Original Article

Phosphorus Balance in Adolescent Girls and the Effect of Supplemental Dietary Calcium†

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Disclosures Page

- Mr. Vorland has nothing to disclose.
- Dr. Martin has nothing to disclose.
- Dr. Weaver has nothing to disclose.
- Dr. Peacock has nothing to disclose.
- Dr. Hill Gallant has nothing to disclose.

Abstract

There are limited data on phosphorus balance and the effect of dietary calcium supplements on phosphorus balance in adolescents. The purpose of this study was to determine phosphorus balance and the effect of increasing dietary calcium intake with a supplement on net phosphorus absorption and balance in healthy adolescent girls. This study utilized stored urine, fecal, and diet samples from a previously conducted study that focused on calcium balance. Eleven healthy girls ages 11-14y participated in a randomized crossover study which consisted of two 3-week periods of a controlled diet with low (817 ± 62 mg/d) or high (1418 ± 35 mg/d) calcium, separated by a 1-week washout period. Phosphorus intake was controlled at the same level during both placebo and calcium supplementation (1531 ± 29 and 1534 ± 30 mg/d, respectively, $p = 0.831$). Mean phosphorus balance was positive by about 200 mg/day and was unaffected by the calcium supplement ($p = 0.826$). Urinary phosphorus excretion was lower with the calcium supplement (535 ± 42 vs 649 ± 41 mg/d, $p = 0.013$), but fecal phosphorus and net phosphorus absorption were not significantly different between placebo and calcium supplement (553 ± 60 vs 678 ± 63 vs mg/d, $p = 0.143$; 876 ± 62 vs 774 ± 64 mg/d, $p = 0.231$, respectively). Dietary phosphorus underestimates using a nutrient database compared with the content measured chemically from meal composites by ~40%. These results show that phosphorus balance is positive in girls during adolescent growth and that a calcium dietary supplement to near the current recommended level does not affect phosphorus balance when phosphorus intake is at 1,400 mg/d, a typical U.S. intake level. This article is protected by copyright. All rights reserved

Introduction

Because of rapid growth in adolescence, the phosphorus recommended dietary allowance (RDA) for girls and boys ages 9-13y is set at 1250 mg/day, nearly twice as high as the RDA of 700 mg/day for adults (1). Data from the National Health and Nutrition Examination Survey (NHANES) show that the average intake of phosphorus in this age group for girls is 1176 mg/day, and 66% of children in this age group meet the estimated average (median) requirement (2). Dietary calcium binds phosphorus in the intestine and impairs its absorption. The interaction between phosphorus and calcium in the intestine in healthy adults demonstrated that the phosphorus binding capacity of calcium carbonate and calcium acetate at approximately 45 mg phosphorus per gram calcium salt (3). Thus, calcium salts are used as phosphate binders to prevent or lower hyperphosphatemia in patients with CKD (4).

Limited information exists on phosphorus balance and the effect of a dietary calcium supplement on phosphorus retention during adolescent growth to inform the phosphorus Dietary Reference Intakes (DRI) (5-7). This deficit in knowledge is particularly important because it is estimated from NHANES data that 24% of females aged 9-13 use supplemental calcium (8).

The aims of this study were to describe phosphorus balance in adolescent girls and determine the effect of a dietary calcium supplement on phosphorus skeletal retention using stored samples from a previous study that examined the effect of particle size of calcium supplementation on calcium balance (9). In addition, because of the uncertainty of the accuracy of dietary phosphorus intake from food composition tables, we tested the relationship between dietary intake estimated from food composition tables and from chemical analyses of the diet during the balance study.

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Materials and Methods

Subjects & Study Design

Stored samples from adolescent girls who participated in calcium balance studies during the summer of 2007 were analyzed for phosphorus content. A detailed description of the original study is described elsewhere (9). Briefly, healthy adolescent girls, ages 11-14, participated in a randomized cross-over study that consisted of two three-week balance studies, separated by a one-week washout period to compare calcium balance in subjects when they were given small particle size calcium carbonate supplements, large particle size calcium carbonate supplements, or a placebo. The original study found no difference in calcium balance between small and large particle size calcium carbonate, but significantly greater positive calcium balance from small particle size than placebo (9). Eleven of the 12 participants who were in the study arm that compared small particle size calcium carbonate with placebo are included in the present analysis. (**Table 1**). One participant was excluded from the present analysis due to insufficient stored fecal sample. Two of the 11 participants included completed only one of the two cross-over periods. Race and ethnicity were self-reported by questionnaire. Height and weight were measured with a stadiometer and scale and used to calculate height-for-age, weight-for-age, and BMI-for-age percentiles from the Centers for Disease Control (CDC) growth charts using the Statistical Analysis Software (SAS) files available online from CDC (10). Sexual maturation stage was determined by breast development using Tanner Sexual Maturity Form by self-assessment (11). The balance studies were conducted in a controlled environment in the form of a summer camp. Participants were fed a controlled diet (containing ~800 mg/d calcium) and randomized to receive either an additional 600 mg/d of elemental calcium from calcium carbonate capsules or placebo (**Figure 1**). The controlled diet consisted of a 4-day cycle menu

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with consistent phosphorus, calcium, sodium, and protein content. Participants were allowed to consume deionized water ad libitum. Diets of differing energy content (1300 kcal/d, 1600 kcal/d, and 1900 kcal/d) were designed to meet the energy requirements of the participants as estimated by the Harris-Benedict equation (12), and weekly body weights were monitored for weight maintenance. During each 3-week balance study, all fecal and urine samples were collected, and participants were closely monitored for diet, fecal, and urine collection compliance. Duplicate diet composites were made at the time of each meal, pooled by 24h, and frozen. Thawed composites were homogenized, freeze-dried (FTS Systems Inc., Stone Ridge, NY), and stored for later analysis. Any uneaten food was offered again to participants during the same 24h period, and complete intake was encouraged. Uneaten food at the end of the 24h period was saved, weighed, and analyzed to determine accurate intake. Polyethylene glycol (PEG), a nonabsorbable fecal marker, was provided with each meal (PEG E3350, Dow Chemical Co., Midland, MI, prepared in capsules by Delavau LLC). Pill counts, urine creatinine, and fecal PEG recovery were used as compliance measures as described previously (9).

Measures

Dietary, Fecal, and Urine Phosphorus Content

Dietary phosphorus was estimated from study menus using Nutrition Data System for Research 2007 (NDSR, Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN), and stored diet composite samples were analyzed for phosphorus. Freeze-dried diet and thawed fecal homogenates (stored at -20°C) were ashed in a muffle furnace (Thermolyne Sybron Type 30400, Dubuque, IA) at 600°C. Ashed diet and fecal samples were diluted with 2% nitric acid. Acidified urine samples (stored at -40°C) were thawed and diluted with 2% nitric acid. Phosphorus was measured in diet, fecal, and urine samples by inductively

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coupled plasma-optical emission spectrophotometry (ICP-OES; Optima 4300DV, Perkin Elmer, Shelton, CT). Daily urinary phosphorus excretion was adjusted based on average daily creatinine excretion (9) for each participant to correct for timing and incomplete sample collection errors.

Phosphorus Balance and Net Absorption Calculations

The first week of each 3-week study was used as an equilibration period to the calcium intake level, and balance calculations were based on the last two weeks of each 3-week study. For each balance period, balance, net absorption, and percent net absorption were calculated. Balance is calculated as dietary phosphorus intake (mg/d) minus urine and fecal phosphorus excretion (mg/d); net absorption as phosphorus intake (mg/d) minus fecal excretion (mg/d); and percent net absorption as net absorption (mg/d) divided by dietary intake (mg/d) x 100.

Statistical Analysis

Repeated measures ANOVA for crossover designs using the PROC MIXED procedure with subject as a random effect was used to compare treatment differences, and included analysis for order and period effects. Unpaired t-tests were used to compare estimated and analytically measured dietary phosphorus and calcium. Statistical significance was set at $\alpha < 0.05$. Statistical Analysis Software (SAS Institute, Cary, NC) version 9.3 was used for all statistical analysis. Results are reported as mean $\pm$ SEM unless otherwise indicated.

Results

The majority of girls were of white race and non-Hispanic ethnicity (**Table 1**). BMI for age was greater than the 50th percentile. All were healthy and were recruited from Indiana, Illinois, and Ohio.

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Phosphorus measured chemically in the daily diet samples from the placebo phase was and from the calcium carbonate was 39% and 40% greater than the phosphorus content estimated by NDSR (p < 0.001), respectively. Calcium measured in the diet samples was not different from estimated calcium content from NDSR in the placebo phase, and was 2% higher than estimated on the calcium phase (p = 0.013, **Table 2**). Dietary phosphorus intake measured chemically did not differ between placebo and calcium (p = 0.611, **Table 2**), and calcium intake was significantly different by design between placebo and calcium phases.

Overall phosphorus balance was not different between calcium and placebo (245 ± 81 mg vs 228 ± 79 mg; p = 0.826) (**Figure 2**). Fecal phosphorus was not significantly different between calcium and placebo (678 ± 63 mg vs 553 ± 60 mg; p = 0.143, NS) while urinary phosphorus was 114 mg/d lower with calcium than with placebo (535 ± 42 mg vs 649 ± 41 mg, p = 0.013). Net phosphorus absorption was not significantly different on calcium compared with placebo expressed as either mg/d (774 ± 64 mg/d vs 876 ± 62 mg/d; p = 0.231, NS) (**Figure 3, A**) or as percent of intake ($53 \pm 4\%$ vs $61 \pm 4\%$; p = 0.186, NS).

As previously reported (7), calcium balance was 307 mg/d higher on calcium compared to placebo, (519 ± 48 mg/d vs 212 ± 46 mg/d; p = 0.002), fecal calcium was higher (857 ± 56 mg/d vs 517 ± 54 mg/d; p = 0.001), while urinary calcium was not significantly different (114 ± 26 mg/d vs 84 ± 26 mg/d; p = 0.079, NS) (**Figure 4**). Net calcium absorption was higher (569 ± 55 mg/d vs 301 ± 53 mg/d; p = 0.004) (**Figure 3, B**).

Serum calcium, phosphate, 25OHD, 1,25-$(OH)_2D_3$, parathyroid hormone (PTH), osteocalcin, alkaline phosphatase (ALP), bone alkaline phosphatase (BAP), and urinary N-terminal telopeptide (NTx) and free deoxypyridinoline (DPD) were not different between the

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placebo and calcium (**Table 3**). Values were within normal ranges on both placebo and calcium supplement.

Discussion

In this 3-week randomized, placebo-controlled crossover balance study, adolescent girls were in positive phosphorus balance of about 200 mg when consuming just over 1,400 mg of phosphorus per day. Because bone contains approximately 85% of the body phosphorus stores (13), the bulk of the retention is presumed to be in the skeleton reflecting the high rate of skeletal growth during adolescence. A calcium supplement of 600 mg/d had no effect on this phosphorus retention, but did increase the calcium retention by over 300 mg/d. In girls ages 9-13, average estimated calcium intake from the diet is 968 mg/d (8), and thus supplements may be used to achieve the calcium RDA of 1300 mg/d (14). The calcium supplement affected phosphorus metabolism in the expected direction by decreasing urinary phosphorus, but the decrease in net phosphorus absorption was not statistically significance. This may be attributable to the higher variability in fecal phosphorus measurements compared to urinary phosphorus.

Urinary phosphorus decreased by 1.9 per 10 mg/d increase in elemental calcium intake in the calcium carbonate period. In comparison, we previously published a study of similar design in moderate-stage CKD patients which showed that there was about 1 mg/d reduction in urinary phosphorus per 10 mg/d increase in elemental calcium (15) in the CKD study urinary phosphorus fell with calcium carbonate supplementation, but there was no increase in fecal phosphorus, net phosphorus absorption, or overall phosphorus balance. A study of pre-dialysis CKD patients also observed a ~1 mg/d reduction in urinary phosphorus per 10 mg/d calcium in patients receiving ~800 mg elemental calcium (16). The difference in reduction in urine phosphorus with

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supplemental calcium between our study in healthy adolescents and the CKD studies probably reflects increased efficiency to retain phosphorus in adolescents compared to adults with CKD. However, the lower amount of the calcium supplement, in the adolescent study which provided only ~600 mg/d may also be a factor.

There are a limited number of phosphorus balance studies in adolescent girls using different levels of phosphorus and calcium intake. Nearly 100 years ago, Sherman et al. (7) performed a series of balance studies in 9-13 year old girls. Calcium intakes ranged from 425 to 1794 mg/d with phosphorus intakes ranging from 886 to 2009 mg/d, and phosphorus balance ranged from -37 to 667 mg/d. Ca:P intake (mass) ratio ranged from 0.48 to 1.03, and there is no relationship between Ca:P intake ratio and phosphorus or calcium balance. Over 50 years later, Greger et al. (5) provided 12.5 to 14.5 year old girls a diet of 1.07 g/d calcium, 0.85 g/d phosphorus and showed that phosphorus balance was positive (48 ± 76 mg/d) as was calcium balance (409 ± 61 mg/d). In a separate study by Greger et al. (6), phosphorus balance was similar to their previous study at 23 ± 110 mg/d. However, the DRIs for phosphorus for ages 9 through 13 years were set based on estimated phosphorus intake to support observed tissue accretion rather than intakes for maximal retention because published studies lacked a range of phosphorus intakes to establish maximal retention as was available for calcium (1). Our study provides additional data at an intake level intermediate to other studies (~1.4 g/d phosphorus), but studies over a wider range of phosphorus intakes will be required to determine the intake that achieves maximal retention.

Because 99% of the body's calcium (17) and 85% of the body's phosphorus (13) reside in bone as hydroxyapatite and assuming that during a three-week balance period retained

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calcium and phosphate are deposited in the skeleton as apatite crystal, the relationship between calcium retention and phosphorus retention measured by balance should mirror the 2.15:1 mass ratio (5:3 molar ratio) of calcium to phosphorus in bone hydroxyapatite. On placebo, the mass ratio of the mean calcium balance to mean phosphorus balance is 1.08 and on calcium supplement is 2.47, which do not agree with the hydroxyapatite 2.15 ratio for placebo, but are close for the calcium supplement. However, apart from two apparent outliers (one with very high calcium balance supplement but with no change in phosphorus balance, and one with very negative phosphorus balance on the calcium supplement) the individual subjects demonstrate that they do follow a slope similar to the expected ratio line (**Figure 5**) although the variation is high and some individuals appear to be higher or lower mineral retainers. This variation probably represents cumulative errors in the balance technique and differences in retention from natural variations in adolescent bone and soft tissue growth rates. It probably also reflects the fact that calcium phosphate is initially deposited in bone with a wide calcium to phosphate ratios (18).

The observation in our study of an underestimation of dietary phosphorus by approximately 40% in mixed meals is consistent with other studies which have found underestimation of phosphorus content in nutrient databases spanning a wide range from ~15-70% (19-24). Recently, Carrigan et al. (21) designed four-day menus to be low or high in phosphorus additives based on the absence or presence of phosphorus additives on food label ingredient lists, then analyzed these diets for phosphorus content and compared measured values with the estimated values from NDSR software and reported that NDSR underestimated phosphorus in these diets by ~14%. Other studies have evaluated the accuracy of nutrient database values for various meat products that list phosphate additives in the ingredients compared with those that do not. Sullivan et al. (22) analyzed the phosphorus content of 38

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chicken products and found on average a 43% underestimation compared to the expected values. Benini et al (23) measured an average ~70% more phosphorus than estimated in ham, roast breast turkey, and roast breast chicken products from Italy. In our study, foods with phosphate additives listed on the ingredient label were matched as closely as possible to items in NDSR that also listed additive phosphate as an ingredient. It is clear that this method is insufficient to accurately estimate phosphorus content in mixed meals. To illustrate the potential impact of this error, we substituted the estimated dietary phosphorus from the nutrient database analysis in the balance calculations in this study. Doing so resulted in calculations of phosphorus balance of -170 mg/d and -163 mg/d for the calcium carbonate and placebo periods, respectively. Without direct chemical analysis of the phosphorus content of the foods used in our balance studies, we would have erroneously concluded that our subjects were in negative phosphorus balance on both placebo and calcium supplement conditions. This underscores the importance of chemical analysis of diet composites in phosphorus balance studies.

This crossover balance study demonstrates that adolescent girls are in positive phosphorus balance of an average 200 mg/d on a diet of ~800 mg/d calcium and ~1400 mg/d phosphorus. When calcium intake is increased from ~800 mg/d to ~1400 mg/d with a calcium carbonate supplement, phosphorus balance is unchanged despite a more positive calcium balance. Increasing dietary calcium levels within a normal dietary range (from typical intake level to around the RDA level) does not negatively impact phosphorus balance when phosphorus intake is at a level typically consumed in the U.S. In addition, we confirm the need to improve estimations of phosphorus in foods in nutrient databases.

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Acknowledgements

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Figure Legends

Figure 1. Randomized crossover study design. Twelve participants enrolled in the study. *1 participant was randomized to the placebo-calcium sequence but did not start until the 2nd phase, so did not receive the placebo; 1 participant randomized to placebo-calcium sequence did not complete the 2nd phase of the crossover; 1 participant randomized to the placebo-calcium sequence is excluded from the present analysis due to insufficient stored fecal sample. Thus, n = 11 are included in this analysis.

Figure 2. Phosphorus balance in healthy adolescent girls on placebo versus calcium carbonate.

Figure 3. A) Net phosphorus absorption in healthy adolescent girls on placebo versus calcium carbonate. **B)** Net calcium absorption in healthy adolescent girls on placebo versus calcium carbonate (n=11), data from Elble 2011 (9).

Figure 4. Calcium balance in healthy adolescent girls on placebo versus calcium carbonate (n=11). Data from Elble 2011 (9).

Figure 5. Phosphorus and calcium balance in individual participants on placebo and calcium supplement. Ca:P mass ratio is given above each point, calculated from 99% Ca balance and 85% P balance. For comparison, the expected 2.15:1 Ca:P mass ratio of bone hydroxyapatite is shown by the dashed line.

Tables

Table 1. Baseline participant characteristics

White/Asian, n	10/1
Hispanic/Other, n	1/10
Age, years	13.5 ± 0.98 (11.3-14.6)
Height for age (percentile)	48.9 ± 29.1 (6.9-97.9)
Weight for age (percentile)	60.1 ± 32.8 (2.9-98.9)
BMI (kg/m^2)	21.3 ± 2.8 (19.4-27.2)
BMI for age (percentile)	62.3 ± 34.2 (6.2-97.1)
Tanner stage, Breast (n for stages 1-5)*	0/2/3/2/2

Mean ± SD. *Data missing for 2 participants

Table 2. Estimated and measured dietary phosphorus and calcium

	Estimated Phosphorus (mg)	Measured Phosphorus (mg)	Estimated Calcium (mg)	Measured Calcium (mg)
Diet with Placebo	1031 ± 5.6	1435 ± 23.5**	784 ± 0.05	817 ± 19.5
Diet with Calcium Carbonate	1037 ± 7.4	1453 ± 28.0**	1384 ± 0.07#	1418 ± 11.1*#

Mean $\pm$ SEM. *$p < 0.05$ measured calcium versus estimated calcium; ** $p < 0.001$ measured phosphorus versus estimated phosphorus; # $p < 0.001$ diet with placebo versus calcium carbonate.

Table 3. Hormone and bone metabolism markers on placebo and calcium supplement

	Placebo	Calcium	*P* value
Serum Ca (mmol/L*)	2.275 (0.02)	2.28 (0.02)	0.764
Serum P (mmol/L*)	1.49 (0.05)	1.42 (0.05)	0.208
25OHD (nmol/L)	62.47 (4.7)	69.09 (5.0)	0.256
1,25(OH)$_2$D$_3$ (pmol/L)	131.45 (8.9)	122.86 (8.9)	0.197
PTH (pmol/L)	2.55 (0.29)	2.40 (0.31)	0.723
Serum Osteocalcin (ug/L)	21.71 (3.6)	24.58 (3.7)	0.494
Serum BAP (ug/L)	73.39 (10.4)	81.25 (10.5)	0.120
Serum ALP (ukat/L)	2.86 (0.40)	2.87 (0.40)	0.958
Urinary NTx (nmol BCE)	3572.54 (1089.4)	4939.19 (1124.9)	0.290
Urinary free-DPD (nmol)	218.17 (35.6)	273.23 (36.8)	0.225

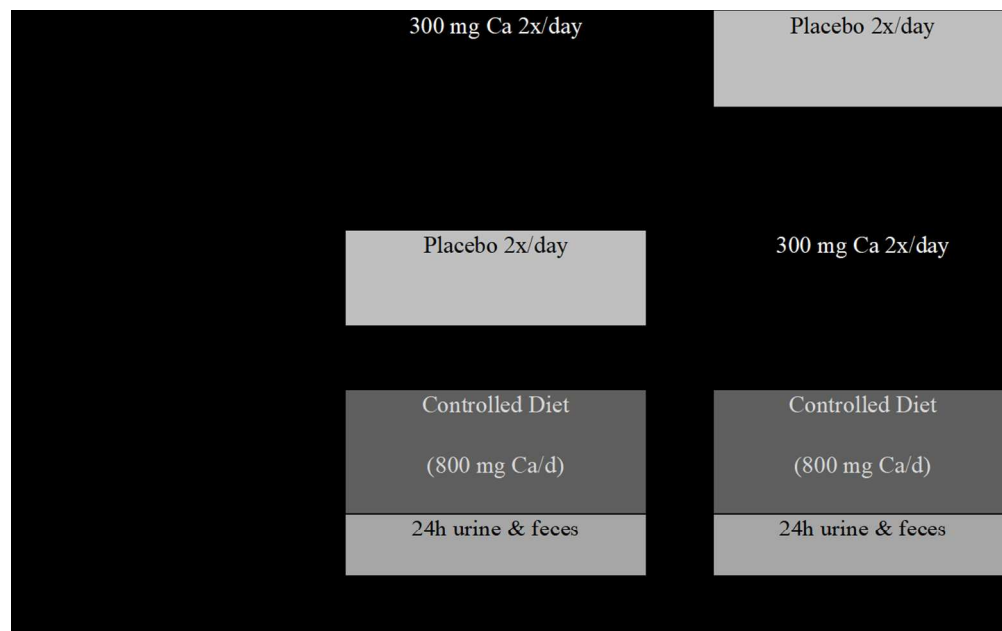Values are presented as Mean (SEM). *To convert to mg/dL, divide Ca by 0.25 and P by 0.323.

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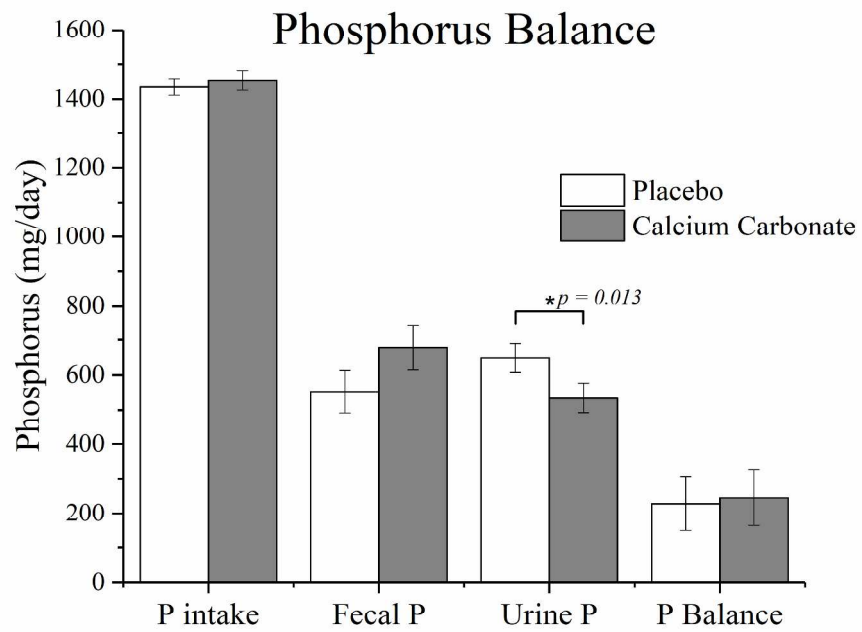
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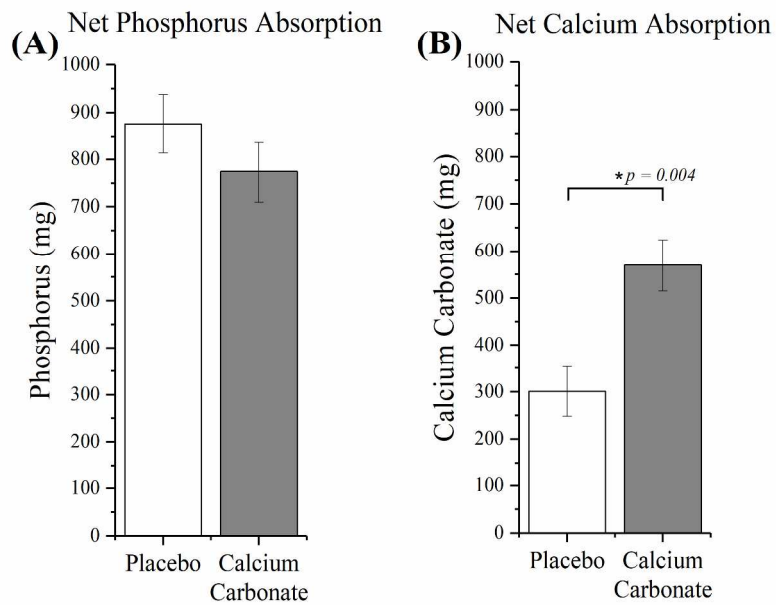
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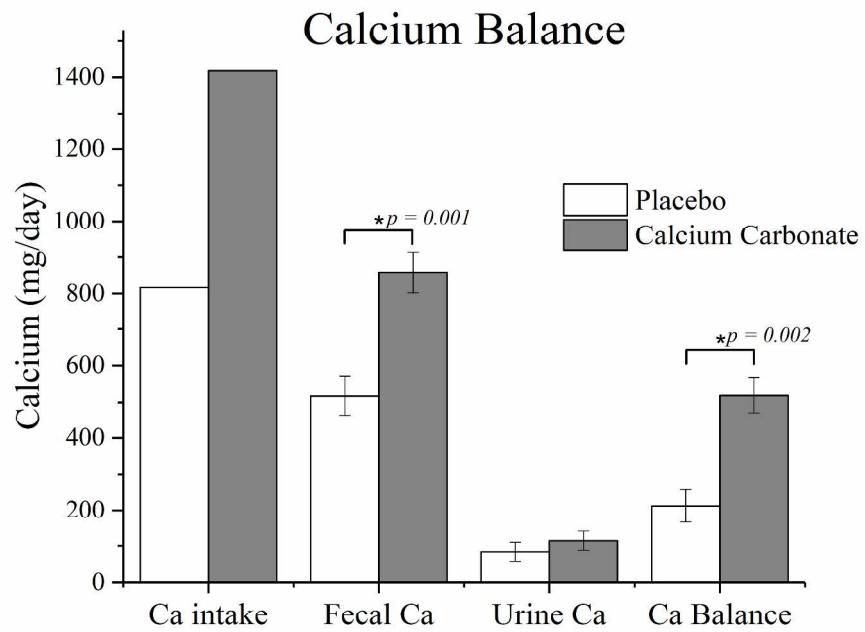
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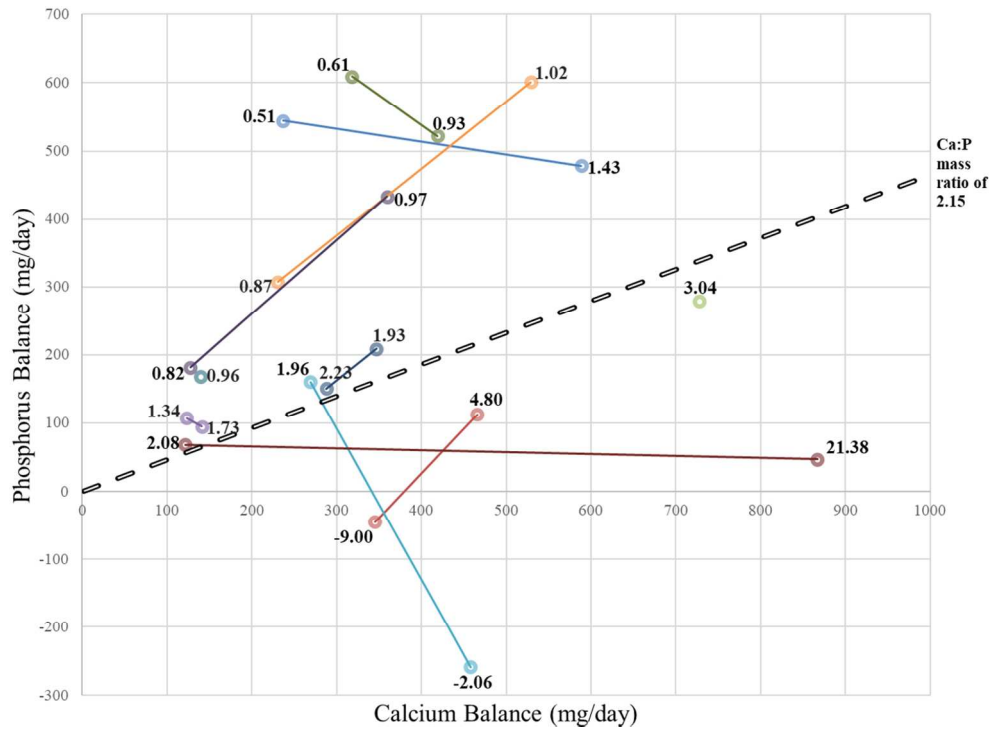
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