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ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 653–658
Journal of Nutritional Biochemistry

Phytase supplementation increases bone mineral density, lean body mass and voluntary physical activity in rats fed a low-zinc diet☆

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Received 5 September 2008; received in revised form 27 February 2009; accepted 23 March 2009

Abstract

Phytic acid forms insoluble complexes with nutritionally essential minerals, including zinc (Zn). Animal studies show that addition of microbial phytase (P) to low-Zn diets improves Zn status and bone strength. The present study determined the effects of phytase supplementation on bone mineral density (BMD), body composition and voluntary running activity of male rats fed a high phytic acid, low-Zn diet. In a factorial design, rats were assigned to ZnLO (5 mg/kg diet), ZnLO +P (ZnLO diet with 1500 U phytase/kg) or ZnAD (30 mg/kg diet) groups and were divided into voluntary exercise (EX) or sedentary (SED) groups, for 9 weeks. SED rats were significantly heavier from the second week, and no catch-up growth occurred in EX rats. Feed intakes were not different between groups throughout the study. ZnLO animals had decreased food efficiency ratios compared to both phytase-supplemented (ZnLO+P) and Zn-adequate (ZnAD) animals ($P<.01$ compared to ZnLO). The ZnLO+P and ZnAD rats ran 56–75 km more total distance than ZnLO rats ($P<.05$), with the ZnLO+P rats running more kilometers per week than the ZnLO rats by Week 6. *In vivo* DEXA analyses indicate that rats fed phytase-supplemented diets had higher lean body mass (LBM) than those fed ZnLO diets; and that rats fed the Zn-adequate diets had the highest LBM. Body fat (%) was significantly lower in EX rats and was both Zn- and phytase insensitive. Rats fed phytase-supplemented diets had higher bone mineral content (BMC), bone area (BA) and BMD than rats fed ZnLO diets; and in rats fed ZnAD diets these indices were the highest. The dietary effects on BMC, BA and BMD were independent of activity level.

We conclude that consuming supplemental dietary phytase or dietary Zn additively enhances Zn status to increase BMD, LBM and voluntary physical activity in rats fed a low-Zn diet. While the findings confirm that bone health is vulnerable to disruption by moderate Zn deficiency in rats, this new data suggests that if dietary Zn is limiting, supplemental phytase may have beneficial effects on LBM and performance activity.
Published by Elsevier Inc.

Keywords: Zinc; Phytase; Bone mineral density; Exercise

1. Introduction

In humans, bone mineral accrual is maximal in adolescence and 90% of peak bone mass is achieved by 18 years of age [1]. Low bone mass increases the risk of fracture [2], and for every standard deviation of decrease in bone mineral density (BMD), fracture risk increases two to three times. Osteopenia is defined as a BMD that is 1 to 2.4 standard deviations below the young-adult, gender-matched mean [3]. Based on this operational definition and on BMD measures, more than 18 million individuals in the United States may have osteopenia [4].

Physical exercise-induced mechanical stress plays an important role in the maintenance and increase of bone mass [5]. Physical activity can modify the risk of bone fracture by improving muscle mass and balance, and by increasing skeletal strength [6,7]. However, the clinical relevance regarding exercise for maintaining or improving BMD in adult men cannot be determined from published studies: some studies in men report significant reductions in fracture risk with high physical activity [8–10], while others do not [11–13]. Nutritional interventions, independent of physical activity interventions, are known to reduce fracture risk by increasing BMD. Attempts to remedy low BMD in men through nutritional interventions have included zinc (Zn), with dietary Zn intake and plasma Zn both having a positive association with BMD [14]. Additionally, dietary Zn has been shown to improve BMD in humans [14–18] and in rats [19–22]. However, the influences of variations in Zn bioavailability and voluntary exercise levels as well as the associated combined effects of these influences on BMD have not been well documented.

Because the bioavailability of Zn in plant-derived food and feed is a function of phytate concentration in the diet [23], supplementation of the diet with microbial phytase is a useful means of improving Zn utilization in monogastric animals [24]. Previous work from our laboratory [25], and that of others [23,24,26], has demonstrated the

☆ This study was funded by the US Army MRMC.
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[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

0955-2863/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.jnutbio.2009.03.015

ability of supplemental dietary phytase to improve micronutrient status, body weight and bone strength in animal models.

It is estimated that half the world's population may be suffering from some degree of Zn deficiency [27]. Stunting, poor cognitive performance and lethargy have been reversed after Zn supplementation. As important roles of Zn nutrition in growth [28,29], bone health [30,31] and exercise [32–34] have been described, we hypothesized that BMD would be decreased in voluntary running rats fed low-Zn diets and that increased dietary Zn, either through supplementation of low-Zn diets or increased bioavailability made possible by phytase supplementation, would reverse this low BMD.

2. Methods and materials

2.1. Animals and diets

Four-week-old Sprague-Dawley rats (male; $n=12$ per diet group; total 72 animals; 125–150 g) from Charles River Laboratories, Inc. (Wilmington, MA, USA), were housed individually in polycarbonate cages in a constant temperature (22±3°C) animal room with a 12-h light/dark cycle. Rats spent 2 weeks in quarantine during which acclimation diets (LM-485 mouse/rat sterilizable diets, Harlan Teklad, Inc., Madison, WI, USA) were provided. Rats were then divided into voluntary exercise (EX) or sedentary (SED) groups based on equal average body weight. Diets (see Table 1) were provided *ad libitum* for 63 days and were either moderately Zn deficient (5 ppm) with (ZnLO+P) or without (ZnLO) microbial phytase (P), or Zn adequate (30 ppm, ZnAD). High phytic acid diets were prepared according to the LM-485 formulation (80 g cellulose/kg of diet) by Research Diets, Inc. (New Brunswick, NJ, USA). Phytase was added to the diet to achieve a final activity of 1500 phytase units (FTU)/kg feed (Natuphos, BASF Corporation, Florham Park, NJ, USA). Throughout the study, rats were weighed weekly (PM 30; Mettler Instruments, Hightstown, NJ, USA) and 24-h feed intakes were determined. Spilled feed was collected carefully and weighed, and feed intakes were corrected accordingly. Following the 9-week diet period, rats were sacrificed using carbon dioxide. Blood was drawn directly from the heart; tibia and fibula from the right leg were harvested and carefully cleaned of all extraneous tissue, wrapped in saline-soaked gauze and stored at 4°C until trace element analyses could be conducted. This study was approved by the Institutional Animal Care and Use Committee (IACUC) at USARIEM, and animals were maintained in accordance with IACUC's guidelines for the care and use of laboratory animals.

2.2. Voluntary running capacity

Rats were arranged in a 3×2 complete factorial design, with half the colony ($n=36$) being assigned to cages equipped with 345-mm (diameter) running wheels (Mini Mitter Co., Bend, OR, USA). EX rats had unrestricted access to the running wheels. Each cage was fitted with a magnetic switch to allow for the counting of wheel revolutions using Vital View 3000 software (Mini Mitter Co.).

2.3. Dual-energy X-ray absorptiometry

After the 63-day feeding trial, rats underwent dual-energy X-ray absorptiometry (DEXA) for determination of lean body mass (LBM), fat mass (FM), bone mineral content (BMC), bone area (BA) and BMD, using a Prodigy fan beam densitometer (GE Lunar, Madison, WI, USA) with a small animal, high-resolution scan module. Small animal software (enCore version 7.53.002, 2003; GE Lunar) was utilized for the determination of all body composition measures. All animals were scanned on the same day by the same operator.

Briefly, rats received an anesthetic by intraperitoneal injection of a 1 ml/kg mixture of 40 mg/ml ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA, USA), 10 mg/ml xylazine (Xyla-Ject; Phoenix Scientific, St. Joseph, MO, USA) and 1.5 mg/ml acepromazine (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA). Once sedation was confirmed, rats were transferred to the densitometer. Animals were scanned dorsally, in an anterior–posterior plane.

Table 1
Diet composition

	ZnLO	ZnLO+P	ZnAD
Cornstarch	495.7[a]	495.7	495.7
Maltodextrin 10	125.0	125.0	125.0
Sucrose	100.0	100.0	100.0
Soy protein	140.0	140.0	140.0
DL-Methionine	1.8	1.8	1.8
Soybean oil	40.0	40.0	40.0
Cellulose BW200	50.0	50.0	50.0
Zn-carbonate added	0.00	0.00	0.058
Zn measured (ICP-OES)[b]	7.28 (0.10)	7.26 (0.37)	38.01 (1.90)
Vitamin mix	10.0	10.0	10.0
Mineral mix[c]	35.0	35.0	35.0
Phytic acid (Na-phytate, Sigma)	3.0	3.0	3.0
Phytase (Natuphos, BASF)	0.00	0.15	0.00
Phytase measured[d]	0.00	0.14	0.00

[a] Expressed as grams per kilogram of feed.
[b] Values expressed as means (±S.D.), for $n=3$ feed samples/diet group as milligrams of zinc per kilogram of diet.
[c] AIN-93 formulation prepared without Zn.
[d] Values expressed as mean for $n=2$ feed samples/diet group.

2.4. Biochemistry

Zn, magnesium (Mg) and iron (Fe) were assessed in tibial bones, serum and feed using a Prodigy High Dispersion Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES; Teledyne Leeman Labs, Hudson, NH, USA) against known standards.

Bone, serum and feed samples were digested 69–70% OmniTrace® nitric acid (VWR, West Chester, VA, USA) and diluted 10-fold with chelex 100 resin (Bio-Rad Laboratories, Hercules, CA, USA)-treated water. Trace element standards, prepared from reference solutions (Fisher Scientific, Pittsburgh, PA, USA) in 7% nitric acid, were used as internal controls. All analysis was conducted in acid-washed glassware.

2.5. Statistical analysis

For body weight and voluntary running data, differences were assessed using analysis of variance (ANOVA) with repeated measures. All other data were analyzed for main effects of exercise, phytase and dietary Zn, as well as interactions, by multifactorial ANOVA, followed by Tukey's *post hoc* tests, using the Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS, Inc., Chicago, IL, USA). Pairwise comparisons were made using Students' unpaired t test. All data are reported as means±S.D. Differences were considered significant at $P<.05$.

3. Results

The initial body weights were not different when the treatment period began (SED rats: 176.9±6.4 g; EX rats: 174.8±6.4 g). Sedentary rats (Fig. 1A) were significantly heavier than voluntary running rats (Fig. 1B) by the second week of study (data pooled for diets, $P<.05$). Final body weights for SED rats were 14–16% greater than for the EX rats, regardless of which diet was consumed. In both SED and EX groups, rats fed the phytase-supplemented diets were significantly heavier than rats fed ZnLO diets by the second week of study. Phytase supplementation of EX rats increased their body weights such that by the fourth week of study they were indistinguishable from rats fed Zn-adequate diets.

The EX rats weighed less than the SED rats, despite consuming similar amounts of feed throughout the study (Fig. 2B), and no catch-up growth occurred in EX rats. Within each diet group, feed intakes over 24 h were not different between SED and EX groups; and within each activity group, dietary Zn intakes did not change the rate of feed consumed. The food efficiency ratios (FERs), expressed as body weight gain/feed intake, assesses utilization of food consumed. FERs decreased significantly in rats consuming ZnLO diets compared to ZnAD diets ($P<.01$); however, no significant differences in FERs were apparent with ZnLO+phytase diet compared to ZnAD diet (data not shown). As expected for rats running 5–7 km/day, the EX rats had lower FERs, compared to SED rats, for 5 out of 7 weeks, i.e., 71% of the time ($P<.05$), independent of diets consumed.

Voluntary running data indicates that varying dietary Zn availability had no observable effects on performance, when expressed as the average kilometer run per day, until close to the end of the study: ZnLO rats ran less kilometers per day than ZnLO+P rats by Week 6 ($P<.05$). Also, rats consuming ZnLO feed ran significantly less over the duration of the study (approx. 56–75 km less total distance) than rats fed either phytase- or Zn-adequate diets ($P<.05$; Fig. 3). The proportion of time spent running in the wheels was not influenced by diet, as the majority of the running was nocturnal (93.7%), and similar for all three diet groups (data not shown).

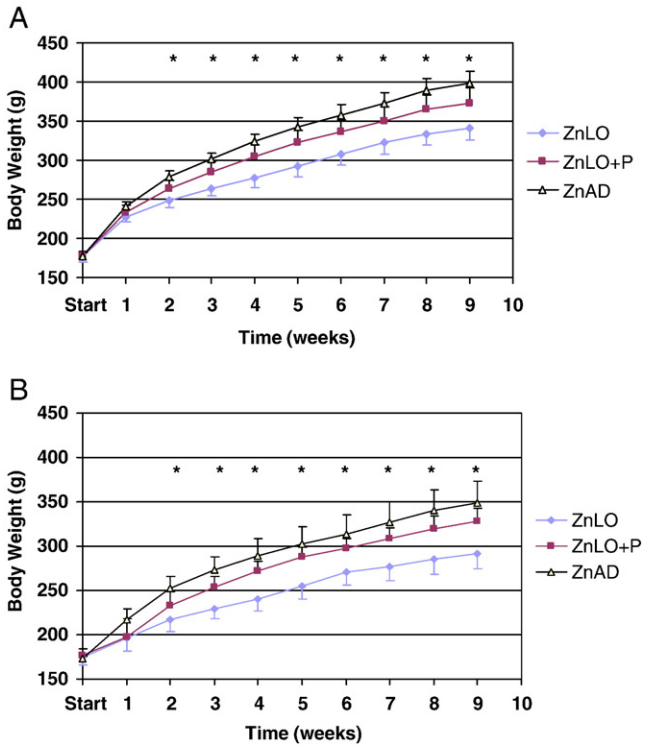

Fig. 1. Effect of dietary Zn on body weight gain in SED (A) and EX (B) rats fed moderately low Zn diets (ZnLO, 5 ppm), ZnLO diets supplemented with phytase (ZnLO+P) or Zn-adequate (ZnAD, 30 ppm) diets *ad libitum*, for a period of 9 weeks. Asterisks (*) indicate significant differences ($P<.05$) between the ZnLO+P and ZnLO groups within either the SED or EX groups. Values are means±S.D., with $n=12$ rats/group.

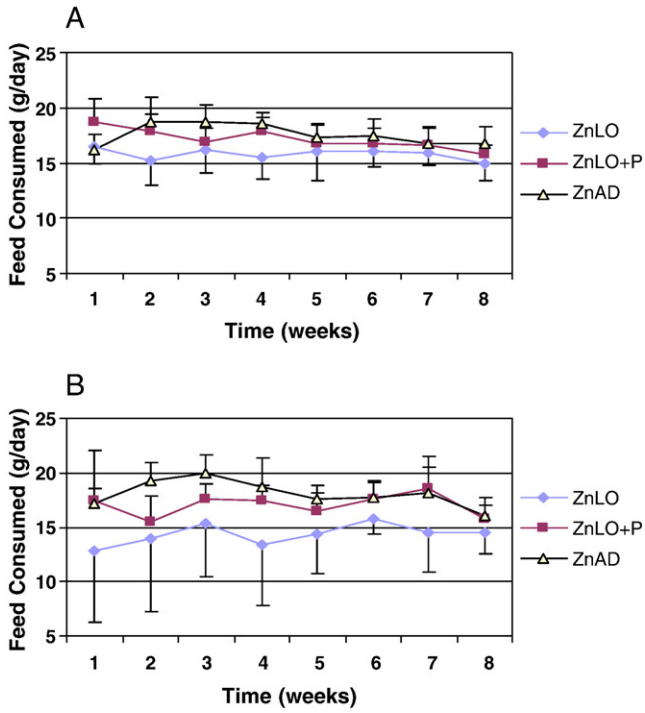

Fig. 2. Feed intakes of SED (A) and EX (B) rats fed moderately low Zn diets (ZnLO, 5 ppm), ZnLO diets supplemented with 1500 FTU phytase/kg feed (ZnLO+P) or Zn-adequate (ZnAD, 30 ppm) diets *ad libitum* for 9 weeks. Values are means±S.D., with $n=12$ rats/group.

Sera and tibial bones were used for the determination of Zn status at the end of the 9-week feeding period (Table 2). Phytase supplementation of ZnLO diets and consumption of ZnAD diets significantly increased serum Zn compared to ZnLO diet (data pooled for activity level, SED+EX; $P<.001$). The interaction between activity and dietary Zn level was significant ($P<.05$). Although serum (or plasma) Zn is commonly used as an indicator of Zn status, bone Zn has been utilized as a more reliable index of Zn status in animal studies [28]. As expected, bone Zn was higher in rats consuming ZnAD diets compared to rats consuming either ZnLO or ZnLO+P diets ($P<.05$). Bone Zn concentrations correlated with Zn levels measured in feed (see Table 1), in both SED ($r=0.94$; $P<.001$) and EX ($r=0.994$; $P<.001$) rats. While the main effect of voluntary running on bone Zn levels was not significant (Table 3), the interaction between activity level and phytase supplementation was significant ($P<.05$). Concentrations of other minerals known to be affected by dietary phytase were investigated as well. Magnesium concentrations in sera and tibial bones were not affected by ZnLO or phytase supplementation, or activity levels (Table 2). In contrast, serum iron was decreased 41% in SED rats fed ZnAD diets ($P<.01$) and by 25% in EX rats fed ZnLO+P diets ($P<.05$), compared to rats fed ZnLO diets.

At study termination, LBM (Fig. 4A) was 11% higher in ZnLO+P rats ($P<.001$), and 19% higher in ZnAD rats, compared to rats fed ZnLO diets (data pooled for activity level, SED+EX rats; $P<.001$). When diet groups were pooled, LBM was significantly lower in rats exposed to running wheels ($P=.011$). For LBM, these were all independent main effects (Table 3). While body fat (%) was significantly lower in EX rats, it was independent of both phytase- and Zn level (Fig. 4B). There was a significant interaction between activity and phytase supplementation for % body fat as determined by two-way ANOVA ($P<.05$). Bone mineral content (Fig. 5A) increased by 9% in ZnLO+P rats ($P<.001$) and by 18% in ZnAD rats ($P<.001$), compared to ZnLO rats (data pooled for activity level, SED+EX rats). When diet groups were pooled, the two-dimensional BMC appears to be significantly decreased by voluntary running activity ($P<.001$). However, when BMC measures are corrected to control for differences in body size, the differences in BMC are no longer significant (mean±S.D. for SED rats=0.0262±0.0023; and for EX rats=0.0255±0.0025 g/g total body weight). Similarly, BA (Fig. 5B) increased by 7% in rats fed ZnLO+P diets ($P<.001$) and by 13% in rats fed ZnAD diets (data pooled for activity level, SED+EX rats; $P<.001$), compared to rats fed ZnLO diets (data pooled for activity level, SED+EX). When diet groups were pooled, the two-dimensional BA appeared to be significantly decreased by activity levels ($P<.001$) — this difference is no longer significant when BA is corrected for total body weight (SED rats=0.1633±0.0137; EX rats=0.1648±0.0118 cm^2/g

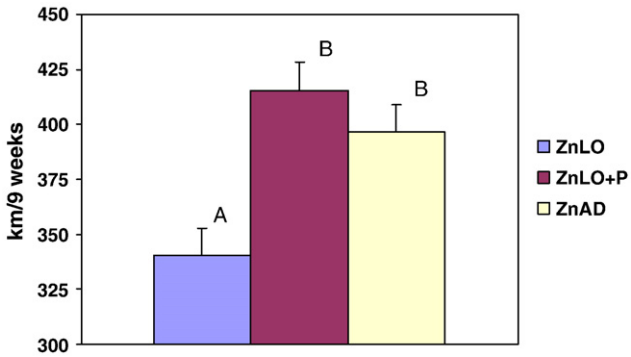

Fig. 3. Voluntary running activity of rats fed moderately Zn-deficient (ZnLO, 5 ppm), ZnLO diets supplemented with phytase (ZnLO+P) or Zn-adequate (ZnAD, 30 ppm) diets *ad libitum*, for a period of 9 weeks. Cages were equipped with voluntary running wheels, and activities of rats monitored continuously. Means of activities were calculated for each rat as kilometers per day and totaled for 9 weeks. Values with different letters differ significantly ($P<.05$); the graph shows means±S.D. for 12 rats/group.

Table 2
Trace element status

	SED			EX		
	ZnLO	ZnLO+P	ZnAD	ZnLO	ZnLO+P	ZnAD
Tibial Ca	102,713 (6,256)	111,334 (7,641)	115,151 (15,588)	111,235 (21,693)	100,583 (19,724)	114,205 (11,294)
Tibial Fe	67.3 (6.9)	69.8 (17.1)	64.3 (11.9)	79.7 (23.0)	63.4 (15.6)	68.0 (13.4)
Tibial Mg	2,064 (152)	2,281 (184)	2,255 (318)	2,331 (455)	2,105 (409)	2,317 (214)
Tibial Zn	62.6 (5.2)^{A}	82.7 (6.1)^{B}	121.7 (16.1)^{C}	80.4 (12.2)^{B}	79.6 (15.7)^{B}	125.9 (11.1)^{C}
Serum Ca	100.5 (4.1)	100.5 (7.5)	103.2 (5.0)	99.8 (2.2)	99.6 (3.2)	98.2 (3.0)
Serum Cu	0.028 (0.027)	0.052 (0.048)	0.040 (0.010)	0.042 (0.033)	0.095 (0.069)	0.059 (0.072)
Serum Fe	3.90 (1.37)^{G}	2.70 (0.78)	2.29 (0.55)^{H}	3.31 (0.58)	2.46 (0.79)^{H}	2.63 (1.02)
Serum Mg	19.2 (2.3)	18.2 (1.7)	18.8 (2.4)	19.1 (0.9)	18.6 (1.0)	18.0 (2.5)
Serum Se	0.63 (0.42)	0.72 (0.39)	0.93 (0.24)	0.80 (0.49)	0.81 (0.40)	0.67 (0.26)
Serum Zn	0.60 (0.12)^{D}	1.02 (0.30)^{E}	1.06 (0.14)^{E}	0.52 (0.15)^{D}	0.78 (0.10)^{F}	1.24 (0.20)^{E}

Effects of varying dietary Zn, phytase and activity levels on Zn, magnesium (Mg), iron (Fe) and calcium (Ca) concentrations in tibiae (μg/g bone) and serum (μg/ml) after 9 weeks on the trial diets. Rats were fed ZnLO (5 ppm), ZnLO+P (5 ppm+phytase) or ZnAD (30 ppm) diets *ad libitum*, and divided into EX or SED groups. Bones underwent digestion in concentrated nitric acid prior to mineral analysis using ICP-OES. Values with different superscripts differ significantly ($P<.05$). Values are means±S.D., for 8–12 tibiae and eight sera per group.

total body weight). Whole-body BMD (Fig. 5C) increased significantly in both Zn-adequate and ZnLO+P rats ($P<.05$, data pooled for activity level, SED+EX) compared to ZnLO rats. However, when pooled for diet, and corrected for differences in total body weight, BMD measured by DEXA (g/cm^2) was not affected by activity level.

4. Conclusions

Previous studies have independently investigated the effects of activity level and the effects of dietary Zn on body composition in growing rats [19,20,22,25,30,35], but this is the first study to examine the interaction of voluntary physical activity with moderate Zn deficiency and phytase supplementation on body composition. This study demonstrates that moderate dietary Zn deficiency negatively affected BMD, with partial reversal occurring with improvements in Zn bioavailability following phytase supplementation. In agreement with bone densitometry studies suggesting that low dietary Zn decreases BMD in rats [19,21,22,35], nonhuman primates [36] and humans [14–18], we found that BMD decreased significantly in rats fed either of the ZnLO diets over the study period, independent of activity levels. Interestingly, supplementation with phytase caused a significant improvement in BMD in ZnLO animals. In SED rats, BMD increased less in rats fed phytase-supplemented diets compared with rats fed Zn-adequate diets. We hypothesized that BMD would be decreased in voluntary running rats fed low-Zn diets. With osteopenia defined as a BMD that is 1 to 2.4 standard deviations below the young-adult, gender-matched mean [3], both EX and SED rats fed ZnLO diets are

Table 3
Main effects and interactions

	Dietary Zn[a]	Phytase[b]	Exercise[c]	Interaction
Tibial Ca	NS	NS	NS	
Tibial Fe	NS	NS	NS	
Tibial Mg	NS	NS	NS	
Tibial Zn	<.001	<.05	NS	EX×Phytase, $P=.012$
Serum Ca	NS	NS	NS	
Serum Cu	NS	NS	<.01	
Serum Fe	<.01	<.01	NS	
Serum Mg	NS	NS	NS	
Serum Se	NS	NS	NS	
Serum Zn	<.001	<.001	NS	EX×Zn, $P=.035$
LBM (g)	<.001	<.001	.011	
Body fat (%)	NS	NS	<.001	EX×Phytase, $P=.039$
Tissue mass (g)[d]	<.01	<.01	<.001	
BMC (g)[e]	<.001	.001	NS	
BA (cm^2)[e]	<.001	<.001	NS	
BMD (g/cm^2)[e]	<.001	<.05	NS	

Effects of varying dietary Zn, phytase and activity levels on Zn concentrations (serum and bone) and body composition (from DEXA) after 9 weeks on the trial diets. Rats were fed ZnLO (5 ppm), ZnLO+P (5 ppm+phytase) or ZnAD (30 ppm) diets *ad libitum*, and divided into EX or SED groups. *P* values are for eight serum samples, 8–12 tibiae and 10–12 DEXA measures per group. The main effect of dietary Zn, dietary phytase and exercise (EX), or the interaction (last column) was considered significant at $P<.05$. NS indicates not significant.

[a] Dietary Zn effects — ZnLO diets (pooled EX and SED rats) *vs.* ZnAD diets (pooled EX and SED rats).

[b] Phytase effects — ZnLO diets (pooled EX and SED rats) *vs.* ZnLO+P diets (pooled EX and SED rats).

[c] Exercise effects — SED rats (pooled ZnLO, ZnLO+P and ZnAD diets) *vs.* EX rats (pooled ZnLO, ZnLO+P and ZnAD diets).

[d] Tissue mass (g) combines fat mass (g) and LBM (g).

[e] Bone densitometric data (BMC, BA, BMD) is corrected for total body weight.

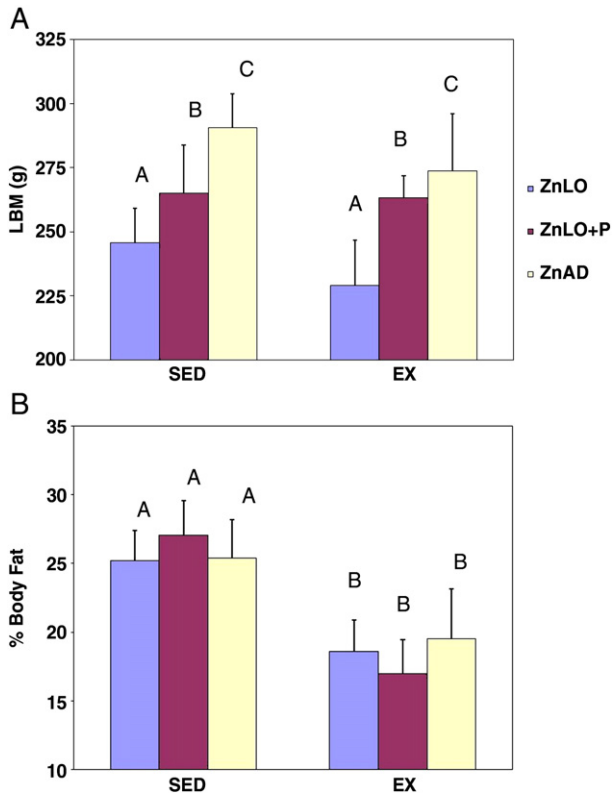

Fig. 4. Effect of varying Zn diets and activity on body composition as assessed using *in vivo* DEXA analysis after 9 weeks on the trial diets. As there was no interaction between phytase, dietary Zn and/or exercise but there were main effects for phytase, dietary Zn and exercise, data were pooled to show means of main effects only. Lean body mass (A) and % body fat (B) were determined for the whole body: for % body fat there was a significant interaction between phytase and exercise as determined by two-way ANOVA, and there was a main effect of exercise. Values are means±S.D. ($n=12$ rats/group). Values with different letters differ significantly ($P<.05$).

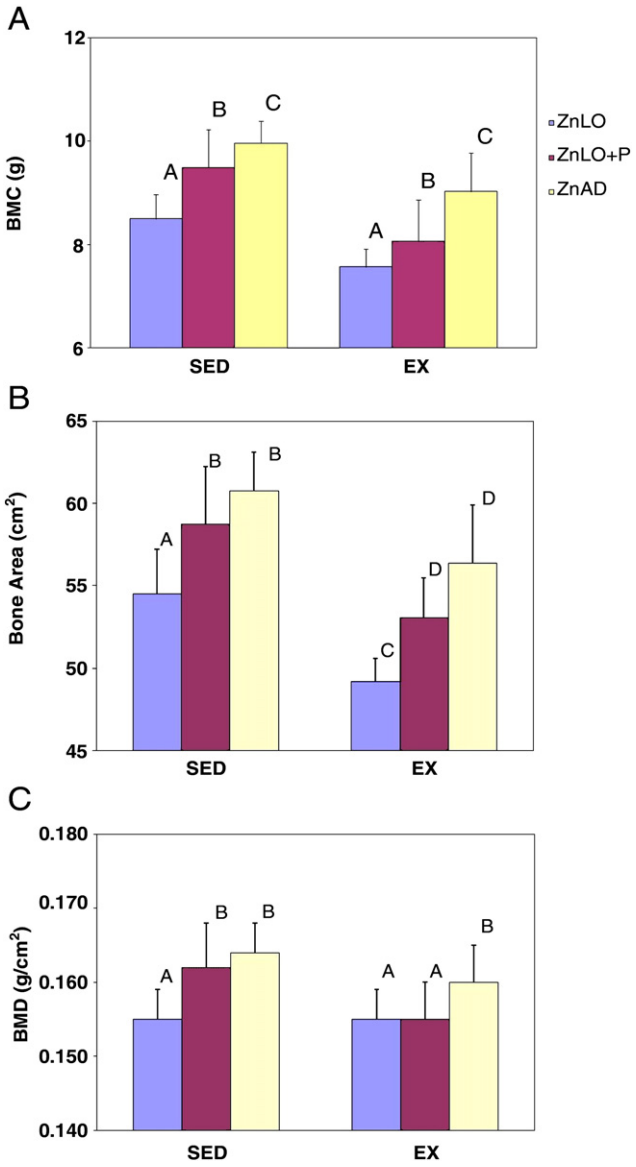

Fig. 5. Effect of varying Zn diets and activity on body composition assessed using *in vivo* DEXA analysis after 9 weeks on the trial diets. BMC (A), BA (B) and BMD (C) were determined for the whole body. As there was no interaction between phytase, dietary Zn and/or exercise but there were main effects for phytase, dietary Zn and exercise, data were pooled to show means of main effects only. Values with different letters differ significantly ($P<.05$). Values are means±S.D. ($n=24–36$ rats/group).

considered osteopenic, having BMD values 1 to 2.4 standard deviations below that of rats consuming ZnAD diets, respectively. We also hypothesized that the increased bioavailability of Zn in the phytase-supplemented diets would reverse the low BMD. The ZnLO+P diets did reduce osteopenia, suggesting that if dietary Zn is limiting, supplemental dietary phytase may have beneficial effects on BMD. These are important findings as low BMD can be markedly affected by dietary Zn, through either bioavailability or level, and the maintenance of Zn adequacy could be important in protecting against osteopenia.

The increase in BMC in response to phytase supplementation is similar to the increase in BMC observed in rats [25] and pigs [24] fed elevated levels of dietary phytase. Comparable increases in BMC have also been observed in rats fed Zn-supplemented feed [22]. In this study, we examined the potential effect of Zn, or its bioavailability, to influence BMC without considering other factors that might impact BMC. It is known that mechanical loading is necessary for maintaining skeletal integrity, but the most effective type, intensity and duration of exercise are not known. Voluntary wheel running is more of an endurance type of training than weight lifting and more suited to the experimental diets used. In addition, moderately Zn-deficient diets (*vs.* frank Zn-deficient diets) are more likely to be found in the United States and Canada [37,38], but longer study periods are required [31], and hence this study was conducted over 63 days, a period equivalent to more than 5 years in human beings [39]. By Week 6 of the study, rats fed both ZnLO+P and ZnAD diets ran more kilometers per week than rats fed ZnLO diets, and by the end of the study both groups ran significantly further than rats fed ZnLO diets. Despite this increase in "performance" resulting from dietary manipulation, no activity-related changes were observed for BMC, BA or BMD measures. Thus, if changes in BMC, or BA, are conditioned more by the diet than by exercise, and are both Zn- and phytase sensitive, this study reinforces the requirements for adequate Zn nutriture during growth periods.

Adding microbial phytase to the diets of these rats significantly improved Zn status in SED rats, as demonstrated by an increase in both serum and tibial Zn concentrations, with no change in the status of other trace minerals, such as Ca, Fe and Mg. In EX rats, phytase supplementation increased serum Zn, but not tibial Zn which has been utilized as a more reliable index of Zn status in animal studies [28]. In addition, the bone mineral data indicates that in EX rats, phytase did not increase Ca, Fe or Mg concentrations. This suggests that the response to added phytase may not be linear in EX rats and that the efficacy of microbial phytase might be compromised in rats running 5–7 km/day. In addition to the improvement of total Zn levels, phytase supplementation had important physiological consequences in animals fed a low-Zn diet. Anabolic effects, as demonstrated by gains in LBM, were apparent with phytase supplementation, and these anabolic effects are accompanied by functional improvements as manifested by increases in running performance. Taken together, the improved Zn status and lack of differences in Ca, Mg and Fe status between dietary treatment groups suggest that the positive effects of phytase supplementation are the result of increased Zn nutriture. In both EX and SED models, the growth-stimulating effect of supplemental phytase is similar to that reported by others in rats [23]. This study is the first work, to the best of our knowledge, to report the effects of dietary phytase on body composition in growing rats using different exercise modes.

As feed intakes were not different in any diet group, the lower body weight and LBM in both EX and SED rats fed ZnLO diets appear to be specific to Zn inadequacy and not a caloric deficit. As noted by Eberle *et al.* [40], there is a possibility that, because the rats had free access to the diets, the observed skeletal effects might be due not to Zn depletion *per se*, but rather to retarded growth and reduced feed intake, with subsequently lowered energy and protein intake in the Zn-deficient animals. However, Fernandez-Madrid *et al.* [41] demonstrated by comparing Zn-deficient rats with pair-fed and *ad libitum*-fed controls that the impairment in protein and collagen synthesis observed in Zn-depleted rats was in fact due to Zn deficiency and not to caloric intake differences. In this study, the feed intakes in the ZnLO rats were not different from the ZnAD rats (Fig. 2); thus, we conclude that the adverse bone measures were specific to Zn inadequacy, *per se*, rather than the result of a reduction of calories consumed. Although the role of trace minerals in bone turnover and metabolism will require further study, our results provide data suggesting that improving the bioavailability of Zn could have profound effects on bone integrity. We hypothesize that the improvements in body composition observed with supplemental phytase may be due to increased expression of Zn metalloenzymes found in bone, including alkaline phosphatase and collagenase.

Feeding animals either ZnLO+P or ZnAD diets limited the accretion of fat mass over the 9-week feeding period, as % body fat

(and absolute fat mass) was similar between dietary treatment groups at the end of the study, in agreement with our earlier studies in SED rats [25]. Wheel running, as a model to study effects of spontaneous activity, has been used extensively to study the effects of chronic activity on body composition. Previous research has demonstrated that long-term (greater than 1 month) access to running wheels results in increased food consumption and decreased body fat, with little change in LBM in adult mice [42]. While our exercise-training regimen was voluntary, the reduced weight gains of the EX rats compared with the SED rats were similar to those reported in the literature for involuntary treadmill-exercise regimens [43]. Under the variable Zn diet conditions of the present study, the differences in body mass between the SED and EX groups is explained more by the difference in % body fat than by differences in LBM. For % body fat, there was a significant main effect of activity ($P<.001$) that was greater than the main effect of activity on LBM ($P=.011$).

In summary, Zn supplementation was more effective than phytase supplementation in preventing osteopenia. The optimal levels of Zn and/or phytase supplementation to prevent osteopenia in exercising rats require further investigation. Clearly, the rat model shows that Zn is essential to bone health and that when Zn is suboptimal for extended periods, there are significant effects on BMD that increase the risk of stress fractures. Additional studies are necessary for establishing the importance of improving Zn status for bone health in other modes of exercise. Although rodent models can contribute valuable information in this area, it is clear that more appropriate human studies are of paramount importance — this study used growing rats, thus any extrapolations to adult humans should be made with prudence. These results also have important implications for mineral supplementation trials in the countries where dietary Zn intakes are limiting, and that supplemental phytase may have beneficial effects on bone mass accrual, body composition and performance levels.

References

[1] Misra M. Bone density in the adolescent athlete. Rev Endocr Metab Disord 2008;9(2):139–44.
[2] Bennell K, Matheson G, Meeuwisse W, Brukner P. Risk factors for stress fractures. Sports Med 1999;28(2):91–122.
[3] Woolf AT, Pfleger B. Burden of major musculoskeletal conditions. Bull World Health Organ 2003;81(9):646–56.
[4] Looker AC, Orwoll ES, Johnston Jr CC, Lindsay RL, Wahner HW, Dunn WL, et al. Prevalence of low femoral bone density in older U.S. adults from NHANES III. J Bone Miner Res 1997;12(11):1761–8.
[5] Chilibeck PD, Sale DG, Webber CE. Exercise and bone mineral density. Sports Med 1995;19(2):103–22.
[6] Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 1994;330(25):1769–75.
[7] Province MA, Hadley EC, Hornbrook MC, Lipsitz LA, Miller JP, Mulrow CD, et al. The effects of exercise on falls in elderly patients. A preplanned meta-analysis of the FICSIT Trials. Frailty and injuries: cooperative studies of intervention techniques. JAMA 1995;273(17):1341–7.
[8] Joakimsen RM, Fønnebø V, Magnus JH, Størmer J, Tollan A, Søgaard AJ. The Tromso study: physical activity and the incidence of fractures in a middle-aged population. J Bone Miner Res 1998;13(7):1149–57.
[9] Kujala UM, Kaprio J, Kannus P, Sarna S, Koskenvuo M. Physical activity and osteoporotic hip fracture risk in men. Arch Intern Med 2000;160(5):705–8.
[10] Michaelsson K, Olofsson H, Jensevik K, Larsson S, Mallmin H, Berglund L, et al. Leisure physical activity and the risk of fracture in men. PLoS Med 2007;4(6):1094–100.
[11] Mussolino ME, Looker AC, Madans JH, Langlois JA, Orwoll ES. Risk factors for hip fracture in white men: the NHANES I epidemiologic follow-up study. J Bone Miner Res 1998;13(6):918–24.
[12] Sorock GS, Bush TL, Golden AL, Fried LP, Breuer B, Hale WE. Physical activity and fracture risk in a free-living elderly cohort. J Gerontol 1988;43(5):M134–9.
[13] Nguyen TV, Eisman JA, Kelly PJ, Sambrook PN. Risk factors for osteoporotic fractures in elderly men. Am J Epidemiol 1996;144(3):255–63.
[14] Hyun TH, Barrett-Connor E, Milne DB. Zinc intakes and plasma concentrations in men with osteoporosis: the Rancho Bernardo Study. Am J Clin Nutr 2004;80(3):715–21.
[15] Angus RM, Sambrook PN, Pocock NA, Eisman JA. Dietary intake and bone mineral density. Bone Miner 1988;4(3):265–77.
[16] New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 1997;65(6):1831–9.
[17] Peretz A, Papadopoulos T, Willems D, Hotimsky A, Michiels N, Siderova V, et al. Zinc supplementation increases bone alkaline phosphatase in healthy men. J Trace Elem Med Biol 2001;15(2-3):175–8.
[18] Relea P, Revilla M, Ripoll E, Arribas I, Villa LF, Rico H. Zinc, biochemical markers of nutrition, and type I osteoporosis. Age Ageing 1995;24(4):303–7.
[19] Hosea HJ, Taylor CG, Wood T, Mollard R, Weiler HA. Zinc-deficient rats have more limited bone recovery during repletion than diet-restricted rats. Exp Biol Med 2004;229(4):303–11.
[20] Jamieson JA, Taylor CG, Weiler HA. Marginal zinc deficiency exacerbates bone lead accumulation and high dietary zinc attenuates lead accumulation at the expense of bone density in growing rats. Toxicol Sci 2006;92(1):286–94.
[21] Otsuka M, Ohshita Y, Marunaka S, Matsuda Y, Ito A, Ichinose N, et al. Effect of controlled zinc release on bone mineral density from injectable Zn-containing beta-tricalcium phosphate suspension in zinc-deficient diseased rats. J Biomed Mater Res 2004;69A(3):552–60.
[22] Seco C, Revilla M, Hernández ER, Gervás J, González-Riola J, Villa LF, et al. Effects of zinc supplementation on vertebral and femoral bone mass in rats on strenuous treadmill training exercise. J Bone Miner Res 1998;13(3):508–12.
[23] Rimbach G, Pallauf J. Enhancement of zinc utilization from phytate-rich soy protein isolate by microbial phytase. Z Ernahrungswiss 1993;32(4):308–15.
[24] Pagano AR, Yasuda K, Roneker KR, Crenshaw TD, Lei XG. Supplemental *Escherichia coli* phytase and strontium enhance bone strength of young pigs fed a phosphorus-adequate diet. J Nutr 2007;137(7):1795–801.
[25] McClung JP, Stahl CH, Marchitelli LJ, Morales-Martinez N, Makin KM, Young AJ, et al. Effects of dietary phytase on body weight gain, body composition, and bone biomechanics in growing rats fed a low zinc diet. J Nutr Biochem 2006;170:190–6.
[26] Yonekura L, Suzuki H. Effects of dietary zinc levels, phytic acid and resistant starch on zinc bioavailability in rats. Eur J Nutr 2005;44(6):384–91.
[27] Cousins RJ. Zinc. In: Bowman BA, Russell RM, editors. Present knowledge in nutrition. 9th ed. Washington, DC: ILSI Press; 2006. p. 445–57.
[28] Hall AG, Kelleher SL, Lonnerdal B, Philipps AF. A graded model of dietary zinc deficiency: effects on growth, insulin-like growth factor-I, and the glucose/insulin axis in weanling rats. J Pediatr Gastroenterol Nutr 2005;41(1):72–80.
[29] MacDonald RS. The role of zinc in growth and cell proliferation. J Nutr 2000;130:1500S–8S.
[30] Ovesen J, Moller-Madsen B, Thomsen JS, Danscher G, Mosekilde L. The positive effects of zinc on skeletal strength in growing rats. Bone 2001;29:565–70.
[31] Scrimgeour AG, Stahl CH, McClung JP, Marchitelli LJ, Young AJ. Moderate zinc deficiency negatively affects biomechanical properties of rat tibiae independently of body composition. J Nutr Biochem 2007;18(12):813–9.
[32] Brun JF, eu-Cambrezy C, Charpiat A, Fons C, Fedou C, Micallef JP, et al. Serum zinc in highly trained adolescent gymnasts. Biol Trace Elem Res 1995;47(1-3):273–8.
[33] Khaled S, Brun JF, Micallel JP, Bardet L, Cassanas G, Monnier JF, et al. Serum zinc and blood rheology in sportsmen (football players). Clin Hemorheol Microcirc 1997;17(1):47–58.
[34] Krotkiewski M, Gudmundsson M, Backstrom P, Mandroukas K. Zinc and muscle strength and endurance. Acta Physiol Scand 1982;116(3):309–11.
[35] Salgueiro MJ, Torti H, Meseri E, Weill R, Orlandini J, Urriza R, et al. Dietary zinc effects on zinc, calcium, and magnesium content in bones of growing rats. Biol Trace Elem Res 2006;110(1):73–8.
[36] Golub MS, Keen CL, Gershwin ME, Styne DM, Takeuchi PT, Ontell F, et al. Adolescent growth and maturation in zinc-deprived rhesus monkeys. Am J Clin Nutr 1996;64(3):274–82.
[37] Gibson RS, Vanderkooy PD, MacDonald AC, Goldman A, Ryan BA, Berry M. A growth-limiting, mild zinc-deficiency syndrome in some southern Ontario boys with low height percentiles. Am J Clin Nutr 1989;49(6):1266–73.
[38] Hambidge KM, Hambidge C, Jacobs M, Baum JD. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediatr Res 1972;6(12):868–74.
[39] Quinn R. Comparing rat's to human's age: how old is my rat in people years? Nutrition 2005;21(6):775–7.
[40] Eberle J, Schmidmayer S, Erben RG, Stangassinger M, Roth HP. Skeletal effects of zinc deficiency in growing rats. J Trace Elem Med Biol 1999;13(1-2):21–6.
[41] Fernandez-Madrid F, Prasad AS, Oberleas D. Effect of zinc deficiency on nucleic acids, collagen, and noncollagenous protein of the connective tissue. J Lab Clin Med 1973;82(6):951–61.
[42] Bell RR, McGill TJ, Digby PW, Bennett SA. Effects of dietary protein and exercise on brown adipose tissue and energy balance in experimental animals. J Nutr 1984;114(10):1900–8.
[43] Huang TH, Lin SC, Chang FL, Hsieh SS, Liu SH, Yang RS. Effects of different exercise modes on mineralization, structure, and biomechanical properties of growing bone. J Appl Physiol 2003;95(1):300–7.