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Title Page:

Title: The effects of acute exercise on bone turnover markers in middle-aged and older adults: a systematic review

Running heading: Acute-exercise and bone turnover markers in middle-aged and older adults

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Abstract

Background: Bone turnover is the cellular machinery responsible for bone integrity and strength and, in the clinical setting, it is assessed using bone turnover markers (BTMs). Acute exercise can induce mechanical stress on bone which is needed for bone remodelling, but to date, there are conflicting results in regards to the effects of varying mechanical stimuli on BTMs.

Objectives: This systematic review examines the effects of acute aerobic, resistance and impact exercises on BTMs in middle and older-aged adults and examine whether the responses are determined by the exercise mode, intensity, age and sex

Methods: We searched PubMed, SCOPUS, Web of Science and EMBASE up to 22nd April 2020. Eligibility criteria included randomised controlled trials (RCTs) and single-arm studies that included middle-aged (50 to 65 years) and older adults (>65 years) and, a single-bout, acute-exercise (aerobic, resistance, impact) intervention with measurement of BTMs. PROSPERO registration number CRD42020145359

Results: Thirteen studies were included; 8 in middle-aged (n= 275, 212 women/63 men, mean age= 57.9 ± 1.5 years) and 5 in older adults (n= 93, 50 women/43 men, mean age= 68.2 ± 2.2 years). Eleven studies included aerobic exercise (AE, 7 middle-aged/4 older adults), and two included resistance exercise (RE, both middle-aged). AE significantly increased C-terminal telopeptide (CTX), alkaline phosphatase (ALP) and bone-ALP in middle-aged and older adults. AE also significantly increased total osteocalcin (tOC) in middle-aged men and Procollagen I Carboxyterminal Propeptide and Cross-Linked Carboxyterminal Telopeptide of Type I Collagen in older women. RE alone decreased ALP in older adults. In middle-aged adults, RE with impact had no effect on tOC or BALP, but significantly decreased CTX. Impact (jumping) exercise alone increased Procollagen Type 1 N Propeptide and tOC in middle-aged women.

Conclusion: Acute exercise is an effective tool to modify BTMs, however, the response appears to be exercise modality-, intensity-, age- and sex-specific. There is further need for higher quality and larger RCTs in this area.

1. Introduction

The skeleton has protective, mechanical and metabolic roles, providing structural support and a site for calcium storage (1-3). Bone should be strong, to prevent fractures, but light, to enable movement in a gravitational environment (1). Bone turnover, the cellular machinery responsible for bone integrity and strength, is a finely balanced process responsive to mechanical loads and hormonal changes (4-6).

Exercise is a non-pharmacological intervention that can improve bone health and reduce the risk of osteoporosis (7-11). The anabolic effects of exercise on osseous tissues are positively associated with the amount of mechanical strain exerted (12). In animals, the strain-adaptive remodelling response requires intermittent and dynamic, but not static, loading (13-18). Additionally, loading periods only need to be very short to stimulate adaptive responses, and that bone formation is threshold-driven and influenced by strain rate, frequency, amplitude and duration of loading (17, 19-23). Altogether, these findings demonstrate that bone requires dynamic (not static) strains (i.e. impact loading) for adaptive responses and, higher physiological rates compared to low rates and applied rapidly, to increase this response (14-16, 19, 24).

In humans, higher impact activities with rapid rates of loading (i.e. tennis/squash) are more osteogenic compared with lower impact sports (i.e. running/cycling) (25-27). Mechanical loads, produced by exercise, change local microenvironments of the canalicular networks within the bone framework via dynamic fluid shifts stimulating local osteocytes and ultimately bone turnover (28-30). Exercise serves varying purposes across the lifespan. In children, exercise is important for optimisation of peak bone mass, whereas, in older adults, exercise serves to maintain/reduce the rate of bone loss (9, 10, 31). However, the search for a relationship between exercise and bone mineral density (BMD) demonstrates contradictory findings, some reporting beneficial effects (7, 11, 32), while others have not (33-35). Moreover, available human data shows that the magnitude of benefit on bone from exercise is inconsistent, often influenced by safety concerns leading to conservatively prescribed training loads (36-40).

To optimise exercise effects on bone health a better understanding of the metabolic responses of bone tissue to various mechanical stimuli is needed. By convention, BMD is widely used as a measure of bone health to predict fracture risk (41), however, it represents a static bone mineral status and cannot

be used to estimate acute bone metabolic changes such as those induced by acute exercise. Therefore, BTMs represent an easy to measure option to assess the dynamic fluctuations in bone turnover (*Table 1*) (42). Using BTMs to describe bone metabolic activity comes with complexities, contributing to the lack of consensus in the literature. Whilst these markers are sensitive, they have high biological variability attributed to differences in i.e. blood sampling, study protocols, effects of feeding and circadian rhythm (42-44). As such, the aims of this systematic review were to 1) examine the effects of acute exercise on BTMs in adults over 50 years of age and to determine if middle-aged and older adults respond differently, and 2) to understand whether these effects were exercise modality-, exercise intensity-, sex- or BTM-specific.

Table 1. Markers of bone turnover that have been used in the exercise literature

2. Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (45) and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) - CRD42020145359.

Fig. 1 Identification screening and selection of studies (PRISMA Flow Diagram)

2.1 Inclusion criteria

The inclusion criteria for studies in brief were: (i) randomised controlled (RCT), cross-sectional or single arm trials including quasi-randomised design; (ii) adults ≥50 years of age, middle-aged adults defined as mean age ≥50 to <65 years and older adults defined as mean age ≥65 years; (iii) intervention of interest includes acute bout or single-bout of exercise; and (v) outcome of interest was BTMs (*see supplementary 1, PICOS protocol*).

2.2 Data extraction

CS and AT performed the literature search (*supplementary 2, search strategy*) and extracted data from the included studies, IL revised discrepancies. The following data were extracted: (i) characteristics of the participants i.e. sample size, sex, age (years), height (centimetres), weight (kilograms) and body mass index (BMI, height/weight^2); (ii) details of the acute exercise bout (intensity, duration, volume, mode); and (ii) details of outcomes of interest (BTMs) measured at baseline and post-acute exercise.

2.3 Quality assessment: Risk of bias and Methodological Index for Non-Randomised Studies

Risk of bias assessments were independently conducted by CS and AT. RCTs were assessed using the Cochrane Collaborations Risk of Bias 2 (ROB2) tool (46). We assessed selection bias (random sequence generation, allocation concealment), performance bias (blinding of participant and personnel), detection bias (outcome assessor blinding), attrition bias (handling of incomplete outcome data) and other bias including baseline imbalance on the primary outcome and selective reporting. All other trials not meeting the criteria for a RCT were assessed using the Methodological Index for Non-Randomised Studies (MINORS) scale (47).

3. Results

We identified 3637 articles. After removal of duplicates, 1465 unique titles and abstracts were screened, and 1421 articles were excluded. The full text of 44 articles was reviewed and a further 31 were excluded, leaving 13 articles for inclusion in our qualitative synthesis (*Fig. 1)*. The authors of four studies were contacted for further information (48-51). One intervention was described in two articles but with different stratification of groups, both articles were included and considered as a single trial (52, 53). Another study had additional analyses published at a later date, both articles were included but considered as a single trial (50, 51). Herein for both of these studies, the first published paper will be referenced.

3.1 Quality assessment

Results of the methodological quality assessments are shown in *Table 2* and *Figure 2*. Only 3 studies were RCTs (54-56) and assessed using the ROB2 tool. All others were assessed using the MINORs scale. No studies achieved a maximum quality score. Scores ranged on the ROB2 (*Figure 2)* and on the MINORs scale (*Table 2*) from 43.8% to 87.5%. The most common source of likely methodological bias using the ROB2 tool was the randomisation process and deviations from the intended study endpoint. Using the MINORs scoring system, the likely source of methodological bias was the absence of unbiased assessment of the study endpoint (n= 10) and prospective calculation of study sample size (n= 8).

Table 2. Quality rating scale (MINORs)

Figure 2. Risk of bias ratings

3.2 Study population and study design

Descriptive characteristics and study outcomes of included studies are described in *Table 3*. Two studies included adults with osteoporosis (untreated) (55, 57), five studies excluded individuals with osteoporosis/conditions affecting bone metabolism (49, 50, 53, 54, 58) and one study included adults with osteopenia (48). Four studies did not state whether they excluded participants with osteoporosis (56, 59-61). Five studies excluded individuals taking medications/supplements that effect bone metabolism (48, 50, 53, 54, 58), one stated except for calcium and vitamin D (55), four studies included participants not taking medications (57, 60-62) and three studies did not refer to medication use (49, 56, 59).

Of the thirteen studies included, eight were in middle-aged (mean age <65 years) (49, 50, 54-56, 59-61) and five were in older adults (mean age >65 years) (48, 53, 57, 58, 62). Sample sizes ranged from 11 to 150 (total combined data of the 13 studies n= 336 [220 women, 116 men]). Participants' age range was 52 to 73 years (mean age 62 ± 6 years) and BMI was 23.5 to 33.1 kg/m^2 (mean BMI 26.85 ± 3.33

171 kg/m^2). Sex-distribution for included studies was predominately women (71%); 77% of middle-aged
172 and 54% of older adults were women.

173 Eleven studies evaluated effects of acute AE exercise on BTMs (seven in middle-aged (49, 50, 55,
174 56, 59-61), and four in older adults (48, 53, 58, 62)). Two studies evaluated effects of acute combined
175 RE and impact (middle-aged adults) (49, 55), one study evaluated the effects of acute impact exercise
176 alone (middle-aged adults) (54), and one study evaluated the effects of acute RE alone (older adults)
177 (57) on changes in BTMs. Only two studies reported that the exercise was supervised (48, 54). Exercise
178 protocols, blood sampling protocols and effects of acute exercise on BTMs have been described in
179 *Table 3* including all reported levels and significant changes.

180 Nine studies reported that exercise and blood sampling were performed in the morning (49, 50, 53-
181 56, 59, 61, 62), one was performed in the afternoon (60), and three did not state the time of the day (48,
182 57, 58). Seven studies were performed in the morning following an overnight fast (49, 50, 53-56, 59),
183 one stated at least 12-hours of fasting (no indication of time) (57), and five studies were not performed
184 in a fasted state (48, 58, 60-62). One study involved a controlled pre-feed (48), and another stated a 2-
185 hour fast after a meal free from milk and cheese (60). Only three studies reported controlling for exercise
186 on preceding days (54, 61, 62). One study mentioned withholding dietary supplements (54). Post-
187 exercise blood sampling varied greatly from one to four timepoints; four studies taking only
188 immediately post (52, 53, 55, 58, 59), the longest taken at 72-hours (61, 62). A range of biochemical
189 assays were used to analyse the circulating BTMs including electrochemiluminescence immunoassay
190 (ECLIA), enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and
191 immunoradiometric assay (IRMA) (*Table 3*).

192

193 **Table 3.** Study characteristics and outcomes

194

195 *3.3 Acute aerobic exercise*

196 3.3.1 Effects on BTMs: middle-aged adults

Two studies reported significant increases in ALP immediately following cycling GXTs performed to exhaustion in men and in middle-aged postmenopausal women (59, 60). BALP also increased (range ~0.7 to 26%) in women after a cycling GXT to exhaustion, and also after moderate intensity walking (46mins, 3-6 METs) (55, 60). Three studies reported significant increase in tOC (range ~13.4 to 18.8%) in men who cycled (GXT to exhaustion; and 75% VO^2_{Peak}, 30mins), and in middle-aged postmenopausal women who jogged (50% HR_{Max} reserve, 45mins) (49, 59, 61). However, three cycling studies reported no change in tOC, one in men (90-95% HR_{Peak}, 30 mins) and two in middle-aged postmenopausal women (70-75% VO^2_{Peak}, 30mins; GXT to exertion) (50, 56, 60). No significant change was reported in P1NP after cycling in middle-aged postmenopausal women (70-75% VO^2_{Peak}, 30mins) (56) or in men (90-95% HR_{Peak}, 30mins) (50). Acute AE was also reported to have no effect on PICP in middle-aged postmenopausal women after jogging (50% HR_{Max} reserve, 45mins) (61).

One study reported that acute AE significantly increased (~16.6%) ß-CTX after cycling in men (90-95% HR_{Peak}, 30mins), however, there was no change in ß-CTX after cycling (75% VO^2_{Peak}, 30mins) or CTX after walking (3-6 METs, 46mins) in middle-aged postmenopausal women (50, 55, 56). Two studies measured ICTP with no significant changes in middle-aged postmenopausal women after jogging (50% HR_{Max} reserve, 45mins) or cycling (to exertion, GXT) (60, 61). SCL was reported to increase following brisk walking in middle-aged postmenopausal women (3-6 METs, 46mins) (55).

3.3.2 Effects on BTMs: older adults

ALP significantly increased in men and women immediately following a treadmill GXT (stopped at 75-85% HR_{Max}) (58). BALP also significantly increased (~12%) immediately following a treadmill GXT (to exertion), but only in men and women who were classed as moderately active (classified using a physical activity questionnaire) and not active based on baseline exercise levels (53). Two studies reported that tOC did not change in women after walking (50% HR_{Max} reserve, 90mins) or in men and women after a treadmill GXT (to exhaustion) (53, 62). PICP was reported to increase in women after walking (50% HR_{Max} reserve, 90mins) (62).

Wherry et al. (48) reported significant increases (range 34.6 to 77.3 %) in CTX levels at all post-exercise time points (peak, 15, 30, 45 and 60mins) in men and women who walked at moderate intensity

(70-80% $HR_{Max,}$ 60mins). In contrast, Maimoun et al (53) reported no significant change in men and women following a maximal GXT (treadmill). Thorsen et al (62) reported a significant decrease (~13.8%) in 1CTP levels at 1hr, but a significant increase (~15.5%) in levels at 72hrs post brisk walking (50% HR_{Max} reserve, 90mins).

3.4 Acute resistance with and without impact, or impact alone exercise

3.4.1 Effects on BTMs: Middle-aged and older adults

The effect of acute RE with and without impact exercises, versus impact only exercise on BTMs greatly varied with a limited number of studies measuring the same BTMs. In studies involving RE+impact, no change was reported in BALP in middle-aged postmenopausal women, or in tOC in middle-aged men (49, 55). On the contrary, impact-only exercise (three forms of jumping, *see Table 3*) significantly increased tOC (double jump group) and P1NP (all groups) immediately post, but at 2-hours tOC significantly decreased (all groups), with P1NP also reducing (non-significant) to below baseline levels (54). The drop in tOC (significant) and P1NP (non-significant) to below baseline levels was consistent with the control group in that study (54). CTX was the only consistent measured bone resorption marker shown to decrease following RE+impact and impact-alone protocols in middle-aged women (54, 55). However, in the impact-alone study, the significant decrease at 2-hours post (not immediately after) was not significantly different to the control group (54). Only one study investigated acute RE in older women (57) and reported a significant decrease in ALP; no other BTMs were measured in this study.

4. Discussion

We report that a) BTM responses to acute exercise vary between middle- and older-aged adults and that the BTM responses may be b) sex-specific and c) altered by exercise mode, intensity and duration. Additionally, responses to acute exercise stimuli may be d) BTM-specific, with some markers being more sensitive than others to the same stimuli. We identified a major gap in the current field with a

small number of studies investigating acute effects of exercise on BTMs in middle-aged adults (n= 8), and even fewer number in older-adults (n= 5).

The application of mechanical stress (i.e. exercise) to the skeleton can preserve and increase BMD, serving as a key intervention in the prevention and management of osteoporosis (8-10). The effect of chronic, long-term exercise training on BMD in older adults is well established, shown to be modality- and intensity-dependent (9, 40, 63, 64). Evidence suggests that walking is of limited value for improving bone health if prescribed without additional loading bearing exercises (37, 40, 63, 65-67). It is well accepted that RE with weight bearing and high impact is safe and effective to optimise bone health in older adults, as they result in high strain rates and peak forces and, reduce falls and fractures (7, 9, 36, 38, 68). In fact, high-velocity power and rapid concentric contractions (inducing higher strain rates on bone) is beneficial for functional performance (i.e. chair rise) in older adults (69-71). Additionally, regular weight-bearing impact, applied in multidirectional patterns, promotes bone maintenance/preservation (63, 72). While the evidence is clear from chronic, long term, exercise training studies what characteristics exercise protocols should consist of for beneficial effects on bone health in adults, the effects of acute exercise are unclear. Available data are conflicting and, as it is not appropriate to measure BMD after a single session, BTMs are used as a surrogate measure (42). Whether various modes of acute exercise with different modifiable characteristics alter bone metabolism differently in middle and older adults is underexplored.

4.1 Age and sex-specific effects on BTM responses to acute exercise

Based on this review, while acute exercise is sufficient to detect responses in BTMs, these responses may be age- and sex-specific, highlighting some possible consideration in the design of future acute exercise studies. For instance, all AE exercise studies investigating the tOC and BALP response in older adults (men and women) report no change after exercise, but some studies in middle-aged adults (men and women) report increases (49, 53, 55, 59-61). Conversely, ALP appears to have similar sensitivity in middle and older aged men and women (50, 58-60) and resorption markers CTX (men and women) and ICTP (women only) appear to increase in older adults, but not middle-aged (48, 55, 60-62). Lastly,

tOC and ß-CTX responses to AE also appears to be more sensitive in middle-aged men than women, suggesting a possible sex-specific response (49, 56, 59-61). Differences in BTM responses between middle- and older-aged adults could be multifactorial, explained by age-related alterations to bone composition and hence bone turnover, and in women, menopausal effects, possibly altering the bone response (6, 73-77). Indeed, underlying bone pathophysiology is different in middle-aged vs older women who, are known to have elevated bone turnover rates, possibly explaining differences in responses (6, 78). Given bone resorption was not significantly altered in some of these studies in women (55, 56, 60, 61) may in fact, be beneficial (not stimulating further the negative balance of the remodelling process), however this is poorly understood and warrants further exploration.

Of note, at baseline, some studies did not report/screen for BMD and/or T-score, as adults are known to be affected by age-related bone composition alterationpowers, particularly women, this should be considered. Some studies excluded individuals with osteoporosis (49, 50, 53, 54), whereas others included adults with osteopenia/osteoporosis (48, 55, 57), possibly influencing BTM responses (79). Some studies in older adults pooled men and women data together (48, 58), only one confirming no sex-interaction in BTM responses (53). As older women are known to have different rates of bone turnover and consequently accelerated bone loss compared to men, bone responses may be altered (or attenuated) thus, men and women should be handled separately, or sensitivity tests performed (35, 73-77, 79).

4.2 BTM responses modulated by exercise mode, intensity, and duration

This review summarises that BTM responses to acute exercise may be modulated by the specific characteristics of the exercise protocol used. For instance, a majority of studies report no change in tOC following AE regardless of intensity (low, moderate, high) (50, 53, 56, 60, 62). However, tOC may be more sensitive only to AE that incorporates loads of greater ground-reaction force increasing in one study after jogging, but not after the majority of studies including cycling or walking protocols (50, 53, 56, 60-62). Whereas, ALP, BALP and PICP increase after cycling and walking, suggesting these markers have higher sensitivity to AE with lower impact (53, 55, 58-60, 62). Indeed, in three separate

studies in middle-aged men utilising cycling protocols the tOC response was different, increasing only after moderate intensity cycling (30mins) and a short duration maximal exertion GXT, but not after high-intensity interval exercise (30mins) (49, 50, 59). This suggests that exercise intensity and duration may be important, but there may be other possible modulating effects on the tOC response, which should be further explored. Markers reflecting bone resorption, CTX and ICTP appear to be more sensitive to AE protocols that are longer (≥ 60mins), not shorter duration (<45mins) (48, 53, 55, 60-62). Whereas, ß-CTX (a different fragment of CTX) responds differently to cycling exercise of same duration (30mins), increasing only after high-intensity, but not moderate-intensity cycling, suggesting that in this instance, intensity may be important (50, 56).

Despite the mounting evidence for the use of RE combined with weightbearing and impact loads distributed in dynamic and novel patterns for optimising bone health effects, little is known about the acute effects and available studies investigating these characteristics is limited. Based on this review, RE with impact does not stimulate a response in markers reflecting bone formation (49, 55). However, one study measured BALP only at immediately post exercise (55), the other measured tOC only up to 2-hours, possibly missing the kinetic response (49). Direct comparison of these study protocols is difficult, one study used core stabilisation bodyweight exercises with small impact exercises (steps, hopping) (55), the other study used power leg press RE (70 to 75% maximal strength) with high impact jumping, thus the impact and mechanical strain load on bone would be very different (49). However, it does appear that high impact exercise alone and RE alone is sufficient to detect a response in BTMs of formation. Indeed, ALP was decreased in one study following a RE regimen of pilates exercises, however, whether this is truly indicative of a bone-response is unclear, and other BTMs were not measured (42, 57, 80). Of note, only the study investigating impact alone using three sessions each containing a different form of jumping, reported increases of tOC and P1NP. P1NP increased for all jumping protocols, but tOC was only increased in the session where participants dropped from a height to an explosive vertical jump, not from jumping directly from the floor (54). Highlighting that, P1NP may be more sensitive than tOC to impact exercise, and that the tOC-specific response may require greater impact loads (ground reaction force) combined with high explosive movements to elicit a response. Based on these studies it appears that CTX decreases with RE combined with impact, and

with impact alone protocols (54, 55). However, while both of these studies were RCTs, the impact only study which was crossover in design report that CTX decreases also in the control condition (54). This decrease was not different to the decrease seen post the impact exercise, indicating that CTX is affected by circadian/diurnal effects (54, 81).

Altogether, the evidence from this review, and from the literature demonstrates that exercise intensity, dynamic, and novelty of new loads (non-habitual nature) placed on the skeleton are important characteristics influencing the bone-exercise response (16, 23, 24, 82, 83). However, only three studies included participants' baseline fitness in the selection criteria (48, 49, 54). Three state (60-62) participants were non-regular exercisers, but one reports participants regularly cycling (1-6km/day, few days a week) (60). As habitual exercise was not considered in a majority of studies, protocols may lack in specificity, and although some used prior testing to define exercise intensity their protocols possibly lack in novelty of new load (12, 24, 84). Indeed, one interesting concept, explored by one study, was the possible effect on the BTM response based on the participants baseline fitness, whereby BALP was only shown to be significantly increased with AE exercise when older adults were further stratified into moderately active, or active groups (53). This possibly suggests that the BALP response in older adults may be dampened, modulated by the participants' baseline fitness, supporting the principle that bone cells have a threshold level of adaptation and the need for consideration of individualised, progressive (graded, based on baseline fitness) and novelty in protocol loads, discussed earlier (12, 24, 84). This should be further explored in future research, as it likely impacts/dampens the BTM-response and therefore a skewness in results.

4.3 BTM-specific responses to acute exercise

To understand if different BTMs thought to reflect the same bone turnover phase have different sensitivities to acute exercise we compared study effects where >1 BTM reflecting the same bone formation or resorption phase was measured within the same study. AE appears to have a limited effect on tOC and P1NP, whereas other markers reflecting bone formation namely ALP, BALP and PICP appear to be more sensitive. Altogether, suggesting that tOC may be the least sensitive BTM of formation and supports the notion that these BTMs may represent different phases of osteoblastic

function or formation (42). Indeed, ALP activity includes serum derived from liver and bone, therefore changes in response of ALP may be non-specific to bone, as such BALP is recommended for its increased specificity (42, 80).

While AE appears to have a limited effect on tOC, one concept to raise about tOC is that it exists in the circulation in a carboxylated (cOC) reflecting more bone mineralisation, and undercarboxylated (ucOC) form, considered the more "bio-active" counterpart, acting as a hormone involved in energy metabolism and possibly a role in muscle maintenance and strength (85-91). When studies measured effects on tOC only, whether there is a shift in favor of cOC, or ucOC, is unclear, as only few studies measured this (49, 50, 56). In these studies, ucOC increased even with null change in tOC in two of them (50, 56). Therefore, regarding tOC, there is much more to be understood.

One study measured >1 BTM reflecting resorption, interestingly SCL, a possible promoter of bone resorption, increased following walking, but not CTX (55, 92). Suggesting, SCL may be more sensitive than CTX, however, blood sampling was performed only once (immediately post) possibly missing peak change in CTX. Of note, SCL increases with age and high levels are associated with long-term physical in-activity/immobilisation (93-96). Additionally, mechanical unloading increases the expression (gene and protein) of SCL, whereas SCL expression decreases with mechanical loading (*in-vivo* and *in-vitro*) (97, 98). Therefore, SCL may be an interesting marker to be included in future studies.

The BTM responses following exercise may be too fast to be a result of new protein being synthesized and secreted by bone. However, there are at least two possible explanations for the rapid alteration of circulating BTMs: 1) it is known that bone responds to fluid shifts (99), which occurs during exercise and as such, it is possible that proteins that were already produced are now released into the circulation at a faster rate and 2) it is plausible that the BTMs are stored in other organs, such as the liver (100), and these are released during exercise. These hypotheses should be tested in future studies.

BTMs are highly dynamic and sensitive, however, investigators should consider factors known to influence BTMs in preparation for testing i.e. circadian/diurnal rhythm, feeding, sleep, smoking, menopause age and exercise (42, 43, 75-77). Some studies were not performed in the fasted state and/or

in the morning (48, 58, 60-62). In addition, blood sampling protocols largely differed between included studies, some sampling only immediately post-exercise, others taking multiple samples up to 2-hours post-exercise, and others up to 72-hours post-exercise. As blood sampling represents only a small "window in time" it is possible that some studies, particularly those that only sampled immediately post-exercise may have missed the peak response of the BTM-kinetics. As such, it is not clear whether there is an "optimal" time to assess BTMs following exercise. It is highly recommended that blood sampling is taken at several time points post-exercise, perhaps immediately after exercise and then every 30-60 minutes up to 2-3 hours post-exercise, to identify the "peak response" of each individual. The data for each time point, in addition to the "peak response" and perhaps the area under the curve should be presented. While there are some ethical considerations for invasive techniques and frequency of venepuncture and/or sampling volume, a better understanding of the time-course response of BTM-kinetics is required. Despite advances in quality assurance, laboratory errors commonly occur in pre-analytical phases i.e. timing of sampling, selection of specimen, collection procedure and, sample transport, temperature and time to storage, thus extra rigor should be employed to ensure accurate and reproducible results (43, 44, 101, 102).

4.7 Limitations and strengths

To our knowledge this is the first systematic review to examine effects of acute exercise on BTMs in adults >50 years of age, highlighting major gaps in the field and considerations for increased rigor in future trials. The current review emphasises that research into the effects of acute exercise on BTMs in middle-aged adults is limited and is even scarcer in older adults. Whilst the number of included studies is low (n = 13), it covers the only available research in this area. Several factors limit the generalizability of the findings; a lack of RCTs, low quality of the evidence, small sample sizes, potential bias in the cohorts, large variance in the exercise and blood sampling protocols, and the use of different assays to detect BTMs. The latter is an important factor that may lead to differences in findings between studies as the sensitivity of each assay may vary. In addition, it will be important for future studies to explore the chronic adaptation of BTMs to exercise training, to identify the optimal frequency, intensity and mode of exercise that should be taken to elicit optimal bone responses.

5. Conclusions

Acute exercise is an effective tool to induce changes in serum BTMs, however, the response appears to be exercise modality-, intensity-, age- and sex-specific. Large variability in study populations, exercise and blood sampling protocols explains conflicting results and as such, future studies should include tight control over factors that influence BTMs. Longer sampling periods of BTMs may assist in understanding the BTMs-kinetic responses. Further high-quality acute exercise studies are needed to identify new mechanistic target pathways for therapeutics and optimising exercise prescription for adults.

6. Declarations

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none to declare

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