The Bone Metabolic Response to Exercise and Nutrition

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DOLAN, E., I. VARLEY, K.E. ACKERMAN, R.M.R. PEREIRA, K.J. ELLIOTT-SALE, and C. SALE. The bone metabolic response to exercise and nutrition. *Exerc. Sport Sci. Rev.*, Vol. 48, No. 2, pp. 49–58, 2020. *Bone (re)modeling markers can help determine how the bone responds to different types, intensities, and durations of exercise. They also might help predict those at risk of bone injury. We synthesized evidence on the acute and chronic bone metabolic responses to exercise, along with how nutritional factors can moderate this response. Recommendations to optimize future research efforts are made.* **Key Words:** bone remodeling, 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 the bone.
- The stimuli (both mechanical and metabolic) created by an acute exercise bout typically elicit an increase in markers indicative of bone resorption, whereas chronic adaptation to exercise training typically results in an increase in bone formation.
- Nutritional status, and acute nutrient intake, can moderate the bone metabolic response to exercise.
- Appropriate use of biomarkers has the potential to progress knowledge on the acute 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.

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 the bone and is considered an effective preventive or treatment strategy for those with conditions characterized by bone loss or increased fracture susceptibility (*e.g.*, osteoporosis) (1). Sports that convey high-impact, multidirectional movement patterns and unaccustomed loads are widely accepted as providing the optimal osteogenic stimulus (2). Conversely, participation in sports involving lower-impact or repetitive loading cycles (such as endurance running) or nonweight 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 bone mineral density (BMD) than nonathletic 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 preemptively identify those at risk for 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) or bone microarchitecture (assessed by high-resolution peripheral quantitative computed tomography) may be used to predict fracture risk (7–9) or to assess intervention efficacy. These outcomes are, however, chronic indicators of the 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)modeling 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. Their full potential cannot be realized currently; however, due to incomplete

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understanding of their physiological relevance, 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 optimize the use of appropriate biomarkers in future studies. In addition, we will describe how nutritional interventions moderate the BMM response to exercise and how this information can elucidate the mechanistic pathways mediating these responses.

THE BONE METABOLIC RESPONSE TO EXERCISE

Provided the nutritional and metabolic environment is favorable, 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). Modeling refers to the isolated formation or resorption of bone at specific sites. In contrast, remodeling is a coupled and synchronized process of bone activation, resorption, reversal, and formation, which is coordinated by teams of bone cells (*i.e.*, osteoblasts, osteoclasts, and osteocytes) termed the basic multicellular unit (BMU). Although some modeling cannot be ruled out, it seems that remodeling is the dominant process through which the bone responds to the mechanical or metabolic stimuli offered by exercise (12,17). An overview of this process is shown in Figure 1.

The Use of Bone (Re)Modeling 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 the Table for an overview of commonly used BMMs). Their potential to elucidate the mechanisms through which the bone responds to exercise is large, but some factors may 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 nonbone 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 bone. As such, their measurement is not necessarily indicative of altered bone activity only. Similarly, osteocalcin (OC) is a small noncollagenous protein synthesized by osteoblasts, which often is used to estimate osteoblast activity and, therefore, bone formation. But both intact and fragmented OC also may be released during bone resorption (20), suggesting that this biomarker may indicate general bone remodeling rather than bone formation specifically. In addition, OC is a nonspecific protein that fulfills a number of extra-skeletal roles, including functions in energy metabolism and muscle activity (21). These extra-skeletal roles are particularly relevant when interpreting the OC response to exercise, given that bioenergetic pathways and muscle activity also are 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 or are difficult to measure accurately. 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* (22), and so results may be misleading. Bone biomarkers often are 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 favor either the loss or gain of the 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 (23). A wide range of potentially confounding factors also may influence BMMs and should be accounted for in study design and interpretation. The bone is responsive to both acute and chronic nutritional stimuli (described in Section "The Influence of Nutrient Intake on the Bone Metabolic Response to Exercise"), and so nutritional status must be carefully controlled in exercise trials. Other factors, including sex, age (24), menstrual cycle phase (25), oral contraceptive use (26), seasonal (27) and circadian (28) variations, genetics (29), various medical conditions and medications (30), injury history, particularly previous fractures (31,32), previous exercise, and preanalytical storage and handling (33) also may influence BMMs.

Nothwithstanding these considerations, the clinical and mechanistic relevance of these biomarkers is well recognized, and in an attempt to optimize their clinical utility, the National Bone Health Alliance advised that all studies should include, as a minimum, measurements of N-terminal propeptide of type 1

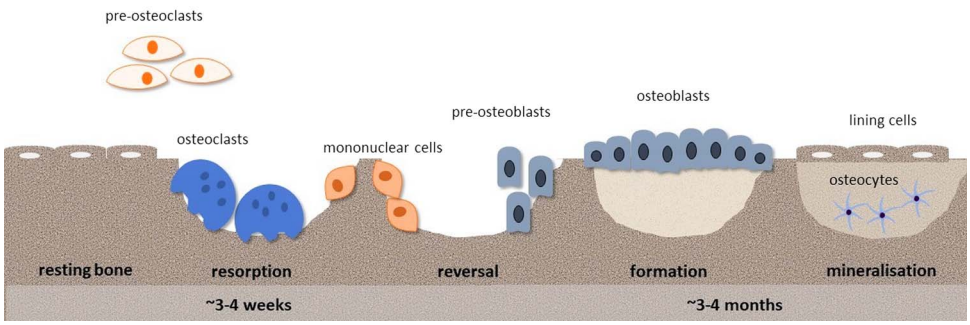

Figure 1. The bone remodeling cycle.

TABLE. Commonly used bone remodeling markers

Abbreviation	Biomarker	Main Activity
Bone Formation		
Bone-ALP	Bone-specific alkaline phosphatase	Bone-ALP is a glycoprotein found on osteoblast cells. Bone-ALP is produced as a result of the breakdown in mineralization inhibitor pyrophosphate.
T-OC	Total osteocalcin	Osteocalcin is a protein secreted by osteoblast cells and is present in two forms. Total osteocalcin is composed of a combination of active (undercarboxylated) and inactive (carboxylated) osteocalcin. Although undercarboxylated (does not bind to hydroxyapatite) and carboxylated (binds to hydroxyapatite) osteocalcin have differing roles, total osteocalcin often has been used as a marker of bone formation, although it is now recognized that this is not really the case. Total OC may provide an indication of general bone metabolism, but it is nonspecific to bone activity and so must be interpreted with caution.
U-OC	Undercarboxylated osteocalcin	Undercarboxylated osteocalcin does not bind to hydroxyapatite during bone formation. The concentration of undercarboxylated osteocalcin is used as a marker of bone formation; the greater the concentration the lower bone formation.
PICP PINP	Carboxyterminal propeptide of type 1 procollagen N-terminal propeptide of type 1 procollagen	PINP and PICP are byproducts of type I collagen synthesis that takes place in osteoblast cells. After collagen synthesis, PINP is removed from the amino terminal, whereas PICP is removed from the carboxy terminal of procollagen. Both are released into the blood stream and are therefore used as markers of bone formation. Recommendations have been made by the IOF and the IFCC to standardize PINP as a marker of bone formation.
Sclerostin		Sclerostin is a glycoprotein secreted primarily by osteocytes that inhibits Wnt signaling by binding to Wnt co-receptor LRP5, ultimately reducing bone formation (18).
Bone Resorption		
Pyr	Pyridinoline	Pyr is produced as a result of mature type I and II collagen breakdown.
Dpd	Deoxypiridinoline	Dpd is produced as a result of mature type I collagen breakdown.
ICTP	Carboxyterminal telopeptide of type 1 procollagen	ICTP is a marker of newly synthesized type I collagen. ICTP is secreted into the blood stream after matrix metalloproteinases during bone resorption.
NTx	Aminoterminal telopeptide of type 1 collagen	NTx is cleaved from type I collagen by cathepsin-K during the process of type I collagen catabolism.
β-CTX-I	C-terminal telopeptide of type 1 collagen	CTX-I is released from osteoclast cells during the degradation of collagen fibrils. Over time, the alpha aspartic acid coverts to beta, thus forming beta-CTX, which is then released into the blood stream. Recommendations have been made by the IOF and the IFCC to standardize CTX as a marker of bone resorption.
TRAP5b	Tartrate-resistant acid phosphatase (isoenzyme 5b)	TRAP5b is an enzyme expressed from immature osteoclast cells. TRAP5b represents osteoclast number rather than actual bone resorption (19) and is unaffected by renal dysfunction.
OPG/RANKL Ratio	Osteoprotegerin/receptor activator NF kappaB ligand	OPG acts as a decoy receptor for RANKL, leading to the prevention of osteoclast precursor development into mature osteoclasts, resulting in the subsequent attenuation of bone resorption. Increased OPG/RANKL ratio would result in a decrease in bone resorption.

procollagen (PINP) and the C-terminal telopeptide of type 1 collagen (β-CTX-I), as indicators of bone formation and resorption (34–36). The decision to focus on two biomarkers was made to allow for greater harmonization, and therefore comparability, of ongoing research efforts. These particular biomarkers were selected based upon the recommendations of an expert working group of the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and Laboratory Medicine, who deemed them to have a relatively smaller biological variability and higher specificity to bone metabolism and to be more responsive to anti-resorptive or anabolic treatments than other available BMMs (reviewed in detail in (35,37)). Considering the currently available information, we agree with this recommendation and support the use of PINP and β-CTX-I as core components of biomarker panels used to investigate the bone metabolic response to exercise and nutrition interventions. We also have weighted our interpretation of the literature toward studies that have measured these biomarkers.

The Bone Metabolic Response to an Acute Bout of Exercise

Increased bone resorption is the initial response to an acute bout of exercise, and increased β-CTX-I has been reported in a number of trials (38–42). This finding is consistent with what we assume about bone remodeling, whereby osteoclast activation, induced by mechanical or metabolic signals, activates the BMU, causing an acute increase in bone resorption. This was shown in response to different exercise types, including treadmill running (41), intense cycling (38), and a fatiguing bilateral jump protocol (42). In contrast, bone formation markers seem to be largely nonresponsive to acute exercise, with most studies reporting no change to serum PINP, even when high-intensity exercise protocols were used (42–45). This finding aligns with the bone remodeling process shown in Figure 2, whereby the BMU is thought to be activated by an initial stimulation of bone resorption, meaning that any change in bone formation would be expected to lag behind that of bone resorption. Despite this, increased PINP has been reported after 60 min of continuous running at intensities ranging from 55%–75% of $\dot{V}O_{2max}$ (41,46), or an unaccustomed football session (19), demonstrating that indicators of bone formation do, sometimes, respond to acute exercise, although this response is less common than that observed in markers indicative of bone resorption. The reason for this inconsistency in response is not entirely clear and further research to better characterize the BMM response to acute bouts of exercise under varying conditions and with longer follow-up postexercise bouts will be of interest.

Exercise intensity and duration seem to be instrumental in determining the BMM response to acute exercise (40), with higher, but not lower, intensity protocols typically eliciting a response. Those studies that observed no response to an acute bout of exercise generally used lower intensity or shorter duration protocols, including 30 min of walking or jogging (18,47), a 30-s Wingate cycling test (48), or water aerobics (18). The bone is

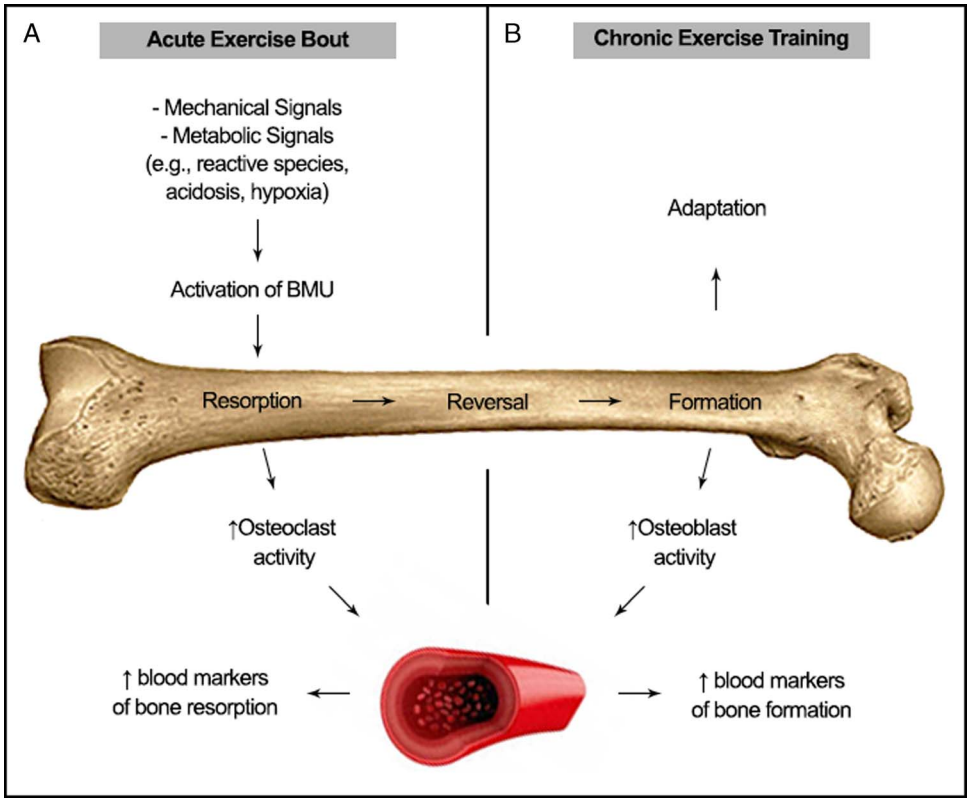

Figure 2. Bone remodeling in response to exercise. Panel A describes how the mechanical and metabolic signals generated by an acute bout of exercise activate the basic multicellular unit (BMU), thus mainly upregulating osteoclast activity, represented by increased blood biomarkers of resorptive activity, for example, β-CTX-1. Panel A relates to Section "The Bone Metabolic Response to an Acute Bout of Exercise," while Panel B relates to Section "The Bone Metabolic Response to Longitudinal Exercise Interventions."

commonly thought to respond only to high-impact or unusual impact loads, but the available studies show that these are not essential to elicit an acute BMM response. For example, cycling tests consistently increase β-CTX-I (38,39,49), despite conveying little to no impact loads. Recently, intensity-matched interval sessions conducted either on a bike or treadmill induced comparable sclerostin responses in men (50) and women (51). This demonstrates that impact was not necessary to elicit this BMM response, which instead must have been stimulated by other, potentially metabolic, factors, such as increased reactive oxygen or nitrogen species (14), acidosis (15), or reduced serum calcium availability (16).

Despite strong evidence that bone resorptive markers, such as β-CTX-I, are responsive to acute exercise, some well-controlled investigations reported no change to any BMM, even though they used high-intensity protocols (45). This finding may relate to the time-specific and transient BMM response to exercise bouts (40,41,52). 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 (41) and a transient (46) β-CTX-I response to treadmill running in the days after 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 use multiple sampling points to characterize the BMM responses to acute exercise, ideally taken over several days, after the exercise. The nature of the temporal BMM response to exercise is, however, incompletely understood, rendering it difficult to identify the most appropriate timing and number of sampling points. In addition, hemoconcentration should be assessed to control for the potentially confounding influence of plasma volume changes on the BMM response to exercise.

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, or both) (54–67), indicating that training might stimulate chronic upregulation of bone formative processes. This aligns with the model shown in Figure 2, panel A, whereby increased bone resorption in response to acute exercise (described in Section "The Bone Metabolic Response to an Acute Bout of Exercise") 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 after a training program (55,57,68,69), and this was typically accompanied by an increase in bone formation, suggesting a metabolic bone profile favoring 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 BMM response to exercise in some situations, 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 with 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 after a period of training, but this does not seem to be the case. Similar to evidence from acute studies (described in Section "The Bone Metabolic Response to an Acute Bout of Exercise"), 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 relation 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 to optimize 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 (1) and older populations (82), which would necessitate an upregulation in bone remodeling, 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 after 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 remodeling (85) (described in Section "Energy availability"). It is also plausible that inadequate recovery during periods of particularly arduous and unaccustomed training may impede the reversal phase of the bone remodeling cycle, thus attenuating its osteogenic potential. These findings highlight the many factors, independent of 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 enhancing understanding of this important research area.

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 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 reported also 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 are considered to provide a suboptimal 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 "Energy availability"), also may 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 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.

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

When considering the BMM response to exercise, it is essential to consider also 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.

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 and Loucks (100) examined the dose-response relation between EA (10, 20, 30, or 45 kcal·kg LBM·d^{-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·kg LBM·d^{-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·d^{-1} (100)). More recently, Papageorgiou *et al.* (101) conducted two independent repeated-measure investigations (reported in the same article), on the influence of 5-d low EA (15 kcal·kg LBM·d^{-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·d^{-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 overrode, the negative effects of restricted energy intake, and further mechanistic elucidation is important. Irrespective of the mechanisms, however, it seems that exercise may somewhat protect bone during periods of energy restriction. This 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 bone alone, although the efficacy of this approach for bone, and other tissues, should be confirmed using randomized controlled investigations.

Reduced bone formation also was reported in cross-sectional studies conducted on energy deficient athletes (103–106), and in clinical populations characterized 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 estrogen-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·d^{-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 after periods of arduous training (83,84,110) (Section "The Bone Metabolic Response to Longitudinal Exercise Interventions") or as identified in cross-sectional investigations of some athletic groups (89,90,92) (Section "The Bone Metabolic Profile of Different Athletic Populations").

Macronutrient Ingestion Pre, During, and Postexercise

Nutrient ingestion before, during, and immediately after exercise can alter the BMM response to that exercise bout. Scott *et al.* (41) investigated preexercise ingestion of a mixed meal, versus fasting, on response to a 60-min treadmill run conducted at 65% of $\dot{V}O_{2max}$. Meal ingestion reduced preexercise β-CTX-I but did not influence its exercise-induced increase, and the authors concluded that preexercise feeding did not meaningfully impact the BMM response to the subsequent exercise bout. However, this study suggested that the stress of the exercise bout overrode the preexercise effect of feeding on β-CTX-I, which, in turn, raised the question of whether or not more continuous nutrient provision throughout the exercise bout would exert a more noticeable effect. This was investigated by Sale *et al.* (53), who provided carbohydrates (CHO) before, during, and after a 120-min treadmill run and reported modestly reduced PINP and β-CTX-I postexercise.

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

These studies demonstrate that feeding around exercise can modulate the bone resorptive response to that exercise bout, with the postexercise period perhaps the most practical and influential opportunity for nutrient provision. Theoretically, this reduction in bone resorption may protect bone during periods of high-intensity or volume training. In contrast, and as described in Section "the Bone Metabolic Response to Exercise," exercise-induced increases in bone resorption are necessary for BMU activation, and it is plausible that attenuated bone resorption during or postexercise could, theoretically, blunt the bone adaptive response. To date, longitudinal studies investigating how acute BMM alterations translate in the long-term are not available, meaning that these potential long-term consequences are hypothetical and require investigation.

The studies described previously were not designed to investigate the independent influence of the three macronutrients (CHO, fats, and proteins) on the BMM response to exercise, and limited data on this topic exist. Protein is a particularly interesting macronutrient in this context, given its relevance to athletic adaptation and performance, along with the many, and potentially conflicting, pathways through which

it influences the 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/vitamin D (116) and so the influence of protein supplementation *per se* could not be isolated. The independent and combined influence of protein, CHO, and fats on the bone metabolic response to exercise represents another exciting avenue for on-going research.

Micronutrient Ingestion

Many micronutrients influence the bone (117), but only the impact of calcium and vitamin D ingestion in conjunction with exercise has been investigated. Vigorous exercise increases parathyroid hormone (PTH) secretion, which in turn activates bone resorption (38,39,118–121). 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 (39,122) or suppressed PTH but no change to β-CTX-I (38) when a calcium supplement was provided during or preexercise. Recently, Kohrt *et al.* (16) conducted an elegant study, investigating the influence of serum iCa availability on the PTH and bone resorptive response to a 60-min, 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 attenuated, 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 prolonged 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 after 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.

FUTURE PERSPECTIVES

It is clear that both acute and chronic exercise can induce a BMM response (summarized in Fig. 2), and these biomarkers have exciting potential to increase our understanding of the relation between exercise and the 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, exist. These knowledge gaps should be filled to progress understanding, and thus practical application, of these exciting biomarkers.

The scientific triad of standardization, harmonization, and population-specific reference ranges were identified as vital steps toward the optimization of BMMs in clinical practice (35,125), 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 (36). But BMMs were unable to differentiate, or to predict, stress fracture occurrence in athletes or military recruits (126–129), and this may be because we cannot currently differentiate between those for whom altered BMM simply reflects the demands of training, and those for whom changes may be pathological. To move toward the practical use of BMMs in sports medicine, validated, population-specific, reference ranges are essential. The specific conditions required to standardize and optimize selected bone biomarkers should be investigated in the design and planning stages of all projects, to ensure that conditions are optimized and that valid information is obtained. For example, β-CTX-I is known to be more significantly influenced by circadian rhythms and nutritional intake than PINP, which is relatively stable in response to these factors (130). As such, the control and standardization approaches adopted for both may differ, depending on the primary objective of the study. Harmonization of future research efforts through including, at a minimum, the reference markers of PINP and β-CTX-I, will allow for greater comparability of future findings, whereas rigorous standardization and control of research design and protocols will allow for a greater isolation of moderating factors.

Most investigations on this topic have relied upon simple dichotomous interpretations of increases/decreases in various BMMs as being either positive or negative. Some care must be taken with this approach, as it does not recognize the complexity of these processes, and the context and magnitude of change must be considered when interpreting BMM results. For example, strategies that attenuate the bone resorptive response to acute exercise are generally considered to be positive, and this may well be the case for highly active individuals at risk of bone loss. But could these same strategies also blunt subsequent anabolic adaptations? Our current understanding of the BMM response to exercise is insufficient to answer this question. Pending a more complete understanding of the physiological relevance of the BMM response to exercise, results should not be extrapolated beyond the limitations of the study, unless accompanied by appropriate clinical or functional outcomes. The length and context of exposure to stimuli, and the temporal nature of BMM responses to exercise, are also important. Transient exposure to various exercise-induced stimuli, including reactive species, acidosis, or glucocorticoids, may well be essential for BMU activation and subsequent remodeling and adaptation. Conversely, prolonged exposure to these same stimuli, as occurs in many situations (*e.g.*, clinical conditions characterized by oxidative stress, low grade metabolic acidosis, or the sustained use of glucocorticoid therapies), is detrimental to the bone.

We do not know how transient changes to individual BMMs translate in the long-term toward changes to BMD and microarchitecture and, ultimately, to bone strength and fracture

susceptibility. Where possible, longitudinal studies should correlate changes in individual BMMs with these chronic indicators 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 optimize the bone health of athletes, as well as those undergoing exercise-based therapeutic interventions.

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