



21 **ABSTRACT:**

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

26 **IN BRIEF:**

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

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

30 **KEY POINTS:**

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

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## 45 1. INTRODUCTION

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

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

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

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

## 74 **2. THE BONE METABOLIC RESPONSE TO EXERCISE**

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

### 87 ***2.1. The use of bone (re)modelling markers in sport and exercise science***

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

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

104 The repeatability of BMM measurement is another important consideration, as some show substantial  
105 inter- and intra-individual variability, and/or, are difficult to accurately measure. For example, the  
106 osteoprotegerin/receptor activator of NF kappaB ligand (OPG/RANKL) ratio is commonly used to  
107 indicate bone resorption, but soluble RANKL is sometimes difficult to accurately measure *in vivo* (20),  
108 and so results may be mis-leading. Bone biomarkers are often described as representing “bone  
109 turnover”. Calculations, such as the uncoupling index, are commonly used to represent the  
110 predominant state of bone metabolism, whereby resorptive activities that are “coupled” with  
111 formation would represent a state of equilibrium, whereas “uncoupling” occurs when formative and  
112 resorptive processes are unbalanced and favour either the loss or gain of bone. Some caution should  
113 be applied when considering such calculations, because BMMs are systemic and cannot indicate bone  
114 activity at any one particular site, which is an issue because the true bone response to loading is largely  
115 site specific (21). A wide range of potentially confounding factors also impact BMMs and must be  
116 accounted for in study design and interpretation. Bone is responsive to both acute and chronic  
117 nutritional stimuli (described in Section 3) and so nutritional status must be carefully controlled in  
118 exercise trials. Other factors, including sex, age (22), menstrual cycle phase (23), oral contraceptive  
119 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 Notwithstanding 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 ( $\beta$ -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 Clinical  
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 responsive 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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -CTX-I, are responsive to acute  
168 exercise, some well-controlled investigations reported no change to any BMM, even though they used

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

### 182 ***2.3. The bone metabolic response to longitudinal exercise interventions***

183 Prolonged exposure to exercise training typically elicits an increase in resting levels of bone formation  
184 markers (either PINP, bone alkaline phosphatase (BALP) or both) (54–67), indicating that training  
185 might stimulate chronic upregulation of bone formative processes. This aligns with the model shown  
186 in Figure 2, whereby increased bone resorption in response to acute exercise (described in Section  
187 2.1) activates the BMU, ultimately leading to an increase in bone formation. Unlike the largely  
188 consistent finding of increased bone formation in response to exercise interventions, markers of bone  
189 resorption are less responsive to training, with most studies reporting no change. A few studies have  
190 reported a reduction in bone resorption markers following a training program (55,57,68,69), and this  
191 was typically accompanied by an increase in bone formation, suggesting a metabolic bone profile  
192 favouring formation. Some studies also reported a concomitant increase in BMD alongside increases



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

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

203 A wide range of exercise types have been investigated, but no one type stands out as being more or  
204 less effective at eliciting a BMM response. It is generally accepted that high-intensity exercise that  
205 conveys large and unaccustomed gravitational or muscular loads is necessary to elicit an osteogenic  
206 response (73). It follows that this type of exercise would induce the largest and most consistent  
207 increase in markers of bone formation following a period of training, but this does not seem to be the  
208 case. Similar to evidence from acute studies (described in Section 2.1), exercise types with lower  
209 impact and repetitive loading cycles (such as treadmill walking/running, step aerobics and yoga  
210 (54,55,57,60,66)) were equally likely to elicit a response in bone formation markers, as those  
211 modalities that exert large muscle or gravitational forces, (such as football training (63), high-impact  
212 jump activities (61,68) resistance training (58,59,62,64) and multi-modal activities (67), including  
213 military combat training (56)). This raises an important question about the relationship between BMM  
214 and chronic bone outcomes, such as mass and strength. It is widely accepted that exercise type is an  
215 important determinant of the bone response to exercise, but this view is not supported by the  
216 available BMM data. Is it possible that exercise type is less important to bone than previously  
217 believed? Or perhaps BMM changes are not necessarily predictive of changes to bone mass, strength

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

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

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

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

#### 245 **2.4. The bone metabolic profile of different athletic populations**

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

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

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

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

271

### 272 **3.1. Energy availability**

273 EA refers to the amount of energy available for physiological processes, after the demands of training  
274 are met (99), and is an important determinant of bone health in athletes. Extensive research shows  
275 that insufficient EA negatively impacts a variety of bone parameters (85), including BMMs. In a  
276 parallel-group study, Ihle & Loucks. (2004) examined the dose-response relation between EA (10, 20,  
277 30 or 45 kcal·kgLBM·day<sup>-1</sup>) and bone metabolism in sedentary, but otherwise healthy, eumenorrheic  
278 young women. Bone formation (OC and carboxyterminal propeptide of type 1 procollagen (PICP)) was  
279 suppressed at all levels of low EA (30, 20 and 10 kcal·kgLBM·day<sup>-1</sup>). This was accompanied by a  
280 significant increase of bone resorption (aminoterminal telopeptide of type 1 collagen (NTX)) during  
281 the lowest EA condition (10 kcal·kg LBM·day<sup>-1</sup> (100)). More recently, Papageougiou et al. (101)  
282 conducted two, independent repeated-measure investigations (reported in the same paper), on the  
283 influence of 5 days low EA (15 Kcal·kg LBM·day<sup>-1</sup>) on bone metabolism in physically active men (study  
284 1) and women (study 2). Low EA increased bone resorption ( $\beta$ -CTX-I) and reduced bone formation  
285 (PINP) in women, but not in men (101). Each of the studies described herein induced low EA through  
286 a combination of exercise and dietary restriction, and so could not distinguish whether restricted  
287 energy intake, or increased energy expenditure, was the dominant cause of altered bone metabolism.  
288 This important point was subsequently investigated, and it seems that low EA (15 kcal·kg LBM·day<sup>-1</sup>)  
289 induced through dietary restriction, but not by exercise-induced energy expenditure, reduces bone  
290 formation (PINP) (102). It is not clear whether this occurred because the benefits of exercise masked,  
291 or over-rode, the negative effects of restricted energy intake, and further mechanistic elucidation is

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

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

### 312 **3.2. Macronutrient ingestion pre, during and post exercise**

313 Nutrient ingestion pre, during and immediately post acute exercise can alter the BMM response to  
314 that exercise bout. Scott et al. (39) investigated pre-exercise ingestion of a mixed meal, versus fasting,  
315 on response to a 60-minute treadmill run conducted at 65% of VO<sub>2max</sub>. Meal ingestion reduced pre-  
316 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  $\beta$ -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  $\beta$ -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  $\beta$ -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

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

### 353 **3.3. Micronutrient ingestion**

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

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

#### 376 **4. FUTURE PERSPECTIVES**

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

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



393 is obtained. For example,  $\beta$ -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  $\beta$ -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 strategies 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

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

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768 **FIGURES:**

769 **Figure 1:** The bone remodelling cycle

770 **Figure 2:** Bone remodelling in response to exercise

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

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