Life Sciences in Space Research 34 (2022) 68–86

Contents lists available at ScienceDirect

Life Sciences in Space Research

journal homepage: www.elsevier.com/locate/lssr

Review article

Skeletal muscle deconditioning during partial weight-bearing in rodents – A systematic review and meta-analysis

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ARTICLE INFO

Keywords:
Hypogravity
Moon
Mars
Musculoskeletal
Atrophy

ABSTRACT

Space agencies are planning to send humans back to the Lunar surface, in preparation for crewed exploration of Mars. However, the effect of hypogravity on human skeletal muscle is largely unknown. A recently established rodent partial weight-bearing model has been employed to mimic various levels of hypogravity loading and may provide valuable insights to better understanding how human muscle might respond to this environment. The aim of this study was to perform a systematic review regarding the effects of partial weight-bearing on the morphology and function of rodent skeletal muscle. Five online databases were searched with the following inclusion criteria: population (rodents), intervention (partial weight-bearing for ≥1 week), control (full weight-bearing), outcome(s) (skeletal muscle morphology/function), and study design (animal intervention). Of the 2,993 studies identified, eight were included. Partial weight-bearing at 20%, 40%, and 70% of full loading caused rapid deconditioning of skeletal muscle morphology and function within the first one to two weeks of exposure. Calf circumference, hindlimb wet muscle mass, myofiber cross-sectional area, front/rear paw grip force, and nerve-stimulated plantarflexion force were reduced typically by medium to very large effects. Higher levels of partial weight-bearing often attenuated deconditioning but failed to entirely prevent it. Species and sex mediated the deconditioning response. Risk of bias was low/unclear for most studies. These findings suggest that there is insufficient stimulus to mitigate muscular deconditioning in hypogravity settings highlighting the need to develop countermeasures for maintaining astronaut/cosmonaut muscular health on the Moon and Mars.

1. Introduction

Skeletal muscle is a mechanosensitive tissue that adapts to mechanical stimuli [Sandri, 2008, Wackerhage et al., 2019]. Along with other mammals, humans and rodents experience disuse-induced deconditioning of skeletal muscle when exposed to settings that completely lack axial mechanical loading such as microgravity (~0g) [Fitts et al., 2010], head-down tilt bed rest [Winnard et al., 2019], and hindlimb unloading [Bodine, 2013]. In both species, deconditioning is characterized by adaptations in lower body skeletal muscle morphology (e.g., reduced muscle volume, mass, and cross-sectional area) [Winnard et al., 2019, Bodine, 2013, Ohira et al., 1992, Harrison et al., 2003, Sandona et al., 2012, Martin et al., 1988, Kraemer et al., 2000, LeBlanc et al., 1992] and function (e.g., reduced strength, rate of force production, and altered neuromuscular function) [Winnard et al., 2019, Shen et al., 2017, Song et al., 2018, Allen et al., 2006]. Exercise countermeasures have been demonstrated to attenuate/prevent disuse-induced deconditioning in and humans [Ploutz-Snyder et al., 2018] and rodents [Fluckey et al., 2002], representing the primary strategy to protect astronauts/cosmonauts' from muscle deconditioning onboard the International Space Station (ISS) [Loerch, 2015].

Space agencies are preparing to send humans to live and work in the hypogravity environments of the Moon (16% Earth's gravity) and Mars (38% Earth's gravity) [Connolly et al., 2018, NASA, 2020]. It is important that the effects of hypogravity on various body systems are understood to inform potential countermeasures. However, two recent systematic reviews have identified only one human study investigating the effect of hypogravity on skeletal muscle [Richter et al., 2017, Swain

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https://doi.org/10.1016/j.lssr.2022.06.007

Available online 29 July 2022

et al., 2021]. Six days of head-up tilt bed rest (weight-bearing at ~20% body weight [simulating Lunar loading]) was associated with a 1.6 ± 1.7% loss of quadriceps muscle volume [Cavanagh et al., 2013]. Together with biomechanical and cardiopulmonary findings, it has been suggested that physiological deconditioning is anticipated to occur in Lunar and Martian hypogravity and that exercise countermeasures are likely required [Richter et al., 2017]. However, it is recognized that there is a general paucity of human evidence regarding the effect of hypogravity on skeletal muscle morphology and function [Richter et al., 2017, Swain et al., 2021].

This lack of evidence has direct implications towards aerospace medical operations for extraterrestrial surface exploration, such as whether exercise countermeasures are required and to what extent [Swain et al., 2021]. Simulated Lunar/Martian extra-vehicular activity (EVA) task performance is significantly associated with muscle function, and strength requirements for ambulatory EVA tasks are being established for Lunar and Martian missions [Ryder et al., 2019, Ade et al., 2014, Taylor et al., 2018]. Hypogravity-induced muscular deconditioning could hinder physically demanding occupational procedures (e.g., moving objects and hill climb/descent) and emergency measures (e.g., body hauling and capsule egress) [Ryder et al., 2019]. This would result in astronauts/cosmonauts either failing to complete tasks or finishing in an unacceptable amount of time [Ryder et al., 2019].

Over the last decade, researchers have established a novel quadrupedal partial weight-bearing (PWB) model in rodents [Wagner et al., 2010, Mortreux et al., 2018]. The PWB model has been designed to reduce forelimb and hindlimb loading to a desired level between 10-80% body weight and maintain this for up to one month [Mortreux and Rosa-Caldwell, 2020]. It has been adopted in several studies which are beginning to elucidate the effects of simulated hypogravity (e.g., Lunar and Martian PWB) on the morphology and function of rodent skeletal muscle [Mortreux et al., 2019]. On the basis that rodent and human skeletal muscle show similar responses during disuse-induce deconditioning through complete unloading, the PWB model has the potential to provide early translational research estimating the effects of hypogravity on human muscle [Qaisar et al., 2020]. The aim of this systematic review was to synthesize animal intervention studies investigating the effect of PWB relative to full weight-bearing on rodent skeletal muscle morphology and function. The objectives were to understand what adaptations occur during PWB (e.g., deconditioning) and how they are mediated by PWB load and exposure duration.

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 Systematic Review Methods Handbook [Winnard et al., Winnard et al., 2021]. A PRISMA checklist can be found in Supplementary Table S1 (https://doi.org/10.5281/zenodo.5728056).

2.1. Search strategy

The following online databases were searched on 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 National Aeronautics and Space Administration (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 using Medical Subject Heading (MeSH) terms on the 19th of May 2021. MeSH terms were selected from previously indexed PWB studies and MeSH hierarchy tables. No specific MeSH term currently exists for PWB. Some PWB studies are indexed under 'hindlimb suspension' (also known as hindlimb unloading [HLU]), which was, therefore, used. Included studies' reference lists and citations were screened for any additional relevant articles. The final search strategy is presented in Table 1. This also includes bone-related terms as these data were collected in parallel and will be reported in a separate systematic

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	(("hypogravity" 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:("hypogravity" 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 ("hypogravity" 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	("hypogravity" 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	(("hypogravity" 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.

review.

2.2. Study eligibility criteria

Search results were exported and stored in the online reference manager Rayyan [Ouzzani et al., 2016]. Following removal of duplicates, each study was screened by two independent reviewers for inclusion/exclusion using an *a priori* PICOS criteria (Table 2). 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 labels 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.5599447). Any disagreements between the two reviewers were resolved initially through discussion. A third reviewer was consulted if the dispute remained unresolved. Data where the PWB and/or control group was combined with another intervention (e.g., radiation) were not eligible but the sham group(s) were.

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 extracted by one reviewer as means, standard deviations, and sample sizes, and were stored using Review Manager (RevMan Version 5, The Cochrane Collaboration) [Cochrane]. 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, 2020]. If no sample size data were stated in the 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 a given outcome (e.g., n = 3-6) were extracted using a conservative approach by using the lowest sample size reported. In Wagner et al. 2010 [Wagner et al., 2010], two full weight-bearing control groups were employed; the age-matched control data were extracted for a larger sample size.

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. Comparisons were made for each outcome and PWB level (PWB20%, PWB40%, and PWB70%) against the normal weight-bearing control group for all exposure durations ≥1-week.

Table 2
PICOS eligibility criteria.

Parameter	Inclusion criteria
Population	Rats or 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	Muscle structure (e.g., limb girth, wet muscle mass, cross-sectional area, and fiber composition) Muscle function (e.g., grip force, torque production, and single fiber twitch characteristics)
Study design	Controlled animal intervention trial

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 calculated only from rodent sub-populations of the same species and sex. Due to the small number of studies comprising meta-analyses, they should be interpreted as preliminary. 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 established from 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]. All comparisons are reported using 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].

2.5. Risk of bias assessment

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool [Hooijmans et al., 2014] was used to assess risk 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 themes of selection, performance, detection, attrition, and reporting biases. However, the SYRCLE's tool addresses RoB at a general level and fails to address RoB relating to specific models such as 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 [Winnard et al., 2019, Richter et al., 2017]. The same strategy was employed in the present review. Two PWB experts were consulted to establish an additional RoB checklist specific to PWB studies (M. Mortreux and M.E. Rosa-Caldwell, personal communication, May 2021) [Swain et al.]. Using an iterative approach, potential checklist items were generated, remarked, and agreed. 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.5550379) [Swain et al.]. The PWB-RoBC was scored using the same method as the SYRCLE's RoB tool (i.e., 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.

3. Results

The final search strategy identified 2,993 articles of which eight met the eligibility criteria (Fig. 1). Study characteristics are presented in Table 3. A full weight-bearing control was employed in all studies, alongside a single or multiple PWB intervention group(s) at loads of PWB20%, PWB40%, and/or PWB70%. Partial weight-bearing exposure duration ranged from 7 to 28 days. Studies used either mature Wistar male rats (n = 4), mature Wistar female rats (n = 1), young/mature BALB/cByJ female mice (n = 2), or young/mature C57Bl/6J female mice (n = 1). Risk of bias, via the SYRLCE's tool, were scored as low (63%), unclear (21%), and high (17%) rounded to the nearest integer (Table 4). Similarly, as per the novel PWB-RoBC, items were scored as low (75%), unclear (23%), and high (2%) (Supplementary Table S3; https://doi.org/10.5281/zenodo.5550379).

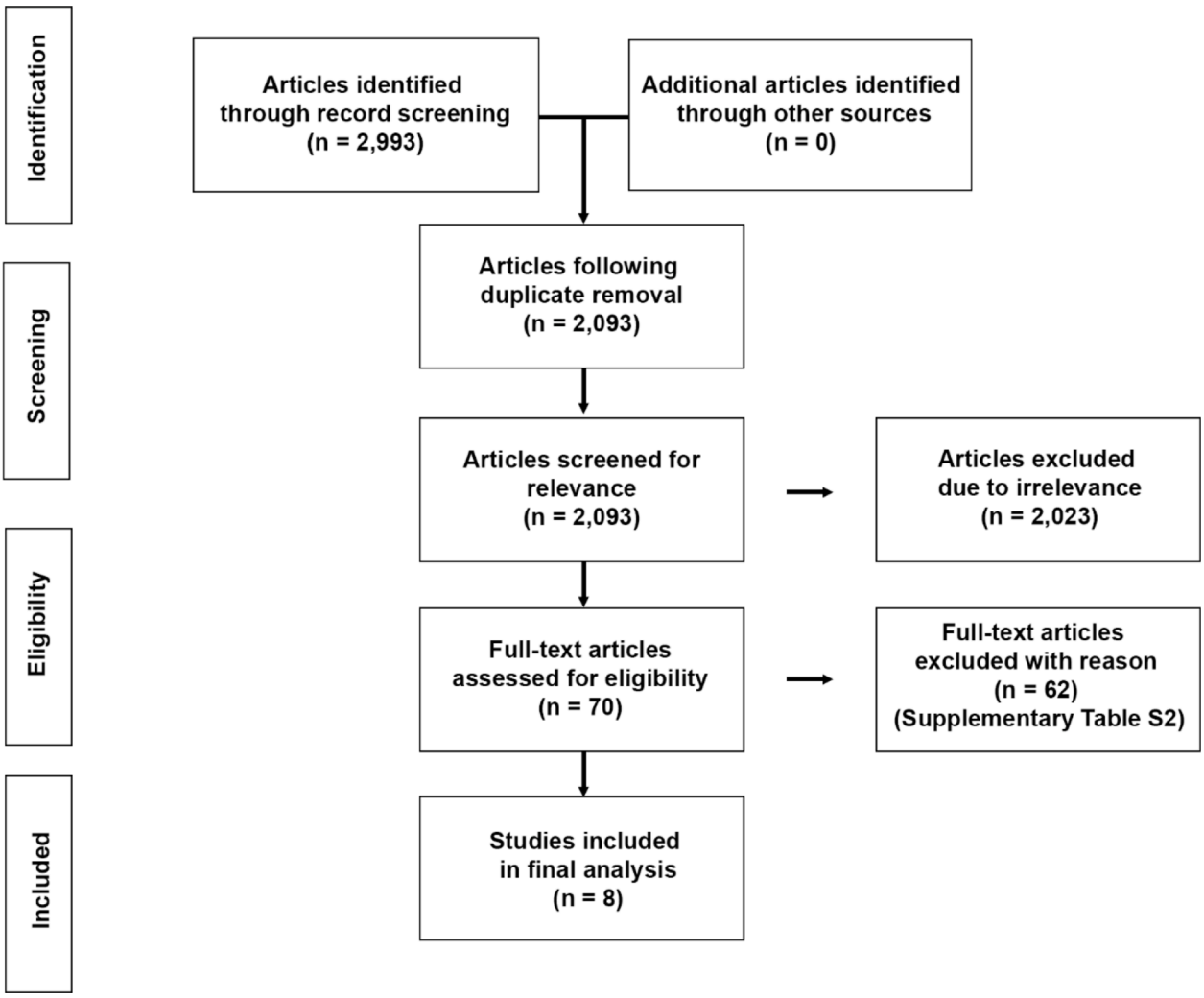

Fig. 1. PRISMA flow diagram [32].

Table 3
Study characteristics.

Reference	Population	Age (weeks)	Control Condition Apparatus	PWB Model Apparatus	Full Weight-Bearing Control	PWB70%	PWB40%	PWB20%	Longest Exposure Duration
Mortreux et al. 2018 [Mortreux et al., 2018]	Wistar male rats	14	NKD, JKT, HNS	JKT + HNS	✓	✓	✓	✓	2 weeks
Mortreux et al. 2019a [Mortreux et al., 2019]	Wistar male rats	14	JKT + HNS	JKT + HNS	✓	✓	✓	✓	4 weeks
Mortreux et al. 2019b [Mortreux et al., 2019]	Wistar male rats	14	JKT + HNS	JKT + HNS	✓	×	✓	×	2 weeks
Mortreux et al. 2020 [Mortreux et al., 2020]	Wistar male rats	14	JKT + HNS	JKT + HNS	✓	✓	✓	✓	4 weeks
Semple et al. 2020 [Semple et al., 2020]	Wistar female rats	14	JKT + HNS	JKT + HNS	✓	×	✓	×	2 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
Ellman et al. 2013 [Ellman et al., 2013]	C57Bl/6J female mice	11	JKT + TW	JKT + TW	✓	✓	✓	✓	3 weeks

PWB = partial weight-bearing (as a percentage of 100% body weight), NKD = naked, JKT = forelimb jacket, HNS = pelvic harness, and TW = tail wrap.

3.1. Calf circumference

The effects of PWB on calf circumference is displayed in Fig. 2. In male rats, PWB20%, PWB40%, and PWB70% cause a rapid decrease in calf circumference by large to very large SMDs. Compared to the full weight-bearing control, the greatest losses occurred by day 7 (PWB20%: -7.7%, PWB40%: -6.0%, and PWB70%: -5.9%) and either plateaued or slightly increased/decreased at days 14 (PWB20%: -7.9%, PWB40%:

Table 4
SYRCLE's risk of bias scoring.

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	Low	Low	Low
Mortreux et al. 2019a [Mortreux et al., 2019]	High	Low	Low	Low	High	Low	Low	Low	Low
Mortreux et al. 2019b [Mortreux et al., 2019]	High	Low	Low	Low	High	Low	Low	Low	Low
Mortreux et al. 2020 [Mortreux et al., 2020]	High	Low	Low	Low	High	Low	Low	Low	Low
Semple et al. 2020 [Semple et al., 2020]	High	Low	Low	Low	High	Low	Low	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
Ellman et al. 2013 [Ellman et al., 2013]	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low

Note: items were scored as low = 63%, unclear = 21%, and high = 17% (rounded to the nearest integer).

-7.2%, and PWB70%: -5.8%) and 28 (PWB20%: -6.1%, PWB40%: -8.5%, PWB70%: -5.7%). Lower levels of PWB tended to cause more pronounced reductions in calf circumference at days 7 and 14. However, for unclear reasons, PWB40% displayed the greatest loss across PWB loads at day 28 whilst PWB20% and 70% were comparable. Female rat calf circumference remained within <1% of the full weight-bearing control at PWB40% at days 7 and 14 (day 28 not investigated).

3.2. Wet muscle mass

Presented herein are the absolute wet muscle mass data for gastrocnemius (Fig. 3), soleus (Fig. 4), and quadriceps (Fig. 5), as they were the most frequently reported outcomes and are of high operational relevance to astronauts/cosmonauts. Other wet muscle mass data are presented in Supplementary Figs. S1-S4 (https://doi.org/10.5281/zenodo.5550222). In male rats, wet muscle mass of the soleus decreased at all PWB loads (20%, 40%, and 70%) and exposure durations (days 7, 14, and 28) compared to the full weight-bearing control by moderate to very large effects. Meta-effects could only be calculated at day 14 but revealed that loss of soleus muscle mass was slightly greater at lower PWB loads (PWB20%: -19.1%, SMD: -2.1; PWB40%: -17.0%, SMD: -2.0; PWB70%: -15.9%, SMD: -1.9) compared to the control. Quadriceps muscle mass also decreased at all PWB loads and exposure durations by moderate to very large effects. Meta-effects could only be calculated at day 14 but again showed that lower PWB loading caused slightly greater losses of quadriceps muscle mass (PWB20%: -19.2%, SMD: -2.3; PWB40%: -17.3%, SMD: -2.1; PWB70%: -12.7%, SMD: 1.5) compared to the control. Gastrocnemius wet muscle mass decreased to a lesser extent during PWB by moderate to large effects typically by days 14 and 28. At day 7, PWB20% decreased by large effects whilst changes at PWB40% and PWB70% were trivial and small, respectively, compared to the control. Meta-effects from day 14 revealed that lower PWB loads caused slightly greater reductions in gastrocnemius muscle mass (PWB20%: -8.9%, SMD: -1.1; PWB40%: -6.4%, SMD: -0.76; PWB70%: -5.9%, SMD: -0.5).

In female mice, soleus and gastrocnemius wet muscle mass declined in PWB20% and PWB40% (PWB70% unavailable) to a greater extent than in male rats. Data were only available following 21-days of PWB exposure but revealed that soleus muscle mass declined more at PWB20% (-35.5%, SMD: -2.5) compared to PWB40% (-25.1%, SMD: 1.7). Interestingly, gastrocnemius muscle mass declined less in PWB20% compared to PWB40% by day 21 (PWB20%: -17.4%, SMD: 2.5; PWB40%: -23.2, SMD: 3.0).

3.3. Muscle fiber composition

The effects of PWB on muscle fiber composition (type 1 fiber percentage) are displayed for the gastrocnemius and soleus (Fig. 6). Changes in the composition of the male rat gastrocnemius across 28 days of PWB exposure were trivial to small at PWB20% (0.2% to 2.4%), PWB40% (-1.8% to 3.4%), and PWB70% (0.7% to 1.0%). Changes in the male rat soleus varied from trivial to small differences at PWB20% (-0.7% to -3.9%) and PWB70% (-1.6% to -2.7%), and small to large differences at PWB40% (-1.8% to -12.1%). Interestingly, the largest change in soleus fiber composition relative to the control group was observed at PWB40% day 14 by two independent studies (-12.1% and -8.4%). Only one study reported muscle fiber composition for female rats and found a 3.1% increase in type 1 fibers within the soleus following 14-days of PWB40%.

3.4. Muscle fiber cross-sectional area

The effects of PWB on the muscle fiber cross-sectional area (CSA) are displayed for the gastrocnemius and gastrocnemius fibers expressing myosin heavy chain (MyHC) 1 and 2 (Fig. 7), and soleus and soleus fibers expressing MyHC 1 and 2 (Fig. 8). In male rats, gastrocnemius CSA declined across 28-days of PWB exposure by large to very large effects at PWB20% (-13.1% to -17.7%) and by small to large effects at PWB40% (-3.5% to -14.0%) and PWB70% (-3.7% to -12.1%) compared to full weight-bearing controls. In female rats, gastrocnemius CSA was only investigated at day 14 during PWB40% and reduced by 14.6%. Similarly, the CSA of gastrocnemius fibers expressing MyHC 1 and 2 were only investigated at PWB40% at day 14 for male and female rats. In both species, CSA reductions in fibers expressing MyHC 2 (male: -20.1%, female: -14.8%) occurred to a greater extent than those expressing MyHC 1 (male: -10.1%, female: -4.3%).

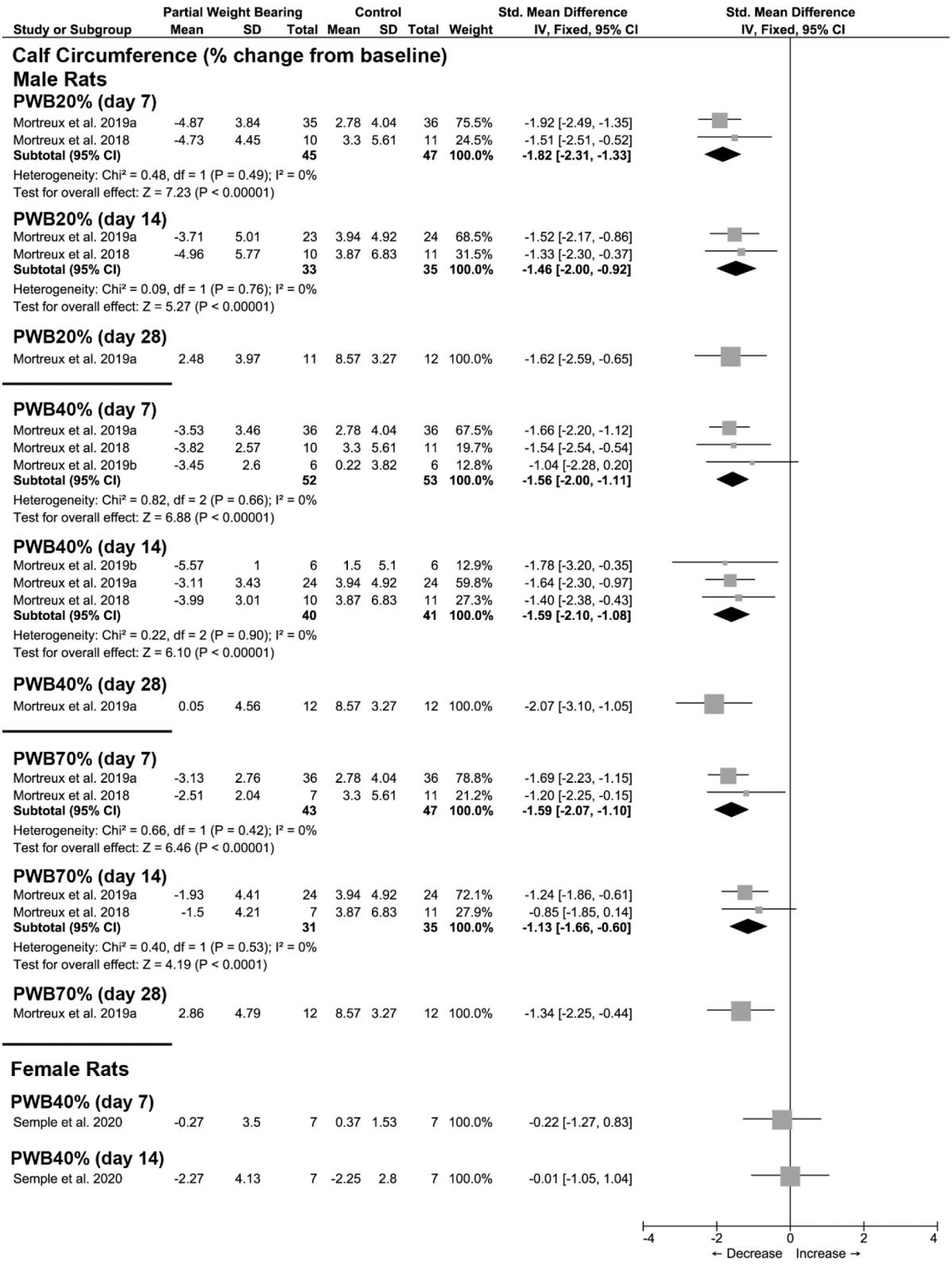

Fig. 2. Effect of Partial Weight-Bearing (PWB) on Calf Circumference (SD = standard deviation, Std. = standardized and CI = confidence interval).

In male rats, soleus CSA declined at all PWB loads and exposure durations. Large to very large effects were observed in PWB20% (-16.5% to -21.0%) and PWB40% (-14.4% to -26.4%), and by moderate to large effects in PWB70% (-10.5% to -13.5%). In female rats, soleus CSA was only investigated at PWB40% following 14-days of exposure and was found decline by 11.8% relative to controls, less than half that seen in male rats (-26.4%) at the same PWB load and time-point. In soleus fibers expressing MyHC 1 and 2, CSA declined by similar extents in male rats (MyHC 1: -26.4%, MyHC 2: 26.7%) at PWB40% following 14-days of exposure (no other PWB load or time-point available). Due to the notable decrease in data variability within the control group, the SMD was larger for soleus MyHC 1 CSA (SMD: -1.6) than MyHC 2 (SMD: -1.0).

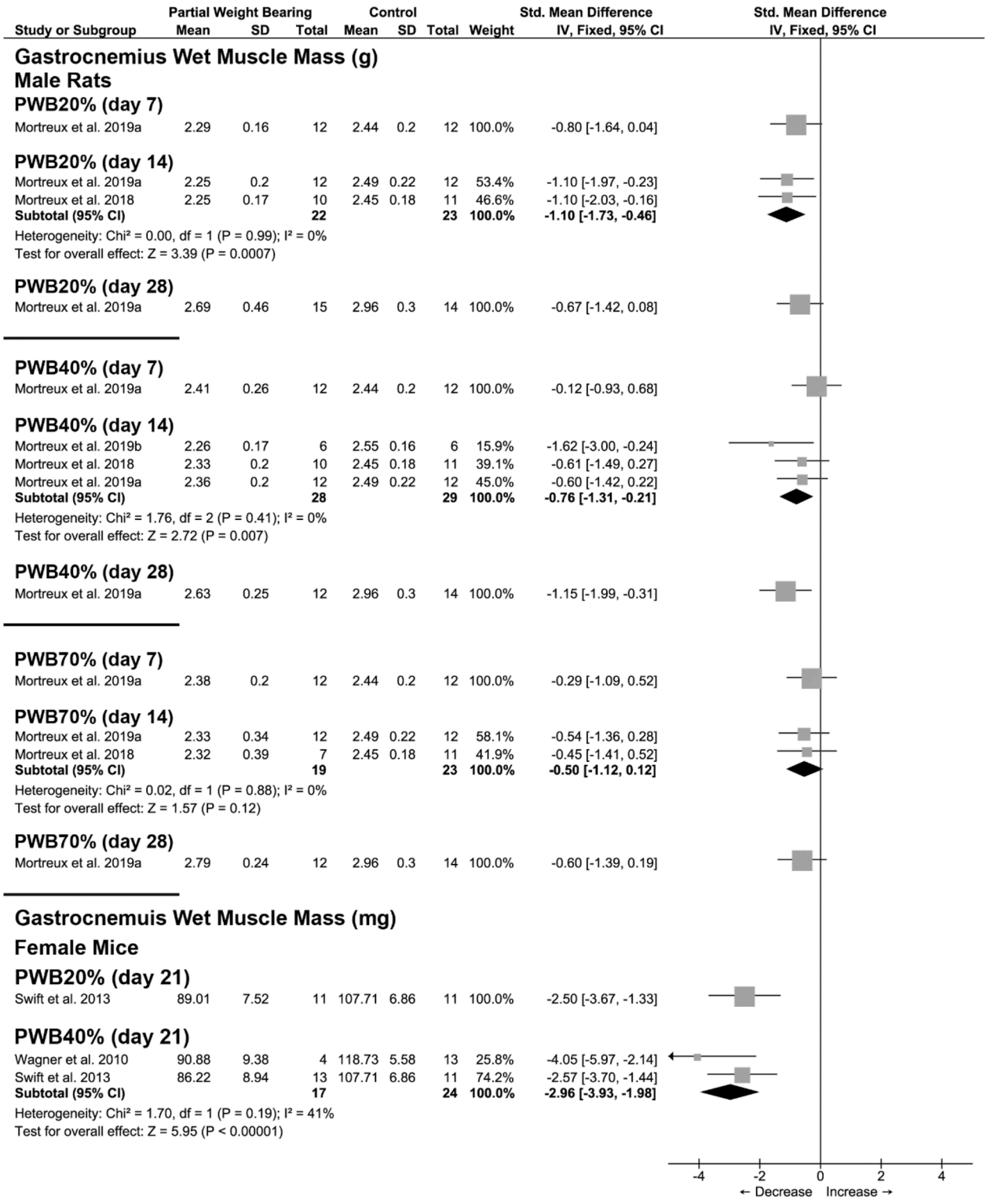

Fig. 3. Effect of Partial Weight-Bearing (PWB) on Gastrocnemius Wet Muscle Mass (SD = standard deviation, Std. = standardized and CI = confidence interval).

In female rats, CSA of the soleus fibers expressing MyHC 1 declined by 14.2% whilst those expressing MyHC 2 were unaffected (-0.2%) relative to the controls. Triceps brachii average fiber CSA are displayed in Supplementary Fig. S5 (https://doi.org/10.5281/zenodo.5550222).

3.5. Rear and front paw grip force

The effects of PWB on grip force are displayed for the rear and front paws (Fig. 9). In male rats, rear paw grip force declined at all PWB loads and exposure durations. Rapid reductions in rear paw grip force (expressed as percentage differences to baseline scores between the PWB and control groups) were observed by day 7 at all PWB levels and was more pronounced at lower loads (PWB20%: -46.3%; PWB40%: -41.3%; PWB70%: -30.8%). At day 14, changes in rear paw grip force were mixed and either increased or decreased from day 7 (PWB20%: -48.4%; PWB40%: -45.2%; PWB70%: -37.0%), but remained impaired relative to

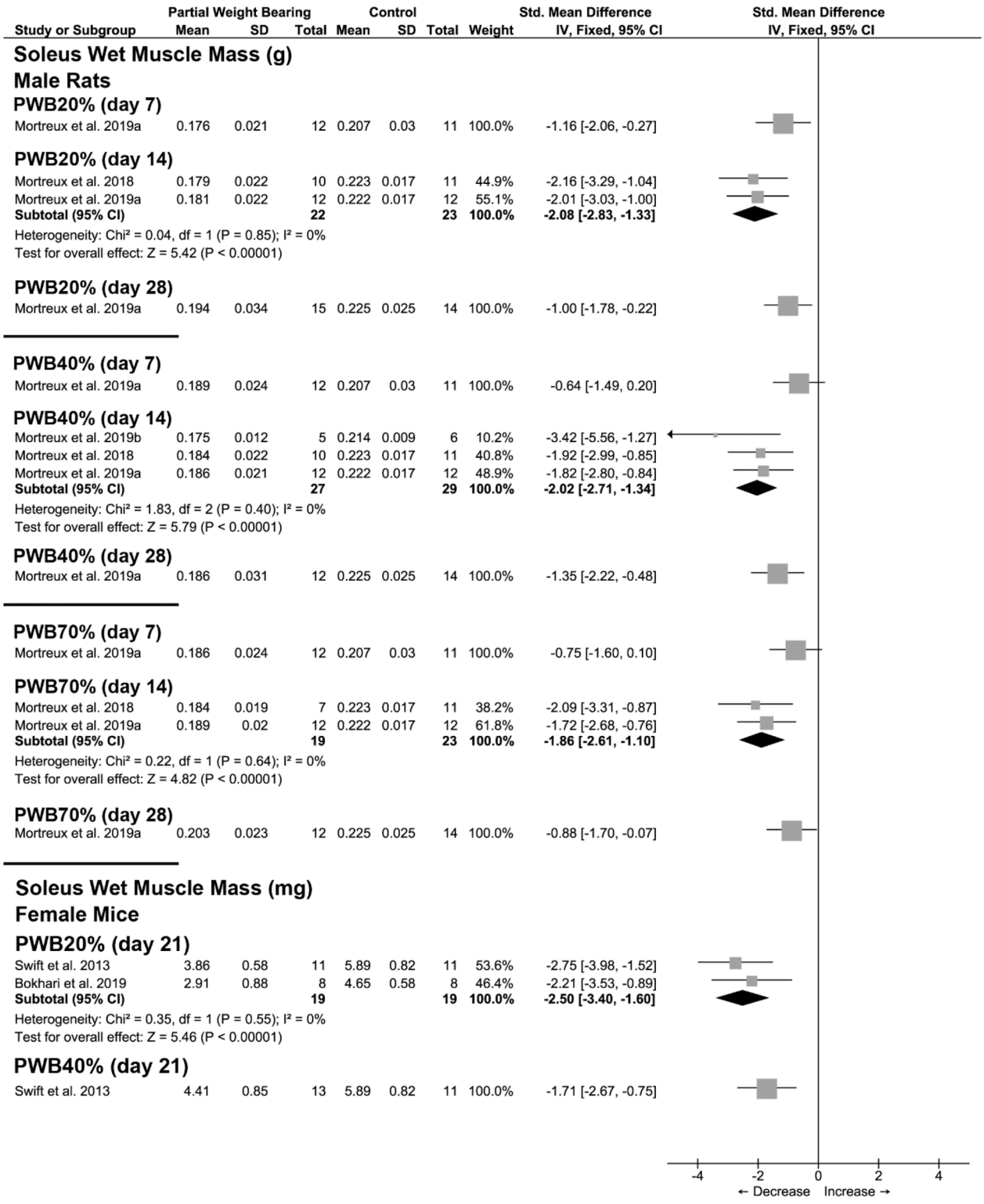

Fig. 4. Effect of Partial Weight-Bearing (PWB) on Soleus Wet Muscle Mass (SD = standard deviation, Std. = standardized and CI = confidence interval).

controls. At day 28, rear paw grip force declined to the greatest extent across all PWB groups (PWB20%: -87.9%; PWB40%: -80.5%; PWB70%: -61.2%). In female rats, rear paw grip force increased by a small effect (4.6%) following 7-days of PWB40% but thereafter decreased by a large effect at day 14 (-11.8%).

Male and female rats following 7 and 14 days of PWB40% displayed differential responses to changes in front paw grip force (no other PWB load of time-point available). Male rats displayed a rapid reduction in front paw grip force following 7-days of exposure (-8.9%) that worsened by day 14 (-20.4%). Female rats displayed no change by day 7, and only a 2.9% reduction following 14-days of PWB40% exposure.

3.6. *Hindlimb nerve-stimulated torque generation*

The effects of PWB on dorsiflexion/plantarflexion torque production and normalized plantarflexion maximum tetanic impulse are displayed

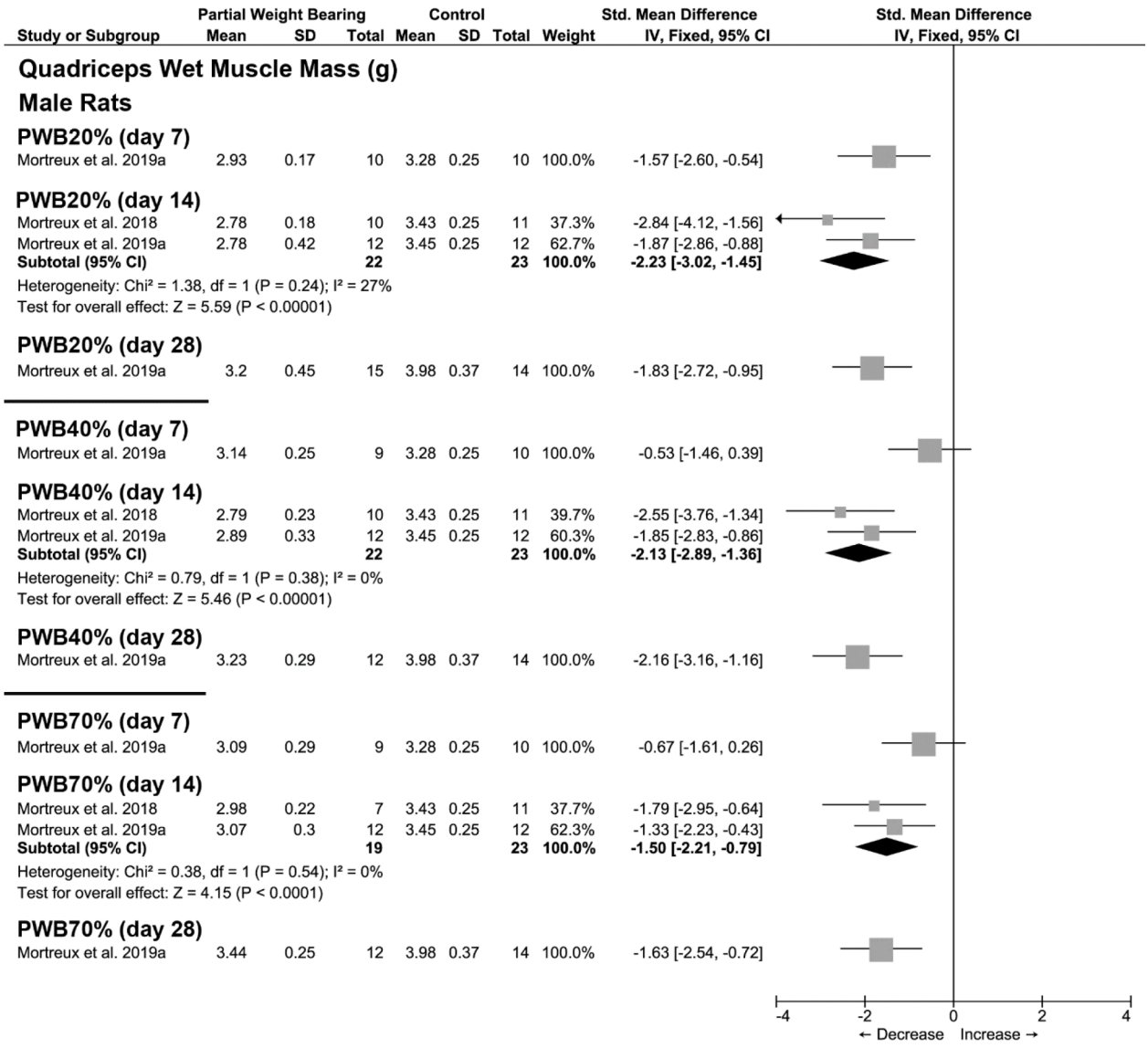

Fig. 5. Effect of Partial Weight-Bearing (PWB) on Quadriceps Wet Muscle Mass (SD = standard deviation, Std. = standardized and CI = confidence interval).

in Fig. 10. In male rats, plantarflexion torque production reduced by very large effects at day 28 (no other time-point available) across all PWB levels (PWB20%: -11.8%; PWB40%: -11.1%; PWB70%: -7.8%). Normalized plantarflexion maximum impulse similarly declined at day 28 across all PWB levels and was most severe at PWB20% (-37.5%) but comparable between PWB40% (-20.0%) and PWB70% (-22.2%). In female rats, dorsiflexion torque production declined by small effects following PWB40% at days 7 (-9.6%) and 14 (-11.4%).

4. Discussion

4.1. Summary of main findings

This study is the first systematic review and meta-analysis regarding the effect of PWB for 1-4 weeks on rodent skeletal muscle morphology and function. The main findings were that most PWB data currently relate to a Wistar male rat population where nearly all morphological (calf circumference, wet muscle mass, and myofiber CSA) and functional (grip force and plantarflexion torque production) parameters declined during PWB relative to normal weight-bearing. Limited evidence was available for female rats and mice, however, they appeared to be less and more susceptible to PWB-induced deconditioning, respectively. Atrophy was more evident in skeletal muscles with an anti-gravity/postural role (e.g., soleus) than those without (e.g., gastrocnemius), particularly in male rats. Preferential atrophy was observed in myofibers expressing MyHC 1 in the soleus, and MyHC 2 in the gastrocnemius, for both male and female rats. The percentage of myofibers expressing MyHC 1 in the male rat soleus reduced, particularly during PWB40% by days 14 and 28, whilst the composition of the gastrocnemius remained largely unchanged. Higher PWB loading (PWB20% → PWB40% → PWB70%) tended to mitigate skeletal muscle deconditioning, but for most outcomes failed to entirely prevent it. However, there were cases where differences between PWB levels were comparable. The largest amount of atrophy commonly occurred within the first 1-2 weeks of exposure before slowing, whilst functional deconditioning appeared to be more progressive. The magnitude of deconditioning varied by outcome, PWB level, exposure duration, and rodent sex and species. There was frequent homogeneity across study findings for a given outcome and risk of bias was low/unclear for almost all studies.

4.2. Comparison of partial weight-bearing findings to rodents and humans during unloading and microgravity

The use of rodents as pre-clinical and/or translational models for

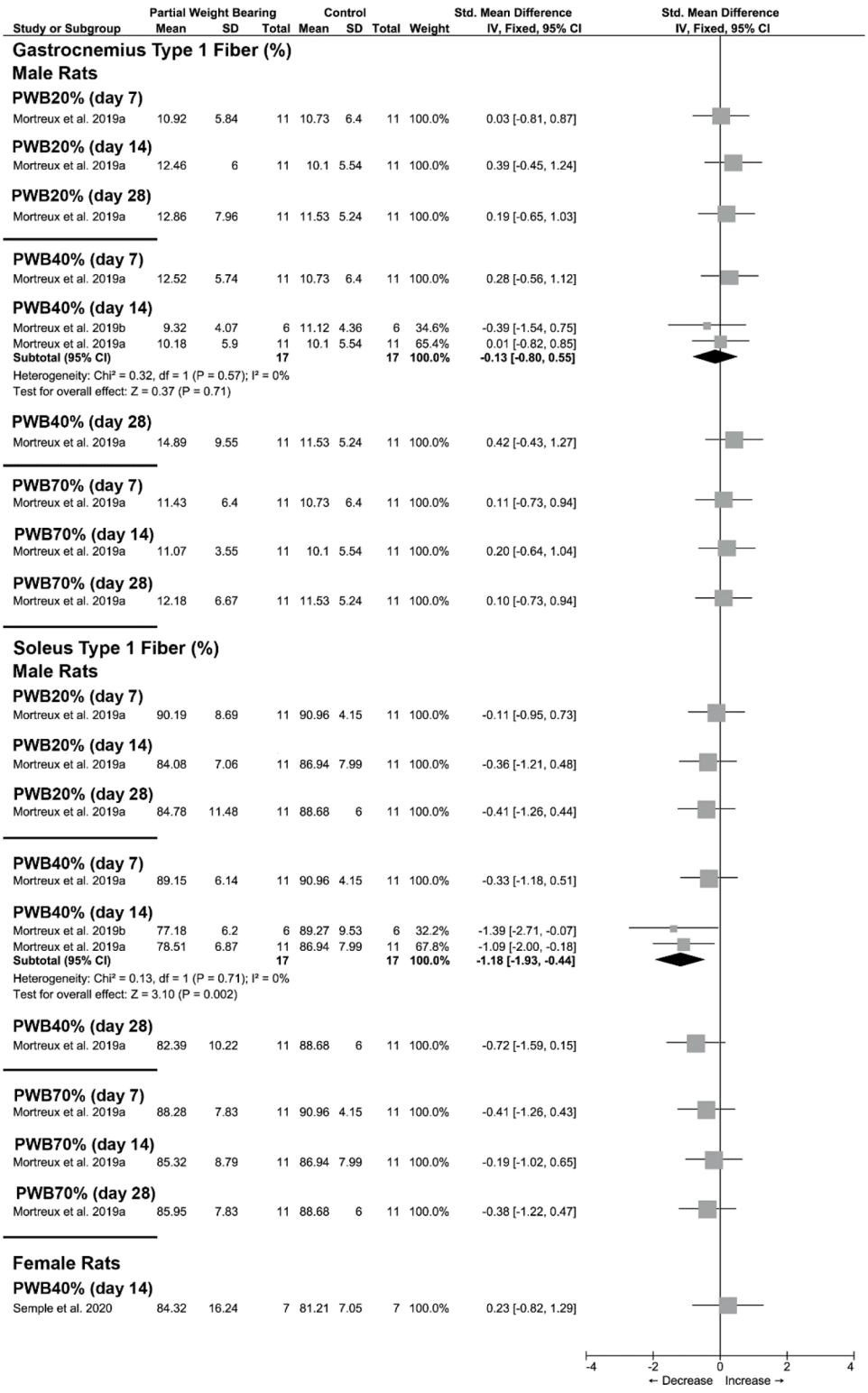

Fig. 6. Effect of Partial Weight-Bearing (PWB) on Gastrocnemius and Soleus Muscle Fiber Composition (SD = standard deviation, Std. = standardized and CI = confidence interval).

human biomedical research has been on-going for decades both in the context of terrestrial and aerospace medicine. This is due to their genetic, anatomical, and physiological similarities to humans [Bryda, 2013, Globus and Morey-Holton, 2016]. Direct comparisons between the present findings and the equivalent in humans are challenging as there is limited evidence concerning morphological and functional adaptations of human skeletal muscle in real/simulated hypogravity [Richter et al., 2017, Swain et al., 2021]. Only one human study has investigated muscle atrophy following exposure to a hypogravity analog (9.5° head-up tilt bed rest) in which quadriceps volume reduced following 6-days of exposure to simulated Lunar gravity (~20% full loading) [Cavanagh et al., 2013]. This response is comparable to rodents exposed to 7-days of PWB20%, where quadriceps wet muscle mass was also reduced. Interestingly, the deconditioning patterns observed during

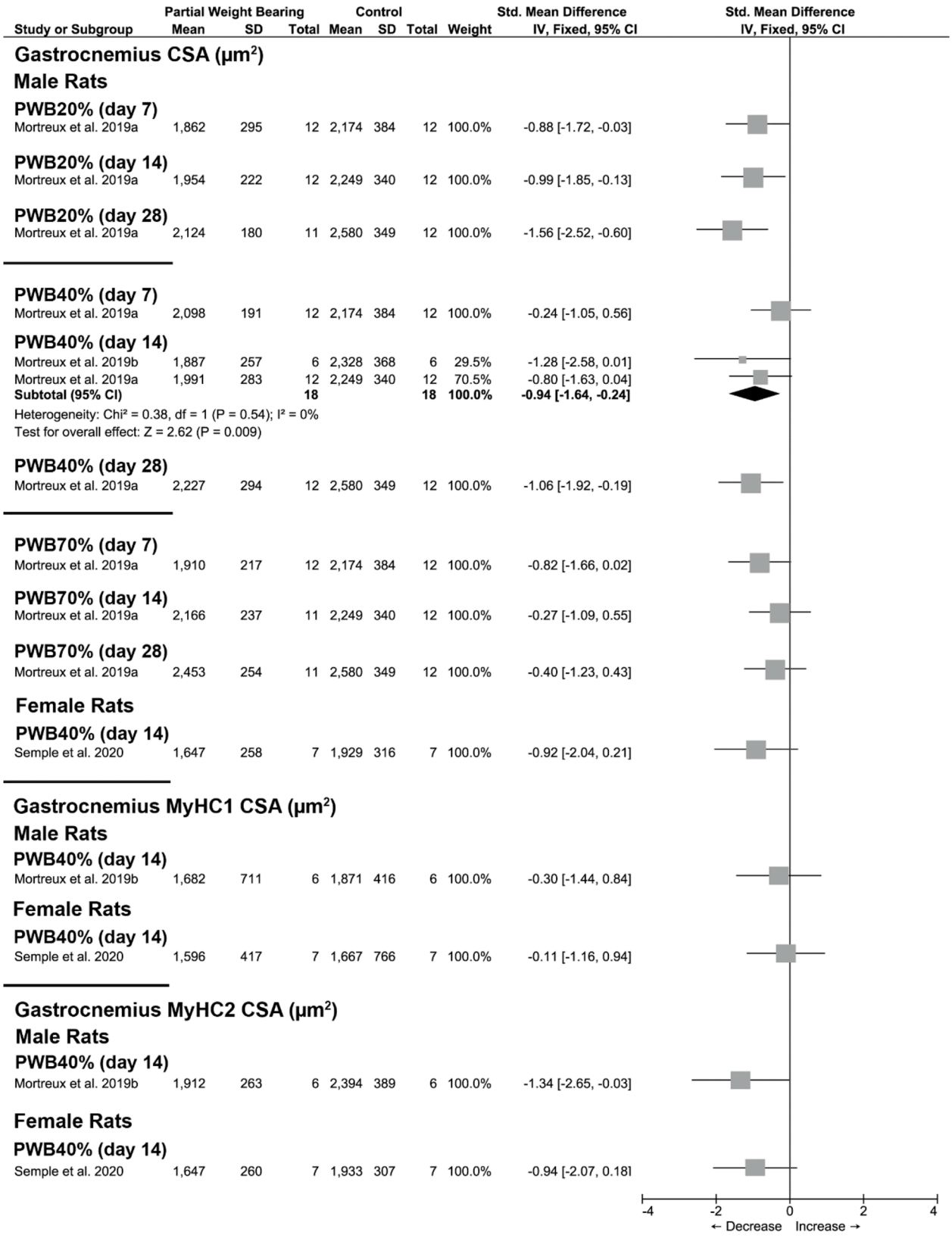

Fig. 7. Effect of Partial Weight-Bearing (PWB) on Gastrocnemius Average Muscle Fiber Cross-Sectional Area (CSA) and Type 1 and 2 Myosin Heavy Chain (MyHC) CSA (SD = standard deviation, Std. = standardized and CI = confidence interval).

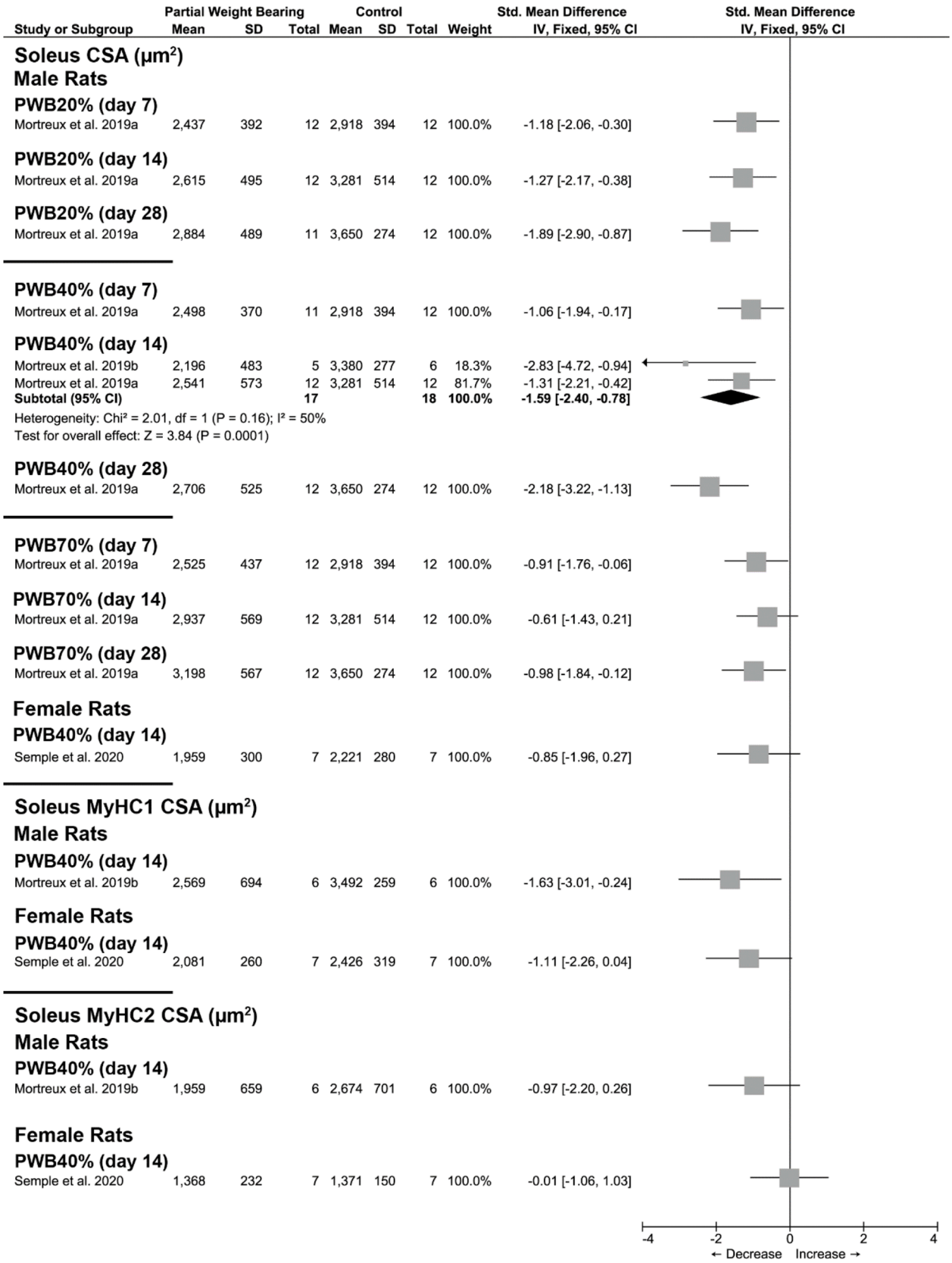

Fig. 8. Effect of Partial Weight-Bearing (PWB) on Soleus Average Muscle Fiber Cross-Sectional Area (CSA) and Type 1 and 2 Myosin Heavy Chain (MyHC) CSA (SD = standard deviation, Std. = standardized and CI = confidence interval).

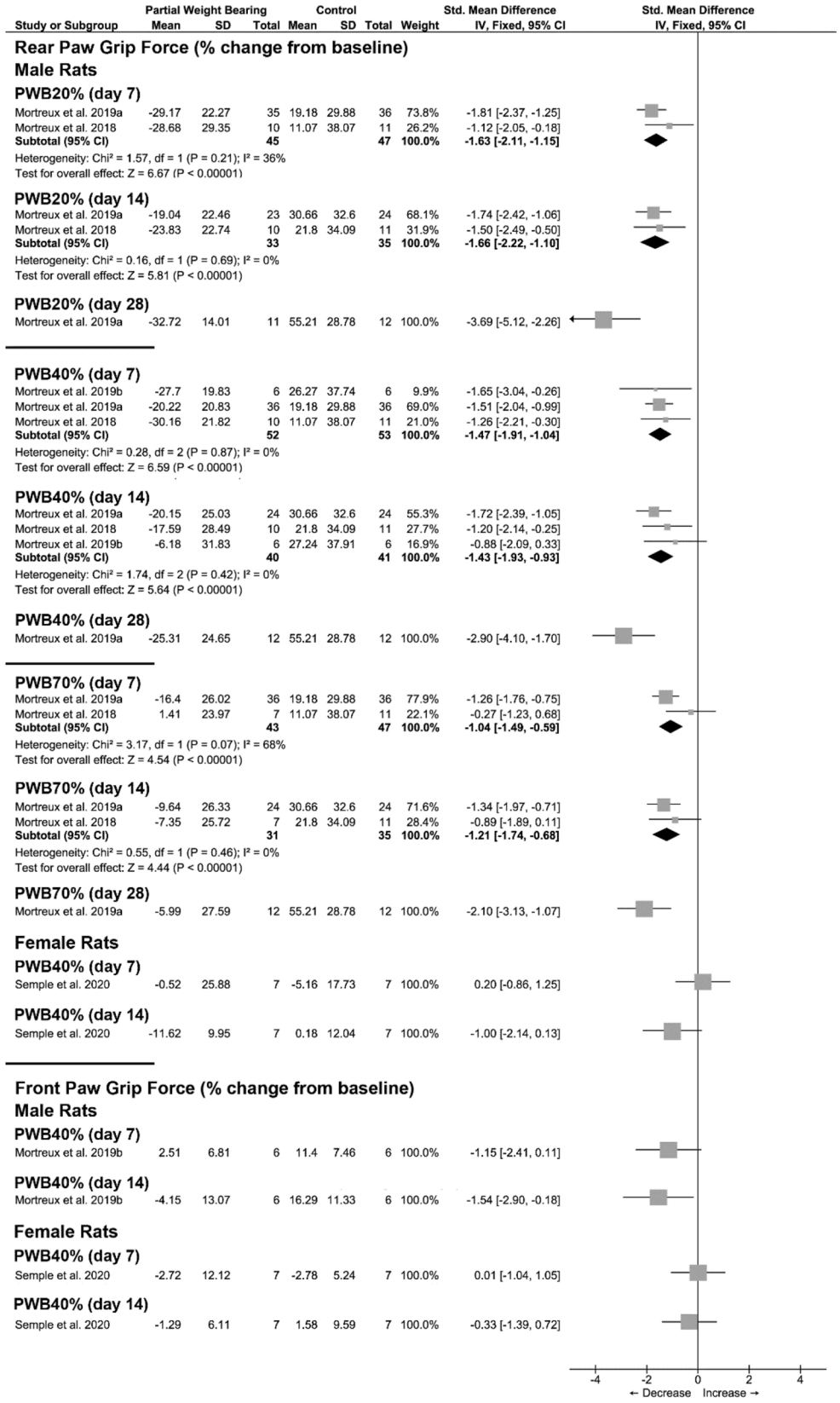

Fig. 9. Effect of Partial Weight-Bearing (PWB) on rear and front paw grip force (SD = standard deviation, Std. = standardized and CI = confidence interval).

PWB resemble those in rodents and humans exposed to microgravity and microgravity analogs [Qaisar et al., 2020].

Muscle atrophy, as determined by reductions in calf circumference, wet muscle mass, and myofiber CSA, occurred during exposure to PWB. Deconditioning was particularly evident in muscles with an anti-gravity/postural function (e.g., soleus and quadriceps), whilst the gastrocnemius was affected to a lesser degree in male rats. Non-standardized mean differences confirm that in female mice during 21

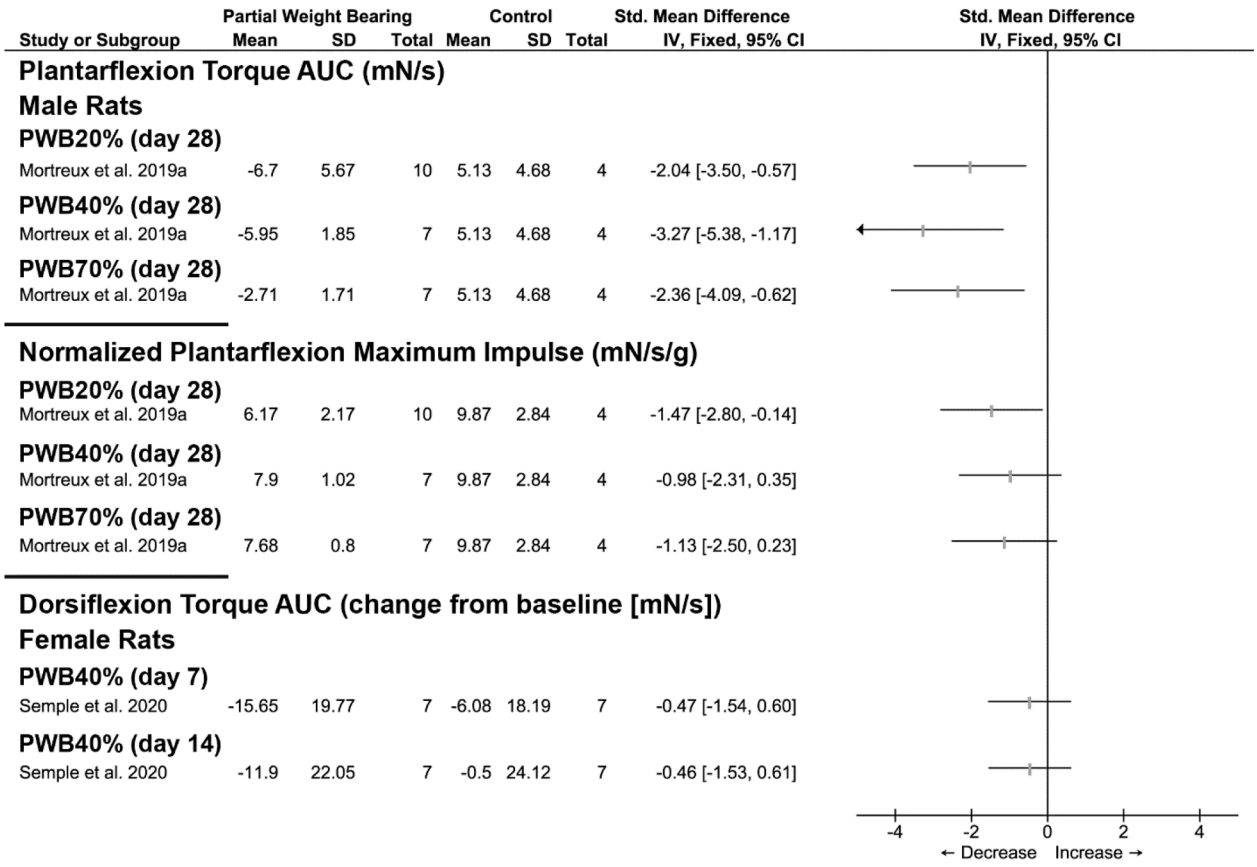

Fig. 10. Effect of Partial Weight-Bearing (PWB) on Nerve-Stimulated Plantarflexion and Dorsiflexion Torque (AUC = area under curve, SD = standard deviation, Std. = standardized and CI = confidence interval).

days of PWB20%, atrophy in the soleus (-34% and -37%) was larger than the gastrocnemius (-17%). However, at PWB40%, the difference was more comparable (soleus: -25%; gastrocnemius: -20% and -23%). It is important to highlight that mice strains (C57BL/6 and BALB/c) are inbred and are genetically homogenous [Beck et al., 2000], whilst the Wistar rat strain is outbred, sharing more genetic variability as seen in human populations [Bryda, 2013, Donovan et al., 2018]. Therefore, rodent species and strain are likely to mediate the deconditioning response during exposure to PWB. Nonetheless, deconditioning of the plantarflexors and quadriceps is comparable to findings from rodents and humans exposed to microgravity and microgravity analogs, where anti-gravity/postural muscles are highly susceptible to atrophy [Winnard et al., 2019, Qaisar et al., 2020, Fitts et al., 2000, Qaisar et al., 2020]. In addition, the rate of atrophy during PWB was rapid, occurring within the first one to weeks of exposure before slowing or plateauing and is akin to what occurs in rodents during exposure to HLU [Bodine, 2013]. However, in human bed-rest studies, muscle atrophy occurs at a slower rate, often taking several weeks to months to reach moderate/large effect sizes [Winnard et al., 2019]. Some studies reported wet muscle mass normalized to rodent's body weight. However, in terms of deconditioning, this parameter can be difficult to interpret due to other variables influencing body weight such as bone loss, food intake, and changes in body composition [Mortreux et al., 2018]. It is important to recognize that in the current review, findings were predominantly from Wistar male rats. Female rat PWB data were limited to one study and displayed slightly different responses to males; mean myofiber CSA reductions were comparable between the soleus (-12%) and gastrocnemius (-15%) whilst calf circumference did not change [Semple et al., 2020]. Sex is known to mediate skeletal muscle deconditioning [Rosa-Caldwell and Greene, 2019, Rosa-Caldwell et al., 2021], therefore, future comparative studies are important to understanding sex-based differences in response to PWB.

During disuse, muscle atrophy has been attributed to the combination of unloading and muscle inactivity, which affects the dynamics of muscle protein turnover [Bodine, 2013, Phillips et al., 2009, Rudrappa et al., 2016]. It is, therefore, important to highlight that the degree of muscle atrophy and PWB have been shown to display moderate to strong linear relationships [Mortreux et al., 2018, Mortreux et al., 2019, Ellman et al., 2013]. This observation may have been distorted in the present review due to the use of Hedges' G statistic, which is sensitive to sample size and variation, which sometimes differed between PWB groups. When PWB data are interpreted as mean differences to the control group, it becomes clearer that higher PWB loading frequently attenuated deconditioning. Therefore, despite rodents having the ability to ambulate and incur mechanical loading, partial reductions in weight-bearing, even just 30% below full loading, still caused notable levels of muscle atrophy in a dose-response fashion across the so-called 'gravity continuum' (0g → 1g) [Swift et al., 2013].

Preferential atrophy of specific fiber-types was observed during PWB within the soleus and gastrocnemius in male and female rats. Myofibers expressing MyHC 1 in the soleus were more atrophied than those expressing MyHC 2. The opposite trend occurred in the gastrocnemius whereby myofibers expressing MyHC 2 atrophied more than those expressing MyHC 2. Previous reviews have highlighted that rodents' often display preferential atrophy of slow-twitch fibers during exposure to spaceflight or weightlessness analogs whilst human fast-twitch fibers appear at least as susceptible, if not more, than slow-twitch fibers [di Prampero and Narici, 2003, Tanaka et al., 2017, Hikida et al., 1989, Bloomfield, 1997]. Therefore, appreciation of species differences is important when considering the potential transferability of findings. In rats following spaceflight, changes in the CSA between different myofiber types within the gastrocnemius have been shown to vary between

the superficial and deep layers [Kraemer et al., 2000]. Therefore, it is important for future research to address whether atrophy of myofibers expressing MyHC 2 in the gastrocnemius is a unique deconditioning feature of PWB or a consequence of the methods used to assess these outcomes.

The proportion of myofibers expressing MyHC 1 in the soleus tended to reduce in male rats during PWB. However, for unclear reasons, this was most pronounced at PWB40% at days 14 and 28 where MyHC 1 expression reduced by 8-12% and 6%, respectively. Data from one study identified that the proportion of myofibers expressing MyHC 1 remained largely unchanged in female rats following PWB, suggesting sex-based differences in this parameter. Similarly, MyHC 1 expression in the male rat gastrocnemius during PWB at all loads remained largely unchanged up to 28 days exposure, showing no more than ~3% difference (no female data available). Previous spaceflight and weightlessness analog studies in rodents and humans generally observe a slow-to-fast MyHC isoform shift in predominantly slow-twitch skeletal muscles (e.g., soleus) [Qaisar et al., 2020, Staron et al., 1998, Shenkman, 2016, Gallagher et al., 2005, Talmadge, 2000]. Characterization of the MyHC spectrum would improve understanding of phenotypic changes during PWB (e.g., [Kraemer et al., 2000]). Furthermore, even when MyHC shifts have not been observed at the protein level, changes in MyHC gene expression at the mRNA level have [Andersen et al., 1999]. These data suggest that susceptible muscles are in a translational state and may require more time before phenotypic changes in MyHC expression can be observed. Implications of the soleus, a postural muscle, increasing the expression of faster MyHC isoforms during PWB is likely to reduce fatigue resistance and impair its anti-gravity function due to differences in MyHC metabolic profiles and contractile properties [Shenkman, 2016, Grichko et al., 2000, Harridge et al., 1996].

Muscle function was impaired in male rats during PWB, whilst female rats were relatively less affected. Grip force is a general measurement of voluntary muscle strength in rodents and rapidly declined (in male rats) for both the rear and front paws during exposure to PWB [Bonetto et al., 2015]. Rear paw grip force was the only measurement to be taken over several exposure durations (in male rats only). Despite a rapid reduction occurring by day 7 and appearing to plateau between days 7 and 14, there was a drastic loss of grip force by day 28 across all PWB loads, indicating a progressive loss of function. The loss of grip force also occurred in a dose-response relationship with PWB load. Rear paw grip force has been shown to decline in rats during exposure to HLU [Song et al., 2018] and spaceflight [Shen et al., 2017], and loss of lower limb function also occurs in humans during bed rest [Winnard et al., 2019, Trappe et al., 2007, Trappe et al., 2008] and spaceflight [Fitts et al., 2010, Tesch et al., 2005]. In addition to voluntary measures of strength, tetanic nerve-stimulated plantarflexion torque decreased by large amounts during PWB at all levels by day 28 in male rats. Similar findings have been demonstrated in rats during HLU [Allen et al., 2006] and in astronauts/cosmonauts following spaceflight [Narici et al., 2003]. No data were available for plantarflexion force in female rats. However, one study measured dorsiflexion torque which declined by ~10% during PWB40% at days 7 and 14, although the original study failed to detect any significant difference to the control. Similarly, Female rats during exposure to 28 days of HLU have been shown to display no significant functional impairments in the dorsiflexors [Winiarski et al., 1987]. Similar to morphological parameters, rear paw grip force and nerve-stimulated plantarflexion force have been demonstrated to be linearly associated with PWB level [Mortreux et al., 2019], highlighting that higher PWB loads are protective but not preventative of functional deconditioning. Furthermore, normalized plantarflexion torque (to triceps surae wet muscle mass) still displayed impairments in function following 28 days of PWB at all loads demonstrating an intrinsic loss of muscle strength. It can be suggested that muscle atrophy caused by PWB was a large contributor to the loss of muscle strength and may explain differences in the rate/magnitude of functional impairments between PWB loads and rodent sexes [Marusic et al., 2021]. However, factors that are associated with intrinsic losses of muscle function (e.g., neuromuscular parameters) remain to be determined during PWB.

The deconditioning patterns of skeletal muscle during PWB resemble those in rodents and humans exposed to microgravity and microgravity analogs. Alongside this, there is emerging evidence in hypogravity settings highlighting that physiological deconditioning is anticipated to occur in humans [Richter et al., 2017, Cavanagh et al., 2013]. Therefore, the present findings provide reason to suggest that in hypogravity environments, such as the Moon and Mars, humans might also experience atrophy and functional impairment of skeletal muscle. However, rodents and humans undergo disuse-induced deconditioning slightly differently. Whilst evidence supports that both rodents and humans show a sustained decrease in the rate of basal protein synthesis during complete unloading, debate exists with regards to changes in proteolysis in humans [Bodine, 2013, Phillips et al., 2009]. It is unclear whether species differences regarding muscle protein turnover during exposure to low levels of mechanical stimuli may lead to significant differences in their susceptibility to hypogravity-induced deconditioning. Therefore, further research is needed to determine the effects of hypogravity on human skeletal muscle morphology and function.

4.3. Findings within the context of Lunar and Martian missions

The International Space Exploration Coordination Group has affirmed that 14 space agencies are interested in expanding human presence in the solar system [NASA, 2020]. It is estimated that the first human-Moon mission will occur in 2024 to begin preparing for the first human-Mars mission by the mid-to-late 2030s. These missions will expose humans to hypergravity (launch and landing), microgravity (transits), and hypogravity (surface exploration). Lunar and Martian missions will differ considerably in terms of transit and surface exploration durations due to their astronomical position in space, orbital paths relative to Earth, and mission objectives [Connolly et al., 2018]. Earth-Moon transit durations will be relatively short (3-5 days) but may increase to several weeks if an orbiting gateway is used [Connolly et al., 2018, Horneck and Comet, 2006]. Lunar surface exploration durations will vary depending on mission objectives and are assumed to range from short-term (3-5 days) to medium (42 days) and long-term (6-month) stays [Connolly et al., 2018]. Earth-Mars transit durations will be much longer, ranging from 6-12 months, with surface exploration durations ranging from short (30 days) to long-term durations (~300-500 days) depending on mission structure estimations [Connolly et al., 2018, Horneck et al., 2006].

In microgravity (~0g), significant morphological and functional deterioration of human skeletal muscle occurs, especially during prolonged exposure [Fitts et al., 2010, Winnard et al., 2019]. Exercise countermeasures have, therefore, been implemented onboard the ISS to protect/attenuate adverse deconditioning of skeletal muscle [Fitts et al., 2010]. Exercise during Moon and Mars transit is likely to be more challenging as small multi-purpose spacecraft such as the Orion have limited upload mass, volume, and power usage/access [Laws et al., 2020]. Whilst it may be less of an issue for short-term Lunar transit, muscle deconditioning due to reduced exercise quality/quantity in long-duration transit to Mars is a major concern [Winnard et al., 2019]. Astronauts/cosmonauts may, therefore, arrive on the Lunar and, particularly Martian, surface in a deconditioned state.

The findings from the present study indicate that Lunar and Martian hypogravities (16% and 38% partial weight-bearing, respectively) will lead to structural and functional deconditioning in muscle compared to Earth's gravity and that the patterns of deconditioning will be similar to those that occur in microgravity. It is important to acknowledge, however, that these findings reflect adaptations in rodents previously exposed to Earth's gravity. Given the effect of mechanical loading on muscle, exposure to hypogravity following microgravity transit may potentially offer some degree of muscular re-conditioning. However, the present findings suggest that the level of protection hypogravity will

provide may be significantly less than that of Earth's gravity. In rodents, it has been demonstrated that 7-days of HLU followed by 7-days of PWB at 40% body mass (simulated mission to Mars) compromises muscular health more than 14-days of 40% PWB [Mortreux et al., 2019]. It remains in question whether muscular health remains compromised during long-term PWB exposure following complete unloading or if it eventually reaches comparable levels to a group exposed to PWB without prior unloading. Nevertheless, whether Lunar/Martian hypogravity is sufficient to uphold the physical capabilities of the crew during surface exploration remains unclear but has critical implications for the requirement of exercise countermeasures aimed at mitigating muscular deconditioning (e.g., resistance exercise). Strength-related parameters are key determinants of extra-vehicular activity (EVA) performance and crew that fall below the minimum physical requirements for any given EVA are expected to either fail the task or finish in an unacceptable amount of time [Ryder et al., 2019, Taylor et al., 2018]. This could become life-threatening in the case of emergency procedures. Taken together, the present findings demonstrate the need to investigate how human muscular health is affected by different levels of partial weight-bearing (e.g., simulated Lunar and Martian gravities) and whether these settings provide a reconditioning effect following a period of complete unloading. This evidence can directly inform countermeasure requirements for extraterrestrial exploration to uphold astronaut performance. A more comprehensive discussion on this matter can be found elsewhere [Swain et al., 2021].

5. Quality of the evidence and overall completeness

The current review aimed to synthesize the effects of PWB on rodent skeletal muscle morphology and function. It is clear from the search recall that this topic remains novel and only a small number of studies presently exist in this area, although a preliminary meta-analyses of multiple outcomes was viable. Only a few recent studies have conducted comprehensive muscle assessments across several PWB levels (PWB20%, PWB40%, and PWB70%) and exposure durations (up to four weeks) [Mortreux et al., 2018, Mortreux et al., 2019], whilst others have reported fewer and sometimes unique muscle outcomes specific to one PWB level and exposure duration. Therefore, the overall completeness of evidence remains limited but is expected to expand once the PWB model becomes increasingly recognized and employed by wider research communities. Current PWB studies are highly homogenous in terms of study methods, however, wider employment of the model may increase their diversity, making inter-study comparisons and pooling data more challenging. It is, therefore, crucial for researchers to consider/agree to use standardized study designs and outcome measurements. Alongside a greater number of partial weight-bearing studies, more standardized methods will improve the statistical power of meta-analyses in future reviews. Lastly, consideration of which rodent outcomes are most important and transferable to humans and aerospace medical operations will help improve their real-world utility for human clinical trials and extraterrestrial exploration.

5.1. Quality of the PWB model

It is important to address whether the PWB model can introduce confounding, for example, by influencing muscle outcomes independent of loading. In male rats, the PWB apparatus (forelimb jacket and hindlimb pelvic harness attached via a chain link) has been shown not to influence muscle outcomes following 28 days of PWB in two normal weight-bearing control groups (with and without the apparatus) [Mortreux et al., 2020]. Additionally, the rat pelvic harness has also been shown to not influence blood pressuring or hindlimb oxygen saturation during PWB [Mortreux et al., 2020]. In female mice, a tail wrap is used instead of a pelvic harness and has been shown to not influence soleus wet muscle mass. However, a small significant decline (3.3%) in the gastrocnemius mass of control animals wearing the PWB apparatus [Ellman et al., 2013]. Therefore, gastrocnemius data from female mice should be interpreted with caution.

Two independent PWB studies in rats have demonstrated that parameters associated with the activation of the hypothalamic-pituitary-adrenal (HPA) axis (e.g., plasma corticosterone, adrenal gland weight, and spleen weight) did not show any indication of chronic stress in response to several weeks of PWB [Semple et al., 2020, Mortreux et al., 2020]. Static weight distribution between the forelimbs and hindlimbs during PWB has been demonstrated in a small sample (n = 3-5) of male rats to differ by small amounts across PWB loads [Mortreux et al., 2020]. Because mechanical loading characteristics have important influence over muscle adaptation, this can introduce bias in forelimb/hindlimb data depending on how rodents manage their weight distribution; future research is being planned to investigate this further [Mortreux et al., 2020].

Rodents in PWB often display a decline in body mass relative to controls which can create bias in outcomes that are influenced by factors other than muscle such as fat-free mass in limb girth measurements and, therefore, these data should be interpreted with caution [Mortreux et al., 2019]. Further, the PWB model can cause slight changes in rodent feeding behavior compared to controls, although this has been shown to be only transient, occurring within the first few days of suspension before stabilizing [Ryder et al., 2019]. The ecological validity of the PWB model relative to real hypogravity settings (e.g., the Moon and Mars) has yet to be established. Hypogravity research is currently limited to ground-based studies. Thus, ensuring that the PWB model can mimic physiological phenomena observed in real hypogravity is relevant to its future application. The Cosmos 2044 missions provided necessary spaceflight data to validate the HLU weightlessness rodent model [Morey-Holton et al., 2005] and similar steps are currently underway to validate the PWB model using the Japan Aerospace Exploration Agency centrifuge onboard the ISS [Shiba et al., 2017].

5.2. Risk of bias

The overall RoB was predominantly low/unclear for both the SYRCLE's RoB tool (low [63%], unclear [21%], and high [17%]) [Hooijmans et al., 2014] (Table 4) and PWB-RoBC (low [75%], unclear [23%], and high [2%]) (Supplementary Table S3; https://doi.org/10.5281/zenodo.5550379). Wagner et al. 2010 [Wagner et al., 2010] was the only study to have at least three or more high RoB and, therefore, data pertaining to this study should be interpreted with caution. In the study, the control group differed from the PWB groups in terms of social and physical housing conditions (group-housed in standard vivarium cages vs. singly housed in custom PWB cages). This would have also compromised the blinding of intervention groups during the experiment due to the intervention and control groups being visually distinguishable. Additionally, the study maintained PWB stability within ±5% of the desired load for only 77% of the study days. The only other high RoBs related to recent studies utilizing rats whereby animals were allocated to intervention groups based on body weight (non-random method) and were unable to maintain caregiver/investigator blinding due to the PWB model requiring daily adjustment to maintain PWB stability. One of the main drawbacks was the infrequent reporting of potential RoB in study manuscripts. Future PWB studies would, therefore, benefit from utilizing the SYRCLE's RoB tool and recently developed PWB-RoBC [Swain et al.] to guide the development and reporting of study methods. This can help improve research transparency, reproducibility, and reduce uncertainties surrounding unclear RoB.

6. Limitations

To ensure a rigorous and transparent review process the present study conformed to methods by PRISMA [Moher et al., 2009], the Cochrane Handbook for Systematic Reviews of Interventions [Cochrane], and the Space Biomedicine Systematic Review Handbook

[Winnard et al, Winnard et al., 2021]. These methods help reduce bias associated with non-systematic reviews. For example, by having clearly defined review aims and objectives, an *a priori* study eligibility criteria, screening multiple online databases with relevant search terms, and using two independent and blinded reviewers to conduct the search screening and RoB scoring. Additionally, meta-analysis was conducted where possible to increase statistical power.

This review is not without limitations. Whilst the present review had access to the raw data from studies by Mortreux et al. that comprised the majority of the present findings, other studies sometimes reported sample sizes as a range (e.g., n = 7-11) where it was not possible to establish what the sample size was for a given outcome. Therefore, a conservative approach was used, extracting the lowest sample size reported. Due to the use of Hedges' G which corrects for small sample bias, this could have had either no effect or slightly underestimated effect sizes. It is recommended that to improve data extraction for readers and reviewers, the mean, standard deviation/error of measurement, and sample size be reported for each outcome measurement by default, either in the manuscript or supplementary materials. Some data had to be extracted through figures which despite the adopted data visualization tool being established as highly reliable and accurate [Aydin and Yassikaya, 2020], can still introduce small errors. Qualitative descriptions of the SMD magnitude (i.e., small, medium, large, very large) and thresholds used for heterogeneity scores are generic and should not be interpreted rigidly.

7. Conclusion

The present systematic review and meta-analysis showed that PWB20% (simulated Lunar gravity), PWB40% (simulated Martian gravity), and up to PWB70% (simulated moderate artificial gravity) causes rapid deconditioning of rodent skeletal muscle at the morphological and functional levels, particularly in those with an anti-gravity/postural role. The greatest changes occurred within the first 7 to 14 days of exposure typically by medium to very large effects. Partial weight-bearing at higher levels frequently attenuated deconditioning but often failed to prevent it from occurring. The hindlimb deconditioning patterns during PWB mimic those observed in rodents during exposure to microgravity/HLU and in the lower limbs of humans during exposure to microgravity/bed rest. These findings provide evidence to suggest that humans within Lunar and Martain gravities may be suspectable to hypogravity-induced deconditioning, particularly in the anti-gravity/postural muscles. Future research toward the long-term effects of hypogravity on the morphological and functional properties of human skeletal muscle is needed to inform countermeasure development for mitigating potential deconditioning and reduce risks to astronaut safety and operational performance.

Data availability

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

Disclosure

The authors declare no conflicts of interest.

Author contributions

P.S., A.W. and N.C. conceived and designed the review; P.S. and J.M.L. performed the literature search and screening; P.S. and M.M. collected study data; P.S., J.M.L. and H.K. conducted risk of bias assessment; P.S. analyzed data; P.S. prepared figures and tables; P.S. drafted the manuscript; P.S., M.M., H.K., E.D., A.W. and N.C. edited and revised the manuscript; P.S., M.M., J.M.L., H.K., E.D., A.W. and N.C. approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This study did not receive any funding.

Acknowledgements

The work of M. Mortreux is supported by the National Aeronautics and Space Administration grants NNX16AL36G and 80NSSC19K1598. The funding sources were not involved in the conduct, analysis, or interpretation of the current review.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lssr.2022.06.007.

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