P1NP and β-CTX-1 responses to a prolonged, continuous running bout in young healthy adult males: a systematic review with individual participant data meta-analysis.

CIVIL, R., DOLAN, E., SWINTON, P.A., SANTOS, L., VARLEY, I., ATHERTON, P.J., ELLIOTT-SALE, K.J. and SALE, C.

2023

P1NP AND β-CTX-1 RESPONSES TO A PROLONGED, CONTINUOUS RUNNING BOUT IN YOUNG HEALTHY ADULT MALES: A SYSTEMATIC REVIEW WITH INDIVIDUAL PARTICIPANT DATA META-ANALYSIS

Rita Civil[*1,2], Eimear Dolan[3], Paul A. Swinton[4], Lívia Santos[2], Ian Varley[2], Philip J. Atherton[5], Kirsty J. Elliott-Sale[2,6], Craig Sale[2,6]

[1] School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, UK.

[2] Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement (SHAPE) Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK.

[3] Applied Physiology & Nutrition Research Group; School of Physical Education and Sport; Rheumatology Division; Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, Brazil.

[4] School of Health Sciences, Robert Gordon University, Aberdeen, UK.

[5] Centre of Metabolism, Ageing & Physiology (CMAP), MRC-Versus Arthritis Centre of Excellence for Musculoskeletal Ageing Research, Nottingham NIHR Biomedical Research Centre, School of Medicine, University of Nottingham, Derby, UK.

[6] Department of Sport and Exercise Sciences, Institute of Sport, Manchester Metropolitan University, Manchester, UK.

[*]Corresponding author:

Professor Craig Sale, Institute of Sport, Manchester Metropolitan University, 99 Oxford Road, Manchester, M1 7EL. Email: c.sale@mmu.ac.uk.

Abstract

BACKGROUND: Circulating biomarkers of bone formation and resorption are widely used in exercise metabolism research, but their responses to exercise are not clear. PURPOSE: To quantify group responses and inter-individual variability of P1NP and β-CTX-1 after prolonged, continuous running (60-120 min at 65-75% VO2max) in young healthy adult males using individual participant data (IPD) meta-analysis. METHODS: The protocol was designed following PRISMA-IPD guidelines. Changes in P1NP and β-CTX-1 relative to baseline were measured during, immediately after, and in the hours and days following exercise. Typical hourly and daily variations were estimated from P1NP and β-CTX-1 changes relative to baseline in non-exercise (control) conditions. Group responses and inter-individual variability were quantified with estimates of the mean and standard deviation of the difference, and the proportion of participants exhibiting an increased response. Models were conducted within a Bayesian framework with random intercepts to account for systematic variation across studies. RESULTS: P1NP levels increased during and immediately after running, where the proportion of response was close to 100% (75% CrI: 99 to 100%). P1NP levels returned to baseline levels within 1 hour and over the next 4 days, showing comparable mean and standard deviation of the difference with typical hourly (0.1 ± 7.6 $ng \cdot ml^{-1}$) and daily (-0.4 ± 5.7 $ng \cdot ml^{-1}$) variation values. β-CTX-1 levels decreased during and up to 4 hours after running with distributions comparable to typical hourly variation (-0.13 ± 0.11 $ng \cdot ml^{-1}$). There was no evidence of changes in β-CTX-1 levels during the 4 days after the running bout, where distributions were also similar between the running data and typical daily variation and (-0.03 ± 0.10 $ng \cdot ml^{-1}$). CONCLUSION: Transient increases in P1NP were likely biological artefacts (*e.g.*, connective tissue leakage) and not reflective of bone formation. Comparable small decreases in β-CTX-1 identified in both control and running data, suggested that these changes were due to the markers' circadian rhythm and not the running intervention. Hence, prolonged continuous treadmill running did not elicit bone responses, as determined by P1NP and β-CTX-1, in this population. REGISTRATION: The protocol for this review was pre-registered on the Open Science Framework prior to implementation (https://osf.io/y69nd).

Key words: bone remodelling, bone markers, exercise, running, inter-individual variability, proportion of response.

Key points:

- It is unclear whether a single running bout produces bone adaptations, but these potential responses were not captured by bone (re)modelling markers P1NP and β-CTX-1.
- There is a need for studies that investigate the acute responses of bone (re)modelling markers to different types of exercise interventions and across different populations, which include a control (non-exercise) group.

Abbreviations

BMD	Bone mineral density
CLIA	Chemiluminescence immunoassay
CrI	Credible intervals
DXA	Dual-energy X-ray absorptiometry
ECLIA	Electro-chemiluminescence assay
ELISA	Enzyme-linked immunosorbent assay
IPD	Individual participant data
P1NP	N-terminal propeptide of type 1 procollagen
PICOS	Population, Intervention, Comparator, Outcomes and Study Design
pQCT	Peripheral quantitative computed tomography
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
PTH	Parathyroid hormone
RIA	Radioimmunoassay
SD	Standard deviation
VO_{2max}	Maximum rate of oxygen consumption
β-CTX-1	C-terminal telopeptide of type 1 collagen

1. Introduction

Weight-bearing exercise is generally considered to be beneficial for bone health and is associated with long-term (*i.e*., months, years) improvements in bone mineral density (BMD) and bone architecture, particularly at load bearing sites [1, 2, 3, 4]. Although the best exercise regimen (*i.e*., type, intensity, duration, and frequency) to optimise bone responses is still not well defined, research suggests that dynamic, high-impact, rapid, multi-directional movement patterns and unaccustomed loads, with a sufficient load intensity, are likely to produce the largest osteogenic stimulus [5, 6, 7, 8]. The effects of endurance running exercise on bone are interesting because, although running produces greater gravitational loading compared to other low-impact activities, such as cycling [9], it also has a repetitive loading cycle and has been associated with a relatively high prevalence of stress fracture injury [10, 11]. Low BMD is prevalent in endurance runners, particularly at non-loaded sites [12], and it seems that beneficial effects of mechanical loading may not counteract the potential negative influences associated with endurance exercise [11], such as micro-damage accumulation and low energy availability [13].

Examining the dynamic bone response to acute running exercise bouts is a logical approach to further investigate the effects of this exercise type on bone, which can be done by measuring changes in bone (re)modelling markers, measured in blood, before and after a running intervention. Almost all studies that have included these measurements, however, were not designed to directly answer this question and did not include a control (non-exercise) group, which makes it difficult to separate running-induced responses from circadian variation [14]. Furthermore, the results from the few studies that have included a running intervention and a control group are inconsistent. Two studies reported no significant differences in bone formation marker P1NP levels 1-24h hours after an intermittent [15] or a continuous [16] bout of running compared to a non-exercise control condition, but Alkahtani *et al*. [17] reported increases in P1NP immediately and 24h after intermittetnt running. In terms of bone resorption, increases in β-CTX-1 have been shown 1h, but not 24h, after intermittent running [15] and 24-96h after continous running [16]. Potential explanations for these discrepant results include differences in

exercise regimen (*i.e.*, duration, intensity, intermittent/continuous) and measurement error (*i.e.*, instrumentation and biological noise), and lack of standardisation of factors such as sleep, diet, physical activity prior and following the running bout. It is also possible that different individuals respond differently to the exercise intervention itself (inter-individual variability).

The extent of inter-individual variation in the bone biomarker response to prolonged continuous running is unknown. The estimation of the typical variation in observed scores derived from measurement or biological noise can be quantified through the variation in scores in control conditions (by including a control group) [18]. For example, when investigating the responses of bone (re)modelling markers to an exercise intervention, the estimation of typical variation would allow quantification of the degree to which the observed changes were affected by factors external to the intervention itself, such as circadian rhythms; and, therefore, would allow quantification of the degree to which the intervention itself may contribute toward the observed variation. Whilst obtaining accurate estimates of these variability assessments is difficult for single studies, individual participant data (IPD) meta-analytic approaches provide better estimates of mean responses with bigger sample sizes and allow for the assessment of effects at the participant level by using the raw data from selected studies [19, 20], and, thus, can determine inter-individual variability.

To better understand bone responses to acute bouts of running, the aims of this study were to (i) evaluate the mean responses of P1NP and β-CTX-1 to a prolonged, continuous running exercise bout in young healthy adult males, (ii) estimate the inter-individual variability in bone (re)modelling marker responses, and (iii) determine to what degree any inter-individual variability was associated with the prolonged, continuous running bout itself (herein termed the intervention response), versus those related to external factors such as circadian variation.

2. Methods

The protocol for this review included all items described in the checklist of Preferred Reporting Items for Systematic Review and Meta-Analysis of Individual Participant Data (PRISMA-IPD) [20, 21]. The protocol for this review was pre-registered on the Open Science Framework prior to implementation (https://osf.io/y69nd).

2.1. Updates from the pre-registered protocol

In the pre-registered protocol, a combined approach of aggregate data and individual participant data meta-analyses was proposed. Because individual participant data were obtained from all running studies, however, the aggregate analysis was deemed unnecessary for the purpose of this investigation. The pre-registered protocol indicated that the statistical model would include the estimation of variability ratio (ratio of standard deviation of inter-individual difference scores relative to measurement error values); this estimation was not included in the statistical approach due to the fact that measurement error values were often as large as variation in the intervention.

2.2. Eligibility Criteria

The PICOS (Population, Intervention, Comparator, Outcomes and Study Design) approach was used to guide the determination of eligibility criteria for this study.

2.2.1. Population

Studies that included young (18-35 years old), healthy (*i.e.*, non-smokers, injury free and not taking medication from any condition known to affect bone metabolism), active males (VO_{2max} ~50 $mL \cdot kg^{-1} \cdot min^{-1}$) were considered for inclusion. Differences in training status are unlikely to influence the responses of bone (re)modelling markers after a running exercise bout [16] and, therefore, participants included young healthy males who were active (*i.e.*, recreationally) or endurance trained (*e.g.*, runners, triathletes). Only male participants were included because most studies in this area have focused upon

young, healthy, adult male populations. Studies in healthy active females are lacking on this topic, a disparity that is considered in the discussion.

2.2.2. Intervention

The term 'intervention' was taken to mean a prolonged, continuous running bout, regardless of whether or not this was the focus of the original studies from which the data were extracted. Studies were considered for inclusion if they included blood sample collections at baseline, before, during, and after prolonged, continuous treadmill running at an intensity of ≥65% VO_{2max} and with a duration of 60-120 min. In order to reduce variation due to circadian rhythms [14, 22] and feeding [23, 24, 25], studies were only included if they were conducted in the morning with a baseline sample collected after an overnight fast and the rest of the samples collected in a fasted state or consuming a non-caloric placebo. Studies were only included in this review if they involved a continuous treadmill running-based exercise bout to control for mechanical loading across studies.

2.2.3. Comparator

Bone (re)modelling marker responses to running were measured by comparing changes in markers during and post-running (*i.e.*, from blood samples taken during and in the hours and days after the running bout) relative to the baseline sample.

To quantify typical variation data from control conditions (resting/non-exercise) were required, however, most of the selected running studies did not include a non-exercise control group, which limited the available data to quantify the typical variation of these markers in resting conditions. For this reason, studies that did not fulfil the exercise intervention criteria but fulfilled the rest of the inclusion criteria (*e.g.*, population, outcomes) and included a control/non-exercise group (with fasted samples collected during the hours and days after baseline) were also used to quantify typical variation.

2.2.4. Outcomes and Prioritisation

P1NP and β-CTX-1 were the primary outcomes of interest for this study as these are the reference markers for bone formation and resorption [26].

2.3. Study design

Any experimental study design that reported the relevant data pre and post a prolonged, continuous running bout or at rest was considered for inclusion, including crossover or parallel group, controlled or uncontrolled, and randomised or non-randomised trials.

2.4. Search strategy and study selection

Studies were identified directly from the list of included articles in a recent systematic review and meta-analysis on the bone (re)modelling marker response to acute exercise interventions [27, 28]. For further details of the protocol, including eligibility criteria, search strategy, study selection and data extraction, of this meta-analysis please refer to Dolan *et al*., [27]. In summary, seven electronic databases were used to source the material: MEDLINE, Embase, Cochrante CENTRAL, Sport Discus, PEDro, LILACS, and IBEC; and were supplemented by citation screening of all selected studies and relevant reviews and book chapters. This search was last updated in May 2022. Additionally, data from a study included in a PhD thesis from the university's research group that fulfilled the inclusion criteria was included [29].

The list of articles selected for inclusion in the investigation by Dolan *et al*. [28], was subsequently screened to identify studies that met the eligibility criteria for the current study. The search strategy and study selection process are illustrated using a modified version of the PRISMA-IPD search flow diagram (**Figure 1)**.

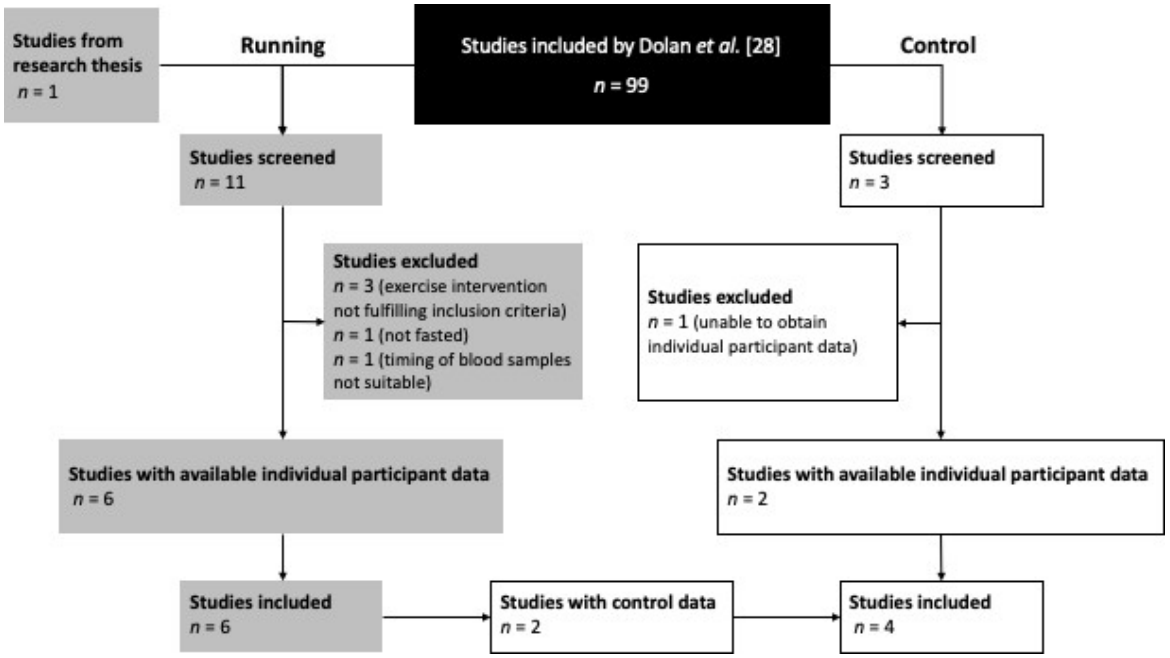

Figure 1. Selection of studies flow diagram. Studies including running data (grey) and studies including control data (white).

2.5. Data extraction and items

Data from selected studies were first extracted into a custom and pre-piloted spreadsheet (**Supplementary file 1)** including: study details (authors; year; study design); participant characteristics (final *n*; training status; age; height; weight; BMI); exercise characteristics (duration; intensity; total work [duration*intensity]); sampling conditions (time of day; diet and exercise standardisation/control before, during and after the intervention, sample handling, assay type); and, if appropriate, intervention group (*e.g.*, trained/recreational participants, placebo).

Anonymised, individual participant raw data were collected from each publication when available (*e.g.*, supplemental material), or directly from study authors, who were contacted via email, with a maximum of two email attempts made over a period of one month. Individual participant data were entered into

codebooks (**Supplementary file 1**) and transformed to the same units when included in the codebook (*i.e.*, for P1NP and β-CTX-1 data ng·ml^{-1} was used).

2.6. Risk of bias assessment in individual studies

The risk of bias for each study was independently assessed in duplicate by two members of the research team using a modified version of the Downs and Black [30] checklist (**Supplementary file 2**). This tool was selected because it provides a comprehensive assessment of the methodological quality of both randomised and non-randomised trials in healthcare research and has been validated as a tool to evaluate the quality of reporting as well as internal and external validity [30]. The modified checklist had a total of 16 items, a maximum score of 20 and was tailored to identify the methodological concerns relevant for this analysis. This tool was not used to exclude any eligible studies.

2.7. Statistical analysis and calculations

Individual participant data meta-analyses were conducted to quantify the responses of P1NP and β-CTX-1 for all available time-points during, immediately after, and following exercise. Responses were quantified based upon estimates of the mean difference (by subtracting each time-point from baseline), the standard deviation (SD) of the difference, and the proportion of participants exhibiting an increased response. All models were conducted within a Bayesian framework with random intercepts to account for systematic variation across individual studies. Change scores relative to baseline were calculated for each participant on an absolute scale (ng·mL^{-1}) with distributional models used to estimate both the mean difference and standard deviation of the difference. Visual exploration of the data identified the existence of heteroscedasticity, with a positive relationship between baseline values and residuals from change scores. Therefore, the baseline value was entered as a predictor of the standard deviation of the difference. Default priors were used for all parameters, including weakly informative Student-t and half Student-t distributions with 3 degrees of freedom for location and variance parameters.

Where estimates showed a mean difference and greater standard deviation of change scores for the exercise group, proportion of positive response was estimated by calculating the amount of the distribution (mean difference plus additional standard deviation of the difference) above zero. Inferences from all analyses were performed on posterior samples generated using the Hamiltonian Markov Chain Monte Carlo method (five chains, 100,000 iterations and 50,000 warmup). Interpretations were based on the median value (0.5-quantile), credible intervals (CrIs) and subjective probabilities calculated from the proportion of the posterior sample that exceeded the relevant value selected. Analyses were performed using the R wrapper package brms [31] interfaced with Stan to perform sampling.

2.7.1. Estimations of typical variation

With the control data, hourly typical variation of P1NP and β-CTX-1 markers was assessed by estimating the mean difference and SD of difference of blood samples taken at rest before a running bout [23, 32] or in a control group [15] compared to baseline. The typical daily variation of P1NP and β-CTX-1 markers was assessed by estimating the mean difference and SD of difference of blood samples taken 24-96 h post-baseline in control (non-exercise) groups [15, 16] compared to the baseline collected on day 1.

3. Results

3.1. Data collection

3.1.1. Running data

From the selected studies by Dolan *et al*. [28], five studies were subsequently selected for inclusion in the analysis of the current study [23, 24, 25, 32, 33]. One study from a PhD thesis of the university's group was also included [29]. In total, six studies, with a total of 87 individuals, were included in the analysis for the running data (**Table 1**). Individual participant data were collected from blood samples measuring P1NP and β-CTX-1 markers at baseline and at all available time-points on each study during and after the running bout (*i.e*., 20 min during running, 30-40 min during running, 30 min post-running,

immediately after, 1 h post-running, 2 h post-running, 3 h post-running, 4 h post-running, 24 h post-running, 48 h post-running, 72 h post-running, and 96 h post-running). For time-points 30 min and 4h post-running, data were only available from one study and, therefore, were not included in the analyses.

Table 1. List of studies included in the analysis.

Study	Running/control data	Bone (re)modelling markers	Assay used	Exercise duration/intensity
Evans *et al*., 2020 [15]	Control	β-CTX-1 P1NP	CLIA (IDS) CLIA (IDS)	-
Lehrskov *et al*., 2020 [32]	Running and control	β-CTX-1 P1NP	CLIA (IDS) CLIA (IDS)	60 min at 75% VO_{2max}
Sale *et al*., 2015 [24]	Running	β-CTX-1 P1NP	ECLIA (Roche) ECLIA (Roche)	120 min at 70% VO_{2max}
Scott *et al*., 2010 [16]	Control	β-CTX-1 P1NP	ECLIA (Roche) RIA (Orion)	-
Scott *et al*., 2011 [33]	Running	β-CTX-1 P1NP	ECLIA (Roche) RIA (Orion)	60 min at 65% or 75% VO_{2max}
Scott *et al*., 2012 [23]	Running and control	β-CTX-1 P1NP	ECLIA (Roche) RIA (Orion)	60 min at 65% VO_{2max}
Townsend *et al*., 2017 [25]	Running	β-CTX-1 P1NP	ECLIA (Roche) ECLIA (Roche)	~75 min at 75% VO_{2max}
Varley, 2014 [29]	Running	β-CTX-1	ELISA (IDS)	120 min at 70% VO_{2max}

CLIA, chemiluminescence immunoassay; ECLIA, electro-chemiluminescence assay; *ELISA,* Enzyme-linked immunosorbent assay; *P1NP, amino-terminal propeptide of type 1 procollagen; RIA, radioimmunoassay; β-CTX-1, carboxy-terminal telopeptide of type 1 collagen.*

3.1.2. Control data

From the six studies included for the running data, two [23, 32] collected blood samples (*i.e.*, 1-3 samples) in resting (control) conditions before the running bout. Three additional studies [15, 16, 17], which fulfilled all inclusion criteria except the intervention characteristics, but included a non-exercise control group were also identified. However, individual participant data were only obtained, and thereby included, from two of these studies [15, 16] for the control data (**Table 1**). For these four studies [15, 16, 23, 32], individual participant data were obtained from blood samples collected at baseline and during a 1-2.5 h period (hourly) and 24-96 h (daily) after the baseline sample in the control conditions/group. These data were used to estimate the hourly and daily P1NP and β-CTX-1 mean difference and SD of the difference in control (resting) conditions.

3.2. Typical hourly and daily variation of P1NP and β-CTX-1

The typical hourly and daily variation in P1NP and β-CTX-1 was determined by the mean difference and SD of the difference in control conditions (**Table 2**). There was limited evidence of a mean difference for hourly (0.06 [95% CrI -7.5 to 5.5] ng·mL^{-1}) and daily (-0.39 [95% CrI -4.3 to 2.9] ng·mL^{-1}) P1NP changes based on median estimates being close to zero and wide CrIs. Slightly higher variation in the hourly changes (±7.6 [95% CrI 6.8 to 8.5] ng·mL^{-1}) were estimated compared with the daily changes (±5.7 [95% CrI 5.1 to 6.5] ng·mL^{-1}). Stronger evidence was obtained for a mean difference for hourly β-CTX-1 changes, with the median and majority of the CrI indicating a decrease (-0.13 [95% CrI -0.34 to 0.06] ng·mL^{-1}). A wide CrI with median close to zero provided limited evidence of a mean difference for daily β-CTX-1 changes (-0.03 [95% CrI -0.54 to 0.30] ng·mL^{-1}). The SD of the difference was consistent between hourly (±0.11 [95% CrI 0.11 to 0.12] ng·mL^{-1}) and daily (±0.10 [95% CrI 0.09 to 0.11] ng·mL^{-1}) changes of β-CTX-1. There was consistent evidence of heteroscedasticity with greater change score magnitudes for those with higher baselines.

Table 2. Hourly and daily typical variation of P1NP and β-CTX-1 in control conditions.

Marker	Studies	Number of participants and observations	Mean difference [95% CrI]	SD of difference [95% CrI]
Hourly				
P1NP (ng·mL^{-1})	Lehrskov *et al*., 2020 [32]; Scott *et al*., 2012 [23]; Evans *et al*., 2020 [15]	Participants *n* = 27 Observations *n* = 58	0.06 [-7.5 to 5.5]	7.6 [6.8 to 8.5] [a]
β-CTX-1 (ng·mL^{-1})	Lehrskov *et al*., 2020 [32]; Scott *et al*., 2012 [23]; Evans *et al*., 2020 [15]	Participants *n* = 27 Observations *n* = 58	-0.13 [-0.34 to 0.06]	0.11 [0.11 to 0.12] [a]
Daily				
P1NP (ng·mL^{-1})	Evans *et al*., 2020 [15]; Scott *et al*., 2010 [16]	Participants *n* = 22 Observations *n* = 52	-0.39 [-4.3 to 2.9]	5.7 [5.1 to 6.5] [a]
β-CTX-1 (ng·mL^{-1})	Evans *et al*., 2020 [15]; Scott *et al*., 2010 [16]	Participants *n* = 22 Observations *n* = 52	-0.03 [-0.54 to 0.30]	0.10 [0.09 to 0.11] [a]

[a] Evidence of heteroscedasticity

3.3. P1NP and β-CTX-1 responses to a prolonged, continuous running bout

3.3.1. Bone formation

In contrast to the control condition (0.06 [95% CrI -7.5 to 5.5] ng·mL^{-1}), there was clear evidence that the levels of circulating P1NP increased during and immediately after the running bout with mean differences of 4.2 [95% CrI 0.2 to 8.8] ng·mL^{-1} at 20 min during the running bout, 9.2 [95% CrI 5.3 to 14.3] ng·mL^{-1} at 30-40 min during the running bout, and 12.0 [95% CrI 8.4 to 16.0] ng·mL^{-1} immediately after the running bout (**Table 3**). Greater SD of the difference was identified only at 30-40 min during (±8.1 [95% CrI 7.1 to 9.4] ng·mL^{-1}) and immediately (±10.2 [95% CrI 9.3 to 11.3] ng·mL^{-1}) after the running bout (**Table 3**) compared to the typical hourly variation (±7.6 [95% CrI 6.8 to 8.5] ng·mL^{-1} (**Table 2**). For these three time-points (20 min during, 30-40 min during and immediately after) the proportion of response was estimated as close to 100% (**Table 3**), indicating that close to all participants would be expected to demonstrate an increase in P1NP levels. From one hour after finishing the running bout and for the next three hours, P1NP returned to "normal" levels, with comparable mean differences and SD of difference (**Table 3**) than the typical hourly variation (**Table 2**). Likewise, for the four days (24-96 h) after the baseline in the running conditions, P1NP mean differences and SD of the difference (**Table 3**) were comparable with the typical daily variation (**Table 2**). The proportion of response was not estimated for these time-points due to these similarities and, therefore, there was a lack of evidence of inter-individual response. There was evidence of heteroscedasticity across all time-points except at 2 h and 72 h post-running.

Table 3. Responses of P1NP bone formation marker to a prolonged, continuous running bout.

P1NP ($ng \cdot mL^{-1}$)	Mean difference [95% CrI]	SD of difference [95% CrI]	*P* of increased variation	Proportion of response
Hourly				
20 min during running (25 observations / 2 studies)	4.2 [0.2 to 8.8]	6.1 [5.2 to 7.3] [a]	0.088	1.0 75% CrI [0.99 to 1.0]
30-40 during running (35 observations / 3 studies)	9.2 [5.3 to 14.3]	8.1 [7.1 to 9.4] [a]	0.700	1.0 75% CrI [0.99 to 1.0]
Immediately after (75 observations / 5 studies)	12.0 [8.4 to 16.0]	10.2 [9.3 to 11.3] [a]	>0.999	1.0 75% CrI [0.90 to 1.0]
1 hour post-running (75 observations / 5 studies)	1.1 [-3.1 to 5.2]	5.0 [4.5 to 5.6] [a]	< 0.001	-
2 hours post-running (60 observations / 4 studies)	0.6 [-3.1 to 4.3]	6.1 [5.5 to 6.8]	0.016	-
3 hours post-running (40 observations / 3 studies)	0.6 [-4.6 to 5.7]	4.6 [3.8 to 5.1] [a]	<0.001	-
Daily				
24 hours post-running (70 observations / 4 studies)	1.4 [-0.5 to 3.5]	5.2 [4.8 to 5.8] [a]	0.172	-
48 hours post-running (40 observations / 3 studies)	0.6 [-2.3 to 3.7]	5.9 [5.2 to 7.1] [a]	0.612	-
72 hours post-running (40 observations / 3 studies)	0.5 [-2.8 to 3.9]	7.8 [6.9 to 9.0]	0.999	-
96 hours post-running (30 observations / 2 studies)	-0.4 [-3.9 to 3.0]	5.4 [4.7 to 6.4] [a]	0.349	-

[a] Evidence of heteroscedasticity. Proportion of response was only calculated where there was strong evidence of a mean difference.

3.3.2. Bone resorption

Although β-CTX-1 blood levels showed a small decrease in mean differences during the running bout and for the four hours after finishing the running bout, the mean and SD of the differences (**Table 4**) were similar to the β-CTX-1 typical hourly variation (-0.13 ± 0.11 $ng \cdot ml^{-1}$) (**Table 2**). For the four days (24-96 h) after the baseline in the running conditions, the distribution of the β-CTX-1 differences (**Table 4**) were similar to the β-CTX-1 typical daily variation (-0.03 ± 0.10 $ng \cdot ml^{-1}$ (**Table 2**). The proportion of response was not estimated for any time-points due to the small mean differences in the running conditions and the similarities in the SD of the difference between running and control conditions. There

363 was evidence of heteroscedasticity across all time-points except for 72 h and 96 h post-running time-
364 points.
365
366 **Table 4.** Responses of β-CTX-1 bone resorption marker to a prolonged, continuous running bout.
367
368
369
370
371
372
373
374
375
376
377
378
379
380

β-CTX-1 ($ng \cdot mL^{-1}$)	Mean difference [95% CrI]	SD of difference [95% CrI]	*P* of increased variation	Proportion of response
Hourly				
20 min during running (25 observations / 2 studies)	-0.09 [-0.55 to 0.37]	0.04 [0.03 to 0.05] [a]	< 0.001	-
30-40 during running (35 observations / 3 studies)	-0.06 [-0.29 to 0.14]	0.10 [0.08 to 0.11] [a]	0.563	-
Immediately after (116 observations / 6 studies)	-0.01 [-0.09 to 0.06]	0.13 [0.12 to 0.14] [a]	0.996	-
1 hour post-running (60 observations / 4 studies)	-0.02 [-0.13 to 0.08]	0.10 [0.09 to 0.12] [a]	0.244	-
2 hours post-running (60 observations / 4 studies)	-0.08 [-0.18 to 0.01]	0.10 [0.09 to 0.11] [a]	0.109	-
3 hours post-running (40 observations / 3 studies)	-0.13 [-0.36 to 0.03]	0.11 [0.10 to 0.13] [a]	0.576	-
Daily				
24 hours post-running (111 observations / 5 studies)	0.01 [-0.02 to 0.04]	0.12 [0.11 to 0.13] [a]	> 0.999	-
48 hours post-running (81 observations / 4 studies)	0.01 [-0.06 to 0.07]	0.15 [0.14 to 0.16] [a]	> 0.999	-
72 hours post-running (81 observations / 4 studies)	0.06 [-0.09 to 0.19]	0.32 [0.29 to 0.35]	> 0.999	-
96 hours post-running (30 observations / 2 studies)	-0.03 [-0.27 to 0.24]	0.09 [0.08 to 0.11]	0.311	-

[a] Evidence of heteroscedasticity. Proportion of response was only calculated where there was strong evidence of a mean difference.

3.4. Risk of bias assessment

The application of the modified Downs and Black [30] checklist (**Supplementary file 2**) resulted in the classification of seven studies as high quality and two studies as moderate quality (**Table 5**). The most common reasons why studies were downgraded were because of lack of details provided regarding the storage and handling of the blood samples [15, 16], the lack of specification of the standardisation of the exact time of the day when the fasted morning baseline was collected [15, 32], and the inadequate

or absent standardisation/monitoring of important nutrition and diet variables [15, 29, 32]. All running studies [23, 24, 25, 29, 32, 33] were downgraded because the during and post running data were not corrected for shifts in plasma volume.

Table 5. Risk of bias in individual studies.

Study	Score	Quality
Evans *et al*., 2020 [15]	17/20	!
Lehrskov *et al*., 2020 [32]	16/20	!
Sale *et al*., 2015 [24]	19/20	+
Scott *et al*., 2010 [16]	19/20	+
Scott *et al*., 2011 [33]	18/20	+
Scott *et al*., 2012 [23]	19/20	+
Townsend *et al*., 2017 [25]	19/20	+
Varley, 2014 [29]	18/20	+

Green circle, high quality; yellow circle, moderate quality

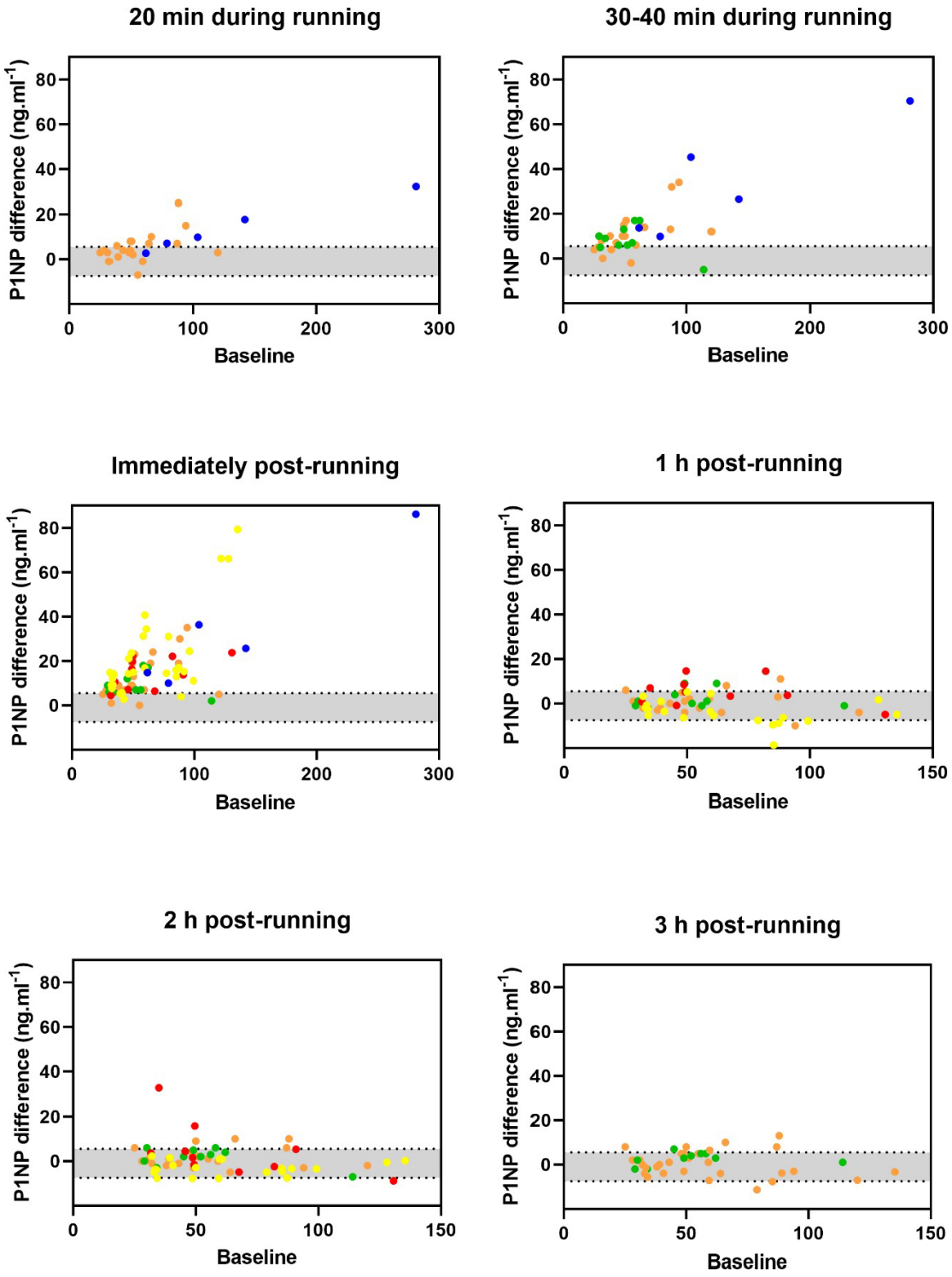

Figure 2. P1NP differences (y axis) from baseline (x axis) during 20 min, 30-40 min, immediately post, 1 h post, 2 h post, and 3 h post a continuous, prolonged running bout. Orange: Scott *et al*., 2011 [33]; blue: Lehrskov *et al*., 2020 [32]; green: Scott *et al*., 2012 [23]; red: Sale *et al*., 2015 [24]; yellow: Townsend *et al*., 2017 [25]. The grey shaded area represents 95% CrI of the mean difference in control conditions (typical hourly variation).

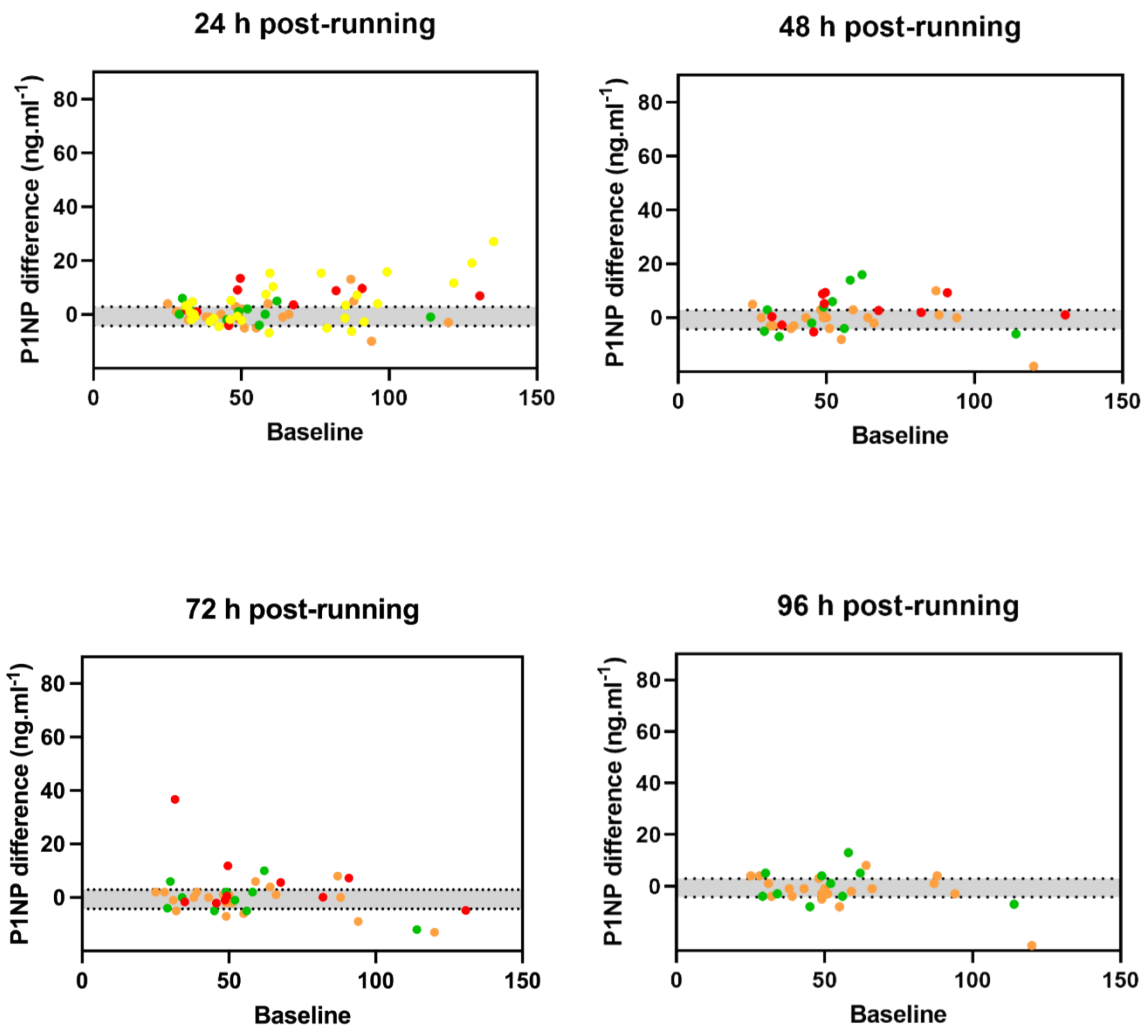

Figure 3. P1NP differences (y axis) from baseline (x axis) 24 h post, 48 h post, 72 h post, and 96 h post a continuous, prolonged running bout. Orange: Scott *et al*., 2011 [33]; green: Scott *et al*., 2012 [23]; red: Sale *et al*., 2015 [24]; yellow: Townsend *et al*., 2017 [25]. The grey shaded area represents 95% CrI of the mean difference in control conditions (typical daily variation).

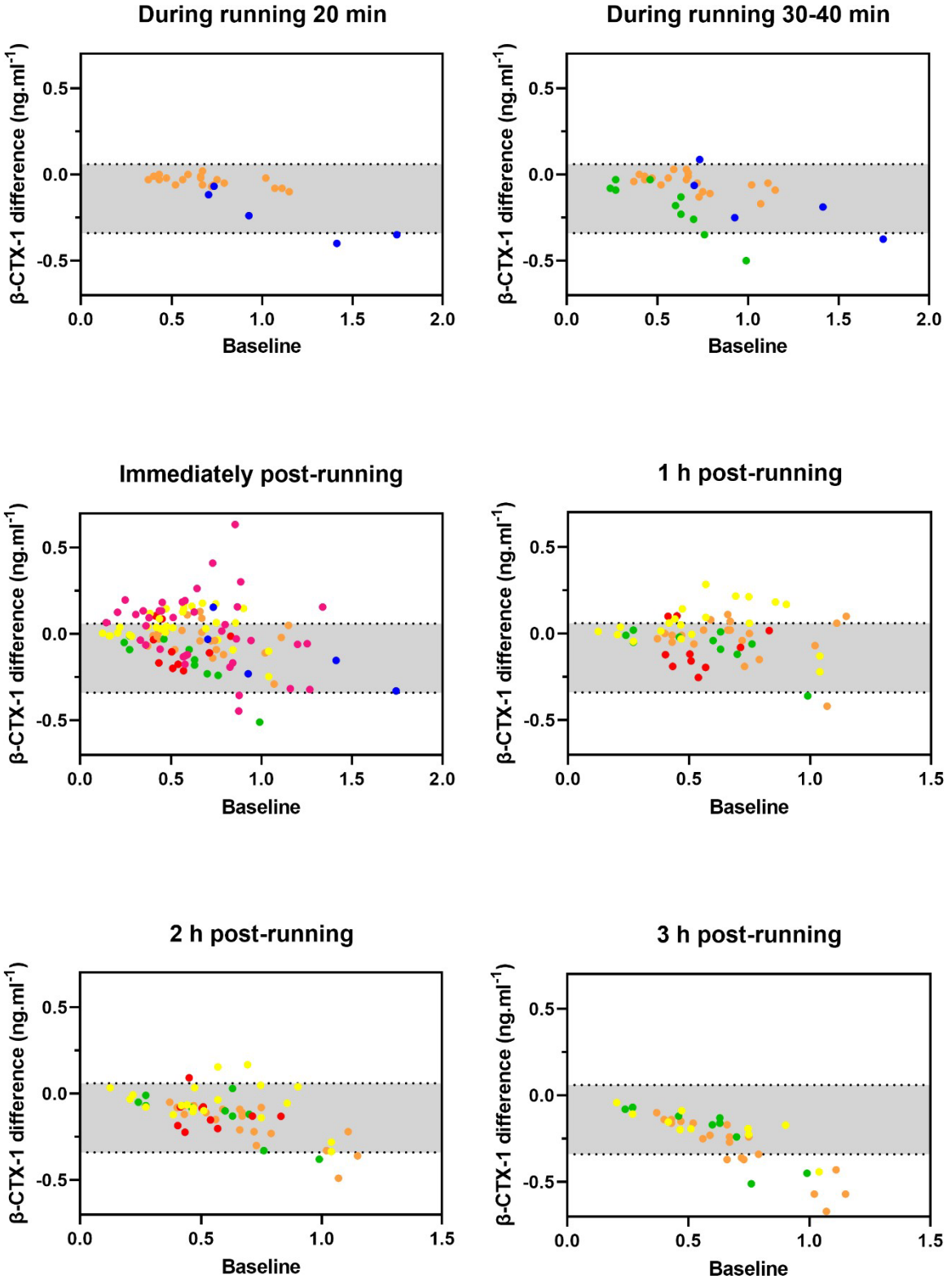

Figure 4. β-CTX-1 differences (y axis) from baseline (x axis) during 20 min, 30-40 min, immediately post, 1 h post, 2 h post, and 3 h post a continuous, prolonged running bout. Orange: Scott *et al*., 2011 [33]; blue: Lehrskov *et al*., 2020 [32]; green: Scott *et al*., 2012 [23]; red: Sale *et al*., 2015 [24]; yellow: Townsend *et al*., 2017 [25] pink: Varley, 2014 [29]. The grey shaded area represents 95% CrI of mean difference in control

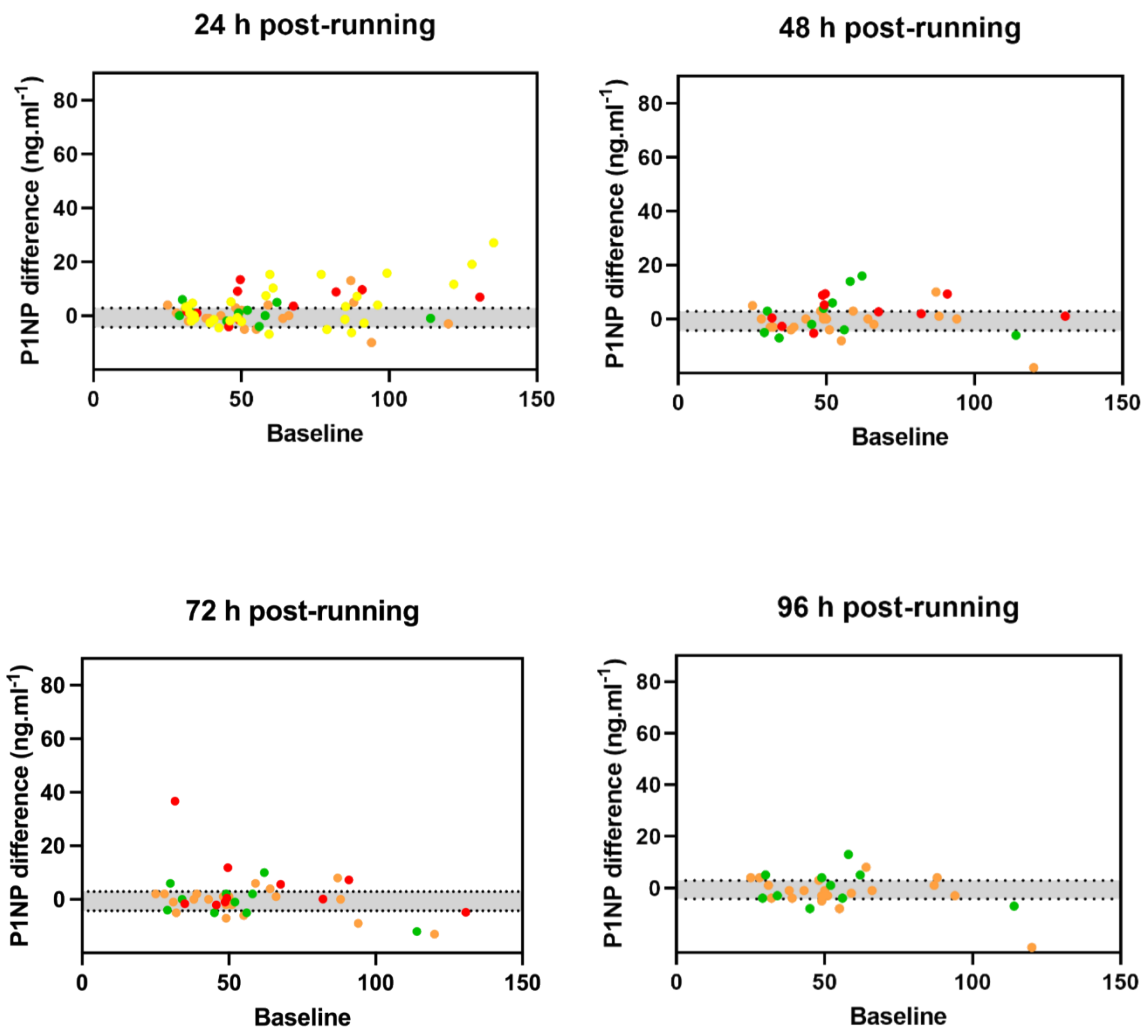

Figure 5. β-CTX-1 differences (y axis) from baseline (x axis) 24 h post, 48 h post, 72 h post, and 96 h post a continuous, prolonged running bout. Orange: Scott *et al*., 2011 [33]; green: Scott *et al*., 2012 [23]; red: Sale *et al*., 2015 [24]; yellow: Townsend *et al*., 2017 [25]; pink: Varley, 2014 [29]. The grey shaded area represents 95% CrI of mean difference in control conditions (typical daily variation).

4. Discussion

The key findings of the study were that: (i) P1NP increased exclusively during and immediately after running, and there was a lack of evidence of changes in β-CTX-1 linked to running, (ii) the inter-individual variability of P1NP and β-CTX-1 change scores were similar between resting (control) conditions and during and after running, except for P1NP levels during and immediately after the running bout, and, therefore, (iii) there was an overall lack of inter-individual response in P1NP and β-CTX-l linked to running, with reported decreases in β-CTX during the hours after running not being attributable to the running intervention.

Increases in P1NP levels were limited to during and immediately after exercise, and this consistently occured within all participants (estimated proportion of response ~100%). Because these changes were sudden and transient, however, it seems unlikely that they reflect any meaningful increase in bone formation. Similar results were reported in the recent meta-analysis by Dolan *et al*., [28], where they pooled the acute responses of bone (re)modelling markers after different exercise interventions, showing increases in P1NP within 15 minutes of the cessation of exercise. It is possible that this transient increase in circulating P1NP could be due to leakage of P1NP from the connective tissue into the circulation or due to haemodynamic shifts. P1NP is not a bone-specific marker and can be affected by the metabolism of collagen from other tissues [26]. Although fluids were provided during the running bout in two studies [24, 33]; shifts in plasma volume were not accounted for in any of the running studies included in this review, which could contribute toward explaining the transiently higher concentrations. Brahm *et al*. [34] reported similar, sudden and transient, increases in C-terminal propeptide of type 1 procollagen (P1CP) in young individuals after a running to exhaustion intervention with a total duration of ~35 minutes, which mirrored changes in plasma volume (showing decreases following the same pattern) and corresponded to increases in haematocrit. They reported no significant changes in P1CP when correcting for plasma volume shifts [34]. As such, it seems plausible that the increases observed herein are due to biological artefact, rather than as representing an actual increase in bone formative processes.

595 The stimulation of bone formation in response to exercise may require a longer period, although how
596 long exactly may be required is currently unknown. Our statistical model based on available data led to
597 the conclusion of no change in P1NP levels in the 1-3 hours and 1-4 days post-running, which indicates
598 that a single prolonged, continuous running bout did not stimulate bone formation, at least up until the
600 fourth day after the running bout. Most studies that have measured bone (re)modelling markers after an
601 acute exercise intervention have only done so for a few days (1-3 days) after the intervention [35, 36,
602 37, 38, 39, 40] and, therefore, there is no data available on longer-term changes of bone formation
603 markers in response to a single exercise session. In contrast, longitudinal studies in healthy adult
604 populations looking at the chronic responses of bone (re)modelling markers to repeated exercise
605 training of various types have consistently shown increased resting levels of bone formation markers,
606 including P1NP [41, 42, 43, 44, 45, 46, 47, 48, 49, 50]. Therefore, bone formation responses measured
607 by changes in P1NP levels might take longer than the 4 days used in studies thus far.
608
609 For circulating levels of β-CTX-1, despite showing small decreases during and in the hours after
610 running, there is no evidence that these changes were caused by the running intervention, given that
611 they were similar in magnitude to the reductions shown in the non-exercise control data. Together, these
612 results suggest that the small decreases in circulating β-CTX-1 shown during and in the hours after
613 running were caused by measurement error rather than as a result of the running intervention. These
614 reductions in β-CTX-1 coincide with the circadian rhythm of this biomarker under fasting conditions
615 [14], peaking in the early morning and declining in the later morning hours [51, 52].
616
617 Furthermore, aggregate meta-analytic evidence [28] suggests that β-CTX-1 responses to exercise are
618 influenced by the type of exercise, with moderate to large increases shown from 15 minutes to 2 hours
619 after long-duration cycling. Increases in β-CTX-1 could be explained by increases in parathyroid
620 hormone (PTH), triggered by reductions in serum calcium, that subsequently stimulates osteoclastic
621 bone resorption [53]. Although this mechanism seems to agree with the β-CTX-1 increases to cycling

622 interventions [54], it does not explain the lack of a response reported herein, given that increased
623 PTH has also been observed in response to similar running bouts as were investigated herein [23, 24, 25,
624 33].

625 For the 1-4 days after the running bout, β-CTX-1 blood levels were also similar to the daily typical
626 variation determined by the control data, indicating that the running intervention did not yield
627 significant responses to β-CTX-1 circulating levels. Similar results were reported by the Dolan *et al*.
628 [28] meta-analysis, which included studies with different designs and exercise interventions, although
629 they showed some evidence of increases in β-CTX-1 at 72 hours post-exercise. The lack of a response
630 (*i.e*., increase) in β-CTX-1 shown herein, could be considered as a beneficial outcome for bone
631 adaptations if it is interpreted as the lack of resorption activity that can lead to bone loss. In contrast,
632 the initial increase in bone resorption markers could be indicative of the activation of the bone
633 (re)modelling cycle [55]. In this case, it could be concluded that a single running bout does not stimulate
634 bone remodelling, at least within the next four days. Bone (re)modelling is, however, a nuanced process
635 that is continuously ongoing at different stages across different skeletal sites and site-specific bone
636 adaptations to exercise interventions might not be reflected by systemic bone (re)modelling markers.
637

638 **4.1. Strengths and limitations**

639 Studies included in this meta-analysis were classified as high quality (n = 8) overall. It should be noted,
640 however, that the inclusion criteria applied herein was thorough and delimited; and, therefore, low
641 quality studies would likely not have met this criterion. In the Dolan *et al*. [28] meta-analysis, which
642 had a less restricted inclusion criteria and included a larger number of studies (n = 99), the general
643 quality of the studies was reported as moderate. While a more inclusive criteria would have allowed the
644 inclusion of a greater number of studies and, thereby, more data points; it would have also added more
645 variability. The aim of this meta-analysis was to investigate the responses of P1NP and β-CTX-1 in
646 very specific conditions by reducing potential sources of variability, such as the type of exercise
647 intervention (*i.e*., impact level, duration, and intensity, intermittent/continuous), participant
648 characteristics (*i.e*., age, sex, and health status) and study design (*i.e*., feeding/fasting conditions, time

of the day). Removing these sources of variability allowed for a better understanding of the inter-individual variability caused by factors external to the intervention itself, such as circadian variation.

This systematic review with individual participant data meta-analysis was not without limitations, including those inherited from the included studies. Although all included studies collected a baseline sample in the morning, the exact time of the day when the fasted baseline sample was taken only varied from 0800-0840 but was not specified in two studies [15, 32]. Similarly, the exact time of the day when the running bout began was different across studies. These factors could have impacted the changes in bone (re)modelling markers; particularly β-CTX-1, which has a more pronounced variation due to its circadian rhythm [14]. Additionally, habitual dietary and nutritional factors, such as energy availability, macronutrient composition of the diet and vitamin D and calcium intakes were not controlled in the included studies, and could have affected P1NP and β-CTX-1 baseline levels (for a review please see [56]). The baseline level of a marker might be an important variable determining the subsequent response to exercise, and potentially to other interventions as well, as the heteroscedasticity shown in the participant data of this meta-analysis suggested that those participants with higher baselines had greater changes for both markers and across time-points and requires further investigation.

Only two studies included a non-exercise control group, which means that the control data used to estimate the typical variation of P1NP and β-CTX-1 in resting conditions was predominantly from different participants (although with similar characteristics). It is possible that the inter-individual variability was greater than if all participant control data had been obtained from the same running participants. Nonetheless, the mean differences and variability (*i.e.*, SD of the difference) in P1NP and β-CTX-1 were similar between the control and running data. For the running data, the running interventions of the included studies had a relatively limited range of durations and intensities, which could have added variability in our results. While exercise duration, intensity, and total work done might modulate bone (re)modelling markers responses [28, 33], the consistency and relevance of these effects is unclear with the current evidence. Nonetheless, the mean differences and variability (*i.e.*, SD of the difference) in P1NP and β-CTX-1 were similar between the control and running data. Another factor

677 that could have increased variability in all data is the measurement error from the instrumentation (*e.g.*,
678 variation of analytical assays used to measure bone biomarkers in the included studies). Various types
679 of assays were used across studies, which have different intra- and inter-assay coefficients of variation,
680 generally ranging from 1.4-4.9% (P1NP) and 2.1-5.3% (β-CTX-1) [57]. This variation can be critical
681 for exercise research given the overall small responses that bone (re)modelling markers exhibit after
682 acute exercise interventions [28]. For example, β-CTX-1 samples analysed using ELISA methods, as
683 in the Varley [29] study, seem to yield higher variability in the data (**Figures 4 and 5**, pink dots)
684 compared to others.
685
686 The current meta-analysis only included studies with a running intervention in young healthy adult
687 males because similar studies in female populations are lacking. Available literature on P1NP and/or β-
688 CTX-1 responses to exercise in females usually involves post-menopausal populations [40, 58, 59].
689 Only a limited number of studies have investigated the acute responses of reference markers P1NP
690 and/or β-CTX-1 to exercise (*e.g.*, jogging, brisk walking with resistance training, football) in young
691 females [40, 60, 61]. No studies have directly compared these responses between males and females;
692 however, in their meta-analysis, Dolan and colleagues [28] showed that sex did not influence exercise-
693 associated changes of markers P1NP and β-CTX-1. In addition, studies in older male populations in
694 this area are also lacking, and older-adults might have different β-CTX-1 responses to aerobic exercise
695 as highlighted in a recent systematic review [62], hence, results from the current study might not
696 translate to older populations.
697
698 **4.2. Implications for future research**
699 The majority of studies included in this meta-analysis did not include a control (non-exercise) group
700 because they were designed to investigate how various factors (*e.g.*, nutrition, exercise intensity) may
701 moderate the bone (re)modelling marker responses to a running bout. Indeed, this study design is
702 commonly used within exercise research and only about a quarter of studies looking at acute exercise
703 responses of bone (re)modelling markers included a control group [28]. It is recommended that non-
704 exercise control groups are included in future studies to quantify the variability of the instrumentation

noise (*i.e.*, from assays) and biological noise (*e.g.*, from circadian rhythms) [18], and to establish if exercise interventions of different kinds produce an effect on bone (re)modelling markers.

Bone (re)modelling markers have not yet been validated or linked to a primary reference measurement because there is no alternative reference measurement system available that can act as a higher order standard or gold standard [57]; and it is not clear whether they can predict changes detected by imaging techniques, such as dual-energy X-ray absorptiometry (DXA) or peripheral quantitative computed tomography (pQCT) [26]. Bone (re)modelling markers are systemic and do not necessarily represent local bone adaptations/changes. Therefore, studies utilising bone (re)modelling markers to investigate the bone responses to acute or short-term exercise interventions will likely be missing key information about the local effects that loading has on the skeleton and they need to be interpreted with understanding of this limitation.

It is important that studies including bone (re)modelling markers adhere to the recommended standardisation guidelines [26, 57, 63], control important factors before the intervention (*e.g.*, nutrition, sleep, physical activity), clearly report the time of the day of all measures, sampling timing, storage, and handling of the samples, and report assay quality control information, which would reduce inter-individual variability and help when making comparisons with other studies. Given the potentially misleading increases in P1NP during and immediately post-running reported herein, studies should also consider shifts in plasma volume and fluid lost or report both adjusted and unadjusted data for changes in plasma volume.

4.3. Summary and conclusions

This individual participant data meta-analysis determined that a prolonged, continuous bout of treadmill running (60-120 min at 65-75% VO_{2max}) does not result in changes in bone (re)modelling, as determined by P1NP and β-CTX-1, in young healthy adult males. Whilst there was evidence of a transient increase of P1NP during and immediately after running, this response was likely caused by biological aspects (*e.g.*, shifts in plasma volume, leakage from other connective tissues) rather than being reflective of

bone formation. Similar small decreases in β-CTX-1 were shown in control and running data, suggesting that these changes were due to the marker's circadian rhythm and not the running intervention. Hence, it remains unclear whether a single running bout produces bone adaptations, but indirect bone (re)modelling markers P1NP and β-CTX-1 markers failed to capture any potential responses. There is a need for individual studies that investigate the acute responses of bone (re)modelling markers to different types of exercise and across different populations, which include a control (non-exercise) group.

Declarations

Availability of data

Data are available on request from the authors.

Competing interests

All authors declare no competing interests relevant to the content of this review.

Funding

This work was completed as part of the PhD programme of work for RC, for which she received funding from the Nottingham Trent University Vice Chancellors Studentship Scheme. ED is financially supported by the *Fundação de Amparo à Pesquisa do Estado do São Paulo* (FAPESP: 2019/05616-6 and 2019/26899-6), which allowed her the time to contribute to this work.

Authors' contributions

RC, ED, and CS conceived the original idea for this article and the protocol was developed by RC, ED, PAS, and CS, with critical input from LS, IV, PJA, and KJES. RC conducted the selection of studies and data extraction. RC and CS evaluated the risk of bias, and PAS conducted all statistical analyses.

RC wrote the initial manuscript draft, which was then revised according to critical input from all authors. All authors read and approved the final manuscript.

Acknowledgements

This study was presented as an oral presentation at the 27th Annual Congress of the European College of Sport Science (30 August -02 September 2022) with the abstract title *P1NP and β-CTX-1 responses to a prolonged, continuous running bout in healthy adult males: a systematic review and individual participant data meta-analysis*. We would like to thank the authors of the selected studies that provided the individual participant data upon request for their collaboration and support.

References

1. Kelley GA, Kelley KS, Tran ZV. Exercise and bone mineral density in men: a meta-analysis. J Appl Physiol. 2000;88(5):1730–6. https://doi.org/10.1152/jappl.2000.88.5.1730
2. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: A review of controlled trials. Bone. 2007;40(1):14–27. https://doi.org/10.1016/j.bone.2006.07.006
3. Marques EA, Mota J, Carvalho J. Exercise effects on bone mineral density in older adults: A meta-analysis of randomized controlled trials. Age. 2012;34(6):1493–515. https://doi.org/10.1007/s11357-011-9311-8
4. Kelley GA, Kelley KS, Kohrt WM. Exercise and bone mineral density in men: A meta-analysis of randomized controlled trials. Bone. 2013;53(1):103–11. https://doi.org/10.1016/j.bone.2012.11.031
5. O'Connor JA, Lanyon LE, MacFie H. The influence of strain rate on adaptive bone remodelling. J Biomech. 1982;15(10):767-81. https://doi.org/10.1016/0021-9290(82)90092-6
6. Lanyon LE, Rubin CT. Static vs dynamic loads as an influence on bone remodelling. J Biomech. 1984;17(12):897-905. https://doi.org/10.1016/0021-9290(84)90003-4
7. Rubin CT, Lanyon LE. Regulation of bone mass by mechanical strain magnitude. Calcif Tissue Int. 1985 Jul;37(4):411-7. https://doi.org/10.1007/BF02553711
8. Turner CH, Robling AG. Designing exercise regimens to increase bone strength. Exerc Sport Sci Rev. 2003 Jan;31(1):45-50. https://doi.org/10.1097/00003677-200301000-00009
9. Rector RS, Rogers R, Ruebel M, Hinton PS. Participation in road cycling vs running is associated with lower bone mineral density in men. Metabolism. 2008;57(2):226–32. https://doi.org/10.1016/j.metabol.2007.09.0055
10. Arendt E, Agel J, Heikes C, Griffiths H. Stress Injuries to Bone in College Athletes. A Retrospective Review of Experience at a Single Institution. Am J Sports Med. 2003;31(6):959–68. https://doi.org/10.1177/0363546503031006360

11. Scofield KL, Hecht S. Bone health in endurance athletes: Runners, cyclists, and swimmers. Curr Sports Med Rep. 2012;11(6):328–34. https://doi.org/10.1249/JSR.0b013e3182779193
12. Hind K, Truscott JG, Evans JA. Low lumbar spine bone mineral density in both male and female endurance runners. Bone. 2006;39(4):880–5. https://doi.org/10.1016/j.bone.2006.03.012
13. Hoenig T, Ackerman KE, Beck BR, Bouxsein ML, Burr DB, Hollander K, Popp KL, Rolvien T, Tenforde AS, Warden SJ. Bone stress injuries. Nat Rev Dis Primers. 2022 Apr 28;8(1):26. https://doi.org/10.1038/s41572-022-00352-y
14. Diemar SS, Dahl SS, West AS, Simonsen SA, Iversen HK, Jørgensen NR. A Systematic Review of the Circadian Rhythm of Bone Markers in Blood. Calcif Tissue Int. 2022 Mar 19. https://doi.org/10.1007/s00223-022-00965-1
15. Evans W, Nevill A, McLaren SJ, Ditroilo M. The effect of intermittent running on biomarkers of bone turnover. Eur J Sport Sci. 2020;20(4):505–15. https://doi.org/10.1080/17461391.2019.1646811
16. Scott JPR, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. The effect of training status on the metabolic response of bone to an acute bout of exhaustive treadmill running. J Clin Endocrinol Metab. 2010;95(8):3918–25. https://doi.org/10.1210/jc.2009-2516
17. Alkahtani SA, Yakout SM, Reginster JY, Al-Daghri NM. Effect of acute downhill running on bone markers in responders and non-responders. Osteoporos Int. 2019;30(2):375–81. https://doi.org/10.1007/s00198-018-4673-8
18. Swinton PA, Hemingway BS, Saunders B, Gualano B, Dolan E. A Statistical Framework to Interpret Individual Response to Intervention: Paving the Way for Personalized Nutrition and Exercise Prescription. Front Nutr. 2018;5(May). https://doi.org/10.3389/fnut.2018.00041
19. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: Rationale, conduct, and reporting. BMJ. 2010;340(7745):521–5. https://doi.org/10.1136/bmj.c221
20. Stewart LA, Clarke M, Rovers M, Riley RD, Simmonds M, Stewart G, et al. Preferred reporting items for a systematic review and meta-analysis of individual participant data: The PRISMA-IPD statement. JAMA - J Am Med Assoc. 2015;313(16):1657–65.

https://doi.org/10.1001/jama.2015.3656

21. Page M, McKenzie J, Patrick B, Boutron I, Hoffmann T, Mulrow C, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst Rev. 2021 Mar 29;10(1):89. https://doi.org/10.1186/s13643-021-01626-4

22. Qvist P, Christgau S, Pedersen BJ, Schlemmer A, Christiansen C. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): Effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. Bone. 2002;31(1):57–61. https://doi.org/10.1016/S8756-3282(02)00791-3

23. Scott JPR, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. Effect of fasting versus feeding on the bone metabolic response to running. Bone. 2012;51(6):990–9. http://doi.org/ 10.1016/j.bone.2012.08.128

24. Sale C, Varley I, Jones TW, James RM, Tang JCY, Fraser WD, et al. Effect of carbohydrate feeding on the bone metabolic response to running. J Appl Physiol. 2015;119(7):824–30. https://doi.org/10.1152/japplphysiol.00241.2015

25. Townsend R, Elliott-Sale KJ, Currell K, Tang J, Fraser WD, Sale C. The effect of postexercise carbohydrate and protein ingestion on bone metabolism. Med Sci Sports Exerc. 2017;49(6):1209–18. https://doi.org/10.1249/MSS.0000000000001211

26. Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards. Osteoporos Int. 2011;22(2):391–420. https://doi.org/10.1007/s00198-010-1501-1

27. Dolan E, Dumas A, Keane KM, Bestetti G, Freitas LHM, Gualano B, et al. The influence of acute exercise on bone biomarkers: protocol for a systematic review with meta-analysis. Syst Rev. 2020;9(1):1–9. https://doi.org/10.1186/s13643-020-01551-y

28. Dolan E, Dumas A, Keane KM, Bestetti G, Freitas LHM, Gualano B, Kohrt WM, Kelley GA, Pereira RMR, Sale C, Swinton PA. The Bone Biomarker Response to an Acute Bout of Exercise: A Systematic Review with Meta-Analysis. Sports Med. 2022; Dec;52(12):2889-08. https://doi.org/10.1007/s40279-022-01718-8

29. Varley, I. Association of genotype with bone metabolism, skeletal adaptation and stress fracture injury occurrence. PhD, Nottingham Trent University. 2014. https://llr.ntu.ac.uk
30. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health. 1998;52(6):377–84. https://doi.org/10.1136/jech.52.6.377
31. Bürkner PC. brms: An R package for Bayesian multilevel models using Stan. Journal of statistical software. 2017; Aug 29;80:1-28. https://doi.org/10.18637/jss.v080.i01
32. Lehrskov LL, Kjeldsen S, Lyngbæk MP, Chirstensen RH, Wedell-Neergaard AS, Søderlund L, et al. Interleukin-6 May not affect bone resorption marker CTX or bone formation marker P1NP in humans. J Endocr Soc. 2020;4(9):1–12. https://doi.org/10.1210/jendso/bvaa093
33. Scott JPR, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. The role of exercise intensity in the bone metabolic response to an acute bout of weight-bearing exercise. J Appl Physiol. 2011;110(2):423–32. https://doi.org/10.1152/japplphysiol.00764.2010
34. Brahm H, Piehl-Aulin K, Ljunghall S. Bone metabolism during exercise and recovery: The influence of plasma volume and physical fitness. Calcif Tissue Int. 1997;61(3):192–8. https://doi.org/10.1007/s002239900322
35. Herrmann M, Müller M, Scharhag J, Sand-Hill M, Kindermann W, Herrmann W. The effect of endurance exercise-induced lactacidosis on biochemical markers of bone turnover. Clin Chem Lab Med. 2007;45(10):1381–9. https://doi.org/10.1515/CCLM.2007.282
36. Guerriere KI, Hughes JM, Gaffney-Stomberg E, Staab JS, Matheny RW. Circulating sclerostin is not suppressed following a single bout of exercise in young men. Physiol Rep. 2018;6(10):1–9. https://doi.org/10.14814/phy2.13695
37. Kouvelioti R, Kurgan N, Falk B, Ward WE, Josse AR, Klentrou P. Response of sclerostin and bone turnover markers to high intensity interval exercise in young women: Does impact matter? Biomed Res Int. 2018 Nov 1;2018:4864952. https://doi.org/10.1155/2018/4864952
38. Clifford T, Ventress M, Allerton DM, Stansfield S, Tang JCY, Fraser WD, et al. The effects of collagen peptides on muscle damage, inflammation and bone turnover following exercise: a

randomized, controlled trial. Amino Acids. 2019;51(4):691–704. https://doi.org/10.1007/s00726-019-02706-5

39. Kouvelioti R, LeBlanc P, Falk B, Ward WE, Josse AR, Klentrou P. Effects of High-Intensity Interval Running Versus Cycling on Sclerostin, and Markers of Bone Turnover and Oxidative Stress in Young Men. Calcif Tissue Int. 2019;104(6):582–90. Available from: https://doi.org/10.1007/s00223-019-00524-1

40. Nelson K, Kouvelioti R, Theocharidis A, Falk B, Tiidus P, Klentrou P. Osteokines and bone markers at rest and following plyometric exercise in pre-and postmenopausal women. Biomed Res Int. 2020 Oct 21;2020:7917309. https://doi.org/10.1155/2020/7917309

41. Fujimura R, Ashizawa N, Watanabe M, Mukai N, Amagai H, Fukubayashi T, et al. Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism. J Bone Miner Res. 1997;12(4):656–62. https://doi.org/10.1359/jbmr.1997.12.4.656

42. Tajima O, Ashizawa N, Ishii T, Amagai H, Mashimo T, Liu LJ, et al. Interaction of the effects between vitamin D receptor polymorphism and exercise training on bone metabolism. J Appl Physiol. 2000;88(4):1271–6. https://doi.org/10.1152/jappl.2000.88.4.1271

43. Adami S, Gatti D, Viapiana O, Fiore CE, Nuti R, Luisetto G, et al. Physical activity and bone turnover markers: A cross-sectional and a longitudinal study. Calcif Tissue Int. 2008;83(6):388–92. https://doi.org/10.1007/s00223-008-9184-8

44. Kim S, Sherk V, Bemben M, Bemben D. Effects Of Short Term Low Intensity Resistance Training With Kaatsu On Bone Metabolic Markers In Untrained Young Men. Med Sci Sport Exerc. 2009;41(5):76–7. https://doi.org/10.1249/01.mss.0000353505.78729.ef

45. Lester ME, Urso ML, Evans RK, Pierce JR, Spiering BA, Maresh CM, et al. Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. Bone. 2009;45(4):768–76. https://doi.org/10.1016/j.bone.2009.06.001

46. Erickson CR, Vukovich MD. Osteogenic index and changes in bone markers during a jump training program: A pilot study. Med Sci Sports Exerc. 2010;42(8):1485–92. https://doi.org/10.1249/MSS.0b013e3181d0fa7a

907 47. Hu M, Finni T, Xu L, Zou L, Cheng S. Effects of resistance training on biomarkers of bone
908 formation and association with red blood cell variables. J Physiol Biochem. 2011;67(3):351–8.
909 https://doi.org/10.1007/s13105-011-0082-6
910 48. Ardawi MSM, Rouzi AA, Qari MH. Physical activity in relation to serum sclerostin, insulin-
911 like growth factor-1, and bone turnover markers in healthy premenopausal women: A cross-
912 sectional and a longitudinal study. J Clin Endocrinol Metab. 2012;97(10):3691–9.
913 https://doi.org/10.1210/jc.2011-3361
914 49. Lutz LJ, Karl JP, Rood JC, Cable SJ, Williams KW, Young AJ, et al. Vitamin D status, dietary
915 intake, and bone turnover in female Soldiers during military training: A longitudinal study. J
916 Int Soc Sports Nutr. 2012;9(1):1. https://doi.org/10.1186/1550-2783-9-38
917 50. Kim SJ, Bemben MG, Knehans AW, Bemben DA. Effects of an 8-month ashtanga-based yoga
918 intervention on bone metabolism in middle-aged premenopausal women: A randomized
919 controlled study. J Sport Sci Med. 2015 Nov 24;14(4):756-68. PMID: 26664272
920 51. Christgau S, Bitsch-Jensen O, Hanover Bjarnason N, Gamwell Henriksen E, Qvist P,
921 Alexandersen P, et al. Serum CrossLaps for monitoring the response in individuals undergoing
922 antiresorptive therapy. Bone. 2000;26(5):505–11.
923 https://doi.org/10.1016/S8756-3282(00)00248-9
924 52. Bjarnason NH, Henriksen EEG, Alexandersen P, Christgau S, Henriksen DB, Christiansen C.
925 Mechanism of circadian variation in bone resorption. Bone. 2002;30(1):307–13.
926 https://doi.org/10.1016/S8756-3282(01)00662-7
927 53. Wherry SJ, Swanson CM, Kohrt WM. Acute catabolic bone metabolism response to exercise
928 in young and older adults: A narrative review. Exp Gerontol. 2022;157(May 2021):111633.
929 https://doi.org/10.1016/j.exger.2021.111633
930 54. Kohrt WM, Wherry SJ, Wolfe P, Sherk VD, Wellington T, Swanson CM, et al. Maintenance
931 of Serum Ionized Calcium During Exercise Attenuates Parathyroid Hormone and Bone
932 Resorption Responses. J Bone Miner Res. 2018;33(7):1326–34.
933 https://doi.org/10.1002/jbmr.3428
934 55. Robling AG, Castillo AB, Turner CH. Biomechanical and molecular regulation of bone

935 remodeling. Annu Rev Biomed Eng. 2006;8:455–98.
936 https://doi.org/10.1146/annurev.bioeng.8.061505.095721
937 56. Dolan E, Varley I, Ackerman KE, Pereira RMR, Elliott-Sale KJ, Sale C. The Bone Metabolic
938 Response to Exercise and Nutrition. Exerc Sport Sci Rev. 2020 Apr;48(2):49-58.
939 https://doi.org/10.1249/JES.0000000000000215
940 57. Bhattoa HP, Cavalier E, Eastell R, Heijboer AC, Jørgensen NR, Makris K, et al. Analytical
941 considerations and plans to standardize or harmonize assays for the reference bone turnover
942 markers PINP and β-CTX in blood. Clin Chim Acta. 2021;515(September 2020):16–20.
943 https://doi.org/10.1016/j.cca.2020.12.023
944 58. Shea KL, Barry DW, Sherk VD, Hansen KC, Wolfe P, Kohrt WM. Calcium supplementation
945 and parathyroid hormone response to vigorous walking in postmenopausal women. Med Sci
946 Sports Exerc. 2014;46(10):2007–13. https://doi.org/10.1249/MSS.0000000000000320
947 59. Prawiradilaga RS, Madsen AO, Jørgensen NR, Helge EW. Acute response of biochemical bone
948 turnover markers and the associated ground reaction forces to high-impact exercise in
949 postmenopausal women. Biol Sport. 2020;37(1):41–8.
950 https://doi.org/10.5114/biolsport.2020.91497
951 60. Tosun A, Bölükbaşi N, Çingi E, Beyazova M, Ünlü M. Acute effects of a single session of
952 aerobic exercise with or without weight-lifting on bone turnover in healthy young women. Mod
953 Rheumatol. 2006;16(5):300–4. https://doi.org/10.1007/s10165-006-0503-5
954 61. Bowtell JL, Jackman SR, Scott S, Connolly LJ, Mohr M, Ermidis G, et al. Short duration small
955 sided football and to a lesser extent whole body vibration exercise induce acute changes in
956 markers of bone turnover. Biomed Res Int. 2016;2016:3574258.
957 https://doi.org/10.1155/2016/3574258
958 62. Smith C, Tacey A, Mesinovic J, Scott D, Lin X, Brennan-Speranza TC, Lewis JR, Duque G,
959 Levinger I. The effects of acute exercise on bone turnover markers in middle-aged and older
960 adults: A systematic review. Bone. 2021;143:115766.
961 https://doi.org/10.1016/j.bone.2020.115766
962 63. Bauer D, Krege J, Lane N, Leary E, Libanati C, Miller P, et al. National Bone Health Alliance

963 Bone Turnover Marker Project: Current practices and the need for US harmonization,
964 standardization, and common reference ranges. Osteoporos Int. 2012;23(10):2425–33.
965 https://doi.org/10.1007/s00198-012-2049-z
966