

## Phytate levels and bone parameters: A retrospective pilot clinical trial

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## 1. ABSTRACT

This study evaluated the relationship between phytate urinary levels and bone characteristics in a large population of postmenopausal women. The study population consisted of 180 postmenopausal women who participated in a descriptive cross-sectional study. A urine sample was collected from each subject to determine phytate levels and the volunteers were divided into two groups according to phytate urinary concentration (i.e., low and high levels). Bone mineral density was determined in the lumbar spine and femoral neck of groups with low and high phytate urinary levels. Urinary levels of phytate were linked to dietary phytate consumption. Hence, bone mineral density values were significantly higher in the lumbar spines and femoral necks of women who consumed high levels of phytate than in women with low urinary phytate concentrations. Higher urinary levels of phytate correlated with higher bone mineral density in the lumbar spine and femoral necks of postmenopausal women. This finding demonstrates the potential use of phytate in the treatment of bone related diseases, as it uses a mechanism of action similar to some bisphosphonates.

## 2. INTRODUCTION

Osteoporosis, a disease characterized by compromised bone strength and an increased risk of fracture (1), remains the most prevalent metabolic bone disease in humans. The measurement of bone mass or bone density is currently the best method for diagnosing osteoporotic patients or those at risk for the disease.

A recent study demonstrated the potential benefits of myo-inositol hexakisphosphate (i.e., InsP6 or phytate) in the treatment of osteoporosis (2). The health benefits of phytate consumption have been known for some time, and the effects of phytate on pathological calcifications such as renal stones (3-8), cardiovascular calcifications (9-12), sialolithiasis (13) and dental calculus (14) have been described.

Early studies of animal models suggested that high doses of phytate (i.e., 200 mg/kg/day to 300 mg/kg/day) resulted in bone affections such as rickets, presumably because the compound reduces the absorption of calcium (15). However, more recent studies have

**Table 1.** Criteria for subject acceptance into the study.

Entrance criteria	Exclusion criteria
Female sex	Menopause of duration superior to 5 years
Presence of menopause	Family history of osteoporosis
Agree to participate in the study and provide informed consent	Weight inferior to 57 kg
	Treatment with bisphosphonates or other drugs related to osteoporosis
	Surgical menopause
	Osteoporosis related diseases

**Table 2.** Demographics of the selected volunteers

Characteristic	Group 1	Group 2
n (number of volunteers)	140	40
Mean age $\pm$ SE (years)	52.4 $\pm$ 0.4	51.6 $\pm$ 0.5
Mean weight $\pm$ SE (kg)	63.8 $\pm$ 0.8	68.3 $\pm$ 2.1 *
Mean body mass index $\pm$ SE (kg/m <sup>2</sup> )	24.1 $\pm$ 0.5	26.2 $\pm$ 0.8 *
Length of menopause (years)	2.8 $\pm$ 0.2	2.4 $\pm$ 0.3

\*  $p < 0.05$  vs group 1: low-phytate consumption group

investigated the effects of calcium magnesium phytate (i.e., a natural salt found in vegetable seeds, commonly known as phytin) on bone mineral density. A recent study (2) of 1,473 individuals demonstrated that bone mineral density levels were higher in subjects who reported consuming phytate-rich foods more than twice per week than in subjects who consumed smaller amounts. The typical phytate content (i.e., as calcium magnesium salt) of a standard Mediterranean diet ranges from 15 mg/kg/day to 20 mg/kg/day. The mentioned study (15) were performed in canine models using phytate sodium salt, which does not occur naturally; however, high doses (i.e., equivalent to 120 mg/kg/day) of sodium phytate have been used in humans to treat hypercalciuria (16) with no reported side effects. Furthermore, human studies indicate that doses of approximately 30 mg/kg/day (i.e., 2 g per day) of phytate in equilibrated diets do not affect mineral balance (17, 18).

Our hypotheses regarding the relationship between phytate consumption and bone mineral density are based on studies of the inhibitory effects of phytate on bone resorption, a process mediated by osteoclasts (19). These effects resemble those exhibited by some bisphosphonates, which are chemically similar to phytates, as the presence of phosphate and phosphonate groups convey a high affinity for calcium crystal surfaces, thereby promoting self-adsorption.

Within the larger group of bisphosphonates, several synthetic compounds have a positive effect on bone mass. The most widely used bisphosphonate, alendronate, is an inhibitor of crystallization and thereby protects against renal stone development (20) and cardiovascular calcification (21). Thus, its mechanism of action is presumably similar to that of phytate. It is well known that alendronate improves bone mineral density levels in postmenopausal women (22-24) and reduces the risk of fracturing the lumbar spine and femoral neck (25-28). In contrast, the use of phytate, a naturally occurring compound, may prevent side effects such as upper gastrointestinal tolerability or osteonecrosis, which are associated with bisphosphonates (29, 30).

Here, we evaluate phytate content in urine samples and describe the effects of phytate consumption on the bone mineral densities of the lumbar spine and femoral neck.

### 3. MATERIALS AND METHODS

#### 3.1. Participants

A descriptive pilot cross-sectional clinical trial was performed on a study population of 180 postmenopausal women from Mallorca (Balearic Islands). All subjects provided informed consent, satisfied the entrance criteria and did not satisfy the exclusion criteria outlined in Table 1. The presence or absence of exclusion criteria was determined by an in depth clinical interview. Weights were measured using standardized scales. Prior to enrollment in the study, a clinical interview was conducted to determine which women had entered menopause. Hormonal determinations were performed in doubtful cases to verify the absence or presence of menopause. Personal and clinical data were collected by the Servicio de Prevencion de Riesgos Laborales (GESMA, Palma de Mallorca Spain). The study protocol was approved by the Balearic Research Ethics Board.

#### 3.2. Urine samples and phytate analysis

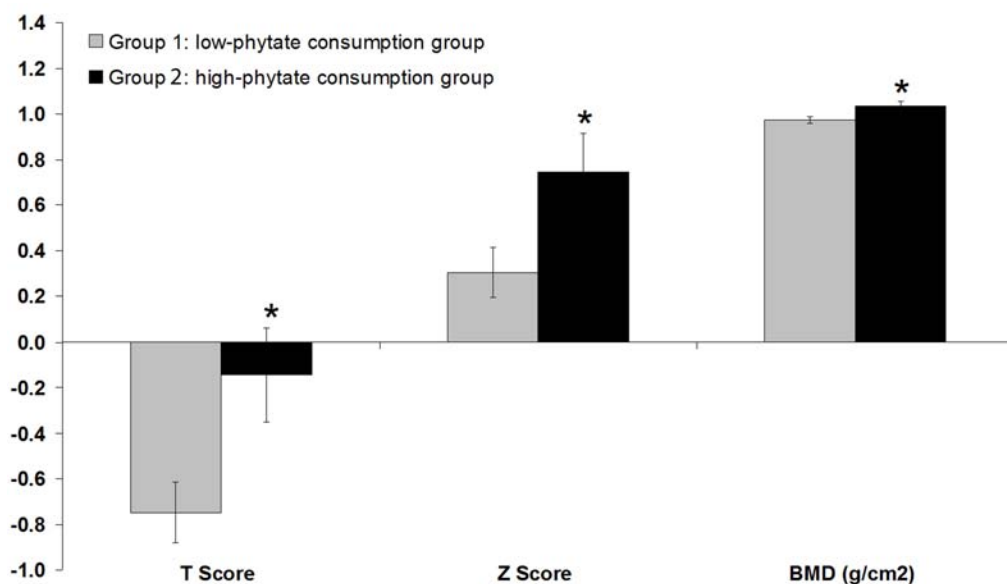
A urine sample was collected from each volunteer, using the following procedure. The first urine of the morning was discarded, and fasting urine was collected 2 hours after the initial urination. The sample was stored at 4°C, transported to the laboratory in a chilled container, and immediately analyzed using previously described methods (31). All individuals consumed an unrestricted diet during the sample collection period. The 180 menopausal women were classified into two groups according to their urinary phytate content. Group 1: Women with a very low consumption of phytate-rich products [i.e., phytate urinary concentrations less than 1.33 microM (0.88 mg/L)]. - Group 2: Women with an acceptable consumption of phytate-rich products [i.e., phytate urinary concentrations equal to or higher than 1.33 microM (0.88 mg/L)]. The demographics of Groups 1 and 2 are shown in Table 2.

#### 3.3. Determination of bone mineral density

The bone mineral densities of the lumbar spine (L2-L4) and femoral neck were determined via dual X-ray absorptiometry (DXA, model Norland Excell bone densitometer, MEC Osteoporosis Bone Densitometry, Minster, OH). We determined the T scores, Z scores and bone mineral densities (g/cm<sup>2</sup>) at both locations. Densitometry was performed by a single technician to avoid interobserver bias.

#### 3.4. Statistics

Values are expressed as mean  $\pm$  SE. Student's t-tests were used to assess differences between means. Conventional Windows software was used for statistical computations. A  $p$  value  $< 0.05$  indicated a significant difference.



**Figure 1.** Values of T score, Z score and BMD according to phytate consumption in lumbar spine (L2-L4). \*  $p < 0.05$  vs group 1: low-phytate consumption group

#### 4. RESULTS

One-hundred and forty women were included in Group 1 (low-phytate concentration) and 40 women were included in Group 2 (high-phytate concentration) (Table 2). The average age of Group 1 was  $52.4 \text{ years} \pm 0.4 \text{ years}$  and the length of menopause  $2.8 \text{ years} \pm 0.2 \text{ years}$ , while the average age of Group 2 was  $51.6 \text{ years} \pm 0.5 \text{ years}$  and the length of menopause was  $2.4 \text{ years} \pm 0.3 \text{ years}$ . There were no statistically significant differences between the groups with regards to age ( $p = 0.269$ ) or length of menopause ( $p = 0.286$ ), although the body mass index was slightly lower in group 1 ( $24.1 \text{ kg/m}^2 \pm 0.58 \text{ kg/m}^2$ ) than in Group 2 ( $26.2 \text{ kg/m}^2 \pm 0.8 \text{ kg/m}^2$ ). Analysis of urine samples revealed a mean phytate concentration of  $0.66 \text{ mg/L} \pm 0.08 \text{ mg/L}$  in Group 1, and  $1.48 \text{ mg/L} \pm 0.08 \text{ mg/L}$  in Group 2.

Examination of the lumbar spine (L2-L4), as shown in Figure 1 and femoral neck, as shown in figure 2, revealed significant differences in T and Z scores when Group 1 was compared with Group 2. Additionally, bone mineral densities were higher in Group 2 when comparing the values with Group 1, both in the lumbar spine ( $1.05 \text{ g/cm}^2 \pm 0.02 \text{ g/cm}^2$  vs  $0.97 \text{ g/cm}^2 \pm 0.01 \text{ g/cm}^2$ ) and in the femoral neck ( $0.88 \text{ g/cm}^2 \pm 0.02 \text{ g/cm}^2$  vs  $0.84 \text{ g/cm}^2 \pm 0.01 \text{ g/cm}^2$ ).

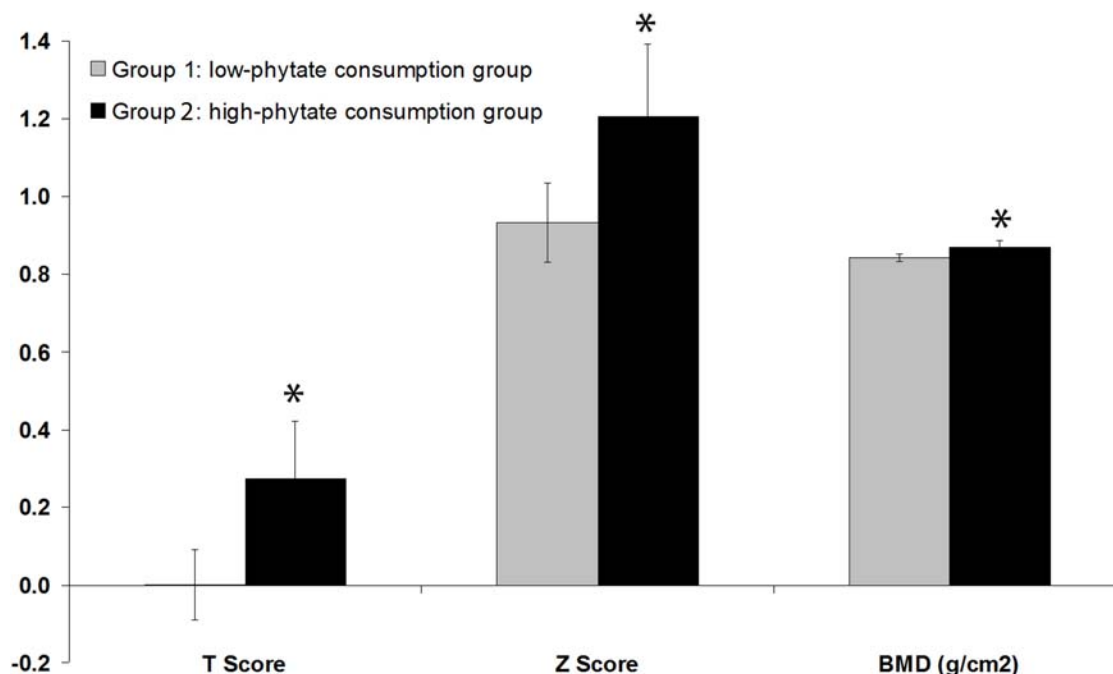
#### 5. DISCUSSION

Our results confirm previous reports (2) of increased bone mineral density in the lumbar spines and femoral necks of women who consume high levels of phytate.

Our observation that women who consume high-phytate foods such as legumes, nuts and whole grains have

better bone mineral densities than other people, coupled with previous reports that phytate consumption can prevent renal stones (3-8) and cardiovascular calcifications (9-12), highlights the importance of the Mediterranean diet in the improvement of health (32). It is important to note that phytate and bisphosphonates, which are both crystallization inhibitors, bind to crystal nuclei or crystal faces to disturb crystal development (33, 34) and thereby protect against pathological calcification and bone resorption. Adsorption of such compounds to crystal faces can also inhibit osteoclast-mediated crystal dissolution (35). However, bisphosphonates interfere with various biochemical intracellular processes when they are internalized by bone-resorbing osteoclasts. Simple non-nitrogen-containing bisphosphonates (e.g., clodronate and etidronate) can be metabolically incorporated into the non-hydrolysable analogues of adenosine triphosphate (ATP). This inhibits ATP-dependent intracellular enzymes and induces osteoclast apoptosis (36). Potent nitrogen-containing bisphosphonates (e.g., pamidronate, alendronate, risedronate, ibandronate and zoledronate) inhibit a key enzyme (i.e., farnesyl pyrophosphate synthase) in the mevalonate pathway and prevent the biosynthesis of isoprenoid compounds that are essential for the posttranslational modification of small guanosine triphosphate (GTP)-binding proteins (GTPases). This results in the loss of osteoclast activity (36, 37). Recently, the effects of phytate on osteoblasts and osteoclast activity have been also described (19).

Phytate has some advantages. Namely, this natural product has been consumed by humans from ancient times and does not appear to cause adverse side effects. Urinary excretion of phytate directly correlates with dietary consumption of this substance (38, 39). Furthermore, the levels of phytate excreted in the urine are closely related to the values detected in biological tissues



**Figure 2.** Values of T score, Z score and BMD, according to phytate consumption in femoral neck. \*  $p < 0.05$  vs group 1: low-phytate consumption group

and plasma (40), which ensures that urinary values correlate with blood concentrations and accurately reflect phytate levels in the diet.

Future studies are needed to confirm our findings and further examine the relationship between phytate consumption and bone mineral density in other groups such as men, menopausal women and people with one or more of the exclusion criteria used in this study.

Thus, higher urinary levels of phytate are linked to increased bone mineral density in postmenopausal women, suggesting that this compound may prove effective in the treatment of bone-related diseases.

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