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Recovery of bone strength in young pigs from an induced short-term dietary calcium deficit followed by a calcium replete diet

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ABSTRACT

This study investigated whether the deficits in bone strength of pre-pubertal pigs, induced by short-term deficits in dietary calcium can be recovered if followed by a calcium-fortified diet. Young pigs were divided into two groups based on diet: a marginal Ca diet (70% of established Ca requirements) or an excess Ca diet (150% of established Ca requirements) for 4 weeks. Each group was then randomly sub-divided into two groups and fed diets with either marginal or excess dietary Ca for 6 weeks in a cross-over design, resulting in four treatment groups: H150–H150, H150–L70, L70–H150, and L70–L70. Animals were DXA scanned at 2-week intervals during the 10-week period to obtain whole body bone mineral content (BMC) and density (BMD). After animals were euthanized, right femurs were collected for this study. Traits such as bone mineral density, mass, volume, area moment of inertia (MI) and the section modulus (SM) were computed from computed tomography (CT) data and failure load was measured from four-point bending tests. DXA results showed significant reduction in BMC (61.6%) and BMD (37.5%) in the (L70–L70) group compared to the (H150–H150) group. DXA results additionally showed that deficiencies induced by the 4-week marginal Ca diet in the (L70–H150) group were not recovered with a subsequent excess Ca diet. While mechanical test results also showed significant reduction (75%) in strength in the L70–L70 group, compared to the H150–H150 group, they revealed no differences between the failure loads of the (L70–H150) group and the (H150–H150) group. Similar results were also found for bone mineral mass and volume, indicating that recovery from a short-term dietary Ca deficiency is possible at the pre-pubertal stage. Furthermore, bone mineral content and bone volume calculated from CT data correlated highly with failure load ($R^2 = 0.78$ and 0.84, respectively), while density, MI and SM only showed weak-to-moderate correlations ($R^2 = 0.40$–0.56), implying that bone mineral mass and volume calculated from CT data are good non-invasive surrogates for strength of growing bones.

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1. Introduction

Although osteoporosis is a serious and growing public health problem [1], comprehensive therapy for treating this disease remains elusive. Attainment of maximal peak bone mass at skeletal maturation has been considered to be the best defense against subsequent debilitating age-related bone loss and fracture risk [2–5]. Dietary calcium (Ca) deficits during childhood may permanently reduce the genetically programmed maximal peak bone mass [2,6–10]. The potential to reverse the adverse effects of short-term calcium deficits in early growth phases on peak bone mass and bone strength by repletion with sufficient calcium intake in later growth phases is an important, but inadequately addressed question. Such a study, which, in humans, would require following subjects from early childhood to beyond peak bone mass to assess the influence of early calcium intake on lifetime fracture risk, is a difficult endeavor [11]. Successful execution of retrospective or prospective observational studies in humans over long periods of time is limited by inaccuracies in dietary intake recall, i.e., inaccuracies of food questionnaires to identify calcium consumption [11] and low compliance rates [12]. Given the limitations involved in human trials, studies with animal models offer advantages in control of diet compliance and the length of time required to assess responses. Peterson et al. [17] used female rats, a commonly used animal model, to study the ability of young bones to recover from the adverse effects of dietary calcium deficits. The deleterious effects of low calcium intake through adolescence in rats were non-reversible, resulting in a sub-optimal peak bone mass [17]. While this particular study did not investigate, separately, the effects in the pre-pubertal stage

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of growth, the need for such a study was recognized by the authors.

Pigs also provide an acceptable animal model for studying the effects of dietary calcium changes on young, developing bones. Porcine digestive and renal functions closely reflect human traits [14]. Porcine bone modeling and remodeling processes, as well as bone density and fracture traits are similar to human bones [13,15,16,18]. Invasive studies requiring euthanasia of test subjects for terminal assessments of bones are more acceptable in porcine models than other animal models [13–16].

The present study has attempted to address the following question using a porcine model: Can a deficit in bone strength induced by short-term restrictions in dietary calcium be recovered in prepubertal pigs fed a calcium-fortified diet? This study also offers an opportunity to compare three techniques used to assess bone mineral. Techniques include: (a) dual energy X-ray absorptiometry (DXA) measurements of the entire animal, (b) computed tomography (CT) scans of the excised right femur, and (c) mechanical tests on the excised right femur.

Whole body bone mineral content (BMC) and areal bone mineral density (BMD) of the young, growing animals were determined using DXA. DXA is a commonly used technique for measuring whole body BMC and BMD, yet there are limitations to this technique [19–21], which can partly be attributed to the two-dimensional areal assessment of bone rather than a true volumetric measure to determine density. Limitations are of particular concern when DXA is used to measure bone mineral in growing animals on account of the greater potential for changes in body size, compared to mature animals. Since the ultimate aim is to achieve adequate bone strength, mechanical tests to measure the ability of a bone to withstand loads offer the "gold standard" for assessing bone integrity. Femurs obtained from pigs were tested for this purpose. However, mechanical tests do not offer clinicians a practical option, therefore non-invasive measures of bone quality are needed. Several such measures have been used in the past like bone mass, bone volume, and structural properties like cross-sectional moment of inertia and section modulus. Hence, we also computed the apparent bone mineral density, bone mineral mass, bone mineral volume, cross-sectional moment of inertia and section modulus using quantitative information from computed tomography (CT) scans of the femur to: (i) study the effects of the short-term Ca deficits on these non-invasive measures of bone quality and (ii) explore how these measures correlated with results from mechanical tests.

2. Materials and methods

Forty young, growing, castrated male pigs (Crossbred 1/2 Duroc × 1/4 Large White × 1/4 Landrace) were used for the study. The pigs weighed 9.2 kg on average and were 39 days old at the start of the trial. Pigs were fed diets with either marginal (L70, i.e., 70% of established Ca requirements [19]) or excess (H150, i.e., 150% of Ca requirements) Ca for a 4-week period. Diets with 70% of Ca requirements were selected to provide dietary conditions, which should reduce bone mineralization without confounding effects of dramatic reductions in growth or induction of spontaneous fractures. Likewise, diets with 150% of Ca requirements were selected, not only to allow maximum growth, but also to allow maximum bone mineralization.

Diets were formulated and mixed at the UW Swine Research and Teaching Center. Lab analyses were used to verify the calcium and phosphorus concentrations of the diets. Dietary requirements for calcium and phosphorus in growing swine have been extensively studied and reviewed [19]. The requirements for growing swine (5–120 kg) were based on dietary amounts of calcium and phosphorus needed to allow maximum growth and feed efficiency with

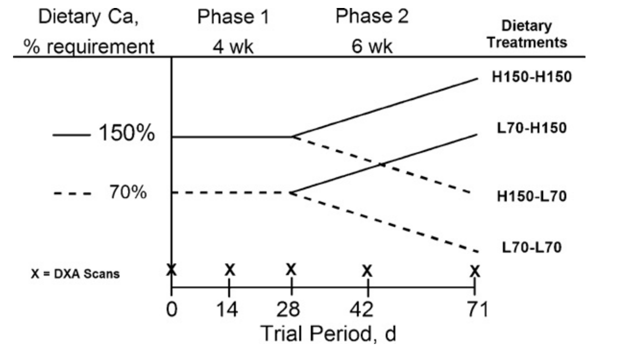

Fig. 1. Treatment group divisions throughout the trial. Horizontal axis indicates number of days in trial period. "x" denotes the days when DXA scans were conducted on animals. Twenty animals per treatment group in phase 1. Eight animals per treatment group in phase 2. (*NRC = National Research Council, Nutrient Requirements of Swine*).

an acknowledgement that at least 10–20% more of each nutrient would be needed to allow maximum bone mineralization. Diets with 150% of Ca requirements are considered the positive control diet for bone mineralization. Eight pigs (four each from L70 and H150 groups) were randomly selected within diet groups and euthanized after the initial 4-week period, henceforth referred to as phase 1, to assess bone mineral content and mechanical properties at that time. The eight pigs euthanized at the end of phase 1 were assumed to represent all pigs at that time. The remaining 32 pigs (eight pigs in each treatment group) continued for an additional 6 weeks (phase 2). After phase 1, the pigs within L70 and H150 phase 1 groups were randomly assigned to diets with either marginal or excess dietary Ca for phase 2 in a cross-over design (Fig. 1). The resulting four treatment groups included H150–H150, H150–L70, L70–H150, and L70–L70 combinations of marginal and excess Ca diets.

Pigs from phase 2 were euthanized and right femurs were collected after completion of the 10-week period (phase 1 + phase 2). Adherent tissue was removed by dissection and the femurs were stored at −20 °C in separate, sealed plastic bags. Data from femurs of eight pigs (phase 2) were lost because of errant storage conditions. Hence, experimental methods and results reported herein relate to responses assessed in excised femurs of 24 (six per treatment group) of the 32 pigs that completed the 10-week trial. Femurs were also obtained from the eight pigs euthanized after phase 1 to confirm diet-induced changes in femur properties after the initial 4-week period. Bones were allowed to thaw to room temperature before CT scans. The bones were re-frozen to −20 °C between CT scans and mechanical tests. The bones were thawed before each test and kept moist during the mechanical tests.

2.1. Dual energy X-ray absorptiometry (DXA)

At designated intervals (days 0, 14, 28, 42 and 71) throughout the trial pigs were scanned with dual energy X-ray absorptiometry (DXA, GE Lunar Prodigy software version 8.10.027) using the pediatric scan mode. Feed was restricted overnight prior to DXA scans. Pigs were anesthetized with halothane during the scan. Scans were analyzed to determine whole body bone mineral content (BMC, g), which is equivalent to the whole body skeletal ash content. Scan analysis also provided estimates of bone mineral density (BMD, g/cm^2), which is the BMC divided by the two-dimensional area (not volume) of skeletal tissue.

On the day following the last DXA scan (day = 28, n = 8, or day = 71, n = 32), pigs were euthanized by exsanguination after an electrical stun. Femurs from the right leg were collected and stored frozen until analysis. Bone mineral content gain (gBMC, g/day) was

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