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The Bone Biomarker Response to an Acute Bout of Exercise:

A Systematic Review with Meta-Analysis

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ABSTRACT

Circulating biomarkers are used to investigate the bone response to an acute bout of exercise, but heterogeneity in factors such as study design, quality, selected biomarkers and exercise and participant characteristics render it difficult to synthesize and evaluate available evidence. **PURPOSE:** To quantify the effects of an acute exercise bout on bone biomarkers, along with the influence of potential moderators such as participant, exercise and design characteristics, using a systematic review and meta-analytic approach. **METHODS:** The protocol was designed in accordance with PRISMA-P guidelines and prospectively published [1]. Seven databases were systematically searched in accordance with pre-defined eligibility criteria. Bayesian three-level hierarchical meta-analysis models were used to explore main effects of acute exercise on bone biomarkers, as well as potential moderating factors. Risk of bias for each individual study was evaluated using a modified version of the Downs and Black checklist, while certainty in each meta-analytic outcome was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. Modelled effect sizes were interpreted according to three metrics including: A) Evidence of an effect (defined by whether, or how much of, the CrI included zero); B) The size of that effect (defined by standard categories, namely threshold values of 0.01, 0.2, 0.5 and 0.8 were used to describe effect sizes as very small, small, medium and large, respectively); and C) The level of certainty in the estimated effect (defined using the GRADE framework). **RESULTS:** Across all designs and categories, a very small effect of exercise on markers of bone resorption ($ES_{0.5}$=0.10 [95%CrI: 0.00 to 0.21] and formation ($ES_{0.5}$=0.06 [95%CrI: 0.02 to 0.09] was found. Moderator analyses indicated that exercise type and impact loading influenced results, with a large effect identified for the bone resorption marker CTX-1 in response to long-duration cycling ($ES_{0.5}$=0.86 [95%CrI: 0.31 to 1.4]). The largest increases in CTX-1 occurred within 2 hours of exercise cessation. Exercise duration, intensity, and total work performed were also found to influence the bone biomarker response, albeit to a smaller degree. Certainty of evidence in most outcomes was deemed to be low or very low. **CONCLUSION:** Markers of bone resorption were most responsive to exercise and this was strongly influenced by exercise type and duration. Long-duration cycling induced a large effect on the resorption marker CTX-1, while other exercise types did not induce a response. All effects related to bone formation markers were very small and transient, calling into question the veracity and physiological relevance of these findings. The lack of a response to resistance or high impact exercise types indicate that these biomarkers may be more useful at investigating potentially osteolytic aspects of exercise, rather than its osteogenic potential. Certainty in all outcomes was low or very low, due to factors including risk of bias, lack of non-exercise controls, inconsistency, imprecision and small-study effects. Better control and standardization of future studies is required to increase certainty in results, and thus to advance understanding of the acute influence of exercise on bone.

Protocol Registration and Publication: This protocol was prospectively registered on the Open Science Framework Registry (https://osf.io/6f8dz) and underwent peer-review prior to conducting the investigation [1].

Keywords: exercise, loading, bone, remodeling, meta-analysis, systematic review

Key Points:

- Circulating bone biomarkers provide information on the current state of bone modelling and remodeling processes (mainly resorption and formation). This means that they have potential to be used as outcomes to investigate how bone responds to an acute exercise bout, along with how factors such as exercise type, duration, intensity or participant age, sex or training status may influence this response.

- A large body of evidence on the bone biomarker response to acute exercise exists, but results are largely inconsistent, with no consensus on the expected direction or magnitude of change. This is unsurprising given the wide range of exercise protocols and study designs employed. A comprehensive systematic review with meta-analysis can contribute toward reaching consensus on the available evidence base.

- The current study meta-analysed data from 88 studies comprising 1401 participants and 1805 effect sizes. Markers of bone resorption were more responsive to exercise than were markers of bone formation, and this was largely influenced by exercise type and duration. A large increase in CTX-1 (a marker of bone resorption) was shown in response to long-duration cycling, but not for any other exercise type. In contrast, the response of all bone formation markers was very small and transient across all investigated categories.

- The lack of a response to resistance or high impact exercise types indicate that these biomarkers may be more useful at investigating potentially osteolytic aspects of exercise, and raises questions about their suitability to investigate the osteogenic potential of different exercise types.

- Certainty in most outcomes was deemed to be low or very low, due to issues related to control and standardization of test procedures, inconsistency and imprecision in outcomes and small-study effects. Improvement of future study designs may be necessary to further advance understanding of this important topic area and recommendations have been made that may increase certainty in future investigations.

1. INTRODUCTION

Exercise interacts with bone via a range of mechanisms [2–4], including the direct influence of mechanical loading [5], activity specific metabolic signals, such as alterations to calcium kinetics [6], redox balance [7] or pH [8], and indirect signals mediated via other tissues, primarily skeletal muscle [9]. The direction and magnitude of these effects, however, vary widely. Activities that convey higher-impact, multi-directional and/or unaccustomed loading patterns convey the greatest osteogenic stimulus, and athletes who train in these modalities commonly have higher BMD and better bone strength indices than controls [10–12] or their counterparts from sports with lower, or repetitive loading cycles [12–15]. As such, guidelines for the use of exercise to improve bone strength generally recommend exercises that convey both ground and joint reaction forces (*e.g.,* impact and resistance-based modalities) [16–18]. Additionally, meta-analytic data indicate that this approach positively influences bone density in a range of populations, including pre [19] and postmenopausal [20] women, older adults [21], individuals with osteoporosis [22] and children [23]. Reported meta-analytic effects have, however, generally been small, and variable. Furthermore, there is evidence that bone may be negatively influenced by high-participation in certain sports, *e.g.,* those that emphasize leanness or that have lower-impact and/or repetitive loading cycles [24–27]. As described by Wherry and colleagues in a recent review [28], exercise provides a complex stimulus to the body, conveying a myriad of signals that may be either catabolic or anabolic to the bone – the influence of sustained exercise training on bone may ultimately depend on which of these processes dominate.

A better understanding of the exercise and participant characteristics that determine whether exercise will positively, negatively, or have no effect on bone is essential to improve exercise-based recommendations to improve bone health. This is, however, a challenging area of investigation, given that static indicators of bone health and function, such as bone mass measured by dual energy x-ray absorptiometry (DXA), microarchitecture as indicated by computed tomography (CT) or magnetic resonance imaging (MRI), are slow to respond to stimuli, with measurable changes taking months or even years to occur [29]. Circulating bone biomarkers provide information on the current state of bone modelling and remodeling processes, (mainly resorption and formation) and, as such, provide a means of identifying response to stimuli well in advance of changes to these more static indicators. Measurement of circulating bone biomarkers are widely used in the clinical setting [30–32]. They are also frequently used to make inferences regarding the bone response to acute or short-term interventions, such as exercise; however, the extent to which they can provide consistent, robust and meaningful information has yet to be established. Recently, our research group narratively synthesized available evidence on the bone biomarker response to exercise [33], and a number of general trends were apparent. For example, an increase in circulating concentrations of biomarkers indicative of bone resorption was the most commonly reported response [34–37], although some studies also reported an increase in markers of bone formation [36,38,39]. There was, however, large variation in most reported outcomes [33], rendering these findings difficult to synthesize and interpret. This ambiguity is unsurprising, given large variation in the design, characteristics and quality of available studies, but it does render onward progression of knowledge difficult. Quantitative synthesis of available data through systematic review and meta-analysis has potential to overcome these issues, and to address important questions in this area. For example, identification of which biomarkers are most likely to respond to exercise, and within which time-frames, along with what exercise characteristics are most likely to elicit a response will not only

advance our mechanistic understanding of how bone responds to exercise, but also inform the design of other studies. Additionally, combined effect estimates are essential to ensuring that future studies are appropriately powered. Finally, a systematic evaluation of potential sources of bias within the existing evidence base, can facilitate the development of recommendations, to inform better standardization and control of future work. A recent systematic review synthesized the bone biomarker response to an acute exercise bout in middle-aged and older adults [40], but to our knowledge no meta-analysis across the entire evidence base exists. Accordingly, the aim of the current investigation was to quantify the effect of exercise on bone biomarkers, along with how various exercise, participant, and study design characteristics may act as moderators, using a systematic review and meta-analytic approach.

2. METHODS

2.1. Overview

This review includes all items described in the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines [41] (checklist in Supplementary File 1) and the full protocol was prospectively peer-reviewed and published [1]. The PICOS (Population, Intervention, Comparator, Outcomes and Study Design) approach was used to guide the determination of eligibility criteria for study selection, and these are summarized in Table 1. Further detail and justification on the parameters of interest are provided in the accompanying codebook (Supplementary File 2), and/or in the published protocol [1].

Table 1: Eligibility Criteria, categorized according to the Population; Intervention; Comparator; Outcomes and Study Design (PICOS).

Population:	Males and females of any age, health or training status.
Intervention:	Single exercise bouts of any type, duration or intensity. Exercise interventions were categorized according to their type (resistance, aerobic, multi-modal, plyometric or calisthenics (including movement therapies such as yoga and tai-chi)), duration (minutes), intensity (percentage of maximum capacity), total work (defined as duration*intensity – arbitrary units) and impact level (high-impact/multi-directional; low-impact/repetitive; moderate-impact/repetitive; or low-impact with high muscular load).
Comparator:	Pre-post change in bone biomarkers following an acute exercise bout. Comparison of pre-post change between intervention and control conditions was not conducted as a prior review of the available evidence base indicated that this research design was infrequently used. Where available, non-exercise control data across the same time periods as the exercise bout were extracted and used to facilitate the interpretation of results.

Outcome:	All biomarkers commonly considered to be indicative of bone metabolism were considered for inclusion (see Supplementary File 2 for a full list of included biomarkers). CTX-1 and P1NP were considered to be the primary biomarkers of interest due to their designation as reference markers of bone resorption and formation [30,32,42]. Where available, biomarkers indicative of calcium metabolism (ionized or albumin adjusted calcium and parathyroid hormone) were extracted and considered as a secondary outcome of this review.
Study Design:	Any experimental study design that included measurement of bone biomarkers before and after an acute bout of exercise were considered for inclusion. This included randomized and non-randomized, parallel-group and cross-over, single or repeated measure experimental designs.

2.2. Search Strategy and Study Selection

Seven electronic databases were searched by ED. These were Medline, Embase, Cochrane CENTRAL, Sport Discus, PEDro, LILACS and IBEC. A combination of free text and database specific subject headings were used, with free text terms used being: bone AND (exercise OR physical activity) AND (biomarkers OR turnover OR remodelling OR formation OR resorption). Searches were limited to human studies, without restricting either the date or language. Only peer-reviewed studies published in scientific journals were considered for inclusion. In line with Cochrane Collaboration recommendations [45], the full strategy for the Medline search was submitted for peer review to an information scientist using the Peer Review for Electronic Search Strategy (PRESS) Guideline Assessment form [46] and that search was then replicated in all other databases (see Supplementary File 3 for the full search strategy used in each database). The Medline and Embase databases were searched using the OVID platform. The final searches were undertaken in December 2020 and results were uploaded to systematic review management software (covidence.org). A three-stage selection strategy was independently undertaken by ED and KK/AD and comprised (1) Title/Abstract Screen (2) Full Text Screen, and (3) Full Text Appraisal. The independent screeners were not blinded to any study information as blinding has previously been reported to neither statistically or clinically impact meta-analysis results [47]. Screeners convened at the end of each screening stage to resolve any discrepancies, which were resolved by discussion, or third-party mediation if required. The database searches were complemented by citation screening of all included studies along with relevant reviews and book chapters (Banfi et al. [43], Dolan et al. [33], Alp [44] and Smith [40]).

2.3. Data Extraction and Coding

Data were independently extracted and coded by at least 2 members of the review team (AD/LHMF and ED/GB). Data were extracted within the following categories: (1) study information (author, year, title, journal, funding and conflict of interest statement, aim, study design overview); (2) participant characteristics (sample size, sex, age, training status, health status, height, body mass, body mass index (BMI); (3) exercise test characteristics (type, intensity, duration, total work done, impact level); (4) blood sampling details (number, timing, whether the

participant was fed or fasted, bone biomarkers measured, sample type (*i.e.*., serum, plasma or urine)); (5) measurement process and inter and intra-assay variability; and (6) main outcomes (mean and standard deviation for each bone biomarker pre and post intervention). A complete description of the coding system applied is described in the accompanying codebook (see Supplementary File 2). If the primary outcomes (mean and standard deviation for each measured biomarker pre and post exercise) were not reported, the corresponding author from the relevant study was contacted to request this information (maximum of two email attempts).

2.4. Data Synthesis

A Bayesian framework was chosen over a frequentist approach as it allows for more flexible modelling and enables results to be interpreted intuitively through reporting of subjective probabilities [48]. The effects of exercise on bone biomarkers were quantified using standardized mean difference effect sizes (dividing by baseline standard deviation and accounting for small sample bias). Some of the included biomarkers act in an inhibitory manner (*e.g.*, sclerostin inhibits formation meaning that higher levels represent a reduction in the process of interest) and this was reflected by multiplying the relevant effect sizes by -1. Three-level random-effects Bayesian hierarchical models were used to pool effect sizes and model average effects (ES), variance within studies, variance between studies (τ^2), and covariance of multiple outcomes (Intraclass correlation coefficient: ICC) reported in the same study (*e.g.*, multiple bone biomarkers and/or single bone biomarkers reported at multiple time-points). Within-study variance is influenced by pre-post correlations [49] that are generally not reported. Therefore, primary data obtained from relevant studies (including those produced in the laboratories of the study team) were used to develop informative priors to model within study variances (Gaussian prior centered at r = 0.85 and range from approximately 0.70 to 0.99). Weakly informative priors (Student-t and half student-t with 3 degrees of freedom for intercepts and variance parameters, respectively) were used for all other model parameters. Inconsistency in models were described by comparing variances across the three levels. Inferences from all analyses were performed on posterior samples generated using the Hamiltonian Markov Chain Monte Carlo method and through use of credible intervals (CrI, 95% intervals for effect sizes and 75% intervals for variance parameters). Modelled effect sizes were interpreted according to the following three categories: A) Evidence of an effect (defined by whether, or how much of, the CrI included zero); B) The size of that effect (standard categories, namely, threshold values of 0.01, 0.2, 0.5 and 0.8 were used to describe effect sizes as very small, small, medium and large respectively [50]) and C) The level of certainty in each meta-analytic outcome (defined using the GRADE framework - see below).

Primary meta-analyses were conducted across outcomes from multiple biomarkers categorized as either: 1) bone formation; 2) bone resorption; 3) general bone remodelling; and 4) calcium metabolism. Sensitivity analyses were then conducted, presenting meta-analysis results for individual biomarkers in each category. Moderator analyses were conducted through meta-regression and selection of specific biomarkers (*i.e.*, P1NP, sclerostin and CTX-1). The moderators investigated included: 1) participant characteristics (age, sex, training status, health status); 2) exercise characteristics (type, duration, intensity, total work done, impact load); and 3) blood sampling characteristics (nutritional status, assay type, sample timing relative to exercise). A more detailed description of all coding categories is described in the accompanying codebook (see Supplementary File 2). Meta-regressions were performed when there was sufficient data including a minimum of four data points per category level, or 10

data points for continuous variables [51]. Small-study effects (publication bias, *etc.*) were visually inspected with funnel plots and quantified with a multi-level extension of Egger's regression-intercept test [52]. The importance of removing outliers to obtain more accurate estimates of meta-analysis parameters was identified in a previous large meta-analysis of exercise related effect sizes [53]. Outlier values were identified by adjusting the empirical distribution by a Tukey g-and-h distribution and obtaining the 0.01- and 0.99-quantiles, with values beyond these points removed prior to further analysis [54]. All analyses were performed using the R wrapper package *brms* interfaced with *Stan* to perform sampling [55].

2.5. Certainty in Cumulative Evidence

Certainty in meta-analytic outcomes was independently assessed in duplicate by ED and AD/KK using the Grading of Recommendations Assessment Development and Evaluation (GRADE) approach [56]. Potential downgrading factors included risk of bias, inconsistency, indirectness, imprecision or the presence of small-study effects. Risk of bias was assessed using a modified version of the Downs & Black Checklist [57]. As described in the published protocol [1], we opted to use this tool due to its flexibility with regard to study designs compared to other commonly used options (*e.g.*, ROB2 or NOS) that are designed to evaluate specific study designs. Modifications to the original tool were made to ensure it provided information directly relevant to this particular investigation. For example, some items were deemed unnecessary, either because they were specifically relevant to longitudinal interventions and therefore not required in an investigation of acute exercise bouts, or because they related to quality of reporting on factors that deemed unlikely to bias the specific outcomes of interest in this review (see Supplementary File 4 for the modified tool employed in this study). Despite our *a-priori* pragmatic decision to include studies that did not include a non-exercise control group, this does reduce certainty as to whether the reported outcomes directly relate to the intervention itself, or instead to some other, non-intervention related factors, *e.g.*, circadian variation [58]. As such, any data-point that did not include a non-exercise control group was downgraded on the basis of indirectness. Both risk of bias and indirectness assessments were conducted for each effect size assessed and the modal value selected. Consistency was ascertained using the meta-analysis results, and based upon visual inspection of effect size estimates, whether credible intervals overlapped, and on assessment of heterogeneity, with outcomes for which between study standard error (τ) was > 90% of the reported effect downgraded. Precision was judged based on the number of outcomes available and on visual analysis of the width of the credible intervals, with intervals that stretched across more than two of the aforementioned effect sized thresholds downgraded. Small-study effects (publication bias, *etc.*) was assessed using Egger's regression-intercept test along with visual inspection of funnel plots. Potential upgrading factors included the presence of large-effects, evidence of dose-response and the presence of plausible residual confounding factors.

2.6. Updates made since the published protocol

Within the original protocol [1], two secondary analyses were proposed including the potential influence of nutritional strategies on the bone biomarker response to exercise, and the bone biomarker response to natural experiments, namely observational studies that examined bone biomarkers before and after an athletic event. Given the amount of data available, and the complexity of analyses required, it was deemed unfeasible to address

these secondary questions within the current manuscript, and instead they will be described in subsequent stand-alone manuscripts. Additionally, some minor modifications were made to our risk of bias tool (see Supplementary File 4), to clarify the scoring. No other adaptations to the published protocol were made.

3. Results

3.1. Study Selection and Characteristics

Following the systematic search and selection, 88 articles comprising a total sample of 1401 participants and 1805 effect sizes were included in the review (see Figure 1 for the search flow diagram). These studies investigated a range of exercise types (aerobic [67.4% of effect sizes]; plyometric [15.4% of effect sizes]; resistance [13.7% of effect sizes]; multi-modal [2.7% of effect sizes]; and calisthenics [0.8% of effect sized]); intensities and durations. Studies were primarily conducted using young healthy male participants (56.8% of studies involved men only, 27.3% of studies involved women only and 15.9% involved mixed groups), with median (IQR) age of 25.2 (22.4 to 31.7 years). The most reported bone biomarkers within each process category were P1NP (formation: 206 outcomes; 37.1%); CTX-1 (resorption: 306 outcomes; 48.9%); total osteocalcin (general: 259 outcomes; 99.2%); and PTH (calcium metabolism: 207 outcomes 57.2%). An overview of all included studies is included in Supplementary File 5.

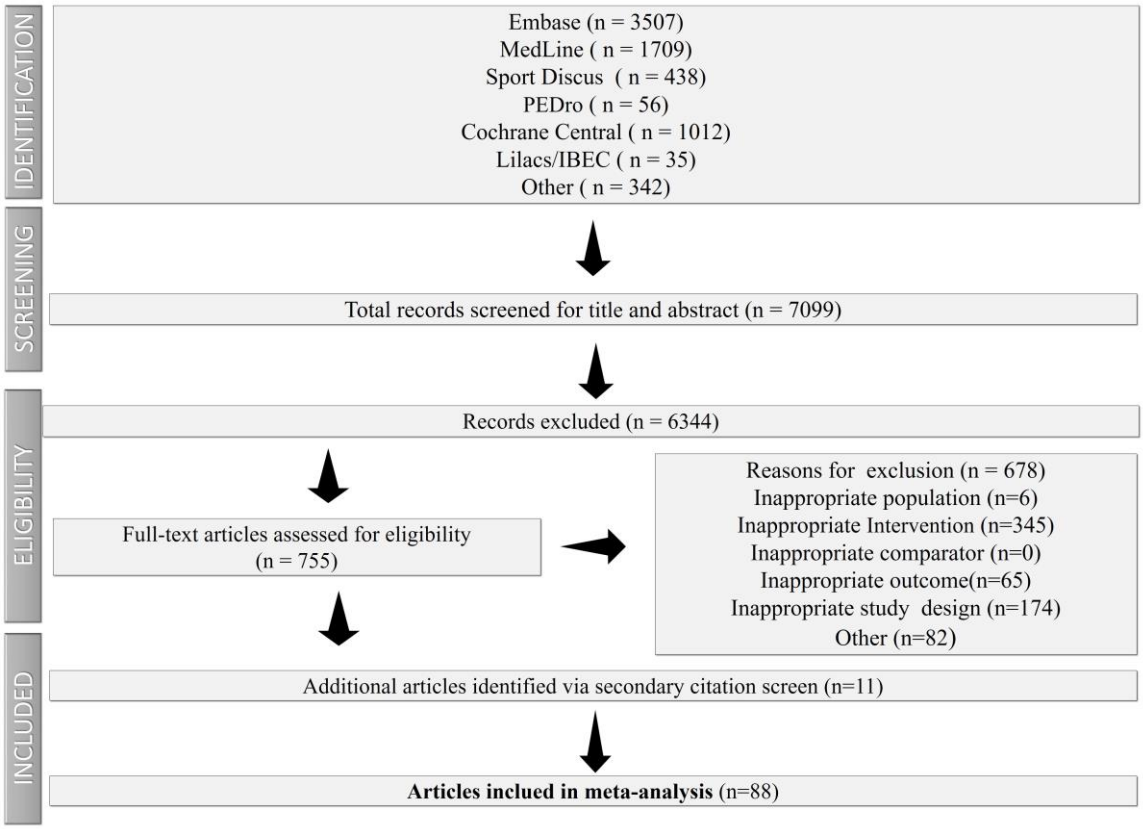

Figure 1. Search Flow Diagram

3.2. Certainty in Evidence

Mode certainty ratings for all studies following assessment of domains 1 (ROB) and 2 (indirectness) were "Moderate" (High = 33; Moderate = 40; Low = 15; Very Low = 0), and "Low" (High = 9; Moderate = 31; Low = 36; Very Low = 12), respectively. Nineteen studies (21.6%) included a non-exercise control group, while common issues arising from the appraisal checklist included: lack of test standardization in relation to time of day (26 studies; 29.5%); nutritional intake (56 studies; 63.6%) or physical activity (44 studies; 50%) in the days preceding the test; lack of familiarization to the exercise test protocol (51 studies; 57.9%) or lack of information on the nutritional conditions under which the exercise test was conducted (32 studies; 36.4%). Meta-analytic outcomes were largely inconsistent, as indicated by between study standard error (τ) values that were generally greater than the effect size estimate. Most outcomes were downgraded due to imprecision, as determined by credible intervals that stretched across more than 2 of our pre-defined standard effect size categories. In addition, all outcomes related to bone resorption and calcium metabolism were downgraded due to apparent small-study effects, as evidenced by substantial right-biased asymmetry in the funnel plots and results from Egger's regression-intercept tests (See Figure 2). Individual funnel plots and Egger's regression-intercept tests for the different bone process categories are presented in Supplementary File 6. Certainty ratings for each individual meta-analytic outcome are described within the relevant sections below, and in the accompanying Supplementary Files 7 – 10.

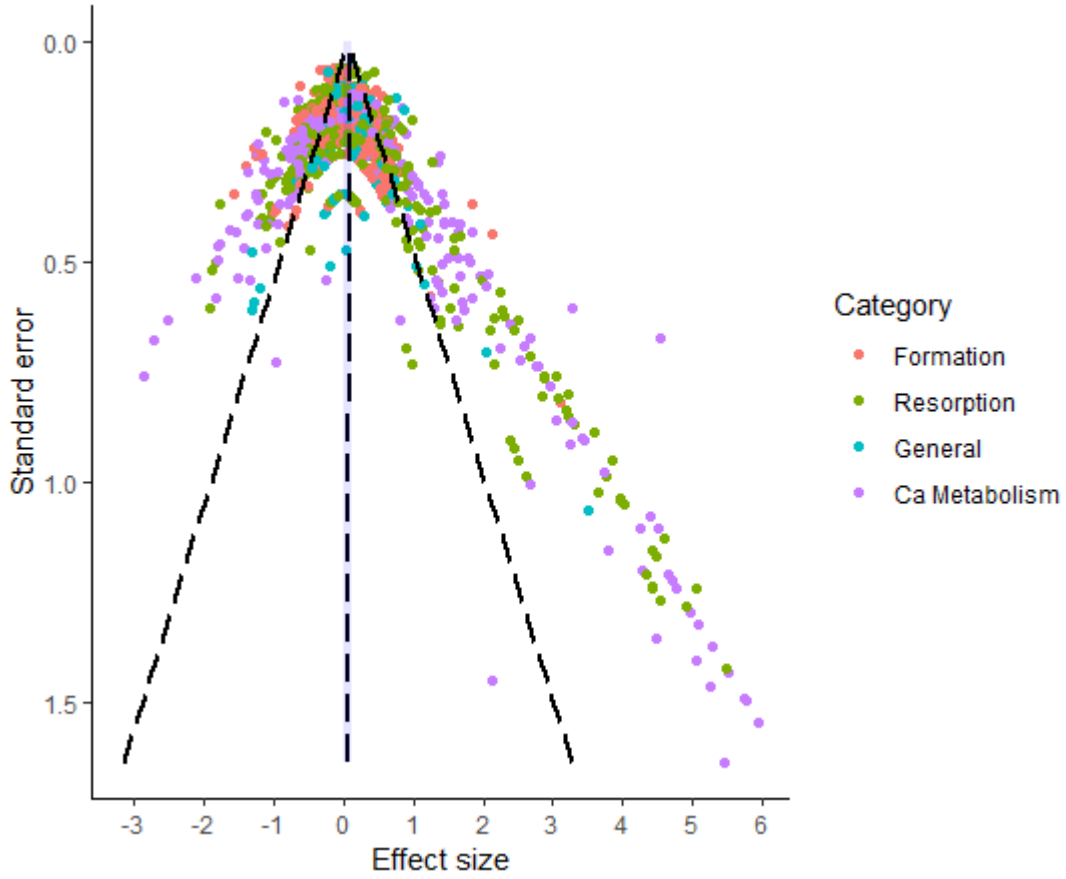

Figure 2: Funnel Plot (all outcomes)

3.3. The influence of exercise on bone resorption

Pooling of bone resorption markers across designs and categories indicated a very small effect of exercise ($ES_{0.5}$=0.10 [95%CrI: 0.00 to 0.21; very low certainty]; Figure 3, Panel A; Supplementary Table 7). Univariate analysis of each biomarker showed that the greatest increases from pre to post exercise bout were obtained for OPG ($ES_{0.5}$=-0.23 [95%CrI: -0.41 to -0.05; very low certainty]), CTX-1 ($ES_{0.5}$=0.16 [95%CrI: -0.01 to 0.34; very low certainty]), and ICTP ($ES_{0.5}$=0.10 [95%CrI: -0.03 to 0.26; very low certainty]) In contrast, CTX-1 control data (*i.e.*, data from studies that included a non-exercise control condition) provided some evidence of decreases across the intervention period ($ES_{0.5}$=-0.15 [95%CrI: -0.44 to 0.10; very low certainty]).

Moderator analyses were conducted with CTX-1, which is considered the reference marker of bone resorption [30,32] and was collected most frequently in the included studies. In relation to sample timing, very small to moderate effects were shown within 15 minutes after cessation of the exercise bout ($ES_{0.5}$=0.15 [95%CrI: -0.05 to 0.36; very low certainty]) and up to 2 hours post-exercise ($ES_{0.5}$=0.40 [95%CrI: -0.13 to 0.97; very low certainty]), while values similar to baseline were shown in samples collected > 2 hours post-exercise. Some evidence of an increase in CTX-1 was also obtained 72 hours after exercise ($ES_{0.5}$=0.23 [95%CrI: -0.05 to 0.53; very low certainty]). Exercise mode and impact level seemed to moderate the circulating CTX-1 concentration, with the largest increases identified from pre to post a bout of aerobic exercise ($ES_{0.5}$=0.25 [95%CrI: 0.01 to 0.49; very low certainty]) and low impact/repetitive loading type ($ES_{0.5}$=0.77 [95%CrI: 0.23 to 1.3; very low certainty]). Further moderator analyses within the aerobic exercise mode identified the greatest increases in CTX-1 following cycling ($ES_{0.5}$=0.86 [95%CrI: 0.31 to 1.4; very low certainty]) and continuous activities ($ES_{0.5}$=0.36 [95%CrI: 0.05 to 0.67; very low certainty]); with greater increases obtained with longer durations ($\beta_{0.5}$=0.14 [95%CrI: 0.09 to 0.19; very low certainty] per 10 mins) and increased total work done ($\beta_{0.5}$=0.27 [95%CrI: 0.21 to 0.34; very low certainty] per 1000 arbitrary units). No clear influence of sex on the CTX-1 response was identified. In contrast, the largest CTX-1 increases following the exercise bout were identified in participants categorized as well-trained, although studies that used prolonged cycling protocols also tended to recruit well-trained athletes and this may have confounded this result. Insufficient data were available to investigate whether age would moderate these results.

3.4. Bone Formation

Pooling of all bone formation markers across all designs and categories showed a very small effect of exercise ($ES_{0.5}$=0.06 [95%CrI: 0.02 to 0.09; low certainty]; Figure 3, Panel C; Supplementary File 8). Univariate analysis of each biomarker showed very small increases in P1NP ($ES_{0.5}$=0.08 [95%CrI: 0.03 to 0.14; low certainty]) and sclerostin ($ES_{0.5}$=0.13 [95%CrI: 0.24 to 0.00; moderate certainty]). No evidence of a change in non-exercise controls was identified ($ES_{0.5}$=-0.03 [95%CrI: -0.08 to 0.02; low certainty]), indicating that bone formation markers were stable over the periods investigated. Moderator analyses were conducted for both P1NP and sclerostin separately. In relation to sample timing, the largest effects for P1NP were shown within 15 minutes of exercise cessation ($ES_{0.5}$=0.19 [95%CrI: 0.10 to 0.28; low certainty), with no evidence of change over 24 to 48 hours. Very small increases were identified pre to post aerobic exercise bouts ($ES_{0.5}$=0.11 [95%CrI: 0.07 to 0.17; moderate certainty]) and similar increases were shown for both low ($ES_{0.5}$=0.09 [95%CrI: -0.02 to 0.20; very low

certainty]) and moderate impact loading ($ES_{0.5}$=0.11 [95%CrI: 0.05 to 0.18; moderate certainty]). There was evidence of small increases in P1NP concentrations with increased work ($\beta_{0.5}$=0.02 [95%CrI: 0.00 to 0.04; low certainty] per 1000 arbitrary units). There was no evidence of a moderating effect of sex or training status, and insufficient data were available to assess the influence of age (Supplementary File 8). In relation to sclerostin, consistently small increases were shown across available moderator analyses (Supplementary File 8). In common with P1NP, very small increases were evident immediately post the exercise bout ($ES_{0.5}$=0.24 [95%CrI: -0.06 to 0.53; low certainty]), but returned to baseline within 15 minutes ($ES_{0.5}$=0.05 [95%CrI: -0.16 to 0.27; very low certainty]). Very small increases were also observed 24 hours post-exercise ($ES_{0.5}$=0.16 [95%CrI: -0.07 to 0.38; very low certainty]), while insufficient data were available to assess proceeding days. There was no evidence of a moderating effect of exercise type, impact level or participant characteristics.

3.5. *General Bone (re)modelling*

There was a very small effect of exercise on total osteocalcin concentrations ($ES_{0.5}$=0.04 [95%CrI: 0.00 to 0.09; low certainty]; Figure 3, Panel D; Supplementary Table 9). Moderator analyses were conducted on total osteocalcin only, small increases were shown immediately following exercise ($ES_{0.5}$=0.08 [95%CrI: 0.00 to 0.17; low certainty]) and up to 2-hours post exercise ($ES_{0.5}$=0.06 [95%CrI: -0.01 to 0.16; very low certainty]). Moderator analyses did not identify clear patterns across categories, but provided evidence of very small increases in osteocalcin with increased work ($\beta_{0.5}$=0.03 [95%CrI: 0.00 to 0.08] per 1000 arbitrary units; low certainty).

3.6. PTH and calcium

A moderate increase in PTH was shown pre to post exercise ($ES_{0.5}$=0.60; 95%CrI: 0.27 to 0.93); very low certainty]. The median point estimate for ionized calcium (iCA) was negative, but the credible intervals were wide and included a range of positive values ($ES_{0.5}$=-0.43 [95%CrI: -1.2 to 0.20; very low certainty]. Moderator analyses were conducted on PTH only, and indicated a large increase in PTH within 15 minutes of finishing the exercise bout ($ES_{0.5}$=1.4 [95%CrI: 0.80 to 1.9; very low certainty], while values were equivalent to baseline at all other time points. Responses varied substantially according to impact level, with low ($ES_{0.5}$=0.95 [95%CrI: 0.01 to 1.9; very low certainty] and moderate ($ES_{0.5}$=0.82 [95%CrI: 0.36 to 1.3; very low certainty] impact exercise types with repetitive loading cycles showing large increases, while exercise protocols that induced low impact but high muscular loads showing some evidence of small decreases ($ES_{0.5}$=-0.26 [95%CrI: -0.50 to -0.04; low certainty]. All results are summarized in Supplementary File 10 and in Figure 3, Panel B.

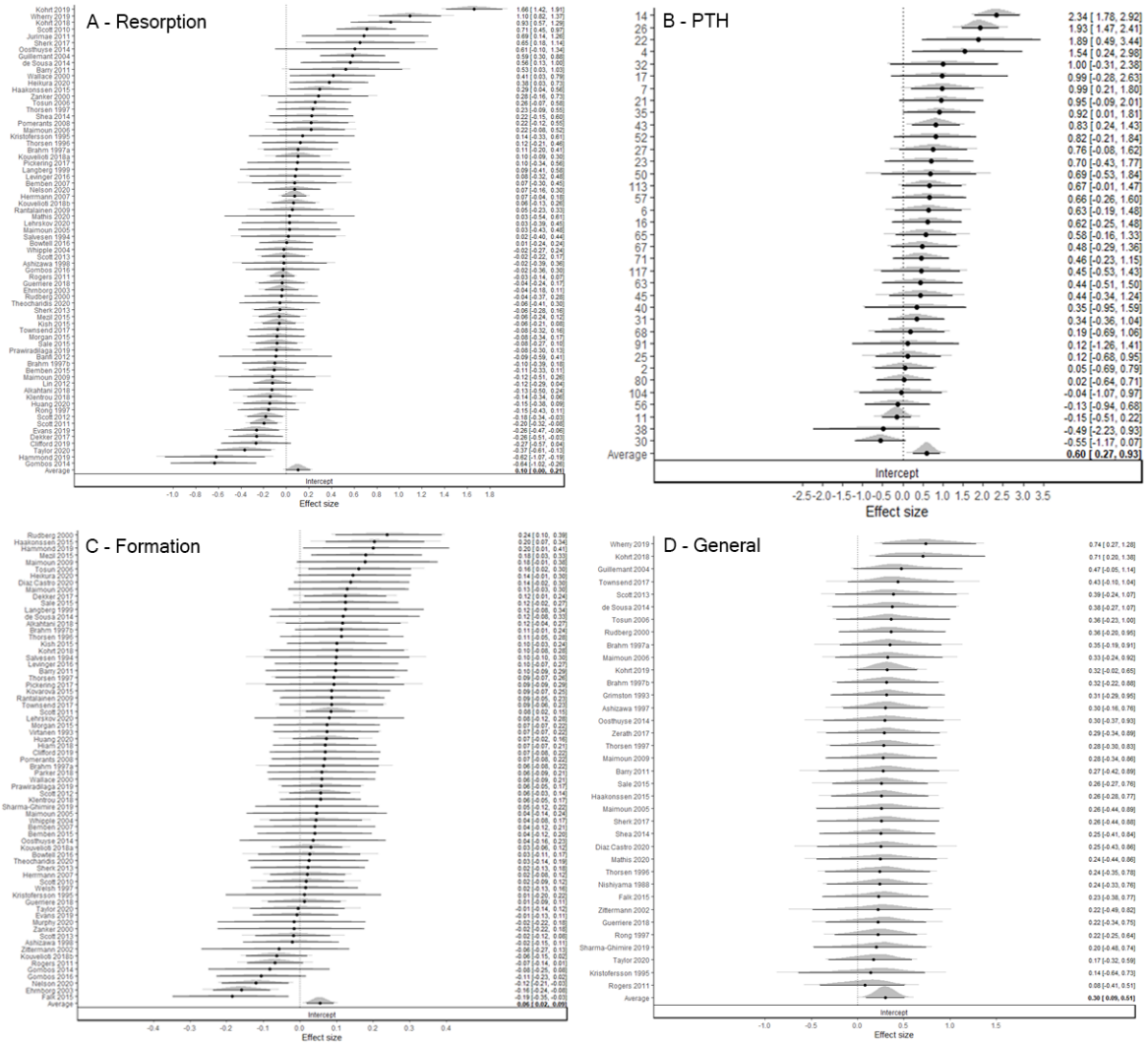

Figure 3: Forest plots illustrating meta-analysis results across the different bone biomarker categories

Legend: Distributions represent "shrunken estimates" based on all relevant effect sizes, the random effects model fitted, and borrowing of information across studies to reduce uncertainty. Black circles and connected intervals represent the median value and 95% credible intervals for the shrunken estimates.

4. Discussion

The key findings from this large and comprehensive systematic review and meta-analysis are as follows: (1) Pooling of outcomes across all designs and categories indicated that an acute exercise bout increased bone resorption and formation markers, but the combined effects were very small and highly variable – moderator analyses revealed the source of some of this variability. (2) Exercise type and impact level influenced the bone resorptive response, and continuous, long-duration cycling induced a large increase in CTX-1, whereas other exercise types did not influence this biomarker. Changes to all bone formation markers were very small and transient, with no major trends identified across the moderating categories investigated (3) The bone biomarker response to exercise was dose-responsive, with increasing durations and the total amount of work done leading to larger increases. (4) The bone biomarker response to exercise is time-sensitive. For example, P1NP and PTH increased immediately post-exercise, but returned to baseline values within 15 minutes, whereas CTX-1 peaked within 15 minutes and 2 hours after the exercise bout; 5) An important caveat to all findings reported herein is that certainty in estimates were low or very low, which was mainly due to a lack of a non-exercise control group against which to compare the exercise response; lack of standardization of factors including nutritional status and time of day; inconsistency and imprecision in observed outcomes, and in the case of outcomes based on bone resorption and calcium metabolism markers, evidence of small-study effects.

4.1. Physiological Interpretation

This systematic review and meta-analysis indicated that bone resorption markers were more responsive to acute exercise than were bone formation markers. Considered collectively, and across all designs, categories and biomarkers, a very small increase in bone resorption was observed, and this was primarily driven by changes in CTX-1 and ICTP. Given that different biomarkers represent different aspects of the bone resorptive process, we chose to focus our moderator analyses on CTX-1 because it is considered to be the reference marker for bone resorption, it was the most frequently measured, and the initial main analysis indicated that it showed the largest increase in response to exercise. Interestingly, non-exercise control data provided some evidence of a reduction in CTX-1 across the same time periods, which is consistent with what is known about its circadian variation, namely that it peaks in the early morning (approximately 05.00), before reaching its nadir at approximately 14.00 [58]. Given that most of the studies included within this review were conducted in the morning, these opposing effects (*i.e.,* an exercise-induced increase versus a natural circadian decline) could indicate that the true effect of exercise is larger than reported herein and highlights the importance of non-exercise control data in studies of this kind (as discussed within the Implications for Research and Practice section).

Increased resorptive activity in response to acute exercise has two, non-mutually exclusive, possible interpretations. It could be that this initial increase in catabolic activity is necessary to activate the bone remodeling cycle [4,59] and that an acute increase in bone resorption could subsequently trigger reversal, and an eventual increase in bone formation, which if sustained could lead to a positive adaptive response of bone to exercise in the long-term. An alternative hypothesis is that, if unchecked, large increases in bone resorptive activity in response to certain exercise types may eventually lead to bone loss, and increased fragility if sustained in the long-term. These contrasting hypotheses have very different practical implications, given that one would suggest that strategies to maximise the initial bone resorptive response to exercise may be to the bone's long-term benefit,

whereas the other would encourage development of strategies to minimize this initial bone resorptive response. In reality, both hypotheses are plausible depending on the circumstances, however our results do favor the latter hypothesis. The most striking outcome from this meta-analysis was that continuous, long-duration cycling induced a large CTX-1 response, while other exercise types had only a very small, or no, effect on this biomarker. Long-duration cycling conveys low-impact, repetitive, loading patterns and is considered to be a "non-osteogenic" exercise type. Indeed, road cyclists are considered to be a group at high risk of low bone mass [60] and a number of studies have reported lower bone mass in cyclists compared to non-athlete control groups [61–63]. As such, it seems plausible that prolonged exposure to exercise stimuli that induce large increases in bone resorption may be detrimental over the longer-term, preventive strategies may be warranted.

A milieu of exercise-induced metabolic changes may have contributed to the identified increases in bone resorption, including pH [8], calcium [6] or redox [7] perturbations. Of these, calcium perturbations has received the most research attention [28]. Exercise-induced reductions to serum calcium may trigger increased PTH secretion, which in turn stimulates osteoclast activation - the subsequent increase in bone resorptive activity releases calcium from the bone, which can then be used to normalize circulating levels. This mechanistic pathway was investigated by Kohrt and colleagues [6], whereby stable serum calcium levels in a group of male cyclists were maintained throughout a 60-minute vigorous cycling bout via intravenous clamp infusion. The maintenance of serum calcium availability attenuated, but did not fully prevent, exercise induced increases in PTH and CTX-1, implying that serum calcium has a role to play in mediating the bone resorptive response to cycling, although other factors (*e.g.,* PO4, pH or redox balance) are also likely to contribute. This perspective is also supported by the results of the current meta-analysis. Ionized calcium declined post-exercise (albeit with wide CrIs that included positive values), whereas PTH increased from pre to post exercise bout. Interestingly, this PTH increase peaked immediately after the exercise bout, and quickly returned to baseline within approximately 15 minutes. In contrast, CTX-1 appeared to peak within the first 2 hours after exercise, which makes sense given that it may have been triggered by an initial increase in PTH. These data highlight the importance of sample timing when interpreting biological data, given that it may not be possible to observe responses in both the "effector" (PTH) and "effectee" (CTX-1) within the same blood sample.

Across all designs, categories and biomarkers, a very small effect of acute exercise on markers indicative of bone formation was shown, and this was primarily driven by very small increases in P1NP and sclerostin. An acute increase in bone formation in response to exercise could imply that exercise can induce modelling-based formation (*i.e.,* formation that is uncoupled to resorption), but timing analyses indicate that this is unlikely. P1NP peaked immediately post-exercise but quickly returned to baseline. P1NP is an indicator of type 1 collagen deposition, and although it seems plausible that acute exercise could activate the process of formation, it is unlikely that new collagen could be formed and deposited within such short time-periods. As such, a true exercise-induced increase in P1NP that is indicative of collagen deposition should not, theoretically, be observed for some time after the exercise bout. Instead, the observed transient increases in P1NP may relate to some biological artefact, such as exercise-induced damage causing a small leak of connective tissue contents into the circulation, or potentially to hemodynamic shifts. Interestingly, increased P1NP is more frequently shown in response to exercise training [64,65], as discussed in our recent narrative review [33]. Biologically, a chronic, as opposed to acute, response of

P1NP to exercise is more plausible given the time required for the formation and deposition of new collagen within bone.

Sclerostin exerts a downregulatory effect on bone formation, through inhibiting the canonical Wnt/β-catenin signaling pathway [66]. If acute exercise promotes bone formation it would therefore be expected that the activity of this osteokine would be reduced, as has been observed in a study that reported reduced osteocyte SOST/sclerostin activity in mechanically stimulated bone [67]. This was not the case, however, and the results of the current meta-analysis indicate that exercise may acutely increase circulating sclerostin levels. In common with P1NP, these increases occurred immediately after the exercise bout, before quickly returning to baseline values and it is plausible to consider that they may have occurred due to similar artefacts, *e.g.*, a release of previously synthesized sclerostin from the osteocytes [68], or to hemodynamic shifts. Thirty-nine percent of available studies corrected their results for plasma volume (PV). Previous studies have reported no difference in bone biomarker outcomes in PV adjusted versus unadjusted analyses [6,69,70], however, it is possible that any potential changes may have been too small to be detected in single studies, and instead may only have been observed when multiple studies were pooled. Very small increases in total osteocalcin across all exercise types were also observed. This osteokine is frequently described as an indicator of bone formation, however, it may also be liberated during bone resorptive processes, and as such, is better described as a general indicator of bone metabolism [71]. It should also be highlighted that osteocalcin fulfils multiple functions, many of which may be influenced by exercise (*e.g.*, glucose regulation [72]) and as such, changes cannot be assumed to relate solely to altered bone metabolism. Indeed, uncarboxylated osteocalcin, which is a better indicator of bone formation, was found in this review to be unaffected by exercise. Considered collectively, the available evidence based on all relevant biomarkers indicates that the very small and transient increases observed may have been spurious, and unlikely to accurately represent changes to bone forming processes.

An interesting finding from this study is that exercise types deemed non-osteogenic (*i.e.*, lower impact activities with repetitive loading cycles) induced the greatest bone biomarker response, and more specifically, a large bone resorptive response. In contrast, little evidence was obtained to support a bone biomarker response to activities that are considered to have the greatest osteogenic potential (*e.g.*, activities with high gravitational or muscular loads). This finding calls into question the validity of these circulating biomarkers to predict or precede an adaptive response in parameters such as bone mass or structure. A number of potential explanations for these findings exist. Total work done, exercise duration and exercise intensity all emerged as likely moderators of the bone biomarker response, and it is possible that the available protocols were not of sufficient time or duration to elicit a response. This explanation seems unlikely, however, given that relatively few, high-impact, loading cycles are required to stimulate a bone response [4,16], meaning that very long, or intense, protocols should not be required, provided the mechanical strain is high enough. It seems, therefore, that circulating bone biomarkers may be more responsive to exercise induced metabolic signals such as pH, Ca^{++} and redox perturbations, most of which are known to be catabolic to bone, than to mechanical signals induced by loading, which are generally considered to be anabolic to bone. Certainly, this theory is speculative and requires empirical testing, but if correct, it would have substantive implications for the way in which commonly used biomarkers are used and implies that they may be more useful to investigate strategies to prevent potentially osteolytic signals (as may occur, for example, during long duration cycling), rather than in investigating the osteogenic potential of different exercise types.

4.2. Study Strengths and Limitations

The main strength of this study is its comprehensiveness and depth of analysis. The inclusion of all available study designs allowed for evaluation of a wide range of potential moderating variables and thus will be applicable to a wide range of situations. The investigation also has a number of limitations, which should be considered when interpreting the results and findings. For example, disparate study designs rendered designation of coding categories difficult. We attempted to be as explicit as possible when defining our coding categories (see codebook in Supplementary File 2), but many were difficult to objectively define and/or were incompletely described within the included articles (*e.g.*, definitions of training status, or categorization of exercise intensity). We also made an *a-priori* decision to be inclusive, and not to exclude any study based on its design. This decision allowed for a systematic evaluation of potential sources of bias within the existing evidence base. It is, however, important to consider that all meta-analyses inherit the limitations of their included studies, and application of the GRADE analysis resulted in an overall low, or very low, level of certainty in most outcomes reported herein. Most of the studies included in this analysis (74%) did not include a non-exercise control group, and this renders it difficult to isolate reported findings to the exercise bout itself. As previously reported [58], and confirmed herein, certain biomarkers, such as CTX-1 have a circadian variation, and failure to account for this (and other potential sources of variation unrelated to the exercise intervention itself) likely impeded accurate effect quantification. Importantly, a lack of standardization of important factors, such as time of day of testing, exercise and feeding practices in the days prior to testing, and the nutritional status of the participants at the time of testing may have introduced considerable noise to these investigations, rendering it difficult to detect small signals. This noise may have contributed (at least in part) to the large variability shown both within and between studies. We investigated a wide range of potential moderating variables, however, imbalances of important moderators may have influenced results and subsequent interpretations. For example, CTX-1 showed large increases in response to long-duration cycling. Highly-trained individuals also appeared to have larger CTX-1 increases than their lesser trained counterparts. But only highly-trained individuals are capable of undergoing a long-duration cycling test, and so it is difficult to separate these findings. Finally, evidence of small-study effects was apparent for outcomes related to bone resorption and calcium metabolism, as evidenced by substantial right-based asymmetry in the funnel plot (Figure 2). This may represent publication bias toward positive findings, or potentially to unusual homogeneity in some samples, potentially leading to an artificial inflation of these effect size estimates [73].

4.3. Implications for Future Research

The results of this investigation have addressed a number of important questions regarding the bone biomarker response to an acute exercise bout, and in turn, these results have opened up new avenues for investigation. Our results indicated that long-duration cycling induces a large increase in CTX-1, which may be deleterious to bone in the long-term, if unmatched by a concomitant increase in processes of bone formation. But the ability of acute changes in bone biomarkers to predict future changes in static bone indicators such as its mass or micro-architecture has yet to be ascertained. Longer-term studies, with multiple sampling points, are required to investigate how these acute changes may translate in the long-term. It is interesting that bone biomarkers seem to be less responsive to exercise types commonly considered to be osteogenic (*e.g.*, jump or resistance-based

modalities) than they were to exercise types generally deemed as non-osteogenic (*e.g.*, cycling). As described above, this result led us to speculate that these biomarkers are more responsive to exercise induced metabolic signals (*e.g.*, calcium, pH or redox perturbations) than to mechanical strain. This hypothesis, however, requires empirical testing.

In order for ongoing studies to be informative, strategies to overcome the prevalent sources of bias inherent within the existing evidence must be implemented. As described above, a lack of standardization of important factors, such as time of day of testing, exercise and feeding practices in the days prior to testing, and the nutritional status of the participants at the time of testing may have introduced considerable noise to these investigations and rigorous standardization of these factors in future work may help to isolate the influence of the exercise bout itself. The use of reporting guidelines that are specific to this type of investigation (*e.g.*, the PRESENT checklist [74]) may be useful in both the design and reporting of future work, while the effect sizes reported herein may facilitate estimation of the samples required to adequately power future work. Importantly, inclusion of a non-exercise control group can further facilitate isolation of reported results to the intervention of interest and we recommend that non-exercise control groups are included in future studies. Finally, sample timing is important. As identified within the current analysis, PTH peaked within 15 minutes of the exercise bout, while CTX-1 seemed to peak within 2 hours post-exercise. As such, and for studies where an increase in bone resorptive activity is expected, repeated sampling for at least 2 hours post exercise is preferable to discrete samples taken immediately post-exercise.

5. Summary and Conclusion:

The primary finding from this review is that only long-duration cycling induces a consistent bone biomarker response, and this is evident only in CTX-1 – a marker of bone resorption. Given that cycling is an unloaded exercise type, this increase was likely triggered by metabolic factors, such as calcium, pH or redox perturbations. The lack of a response to resistance, or high impact exercise types indicate that these biomarkers may be more useful at investigating potentially osteolytic aspects of exercise, and raises questions about their capacity to investigate the osteogenic potential of different exercise types. Very large between and within-study variability was shown, which may have been influenced by a combination of controllable factors, including a lack of standardization and non-exercise control groups. Enhanced harmonization of ongoing research efforts may facilitate these barriers to be overcome, and lead to more efficient and informative use of these biomarkers in the future.

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None of the authors declare any conflicts of interest relevant to the content of this review.

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