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ORIGINAL CONTRIBUTION

Mono- and polyphosphates have similar effects on calcium and phosphorus metabolism in healthy young women

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Abstract
Purpose Phosphate (Pi) salts, often mono- (MP) or polyphosphates (PP), are commonly used as additives in the food industry. Previous studies have shown that the effects of MP and PP on calcium (Ca) and phosphorus (P) metabolism may differ. The aim of this study was to determine whether the effects of MP and PP salts differ on markers of Ca and P metabolism in young women.
Methods Fourteen healthy women 19–31 years of age were randomized into three controlled 24-h study sessions, each subject serving as her own control. During each session, the subjects received three doses of MP, PP or a placebo with meals in randomized order. Both Pi salts provided 1,500 mg P/d, and the diet during each session was identical. Markers of Ca and P metabolism were followed six times over 24 h.
Results During both MP and PP sessions, we found an increase in serum phosphate (S-Pi, $p = 0.0001$), urinary phosphate (U-Pi, $p = 0.0001$) and serum parathyroid hormone (S-PTH, $p = 0.048$ MP, $p = 0.012$ PP) relative to the control session. PP decreased U-Ca more than did MP ($p = 0.014$).
Conclusions The results suggest that PP binds Ca in the intestine more than does MP. Based on the S-Pi, U-Pi and S-PTH results, both Pi salts are absorbed with equal efficiency. In the long run, increased S-PTH, caused by either an MP or PP salt, could have negative effects on bone metabolism.

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Introduction

Chronic renal disease leads to impaired phosphate (Pi) excretion and hyperphosphataemia, which is associated with progression of secondary hyperparathyroidism, renal osteodystrophy [1], and increased mortality and morbidity in dialysis patients [2, 3]. Therefore, dietary P restriction is an essential part of the treatment in renal patients [4]. In addition, high intake of P may have negative effects on bone metabolism [5, 6] and cardiovascular health [7–9], even in healthy individuals. However, according to European Food Safety Authority (EFSA), P-induced adverse effects on bone have not been observed in healthy humans [10]. Dietary P is well absorbed from the intestine, and it increases serum phosphate (S-Pi) and serum parathyroid hormone (S-PTH) concentration within hours [5, 6].

In Western countries, P intake exceeds dietary recommendations 2- to 3-fold [11–14]. In addition to several natural sources of P (especially dairy products, meats, whole grains, nuts and egg), the use of P additives in the food industry is common and further increases P intakes [15, 16]. P from inorganic additives is absorbed almost completely [17] and may have effects that differ from those of natural P [18, 19]. It has been estimated that in the USA, P additives may add as much as 1 g of P to the diet, depending on food choices [17]. Based on a recent report, a haemodialysis patient in Europe approaches at least 100–300 mg of extra P from additives [20]. The use of P additives makes it difficult to estimate the P intakes of individual patients and of the general public, since the increased amounts of P in foods as additives are not properly known.

Several Pi salts are used as additives, based on their technical properties [15]. P additives are added to foods for several technical reasons, for example, to retain moisture, enhance colour, improve shelf-life or to emulsify. Various compounds are used in different food groups. Based on food labels, sodium polyphosphates at least in Finland are often used in meats and meat products, and sodium monophosphates in processed cheeses [21]. Still, the use of P compounds in different food groups may be more complex in other countries [15].

Previous studies have shown differences between the effects of MP and PP salts on Ca metabolism and kidney function. In rats fed a diet containing PP, development of nephrocalcinosis was more severe than in rats fed an MP diet, despite equal amounts of P intake in both diets [22, 23]. The only study in humans comparing the effects of MP and PP was an early experimental study in young men [24]. In this study, only the PP supplement decreased Ca absorption, but MP ingestion led to higher urinary excretion of cAMP than did PP ingestion, indicating increased PTH excretion in the MP diet [24]. However, a direct method for measuring S-PTH concentration was not available at the time the study was conducted. Furthermore, to our knowledge, no studies comparing the effects of different Pi compounds on female subjects have been undertaken. Therefore, since the use of Pi additives is common and data regarding the absorbability and effects of different Pi compounds on Ca metabolism is lacking, we compared the effects of MP and PP supplements on Ca and P metabolism in healthy young females. We hypothesized that the PP supplement would decrease Ca absorption, and consequently, increase the S-PTH concentration more than would MP supplements.

Subjects and methods

Fourteen healthy female volunteers, 19–31 years of age, participated in three separate 24-h sessions, with each subject serving as her own control. The sessions were attended at 7-day intervals in randomized order. The subjects had no medications or illnesses known to affect Ca or bone metabolism. Seven of the subjects used oral contraceptives. The basic characteristics of the participants are shown in Table 1. For estimation of habitual Ca, P and energy intakes, the volunteers kept a 4-day food record before the experiment.

Before presenting at the research unit, the subjects fasted overnight. During each session, the subjects ingested either an MP, PP or placebo supplement during three meals. The MP supplement contained sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}2H_2O$, E339, Merck Eurolab, Germany) and the PP sodium tripolyphosphate ($Na_5P_3O_{10}$, E452, Six Oy, Finland). The Pi and placebo drinks were divided into three doses and served at 08.00, 12.00 and 16.00 hours with meals. The Pi supplements contained 1,500 mg/d of P, each dose containing 500 mg of P. The meals served during each study session were identical, providing 340 mg of Ca and 500 mg of P. The P dose of 1,500 mg/d was chosen, based on previous short-term studies, in which we detected an effect on S-PTH concentration with a similar P dose [5, 6]. Furthermore, a P dose of 2,000 mg (the supplement + diet) is easily achieved in a normal diet, and closely corresponds to the average P intake in men [12].

Table 1 Basic characteristics of subjects ($n = 14$)

Variable	Mean	SD
Age (years)	23	4.4
Height (cm)	165	5.5
Weight (kg)	59	5.8
BMI (kg/m^2)	21.7	1.6
Habitual energy intake (MJ/d)	7.3	1.2
Habitual dietary P intake (mg/d)	1,580	415
Habitual dietary Ca intake (mg/d)	1,040	320

Since the Pi supplements also contained Na, the Na intakes were higher during the MP and PP sessions than during the placebo session. The total intake of Na (diet + supplements) during the placebo session was 1.4 g, during the MP session 2.5 g, and during the PP session 3.2 g. No additional meals or snacks were allowed, but tap water was provided ad libitum. The meals were served at 08.00, 12.00, 14.00, 16.00 and 20.00 hours. The intakes of selected nutrients during the study sessions are presented in Table 2.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Ethics Committee of the Faculty of Agriculture and Forestry. Written informed consent was obtained from all subjects prior to their inclusion in the study.

Sampling

Blood samples were taken anaerobically at 08.00, 12.00, 14.00, 16.00 and 18.00 hours, and again at 08.00 hours on

Table 2 Dietary intakes of selected nutrients from meals during the study sessions

Variable	Intake
Energy (kJ)	8,100
Calcium (mg)	340
Phosphorus (mg)	500
Sodium (mg)	1,370
Fibre (g)	20

the following morning. The blood samples were taken immediately before meals, except at 18.00 hours, when they were taken immediately after the meal for practical reasons. Serum was separated from the blood and stored at −20 °C until analysed. The 24-h urine collections were obtained on the study day from 08.00 to 08.00 hours the following morning. The urine and separated serum samples were stored at −20 °C until analysis.

Laboratory methods

The serum ionized calcium (S-iCa) concentration was analysed from anaerobically handled serum samples with an ion selective analyser (Microlyte 6; Thermo Electron Oy, Vantaa, Finland) within 90 min of sample collection. The intra-assay CV% for S-iCa was 1.6. In the other measurements, all samples from the same person were analysed in the same assay in randomized order. The serum phosphate (S-Pi) and creatinine (S-Crea) concentrations and excretions of urinary calcium (U-Ca), phosphate (U-Pi) and creatinine (U-Crea) were measured spectrometrically with an autoanalyser (Konelab 20, Thermo Electron). The intra- and interassay CV% of these analyses were 1.7 and 2.4, respectively. The serum intact PTH concentration was measured by radioimmunoanalysis, using Allegro intact PTH Assay Kits (Nichols Institute, San Juan Capistrano, CA, USA). The intra- and interassay CV% were 2.9 and 5.1, respectively.

Statistical analysis

The data are expressed as mean ± SEM (standard error of the mean). For serum variables, the area under the curve (AUC) for the difference from the morning fasting value was calculated. AUC is a summary measure commonly used in analysing data in serial measurements [25]. To exclude the effect of the first fasting sample, the deltas of the 0-h sample to the 24-h sample were used in the analyses. The original formula for calculating AUC can be found in, that is [25]. The modified formula used in the present study to calculate AUC using the deltas of the first fasting sample (0 h) to the following samples is as follows:

$$\mathrm{AUC} = \frac{1}{2}\sum_{i=0}^{k-1}(t_{i+1} - t_i)(y_i + y_{i+1} - 2y_0)$$

k = number of samples, t = sampling time, y_i = value.

The results of U-Ca and U-Pi were corrected for U-Crea excretion before the statistical analyses. Non-normal distributions were normalized by logarithmic transformations. Analysis of variance (ANOVA) with repeated measures was used to compare the study sessions. If the sphericity assumption was violated, a Huynh–Feldt adjustment was applied. Contrast analysis was used for pairwise comparisons between study sessions. Analyses were conducted with PASW Statistics 17.0.2 (SPSS Inc., Chicago, IL, USA) in a Windows environment. We regarded $p < 0.10$ as a trend and $p < 0.05$ as a statistically significant difference.

Results

The S-Pi concentrations differed among the study sessions ($p = 0.0001$, ANOVA) (Fig. 1). Both MP ($p = 0.0001$) and PP ($p = 0.0001$) increased S-Pi compared with the control session. The difference between the MP and PP sessions in S-Pi was not statistically significant ($p = 0.18$). Differences were found in the U-Pi excretion among the study sessions ($p = 0.0001$, ANOVA) (Fig. 1). Both MP and PP increased U-Pi, compared with the control session ($p = 0.0001$, both). No differences between the MP and PP sessions in U-Pi excretion were found ($p = 0.33$).

The S-iCa concentrations did not differ among study sessions ($p = 0.29$, ANOVA) (Fig. 2). The U-Ca excretion differed between the study sessions ($p = 0.001$, ANOVA) (Fig. 2). Only PP decreased the U-Ca in a statistically significant manner ($p = 0.002$). However, the U-Ca excretion tended to decrease in the MP session relative to the control session ($p = 0.069$). The PP decreased the U-Ca more than did the MP ($p = 0.014$).

The S-PTH concentrations differed between the study sessions ($p = 0.019$, ANOVA) (Fig. 3). Both MP ($p = 0.048$) and PP ($p = 0.012$) increased the S-PTH compared with the control session. The increase in S-PTH was equal with both Pi salts ($p = 0.74$). None of the subjects reported gastrointestinal symptoms due to the P supplements during the study sessions.

Discussion

In this short-term study, both Pi salts, commonly used in the food industry, affected Pi metabolism (S-Pi, U-Pi and S-PTH) in a similar manner and were absorbed equally well. The only statistically significant difference between the MP and PP sessions was found in U-Ca, which PP decreased more than did MP. As a consequence of the increased level of S-PTH in both Pi sessions, a decrease in U-Ca in both sessions would have been expected. We consider it likely that MP ingestion also decreases U-Ca, even though in this study the decrease relative to the control session reached only the level of a trend. The supplements did not affect S-iCa, even though in our previous intervention studies similar amounts of P decreased S-iCa [5, 6].

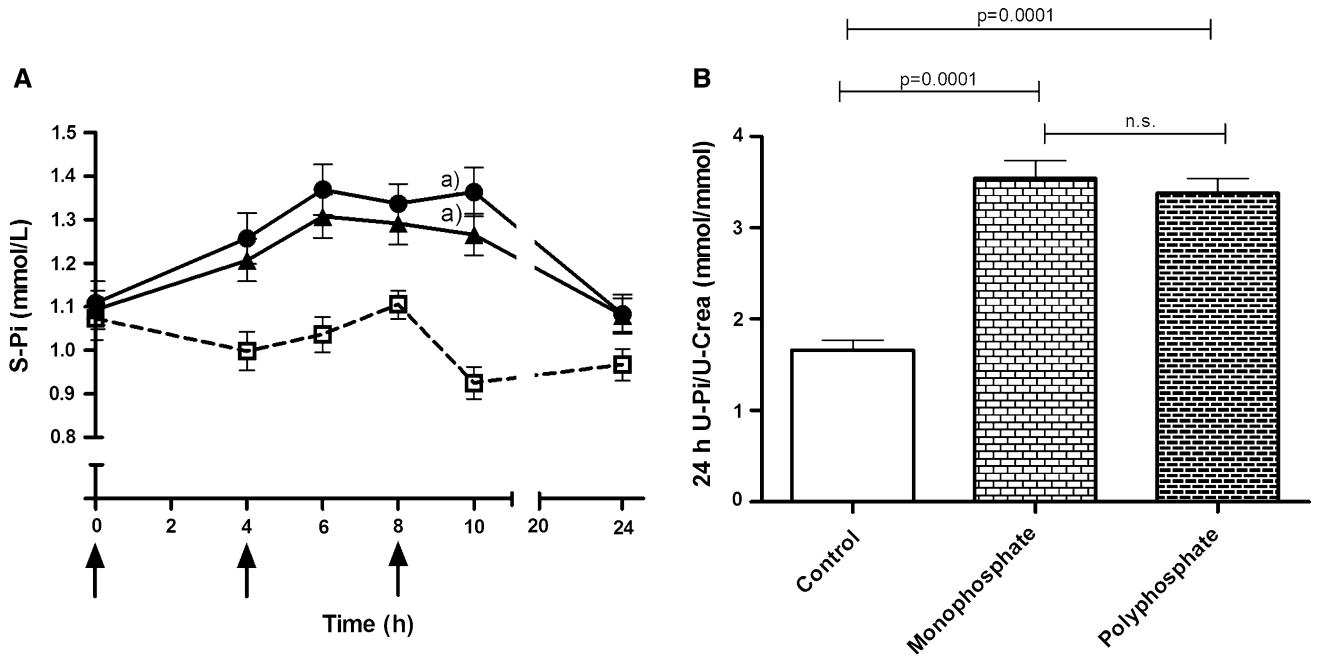

Fig. 1 Changes in serum phosphate (S-Pi) concentration (**a**) and 24-h urinary excretion of phosphate (U-Pi/Crea) (**b**) during the three study sessions. **a** control (*empty square*), monophosphate (*filled circle*), polyphosphate (*filled triangle*). The supplement administration times are indicated with an *arrow*. The supplements affected the area under the curve values of S-Pi (ANOVA, $p = 0.0001$) and 24-h U-Pi/Crea (ANOVA, $p = 0.0001$); [a]significantly different from control session

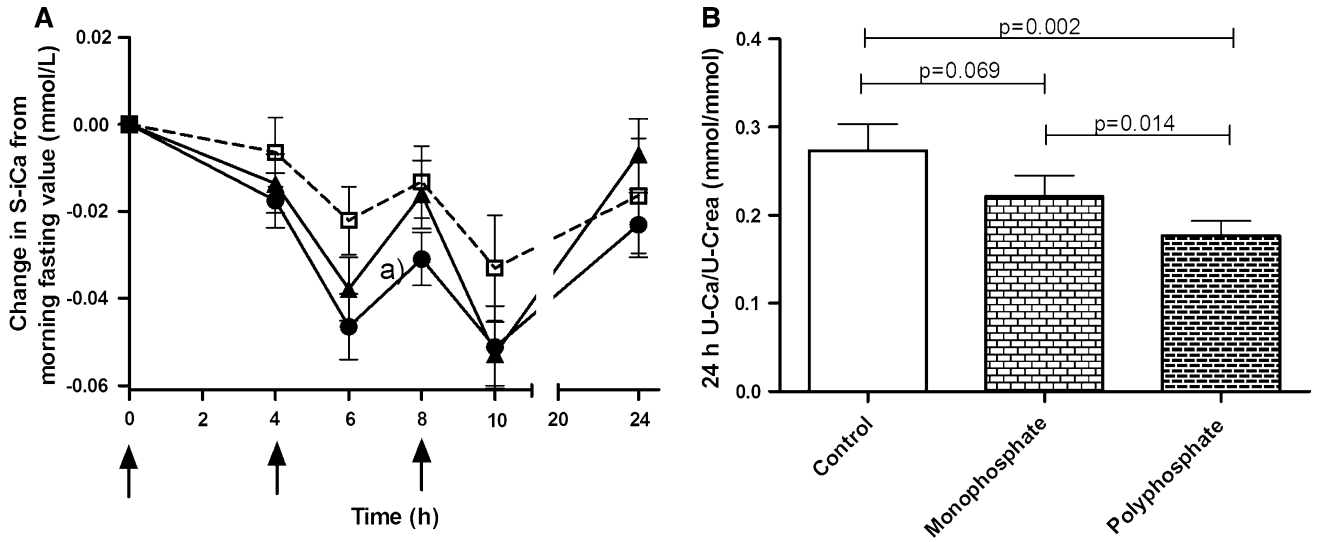

Fig. 2 Changes in serum ionized calcium (S-iCa) concentration (**a**) and 24-h urinary excretion of calcium (U-Ca/Crea) (**b**) during the three study sessions. **a** Control (*empty square*), monophosphate (*filled circle*), polyphosphate (*filled triangle*). The supplement administration times are indicated with an *arrow*. The supplements did not affect the area under the curve values of S-iCa (ANOVA, $p = 0.29$), but affected the 24-h U-Ca/Crea (ANOVA, $p = 0.001$); [a]significantly different from control session

The larger decrease in U-Ca in the PP session than in the MP session was likely due to PP binding more Ca in the intestine than did MP [26]; hence, less Ca was absorbed in the PP session than in the MP session. To verify this conclusion, faecal collections would have been necessary. However, also in the study of Zemel and Linkswiler in young men [24], the authors concluded that the greater Ca loss at the PP diet was due to a decrease in Ca absorption. In that study, no difference in U-Ca excretion between the MP and PP diets was observed, which is in contrast to our results. Based on differences in cAMP excretion (indicating differences in PTH secretion), the differences in U-Ca would have been expected, since Ca intake was the same during both diets. In the study of Zemel and Linkswiler

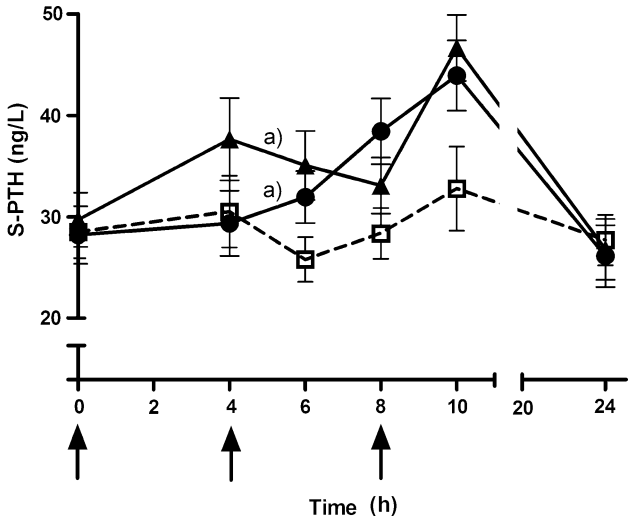

Fig. 3 Changes in serum parathyroid hormone (S-PTH) concentration during the three study sessions. Control (*empty square*), monophosphate (*filled circle*), polyphosphate (*filled triangle*). The supplement administration times are indicated with an *arrow*. The supplements affected the area under the curve values of S-PTH (ANOVA, $p = 0.019$); [a]significantly different from control session

[24], potassium (K) intakes differed between the MP and PP diets, because the MP salt was a monobasic potassium phosphate, and the PP salt a sodium phosphate. The difference in K intake may explain the U-Ca results in that study, since K increases the retention of Ca [27].

Both Pi salts increased S-PTH, which is in line with our previous intervention studies conducted with P additives [5, 6]. Hence, both MP and PP affect PTH secretion similarly, even though previously MP increased U-cAMP more than did PP [24]. Since cAMP excretion is an indirect measure of PTH secretion, we consider our results more accurate. The rise in S-PTH may partly have been due to the Ca-binding capacity of Pi, especially PP. In the present study, however, we were unable to detect a difference in PTH secretion between the MP and PP sessions. It is possible that more samples would have been required to detect all the differences in the serum measurements. As no samples were taken between 0–4 and 10–24 h, we do not know what happened in S-PTH, S-Pi and S-iCa during these periods. With more frequent sampling we also would have seen the diurnal variation of serum measurements in more detail.

If PTH secretion increased more in the PP session than in the MP session, a larger decrease in U-Ca in the PP session would have been expected, because PTH decreases Ca excretion. In U-Pi, a higher level of excretion would have been expected in the PP session if the S-PTH increased more during the PP than the MP session. However, if MP were absorbed in the gut slightly more efficiently than PP, this difference in U-Pi could have disappeared. To summarize, two mechanisms may have contributed to the difference in U-Ca between the MP and PP sessions: Reduced intestinal Ca absorption itself and possibly also increased PTH secretion.

Na intake may also increase PTH secretion by increasing Ca excretion [28]. Na intake may increase urinary calcium excretion, which could lead to a need for maintaining S-Ca by an increase in PTH secretion. We previously showed that a dose of 1.4 g Na as NaCl does not affect PTH secretion [5]. In this study, the difference in Na intakes was highest (1.8 g) between the PP and control sessions, the differences between the control and MP sessions and the MP and PP sessions being 1.1 and 0.7 g, respectively. Theoretically, Na intake could have contributed to the S-PTH increase in the PP session. In that case, higher Ca excretion would have been expected at the PP session. However, since Ca excretion was lowest in the PP session, we consider it unlikely that the difference in Na intakes would have explained the rise in S-PTH in the Pi sessions.

The use of sodium phosphates in the food industry increases both the P and Na contents of the product, but since the amounts of Pi added (and possibly other Na-containing additives) are not required to be listed on food labels, the contents of these nutrients are unknown. Based on the present study, ingestion of PP-containing meat products could more likely interfere with Ca balance than MP-containing processed cheese not only due to the higher Ca content of cheese products but to the Ca-binding property of the PP compound.

Based on this short-term intervention study, we conclude that MP and PP have similar effects on Ca and P metabolism. Both MP and PP are absorbed efficiently and increase S-PTH, which may have a negative effect on bone. PP may bind Ca in the intestine more than does MP. Our results await confirmation in a longer-term setting.

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Conflict of interest None of the authors have conflicts of interest.

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