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Review article

# Bone deconditioning during partial weight-bearing in rodents – A systematic review and meta-analysis

Patrick Swain [a,*], Marie Mortreux [b], Jonathan M. Laws [a], Harry Kyriacou [c], Enrico De Martino [a], Andrew Winnard [a], Nick Caplan [a]

[a] Aerospace Medicine and Rehabilitation Laboratory, Faculty of Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne, United Kingdom
[b] Harvard Medical School, Department of Neurology, Beth Israel Deaconess Medical Center Boston, MA, United States
[c] School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom

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ABSTRACT

Space agencies are preparing to send humans to the Moon (16% Earth's gravity) and Mars (38% Earth's gravity), however, there is limited evidence regarding the effects of hypogravity on the skeletal system. A novel rodent partial weight-bearing (PWB) model may provide insight into how human bone responds to hypogravity. The aim of this study was to perform a systematic review investigating the effect of PWB on the structure and function of rodent bone. Five online databases were searched with the following inclusion criteria: population (rodents), intervention (PWB for ≥1-week), control (full weight-bearing), outcomes (bone structure/function), and study design (animal intervention). Of the 2,993 studies identified, eight were included. The main findings were that partial weight-bearing exposure for 21–28 days at 20%, 40%, and 70% of full loading causes: (1) loss of bone mineral density, (2) loss of trabecular bone volume, thickness, number, and increased separation, (3) loss of cortical area and thickness, and 4) reduced bone stiffness and strength. These findings predominately relate the tibia/femur of young/mature female mice, however, their deconditioning response appeared similar, but not identical, to male rats. A dose-response trend was frequently observed between the magnitude of deconditioning and PWB level. The deconditioning patterns in PWB resembled those in rodents and humans exposed to microgravity and microgravity analogs. The present findings suggest that countermeasures against bone deconditioning may be required for humans exploring the Lunar and Martian surfaces.

**New & Noteworthy:** Partial weight-bearing causes bone deconditioning at the structural and functional levels in rodents. Higher levels of weight-bearing frequently attenuated deconditioning but did not always prevent it. Deconditioning patterns resembled those that occur in rodents and humans exposed to microgravity and microgravity analogs. This evidence suggests that bone deconditioning may occur in humans on the surface of the Moon and Mars, in a similar manner that occurs in astronauts onboard the International Space Station.

## 1. Introduction

Space agencies are preparing to expand human presence beyond low-Earth orbit through crewed missions to the Moon (16% Earth's gravity) and Mars (38% Earth's gravity) (ISECG, 2018). These journeys will expose astronauts/cosmonauts to microgravity during transit (Moon: 3–5 days or weeks/months if an orbital gateway is used; Mars: 6–12 months) and upon planetary arrival, they will then live and work in hypogravity for weeks/months/years, depending on mission objectives (Horneck et al., 2006; Connolly et al., 2018; Horneck and Comet, 2006). Prolonged mechanical unloading of the skeletal system, such as in microgravity and microgravity analogs (e.g., head-down tilt bed rest), disrupts the dynamic coupling between bone formation and resorption, particularly in weight-bearing regions (Stavnichuk et al., 2020). This results in bone deconditioning, characterized by reduced bone mineral density (BMD) and diminished trabecular and/or cortical geometry and microarchitecture (Stavnichuk et al., 2020; Coulombe et al., 2020; Nagaraja and Risin, 2013b; Grimm et al., 2016). Mathematical modeling suggests that bones may demineralize and become mechanically weaker in response to Lunar and Martian hypogravity (Keller and Strauss, 1992; Lewandowski et al., 2008). However, despite head-up tilt bed rest being

* Corresponding author at: Aerospace Medicine and Rehabilitation Laboratory Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom.
*E-mail address:* patrick.swain@northumbria.ac.uk (P. Swain).

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established as a long-term hypogravity analog in 2013 (Cavanagh et al., 2013), recent systematic reviews have failed to identify any controlled experimental research in humans investigating the effect of hypogravity on bone health (Richter et al., 2017; Swain et al., 2021).

The importance of this topic is emphasized by the risks associated with bone deconditioning in astronauts/cosmonauts. As highlighted by the National Aeronautics and Space Administration (NASA) Bioastronautics Roadmap, these include: (1) early-onset osteopenia and osteoporosis, (2) increased risk of bone fracture, (3) increased risk of fascia, tendon, ligament, and joint overuse, injury, or dysfunction, (4) impaired and incomplete bone healing following fracture, (5) neurological damage caused by a fracture in close proximity to the nerves, and (6) altered urinary biochemistry leading to renal stone formation (NASA, 2005). Knowledge of how bone adapts to different levels of hypogravity can, therefore, help support clinical decision-making and medical operations, such as the requirement for countermeasure(s) (e.g., resistance exercise and/or pharmaceutical supplementation) on the Moon and Mars (Grimm et al., 2016). Terrestrial medicine may also benefit from this information as various clinical conditions (e.g., stroke and cerebral palsy) can lead to reduced mobility and altered weight-bearing for prolonged durations.

Researchers have recently established a novel rodent partial weight-bearing (PWB) model to simulate hypogravity loading (Wagner et al., 2010; Mortreux et al., 2018). This was initially developed for mice in 2010 (Wagner et al., 2010) and has been recently adapted for rats in 2018 (Mortreux et al., 2018). The PWB model has been applied by several studies and is beginning to elucidate the effects of simulated Lunar gravity (PWB20%), Martian gravity (PWB40%), and moderate artificial gravity (PWB70%) on the rodent skeletal system (Ko et al., 2020). For decades, rodents have been used in biomedical research as pre-clinical and/or translational models due to their genetic, anatomical, and physiological similarities to humans (Coulombe et al., 2020; Nagaraja and Risin, 2013b; Grimm et al., 2016; Fu et al., 2021). For example, rodent models are widely employed for investigating age-related and unloading-induced osteoporosis (Syed and Melim, 2011; Lau and Guo, 2011; Globus and Morey-Holton, 2016). Importantly, rodent, monkey, and human skeletons display similar patterns of bone deconditioning during periods of complete unloading (Stavnichuk et al., 2020; Coulombe et al., 2020; Nagaraja and Risin, 2013b; Grimm et al., 2016; Fu et al., 2021; Spector et al., 2009; Leblanc et al., 1990; Nagaraja and Risin, 2013a; Lloyd et al., 2014; Maupin et al., 2019; Zhang et al., 2013). Therefore, given the paucity of human evidence on this topic, rodent PWB experiments may provide valuable insight regarding the potential effects of hypogravity on human bone and potential countermeasure requirements on the Moon and Mars. The aim of this study was to, therefore, perform a systematic review to determine the effects of PWB, relative to full weight-bearing, on the structure and function of the rodent skeletal system. The objectives were to establish whether skeletal deconditioning occurs and whether the magnitude of deconditioning is mediated by the PWB load.

## 2. Materials and method

This study conformed to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009) and the Space Biomedicine Methods Handbook (Winnard et al., 2021; Winnard et al., 2020). A PRISMA checklist can be found in Supplementary Table S1 (https://doi.org/10.5281/zenodo.5728034). This review follows the same methods described in our previous manuscript regarding the effect of PWB on rodent muscle (Swain et al., 2021).

### 2.1. Search strategy

The following online databases were searched from inception to the 18th of June 2020 using key search terms and Boolean logic: PubMed, Scopus, EMBASE, MEDLINE, and Web of Science. Pre-scoping searches were performed in the NASA Technical Reports Server (NTRS), the NASA Life Science Data Archive (LSDA), and the Cochrane Collaboration Library, but were not included in the final search due to a lack of relevant findings. SPORTDiscus was searched (27 hits) but not used as all eligible studies had been identified from other database searches. A second search was performed in PubMed on the 19th of May 2021 using Medical Subject Heading (MeSH) terms selected from previously indexed PWB studies and MeSH hierarchy tables. No MeSH term existed for PWB, however, some PWB studies are indexed within the 'hindlimb suspension' MeSH term vocabulary, which was therefore used. Included studies' reference lists and citations were screened for any additional relevant articles. The final search strategy for each database is presented in Table 1. This also includes muscle-related terms as these data were collected in parallel.

### 2.2. Study eligibility criteria

Search results were exported and stored in the online reference manager Rayyan (Ouzzani et al., 2016). Following the removal of duplicates, each study was screened by two independent reviewers for inclusion using the PICOS criteria (Table 2). Structural and functional bone outcomes were prioritized, as these are the most relevant to understanding the integrity of bone tissue (metabolic outcomes were not included). Trabecular and cortical microarchitecture/geometry outcome eligibility was determined based on the default set of variables that rodent studies are recommended to report, as per assessment guidelines for microcomputed tomography (Bouxsein et al., 2010). Data where the PWB and/or control group was combined with another intervention (e.g., radiation) were not eligible, but the sham groups were. A two-stage process was used for eligibility screening:

- Stage one involved assessing studies by title and abstract (full text if unclear) for relevance in accordance with the PICOS criteria, with potentially relevant studies labeled as 'maybe' within Rayyan.
- Stage two involved screening all 'maybe' studies in full text for final inclusion/exclusion, with any reasons for exclusion agreed and logged (Supplementary Table S2; https://doi.org/10.5281/zenodo.5599399). Any disagreements were resolved initially through discussion, and if the dispute remained unresolved, a third reviewer was consulted.

### 2.3. Data extraction

All included studies were downloaded, and the study characteristics (rodent species, sex, age, control condition, intervention, PWB level(s), and longest exposure duration) were extracted. Eligible outcome data were manually collected by one reviewer in the form of means, standard deviations, and sample sizes, and were stored using Review Manager (RevMan Version 5, The Cochrane Collaboration) (Cochrane, 2019). Data reported as a standard error of measurement were converted to standard deviations. Available data were initially retrieved from the manuscript and/or supplementary materials. Where data could not be retrieved, such as in figures or missing data, the corresponding and/or lead authors were contacted. Where authors were unable to provide data or respond, WebPlotDigitizer (Version 4.3) was used to determine figure data. This tool has been demonstrated to have excellent reliability ($r = 0.99$) and accuracy ($r = 0.93$) (Aydin and Yassikaya, 2022). If no sample size data were stated in a table/figure caption, the sample size reported in the methods for the respective group was used. Sample sizes that were reported as a range for an outcome (e.g., $n = 3$–6) were extracted using a conservative approach; the lowest sample size reported was used. Two control groups were used in Wagner et al. 2010 (Wagner et al., 2010); the age-matched control data were extracted for a higher sample size. Macias et al. (2016) investigated the effect of PWB in combination with ration and used two PWB sham groups (X-ray G/6 sham and Si G/6 sham), both with independent full weight-bearing control groups (X-ray

**Table 1**
Search strategy.

| Database | Search string | Filter (s) | Hits | Date |
|---|---|---|---|---|
| PubMed (MeSH) | ("Gravity, Altered"[Mesh] OR "Hindlimb Suspension"[*Mesh*]) AND "Musculoskeletal System"[Mesh] AND "Murinae"[Mesh] | None | 1495 | 19th of May 2021 |
| PubMed | (("partial gravity" OR "reduced gravity" OR hypogravity OR "quadrupedal unloading" OR "partial weight bearing" OR "partial weight-bearing" OR "partial weightbearing" OR "martian gravity analog" OR "martian-gravity analog" OR "lunar gravity analog" OR "lunar gravity-analog") AND (musculoskeletal OR muscle OR bone OR skeleton OR skeletal OR strength OR grip)) AND (rat OR mice OR rodent OR animal OR murine) | None | 1077 | 18th of June 2020 |
| Web of Science | TOPIC: ("partial gravity" OR "reduced gravity" OR hypogravity OR "quadrupedal unloading" OR "partial weight bearing" OR "partial weight-bearing" OR "partial weightbearing" OR "martian gravity analog" OR "martian-gravity analog" OR "lunar gravity analog" OR "lunar gravity-analog") AND TOPIC: (musculoskeletal OR muscle OR bone OR skeleton OR skeletal OR strength OR grip) AND TOPIC: (rat OR mice OR rodent OR animal OR murine) | None | 71 | 18th of June 2020 |
| Scopus | (TITLE-ABS-KEY ("partial gravity" OR "reduced gravity" OR hypogravity OR "quadrupedal unloading" OR "partial weight bearing" OR "partial weight-bearing" OR "partial weightbearing" OR "martian gravity analog" OR "martian-gravity analog" OR "lunar gravity analog") AND TITLE-ABS-KEY (musculoskeletal OR muscle OR bone OR skeleton OR skeletal OR strength OR grip) AND TITLE-ABS-KEY (rat OR mice OR rodent OR animal OR murine)) | None | 128 | 18th of June 2020 |
| MEDLINE | ("partial gravity" OR "reduced gravity" OR hypogravity OR "quadrupedal unloading" OR "partial weight bearing" OR "partial weight-bearing" OR "partial weightbearing" OR "martian gravity analog" OR "martian-gravity analog" OR "lunar gravity analog" OR "lunar gravity-analog") AND (musculoskeletal OR muscle OR bone OR skeleton OR skeletal OR strength OR grip) AND (rat OR mice OR rodent OR animal OR murine) | None | 119 | 18th of June 2020 |
| EMBASE | (("partial gravity" or "reduced gravity" or hypogravity or "quadrupedal unloading" or "partial weight bearing" or "partial weight-bearing" or "partial weightbearing" or "martian gravity analog" or "martian-gravity analog" or "lunar gravity analog" or "lunar gravity-analog") and (musculoskeletal or muscle or bone or skeleton or skeletal or strength or grip) and (rat or mice or rodent or animal or murine)).af. | None | 103 | 18th of June 2020 |

Note: Scopus only allows a limited number of search terms per search box.

**Table 2**
PICOS eligibility criteria.

| Parameter | Inclusion criteria |
|---|---|
| *Population* | Rats and mice (no sex or breed restriction) |
| *Intervention* | Quadrupedal partial weight-bearing (between 10% and 80% full loading) for ≥1-week |
| *Comparison* | Full weight-bearing control |
| *Outcomes* | 1) Areal BMD and trabecular/cortical volumetric BMD<br>2) Trabecular architecture/geometry (BV/TV, Tb.Th, Tb.N, Tb.Sp)<br>3) Cortical architecture/geometry (Tt.Ar, Ct.Ar, Ct.Ar/Tt.Ar, Ct.Th)<br>4) Three-point bending and compression testing outcomes (e.g., Young's modulus, stiffness, ultimate load, and failure load) |
| *Study design* | Controlled animal intervention trial |

Where: BMD = bone mineral density, BV/TV = bone volume fraction, Tb.Th = trabecular thickness, Tb.*N* = trabecular number, Tb.Sp = trabecular separation, Tt.Ar = total cross-sectional area inside the periosteal envelope, Ct.Ar = cortical bone area, Ct.Ar/Tt.Ar = cortical area fraction, Ct.Th = cortical thickness (Bouxsein et al., 2010).

1G sham and Si 1G sham). Therefore, the sham data were extracted and termed 'X-ray sham' and 'Si sham'. To maintain consistency with all other studies, terminal data from Mortreux et al. (2018) and Ko et al. (2020) were collected, except for trabecular BMD, which was the only outcome where male rat data could be pooled.

### 2.4. Data analysis

Standardized mean differences (SMD) were calculated using the Hedges' G effect size statistic (Deeks and Higgins, 2010). Hedges' G adjusts for small sample bias, which was commonplace among the eligible studies. Individual comparisons were made for each outcome and PWB level (PWB20%, PWB40%, and PWB70%) against the full weight-bearing control group. Where there were two or more independent reports of the same outcome at a given PWB level and exposure duration, weighted meta-effect sizes using a fixed-effect inverse variance model (with 95% confidence intervals) and heterogeneity ($I^2$) were calculated using RevMan 5 (Deeks and Higgins, 2010). Statistical significance was set at $P < 0.05$. To minimize anticipated heterogeneity, meta-effects were only calculated from rodent sub-populations of the same species and sex. The magnitude of the SMD was qualitatively described using the following thresholds: 0.2 (small), 0.5 (medium), 0.8 (large), and 1.3 (very large) (Rosenthal, 1996). Thresholds for heterogeneity were guided by the Cochrane Handbook for Systematic Reviews of Interventions: 0–40% (might not be important), 30–60% (may represent moderate heterogeneity), 50–90% (may represent substantial heterogeneity), and 75–100% (considerable heterogeneity) (Cochrane, 2019). All comparisons are presented in the format of forest plots generated by RevMan 5. Assessment of reporting bias (e.g., via funnel plots) was not performed as the sample size for all given meta-analyses failed to reach the minimum requirement for adequate statistical power ($n = 10$) as outlined by the Cochrane Handbook for Systematic Reviews of Interventions (Cochrane, 2019).

### 2.5. Risk of bias assessment

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool (Hooijmans et al., 2014) was used to assess several risks of bias (RoB) at the study level. This tool uses a three-point bias ranking system (low risk, high risk, or unclear risk) for nine checklist items relating to the main themes of selection, performance, detection, attrition, and reporting biases. However, the SYRCLE tool addresses RoB at a general level and fails to address RoB relating to specific models (e.g., PWB). Previous aerospace medical systematic reviews have dealt with model specific RoB through consultation with academics in the aerospace industry, creating tools assessing bed rest quality and the ecological validity of human hypogravity simulation methods for example (Richter et al., 2017; Winnard et al., 2019). The same strategy

was employed in the present review. Two PWB experts were consulted to establish an additional RoB tool specific to PWB studies (M. Mortreux and M.E. Rosa-Caldwell, personal communication, May 2021). Using an iterative approach, potential checklist items were generated, remarked, and agreed (Swain et al., 2021). A total of 16 checklist items were compiled into the final 'PWB RoB checklist' (PWB-RoBC) (Supplementary Table S3; https://doi.org/10.5281/zenodo.5599188) (Swain et al., 2021). The PWB-RoBC was scored using the same method as the SYRCLE's RoB tool (high, low, or unclear RoB). Two independent assessors scored all eligible studies using the SYRCLE tool and PWB-RoBC. Any disagreements were initially resolved via consensus-oriented discussion, and if the conflict remained unresolved, through consultation with a third assessor. (Wagner et al. (2010) was flagged for having several high RoBs, therefore, sensitivity analyses were performed in all applicable analyses by excluding this study and assessing what difference this had on the original meta-effect and heterogeneity values.

## 3. Results

The final search strategy identified 2993 articles, of which eight met the eligibility criteria (Fig. 1). Study characteristics are presented in Table 3. All employed a full weight-bearing control and either a single or multiple PWB intervention group(s) at loads of PWB20%, PWB40%, and/or PWB70%. Partial weight-bearing exposure duration ranged from 14 to 28 days. Six studies used young/mature female mice with BALB/cByJ ($n = 4$) or C57Bl/6 J ($n = 2$) strains, and two studies used mature Wistar male rats. Risk of bias, via the SYRCLE tool were predominantly scored as unclear (49%) or low (44%); only 7% of items were scored as high (Table 4). Similarly, as per the novel PWB-RoBC, RoBs were scored as unclear (36%) or low (62%); only 2% of items were scored as high (Supplementary Table S3; https://doi.org/10.5281/zenodo.5599188).

### 3.1. Dual-energy X-Ray absorptiometry (DXA)

Partial weight-bearing at all loads (PWB20%, PWB40%, and PWB70%) caused significant reductions in areal BMD (aBMD) at the total body (excluding the head) and hindlimb (femoral neck to ankle) levels following 21-days of exposure in female mice (Fig. 2). The magnitude of deconditioning displayed a dose-response trend with PWB load, however, there was considerable heterogeneity in the magnitude of effects between the two studies that reported aBMD.

### 3.2. Peripheral quantitative computed tomography (pQCT)

Two studies observed reductions in trabecular volumetric BMD (vBMD) at the proximal tibia in male rats during PWB20%, PWB40%, and PWB70% following 28-days of exposure (Fig. 3). Reductions occurred in a dose-response manner with PWB load and were progressive with increasing exposure duration (day 7 < day 14 < day 28). By contrast, following 28-days of exposure, cortical vBMD at the tibia mid-diaphysis in male rats slightly increased by a small effect at PWB20%, whilst PWB40% and PWB70% were unaffected (Fig. 3).

All supplementary figures (S1–5) are available at https://doi.org/10.5281/zenodo.5599117. Total vBMD at the proximal tibia metaphysis in female mice reduced by similar amounts following 21-days of exposure to PWB20% and PWB40% (very large effects) (PWB70% not reported by any study) (Supplementary Fig. S1) At the tibia mid-diaphysis, the cross-sectional moment of inertia reduced by a medium effect at PWB20% and a small effect at PWB40% (Supplementary Fig. S1).

### 3.3. Microcomputed tomography (Trabecular)

The femoral distal metaphysis and fourth lumbar vertebra (L4) displayed signs of diminished trabecular geometry and microarchitecture

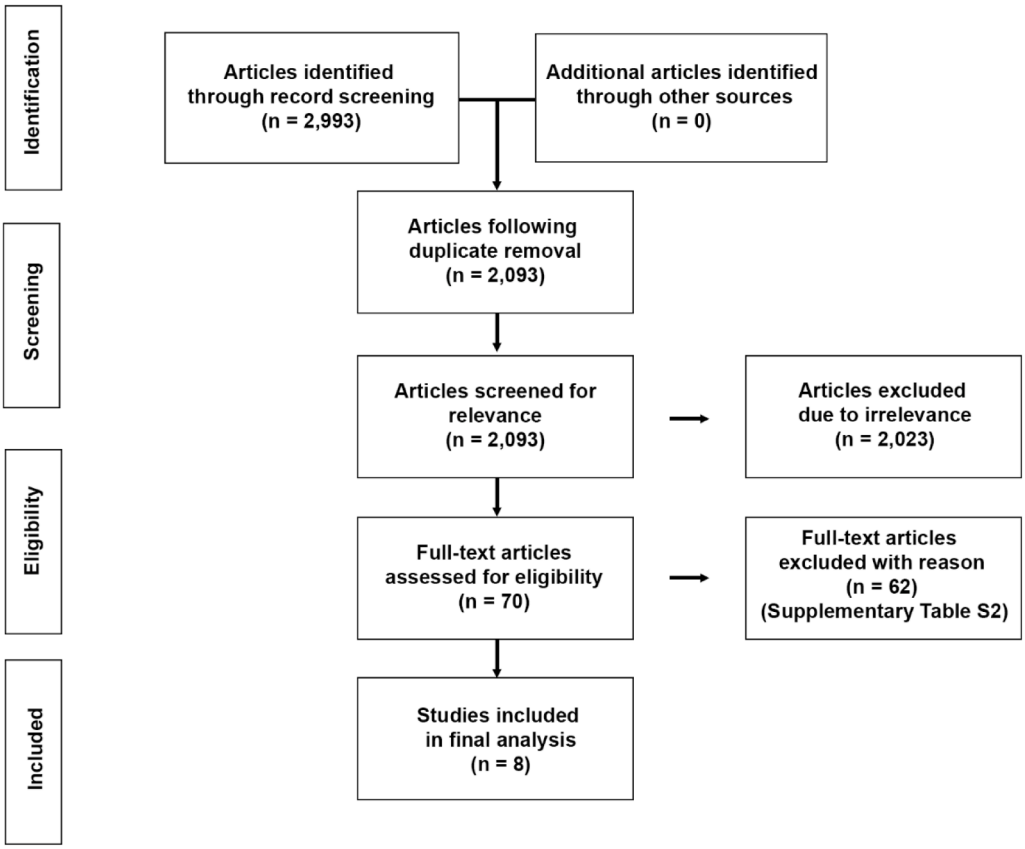


**Fig. 1.** PRISMA flow diagram.

**Table 3**
Study characteristics.

| Reference | Population | Age (weeks) | Control condition apparatus | PWB model apparatus | Full weight-bearing control | PWB 70% | PWB 40% | PWB 20% | Longest exposure duration |
|---|---|---|---|---|---|---|---|---|---|
| Mortreux et al. 2018 (Mortreux et al., 2018) | Wistar male rats | 14 | NKD, JKT, HNS | JKT + HNS | ✓ | ✓ | ✓ | ✓ | 2 weeks |
| Ko et al. 2020 (Ko et al., 2020) | Wistar male rats | 14 | JKT + HNS | JKT + HNS | ✓ | ✓ | ✓ | ✓ | 4 weeks |
| Swift et al. 2013 (Swift et al., 2013) | BALB/cByJ female mice | 17 | Unclear | JKT + TW | ✓ | × | ✓ | ✓ | 3 weeks |
| Wagner et al. 2010 (Wagner et al., 2010) | BALB/cByJ female mice | 10 | NKD | JKT + TW | ✓ | × | ✓ | × | 3 weeks |
| Macias et al. 2016 (Macias et al., 2016) | BALB/cByJ female mice | 17 | Unclear | JKT + TW | ✓ | × | × | ✓ | 3 weeks |
| Bokharki et al. 2019 (Bokhari et al., 2019) | BALB/cByJ female mice | 16 | NKD | JKT + TW | ✓ | × | × | ✓ | 3 weeks |
| Ellman et al. 2013 (Ellman et al., 2013) | C57Bl/6 J female mice | 11 | JKT + TW | JKT + TW | ✓ | ✓ | ✓ | ✓ | 3 weeks |
| Spatz et al. 2017 (Spatz et al., 2017) | C57Bl/6 J female mice | 11 | Unclear | JKT + TW | ✓ | ✓ | ✓ | ✓ | 3 weeks |

Where: PWB = partial weight-bearing (as a percentage of 100% body weight), NKD = naked, JKT = forelimb jacket, HNS = pelvic harness, and TW = tail wrap. Note: see forest plots for exact sample sizes, which varied PWB group and outcome measurement. Note: rodent age at the start of PWB is presented.

**Table 4**
Risk of bias scoring (via SYRCLE's tool).

| Reference | Selection bias *Sequence generation* | *Baseline characteristics* | *Allocation concealment* | Performance bias *Random housing* | *Blinding* | Detection bias *Random outcome assessment* | *Blinding* | Attrition bias *Incomplete outcome data* | Reporting bias *Selective outcome reporting* |
|---|---|---|---|---|---|---|---|---|---|
| **Mortreux et al. 2018** (Mortreux et al., 2018) | High | Low | Low | Low | High | Low | Unclear | Low | Low |
| **Ko et al. 2020** (Ko et al., 2020) | High | Low | Low | Low | High | Low | Unclear | Low | Low |
| **Swift et al. 2013** (Swift et al., 2013) | Unclear | Low | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Low |
| **Wagner et al. 2010** (Wagner et al., 2010) | Low | Low | Unclear | High | High | Unclear | Unclear | Low | Low |
| **Macias et al. 2016** (Macias et al., 2016) | Unclear | Low | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Low |
| **Bokhari et al. 2019** (Bokhari et al., 2019) | Low | Low | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Low |
| **Ellman et al. 2013** (Ellman et al., 2013) | Unclear | Low | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Low |
| **Spatz et al. 2017**(Spatz et al., 2017) | Unclear | Low | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Low |

Note: items scored as low = 44%, unclear = 47%, and high = 8% (rounded to the nearest integer).

following 21-days of PWB exposure in female mice (Fig. 4). Trabecular bone volume fraction (BV/TV) significantly reduced at PWB20% (large effect) and to a lesser degree at PWB40% (medium effect), whilst PWB70% declined by a small non-significant effect. The magnitude of BV/TV loss increased as PWB load decreased in a dose-response manner (Fig. 4). Trabecular number (Tb.N) significantly reduced at PWB20% (large effect), but was not significantly different at PWB40% or PWB70%, following 21-days of exposure (Fig. 4). Trabecular thickness (Tb.Th) significantly reduced at all PWB loads by large/very large effects, with lower PWB loading causing greater declines in a dose-response manner (Fig. 4). Trabecular separation (Tb.Sp) increased at PWB20% (very large effect), the meta-effect at PWB40% displayed a non-significant trend for a medium increase ($P = 0.07$), and PWB70% increased by a medium effect (Fig. 4). At the fourth lumbar vertebra (L4), a single study employing PWB20% observed reductions in BV/TV and Tb.Th by large and very large effects, respectively, whilst Tb.N reduced by a small effect (Fig. 4). Sensitivity analysis for trabecular parameters, via the removal of Wagner et al. 2010 (Wagner et al., 2010) in all applicable meta-analyses at PWB40% (no other PWB loads investigated in this study), had trivial impact on BV/TV and Tb.N meta-effects, however, caused a small increase for Tb.Th (SMD: −1.68 to −1.46) (Supplementary Table S4; https://doi.org/10.5281/zenodo.5599264). In addition, heterogeneity remained at 0% for BV/TV and Tb.N, however, decreased for Tb.Th ($I^2$: 46% to 0%).

One study measured trabecular geometric and microarchitectural outcomes in male rats (Fig. 5). At the femoral distal metaphysis following 28-days exposure, BV/TV reduced at all PWB loads, with the largest decline occurring at PWB20% (very large effects), whilst PWB40% and PWB70% declined by medium and large effects, respectively (Fig. 5). Tb.Th reduced at all PWB loads, with PWB20% causing the greatest reductions (very large effect), whilst PWB40% and PWB70% displayed similar losses by large effects (Fig. 5). Tb.N reduced by medium effects at PWB20%, and by small effects at PWB40% and PWB70% (Fig. 5).

### 3.4. Microcomputed tomography (Cortical)

Cortical geometric parameters have been assessed in female mice at the femoral and tibial mid-diaphysis (Fig. 6) and femoral distal metaphysis (Supplementary Fig. S2), following 21-days of PWB exposure. At the femoral mid-diaphysis, the total cross-sectional area inside the periosteal envelope (Tt.Ar) remained significantly unaffected at PWB20% or PWB70%, but was significantly reduced at PWB40% (medium effect) (Fig. 6). Cortical area (Ct.Ar) significantly reduced at all

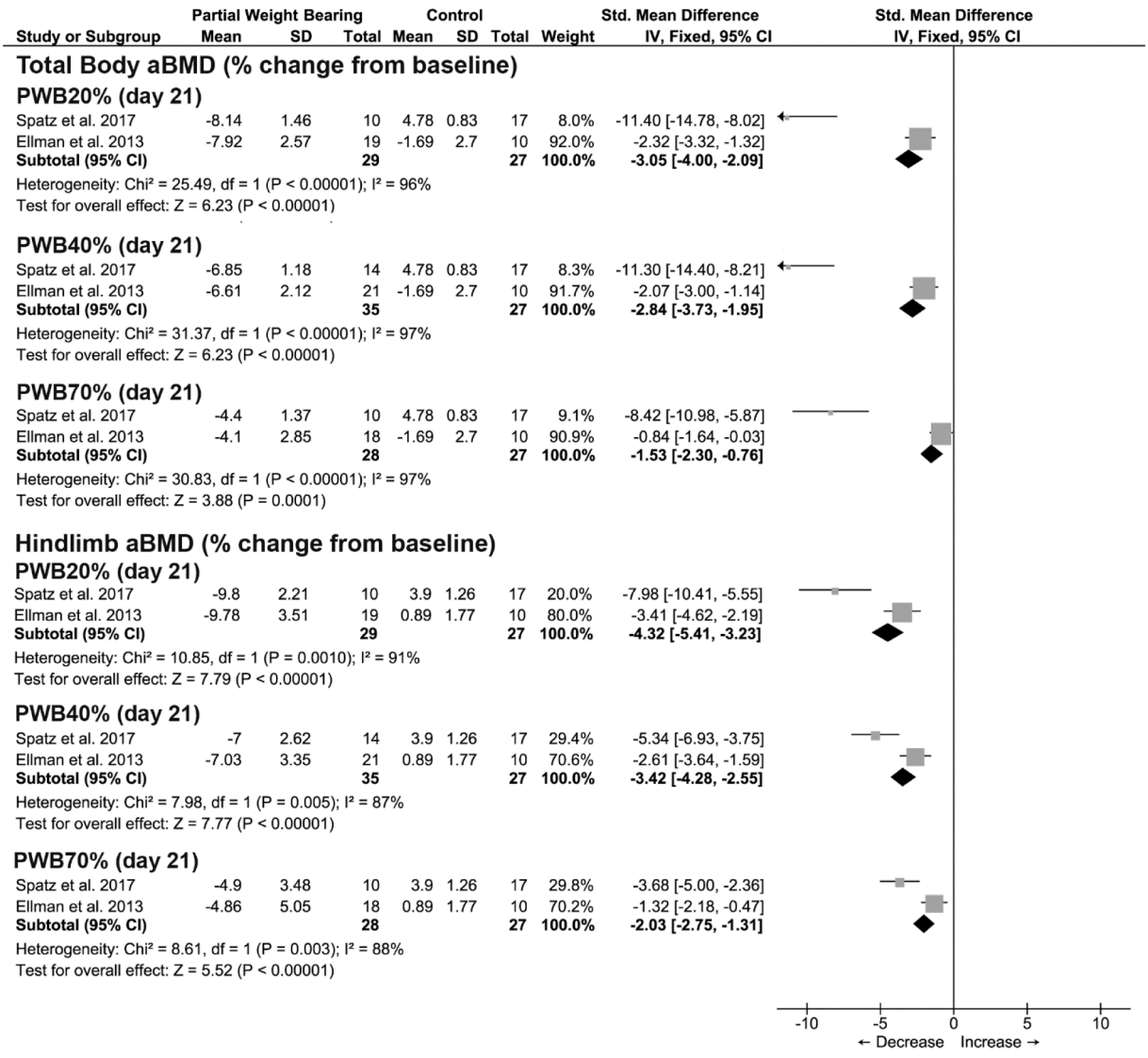


**Fig. 2.** The effect of partial weight-bearing (PWB) on total body and hindlimb areal bone mineral density (aBMD) in female mice.

PWB loads by large to very large effects, however, there was considerable heterogeneity across study findings (Fig. 6). In the two studies which investigated PWB across several loads concomitantly, both identified that lower PWB loading caused greater Ct.Ar reductions at the mean difference level. Cortical area fraction (Ct.Ar/Tt.Ar) significantly reduced by very large effects at all PWB levels, with the greatest reductions occurring at PWB20%, whilst PWB40% and PWB70% displayed more comparable losses, however, with significant heterogeneity (Fig. 6). Cortical thickness (Ct.Th) reduced at PWB20% by very large effects, and by lesser amounts at PWB40% (very large effect) and PWB70% (large effect) (Fig. 6). At the tibia mid-diaphysis, one study reported data for Ct.Ar and Ct.Th, both of which reduced by large effects at PWB20% and small effects at PWB40% (Fig. 6). Sensitivity analysis had trivial influence on the Tt.Ar meta-effect or heterogeneity score at PWB40%, however, caused a small increase for Ct.Ar (SMD: -1.03 to -0.73) and a reduction in heterogeneity ($I^2$: 72% to 38%) (Supplementary Table S4; https://doi.org/10.5281/zenodo.5599264).

At the femoral distal metaphysis following 21-days of exposure to PWB in female mice, Tt.Ar reduced at PWB20% by large effects, PWB40% by small effects, and was unaffected at PWB70% (Supplementary Fig. S2). Cortical area reduced at all PWB loads by very large effects, with PWB20% showing the greatest decline, whilst PWB40% and PWB70% had comparable losses (Supplementary Fig. S2). Similarly, Ct.Ar/Tt.Ar reduced by very large effects at all PWB loads, with PWB20% having the greatest declines, however, PWB70% was slightly more affected than PWB40% (Supplementary Fig. S2). Cortical thickness reduced at all PWB loads by very large effects, PWB20% had the greatest loss, whilst PWB40% and PWB70% displayed comparable declines, but with considerable heterogeneity (Supplementary Fig. S2). Sensitivity analysis reduced the meta-effect SMD for Ct.Th at PWB40% from -1.50 to -1.19, but had trivial effects on heterogeneity ($I^2$: 86% to 79%) (Supplementary Table S4; https://doi.org/10.5281/zenodo.5599264).

In male rats following 28-days of PWB exposure, Tt.Ar at the femoral mid-diaphysis remained unaffected at PWB20%, however declined by large effects at PWB40%, and increased by a medium effect at PWB70% (Fig. 7). Cortical area reduced by a medium effect at PWB20% and to a slightly greater degree at PWB40% (large effect), whilst PWB70% displayed a small positive effect (Fig. 7). Cortical area fraction declined at PWB20% and PWB70% by large effects of similar magnitudes, however it increased at PWB40% by a small effect (Fig. 7). Cortical thickness reduced at PWB20% and PWB70% by large effects, with PWB20% showing greater losses, but was unaffected at PWB40% (Fig. 7).

### 3.5. *Mechanical properties*

The biomechanical characteristics of the female mouse femoral and

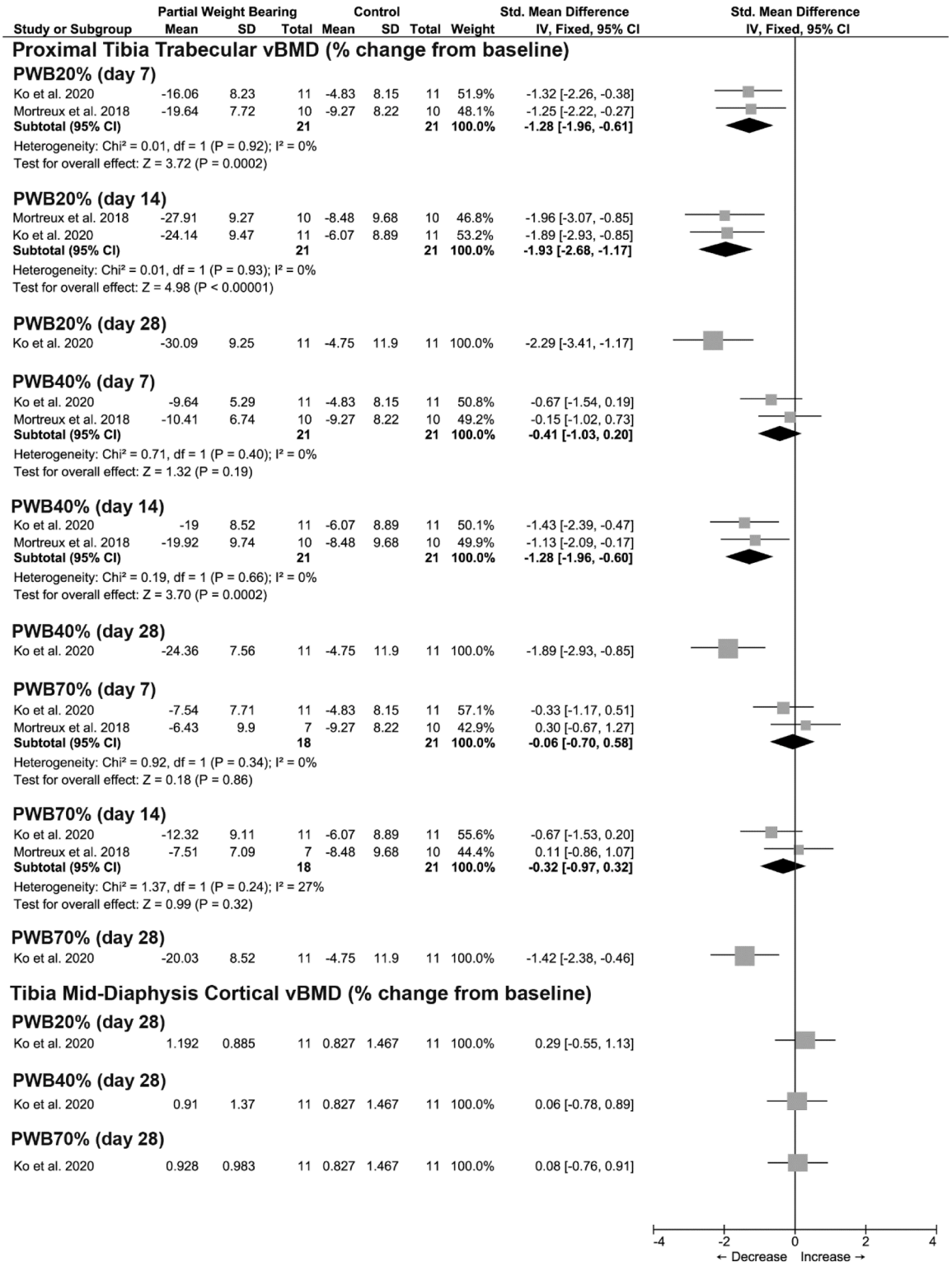


**Fig. 3.** The effect of partial weight-bearing (PWB) on trabecular and cortical volumetric bone mineral density (vBMD) at the proximal tibia and tibia mid-diaphysis, respectively, in male rats.

tibial diaphysis via three-point bending to failure following 21-days of PWB exposure are presented in Fig. 8. At the femoral diaphysis, estimated Young's modulus remained unaffected or slightly increased, whilst stiffness and maximum force significantly decreased at all PWB loads by large/very large effects. The trend between PWB load and the magnitude of reduction was unclear due to considerable heterogeneity between study findings. However, in the two studies which investigated PWB across several loads simultaneously, both identified that PWB20% caused greater biomechanical impairments in maximum force, whilst PWB40% and PWB70% displayed similar losses. Reductions in stiffness appeared to be more pronounced at PWB40% and PWB70% compared to PWB20%. Sensitivity analysis could only be performed for the femoral diaphysis stiffness at PWB40% and had a trivial impact on the meta-effect (SMD: -1.97 to -2.02) but increased heterogeneity ($I^2$: 31% to

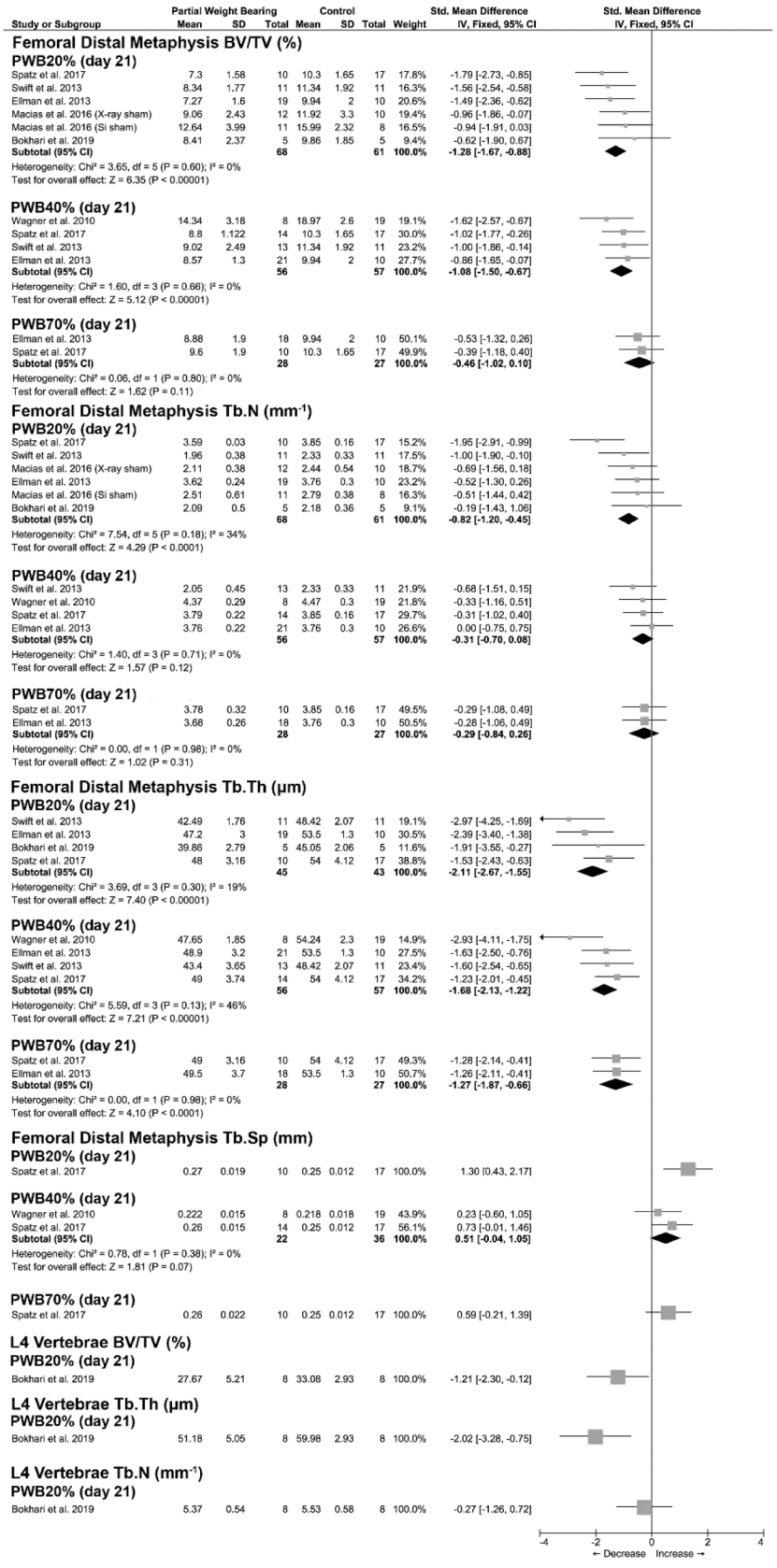


**Fig. 4.** The effect of partial weight-bearing (PWB) on femoral distal metaphysis and L4 vertebrae trabecular geometry and microarchitecture in female mice. Bone volume fraction = BV/TV, trabecular number = Tb.N, trabecular thickness = Tb.Th, and trabecular separation = Tb.Sp.

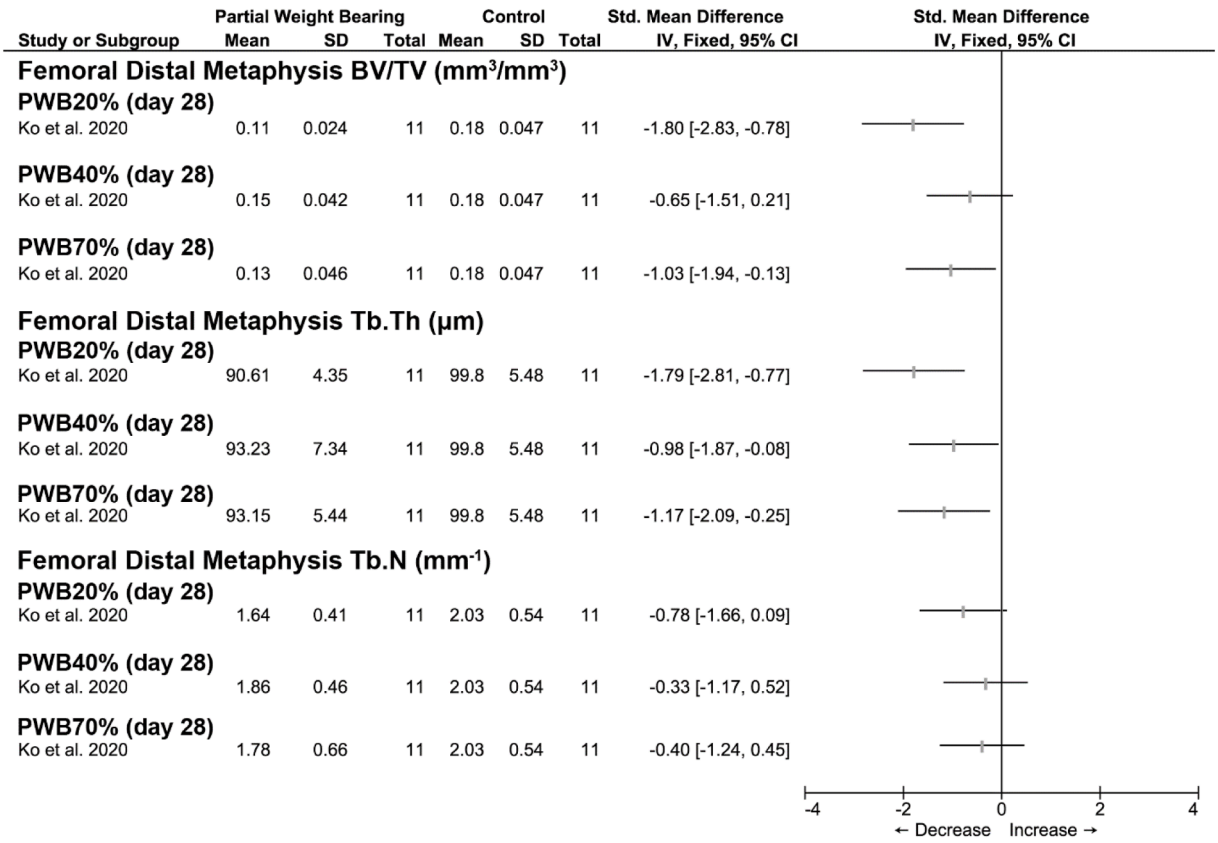


**Fig. 5.** The effect of partial weight-bearing (PWB) on femoral distal metaphysis trabecular geometry and microarchitecture in male rats. Bone volume fraction = BV/TV, trabecular thickness = Tb.Th, and trabecular number = Tb.N.

64%) (Supplementary Table S4; https://doi.org/10.5281/zenodo.5599264). One study measured maximum moment at PWB40% and observed a very large reduction. At the tibia diaphysis, estimated Young's modulus and maximal stress reduced by similar amounts at PWB20% and PWB40% (small effects). Stiffness and maximum force reduced at PWB20% by large/very large effects and to a lesser degree in PWB40% (small/medium effects).

Additional three-point bending to failure parameters for the femoral diaphysis are presented in Supplementary Fig. S3. Work-to-failure decreased at PWB20% by medium effects and to a greater degree at PWB40% (large effect), however, was unaffected at PWB70%. Post-yield displacement was unaffected at any PWB load. Energy-to-ultimate increased by a medium effect at PWB20%, whilst post-yield energy and energy-to-fracture were unaffected. At PWB40% post-yield bending work was unaffected, whilst yield moment reduced by a large effect at PWB40%.

Biomechanical properties of the male rat femoral diaphysis following 28-days of PWB exposure are presented in Supplementary Fig. S4. Estimated Young's modulus and stiffness reduced by small and medium effects, respectively, across all PWB loads by similar magnitudes. Maximum moment and fail moment reduced in a dose-response manner with PWB load.

Compression-to-failure of the female mouse femoral neck and L4 vertebra following 21-days of PWB exposure are presented in Supplementary Fig. S5. At PWB20%, femoral neck stiffness was unaffected, however, with moderate heterogeneity (one study identified a medium decrease and two identified small/medium increases). Significant reductions occurred in load-to-failure and maximum force at PWB20% (very large effects). A single study also identified a medium reduction in maximum force at PWB40%. The L4 vertebra displayed small reductions in stiffness and ultimate load at PWB20% (no data available for PWB40% or PWB70%).

## 4. Discussion

### 4.1. Summary of main findings

The present study was the first systematic review and meta-analysis investigating the effects of PWB on rodent bone. Current evidence indicates that exposure to 21–28 days of PWB causes bone deconditioning characterized by a loss of BMD, diminished trabecular and cortical geometry and microarchitecture, and impaired mechanical function in young/mature female mice and mature male rats. These findings predominantly relate the femur and tibia. Importantly, many of the affected bone parameters reduced by greater amounts at lower PWB loads (PWB20% > PWB40% > PWB70%). Higher PWB loads, notably PWB70%, were able to prevent some, but not all, deconditioning. The patterns of deconditioning between mice and rats were difficult to establish due to the limited number of studies, however, they appeared similar but not identical. Risk of bias was predominantly low or unclear across studies.

### 4.2. Comparison of PWB findings to rodents and humans during complete unloading

Whole body and hindlimb aBMD were observed to decline in a dose-response manner with PWB load in female mice. Considerable heterogeneity was, however, identified between the two studies comprising these data. This may be due to the use of a figure extraction tool as data from Spatz et al. (Spatz et al., 2017) were more challenging to accurately measure relative to Ellman et al. (Ellman et al., 2013) because of the figure formating. Nonetheless, DXA-derived measurements (i.e. aBMD) cannot distinguish between trabecular and cortical bone compartments, which have previously been shown to display differential responses to unloading and have distinct mechanical properties (Bloomfield et al.,

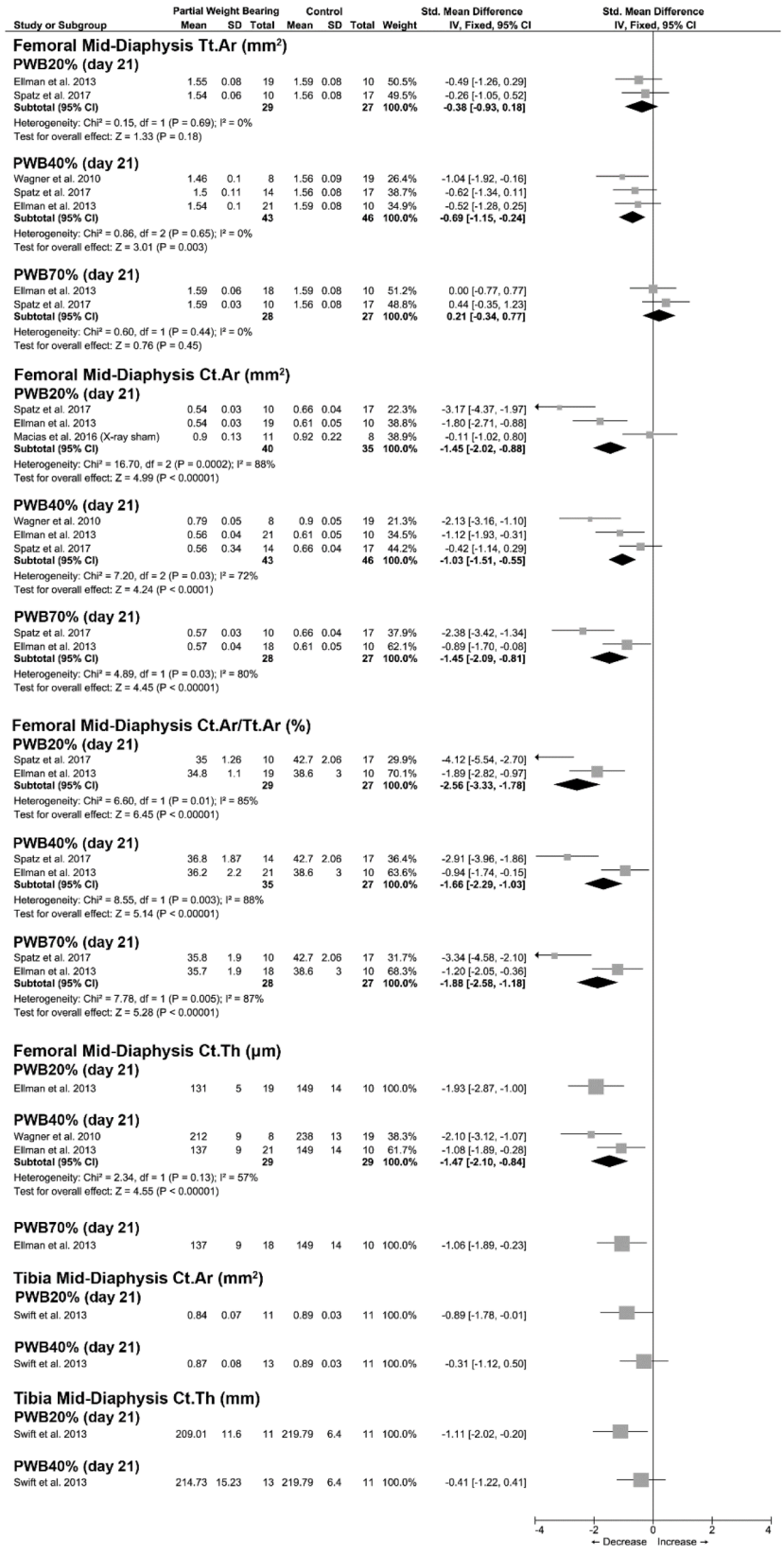


**Fig. 6.** The effect of partial weight-bearing (PWB) on femoral and tibial mid-diaphysis cortical geometry in female mice. Total bone area = Tt.Ar, cortical area = Ct.Ar, cortical area fraction = Ct.Ar/Tt.Ar, and cortical thickness = Ct.Th.

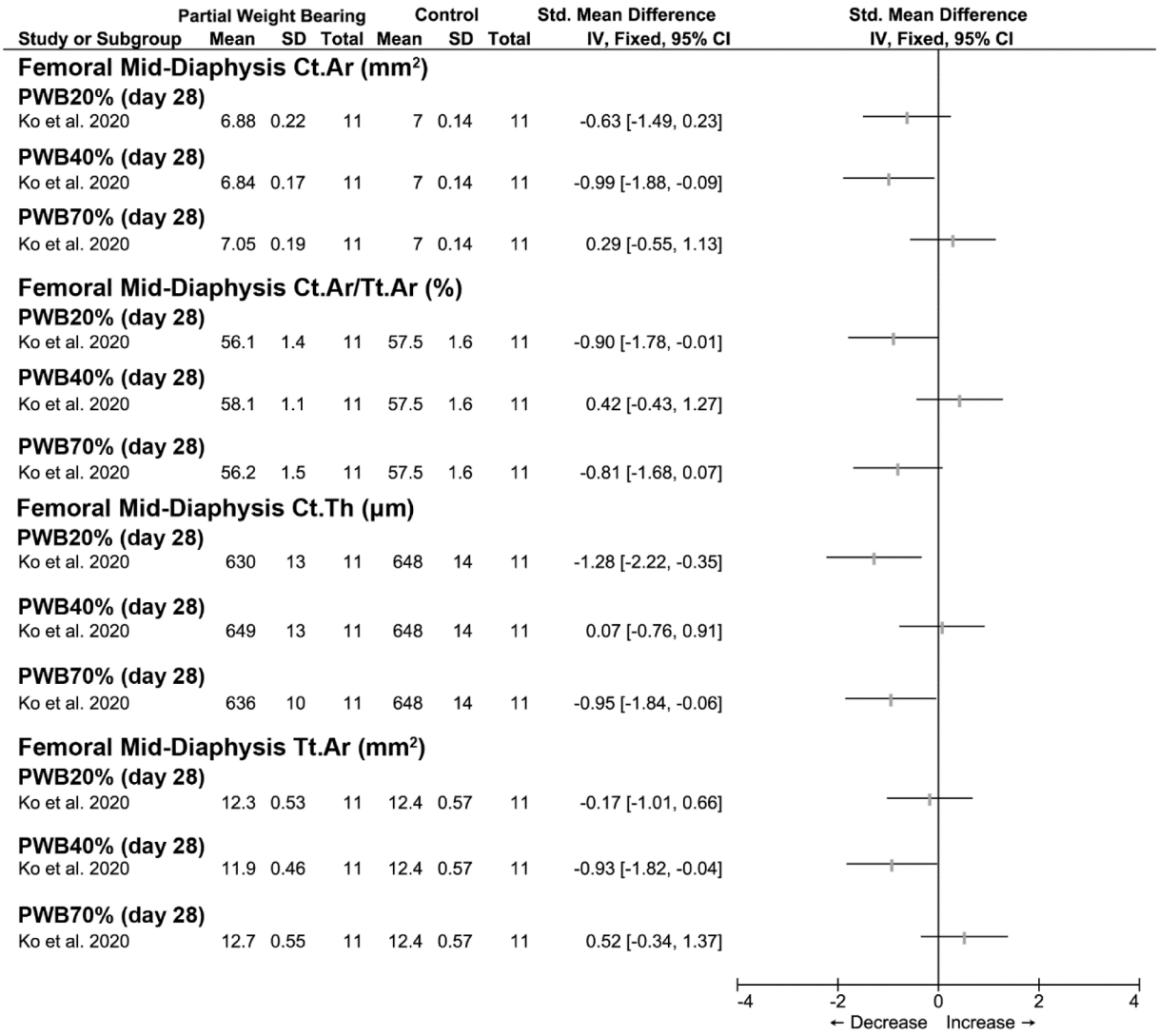


**Fig. 7.** The effect of partial weight-bearing on femoral mid-diaphysis cortical parameters in male rats. Total bone area = Tt.Ar, cortical area = Ct.Ar, cortical area fraction = Ct.Ar/Tt.Ar, and cortical thickness = Ct.Th.

2002). Two studies (using male rats) assessed BMD via pQCT and revealed that PWB exposure caused a preferential loss of trabecular vBMD at the proximal tibia, whilst cortical vBMD at the tibial mid-diaphysis appeared less affected. This is consistent with previous findings in male rats exposed to hindlimb unloading (HLU) (Bloomfield et al., 2002; Swift et al., 2010). Similarly, following 4–6-months of spaceflight onboard the International Space Station (ISS), astronauts/cosmonauts have shown a preferential loss of trabecular vBMD in the tibia and femoral neck (Vico et al., 2000; Lang et al., 2006). More recently, comparable losses in the trabecular and cortical regions in the distal tibia have been observed in ISS astronauts/cosmonauts, possibly due to countermeasure advancements (Vico et al., 2017). During bed rest, a preferential loss of cortical vBMD in the femur is seen within the first one to two months of exposure, with longer-durations being shown to cause preferential loss of trabecular vBMD in line with spaceflight studies (Rittweger et al., 2009; Cervinka et al., 2014). Recent systematic reviews have failed to identify any experimental evidence regarding bone loss in humans during exposure to hypogravity (Richter et al., 2017; Swain et al., 2021). However, mathematical modeling estimates that bone loss will occur within Lunar and Martian hypogravity in proportion to gravitational field strength (Lewandowski et al., 2008), which is supported by the present rodent evidence. In addition, whilst associational data were not reviewed, original PWB studies have identified strong linear relationships between PWB load and whole body/hindlimb aBMD in female mice ($r = 0.65$) and trabecular vBMD in male rats ($r = 0.65$) (Mortreux et al., 2018).

Static histomorphometry findings reported in an original PWB study suggest that in male rats, the disruption in trabecular bone homeostasis is driven by reductions in osteoblast activity (bone formation), whilst osteoclast activity (bone resorption) remains unaffected (Ko et al., 2020). Similar responses have previously been observed in rats exposed to HLU (Dehority et al., 1999; Basso et al., 2005) and spaceflight (Fu et al., 2021), however, the mechanisms underlying reduced osteoblast activity within PWB remain unclear. Future cellular and molecular research would, therefore, yield important findings to understanding the etiology of unloading-induced and PWB-induced bone deconditioning. In contrast to rats, a recent systematic review and meta-analysis identified that astronauts/cosmonauts display increased levels of bone resorption biomarkers (plateauing at ~40-days exposure), whilst bone formation biomarkers remain unchanged or decreased initially, before gradually increasing with longer exposure durations (Stavnichuk et al., 2020). Similarly, during bed rest, bone resorption markers increase whilst bone formation markers are less/unaffected (Hargens and Vico, 2016). Adult mice display similar mechanisms underlying bone loss to humans during periods of unloading (Globus and Morey-Holton, 2016). However, whilst PWB studies in mice have identified reduced bone

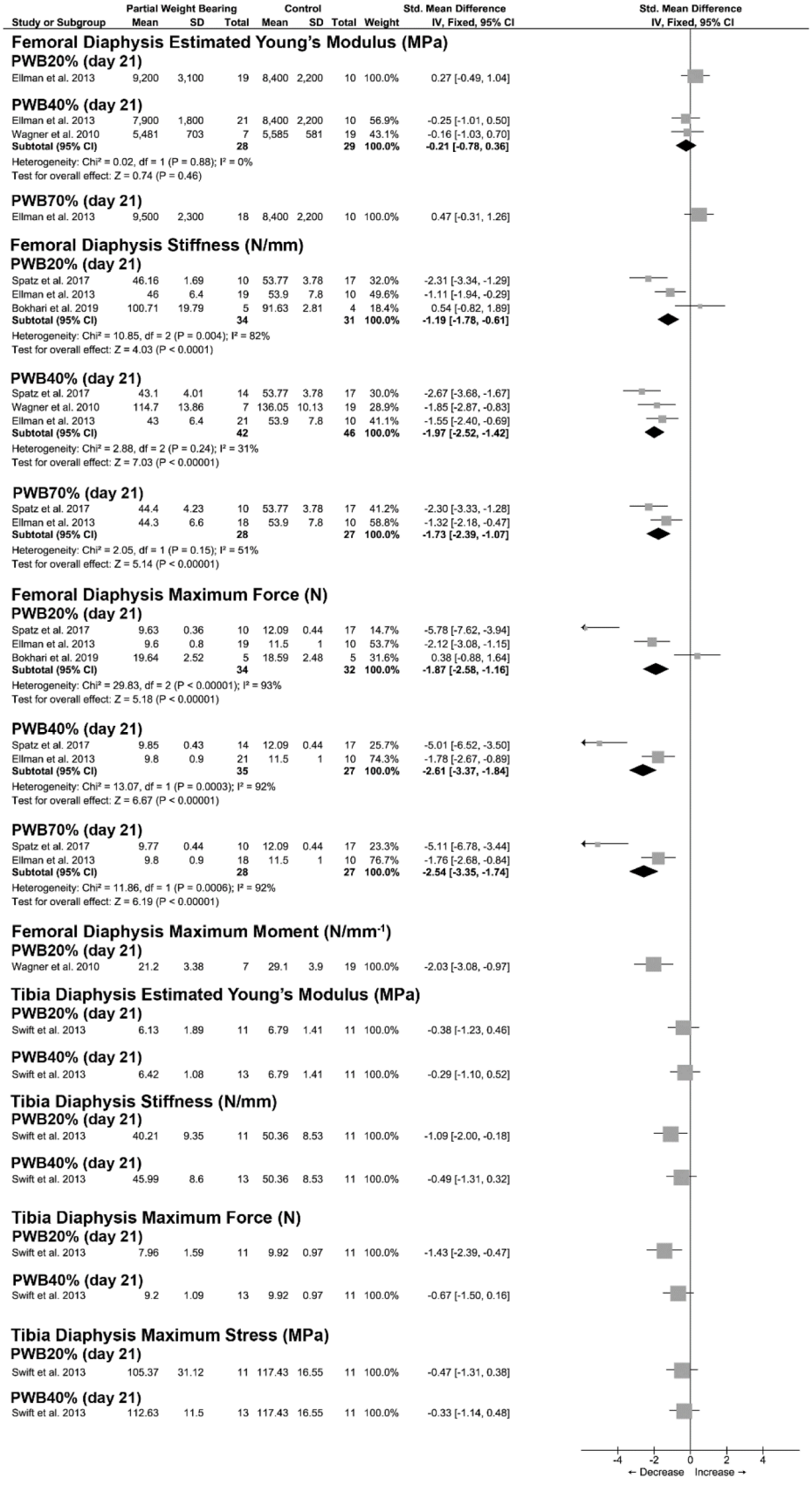


**Fig. 8.** The effect of partial weight-bearing (PWB) on femoral and tibial diaphysis mechanical properties via three-point bending to failure in female mice.

formation rates in the trabecular, endocortical, and periosteal bone regions (Bokhari et al., 2019; Swift et al., 2013), current histology data are limited to Wagner et al. (2010) (Wagner et al., 2010) which was found to have several high RoBs (Table 4 and Supplementary Table S3; https://doi.org/10.5281/zenodo.5599188).

Microarchitectural and geometric characteristics of trabecular and cortical tissue contribute to bone fragility independently of BMD and, thus, changes to their shape/structure have important mechanical implications (Bouxsein, 2005; Ulrich et al., 1999; Samelson et al., 2019; Mikolajewicz et al., 2020). Both compartments diminished during PWB exposure, characterized by a loss of trabecular bone volume, thickness, and number, with an increase in separation, alongside reductions in cortical area and thickness. These findings predominantly relate to the femoral distal metaphysis (trabecular parameters) and femoral mid-diaphysis (cortical parameters) in female mice. Female mice also displayed a loss of trabecular bone volume and thickness in the L4 vertebrae and reduced cortical area and thickness in the tibia mid-diaphysis and femoral distal metaphysis. In male rats, the original study failed to identify any significant differences in cortical parameters during PWB (Ko et al., 2020), however, SMDs calculated in the present review suggest there were moderate/large reductions in cortical area (at PWB20% and PWB40%) and thickness (at PWB20% and PWB70%) at the femoral mid-diaphysis. The discrepancy in statistical interpretation (null-hypothesis significance testing vs. individual SMDs) may explain this conflict. Therefore, future PWB bone research is required with larger samples for increased statistical power. Nonetheless, of particular importance was that the most severe deconditioning was observed in rodents exposed to PWB20%, whilst those in PWB40% and PWB70% were partially or fully protected against diminished microarchitecture/geometry depending on the outcome. This is supported by an original PWB study in mice that identified moderate correlations between PWB load and trabecular bone volume ($r = 0.45$), trabecular thickness ($r = 0.52$), cortical area ($r = 0.49$), and cortical thickness ($r = 0.50$) (Ellman et al., 2013).

Similar patterns of microarchitectural/geometric deconditioning have been observed in mice exposed to HLU, such as in the femora (reduced trabecular bone volume, thickness, number and increased separation, and reduced cortical area and thickness) and L4 vertebrae (reduced trabecular bone volume and thickness) (Ellman et al., 2013; Swift et al., 2013; Sankaran et al., 2017; Cabahug-Zuckerman et al., 2016; Teguh et al., 2021). To date, two comparative studies have compared PWB to HLU (Ellman et al., 2013; Swift et al., 2013). Female mice exposed to either PWB20% or HLU displayed loss of trabecular bone volume and thickness, although trabecular number remained unaffected following 21-days of HLU, whilst it reduced in PWB20%, and cortical area and thickness declined by greater amounts in PWB20% (Ellman et al., 2013; Swift et al., 2013). It is important to recognize that PWB and HLU are separate models, and it has been argued that PWB at 0% loading would not be equivalent to HLU due to factors such as cephalad fluid shifts (Ellman et al., 2013), however, this remains to be experimentally validated. Nevertheless, following exposure to microgravity (via spaceflight), a recent systematic review and meta-analysis identified that rodents (mice and rats) and primates (rhesus monkeys) also display reduced trabecular bone volume, thickness, and number, and increased separation and reduced cortical area (findings for cortical thickness were heterogeneous) (Fu et al., 2021). Likewise, astronauts/cosmonauts display reduced trabecular bone volume, cortical area, and thickness (at the distal tibia) following 4–6-months of spaceflight, however, trabecular number, thickness, and separation were found to be unaffected (Vico et al., 2017). During bed rest, male humans are observed to have reduced trabecular number and cortical area and thickness, whilst trabecular bone area, thickness, and separation increased at the distal tibia following 59-days of exposure (Belavy et al., 2011). In contrast, following 43-days of bed rest in females, cortical thickness reduced and trabecular bone volume declined only 3-days post bed rest at the distal tibia, whilst trabecular number, thickness, and separation remained unaffected (Armbrecht et al., 2005). Taken together, whilst diminished/altered trabecular and cortical microarchitecture/geometry is evident during PWB, HLU, spaceflight, and bed rest, the characteristics of such deconditioning may not be identical between settings, species, or sex. Nonetheless, both trabecular and cortical microarchitecture/geometry are important determinants of bone fragility, as they are associated with an increased risk of bone fracture and are diminished in fracture patients (Bouxsein, 2005; Ulrich et al., 1999; Samelson et al., 2019; Mikolajewicz et al., 2020).

The present evidence supports that PWB, particularly at lower loads, leads to defining characteristics of osteoporosis through a loss of bone density, volume, area, and diminished microarchitecture (Kalpakcioglu et al., 2008). Such adaptations are likely attributable to mechanical impairments. Following PWB exposure in female mice and male rats, the present review identified that both the tibial and femoral mid-diaphyses had reduced flexural rigidity and strength. In addition, the femoral neck became mechanically weaker, whilst the L4 vertebra was affected to only small degrees. The limited evidence and heterogenous findings make it difficult to determine whether higher PWB loads attenuated/prevented mechanical impairments in the femoral mid-diaphysis (female mice), however, deficiencies were still evident by large degrees at PWB70%. Evidence for the tibia mid-diaphysis (female mice) and femoral mid-diaphysis (male rats) were limited to one study each, however, both revealed that lower PWB loading causes more severe biomechanical impairments. Studies employing hindlimb unloading have similarly observed biomechanical impairments in weight-bearing skeletal bones (Ellman et al., 2013; Swift et al., 2013). Two comparative studies have, however, found mixed evidence when comparing differences between PWB and HLU (Ellman et al., 2013; Swift et al., 2013). For example, greater reductions in femoral neck ultimate load occurred in rodents exposed to HLU relative to PWB40%, but not PWB20%, whilst comparable losses were observed in stiffness and maximum force at the femoral diaphysis, but were more severe at tibial diaphysis in PWB20% (Ellman et al., 2013; Swift et al., 2013). It is challenging to determine whether differences between PWB and HLU are caused by loading condition independent of the model. Future research should, therefore, aim to compare PWB0% to HLU to better understand the differences in these unloading models. Nevertheless, in humans, finite element analysis has shown impaired strength in weight-bearing bones following periods of disuse (via the use of crutches) (Kazakia et al., 2014) and exposure to long-duration spaceflight (Vico et al., 2017; Keyak et al., 2009).

### 4.3. Findings within the context of lunar and Martian missions

Development of early-onset osteopenia/osteoporosis during/following spaceflight has been a longstanding concern for astronauts/cosmonauts, even when countermeasures are employed (Vico et al., 2017; Keyak et al., 2009). Increased risk of bone fracture, impaired and incomplete fracture healing, neurological damage caused by injured joints/vertebrae, and renal stone formation are some of the major risks to astronauts/cosmonaut health (NASA, 2005). The success of missions to the Moon or Mars will largely depend on crew members being physically capable. There is a concern, however, that they will arrive on these terrestrial bodies in a deconditioned state due to prolonged microgravity exposure, and that fragile bones may fracture when exposed to hypogravitational loading during work-related activities (e.g., bending and lifting objects) or accidents (e.g., falling) (Lewandowski et al., 2018). In addition, factors such as post-spaceflight sensorimotor impairments (increasing the risk of falling) (Ozdemir et al., 2018; Wood et al., 2015) and altered postural control in hypogravity (Ritzmann et al., 2015), could further amplify fracture risk. Microgravity-induced deconditioning is likely to be less of an issue for Lunar missions, due to the relatively short transit period (3–5 days) but may become more of a hazard if an orbital gateway is used which could increase microgravity exposure durations to weeks/months. Transit to Mars, however, will

take 6–12-months depending on the mission profile (Horneck et al., 2006; Connolly et al., 2018), where it is predicted that 62–100% or 33% of astronauts/cosmonauts will develop osteopenia or osteoporosis, respectively (Axpe et al., 2020). Additionally, previous studies have demonstrated that 4–6 months of spaceflight significantly reduces bone strength, even when countermeasures are employed (Vico et al., 2017; Keyak et al., 2009). This heightens concerns regarding astronaut/cosmonaut bone health as multi-purpose crew vehicles (e.g., NASA's Orion capsule) have major volume and mass constraints (Laws et al., 2020), limiting the capacity for large exercise countermeasures as used onboard the ISS (Korth, 2015).

The present PWB findings suggest that exposure to mechanical loads equivalent to Lunar (PWB20%), Martian (PWB40%), and moderate artificial hypogravity (PWB70%) may also cause bone deconditioning in humans. This is based on the observation that rodents, monkeys, and humans share similarities in the patterns of regional bone deconditioning during unloading and spaceflight that are comparable to those in PWB (Stavnichuk et al., 2020; Fu et al., 2021; Nagaraja and Risin, 2013). Further, the present findings support early mathematical modeling which predicts that bone loss will occur in humans exposed to Lunar and Martian hypogravity (Keller and Strauss, 1992). The progressive loss of trabecular vBMD observed at all PWB loads in male rats from 7 to 28 days suggests that increasing hypogravity exposure may become progressively more hazardous to the integrity of astronauts/cosmonauts' bone due to the association between BMD and risk of fracture (Marshall et al., 1996). Risk assessment modeling has predicted that the probability of fracture on the Moon and Mars increases with longer mission lengths (Lewandowski et al., 2008). Therefore, as exploration of the Lunar and Martian surfaces are expected to last up to 6-months and ~10–16-months, respectively (Horneck et al., 2006; Connolly et al., 2018), countermeasures may be required for long-duration surface missions to protect astronaut/cosmonaut bone health. Taken together, these findings justify the requirement for future studies to investigate the long-term effects of hypogravity exposure on bone structure and function in human models (e.g., head-up tilt bed rest) (Cavanagh et al., 2013; Barr et al., 2016). Understanding the time-course and magnitude of effects at various levels of hypogravity can help inform countermeasure strategies to mitigate bone deconditioning during long-term Lunar or Martian habitation. It is also crucial to understand the effects of hypogravity (during surface exploration) on bone following prolonged microgravity exposure (during transit), to better mimic exploration mission demands (Mortreux et al., 2019). This is critical, as the reviewed studies directly transitioned rodents from full weight-bearing to PWB. It remains plausible that hypogravity may cause some degree of bone reconditioning following a period of microgravity exposure due to the increased level of weight-bearing. However, the present findings indicate that any protection offered will remain significantly below that provided by Earth's gravity. Lastly, early-onset osteopenia/osteoporosis and risk of bone fracture is not only a concern to astronauts/cosmonauts' health during exploration-class missions, but also upon their return to Earth. Longitudinal studies have observed that in astronauts/cosmonauts following 4–6-months of spaceflight, non-weight-bearing bones progressively deteriorate up to a year post-spaceflight (Vico et al., 2017) and that the recovery of BMD to pre-flight values can take up to 3-years (Sibonga et al., 2007).

## 5. Quality of the evidence and overall completeness

Studies investigating the effects of PWB on bone remain limited and is one of the main drawbacks of the present review. Some studies, however, have reported an abundance of bone parameters, capturing a comprehensive picture of what adaptations occur across several PWB loads. Yet, these findings are somewhat constrained to the rodent population investigated. This is important to note when interpreting the present findings as they are derived from young/mature female mice (6 studies) and mature male rats (2 studies). It has yet to be directly established whether the skeletal response to PWB is influenced by age or sex, but it is important nonetheless to identify whether these, among other factors, augment PWB-induced skeletal deconditioning which may have implications to astronaut bone health on the Moon/Mars. Additionally, not all PWB studies have provided comprehensive bone assessments, likely as it was not their main objective. This then created the issue that many outcomes could not be pooled for increased statistical power, but only reported as individual effect sizes.

Given that PWB is a novel method, it is anticipated that it will become more widely employed by the research community due to the contemporary relevance of PWB to human space exploration. This will act to support future systematic reviews, where a larger number of studies will be able to be synthesized. To this end, it would be beneficial for future researchers to consider using/agreeing standardized study designs, rodent populations, and outcomes to ensure that the benefits of meta-analysis can be fully utilized. As PWB can be investigated across a spectrum (>0 g to <1 g), with more PWB research, meta-regression could also be applied in future systematic reviews, allowing the relationship between PWB load, exposure duration, and magnitude of bone deconditioning to be modeled.

### 5.1. Quality of the PWB model

An important consideration regarding the quality of the PWB model is whether it affects bone outcomes independent of the loading condition. One study using female mice observed significant differences in several mechanical parameters (femoral stiffness, yield moment, ultimate moment, and estimated Young's modulus) between two full weight-bearing control groups with and without the PWB apparatus (Wagner et al., 2010). However, given that the groups also differed in respect to housing (single vs. group housed) and feeding (ad libitum vs. pair-fed) conditions, it is difficult to determine whether the PWB model independently caused these changes. Similarly, in another PWB study using female mice, two full weight-bearing controls with and without the apparatus were shown to have several significantly different cortical parameters, but similar femoral mechanical properties. However, the housing conditions were also not standardized between the control groups (singly vs. group housed) (Ellman et al., 2013). It has recently been demonstrated that social housing conditions (single vs. paired housing) significantly affects immune and stress parameters but not musculoskeletal structure in mice following 30-days of HLU (Tahimic et al., 2019). However, other research in mice has found that stress can adversely affect bone metabolism and structure (Azuma et al., 2015). Furthermore, elevated adrenocortical activity has been observed in singly housed mice during PWB20%, PWB40%, and PWB70%, relative to full weight-bearing controls (Ellman et al., 2013), suggesting that the mouse PWB model may amplify bone loss through stress-related mechanisms independent of the loading condition.

During full-weight bearing, male rats bear ~65% of their weight on their hindlimbs (Mortreux et al., 2020). However, during PWB, it has been demonstrated within a small sample ($n$ = 3–5) that the relative weight-bearing load in the forelimbs was equivalent or slightly higher than that in the hindlimbs (Mortreux et al., 2020). Whilst the sample size limits the generalizability of these data to other rats and also mice, it is nonetheless important to note that discrepancies in relative forelimb and hindlimb loading between PWB groups can bias bone outcomes, as they are sensitive to mechanical stimuli; future research is planned to investigated this further (Ellman et al., 2013). Overall, PWB in mice and rats is a novel method and its limitations must be considered when interpreting the present findings and for future PWB studies to improve upon.

### 5.2. Risk of bias

In accordance with the SYRCLE's RoB tool and the novel PWB-RoBC tool, overall RoB was low/unclear (SYRCLE: low [44%], unclear [47%],

high [8%]; PWB-RoBC: low [62%], unclear [36%], high [2%]). Of the items that were scored high RoB, two studies (Mortreux et al., 2018; Ko et al., 2020) used a non-random approach to allocate rodents to the experimental and control groups. Rodents were assigned to maintain an equal distribution of body weights across groups at baseline. This method minimizes the chance of baseline imbalances occurring due to randomization and avoids having significant between-group differences independent of the intervention effect, as there is a strong relationship between rodent body weight and musculoskeletal properties (Tamaki and Uchiyama, 1995). To avoid making this trade-off, however, researchers have recommended that randomization should be performed and data be analyzed with and without adjustment for pre-determined prognostic covariates (e.g., body weight) (De Boer et al., 2015). The same two studies were also confirmed to be unable to fully blind caregivers/investigators to the PWB load during the experiment, as it required daily monitoring to maintain stability within ±5%. This limitation is, however, commonplace in the related HLU model, where the intervention and control groups are visually distinct and unable to be blinded. All other high RoBs were from the study of Wagner et al. (Wagner et al., 2010), that did not standardize physical or social housing conditions (compromising blinding of researchers/caregivers) and only maintained PWB within ±5% of the desired load on 77% of the study days. Sensitivity analysis via the removal of this study from all applicable meta-effects had either trivial influence on the findings or reduced the magnitude of the meta-effect by a small amount, none of which influenced the present conclusions. Where small differences were observed between original and sensitivity analyses, the effect size magnitude became less negative, suggesting that the study may have overestimated the effect of PWB40%. Findings from Wagner et al., 2010, therefore, should be interpreted with caution. It is also important to note that the sham-irradiated mice from Macias et al. (2016) (reported in this review) were removed from their cages on two occasions and transported to a radiation facility to maintain consistency with the radiated intervention groups. Though this procedure exposed mice to full weight-bearing, they were placed under the effects of anesthesia during transit and irradiation periods to minimize non-PWB ambulatory activities and thus was considered low RoB. The high frequency at which RoB items were scored as unclear warrants improved reporting standards, which can be aided with pre-established checklists. The SYRCLE's RoB tool can be used as a foundational guide. However, use of the PWB-RoBC checklist, which was designed in the present review specifically for PWB studies, is recommended when planning and reporting future study methods (Swain et al., 2021). This should help improve reporting transparency, reduce heterogeneity of methods between similar studies, and minimize preventable RoBs.

## 6. Limitations

The limitations of the present review include those described in a separate systematic review concerning the effect of PWB on muscle outcomes (Swain et al., 2021). In brief, the main drawbacks were that studies often reported sample sizes as a range, and, thus, were used conservatively, only extracting the smallest possible size. However, this can lead to underestimations of the SMD, as Hedge's G was used, which adjusts for small sample bias. Additionally, some data were only presented graphically in the original study, and where authors were unable to provide raw data, had to be extracted using WebPlotDigitizer. Despite this tool having near perfect reliability and accuracy (Ouzzani et al., 2016), this can still introduce errors.

## 7. Conclusion

Partial weight-bearing causes bone deconditioning in rodents characterized by a loss of BMD, specifically in the trabecular compartment, diminished trabecular and cortical microarchitecture/geometry, and impaired bone stiffness and strength. Deconditioning was frequently observed to be more severe at lower PWB loads (PWB20% > PWB40% > PWB70%), the patterns of which resemble those that occur during complete unloading in rodents (e.g., HLU and spaceflight) and humans (e.g., bed rest and spaceflight). Age- and sex-based responses to PWB remain unclear due to the limited evidence-base, however, deconditioning patterns between female mice and male rats (the only two populations currently employed in PWB studies) appeared similar, but not identical. These early findings have potential implications for astronaut/cosmonaut bone health on the surface of the Moon or Mars. Future research regarding the effects of hypogravitational loading on human bone is recommended and will help inform countermeasure strategies for the exploration of extraterrestrial bodies.

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## Data availability

All data are stored in RevMan and can be found at: https://doi.org/10.5281/zenodo.5727984.

## CRediT authorship contribution statement

**Patrick Swain:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Marie Mortreux:** Data curation, Writing – review & editing. **Jonathan M. Laws:** Data curation, Formal analysis, Writing – review & editing. **Harry Kyriacou:** Formal analysis, Writing – review & editing. **Enrico De Martino:** Writing – review & editing. **Andrew Winnard:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **Nick Caplan:** Conceptualization, Project administration, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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## Supplementary materials

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