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1 **The Bone Biomarker Response to an Acute Bout of Exercise:**

2 **A Systematic Review with Meta-Analysis**

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26 **ABSTRACT**

27 Circulating biomarkers are often used to investigate the bone response to an acute bout of exercise, but
28 heterogeneity in factors such as study design, quality, selected biomarkers and exercise and participant
29 characteristics render it difficult to synthesize and evaluate available evidence. **PURPOSE:** To quantify the
30 effects of an acute exercise bout on bone biomarkers, along with the influence of potential moderators such as
31 participant, exercise and design characteristics, using a systematic review and meta-analytic approach.
32 **METHODS:** The protocol was designed in accordance with PRISMA-P guidelines and prospectively published.
33 Seven databases were systematically searched in accordance with pre-defined eligibility criteria. Bayesian three-
34 level hierarchical meta-analysis models were used to explore main effects of acute exercise on bone biomarkers,
35 as well as potential moderating factors. Modelled effect sizes were interpreted according to three metrics namely:
36 A) Evidence of an effect (defined by whether, or how much of, the CrI included zero); B) The size of that effect
37 (threshold values of 0.01, 0.2, 0.5 and 0.8 were used to describe effect sizes as very small, small, medium and
38 large, respectively); and C) The level of certainty in the estimated effect (defined using the GRADE framework).
39 **RESULTS:** Pooling of outcomes across all designs and categories indicated that an acute bout of exercise
40 increased bone resorption ($ES_{0.5}=0.10$ [95%CrI: 0.00 to 0.20] and formation ($ES_{0.5}=0.05$ [95%CrI: 0.01 to 0.08]
41 markers, but the effects were very small and highly variable Moderator analyses revealed the source of some of
42 this variability and indicated that exercise type and impact loading influenced the bone resorptive response. A
43 moderate increase in CTX-1 was observed in response to cycling ($ES_{0.5}=0.65$ [95%CrI: 0.20 to 0.99]), with greater
44 durations and more work leading to larger CTX-1 increases. CTX-1 response peaked within 15 minutes and 2
45 hours after the exercise bout. Other exercise types did not influence CTX-1. Changes to all bone formation
46 markers were very small and transient, with the very small increases returning to baseline within 15 minutes of
47 exercise cessation. No major trends for bone formation markers were identified across any of the moderating
48 categories investigated. Certainty of evidence in most outcomes was deemed to be low or very low.
49 **CONCLUSION:** The large influence of an acute bout of prolonged cycling on the bone resorption marker CTX-
50 1, alongside the lack of a response of any biomarker to resistance or high-impact exercise types, indicate that these
51 biomarkers may be more useful at investigating potentially osteolytic aspects of exercise, and raises questions
52 about their suitability to investigate the osteogenic potential of different exercise types, at least in the short term
53 and in response to a single exercise bout. Certainty in all outcomes was low or very low, due to factors including
54 risk of bias, lack of non-exercise controls, inconsistency, imprecision and small-study effects.

55 **Protocol Registration and Publication:** This investigation was prospectively registered on the Open Science
56 Framework Registry (<https://osf.io/6f8dz>) and the full protocol underwent peer-review prior to conducting the
57 investigation.

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63 **Key Points:**

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- 65 • Circulating bone biomarkers are frequently used as outcomes in studies investigating the bone response
66 to acute exercise, but results are largely inconsistent, with no consensus on the expected direction and
67 magnitude of change of specific biomarkers.
 - 68 • This meta-analysis indicated a moderate increase in the bone resorption marker CTX-1 only in response
69 to an acute bout of activities with low impact and repetitive loading cycles (e.g., cycling), with greater
70 durations and more work leading to larger increases. In contrast, the response of all bone formation
71 markers was very small and transient across all investigated categories.
 - 72 • The lack of a response to a single bout of resistance or high impact exercise types indicate that these
73 biomarkers may be more useful at investigating potentially osteolytic aspects of acute exercise bouts,
74 and raises questions about their suitability to investigate the osteogenic potential of different exercise
75 types, at least in the short term.
 - 76 • Certainty in most outcomes was deemed to be low or very low, due to issues related to control and
77 standardization of test procedures, inconsistency and imprecision in outcomes and small-study effects.

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101 **1. INTRODUCTION**

102 Exercise interacts with bone via a range of mechanisms [1–3], including the direct influence of mechanical loading
103 [4], activity specific metabolic signals, such as alterations to calcium kinetics [5], redox balance [6] or pH [7],
104 and indirect signals mediated via other tissues, primarily skeletal muscle [8]. The direction and magnitude of these
105 effects, do however, vary widely. Activities that convey higher-impact, multi-directional and/or unaccustomed
106 loading patterns convey the greatest osteogenic stimulus, and athletes who train in these modalities commonly
107 have higher bone mineral density (BMD) and better bone strength indices than controls [9–11] or their
108 counterparts from sports with lower, or repetitive loading cycles [11–14]. As such, guidelines for the use of
109 exercise to improve bone strength generally recommend exercises that convey both ground and joint reaction
110 forces (*e.g.*, impact and resistance-based modalities) [15–17]. Meta-analytic data indicate that this approach
111 positively influences bone density in a range of populations, including pre [18] and postmenopausal [19] women,
112 older adults [20], individuals with osteoporosis [21] and children [22]. Reported meta-analytic effects have,
113 however, generally been small and variable. Furthermore, there is evidence that bone may be negatively
114 influenced by high-participation in certain sports, *e.g.*, those that emphasize leanness or that have lower-impact
115 and/or repetitive loading cycles [23–26]. As described by Wherry and colleagues in a recent review [27], exercise
116 provides a complex stimulus to the body, conveying a myriad of signals that may be either catabolic or anabolic
117 to the bone and the influence of sustained exercise training on bone may ultimately depend on which of these
118 processes dominate.

119 A better understanding of the exercise and participant characteristics that determine whether exercise will
120 positively, negatively, or have no effect on bone is essential to improve exercise-based recommendations to
121 improve bone health. This is, however, a challenging area of investigation, given that static indicators of bone
122 health and function, such as bone mass measured by dual energy x-ray absorptiometry (DXA), microarchitecture
123 as indicated by computed tomography (CT) or magnetic resonance imaging (MRI), are slow to respond to stimuli,
124 with measurable changes taking months or even years to occur [28]. Circulating bone biomarkers provide
125 information on the current state of bone modelling and remodeling, (mainly resorption and formation) and, as
126 such, provide a means of identifying response to stimuli well in advance of changes to static indicators.
127 Measurement of circulating bone biomarkers are widely used in the clinical setting [29–31]. They are also
128 frequently used to make inferences regarding the bone response to acute or short-term interventions, such as
129 exercise; however, the extent to which they can provide consistent, robust and meaningful information has yet to
130 be established. Recently, our research group narratively synthesized available evidence on the bone biomarker
131 response to acute exercise bouts and to chronic exercise training [32], and a number of general trends were
132 apparent. For example, an increase in circulating concentrations of biomarkers indicative of bone resorption was
133 the most commonly reported response to an acute exercise bout [33–36], although some studies also reported an
134 increase in markers of bone formation [35,37,38]. There was, however, large variation in most reported outcomes
135 [32], rendering these findings difficult to synthesize and interpret. This ambiguity is unsurprising, given large
136 variation in the design, characteristics and quality of available studies, but it does render onward progression of
137 knowledge difficult. Quantitative synthesis of available data through systematic review and meta-analysis has
138 potential to overcome these issues, and to address important questions in this area. For example, identification of
139 which biomarkers are most likely to respond to acute exercise, and within which time-frames, along with what

140 exercise characteristics are most likely to elicit a response will not only advance our mechanistic understanding
 141 of how bone responds to exercise, but also inform the design of future studies. Additionally, combined effect
 142 estimates are essential to ensuring that future studies are appropriately powered. Finally, a systematic evaluation
 143 of potential sources of bias within the existing evidence base, can facilitate the development of recommendations,
 144 to inform better standardization and control of future work. A recent systematic review synthesized the bone
 145 biomarker response to an acute exercise bout in middle-aged and older adults [39], but to our knowledge no meta-
 146 analysis across the entire evidence base exists. Accordingly, the aim of the current investigation was to quantify
 147 the effect of exercise on bone biomarkers, along with how various exercise, participant, and study design
 148 characteristics may act as moderators, using a systematic review and meta-analytic approach.

149

150 2. METHODS

151 2.1. Overview

152 This review includes all items described in the Preferred Reporting Items for Systematic Review and Meta-
 153 Analysis (PRISMA) 2020 guidelines [40] (checklist in Supplementary File 1) and the full protocol was
 154 prospectively peer-reviewed and published [41]. The PICOS (Population, Intervention, Comparator, Outcomes
 155 and Study Design) approach was used to guide the determination of eligibility criteria for study selection, and
 156 these are summarized in Table 1. Further detail and justification on the parameters of interest are provided in the
 157 accompanying codebook (Supplementary File 2), and/or in the published protocol [41].

158

159 **Table 1:** Eligibility Criteria, categorized according to the Population; Intervention; Comparator; Outcomes and
 160 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). C-terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N propeptide (P1NP) were the primary biomarkers of interest due to their designation as reference markers of bone resorption and formation [29,31,42]. 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. When studies used a controlled design with nutritional intervention (e.g., comparing the effects of calcium supplementation versus placebo on the bone biomarker response to exercise) only the data from the placebo or control condition was extracted.

161

162 *2.2. Search Strategy and Study Selection*

163 Seven electronic databases were searched by ED. These were MEDLINE, Embase, Cochrane CENTRAL,
164 SPORTDiscus, PEDro, LILACS and IBEC. A combination of free text and database specific subject headings
165 were used, with free text terms used being: bone AND (exercise OR physical activity) AND (biomarkers OR
166 turnover OR remodelling OR formation OR resorption). Searches were limited to human studies, without
167 restricting either the date or language. Only peer-reviewed studies published in scientific journals were considered
168 for inclusion. In line with Cochrane Collaboration recommendations [43], the full strategy for the Medline search
169 was submitted for peer review to an information scientist using the Peer Review for Electronic Search Strategy
170 (PRESS) Guideline Assessment form [44] and that search was then replicated in all other databases (see
171 Supplementary File 3 for the full search strategy used in each database). The Medline and Embase databases were
172 searched using the OVID platform. The final searches were undertaken in May 2022 and results were uploaded
173 to systematic review management software (covidence.org). A three-stage selection strategy was independently
174 undertaken by ED and KK/AD and comprised (1) Title/Abstract Screen (2) Full Text Screen, and (3) Full Text
175 Appraisal. The independent screeners were not blinded to any study information as blinding has previously been
176 reported to neither statistically nor clinically impact meta-analysis results [45]. Screeners convened at the end of
177 each screening stage to resolve any discrepancies, which were resolved by discussion, or third-party mediation if
178 required. The database searches were complemented by citation screening of all included studies (backward
179 snowball technique) along with relevant reviews and book chapters (Banfi et al. [46], Dolan et al. [32], Alp [47],
180 Smith [39] and Wherry [27]).

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184 2.3. *Data Extraction and Coding*

185 Data were independently extracted and coded by at least 2 members of the review team (AD/LHMF and ED/GB).
186 Data were extracted within the following categories: (1) study information (author, year, title, journal, funding
187 and conflict of interest statement, aim, study design overview); (2) participant characteristics (sample size, sex,
188 age, training status, health status, height, body mass, body mass index (BMI); (3) exercise test characteristics
189 (type, intensity, duration, total work done, impact level); (4) blood sampling details (number, timing, whether the
190 participant was fed or fasted, bone biomarkers measured, sample type (*i.e.*, serum, plasma or urine)); (5)
191 measurement process and inter and intra-assay variability; and (6) main outcomes (mean and standard deviation
192 for each bone biomarker pre and post intervention). A complete description of the coding system applied is
193 described in the accompanying codebook (see Supplementary File 2). If the primary outcome (mean and standard
194 deviation for each measured biomarker pre and post exercise) was not reported, the corresponding author from
195 the relevant study was contacted to request this information.

196
197 2.4. *Data Synthesis*

198 A Bayesian framework was chosen over a frequentist approach as it allows for more flexible modelling and
199 enables results to be interpreted intuitively through reporting of subjective probabilities [48]. The effects of
200 exercise on bone biomarkers were quantified using standardized mean difference effect sizes (dividing by baseline
201 standard deviation and accounting for small sample bias). Some of the included biomarkers act in an inhibitory
202 manner (*e.g.*, sclerostin inhibits formation meaning that higher levels represent a reduction in the process of
203 interest) and this was reflected by multiplying the relevant effect sizes by -1. Three-level random-effects Bayesian
204 hierarchical models were used to pool effect sizes and model average effects (ES), variance within studies,
205 variance between studies (τ^2), and covariance of multiple outcomes (Intraclass correlation coefficient: ICC)
206 reported in the same study (*e.g.*, multiple bone biomarkers and/or single bone biomarkers reported at multiple
207 time-points). Within-study variance is influenced by pre-post correlations [49] that are generally not reported.
208 Therefore, primary data obtained from relevant studies (including those produced in the laboratories of the study
209 team) were used to develop informative priors to model within study variances (Gaussian prior centered at $r =$
210 0.85 and range from approximately 0.70 to 0.99). Weakly informative priors (Student-t and half student-t with 3
211 degrees of freedom for intercepts and variance parameters, respectively) were used for all other model parameters.
212 Inconsistency in models were described by comparing variances across the three levels. Inferences from all
213 analyses were performed on posterior samples generated using the Hamiltonian Markov Chain Monte Carlo
214 method and through use of credible intervals (CrI, 95% intervals for effect sizes and 75% intervals for variance
215 parameters). Modelled effect sizes were interpreted according to the following three categories: A) Evidence of
216 an effect (defined by whether, or how much of, the CrI included zero); B) The size of that effect (standard
217 categories, namely, threshold values of 0.01, 0.2, 0.5 and 0.8 were used to describe effect sizes as very small,
218 small, medium and large respectively [50]) and C) The level of certainty in each meta-analytic outcome (defined
219 using the GRADE framework - see below).

220 Primary meta-analyses were conducted across outcomes from multiple biomarkers categorized as either: 1) bone
221 formation; 2) bone resorption; 3) general bone remodelling; and 4) calcium metabolism. Sensitivity analyses were

222 then conducted, presenting meta-analysis results for individual biomarkers in each category. Moderator analyses
223 were conducted through meta-regression and selection of specific biomarkers (*i.e.*, P1NP, sclerostin and CTX-1).
224 The moderators investigated included: 1) participant characteristics (age, sex, training status, health status); 2)
225 exercise characteristics (type, duration, intensity, total work done, impact load); and 3) blood sampling
226 characteristics (nutritional status, assay type, sample timing relative to exercise). A more detailed description of
227 all coding categories is described in the accompanying codebook (see Supplementary File 2). Meta-regressions
228 were performed when there was sufficient data including a minimum of four data points per category level, or 10
229 data points for continuous variables [51]. Small-study effects (publication bias, *etc.*) were visually inspected with
230 funnel plots and quantified with a multi-level extension of Egger's regression-intercept test [52]. The importance
231 of removing outliers to obtain more accurate estimates of meta-analysis parameters was identified in a previous
232 large meta-analysis of exercise related effect sizes (ES) [53]. Outlier values were identified by adjusting
233 the empirical distribution by a Tukey *g*-and-*h* distribution and obtaining the 0.01- and 0.99-quantiles, with values
234 beyond these points removed prior to further analysis [54]. All analyses were performed using the R wrapper
235 package *brms* interfaced with *Stan* to perform sampling [55].
236

237 2.5. Certainty in Cumulative Evidence

238 Certainty in meta-analytic outcomes was independently assessed in duplicate by ED and AD/KK using the
239 Grading of Recommendations Assessment Development and Evaluation (GRADE) approach [56]. Potential
240 downgrading factors included risk of bias, inconsistency, indirectness, imprecision or the presence of small-study
241 effects. Risk of bias was assessed using a modified version of the Downs & Black Checklist [57]. As described
242 in the published protocol [41], we opted to use this tool due to its flexibility with regard to study design compared
243 to other commonly used options (*e.g.*, the Cochrane risk of bias tool (ROB2) or the Newcastle Ottawa Scale
244 (NOS)) that are designed to evaluate specific study designs. The original tool was modified to ensure it provided
245 information directly relevant to this particular investigation. For example, some items were deemed unnecessary,
246 either because they were specifically relevant to longitudinal interventions and therefore not required in an
247 investigation of acute exercise bouts, or because they related to quality of reporting on factors deemed unlikely to
248 bias the specific outcomes of interest in this review (see Supplementary File 4 for the modified tool employed in
249 this study). Despite our *a-priori* pragmatic decision to include studies that did not include a non-exercise control
250 group, this does reduce certainty as to whether the reported outcomes directly relate to the intervention itself, or
251 instead to some other, non-intervention related factors, *e.g.*, circadian variation [58]. As such, any data-point that
252 did not include a non-exercise control group was downgraded on the basis of indirectness. Both risk of bias and
253 indirectness assessments were conducted for each effect size assessed and the modal value selected.
254 Consistency was ascertained using the meta-analysis results, and based upon visual inspection of effect size
255 estimates, whether credible intervals overlapped, and on assessment of heterogeneity, with outcomes for which
256 between study standard error (τ) was $> 90\%$ of the reported effect downgraded. Precision was judged based on
257 the number of outcomes available and on visual analysis of the width of the credible intervals, with intervals that
258 stretched across more than two of the aforementioned effect size thresholds downgraded. Small-study effects
259 (publication bias, *etc.*) were assessed using Egger's regression-intercept test along with visual inspection of funnel

260 plots. Potential upgrading factors included the presence of large-effects, evidence of dose-response and the
261 presence of plausible residual confounding factors.

262

263 *2.6. Updates made since the published protocol*

264 Within the original protocol [41], two secondary analyses were proposed including the potential influence of
265 nutritional strategies on the bone biomarker response to exercise, and the bone biomarker response to natural
266 experiments, namely observational studies that examined bone biomarkers before and after a real-life athletic
267 event. Given the amount of data available, and the complexity of analyses required, it was deemed unfeasible to
268 address these secondary questions within the current manuscript, and instead they will be described in subsequent
269 stand-alone manuscripts. Additionally, some minor modifications were made to our risk of bias tool (see
270 Supplementary File 4), to clarify the scoring. No other adaptations to the published protocol were made.

271

272 **3. Results**

273 *3.1. Study Selection and Characteristics*

274 Following the systematic search and selection, 99 articles comprising a total sample of 1530 participants and 1964
275 effect sizes were included in the review (see Figure 1 for the search flow diagram) [5,33,62–71,34,72–81,35,82–
276 91,36,92–101,37,102–111,38,112–121,59,122–131,60,132–141,61,142–150]. These studies investigated a range
277 of exercise types (aerobic [67.7% of effect sizes]; plyometric [15.2% of effect sizes]; resistance [13.1% of effect
278 sizes]; multi-modal [3.3% of effect sizes]; and calisthenics [0.8% of effect sizes]); intensities and durations.
279 Studies were primarily conducted using young healthy male participants (55.6% of studies involved men only,
280 27.3% of studies involved women only and 17.2% involved mixed groups), with median (interquartile range) age
281 of 25.2 (22.4 to 31.7 years). The most reported bone biomarkers within each process category were PINP
282 (formation: 215 outcomes; 36.1%); CTX-1 (resorption: 323 outcomes; 60%); total osteocalcin (general: 267
283 outcomes; 99.3%); and parathyroid hormone (PTH) (calcium metabolism: 238 outcomes 57.5%). An overview of
284 all included studies is included in Supplementary File 5.

285

286 *3.2. Certainty in Evidence*

287 Mode certainty ratings for all studies following assessment of domains 1 (ROB) and 2 (indirectness) were
288 “Moderate” (High = 37; Moderate = 46; Low = 16; Very Low = 0), and “Low” (High = 9; Moderate = 36; Low
289 = 41; Very Low = 13), respectively. Twenty studies (20.2%) included a non-exercise control group, while
290 common issues arising from the appraisal checklist included: lack of test standardization in relation to time of day
291 (29 studies; 29.3%); nutritional intake (66 studies; 66.7%) or physical activity (51 studies; 51.5%) in the days
292 preceding the test; lack of familiarization to the exercise test protocol (57 studies; 57.6%) or lack of information
293 on the nutritional conditions under which the exercise test was conducted (34 studies; 34.3%). Meta-analytic
294 outcomes were largely inconsistent, as indicated by between study standard error (τ) values that were generally
295 greater than the effect size estimate. Most outcomes were downgraded due to imprecision, as determined by

296 credible intervals that stretched across more than 2 of our pre-defined effect size categories. In addition, all
297 outcomes related to bone resorption and calcium metabolism were downgraded due to apparent small-study
298 effects, as evidenced by substantial right-biased asymmetry in the funnel plots and results from Egger's
299 regression-intercept tests (See Figure 2 and Supplementary File 6). Certainty ratings for each individual meta-
300 analytic outcome are described within the relevant sections below, and in the accompanying Supplementary Files
301 7 – 10.

302

303 3.3. The influence of acute exercise on bone resorption

304 Pooling of bone resorption markers across designs and categories indicated a very small effect of exercise
305 ($ES_{0.5}=0.10$ [95%CrI: 0.00 to 0.20; very low certainty]; Figure 3, Panel A; Supplementary Table 7). Univariate
306 analysis of each biomarker showed that the greatest increases from pre to post exercise bout were obtained for
307 osteoprotegerin (OPG) ($ES_{0.5}=0.20$ [95%CrI: 0.04 to 0.38; very low certainty]), CTX-1 ($ES_{0.5}=0.14$ [95%CrI: -
308 0.01 to 0.31; very low certainty]), and carboxy-terminal telopeptide of type 1 collagen (ICTP) ($ES_{0.5}=0.10$
309 [95%CrI: -0.03 to 0.26; very low certainty]) In contrast, CTX-1 control data (*i.e.*, data from studies that included
310 a non-exercise control condition) provided some evidence of decreases across the intervention period ($ES_{0.5}=-$
311 0.15 [95%CrI: -0.41 to 0.09; very low certainty]).

312

313 Moderator analyses were conducted with CTX-1, which is considered the reference marker of bone resorption
314 [29,31] and was collected most frequently in the included studies. In relation to sample timing, very small to
315 moderate effects were shown within 15 minutes after cessation of the exercise bout ($ES_{0.5}=0.15$ [95%CrI: -0.05
316 to 0.34; very low certainty]) and up to 2 hours post-exercise ($ES_{0.5}=0.36$ [95%CrI: -0.09 to 0.86; very low
317 certainty]), while values similar to baseline were shown in samples collected > 2 hours post-exercise. Some
318 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;
319 very low certainty]). Exercise mode and impact level seemed to moderate the circulating CTX-1 concentration,
320 with the largest increases identified from pre to post an acute bout of aerobic exercise ($ES_{0.5}=0.23$ [95%CrI: 0.02
321 to 0.48; very low certainty]) and low impact/repetitive loading type ($ES_{0.5}=0.56$ [95%CrI: 0.08 to 1.0; very low
322 certainty]). Further moderator analyses within the aerobic exercise mode identified the greatest increases in CTX-
323 1 following cycling ($ES_{0.5}=0.65$ [95%CrI: 0.20 to 0.99; very low certainty]) and continuous activities ($ES_{0.5}=0.35$
324 [95%CrI: 0.07 to 0.65; very low certainty]); with greater increases obtained with longer durations ($\beta_{0.5}=0.15$
325 [95%CrI: 0.11 to 0.20; very low certainty] per 10 mins) and increased total work done ($\beta_{0.5}=0.27$ [95%CrI: 0.21
326 to 0.35; very low certainty] per 1000 arbitrary units). No clear influence of sex on the CTX-1 response was
327 identified. In contrast, the largest CTX-1 increases following the exercise bout were identified in participants
328 categorized as well-trained as opposed to sedentary or recreationally trained participants, although studies that
329 used prolonged cycling protocols also tended to recruit well-trained athletes and this may have confounded this
330 result. Insufficient data were available to investigate whether age would moderate these results.

331

332

333

334 3.4. *The influence of acute exercise on bone formation*

335 Pooling of all bone formation markers across all designs and categories showed a very small effect of exercise
336 ($ES_{0.5}=0.05$ [95%CrI: 0.01 to 0.08; low certainty]; Figure 3, Panel C; Supplementary File 8). Univariate analysis
337 of each biomarker showed very small increases in P1NP ($ES_{0.5}=0.08$ [95%CrI: 0.03 to 0.13; low certainty]), B-
338 ALP ($ES_{0.5}=0.05$ [95%CrI: -0.01 to 0.10; low certainty]; and sclerostin ($ES_{0.5}=0.13$ [95%CrI: 0.03 to 0.22;
339 moderate certainty]). No evidence of a change in non-exercise controls was identified ($ES_{0.5}=-0.03$ [95%CrI: -
340 0.08 to 0.02; low certainty]), indicating that bone formation markers were stable over the periods investigated.
341 Moderator analyses were conducted for both P1NP and sclerostin separately. In relation to sample timing, very
342 small P1NP increases were shown within 15 minutes of exercise cessation ($ES_{0.5}=0.18$ [95%CrI: 0.10 to 0.27; low
343 certainty]), with no evidence of change over 24 to 48 hours. Very small increases were identified pre to post aerobic
344 exercise bouts ($ES_{0.5}=0.10$ [95%CrI: 0.06 to 0.16; moderate certainty]) and similar increases were shown for both
345 low ($ES_{0.5}=0.08$ [95%CrI: -0.02 to 0.18; very low certainty]) and moderate impact loading ($ES_{0.5}=0.10$ [95%CrI:
346 0.05 to 0.17; moderate certainty]). No evidence of any changes to P1NP were observed in response to high-impact
347 or multi-directional activities ($ES_{0.5}=-0.03$ [95%CrI: -0.31 to 0.40; very low certainty]). Insufficient data was
348 available to evaluate response to resistance training. There was evidence of very small increases in P1NP
349 concentrations with increased work ($\beta_{0.5}=0.02$ [95%CrI: 0.00 to 0.04; low certainty] per 1000 arbitrary units).
350 There was no evidence of a moderating effect of sex or training status, and insufficient data were available to
351 assess the influence of age (Supplementary File 8). In relation to sclerostin, consistently small increases were
352 shown across available moderator analyses (Supplementary File 8). In common with P1NP, small increases were
353 evident immediately post the exercise bout ($ES_{0.5}=0.21$ [95%CrI: -0.03 to 0.46; low certainty]), but returned to
354 baseline within 2 hours ($ES_{0.5}=0.07$ [95%CrI: -0.08 to 0.24; very low certainty]). Very small increases were also
355 observed 24 hours post-exercise ($ES_{0.5}=0.15$ [95%CrI: -0.04 to 0.36; very low certainty]), while insufficient data
356 were available to assess proceeding days. There was no evidence of a moderating effect of exercise type, impact
357 level or participant characteristics.

358

359 3.5. *The influence of acute exercise on general bone (re)modelling*

360 There was a very small effect of exercise on total osteocalcin concentrations ($ES_{0.5}=0.04$ [95%CrI: 0.00 to 0.08;
361 low certainty]; Figure 3, Panel D; Supplementary Table 9). Moderator analyses were conducted on total
362 osteocalcin only, small increases were shown immediately following exercise ($ES_{0.5}=0.06$ [95%CrI: 0.00 to 0.13;
363 low certainty]) and up to 2-hours post exercise ($ES_{0.5}=0.05$ [95%CrI: -0.01 to 0.13; very low certainty]). Moderator
364 analyses did not identify clear patterns across categories, but provided evidence of very small increases in
365 osteocalcin with increased work ($\beta_{0.5}=0.03$ [95%CrI: 0.01 to 0.07] per 1000 arbitrary units; low certainty).

366

367 3.6. *The influence of acute exercise on PTH and calcium*

368 A moderate increase in PTH was shown pre to post exercise ($ES_{0.5}=0.61$; 95%CrI: 0.27 to 0.90); very low
369 certainty]. The median point estimate for ionized calcium (iCA) was negative, but the credible intervals were wide
370 and included a range of positive values ($ES_{0.5}=-0.14$ [95%CrI: -0.73 to 0.43; very low certainty]). Moderator
371 analyses were conducted on PTH only, and indicated a large increase in PTH within 15 minutes of finishing the

372 exercise bout ($ES_{0.5}=1.3$ [95%CrI: 0.79 to 1.8; very low certainty], while values were equivalent to baseline at all
373 other time points. Responses varied substantially according to impact level, with low ($ES_{0.5}=0.75$ [95%CrI: 0.01
374 to 1.5; very low certainty] and moderate ($ES_{0.5}=0.99$ [95%CrI: 0.46 to 1.4; very low certainty] impact exercise
375 types with repetitive loading cycles showing moderate to large increases, while exercise protocols that induced
376 low impact but high muscular loads showing some evidence of small decreases ($ES_{0.5}=-0.25$ [95%CrI: -0.46 to -
377 0.08; low certainty]. Small reductions to PTH were observed following bouts of resistance exercise ($ES_{0.5}=-0.28$
378 [95%CrI: -0.52 to -0.06; very low certainty]. All results are summarized in Supplementary File 10 and in Figure
379 3, Panel B.

380

381 4. Discussion

382 The key findings from this large and comprehensive systematic review and meta-analysis are as follows: 1)
383 Pooling of outcomes across all designs and categories indicated that an acute exercise bout increased bone
384 resorption and formation markers, but the combined effects were very small and highly variable. Moderator
385 analyses revealed the source of some of this variability. 2) Exercise type and impact level influenced the bone
386 resorptive response, and cycling induced a moderate increase in CTX-1, with longer durations and more work
387 done leading to larger increases. Other exercise types did not influence this biomarker. Changes to all bone
388 formation markers were very small and transient, with no major trends identified across the moderating categories
389 investigated. 3) The bone biomarker response to exercise is time-sensitive. For example, PINP and PTH increased
390 immediately post-exercise, but returned to baseline values within 15 minutes, whereas CTX-1 peaked within 15
391 minutes and 2 hours after the exercise bout; 4) An important caveat to all findings reported herein is that certainty
392 in estimates were low or very low, which was mainly due to a lack of a non-exercise control group against which
393 to compare the exercise response; lack of standardization of factors such as nutritional status and time of day;
394 inconsistency and imprecision in observed outcomes, and in the case of outcomes based on bone resorption and
395 calcium metabolism markers, evidence of small-study effects.

396

397 4.1. Physiological Interpretation

398 This systematic review and meta-analysis indicated that in the short-term bone resorption markers were more
399 responsive to acute exercise than were bone formation markers. Considered collectively, and across all designs,
400 categories and biomarkers, a very small increase in bone resorption was observed, and this was primarily driven
401 by changes in CTX-1 and ICTP. Given that different biomarkers represent different aspects of the bone resorptive
402 process, we chose to focus our moderator analyses on CTX-1 because it is considered the reference marker for
403 bone resorption and was the most frequently measured. Interestingly, non-exercise control data provided some
404 evidence of a reduction in CTX-1 across similar time periods as the acute exercise bouts investigated, which is
405 consistent with what is known about its circadian variation, namely that it peaks in the early morning
406 (approximately 05.00), before reaching its nadir at approximately 14.00 [58]. Given that most of the studies
407 included within this review were conducted in the morning, these opposing effects (*i.e.*, an exercise-induced
408 increase versus a natural circadian decline) could indicate that the true effect of exercise is larger than reported

409 herein and highlights the importance of non-exercise control data in studies of this kind (as discussed within the
410 Implications for Research and Practice section).

411 Increased resorptive activity in response to acute exercise has two, non-mutually exclusive, possible
412 interpretations. It could be that this initial increase in catabolic activity is necessary to activate the bone remodeling
413 cycle [3,151] and that an acute increase in bone resorption could subsequently trigger reversal, and an eventual
414 increase in bone formation, which if sustained could lead to a positive adaptive response of bone to exercise in
415 the long-term. An alternative hypothesis is that, if unchecked, large increases in bone resorptive activity in
416 response to certain exercise types may eventually lead to bone loss, and increased fragility if sustained in the long-
417 term. These contrasting hypotheses have very different practical implications, given that one would suggest that
418 strategies to maximise the initial bone resorptive response to exercise may be to the bone's long-term benefit,
419 whereas the other would encourage development of strategies to minimize this initial bone resorptive response.
420 In reality, both hypotheses are plausible depending on the circumstances, however our results do favor the latter.
421 The most striking outcome from this meta-analysis was that cycling induced a moderate CTX-1 response, with
422 longer durations and more total work done leading to larger increases, while other exercise types had only a very
423 small, or no, effect on this biomarker. Long-duration cycling conveys low-impact, repetitive, loading patterns and
424 is considered to be a “non-osteogenic” exercise type. Indeed, road cyclists are considered to be a group at high
425 risk of low bone mass [152] and a number of studies have reported lower bone mass in cyclists compared to non-
426 athlete control groups [153–155]. As such, it seems plausible that prolonged exposure to exercise stimuli that
427 induce large increases in bone resorption may be detrimental over the longer-term, and that preventive strategies
428 may be warranted.

429 A milieu of exercise-induced metabolic changes may have contributed to the identified increases in bone
430 resorption, including pH [7], calcium [5] or redox [6] perturbations. Of these, calcium perturbations has received
431 the most research attention [27]. Exercise-induced reductions to serum calcium may trigger increased PTH
432 secretion, which in turn stimulates osteoclast activation. The subsequent increase in bone resorptive activity
433 releases calcium from the bone, which can then be used to normalize circulating levels. This mechanistic pathway
434 was investigated by Kohrt and colleagues [5], whereby stable serum calcium levels in a group of male cyclists
435 were maintained throughout a 60-minute vigorous cycling bout via intravenous clamp infusion. The maintenance
436 of serum calcium availability attenuated, but did not fully prevent, exercise induced increases in PTH and CTX-
437 1, implying that serum calcium has a role to play in mediating the bone resorptive response to cycling, although
438 other factors (*e.g.*, phosphate, pH or redox balance) are also likely to contribute. This perspective is also supported
439 by the results of the current meta-analysis. Ionized calcium declined post-exercise (albeit with wide CrIs that
440 included positive values), whereas PTH increased from pre to post exercise bouts that involved low or moderate
441 impact repetitive loading cycles. Interestingly, this PTH increase peaked immediately after the exercise bout, and
442 quickly returned to baseline within approximately 15 minutes. In contrast, CTX-1 appeared to peak within the
443 first 2 hours after exercise, which makes sense given that it may have been triggered by an initial increase in PTH.
444 These data highlight the importance of sample timing when interpreting biological data, given that it may not be
445 possible to observe responses in both the “effector” (PTH) and “effectee” (CTX-1) within the same blood sample.

446 Across all designs, categories and biomarkers, a very small effect of acute exercise on markers indicative of bone
447 formation was shown, and this was primarily driven by very small increases in P1NP and sclerostin. An acute

448 increase in bone formation in response to exercise could imply that exercise can induce modelling-based formation
449 (*i.e.*, formation that is uncoupled to resorption), but timing analyses indicate that this is unlikely. P1NP peaked
450 immediately post-exercise but quickly returned to baseline. P1NP is an indicator of type 1 collagen deposition,
451 and although it seems plausible that acute exercise could activate the process of formation, it is unlikely that new
452 collagen could be formed and deposited within such short time-periods. As such, a true exercise-induced increase
453 in P1NP that is indicative of collagen deposition should not, theoretically, be observed for some time after the
454 acute exercise bout. Instead, the observed transient increases in P1NP may relate to some biological artefact, such
455 as exercise-induced damage causing a small leak of connective tissue contents into the circulation, or potentially
456 to hemodynamic shifts. Interestingly, increased P1NP is more frequently shown in response to exercise training
457 [156,157], as discussed in our recent narrative review [32]. Biologically, a chronic, as opposed to acute, response
458 of P1NP to exercise is more plausible given the time required for the formation and deposition of new collagen
459 within bone.

460 Sclerostin exerts a downregulatory effect on bone formation, through inhibiting the canonical Wnt/ β -catenin
461 signaling pathway [158]. If acute exercise promotes bone formation it would be expected that the activity of this
462 osteokine would be reduced, as has been observed in a study that reported reduced osteocyte sclerostin gene
463 activity in mechanically stimulated bone [159]. This was not the case, however, and the results of the current
464 meta-analysis indicate that exercise may acutely increase circulating sclerostin levels. In common with P1NP,
465 these increases occurred immediately after the exercise bout, before quickly returning to baseline values and it is
466 plausible to consider that they may have occurred due to similar artefacts, *e.g.*, a release of previously synthesized
467 sclerostin from the osteocytes [118], or to hemodynamic shifts. Thirty-nine percent of available studies corrected
468 their results for plasma volume (PV). Previous studies have reported no difference in bone biomarker outcomes
469 in PV adjusted versus unadjusted analyses [5,116,122], however, it is possible that any potential changes may
470 have been too small to detect in single studies, and instead may only have been observed when multiple studies
471 were pooled. Very small increases in total osteocalcin across all exercise types were also observed. This osteokine
472 is frequently described as an indicator of bone formation, however, it may also be liberated during bone resorptive
473 processes, and as such, is better described as a general indicator of bone metabolism [160]. It should also be
474 highlighted that osteocalcin fulfils multiple functions, many of which may be influenced by exercise (*e.g.*, glucose
475 regulation [161]) and as such, changes cannot be assumed to relate solely to altered bone metabolism. Indeed,
476 uncarboxylated osteocalcin, which is a better indicator of bone formation, was found in this review to be
477 unaffected by exercise. Considered collectively, the available evidence based on all relevant biomarkers indicates
478 that the very small and transient increases observed may have been spurious, and unlikely to accurately represent
479 changes to bone forming processes.

480 An interesting finding from this study is that exercise types deemed non-osteogenic (*i.e.*, lower impact activities
481 with repetitive loading cycles) induced the greatest bone biomarker response, and more specifically, a large bone
482 resorptive response. In contrast, little evidence was obtained to support a bone biomarker response to activities
483 that are considered to have the greatest osteogenic potential (*e.g.*, activities with high gravitational or muscular
484 loads). This finding calls into question the validity of these circulating biomarkers to predict or precede an adaptive
485 response in parameters such as bone mass or structure. A number of potential explanations for these findings exist.
486 Total work done, exercise duration and exercise intensity all emerged as likely moderators of the bone biomarker

487 response, and it is possible that the available protocols were not of sufficient time or duration to elicit a response.
488 This explanation seems unlikely, however, given that relatively few, high-impact, loading cycles are required to
489 stimulate a bone response [3,15], meaning that very long, or intense, protocols should not be required, provided
490 the mechanical strain is high enough. It seems, therefore, that circulating bone biomarkers may be more responsive
491 to exercise induced metabolic signals such as pH, Ca⁺⁺ and redox perturbations, most of which are known to be
492 catabolic to bone, than to mechanical signals induced by loading, which are generally considered to be anabolic
493 to bone. Certainly, this theory is speculative and requires empirical testing, but if correct, it would have substantive
494 implications for the way in which commonly used biomarkers are used and implies that they may be more useful
495 to investigate strategies to prevent potentially osteolytic signals (as may occur, for example, during long duration
496 cycling), rather than in investigating the osteogenic potential of different exercise types.

497

498 4.2. Study Strengths and Limitations

499 The main strength of this study is its comprehensiveness and depth of analysis. The inclusion of all available study
500 designs allowed for evaluation of a wide range of potential moderating variables and thus will be applicable to a
501 wide range of situations. The investigation also has a number of limitations, which should be considered when
502 interpreting the results and findings. For example, disparate study designs rendered designation of coding
503 categories difficult. We attempted to be as explicit as possible when defining our coding categories (see codebook
504 in Supplementary File 2), but many were difficult to objectively define and/or were incompletely described within
505 the included articles (*e.g.*, definitions of training status, or categorization of exercise intensity). We also made an
506 *a-priori* decision to be inclusive, and not to exclude any study based on its design. This decision allowed for a
507 systematic evaluation of potential sources of bias within the existing evidence base. It is, however, important to
508 consider that all meta-analyses inherit the limitations of their included studies, and application of the GRADE
509 analysis resulted in an overall low, or very low, level of certainty in most outcomes reported herein. Most of the
510 studies included in this analysis (74%) did not include a non-exercise control group, and this renders it difficult
511 to isolate reported findings to the exercise bout itself. As previously reported [58], and confirmed herein, certain
512 biomarkers, such as CTX-1 have a circadian variation, and failure to account for this (and other potential sources
513 of variation unrelated to the exercise intervention itself) likely impeded accurate effect quantification. Importantly,
514 a lack of standardization of important factors, such as time of day of testing, exercise and feeding practices in the
515 days prior to testing, and the nutritional status of the participants at the time of testing may have introduced
516 considerable noise to these investigations, rendering it difficult to detect small signals. This noise may have
517 contributed (at least in part) to the large variability shown both within and between studies. We investigated a
518 wide range of potential moderating variables, however, imbalances of important moderators may have influenced
519 results and subsequent interpretations. For example, CTX-1 showed large increases in response to long-duration
520 cycling. Highly-trained individuals also appeared to have larger CTX-1 increases than their lesser trained
521 counterparts. But only highly-trained individuals are capable of undergoing a long-duration cycling test, and so it
522 is difficult to separate these findings. Finally, evidence of small-study effects was apparent for outcomes related
523 to bone resorption and calcium metabolism, as evidenced by substantial right-based asymmetry in the funnel plot
524 (Figure 2). This may represent publication bias toward positive findings, or potentially to unusual homogeneity
525 in some samples, potentially leading to an artificial inflation of these effect size estimates [162].

527 The results of this investigation have addressed a number of important questions regarding the bone biomarker
528 response to an acute exercise bout, and in turn, these results have opened up new avenues for investigation. Our
529 results indicated that long-duration cycling induces a large increase in CTX-1, which may be deleterious to bone
530 in the long-term, if unmatched by a concomitant increase in processes of bone formation. But the ability of acute
531 changes in bone biomarkers to predict future changes in static bone indicators such as its mass or micro-
532 architecture has yet to be ascertained. Longer-term studies, with multiple sampling points, are required to
533 investigate how these acute changes may translate in the long-term. It is interesting that bone biomarkers seem to
534 be less responsive to exercise types commonly considered to be osteogenic (*e.g.*, jump or resistance-based
535 modalities) than they were to exercise types generally deemed as non-osteogenic (*e.g.*, cycling). As described
536 above, this result led us to speculate that these biomarkers are more responsive to exercise induced metabolic
537 signals (*e.g.*, calcium, pH or redox perturbations) than to mechanical strain. This hypothesis, however, requires
538 empirical testing.

539 In order for ongoing studies to be informative, strategies to overcome the prevalent sources of bias inherent within
540 the existing evidence must be implemented. As described above, a lack of standardization of important factors,
541 such as time of day of testing, exercise and feeding practices in the days prior to testing, and the nutritional status
542 of the participants at the time of testing may have introduced considerable noise to these investigations and
543 rigorous standardization of these factors in future work may help to isolate the influence of the exercise bout itself.
544 The use of reporting guidelines that are specific to this type of investigation (*e.g.*, the PRESENT checklist [163])
545 may be useful in both the design and reporting of future work, while the effect sizes reported herein may facilitate
546 estimation of the samples required to adequately power future work. Importantly, inclusion of a non-exercise
547 control group can further facilitate isolation of reported results to the intervention of interest and we recommend
548 that non-exercise control groups are included in future studies. Finally, sample timing is important. As identified
549 within the current analysis, PTH peaked within 15 minutes of the exercise bout, while CTX-1 seemed to peak
550 within 2 hours post-exercise. As such, and for studies where an increase in bone resorptive activity is expected,
551 repeated sampling for at least 2 hours post exercise is preferable to discrete samples taken immediately post-
552 exercise.

553

554 5. Summary and Conclusion:

555 The primary finding from this review is that a single bout of exercise with low-impact repetitive loading cycles,
556 *e.g.*, cycling induced a moderate increase in the bone resorption marker CTX-1, with greater durations and more
557 work leading to larger increases. Given that these exercise modalities are unloaded, this increase was likely
558 triggered by metabolic factors, such as calcium, phosphate, pH or redox perturbations. The lack of a response of
559 any biomarker to a single bout of resistance, or high impact exercise types indicate that these biomarkers may be
560 more useful at investigating potentially osteolytic aspects of exercise, and raises questions about their capacity to
561 investigate the osteogenic potential of different exercise types, at least in the short-term. Very large between and
562 within-study variability was shown, which may have been influenced by a combination of controllable factors,
563 including a lack of standardization and non-exercise control groups. Enhanced harmonization of ongoing research

564 efforts may facilitate these barriers to be overcome, and lead to more efficient and informative use of these
565 biomarkers in the future.

566

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570

571 **Conflicts of Interest:**

572 Eimear Dolan, Alina Dumas, Karen M. Keane, Giulia Bestetti, Luisa Helena Mavalli Freitas, Bruno Gualano,
573 Wendy M. Kohrt, George A. Kelley, Rosa Maria Rodrigues Pereira, Craig Sale and Paul A. Swinton declare that
574 they have no conflict of interest relevant to the content of this review.

575

576 **Author Contributions:**

577 ED and CS conceived the original idea for this article and the protocol was developed by ED, PAS, CS and
578 GAK, with ongoing critical input from WMK, BG and RMRP. ED conducted the searches and ED, AD and KK
579 selected the studies. Data were extracted by ED, AD, GB and LHMF. ED, AD and KK evaluated the risk of bias
580 of each study. PAS conducted all statistical analyses, with ongoing critical input from GEK. ED wrote the initial
581 manuscript draft, which was then edited in accordance with ongoing critical input from all authors. All authors
582 read and approved the final manuscript.

583

584 **Data Availability Statement:**

585 The datasets generated during and/or analyzed during the current study are available from the corresponding
586 author on reasonable request.

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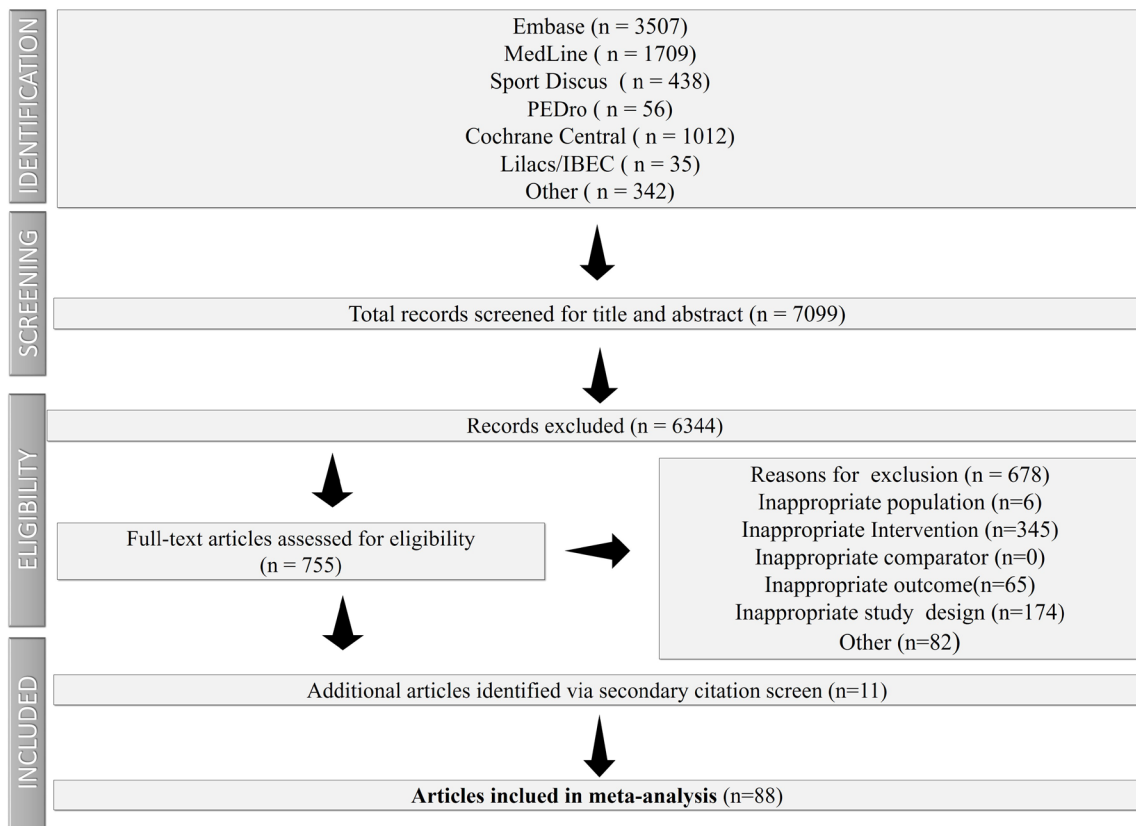
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595 FIGURES:

596 **Figure 1:** Search Flow Diagram



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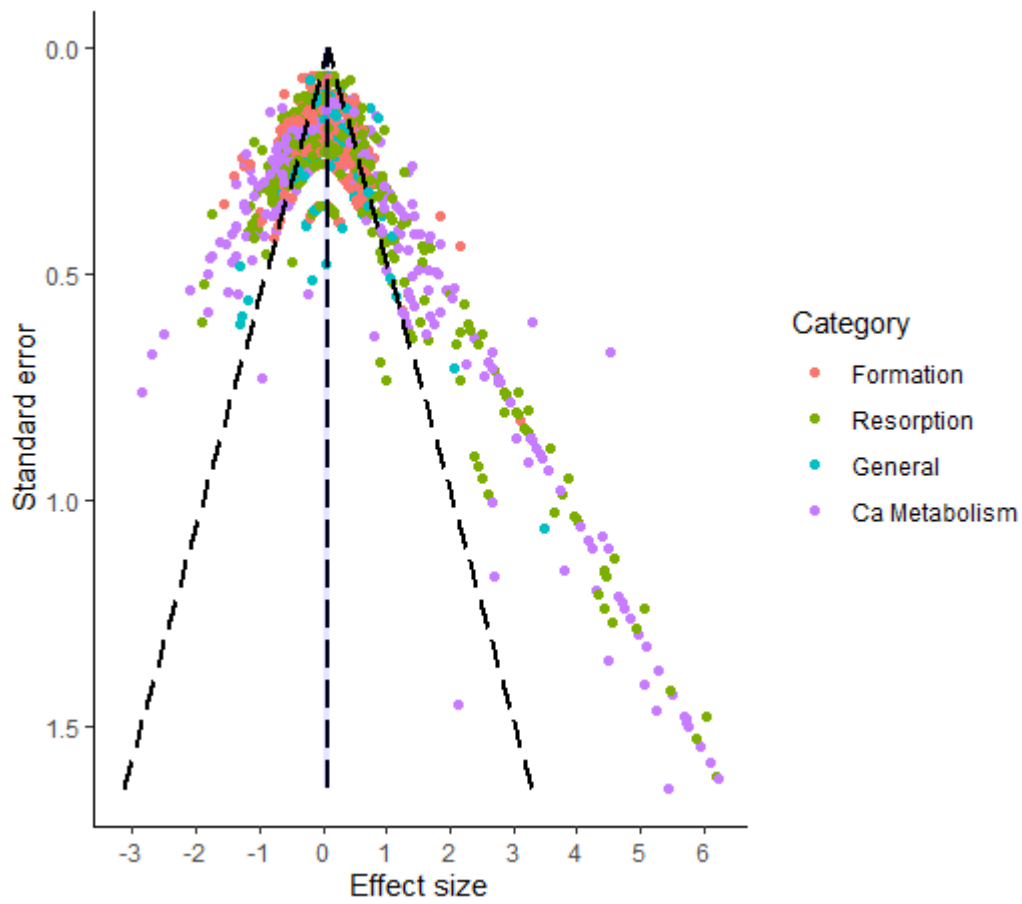
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611 **Figure 2:** Funnel plot (all outcomes)



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613 Legend: Funnel plot providing a visual tool to assess potential small-study effects. Each point represents a
614 calculated effect size from an individual outcome within a study. Centre vertical line represents the pooled
615 mean effect size obtained from meta-analysis including all outcomes. Diagonal lines represent 'pseudo 95%
616 confidence limits' indicating expected distribution in the absence of small study-effects.

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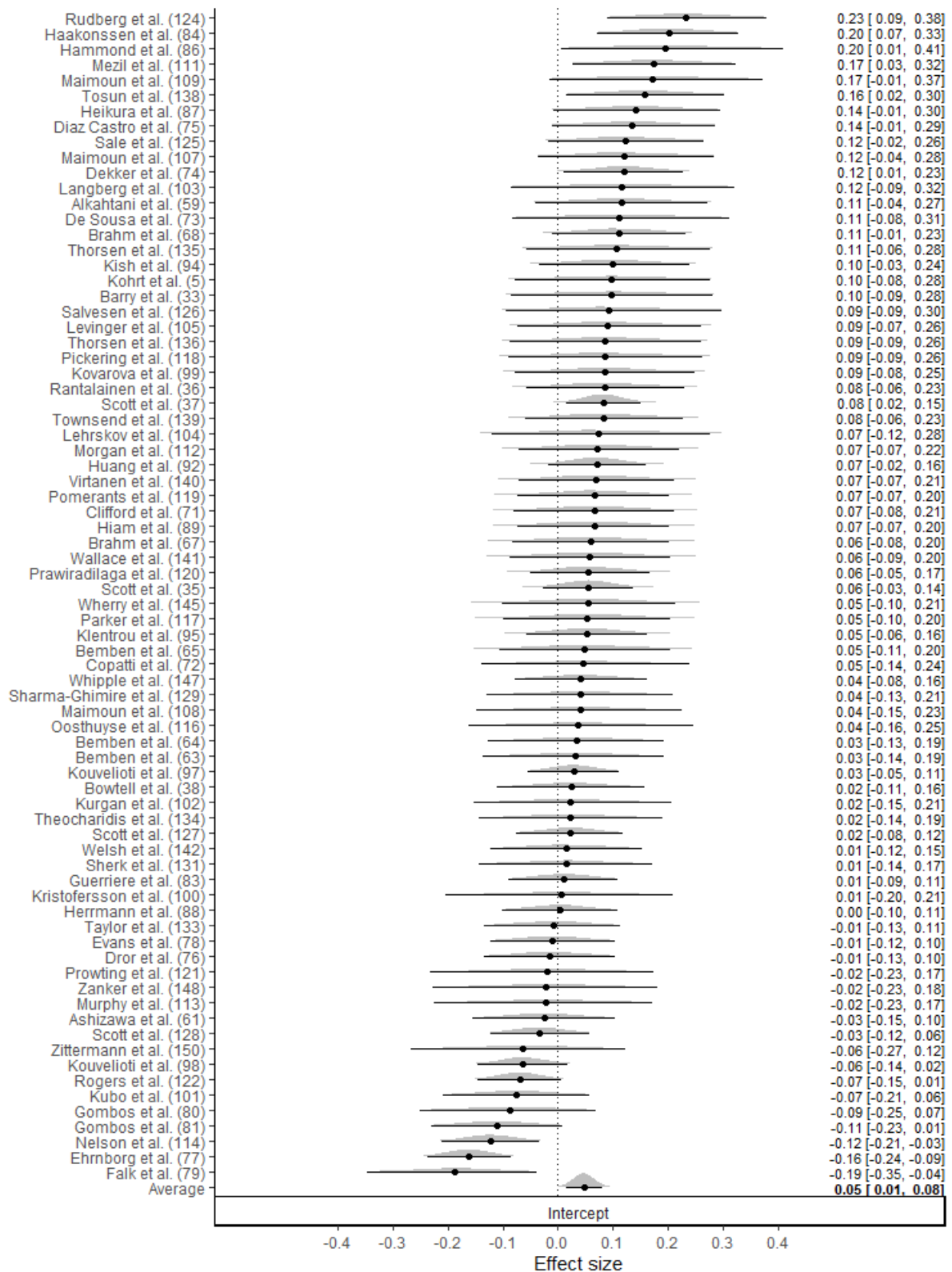
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629 **Figure 3:** Forest plots illustrating meta-analysis results across the different bone biomarker categories

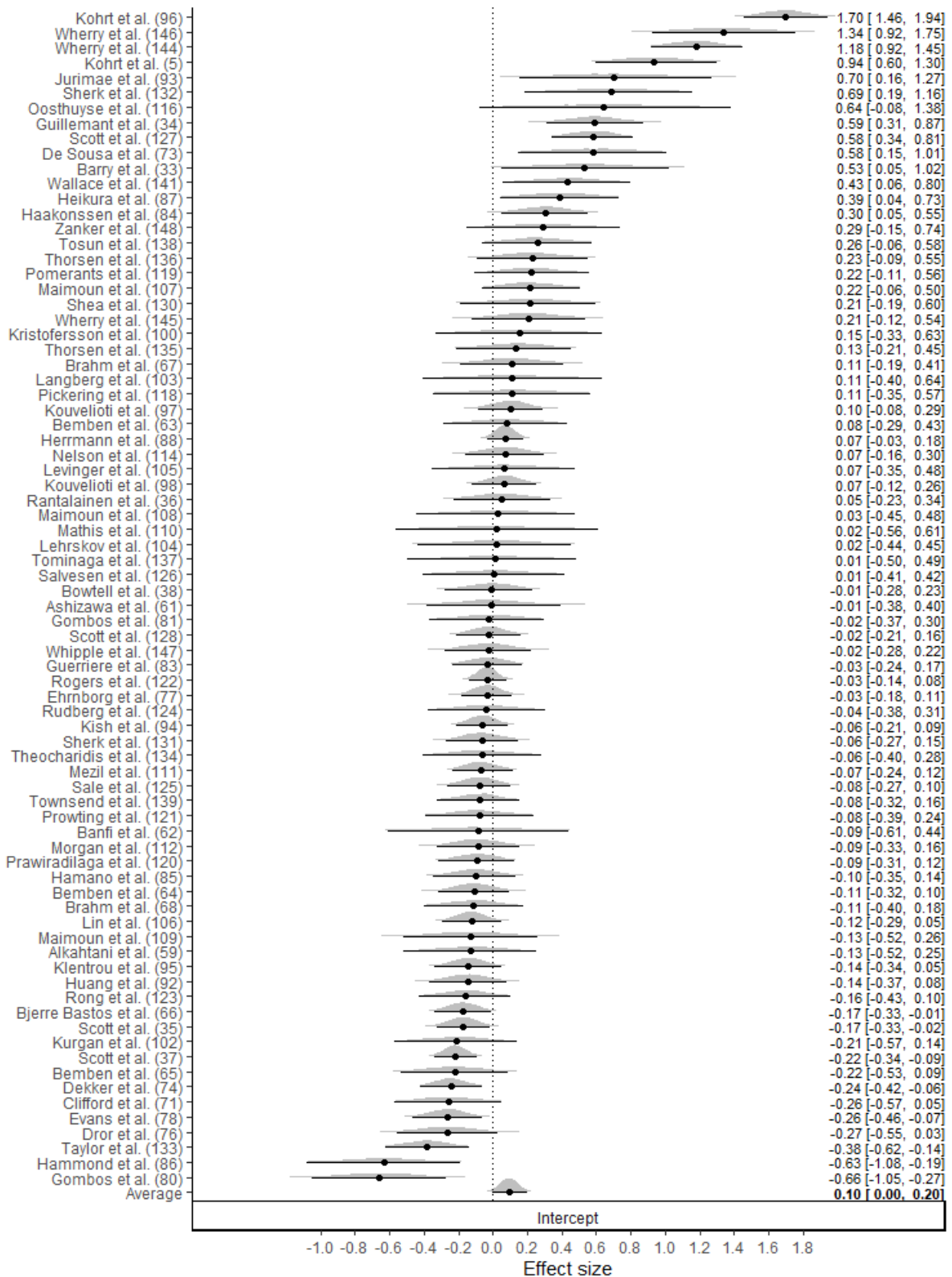
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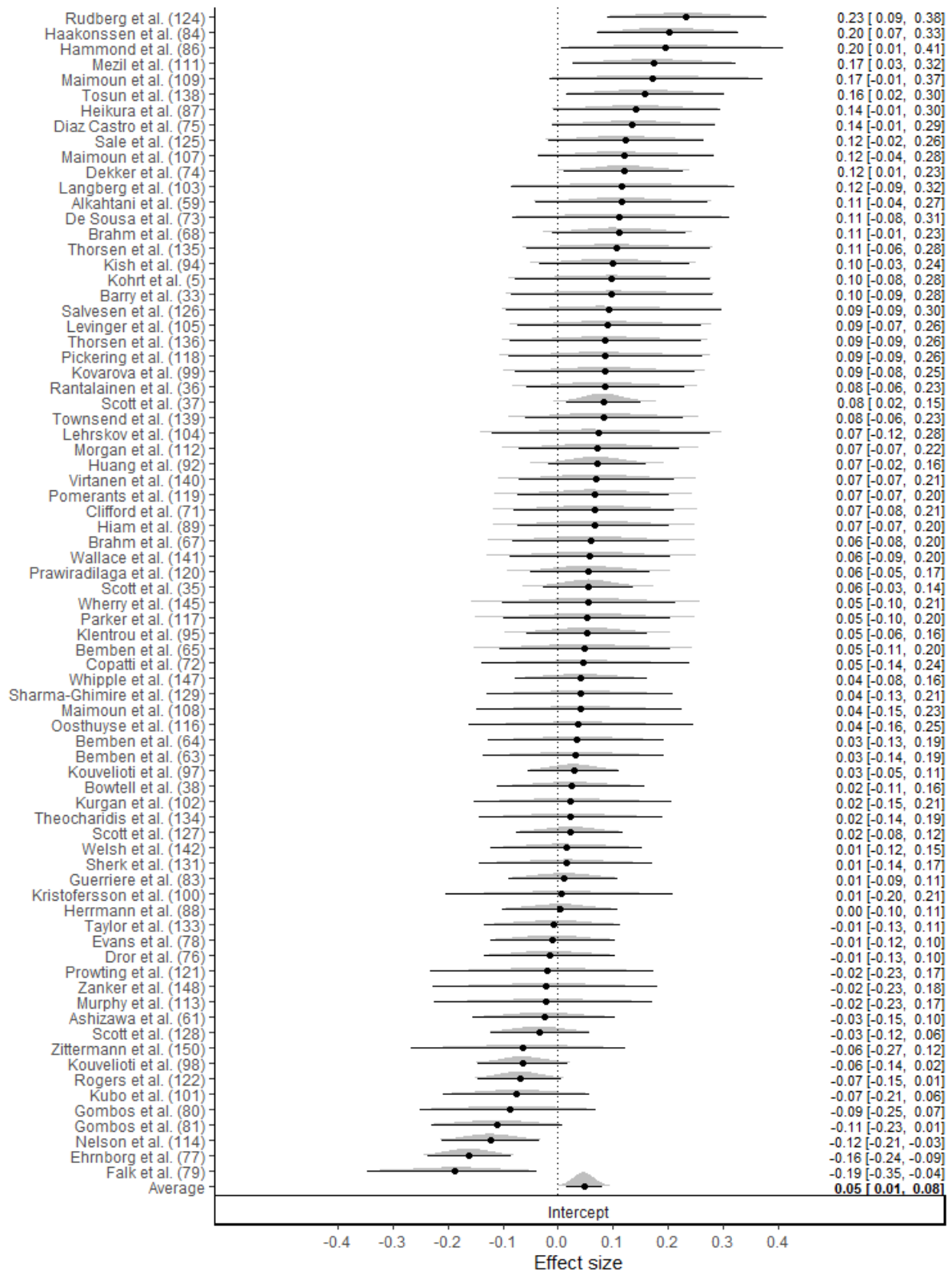


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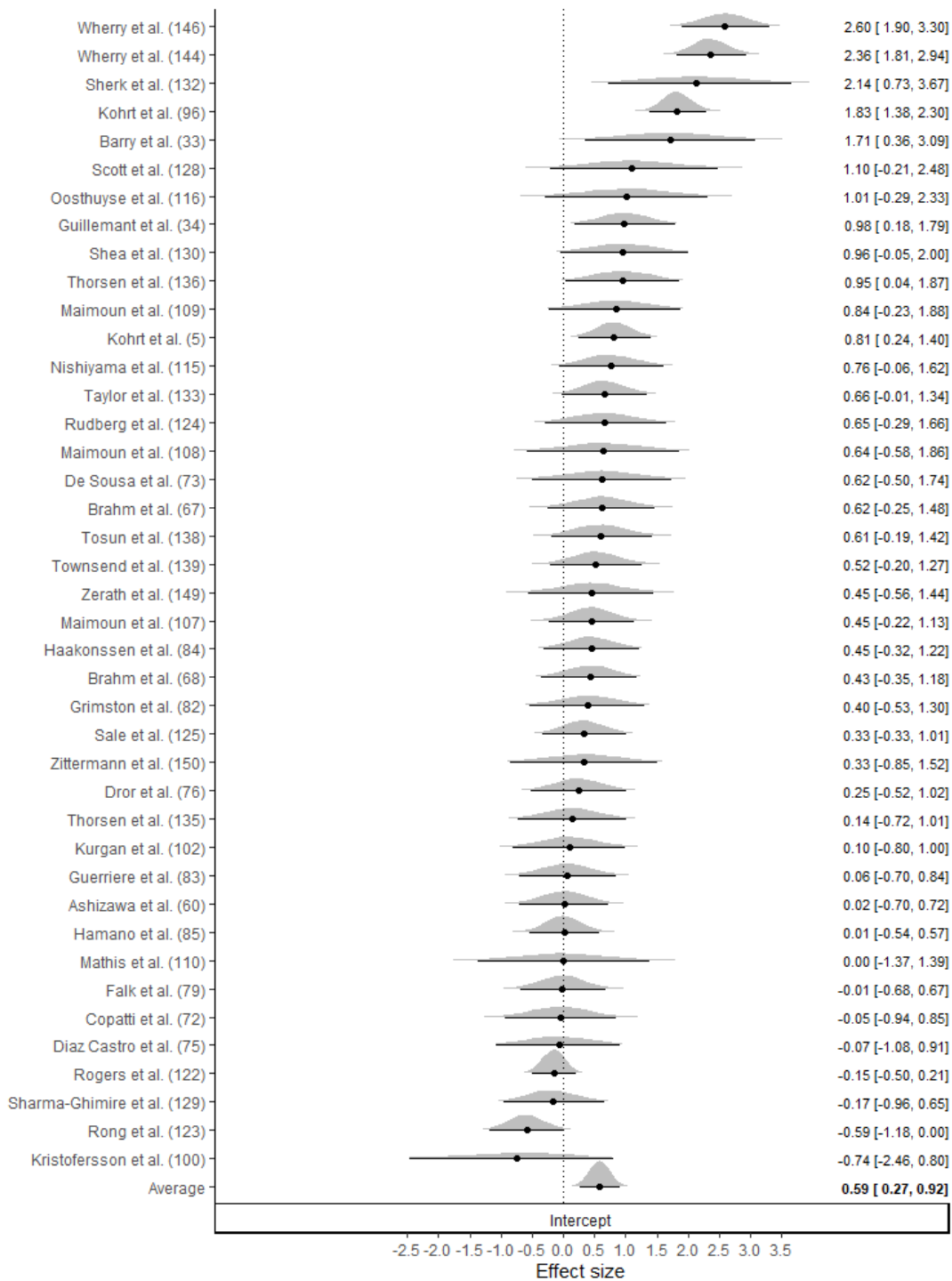


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646 Legend: Distributions represent “shrunken estimates” based on all relevant effect sizes, the random effects
 647 model fitted, and borrowing of information across studies to reduce uncertainty. Black circles and connected
 648 intervals represent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

649

650

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