Abstract

It is important for athlete and public health that we continue to develop our understanding of the effects of exercise and nutrition on bone health. Bone turnover markers (BTMs) offer an opportunity to accelerate the progression of bone research by revealing a bone response to exercise and nutrition stimuli far more rapidly than current bone imaging techniques. However, the association between short-term change in the concentration of BTMs and long-term bone health remains ambiguous. Several other limitations also complicate the translation of acute BTM data to applied practice. Importantly, several incongruencies exist between the effects of exercise and nutrition stimuli on short-term change in BTM concentration compared to long-term bone structural outcomes to similar stimuli. There are many potential explanations for these inconsistencies, including that short-term study designs fail to encompass a full remodeling cycle. The current article presents the opinion that data from relatively acute studies measuring BTMs may not be able to reliably inform applied practice aiming to optimise bone health. Important factors to consider when interpreting or translating BTM data are discussed.

Keywords

bone metabolism; bone health; bone remodeling; nutrition; exercise.

Introduction

There is a growing need within sports and exercise science to improve our understanding of how exercise and nutrition influence bone health. Osteoporosis is a disease characterised by low bone mineral density (BMD) and millions suffer osteoporotic fracture each year (primarily the elderly and post-menopausal women), costing $17.9 and £4 billion to US and UK healthcare systems, respectively (Clynes et al., 2020). Low BMD is also prevalent in endurance-based athletes, in 28% of adolescent female runners (Barrack et al., 2008) and 89% of male masters cyclists (Nichols & Rauh, 2011), increasing the risk of bone injury and early onset osteoporosis. For example, it has been shown that up to 21% of female distance runners experience at least one bone stress injury per year (Barrack et al., 2014; Hutson et al., 2021b; Scofield & Hecht, 2012). Exercise and nutrition are known to influence bone health; however, it takes at least several months for stimuli to result in observable change in bone mass using the gold standard method of dual-energy x-ray absorptiometry (DXA) (Ahola et al., 2009). Therefore, high quality studies measuring bone using DXA bear a high time demand, the effects of specific practices are difficult to categorically confirm, and research progression is slow. Bone turnover markers (BTMs) offer the potential to reveal bone responses immediately post-exercise (acute) and within days (short-term) of a given exercise or nutrition intervention in the rested state (Smith et al., 2021). For this reason, it is tempting to consider that BTMs may be used to accelerate bone research in sport and exercise science. This article provides an important opinion on the extent to which acute and short-term BTM responses to exercise and nutrition intervention may be relied upon to inform applied practice aiming to optimise bone health during developmental and older years in athletes and non-athletes.

Bone turnover markers

BTMs are typically products or signalling molecules released into the circulation during one or more stages of osteoblastic bone formation or osteoclastic bone resorption (Shetty et al., 2016), see Table 1 for more detail on specific markers. They are often measured in plasma,

serum or urine to determine the rate of these processes on a systemic level at the time of measurement. The fact that studies measuring BTMs can be much shorter in duration compared to studies using imaging techniques allows for tighter control of extraneous variables, reduces participant burden and lowers the risk of participant dropout. Nevertheless, several laboratory visits are required under strict control. BTMs also provide mechanistic information regarding bone cell activity and data may be used to inform and justify larger scale long-term intervention studies. Furthermore, BTMs do not necessarily incur the same equipment purchase and maintenance costs of radiological scanning. These are some of the factors that have led to a growth in the use of BTMs in sport and exercise science; however, ambiguity remains over their association with bone mass change (Bennell et al., 1997).

A bone remodeling cycle begins with osteoclastic bone resorption lasting up to 27 days, followed by several days of reversal until coupled osteoblast activity forms new osteoid bone in the resorptive cavity, which then becomes mineralised, with the entire cycle lasting >100 days (Agerbæk et al., 1991). BTMs provide a snapshot of the rate of bone formation and resorption at the time of measurement and typicalpre-test-post-test study designs are much too short in duration to capture a complete cycle at any remodeling site initiated during the intervention. Outcomes will also be influenced by remodeling cycles that were initiated prior to study entry. Detailed reviews of the many available BTMs and associated limitations exist elsewhere (Hlaing & Compston, 2014; Vasikaran et al., 2011). BTMs are measured systemically whereas bone remodeling is highly localised and site-specific, at least in response to mechanical loading (Hart et al., 2017), and some can lack specificity to either the process of formation or resorption (or even bone tissue itself) (Table 1). Unlike the loss or gain of bone tissue measured via imaging techniques, there is no consensus as to what constitutes a meaningful change in any BTM in response to exercise or nutrition intervention. Several factors are known to influence the accurate measurement of BTMs, including circadian and seasonal variation, diet and exercise, disease and medication, hormonal status, intrinsic day-to-day variations, renal function, blood flow, and sampling procedures and type (blood or urine)

(Hlaing & Compston, 2014). A summary of BTMs most frequently used in the studies cited herein is provided in Table 1; however, for the purpose of this article, findings will mostly be described in terms of the processes of bone formation and resorption rather than the specific marker(s) measured.

Table 1: A summary of several bone turnover markers commonly used in exercise and nutrition research.

Marker (abbreviation)	Origin	Main activity	Comments
Bone formation			
Amino-terminal propeptide of type I collagen (P1NP)	N-terminal extension peptide of type 1 collagen precursor molecule	P1NP and P1CP are both cleaved from newly synthesised type 1 collagen following secretion into the extracellular space and released into the bloodstream.	P1NP is the international reference standard marker of bone formattion and the most used marker of bone formation in the studies cited in the current article. P1NP and P1CP are formed following the synthesis of newly formed type 1 collagen in other tissues (e.g., skin, dentin, cornea, vessels, fibrocartilage, and tendons) as well as bone.
Carboxy-terminal propeptide of type 1 collagen (PICP)	C-terminal extension peptide of type 1 collagen precursor molecule		
Osteocalcin (OC)	Non-collagenous protein secreted by osteoblasts.	OC encompasses both carboxylated (cOC) and undercarboxylated (ucOC) forms. cOC osteocalcin binds to hydroxyapatite and increased concentrations have previously been used as a marker of increased bone formation. ucOC does not bind to hydroxyapatite. It is predominantly released into the circulation and is proposed to have various endocrine functions. It has been used as a marker of bone formation, such that increased levels reflect decreased bone formation.	cOC fragments bound to hydroxyapatite within the bone matrix are released into the circulation during bone formation. It is suggested that ucOC is involved in several processes in an endocrine manner, including glucose homeostasis. OC may be measured in its various forms, or as total OC, but it may be that none are markers of bone formation specifically, and may be influenced by bone formation, resorption, and several other metabolic processes.
Bone alkaline phosphatase (BAP)	Bone specific isoform of a membrane-bound glycoprotein. Found on outer surface of osteoblasts.	Hydrolysis of mineralisation inhibitor pyrophosphate and adenosine triphosphate, forming inorganic phosphate accumulation and promoting hydroxyapatite mineralisation.	BAP is considered a highly bone specific marker of bone formation, however, available assays exhibit some cross-reactivity with other alkaline phosphatase isoforms (e.g., liver).
Bone resorption			

Carboxyterminal telopeptide of type 1 collagen, β-isomer (β-CTx) Amino-terminal telopeptide of type 1 collagen (NTx)	Telopeptides found on the C-terminal and N-terminal of tropocollagen molecules.	Form crosslinks between peptides within, or of adjacent, tropocollagen molecules and are cleaved and released into the circulation during collagen breakdown.	β-CTx is the international reference standard marker of bone resorption and the most used marker of bone resorption in the studies cited in the current article. β-CTx and NTx are involved in crosslink formation in other collagen-based structures. Other collagen telopeptide bone markers exist that reflect different types of crosslinks (e.g., carboxyterminal cross-linked telopeptide of type 1 procollagen; 1CTP) and various isoforms of specific crosslinks (e.g., α-CTx).
Pyridinoline (Pyd or Pyr) Deoxypyridinoline (Dpd or D-Pyr)	Pyridinium crosslink compounds formed during extracellular maturation of collagen fibrils	Pyd and Dpd mechanically stabilise collagen by bridging collagen peptides and are released into the circulation during resorption as mature crosslinked collagens are broken down.	Pyd and Dpd are formed in various other tissues of the body that also contain collagen.

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Effects of exercise on bone

The effects of habitual exercise on bone health are well documented (Santos et al., 2017). Cross-sectional (Nilsson et al., 2009; Tenforde & Fredericson, 2011; Varley et al., 2021) and longitudinal intervention studies (Evans et al., 2012; Nilsson et al., 2012; Weidauer et al., 2012) have repeatedly shown the benefit of weight bearing exercise (including running) on BMD and bone structure. However, the BTM response to running has been shown to be variable, with both bone formation and resorption markers shown to increase (Scott et al., 2011), decrease (Zittermann et al., 2002) and remain unchanged (Nishiyama et al., 1988) in the hours following a running bout. Moreover, a recent systematic review and meta-analysis showed no change in commonly studied BTMs in response to running (Civil et al., 2023). Systematic reviews of the literature have concluded that non-weight bearing exercise (cycling and swimming) does not benefit BMD (Gomez-Bruton et al., 2016; Nagle & Brooks, 2011; Olmedillas et al., 2012). Non-weight bearing exercise interventions tend to result in a moderate post-exercise increase in bone resorption; however, there is significant variability in this response with effect sizes indicating a very low certainty (Dolan et al., 2020). For example, bone formation has been shown to increase (Herrmann et al., 2007; Rong et al., 1997), decrease (Herrmann et al., 2007), and remain unchanged (Guillemant et al., 2004; Pomerants et al., 2008) in response to ergometer-based cycling.

The reason for the varied response could be multi-factorial and include the following: lack of control over diet or exercise, history of physical activity, population studied, other tissues releasing studied markers, systemic measure of tissue which exhibits site-specific adaptations. Another reason for the inconsistency in the findings could be the time taken for bone markers to significantly increase in concentration following an exercise bout being greater than the time-period of follow-up (typically a maximum of 72 hours). Alternatively, an insufficient period of pre-intervention standardisation may have been employed, such that the increase or decrease in BTMs being captured during the measurement window could have been activated by a stimulus incurred well before the start of the study. The inability of studies

111 to follow-up for longer than 72 hours is likely due to practical and logistical reasons. However,
112 it could be theorised that the effects of an exercise bout are not evident until >72 hours-post
113 intervention. For example, it is unlikely that bone formation marker P1NP would increase in
114 the 72 hours post exercise because it is a marker of type 1 collagen deposition, which is
115 unlikely to be formed and deposited in a short space of time (Dolan et al., 2020). The observed
116 increase in P1NP seen in some studies could be a result of leakage from other tissues
117 containing collagen (Civil et al., 2023) or due to changes in plasma volume (Brahm et al.,
118 1996) that are not typically accounted for. Therefore, literature may be making erroneous
119 conclusions regarding the effects of acute exercise on bone health due to the methodologies
120 employed not adequately capturing the full bone metabolic response.

121 **Effects of low energy availability and low carbohydrate high fat diets on bone**

122 Nutritional practices can also influence bone health and the effects of various interventions on
123 BMD and BTMs have been investigated (Palacios, 2006; Sale & Elliott-Sale, 2019). Energy
124 and macronutrient (particularly carbohydrate) demands of athletes vary between and within
125 days and this is a key driver of dietary intake, such that a degree of periodisation in energy
126 and carbohydrate intake is typically recommended (Stellingwerff et al., 2019). Planned and
127 unplanned bouts of low energy availability (LEA) and low carbohydrate diets (with or without
128 high fat) have been observed in various groups of athletes; thus, the bone response has been
129 of specific interest. It has been hypothesised that LEA and low carbohydrate high fat (LCHF)
130 have detrimental effects on bone health (Garofalo et al., 2023; Hutson et al., 2021a). This
131 raises ethical issues (in addition to the practical difficulties) of prolonged dietary control and
132 standardisation of LEA and LCHF. Therefore, when measuring bone imaging outcomes in
133 humans, investigations into LEA and LCHF have tended to employ observational or cross-
134 sectional designs (Garofalo et al., 2023; Hutson et al., 2021a). No gold standard measure
135 exists for LEA, so surrogate markers of LEA such as menstrual function or cumulative risk
136 score are utilised for group comparisons (Ackerman et al., 2011a; Heikura et al., 2018);
137 creating a demand for highly controlled short-term studies to support conclusions. In contrast,

the effects of various nutrition interventions hypothesised to improve bone health (e.g., increased protein, vitamin D, and calcium intake) have been extensively examined in well-controlled prospective studies utilising bone imaging techniques (Mitchell et al., 2015). Studies have begun to characterise the BTM response to <7 days of LEA or LCHF in men and women (Anton-Solanas et al., 2016; Clayton et al., 2020; Ihle & Loucks, 2004; Murphy et al., 2021; Papageorgiou et al., 2017; Papageorgiou et al., 2018; Zanker & Swaine, 2000) and these will be the focus of this section.

It is often described that short (decreased bone formation and, sometimes, increased bone resorption in the resting and fasted state) and longer-term bone outcomes to LEA (lower BMD and differences in cortical bone geometry and trabecular microarchitecture) are both detrimental to bone health (Hutson et al., 2021a; Murphy et al., 2021; Papageorgiou et al., 2017). However, the following evidence suggests that there is an array of confounding factors that impact this congruency. For example, men are more robust in defending against the effects of a standardised bout of short-term LEA compared to women (Papageorgiou et al., 2017). Nevertheless, similarly high rates of LEA and low BMD exist in men and women participating in sports emphasising leanness and there is growing evidence to support that male athletes with LEA have impaired bone health (De Souza et al., 2019; Mountjoy et al., 2023; Viner et al., 2015). Short-term LEA induced by treadmill running may not impact bone formation and resorption (Papageorgiou et al., 2018). However, a large body of evidence shows that female distance runners who exhibit symptoms of chronic LEA have impaired bone health (Hutson et al., 2021a). Carbohydrate restriction, independent of LEA, has been shown to decrease bone formation and increase bone resorption within 6 days of a LCHF diet in elite racewalkers (Fensham et al., 2022). Comparatively, a recent systematic review in overweight and obese populations found no evidence of negative effects of longer-term LCHF on BMD, although existing human studies are lacking in robust design and statistical power (Garofalo et al., 2023). There are also no robust long-term data in athletic populations by which to compare. Furthermore, 4 days caloric restriction of -630 ± 50 kcals.day^{-1} from estimated

energy requirement reduced bone formation but had no effect on bone resorption in healthy young women (Ihle & Loucks, 2004). However, 12 months caloric restriction of -280 ± 29 kcals.day^{-1} from estimated energy requirement had no effect on bone formation but increased bone resorption and caused loss of BMD in young healthy men and women, with no difference between sexes (Villareal et al., 2016). A far greater energy deficit can be accumulated over more prolonged periods of energy restriction (e.g., 12-months compared to 4-days) even if the daily deficit is less severe, and this likely contributed to the differences identified between the studies by Ihle & Loucks and Villareal and colleagues. The comparisons presented suggest that whilst short-term studies measuring BTMs may have the potential to identify a bone response, they do not reliably predict how bone mass (or even bone metabolism) will change during a similar but more prolonged intervention and should not be used as evidence upon which to base applied practice aiming to optimise bone health.

Findings of impaired bone health in women exhibiting symptoms of severe chronic LEA are highly consistent (Ackerman et al., 2011b; Ackerman et al., 2012; Hutson et al., 2021a; Singhal et al., 2019). This does not necessarily mean that a decrease in bone formation and an increase in bone resorption in response to severe acute LEA (or LCHF) is detrimental. It is plausible that an acute and transient bout of LEA might accelerate bone adaptation by initiating greater resorptive activity which, provided adequate energetic recovery, may be followed by an equivalent increase in bone formation, as per a typical remodeling cycle. However, the typical pre-test-post-test design of acute studies fails to capture a complete bone remodeling response and energy status is fixed.

A recent study haa performed repeated post-exercise BTM measurement for up to 3-hours (Fensham et al., 2022); however, it would be difficult to maintain appropriate standardisation for the duration of an entire bone remodeling cycle. Interestingly, Fensham and colleagues showed elevated post-exercise bone resorption for up to 3-hours following 6 days of LEA and LHFC compared to a control diet. It was suggested that these changes were unfavourable, but it is intriguing to consider the bone health result assuming an equal and opposite bone

formation response in following the days, weeks or months. In this view, parallels may be drawn with acute “train-low” strategies which have been shown to augment exercise stress and specific adaptations in muscle tissue provided daily energy status is not compromised (Hansen et al., 2005). A hypothetical benefit of carbohydrate or energy periodisation could help to explain why an observational study failed to show prospective losses in BMD over 12-months in women exhibiting symptoms of long-term LEA (Singhal et al., 2019). There is also little evidence that intermittent fasting protocols negatively impact bone health and, on the contrary, some might even protect against bone loss during weight loss (Clayton et al., 2023). It is not clear exactly how long or how many samples would be required to characterise a full bone remodeling response to an acute stimulus of LEA (or indeed any nutrition or exercise stimulus), but it would likely become very expensive and difficult to maintain appropriate control and standardisation. Considering that months of repeat samples could be required, the time and cost benefit of measuring BTMs instead of using imaging techniques might all but disappear.

Conclusion

BTMs can be valuable tools for research and practice, particularly for monitoring an individual’s ongoing bone metabolic activity throughout a prolonged and consistent exercise or nutrition intervention. However, the opinion presented herein is that pre-post change in BTM concentration immediately following exercise or following several days of exercise or nutrition intervention should not be relied upon to inform applied practice, where the goal is to optimise bone health. A summary of the factors that should be considered when using and interpreting acute BTMs is presented in Table 2. Highly controlled short-term studies may still be useful to accelerate bone research by informing longer-term follow-up studies with greater efficiency. Regular measurement of BTMs in combination with imaging techniques during long-term prospective research will help to build a better understanding of how these markers relate to structural change in response to exercise and nutrition intervention.

Table 2: Considerations regarding the use and interpretation of bone turnover markers (BTMs) in applied exercise and nutrition research and practice.
• Implement rigorous control measures and standardisation procedures for as long as feasibly possible preceding BTM measurement, considering the potential lasting influence of prior exercise or dietary practices on bone remodelling and the potential
• There is no consensus regarding what represents a meaningful change in BTM concentrations
• Longitudinal monitoring of BTMs (with as many repeat measurements as feasibly possible) should be preferred to cross-sectional or pre-post comparisons
• Integrate BTM measurement with imaging techniques during longitudinal monitoring
• Research aiming to make inferences regarding bone health should use imaging techniques for primary outcome measures
• Avoid concluding a beneficial, detrimental or null effect of exercise or nutrition intervention based on BTM data alone
• Avoid relying solely on BTM outcomes to inform applied exercise or nutrition practice aiming to impact bone health

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All authors made substantial contributions to 1) the conception or design of the work, 2) drafting the work or revising it critically for important intellectual content, and 3) final approval of the version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. MH and IV both contributed to conceptualization, writing the original draft, and review and editing.

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