics (e.g. intervention, timing, duration)	Type, frequency, duration, intensity of the intervention defined as “Exercise Therapy” OR “Physical Activity”	

35.	Outcome measures	<p>Outcomes of the cartilage quality. The following hierarchy is used for each outcome.</p> <ul style="list-style-type: none"> <li>• Histology (e.g. Mankin gradin scale and OARSI grading system)</li> <li>• Immunochemistry (e.g. gag content, or cartilage thickness)</li> <li>• Cartilage-sensitive MRI (e.g. T2 mapping)</li> <li>• Gross macroscopic degenerative changes of cartilage (e.g. India ink staining)</li> <li>• Biomechanics (e.g. stiffness of articular cartilage)</li> </ul>	
36.	Other (e.g. drop-outs)	Number and reason of 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	(a) Alessio Bricca and Carsten Juhl will independently assess the risk of bias/study quality in each study and (b) disagreements will be discussed between the two reviewers until consensus is reach.	
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)	<input checked="" type="checkbox"/> By use of <a href="#">SYRCLE's Risk of Bias tool<sup>4</sup></a> <input type="checkbox"/> By use of SYRCLE's Risk of Bias tool, adapted as follows: <input type="checkbox"/> By use of <a href="#">CAMARADES' study quality checklist, e.g.<sup>22</sup></a> <input type="checkbox"/> By use of CAMARADES' study quality checklist, adapted as follows: <input type="checkbox"/> Other criteria, namely:	
Collection of outcome data			
39.	For each outcome measure, define the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement)	<ul style="list-style-type: none"> <li>• Scoring systems for macroscopic grading of cartilage damage [semi- continuous]</li> <li>• Scoring systems for macroscopic grading of osteophytes [semi-continuous]</li> <li>• Microscopic scoring of cartilage alterations (Mankin) [semi-continuous]</li> <li>• Microscopic scoring via immunohistochemistry (cell apoptosis/collagen denaturation) [continuous]</li> <li>• Microscopic scoring via histomorphometry (e.g. gag content, or cartilage thickness) [continuous]</li> </ul> <p>If the SD is not presented in the included articles then SD will be estimated from the standard error (SE), 95% CI, the p-value or the IQR.</p>	
40.	Methods for data extraction/retrieval (e.g. first extraction from graphs using a digital screen ruler, then contacting authors)	Data will be extracted of Alessio Bricca and Carsten Juhl indenpendtly. Data will firstly be extracted from tables in the published manuscript – secondly measuring numbers from graphs.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	Alessio Bricca and Carsten Juhl. Disagreements will be discussed between the two reviewers until consensus is reach. Authors will be contacted if the data cannot be extracted from the published manuscript.	
Data analysis/synthesis			

42.	Specify (per outcome measure) how you are planning to combine/compare the data (e.g. descriptive summary, meta-analysis)	For outcome measures where a meta-analysis is not possible a qualitative data synthesis of the results from individual studies will be performed.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Meta-analysis will be performed if sufficient data from more than 3 studies can be included	
<i>If a meta-analysis seems feasible/sensible, specify (for each outcome measure):</i>			
44.	The effect measure to be used (e.g. mean difference, standardized mean difference, risk ratio, odds ratio)	Standardized mean differences (SMD) with 95% CIs will be calculated for outcome measures of continuous and semi-continuous scales	
45.	The statistical model of analysis (e.g. random or fixed effects model)	A random-effects model will be conducted as heterogeneity is expected due to differences in animal model, interventions, outcome measures etc.	
46.	The statistical methods to assess heterogeneity (e.g. $I^2$ , Q)	$I^2$ statistic measuring the proportion of variation (i.e., inconsistency) in the combined estimates due to between study variance.	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Subgroup analysis will be performed on Study design (RCT, CCT) - Animal species (mice, rats, rabbits etc.) – Type of intervention (running, swimming, jumping etc.) – exercise intensity (low, moderate, high) - exercise dose (frequency, duration, intensity).	
48.	Any sensitivity analyses you propose to perform	Sensitivity analysis restricting to (1) RCT study and (2) small animals such as mice, rats, hamsters and guinea pigs.	
49.	Other details meta-analysis (e.g. correction for multiple testing, correction for multiple use of control group)	If several intervention groups is compare to one control group the number of animals in the control group will be divided by the number in the intervention group.	
50.	The method for assessment of publication bias	The publication bias (small study bias) will be investigated using a funnel plot based on the SMD and SE (SMD). Furthermore the publication bias will be tested with Eggers test.	
Final approval by (names, affiliations):		Alessio Bricca	Date: 22-10-2015