Small changes in exercise, not nutrition, often result in measurable changes in bone

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Abstract
Skeletal injuries in the equine athlete are a tremendous concern with both economic and animal welfare implications. As a result, much research has focused on improving bone quality through nutritional and exercise interventions. With the recent utilization of biochemical markers, changes in bone metabolism can be monitored. This study examined and compared the response of bone markers and estimates of bone mineral content, in studies with nutritional interventions, with those utilizing exercise interventions. The post hoc analyses suggest that nutritional interventions result in less change to bone markers and bone mineral content than exercise treatments. Of the bone markers examined, osteocalcin correlates most strongly to estimates of bone quality while keratin sulphate, an indicator of cartilage turnover, showed the least correlation. Comparing the results of this study with other published studies, similar findings were observed, suggesting that small alterations in exercise play a greater role in affecting measurable changes in bone metabolism and quality of the equine athlete than do small changes in nutrition.

Keywords: equine; bone metabolism; nutrition; osteocalcin; RBAE

Introduction
Skeletal injuries in performance horses are quite common. Research investigating ways to decrease injuries has been limited due to the difficulty in non-invasively measuring bone response to a given treatment. With the recent use of biochemical markers of bone turnover, more projects are being conducted and advancements in improving bone health are occurring. However, such assays are expensive and researchers often need to make decisions as to when their use is justified. The purpose of this paper is to review the responses of bone markers in nutrition studies and compare them with the responses in exercise studies. The objectives are to determine whether the response of bone markers is similar to estimates of bone mineral content or quality and to determine whether bone is more likely to respond to dietary or exercise interventions.

Materials and methods
The results of studies by the authors in the last 9 years using the marker of bone formation, osteocalcin (OC), as well as markers of bone resorption, including cross-links of both pyridinoline (PYD) and deoxypyridinoline (DPD), and carboxy-terminal PYD cross-linked telopeptides of type I collagen (ICTP), were compiled, summarized and compared with estimates of bone quality.

In the various studies, OC concentrations were determined using Novocalcin (Metra Biosystems, Mountain View, CA) or Metra Osteocalcin (Quidel Corp., Santa Clara, CA), which are ELISAs or an RIA kit from INCSTAR Corporation (Stillwater, MN). Urinary DPD, as well as serum PYD and DPD cross-links, was quantified using an ELISA (Serum PYD, Metra Biosystems, Mountain View, CA). Serum total PYD concentrations were analysed using Pyrilinks-D ELISA (Quidel Corp., San Diego, CA). An RIA kit was used to measure concentrations of ICTP in serum (ICTP, 125I RIA Kit, Diasorin, Stillwater, MN). Additionally, though not considered to be measures of bone metabolism but being implicated as potentially influencing them, concentrations of serum 25-hydroxyvitamin D (Vit D) and C-terminal parathyroid hormone (PTH) were measured in two studies using an RIA kit (INCSTAR Corporation, Stillwater, MN). Likewise,
keratin sulphate (KS), used as an indicator of cartilage turnover, was quantified using an ELISA with an anti-KS monoclonal antibody (ICN Pharmaceuticals, Costa Mesa, CA) and with a standard provided by R.J. Todhunter (Cornell University, Ithaca, NY).

While bone quality is difficult to assess in vivo, diagnostic imaging techniques have been used to provide a means of determining such. Mineral content of third metacarpi was estimated in horses through radiographic photodensitometry. Radiographs were taken with an aluminum step wedge penetrometer attached to each radiographic cassette. The penetrometer image was used to standardize the readings obtained from each radiograph. The radiographs were scanned at the nutrient foramen of the third metacarpal with a video densitometer. A logarithmic regression was formed using the thickness of steps on the aluminum penetrometer to determine bone optical density in radiographic bone aluminum equivalence (RBAE, expressed in mm Al) at the maximum optical density reading of both cortices and for each view of the metacarpi. In studies where bovine subjects were used as a model for horses, computed tomography was used on limbs harvested from sacrificed animals.

For the purpose of analyses, the studies were grouped by whether they had a nutritional or exercise component that was hypothesized to impact bone. The studies involving both differences in diets and alterations in the amount or type of exercise were categorized according to whether alterations in bone or bone markers were linked by time to the differences in nutrition or the change in exercise. For each study, it was determined which markers of bone metabolism were used and if any measurements of bone mineral content were made. For the measured variables, it was determined whether treatment differences were noted at \( P \leq 0.05 \), if there were trends at \( P \leq 0.1 \), or whether no treatment differences were observed at \( P > 0.1 \).

Results

Of seven nutritional studies using OC concentrations to examine dietary impacts on bone, five showed no differences, while two reported a trend for differences (Table 1). Of three nutritional studies that evaluated PYD and DPD concentrations, one showed a treatment difference, one showed a trend for a difference, and one showed no difference. Of four nutritional studies that evaluated ICTP concentrations, only one showed a treatment difference. Only three of the nutritional studies made an evaluation of bone quality (either mineral content or breaking strength) and one reported no difference while two showed trends for differences in at least one measured parameter of bone quality.

In contrast, in eight studies examining the impact of exercise on OC, six showed differences, one showed a trend for a difference, and one reported no difference. Three studies evaluated either PYD or DPD in response to alterations in exercise, and two studies reported differences, while one did not. Two studies used ICTP and one showed a treatment difference, while the other did not. Seven of the studies evaluated bone quality and all of them reported a difference in at least one measured parameter.

Only one study evaluated Vit D and PTH in response to an alteration in diet, with no treatment differences being observed in either parameter despite a trend for a treatment difference in the measurement of bone quality.

Table 1  Studies evaluating markers of bone metabolism in response to alterations in nutrition or exercise

<table>
<thead>
<tr>
<th>Study</th>
<th>Parameter altered</th>
<th>OC</th>
<th>DPD/PYD</th>
<th>ICTP</th>
<th>Bone quality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutritional study</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nielsen 1998b</td>
<td>Ca, P</td>
<td>No</td>
<td>T</td>
<td></td>
<td>T</td>
</tr>
<tr>
<td>Fenton 1999</td>
<td>Glucosamine</td>
<td>No</td>
<td></td>
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<tr>
<td>Lang 2001a</td>
<td>Supplemental Si</td>
<td>T</td>
<td>T</td>
<td>No</td>
<td></td>
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<tr>
<td>Lang 2001b</td>
<td>Supplemental Si</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Nielsen 2002</td>
<td>Anabolic supplement</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Woodward 2005</td>
<td>Omega-3 fatty acids</td>
<td>No</td>
<td></td>
<td>No</td>
<td></td>
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<tr>
<td>Turner 2005*</td>
<td>Supplemental Si</td>
<td>T</td>
<td>Yes</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td><strong>Exercise study</strong></td>
<td></td>
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<tr>
<td>Nielsen 1998a</td>
<td>Onset of training</td>
<td>Yes</td>
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<td>Yes</td>
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<tr>
<td>Nielsen 1998b</td>
<td>Onset of training</td>
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<tr>
<td>Fenton 1999</td>
<td>Longeing</td>
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<tr>
<td>Hoekstra 1999</td>
<td>Stalling/pastured</td>
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<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Bell 2001</td>
<td>Stalling/pastured</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Nielsen 2002</td>
<td>Weight carrying</td>
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<td></td>
<td>Yes</td>
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<tr>
<td>Hiney 2004*</td>
<td>Stalling/forced exercise</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Hiney 2002, 2004b</td>
<td>Stalling/forced exercise</td>
<td>T</td>
<td>Yes</td>
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</tbody>
</table>

* indicates juvenile bovine model instead of equine.
Exercise causes changes in bone

bone mineral (Table 2). While a difference in Vit D was observed when exercise was altered in one study, it was not observed in another similar study. When Vit D was altered in the first study, PTH did not differ. However, in the second study using a similar training protocol, Vit D remained unchanged while there was a trend for a change in PTH, though differences were seen in response to exercise in both the studies. While there was a trend for KS to be altered in response to a nutritional treatment (no measurement of bone quality made), two studies failed to show differences in KS in response to different exercise protocols. Despite no differences in KS, one of the studies on exercise reported significant differences in estimates of bone mineral content.

Discussion

This post hoc analysis suggests that dietary treatments usually failed to result in differences in markers of bone metabolism. In five out of seven nutritional studies, no differences (P < 0.05) were seen in any of the biochemical markers. Additionally, no differences (P < 0.05) in measurements of bone quality were seen in the three studies that evaluated such, though modest trends for treatment differences were noted in two of them. In contrast, variations in exercise resulted in differences in bone marker concentrations and bone quality in seven of eight studies.

Osteocalcin appears to correspond quite strongly with estimates of bone quality. Not one study reported a significant difference in bone quality or OC while not finding a significant difference in the other – regardless of whether it was influenced by nutrition or exercise.

Markers of bone resorption did not agree with estimates of bone quality to the same degree. One study even found one marker of resorption being altered, while the other marker of resorption remained unchanged. Both PYD/DPD and ICTP agreed with the assessment of bone quality twice (thrice for PYD/DPD if a trend for a difference is counted as being significant) and disagreed once. Interestingly, the study in which DPD disagreed with the assessment of bone quality was a study in which juvenile calves were used. While the differences in bone were remarkable, the failure of DPD to be significantly altered may be related to a species specificity issue with the assay kit. Variability was high and repeatability between duplicates was low.

While Vit D and PTH can impact bone, their serum concentrations did not strongly agree with the changes in bone quality. For this reason, their use was discontinued in further studies. Both KS and an estimate of bone quality were only measured in one study and the results did not agree. Considering it is used as a measure of cartilage turnover, it is not surprising that it does not appear to agree with assessments of bone mineral content.

A review of papers presented at the 2001 and 2003 Equine Nutrition and Physiology Symposia using markers of bone formation reveals similar findings. Three of five studies failed to find alterations in either bone metabolism markers or bone quality in response to alterations in diet. The studies evaluated increased concentrations of trace minerals, supplemental manganese and inorganic mineral supplementation. Concentrations of ICTP, but not OC, were altered in response to feeding high Ca, P and Mg. Peterson et al. found that OC was altered when young horses were fed a diet formulated for rapid growth as compared with slow growth. Unfortunately, no measurements of bone quality were reported in either of the latter two studies.

The two studies that examined the response of horses to alterations in exercise reported that both bone metabolism markers and bone quality were altered. These studies examined forced exercise before the onset of initial training and disuse associated with stalling.

A review of papers of the 2005 Equine Science Society found few reports of markers of bone metabolism, despite papers monitoring changes in estimates of bone quality. For example, Spooner et al. compared estimates of mineral content of the third metacarpus in young horses that were fed two

<table>
<thead>
<tr>
<th>Parameter altered</th>
<th>Vit D</th>
<th>PTH</th>
<th>KS</th>
<th>Bone quality</th>
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<td>Nutritional study</td>
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<td>Nielsen 1998a</td>
<td>Onset of training</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Bell 2001</td>
<td>Stalling/pastured</td>
<td>No</td>
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<td>Yes</td>
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Yes, P ≤ 0.05; T, trend with P ≤ 0.1; No, P > 0.1.
different concentrations of dietary protein and found no differences due to treatment. Stephens et al. reported differences in the geometry of the third metacarpus of horses due to exercise but no differences in the bone was observed in response to dietary differences, including varied calcium and phosphorus concentrations and different calcium:phosphorus ratios. These papers support the contention that exercise plays a much greater role in influencing bone quality than does nutrition, as does the paper by Inoue et al. that found changes in bone markers shortly after the onset of training.

Reviewing the literature on a larger scale through numerous database searches reveals other supporting studies. Price et al. exercised 2-year-olds on a treadmill at speeds up to 14 m s \(^{-1}\) thrice weekly for 1 year. While carboxy-terminal propeptide of type I procollagen (PICP), bone-specific alkaline phosphatase (BAP), and ICTP decreased in all horses over time (likely reflective of normal age-related changes), differences in each marker over time compared with controls suggest an increase in bone turnover in the exercised animals. Interestingly, while the authors suggest that each marker holds promise for continued use, they admit the use of ICTP in humans has been criticized. Looking at nutritional factors, a study by de Behr et al. reports no difference in BAP, OC or hydroxyproline in adult horses fed a low (0.8) Ca:P ratio, although the authors reported high inter-animal variability.

Several studies conducted at the University of Florida should also be considered. First, Graham et al. supplemented the diets of yearlings with lysine and threonine. While growth rates increased in supplemented animals, no differences in bone quality estimates (RBAE) were observed. In four other experiments (reported as two studies) when yearlings were supplemented with trace minerals and compared with those fed deficient amounts of trace minerals (according to NRC 1989), differences in RBAE were only observed in two experiments. In one of the experiments reported to show a difference, the difference was observed only in one cortex. Similarly, no differences in RBAE were observed when yearlings were fed either a mixed-grain or a corn-based concentrate or when given restricted versus ad libitum access to concentrate. Finally, when low-starch concentrates were fed to yearlings, even to the extent of reducing growth, no adverse effect on bone mineral deposition was observed.

The rapid response of markers of bone metabolism brings up an interesting point. Bone formation and resorption appear to be able to be up- or down-regulated quite quickly in response to changes in the amount of load placed upon bone. This is supported by the study of Lang et al., which reported OC concentrations doubled from the day of birth to 15 days post-partum and then gradually decreased through the 2-year study. It was hypothesized that the dramatic increase in OC in the newborn foal was due to an increase in bone formation accompanying loading of the skeleton of foals after birth. Additional support for rapid and transient changes in markers of bone formation comes from the study by Hoekstra et al. They reported lowered serum OC concentrations in stalled horses compared with pastured horses just 2 weeks after stalled horses were removed from pasture. Likewise, an increase in urinary DPD was noted in stalled horses 28 days after the horses were assigned to their respective treatments. Combined, these responses reveal a decrease in bone formation coupled with an increase in bone resorption. However, both responses were transient and went back to baseline values at the next measurement. In contrast, mineral content of the third metacarpus, as estimated through radiographic photodensitometry, had also decreased by day 28 of the study but remained low throughout the duration of the 140-day study. That study demonstrates two important points. First, if not measured at the right interval, dramatic changes in bone markers may be missed. This study also clearly points out that once the equine skeleton becomes adapted to a new strain threshold (relative to the load placed upon the skeleton), markers of bone metabolism return to baseline concentrations as bone formation matches up with bone resorption. Unfortunately, without taking some type of measurement to assess bone quality or the amount of mineral present, one cannot discern whether the skeleton is of adequate strength. Thus, the use of bone markers appears to be limited to telling whether a treatment is impacting bone – but use of bone markers cannot fully reveal the impact that the treatment has on skeletal strength. Similarly, while Vit D and PTH can have an impact on bone, neither would adequately reveal changes to skeletal strength. While KS may serve as a marker to reveal changes in cartilage turnover, there was limited value to using it in the examined studies.

Conclusions

Markers of bone metabolism often failed to reveal treatment differences in nutritional studies. This failure has caused some to question the usefulness of these markers. However, in studies examining changes in exercise protocols, differences in concentrations of these bone markers were often seen. These results suggest that exercise plays a much greater role in bone metabolism than do differences in nutrition, particularly if the nutritional differences are relatively
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minor. The horse appears capable of altering its absorption of nutrients to maintain bone health to a much greater degree than its ability to maintain bone strength if not provided with adequate exercise. Thus, it should not be considered a failure of bone metabolism markers when treatment differences are not detected in nutritional studies. Instead, it is likely a failure of imposed treatments in nutritional studies to create a difference. Another way to view this is that as long as nutritional differences are not dramatic, horses appear to be extremely successful in adjusting their absorption and utilization of nutrients to accommodate their skeletal needs.

While these markers do seem capable of detecting treatment differences when they truly exist, their use can still be questioned. While OC appears to be somewhat reliable, the usefulness of PYD/DPD and ICTP may be less so. The assays are expensive to use and changes in concentrations can be missed if samples are not taken at the correct time. Such fluctuations in serum concentrations require relatively frequent sampling to ensure that any changes in concentration are recorded. However, the expense of the assay kits usually limits how often it can be done. Furthermore, since monitoring potential changes in bone metabolism will provide limited evidence as to the quality of bone, it is suggested that other direct measurements still be taken at the same time.

References


