# Systematic Review Protocol for Animal Intervention Studies

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<table>
<thead>
<tr>
<th>Item #</th>
<th>Section/Subsection/Item</th>
<th>Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>Title of the review</td>
<td>Effect of anti-diabetic drugs on bone: a systematic literature review and meta-analysis of animal studies</td>
</tr>
</tbody>
</table>
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| 5.     | Funding sources/sponsors | None |
| 6.     | Conflicts of interest | None |
| 7.     | Date and location of protocol registration |  |
| 8.     | Registration number (if applicable) |  |
| 9.     | Stage of review at time of registration |  |

## B. Objectives

**Background**

Diabetes mellitus is associated with increased fracture risk [1, 2], and the mechanisms behind deleterious effects of diabetes on bone health are not well explored. Increasing evidence suggested that anti-diabetic drugs might have significant role on the skeletal system [3]. For instances, thiazolidinediones increases the bone loss and risk of fracture possibly through PPARγ activation in bone marrow cells and hamper the osteoblastogenesis via decreasing Runx2 transcription factor, IGF-1 and Wnt signalling pathways [4, 5]. On the other hand, metformin and sulfonylureas shows neutral or positive effect on bone health and reduced risk of fracture [6,7]. In addition,
Results from the animal and human studies create controversy over insulin safety profile on bone health. Incretin based therapy (GLP-1 receptor agonist and DPP-4 inhibitors) and SGLT2 inhibitors are currently available marketed anti-diabetic drugs. Data from animal studies suggested that incretin based therapy play an important role in the regulation of bone turnover [8, 9]. SGLT2 inhibitors may cause bone loss or increased risk of fracture might be due to decrease bone mineral density (BMD), altered calcium, phosphate and sodium concentration [10, 11]. Therefore, aim of this systematic literature review is to accumulate data from animal studies and provide better information about the safety concern of anti-diabetic medication.

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<tr>
<th>Research question</th>
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<tbody>
<tr>
<td>11. Specify the disease/health problem of interest</td>
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<tr>
<td>12. Specify the population/species studied</td>
</tr>
<tr>
<td>13. Specify the intervention/exposure</td>
</tr>
<tr>
<td>14. Specify the control population</td>
</tr>
<tr>
<td>15. Specify the outcome measures</td>
</tr>
<tr>
<td>16. State your research question (based on items 11-15)</td>
</tr>
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</table>

### 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:]

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: [insert file name]

19. Identify other sources for study identification [Reference lists of included studies] [Reference lists of relevant reviews] [Books] [Conference proceedings, namely:]

20. Define search strategy for these other sources **Search string for bone:** "Skeleton"[Mesh] OR bone[Title/Abstract] OR "osteogenesis"[MeSH Terms] OR ("osteogenesis"[MeSH Terms] OR "osteogenesis"[All Fields] OR "bone"[All Fields] AND "formation"[All Fields]) OR "bone formation"[All Fields]) OR ("osteogenesis"[MeSH Terms] OR "osteogenesis"[All Fields] OR "bone"[All Fields] AND "formation"[All Fields]) OR "bone formation"[All Fields])

**Search string for intervention:**


**Search String for diabetes:**


**Search String for animals:** [12]

<table>
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<tr>
<th>Study selection</th>
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<tr>
<td><strong>21.</strong> Define screening phases (e.g. pre-screening based on title/abstract, full text screening, both)</td>
<td>First Pass: Screening based on title and abstract</td>
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<tr>
<td><strong>22.</strong> Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved</td>
<td>Three independent reviewer (MA, PV and SV) Discrepancies will be resolved by contacting (PG, AK and MS)</td>
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<tr>
<td><strong>23.</strong> Type of study (design)</td>
<td>Inclusion criteria: Pre-clinical studies with control group Exclusion criteria: Review papers, opinion papers, non-diabetic studies, non-interventional studies</td>
</tr>
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</table>
| 24. | Type of animals/population *(e.g. age, gender, disease model)* | Inclusion criteria: Experimental animals with diabetes  
Exclusion criteria: Human and in-vitro studies |
| 25. | Type of intervention *(e.g. dosage, timing, frequency)* | Inclusion criteria: Any anti-diabetic medication in any dose, duration and frequency  
Exclusion criteria: other than anti-diabetic medication |
| 26. | Outcome measures | Inclusion criteria: Blood glucose level and bone biomarkers  
Exclusion criteria: N/A |
| 27. | Language restrictions | Inclusion criteria: English language papers  
Exclusion criteria: None |
| 28. | Publication date restrictions | Inclusion criteria: No restriction  
Exclusion criteria: N/A |
| 29. | Other | Inclusion criteria: N/A  
Exclusion criteria: N/A |
| 30. | Sort and prioritize your exclusion criteria per selection phase | Selection phase: first pass based on title/abstract  
1. Non-diabetic studies  
2. other interventions  
3. Review or non original papers  
4. Not English  
Selection phase: Second pass based on full text  
1. Not an original paper  
2. No data regarding bone  
3. No control group |
| 31. | Study characteristics to be extracted *(for assessment of external validity, reporting quality)* | First author, title, year, journal |
| 32. | Study design characteristics *(e.g. experimental groups, number of animals)* | Experimental setting  
Experimental groups  
Number of animals per group  
Type of animal model |
| 33. | Animal model characteristics *(e.g. species, gender, disease induction)* | Species  
Gender  
Diseased models *(chemical induced such as streptozotocin, alloxane; Spontaneous autoimmune models; Genetically induced models)* |
| 34. | Intervention characteristics *(e.g. intervention, timing, duration)* | Name of the interventions  
Dose, duration, frequency and route of administration |
| 35. | Outcome measures | Blood glucose level and bone biomarkers *(osteocalcin; RANKL; OPG; CTX; PINP; ALP; TRAP; calcium and sclerostin)* |
| 36. | Other *(e.g. drop-outs)* | |
| 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) Two independent reviewers (MA and PV) will assess risk of bias of included studies.  
(b) Discrepancies will be resolved by contacting (SV, PG, AK and MS). |
### Define criteria to assess internal validity of included studies

- **Option:** 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:

### Collection of outcome data

#### For each outcome measure, define the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement)
- **Blood glucose level (mg/dl or mmol/L)** and bone biomarkers (unit as per considerable markers)

#### Methods for data extraction/retrieval (e.g. first extraction from graphs using a digital screen ruler, then contacting authors)
- Extract data from table, text or figures
- For Incomplete or unavailable data respective authors will be contacted and if authors failed to respond then study will be excluded.

#### Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved
- **(a)** Two independent reviewers (MA and PV) will extract data.
- **(b)** Discrepancies will be resolved by contacting (SV, PG, AK and MS).

### Data analysis/synthesis

#### Specify (per outcome measure) how you are planning to combine/compare the data (e.g. descriptive summary, meta-analysis)
- Meta-analysis

#### Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed
- If data from more than three studies homogeneous in nature then meta-analysis will be performed.

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

#### The effect measure to be used (e.g. mean difference, standardized mean difference, risk ratio, odds ratio)
- Mean difference or standard mean difference and 95% confidence interval will be used.

#### The statistical model of analysis (e.g. random or fixed effects model)
- Random effect model

#### The statistical methods to assess heterogeneity (e.g. I^2, Q)
- I^2

#### Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)
- Species
- Gender
- Diabetes duration
- Duration of drug treatment
- Type of intervention

#### Any sensitivity analyses you propose to perform
- To be determined

#### Other details meta-analysis (e.g. correction for multiple testing, correction for multiple use of control group)
- If applicable, we will perform a Bonferroni correction for testing multiple subgroups. If one or more subgroup analyses cannot be performed due to insufficient data, the p-value will be adjusted accordingly. Also correction for multiple use of control groups will be performed by dividing the number of animals in the control group by the number of comparisons performed with this control group.
References: