The Bone Metabolic Response to Exercise and Nutrition

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ABSTRACT:

Bone (re)modelling markers can help determine how bone responds to different types, intensities and durations of exercise. They might also help predict those at risk of bone injury. We synthesised evidence on the acute and chronic bone metabolic responses to exercise, along with how nutritional factors can moderate this response. Recommendations to optimise future research efforts are made.

IN BRIEF:

Bone (re)modelling markers elucidate the dynamic bone response to exercise and if used appropriately have large potential to progress understanding.

KEY WORDS: bone remodelling, resorption, formation, exercise, turnover, loading, metabolism.

KEY POINTS:

- Bone (re)modeling markers (BMMs) are products of bone proteins or cells, and represent processes involved in either the formation or resorption of bone.
- The stimuli (both mechanical and metabolic) created by an acute exercise bout, typically elicits an increase in markers indicative of bone resorption (*e.g.,* β-CTX-1), whilst adaptation to exercise training typically results in an increase in bone formation (*e.g.,* PINP).
- Nutritional status, and acute nutrient intake, can moderate the bone metabolic response to exercise.
- Appropriate use of these biomarkers, in well-controlled settings, has the potential to progress knowledge on the acute, or short-term, responses of bone to exercise and nutritional stimuli, and so to contribute toward the development of strategies to protect or enhance the bone health of exercising individuals.

1. INTRODUCTION

The bone response to exercise is complex and influenced by multiple factors, including nutrition; training status; age; genetics and the characteristics of the specific exercise stimulus. Exercise is typically beneficial to bone, and sports that convey high-impact, multi-directional movement patterns, and unaccustomed loads, are widely accepted as providing the optimal osteogenic stimulus (1). Accordingly, exercise is considered an effective preventive or treatment strategy for those with conditions characterised by bone loss, or increased fracture susceptibility (*e.g.,* osteoporosis) (2). Conversely, participation in sports involving lower-impact and/or repetitive loading cycles (such as endurance running) or non-weight bearing sports (such as cycling and swimming) do not typically elicit skeletal benefits (3,4). Indeed some groups of athletes (*e.g.,* cyclists and jockeys) have lower BMD than non-athletic controls, implying a negative influence of some types, or volumes, of exercise on bone (5,6).

Much remains unknown about factors influencing the bone response to acute and chronic exercise, or how to pre-emptively identify those at risk of bone injuries. To elucidate these factors, objective and quantifiable indicators of bone strength and function are essential. BMD [assessed by dual energy x-ray absorptiometry (DXA)] or bone microarchitecture [assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT)] may be used to predict fracture risk (7–9) or to assess intervention efficacy. These outcomes are, however, chronic indicators of bone, which responds slowly to stimuli. Measurable changes can take months, or even years, to occur; and so acute or shorter-duration responses cannot be detected using these measures.

In contrast, bone (re)modelling markers (BMMs) provide information about dynamic bone activity and can indicate the acute or short-term response to stimuli, and their potential to progress knowledge on this topic is large. This potential cannot currently be realised, however, due to incomplete understanding of their physiological relevance, however, along with large heterogeneity in study design and characteristics. The aim of this review is to consolidate understanding of the acute and

chronic BMM response to exercise, and to make recommendations to optimise the use of appropriate biomarkers in future studies. Additionally, we will describe how nutritional interventions moderate the BRM response to exercise, and how this information can elucidate the mechanistic pathways mediating these responses.

2. THE BONE METABOLIC RESPONSE TO EXERCISE

Provided the nutritional and metabolic environment is favourable, the primary stimulus for bone anabolism is physical loading (10,11), with bone responding to the magnitude, rate, number and direction of activity-induced loading-cycles (12). As such, different exercise modalities exert distinct loading patterns and activity-specific mechanotransductive signals (13). Various metabolic signals also influence the bone response to exercise, such as reactive oxygen/nitrogen species (14), altered pH (15) and serum calcium availability (16). Modelling refers to the formation or resorption of bone at specific sites. In contrast, remodelling is a coupled and synchronized process of of bone activation, resorption, reversal and formation, which is co-ordinated by teams of bone cells (*i.e.,* osteoblasts, osteoclasts and osteocytes) termed the basic multicellular unit (BMU). Although some modelling cannot be ruled out, it seems that remodelling is the dominant process through which bone responds to the mechanical or metabolic stimuli offered by exercise (12,17). An overview of this process is shown in Figure 1.

2.1. The use of bone (re)modelling markers in sport and exercise science

BMMs are products of bone proteins or cells and mostly represent processes involved in either the formation or resorption of bone (see Table 1 for an overview of commonly used BMMs). Their potential to elucidate the mechanisms through which bone responds to exercise is large, but some factors impede this interpretation, if not considered in study design and interpretation. It is important to understand that many BMMs (*e.g.,* PINP, OC, OPG and PYD) are non-bone-specific, which renders mechanistic interpretation challenging. For example, some biomarkers (e.g., PINP, PYR, DYP and ICTP)

are products of collagen metabolism, which is the main structural protein of many connective tissues, and not only the bone. As such, their measurement is not necessarily indicative of altered bone activity only. Similarly, osteocalcin (OC) is a small non-collagenous protein synthesised by osteoblasts, which is often used to estimate osteoblast activity and, therefore, bone formation. But both intact and fragmented OC may also be released during bone resorption (18), suggesting that this biomarker may indicate general bone remodelling rather than bone formation specifically. Additionally, OC is a non-specific protein that fulfils a number of extra-skeletal roles, including functions in energy metabolism and muscle activity (19). These extra-skeletal roles are particularly relevant when interpreting the OC response to exercise, given that bioenergetic pathways and muscle activity are also upregulated by exercise. Thus, it is difficult to attribute any changes in circulating OC to altered bone activity.

The repeatability of BMM measurement is another important consideration, as some show substantial inter- and intra-individual variability, and/or, are difficult to accurately measure. For example, the osteoprotegerin/receptor activator of NF kappaB ligand (OPG/RANKL) ratio is commonly used to indicate bone resorption, but soluble RANKL is sometimes difficult to accurately measure *in vivo* (20), and so results may be mis-leading. Bone biomarkers are often described as representing "bone turnover". Calculations, such as the uncoupling index, are commonly used to represent the predominant state of bone metabolism, whereby resorptive activities that are "coupled" with formation would represent a state of equilibrium, whereas "uncoupling" occurs when formative and resorptive processes are unbalanced and favour either the loss or gain of bone. Some caution should be applied when considering such calculations, because BMMs are systemic and cannot indicate bone activity at any one particular site, which is an issue because the true bone response to loading is largely site specific (21). A wide range of potentially confounding factors also impact BMMs and must be accounted for in study design and interpretation. Bone is responsive to both acute and chronic nutritional stimuli (described in Section 3) and so nutritional status must be carefully controlled in exercise trials. Other factors, including sex, age (22), menstrual cycle phase (23), oral contraceptive use (24), seasonal (25) and circadian (26) variations, genetics (27), various medical conditions and

120 medications (28) and injury history, particularly previous fractures (29,30), prior exercise and pre-
121 analytical storage and handling (31) may also influence BMMs.

122 Nothwithstanding these considerations, the clinical and mechanistic relevance of these biomarkers is
123 well recognised, and in an attempt to optimise their clinical utility, the National Bone Health Alliance
124 (NBHA) advised that all studies should include, as a minimum, measurements of N-terminal
125 propeptide of type 1 procollagen (PINP) and the C-terminal telopeptide of type 1 collagen (β-CTX-I),
126 as indicators of bone formation and resorption (32–34). The decision to focus on two biomarkers was
127 made to allow for greater harmonisation, and therefore comparability, of ongoing research efforts.
128 These particular biomarkers were selected based upon the recommendations of an expert working
129 group of the International Osteoporosis Foundation and the International Federation of Clincial
130 Chemistry and Laboratory Medicine, who deemed them to have a relatively smaller biological
131 variability, higher specificity to bone metabolism and to be more responsivene to anti-resorptive or
132 anabolic treatments, than other available BRMs (reviewed in detail in 33,35). Considering the currently
133 available information, we concur with this recommendation, and support the use of PINP and β-CTX-
134 I as core components of biomarker panels used to investigate the bone metabolic response to exercise
135 and nutrition interventions.

136

137 ***2.2. The bone metabolic response to an acute bout of exercise***

138 Increased bone resorption is the initial response to an acute bout of exercise, and increased β-CTX-I
139 has been reported in a number of trials (36–40). This finding is consistent with what we assume about
140 bone remodelling, whereby osteoclast activation, induced by mechanical or metabolic signals,
141 activates the BMU, causing an acute increase in bone resorption. This was shown in response to
142 different exercise types, including treadmill running (39), intense cycling (36) and a fatiguing bilateral
143 jump protocol (40). In contrast, bone formation markers seem to be largely non-responsive to acute

144 exercise, with most studies reporting no change to serum PINP, even when high-intensity exercise
145 protocols were used (40–43). This finding aligns with the bone remodelling process shown in Figure 2,
146 whereby the BMU is thought to be activated by an initial stimulation of bone resorption, meaning that
147 any change in bone formation would be expected to lag behind that of bone resorption. Despite this,
148 increased PINP has been reported following 60 minutes of continuous running at intensities ranging
149 from 55 – 75% of VO_{2max} (39,44), or an unaccustomed football session (45), demonstrating that
150 indicators of bone formation do, sometimes, respond to acute exercise, although this response is less
151 common than that observed in markers indicative of bone resorption. The reason for this
152 inconsistency in response is not entirely clear and further research to better characterise the BMM
153 response to acute bouts of exercise under varying conditions and with longer follow-ups post exercise
154 bout will be of interest.

155 Exercise intensity and duration seem to be instrumental in determining the BMM response to acute
156 exercise (38), with higher, but not lower, intensity protocols typically eliciting a response. Those
157 studies that observed no response to an acute bout of exercise generally used lower intensity and/or
158 shorter duration protocols, including 30 minutes of walking or jogging (46,47), a 30 second Wingate
159 cycling test (48) or water aerobics (46). Bone is commonly thought to respond only to high-impact or
160 unusual impact loads, but the available studies show that these are not essential to elicit an acute
161 BMM response. For example, cycling tests consistently increase β-CTX-I (36,37,49), despite conveying
162 little to no impact loads. Recently, intensity-matched interval sessions conducted either on a bike or
163 treadmill induced comparable sclerostin responses in men (50) and women (51). This demonstrates
164 that impact was not necessary to elicit this BMR response, which instead must have been stimulated
165 by other, potentially metabolic, factors, such as increased reactive oxygen or nitrogen species (14),
166 acidosis (15) or reduced serum calcium availability (16).

167 Despite strong evidence that bone resorptive markers, such as β-CTX-I, are responsive to acute
168 exercise, some well-controlled investigations reported no change to any BMM, even though they used

high-intensity protocols (43). It is important to keep in mind, that the BMM response to exercise is time-specific and transient (38,39,52). For example, some studies reported changes to BMM concentrations as the area under the curve of multiple sampling points (53), but the response was not apparent when based on single sampling points. Similarly, both a sustained (39) and a transient (44) β-CTX-I response to treadmill running in the days following an acute exercise bout were reported, with exercise intensity the apparent differentiator between these two responses. Thus, studies that use single sampling points may well miss transient or time-specific changes, consequently impacting mechanistic interpretation of findings. Future studies should seek to use multiple sampling points to characterise the BRM responses to acute exercise, ideally taken over several days, post-exercise. The nature of the temportal BRM response to exercise is, however, incompletely understood, rendering it difficult to identify the most appropriate timing and number of sampling points. Additionally, hemoconcentration should be assessed and accounted for in order to control for the potentially confounding influence of plasma volume changes on the bone biomarker response to exercise.

2.3. The bone metabolic response to longitudinal exercise interventions

Prolonged exposure to exercise training typically elicits an increase in resting levels of bone formation markers (either PINP, bone alkaline phosphatase (BALP) or both) (54–67), indicating that training might stimulate chronic upregulation of bone formative processes. This aligns with the model shown in Figure 2, whereby increased bone resorption in response to acute exercise (described in Section 2.1) activates the BMU, ultimately leading to an increase in bone formation. Unlike the largely consistent finding of increased bone formation in response to exercise interventions, markers of bone resorption are less responsive to training, with most studies reporting no change. A few studies have reported a reduction in bone resorption markers following a training program (55,57,68,69), and this was typically accompanied by an increase in bone formation, suggesting a metabolic bone profile favouring formation. Some studies also reported a concomitant increase in BMD alongside increases

in bone formation markers (59,68,70,71), indicating that training can be osteogenic, and that this can be monitored by BMMs.

In common with the studies investigating the BMM response to acute bouts of exercise, efforts have been made to identify how various exercise characteristics, including type, intensity and total work done, influence their response to training. Studies that matched training volume, but varied intensity, reported either a larger (59) or similar (72) response in bone formation markers (serum OC and B-ALP) when a higher intensity protocol was employed. These inconsistent results suggest that, although exercise intensity may well influence the BRM response to exercise, it is unlikely to be the predominant factor. Instead the total amount of work done (to which intensity will certainly contribute) may be a more important factor.

A wide range of exercise types have been investigated, but no one type stands out as being more or less effective at eliciting a BMM response. It is generally accepted that high-intensity exercise that conveys large and unaccustomed gravitational or muscular loads is necessary to elicit an osteogenic response (73). It follows that this type of exercise would induce the largest and most consistent increase in markers of bone formation following a period of training, but this does not seem to be the case. Similar to evidence from acute studies (described in Section 2.1), exercise types with lower impact and repetitive loading cycles (such as treadmill walking/running, step aerobics and yoga (54,55,57,60,66)) were equally likely to elicit a response in bone formation markers, as those modalities that exert large muscle or gravitational forces, (such as football training (63), high-impact jump activities (61,68) resistance training (58,59,62,64) and multi-modal activities (67), including military combat training (56)). This raises an important question about the relationship between BMM and chronic bone outcomes, such as mass and strength. It is widely accepted that exercise type is an important determinant of the bone response to exercise, but this view is not supported by the available BMM data. Is it possible that exercise type is less important to bone than previously believed? Or perhaps BMM changes are not necessarily predictive of changes to bone mass, strength

or microarchitecture? The available evidence does not allow this question to be answered, but in order to optimise the use of BMMs in sport and exercise science, it should be addressed in future investigations.

Individual participant characteristics, such as age, health and training status, are also important when considering the bone metabolic response to exercise. Many of the investigations that reported no response to exercise training were conducted on older adults (74–76), children with type 2 diabetes (77) and breast cancer survivors (78). It seems plausible to suggest, therefore, that age, or health-related factors, may have influenced these results. Indeed "anabolic resistance" has been reported to be a consequence of senescence, and refers to a blunted response to anabolic stimuli, such as exercise or protein (79,80). Similarly "osteogenic resistance" to bone loading programs in older adults has been proposed (81), which may be due to various age-associated physiological changes, such as reduced sex hormone content, although direct evidence to support or refute this hypothesis does not currently exist. Having said that, exercise interventions were osteogenic in postmenopausal (2) and older populations (82), which would necessitate an upregulation in bone remodelling, suggesting that while age and hormonal changes may attenuate exercise-induced osteogenesis, they do not block it.

Most of the investigations described in this review indicate that exercise training triggers an increase in bone formation activities, and so should be osteogenic. But circulating OC and B-ALP decreased following a period of intensive training in two groups of military recruits (83,84), showing that training can, in certain situations, suppress bone formation. This finding likely relates to the amount of energy available to support bone remodelling (85) (described in Section 3.1). It is also plausible that inadequate recovery during periods of particularly arduous and unaccustomed training may impede the reversal phase of the bone remodelling cycle, thus attenuating its osteogenic potential. These findings highlight the many factors, independent to the actual exercise itself, that may moderate the bone metabolic response to exercise training. This complexity makes it difficult to isolate, and so to investigate, any one individual factor. Recognition of this challenge is essential to the design and

interpretation of appropriately-controlled studies that are capable of really enhancing understanding of this important research area.

2.4. The bone metabolic profile of different athletic populations

Cross-sectional studies of different athletic groups provide insight into the influence of habitual training practices on bone metabolism. As expected, increased BMMs (both formation and resorption), alongside increased BMD and/or enhanced geometry, have been reported in athletes participating in sports involving high mechanical loading, including gymnasts (86), decathletes (87) and football players (88). Altered bone metabolism was also reported in athletes involved in lower-impact sports (*e.g.*, swimming (89), cycling (90,91) and horse-racing (92)), but these typically presented as either decreased bone formation (89,91) or increased bone resorption (90,92), suggesting overall bone loss. Low-impact sports such as these are considered to provide a sub-optimal stimulus to bone, although it is not clear if this is due to the lower mechanotransductive signals provided by low-impact and repetitive loading cycles, or whether other factors, such as an insufficient energy availability (EA; described in Section 3.1), may also influence this response.

The finding of altered bone metabolism in athletic groups is not consistent across the literature; no BMM differences were shown between controls and female athletes involved in high-impact sports (93), rhythmic gymnasts (94) and male master runners and speed/power athletes (95). Adapted BMD and/or bone microarchitecture was, however, reported in these studies, suggesting that bone was impacted by sports participation. This might suggest that BMMs are not necessarily indicative of altered bone mass or microarchitecture. On the other hand, many of the studies described herein were based upon single sampling points and given the temporal BMM responses to exercise and training it is possible that upregulated metabolism was not detected.

3. THE INFLUENCE OF NUTRIENT INTAKE ON THE BONE METABOLIC RESPONSE TO EXERCISE

When considering the BMM response to exercise, it is essential to also consider the nutritional environment within which that response took place. Bone is acutely responsive to nutrient intake (96–98) and studies investigating the impact of nutritional interventions on the bone metabolic response to exercise can be used to identify the mechanistic pathways underpinning this response, and to inform the development of nutritional interventions to improve bone health.

3.1. Energy availability

EA refers to the amount of energy available for physiological processes, after the demands of training are met (99), and is an important determinant of bone health in athletes. Extensive research shows that insufficient EA negatively impacts a variety of bone parameters (85), including BMMs. In a parallel-group study, Ihle & Loucks. (2004) examined the dose-response relation between EA (10, 20, 30 or 45 kcal·kgLBM·day^{-1}) and bone metabolism in sedentary, but otherwise healthy, eumenorrheic young women. Bone formation (OC and carboxyterminal propeptide of type 1 procollagen (PICP)) was suppressed at all levels of low EA (30, 20 and 10 kcal·kgLBM·day^{-1}). This was accompanied by a significant increase of bone resorption (aminoterminal telopeptide of type 1 collagen (NTX)) during the lowest EA condition (10 kcal·kg LBM·day^{-1} (100)). More recently, Papageougiou et al. (101) conducted two, independent repeated-measure investigations (reported in the same paper), on the influence of 5 days low EA (15 Kcal·kg LBM·day^{-1}) on bone metabolism in physically active men (study 1) and women (study 2). Low EA increased bone resorption (β-CTX-I) and reduced bone formation (PINP) in women, but not in men (101). Each of the studies described herein induced low EA through a combination of exercise and dietary restriction, and so could not distinguish whether restricted energy intake, or increased energy expenditure, was the dominant cause of altered bone metabolism. This important point was subsequently investigated, and it seems that low EA (15 kcal·kg LBM·day^{-1}) induced through dietary restriction, but not by exercise-induced energy expenditure, reduces bone formation (PINP) (102). It is not clear whether this occurred because the benefits of exercise masked, or over-rode, the negative effects of restricted energy intake, and further mechanistic elucidation is

important. Irrespective of the mechanisms, however, it seems that exercise may protect bone during periods of energy restriction, which has implications for interventions designed to protect bone during such periods, suggesting that the focus should be on increasing dietary intake, rather than on reducing exercise. The benefits of this strategy may extend beyond the bone alone, although the efficacy of this approach for bone, and for other tissues should be confirmed using randomised controlled investigations.

Reduced bone formation was also reported in cross-sectional studies conducted on energy deficient athletes (103–106), and in clinical populations characterised by extreme energy deficiency (*e.g.,* anorexia nervosa) (107–109). This likely occurs in an attempt to preserve energy for more immediately essential physiological processes (99). Reduced bone formation was accompanied by reduced resorption in energy and oestrogen-deficient exercising women (103), adolescent boys with anorexia (108), fasted lightweight male rowers (104) and energy-deficient amenorrheic and oligomenorrheic women (105). In contrast, extremely low EA simultaneously increased bone resorption, and decreased formation, in severe restriction trials (10 kcal·kg LBM·day^{-1}, (100)) and in some studies of patients with anorexia nervosa (107,109). Such a bone profile has particularly negative consequences for bone, should it persist for prolonged periods of time. Evidence of disrupted bone metabolism in response to low EA has implications for research in this area and likely accounts, at least in part, for findings described earlier in this review, including reduced bone formation following periods of arduous training (83,84,110) (Section 2.2) or as identified in cross-sectional investigations of some athletic groups (89,90,92) (Section 2.3).

3.2. Macronutrient ingestion pre, during and post exercise

Nutrient ingestion pre, during and immediately post acute exercise can alter the BMM response to that exercise bout. Scott et al. (39) investigated pre-exercise ingestion of a mixed meal, versus fasting, on response to a 60-minute treadmill run conducted at 65% of VO_{2max}. Meal ingestion reduced pre-exercise β-CTX-I, but did not influence its exercise induced increase, and the authors concluded that

317 pre-exercise feeding did not meaningfully impact the BMM response to the subsequent exercise bout.
318 However, this study suggested that the stress of the exercise bout over-rode the pre-exercise effect
319 of feeding on β-CTX-I, which, in turn, raised the question of whether or not more continuous nutrient
320 provision throughout the exercise bout would exert a more noticeable effect. This was investigated
321 by Sale et al. (53), who provided CHO before, during, and after a 120 minute treadmill run and reported
322 modestly reduced PINP and β-CTX-I post-exercise.

323 Studies investigating nutrient ingestion pre- and during exercise are limited by practical considerations
324 related to the type and volume of nutrients that can be ingested without impacting exercise
325 performance. The post-exercise period is thus more amenable to feeding interventions. Townsend et
326 al. (111) investigated the influence of a combined CHO/protein supplement following a fatiguing
327 treadmill run and reported a suppression of the β-CTX-I response when compared to the control trial,
328 along with a smaller, but statistically significant, increase in PINP concentrations at 3 and 4 hours post-
329 exercise (111).

330 These studies demonstrate that feeding around exercise can modulate the bone resorptive response
331 to that exercise bout, with the post-exercise period perhaps the most practical and influential
332 opportunity for nutrient provision. Theoretically, this reduction in bone resorption may protect bone
333 during periods of high intensity and/or volume training. On the other hand, and as described in Section
334 2, exercise-induced increases in bone resorption are necessary for BMU activation, and it is plausible
335 that attenuated bone resorption during or post-exercise, could, theoretically, blunt the bone adaptive
336 response. To date, longitudinal studies investigating how acute BMM alterations translate in the long-
337 term are not available, meaning that these potential long-term consequences are hypothetical and
338 require investigation.

339 The studies described above were not designed to investigate the independent influence of the three
340 macronutrients (carbohydrates, fats and proteins) on the BMM response to exercise, and limited data
341 on this topic exist. Protein is a particularly interesting macronutrient in this context, given it's

relevance to athletic adaptation and performance, along with the many, and potentially conflicting, pathways through which it influences bone. The available evidence indicates that protein is a bone-protective nutrient (112) and largely positive, albeit somewhat inconsistent, results have been reported in studies investigating the influence of protein supplementation in conjunction with exercise-training on bone metabolism in healthy men and women (113), overweight and obese individuals (114,115) and postmenopausal women (116). No change (114), a trend toward increased formation and resorption (113) and increased bone formation only (115,116) were reported. However, the latter two studies provided a combined protein/calcium supplement (115) or protein/CHO/calcium/Vit D (116) and so the influence of protein supplementation *per se* could not be isolated. The independent and combined influence of protein, carbohydrates and fats on the bone metabolic response to exercise represents another exciting avenue for on-going research.

3.3. Micronutrient ingestion

Many micronutrients influence bone (117), but only the impact of calcium and vitamin D ingestion in conjunction with exercise has been investigated. Vigorous exercise increases PTH secretion, which in turn activates bone resorption (36,37,118–121). This increase in PTH secretion may be induced, at least in part, by a reduction of serum ionized calcium (iCa). Therefore, strategies to protect serum calcium availability during exercise may influence the bone metabolic response to that exercise bout. This hypothesis is supported by studies that showed suppressed PTH and β-CTX-I (37,122), or suppressed PTH but no change to β-CTX-I (36) when a calcium supplement was provided during and/or pre-exercise. Recently, Kohrt and colleagues (16) conducted an elegant study, investigating the influence of serum iCa availability on the PTH and bone resorptive response to a 60 minute, vigorous cycling protocol. A clamp was used to provide a variable iCa infusion throughout the exercise test, thus preventing an exercise-induced decline in serum iCa. This maintenance of serum iCa availability attenutated, but did not fully prevent, exercise-induced increases in PTH and β-CTX-I (16), demonstrating that calcium disruption, at least partially, regulates the bone resorptive response to

exercise. The underlying causes of exercise induced calcium disruptions are not entirely clear. Dermal calcium losses due to sweating may contribute, but these losses are small (apart from during very prolonged and/or intense exercise perhaps), and are unlikely to largely impact either serum calcium availability or the β-CTX response to exercise (123). Further research is certainly required to elucidate the underlying mechanism, particularly given that calcium supplementation may be protective to athletic bone health. In further support of this, reduced β-CTX-I levels, along with enhanced tibial bone properties, were reported following 6 months of combined calcium and vitamin D supplementation in a group of young horse-racing jockeys (124); of note, the study was not designed to investigate the independent influence of calcium or vitamin D.

4. FUTURE PERSPECTIVES

It is clear that both acute and chronic exercise can induce a BMM response (summarised in Figure 2) and these biomarkers have exciting potential to increase our understanding of the complex relation between exercise and bone. Currently, important gaps in our understanding of the different factors that regulate the bone response to exercise, and a lack of data on BMMs predictive ability exists. These knowledge gaps should be filled to progress understanding, and thus practical application, of these exciting biomarkers.

The scientific triad of standardisation, harmonisation and population specific reference ranges were identified as vital steps toward the optimisation of BMMs in clinical practice (33,125), and the same is true for their use within sport and exercise science and medicine. Elevated bone metabolism within the clinical setting is indicative of increased fracture risk (34). But BMMs were unable to differentiate, or to predict, stress fracture occurrence in athletes or military recruits (126–129). In order to move toward the practical use of BMMs in sports medicine, validated, population specific, reference ranges are essential. This will allow differentiation between those for whom altered BRM simply reflects the demands of training, and those for whom changes may be pathological. The specific conditions required to standardise and optimise selected bone biomarkers should be investigated in the design and planning stages of all projects, to ensure that conditions are optimised and that valid information

393 is obtained. For example, β-CTX-I is known to be more significantly influenced by circadian rhythms
394 and nutritional intake than PINP, which is relatively stable in response to these factors (130). As such,
395 the control and standardisation approaches adopted for both may differ, depending on the primary
396 objective of the study. Harmonisation of future research efforts through including, at a minimum, the
397 reference markers of PINP and β-CTX-I, will allow for greater comparability of future findings, while
398 rigorous standardisation and control of research design and protocols will allow for a greater isolation
399 of moderating factors.
400 Most investigations on this topic have relied upon simple dichotomous interpretations of
401 increases/decreases in various BMMs as being either positive or negative. Some care must be taken
402 with this approach, as it does not recognise the complexity of these processes, and the context and
403 magnitude of change must be considered when interpreting BMM results. For example, strategies that
404 attenuate the bone resorptive response to acute exercise are generally considered to be positive, and
405 this may well be the case for highly-active individuals at risk of bone loss. But could these same
406 stratgies also blunt subsequent anabolic adaptations? Our current understanding of the BMM
407 response to exercise is insufficient to answer this question. Pending a more complete understanding
408 of the physiological relevance of the BRM response to exercise, results should not be extrapolated
409 beyond the delimitations of the study, unless accompanied by appropriate clinical or functional
410 outcomes. The length and context of exposure to stimuli, and the temporal nature of BRM responses
411 to exercise is also very important. Transient exposure to various exercise-induced stimuli, including
412 reactive species, acidosis, or glucocorticoids, may well be essential for BMU activation and subsequent
413 remodelling and adaptation. Conversely, prolonged exposure to these same stimuli, as occurs in many
414 situations (*e.g.,* clinical conditions characterised by oxidative stress, low grade metabolic acidosis or
415 the sustained use of glucocorticoid therapies) are detrimental to bone.
416 We do not know how transient changes to individual BRMs translate in the long-term toward changes
417 to BMD and microarchitecture and, ultimately, to bone strength and fracture susceptibility. Where
418 possible, longitudinal studies should correlate changes in individual BRMs with these chronic

indicators in order to estimate their predictive ability. Careful consideration of these, and other factors described in this review, may enhance the use of these biomarkers in ongoing investigations, thus providing a platform upon which evidence-based practical and clinical recommendations may be made to enhance the bone health of athletes, as well as those undergoing exercise-based therapeutic interventions.

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FIGURES:

Figure 1: The bone remodelling cycle

Figure 2: Bone remodelling in response to exercise

Legend: ***Panel A*** describes how the mechanical and metabolic signals generated by an acute bout of exercise activate the basic multicellular unit (BMU), thus mainly upregulating osteoclast activity, represented by an increased blood biomarkers of resorptive activity, *e.g.,* β-CTX-1 (section 2.1). Through the process of reversal, this increased bone resorptive activity induces a coupled elevation in osteoblast activity, as is evident by increased resting blood bone formation markers following a period of exercise training (***Panel B***; section 2.2).