Effect of Dietary Calcium and Phosphorus Levels on the Utilization of Iron, Copper, and Zinc by Adult Males$^{1,2}$

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ABSTRACT Iron, copper, and zinc utilization were examined in nine adult males fed a moderate calcium-moderate phosphorus diet (MCDP), a moderate calcium-high phosphorus diet (MCaHP), and a high calcium-high phosphorus diet (HCaHP) during a 39-day balance study. The moderate and high calcium diets contained 780 mg and 2382 mg calcium daily, respectively. The moderate and high phosphorus diets contained 843 and 2442 mg phosphorus daily, respectively. The calcium supplements were fed as calcium gluconate, while the phosphorus supplements were fed as glycerol phosphate. Subjects lost more iron and copper in their feces and apparently retained less iron and copper when fed the HCaHP diet than when fed the other two diets, but these effects were not statistically significant. Urinary iron and copper excretion were significantly affected by the dietary treatments. Dietary treatments had no effect on subjects' fecal and urinary losses of zinc nor on their apparent retention of zinc. Plasma iron, zinc, copper, and transferrin levels and serum ferritin levels were not affected by the dietary treatments. J. Nutr. 112: 136-143, 1982.

INDEXING KEY WORDS: dietary calcium • dietary phosphorus • zinc • copper • iron • human balance study

Many investigators have studied the effects of various forms of dietary phosphorus and calcium on the utilization of trace elements, particularly iron and zinc, in a variety of species. The results of these studies have not been consistent. The variable results may be attributed to differences in the dietary forms of phosphorus, orthophosphates versus polyphosphates, and inorganic salts versus organic complexes, (1); the solubility of phosphorus and calcium salts (2, 3); the method of incorporating calcium and phosphorus supplements into the diets (4); the level and ratio of dietary calcium and phosphorus (2, 5-8); the levels of dietary iron and zinc (2, 7, 9-11); the levels of other dietary factors which may have affected iron and zinc absorption (12-14); the species and physiological state of the test animals (2, 8, 15, 16); and the methods used to assess iron and zinc status (15, 17-19).

A few investigators have studied the effects of dietary calcium and phosphorus levels on the utilization of iron and zinc by human subjects. Peters et al. (13) and Monsen and Cook (20) observed the effects of phosphate salts on the absorption of $^{56}$Fe. Pécout et al. (21) studied the response of serum zinc levels to 50 mg doses of zinc with and without supplements of phosphorus. Spencer et al. (22) reported the effects of phosphorus supplements on the retention of copper and zinc by one subject, and the effects of calcium supplements on the retention of copper, zinc, and $^{65}$Zn by several subjects (22, 23).

The main purposes of this study were to:

$a)$ determine the effects of varying dietary phosphorus levels alone and in combination with varying dietary calcium levels on iron,

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copper and zinc metabolism in human subjects and b) to assess the effects of varying the ratio of P:Ca in the diets (1:1 to 3:1) on iron, copper, and zinc metabolism in human subjects.

METHODS

Experimental design. The 39-day metabolic study consisted of a three-day orientation period followed by three 12-day experimental periods. The dietary treatments were a moderate calcium-moderate phosphorus diet (MCaMP), a moderate calcium-high phosphorus diet (MCaHP) and a high calcium-high phosphorus diet (HCaHP). The moderate and high calcium diets contained 780 mg and 2382 mg calcium, daily, respectively. The moderate and high phosphorus diets contained 843 mg and 2442 mg phosphorus, daily, respectively.

Three subjects were randomly assigned to each of the three treatments during each period. Subjects consumed the diets in random order. They consumed the same diet during the initial 3-day orientation period as they consumed during the first experimental period.

Subjects. Nine male college students were chosen as subjects after extensive interviews by the investigators. Subjects were free of any known metabolic disorders as determined by medical history and by examination by a physician prior to the study. Their mean age was 23.6 ± 2.5 (SD) years; their mean height was 179 ± 6 cm; their mean weight was 72 ± 10 kg.

Subjects continued their normal daily routine during the study but refrained from any unusual physical activity. Subjects were required to eat all food provided. They consumed their meals at the metabolic facility during certain supervised time periods while one of the investigators was present. They were also required to weight themselves daily before breakfast and to collect all excreta in designated containers.

Subjects gave informed consent in a manner approved by a University of Wisconsin committee on the use of human subjects.

Diet. The foods included daily in the two menus (A and B), were: 280 g lemon carbonated beverage; 250 g lemonade made from frozen concentrate; 200 g orange juice made from frozen concentrate in menu A or 200 g canned apply juice in menu B; 150 g Italian bread; 126 g fried apple turnover in menu A or 126 g fried cherry turnover in menu B; 100 g canned applesauce in menu A or 100 g bottled grapefruit sections in menu B; 100 g (10% fat) ground beef; 100 g canned tomato juice; 100 g frozen wax beans in menu A or 100 g frozen green beans in menu B; 100 g canned pineapple chunks; 100 g frozen blueberries in menu A or 100 g canned pears in menu B; 70 g margarine; 60 g pound cake; 60 g half-and-half cream; 56 g jelly; 35 g potato chips in menu A or 45 g pretzels in menu B; 40 g dry enriched rice; 30 g frozen onions; 30 g frozen carrots; 30 g frozen celery; 30 g dehydrated potato flakes; 30 g canned cheddar cheese soup in menu A or 30 g canned cream of mushroom soup in menu B; 2.0 g salt and 0.4 g pepper. The weight of each portion of food, except salt and pepper, was accurate to the nearest 0.1 g. The weight of the portions of salt and pepper varied less than ±5%.

Foods were purchased in case lots to ensure uniformity. Deionized water was used for cooking drinking. All foods were stored, prepared and served with plastic and paper utensils and containers to help prevent trace element contamination.

The diet, which was calculated to provide about 3050 kilocalories daily, was supplemented with margarine, sugar, and candy as necessary to maintain subjects' weights. The diets were calculated to contain about 58 g of protein daily and 100% of the Recommended Dietary Allowances (RDA) for iron, thiamine, niacin, and vitamins A and C and 83% of the RDA for riboflavin for males 19 to 22 years of age (24).

The diet was supplemented daily with 400 IU of vitamin D. Zinc level in this basal diet was low, thus 2.0 mg of zinc as zinc sulphate in solution was given at the noon meal. Tablets which contained 27 mg of magnesium gluconate were administered to subjects at each meal.

Subjects were given calcium gluconate in solution in equal portions with each meal to achieve the desired level of calcium in the diets. Subjects were given glycerol phosphate
in gelatin capsules with each meal as a part of the MCaHP and HCaHP treatments. Subjects were given an equal number of empty gelatin capsules with each meal as a part of the MCaMP treatment. Composites of a total day’s food for both menus were prepared three times prior to the study and once during each experimental period. The actual analyzed mineral contents of the menus are shown in Table 1. Besides the food composites, all mineral, vitamin, and caloric supplements were analyzed for their mineral content and the average mineral intakes of subjects during each dietary treatment were calculated.

Sample collection. All fecal, urine and food samples were collected and stored in acid washed containers. Details concerning procedures to prevent trace element contamination have been reported elsewhere (12).

Brilliant blue dye was administered as a fecal marker before breakfast on days five and eleven of each period and feces were composited accordingly. A recent report indicates that the movement of brilliant blue and opaque pellets through the gut are correlated (25). Although brilliant blue is not a perfect marker, it is free of zinc, copper, and iron contamination (analyses performed in our laboratory) and gives a convenient indication of gut transit of some dietary components.

Daily urine composites were made from 24-hour urine collections for each subject, and samples were acidified with hydrochloric acid (2 mls HCl per 100 mls urine). Six-day urine composites for days 5 through 10 of each experimental period were prepared from the daily composites and frozen. Venous blood samples were collected from subjects after an overnight fast twice during each experimental period with Vacutainer tubes designed for use in zinc analysis (Becton-Dickinson, Rutherford, NJ 07070).

Analyses. Food and fecal composites were homogenized in acid-rinsed stainless steel blenders. Aliquots of food and fecal samples were ashed as described by Hegsted et al. (26) and analyzed for iron, zinc and copper content by atomic absorption spectrophotometry (Model 372, Perkin Elmer, Norwalk, CN 06856). Bovine liver standard, which was obtained from the National Bureau of Standards, was ashed in triplicate and analyzed in the same manner as the food and fecal samples. Liver samples were determined to contain 131 ± 5 μg zinc/g tissue, 188 ± 2 μg copper/g tissue and 261 ± 3 μg iron/g tissue. The certified values for zinc, copper and iron were 130 μg, 193 μg and 270 μg, respectively.

The zinc content of the six-day urine composites were determined directly by atomic absorption spectrophotometry. The recovery of added zinc to the urine samples ranged from 97% to 99%. Copper and iron content of urine samples were determined with atomic absorption spectrophotometry by standard additions to samples which were wet ashed with nitric acid, concentrated, and brought to a known volume with deionized water.

Creatinine content of fresh urine samples was determined daily using an alkaline pi-

### Table 1

**Mineral composition of the diets**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Menu A</th>
<th>Menu B</th>
<th>MCaMP</th>
<th>MCaHP</th>
<th>HCaHP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg)</td>
<td>(mg)</td>
<td>(mg)</td>
<td>(mg)</td>
<td>(mg)</td>
</tr>
<tr>
<td>Calcium</td>
<td>301 ± 12</td>
<td>366 ± 13</td>
<td>780</td>
<td>780</td>
<td>2382</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>853 ± 11</td>
<td>836 ± 32</td>
<td>843</td>
<td>2442</td>
<td>2443</td>
</tr>
<tr>
<td>Iron</td>
<td>18.54 ± 0.50</td>
<td>18.92 ± 0.67</td>
<td>17.40</td>
<td>17.51</td>
<td>17.65</td>
</tr>
<tr>
<td>Copper</td>
<td>1.32 ± 0.15</td>
<td>1.42 ± 0.13</td>
<td>1.39</td>
<td>1.40</td>
<td>1.50</td>
</tr>
<tr>
<td>Zinc</td>
<td>8.08 ± 0.48</td>
<td>8.43 ± 0.31</td>
<td>10.41</td>
<td>10.43</td>
<td>10.44</td>
</tr>
</tbody>
</table>

† Mineral content of diet composites. Means ± SD (n = 6).

‡ Average mineral intake of subjects from basal diet and from caloric, mineral and vitamin supplements.
cbrate method (27). The calcium and phosphorus content of food composites and all supplements were determined on ashed samples with atomic absorption spectrophotometry and with the colorometric procedure described by Fiske and Subbarow (28), respectively.

Plasma zinc, iron, and copper levels were determined on samples diluted 3-fold with deionized water and analyzed with atomic absorption spectrophotometry. Glycerol was added to standards to equilibrate flow rates.

Plasma transferrin was determined on serum samples by Mancini's et al. (29) single radial immunodiffusion method using prepared plates (M-partigen® Behring Diagnostics, American Hoechst Corporation, Somerville, NJ 08876). One serum sample was analyzed on six different plates. The coefficient of variation was 3.3%. Serum ferritin was assayed by a double-antibody radioimmunoassay (Gamma Dab 125I Ferritin Radioimmunoassay kit, Clinical Assays, Cambridge, MA 02139).

The effect of dietary treatments on fecal and urinary losses, serum levels and apparent retention of iron, zinc and copper were evaluated by two-way analysis of variance (30). Significant differences between individual treatment means were compared by Duncan's Multiple range test (31). Pearson correlation factors were calculated.

RESULTS

Excretion of fecal iron was greatest when subjects consumed the HCaHP diet (table 2). Subjects' average apparent absorption of iron from the MCaMP, MCaHP and HCaHP diets were 4.1%, 5.7%, and -1.2% respectively. Subjects excreted significantly more iron in their urine (P < 0.05) when fed the HCaHP diet than when fed the other two diets. Average apparent retention of iron was positive when subjects consumed the MCaMP and MCaHP diets, but average apparent retention of iron was negative when subjects consumed the HCaHP diet. This effect was not statistically significant.

Mean fecal copper losses were slightly elevated when subjects were fed the HCaHP diet as compared to when they consumed the MGaMP or MCaHP diets (table 2). The difference was not statistically significant as the P < 0.05 level. Subjects' average apparent absorption of copper from the MCaMP, MCaHP and HCaHP diets were 7.7%, 10.4%, and 5.2%, respectively. Urinary losses of copper were significantly higher (P < 0.05) when subjects consumed the diets high in phosphorus as compared to when they consumed the MCaMP diet. Daily urine volumes were significantly correlated (r = 0.44, P < 0.05) to daily urinary copper losses.

Dietary calcium and phosphorus levels did not affect subjects' urinary or fecal losses of zinc (table 2). Average apparent absorption of zinc was -4.6%, -5.2% and -5.0% when subjects were fed the MCaMP, MCaHP and HCaHP diets, respectively. The dietary intake of zinc at 10.4 mg/day was insufficient to maintain subjects in positive zinc balance regardless of the dietary treatment.

Fecal excretions of zinc and iron by these subjects were positively correlated (r = 0.52, P < 0.01). Similarly, retentions of zinc and iron by these subjects were positively correlated (r = 0.49, P < 0.01).

Urinary creatinine excretion was not affected by the dietary treatments. The day-to-day variations in urinary creatinine excretion by individual subjects were moderate. The coefficient of variation for urinary creatinine excretion by individuals (n = 18 days) ranged from 6.3% to 13.1%.

Blood samples were collected twice during each treatment period. Plasma iron, copper, and zinc and serum ferritin levels were determined on samples collected on both collection days of each period; plasma transferrin levels were determined only on samples collected during the second blood collection day of each period (table 3).

The dietary treatments had no significant effect on plasma iron levels or plasma transferrin levels (table 3). Plasma iron levels ranged from 96 to 198 μg/100 ml during the study; plasma transferrin levels ranged from 232 to 320 mg/100 ml during the study.

Serum ferritin levels were lower when subjects consumed the HCaHP diet rather than the other two diets but this effect was not statistically significant at the P < 0.05 level. Subjects varied greatly in regard to their ferritin levels, which ranged from 42 to 225 ng/ml during the study. Plasma transferrin levels
TABLE 2

The effect of dietary calcium and phosphorus on the excretion and retention of iron, copper and zinc by adult male subjects

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MCAHP</th>
<th>MCAHP</th>
<th>HCAHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal losses, mg/day</td>
<td>16.70 ± 1.41</td>
<td>16.52 ± 1.22</td>
<td>17.87 ± 1.62</td>
</tr>
<tr>
<td>Urinary losses mg/day</td>
<td>0.14 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.22 ± 0.08</td>
</tr>
<tr>
<td>µg/g creatinine</td>
<td>85 ± 25</td>
<td>84 ± 20</td>
<td>127 ± 52</td>
</tr>
<tr>
<td>Apparent retention, mg/day</td>
<td>0.56 ± 1.44</td>
<td>0.84 ± 1.34</td>
<td>-0.44 ± 1.57</td>
</tr>
<tr>
<td>Copper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal losses, mg/day</td>
<td>1.28 ± 0.12</td>
<td>1.26 ± 0.15</td>
<td>1.42 ± 0.17</td>
</tr>
<tr>
<td>Urinary losses, mg/day</td>
<td>51 ± 8</td>
<td>66 ± 10</td>
<td>67 ± 10</td>
</tr>
<tr>
<td>µg/g creatinine</td>
<td>30 ± 5</td>
<td>37 ± 7</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>Apparent retention, mg/day</td>
<td>0.06 ± 0.14</td>
<td>0.08 ± 0.14</td>
<td>0.01 ± 0.17</td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal losses, mg/day</td>
<td>10.89 ± 0.76</td>
<td>10.97 ± 1.43</td>
<td>10.97 ± 1.72</td>
</tr>
<tr>
<td>Urinary losses, mg/day</td>
<td>0.78 ± 0.28</td>
<td>0.78 ± 0.25</td>
<td>0.73 ± 0.43</td>
</tr>
<tr>
<td>mg/g creatinine</td>
<td>0.44 ± 0.14</td>
<td>0.45 ± 0.22</td>
<td>0.41 ± 0.24</td>
</tr>
<tr>
<td>Apparent retention, mg/day</td>
<td>-1.26 ± 0.81</td>
<td>-1.32 ± 1.55</td>
<td>-1.26 ± 1.75</td>
</tr>
</tbody>
</table>

1 Means ± SD (n = 9). 2 Apparent retention = intake — fecal losses — urinary losses. * Means in row with different superscripts are significantly different the P < 0.05 level.

were correlated to serum ferritin levels (r = 0.44, P < 0.05), but neither plasma transferrin nor serum ferritin levels were correlated to plasma iron levels.

The subjects’ plasma copper levels ranged from 76 to 135 µg/100 ml during the study (Table 3). Just as the dietary treatments had no effect on plasma iron or copper levels, dietary calcium and phosphorus levels had no effect on plasma zinc levels (Table 3). Plasma zinc levels ranged from 74 to 120 µg/100 ml when subjects were fed the MCAHP diet, from 55 to 105 µg/100 ml when subjects were fed the MCAHP diet, and from 67 to 116 µg/100 ml when subjects were fed the HCAHP diet. Only one subject had plasma zinc levels below 70 µg/100 ml. Plasma zinc levels were significantly correlated to fecal zinc losses (r = 0.51, P < 0.01).

DISCUSSION

Investigators have demonstrated that high levels of phosphorus administered with a

TABLE 3

The effect of dietary calcium and phosphorus on iron, zinc and copper status of adult male subjects

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MCAHP</th>
<th>MCAHP</th>
<th>HCAHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma iron, µg/100 ml</td>
<td>146 ± 21</td>
<td>139 ± 19</td>
<td>146 ± 17</td>
</tr>
<tr>
<td>Plasma transferrin, µg/100 ml</td>
<td>274 ± 25</td>
<td>278 ± 20</td>
<td>274 ± 27</td>
</tr>
<tr>
<td>Serum ferritin, ng/ml</td>
<td>108 ± 49</td>
<td>104 ± 50</td>
<td>96 ± 41</td>
</tr>
<tr>
<td>Plasma copper, µg/100 ml</td>
<td>105 ± 17</td>
<td>99 ± 12</td>
<td>100 ± 8</td>
</tr>
<tr>
<td>Plasma zinc, µg/100 ml</td>
<td>89 ± 12</td>
<td>85 ± 12</td>
<td>84 ± 12</td>
</tr>
</tbody>
</table>

1 Means ± SD, n = 18. 2 Means ± SD, n = 9.
meal had little effect on the retention of iron by 13 human subjects (20) or on the retention of copper and zinc by one human subject (22). Our work supports these findings. Adverse effects of high dietary phosphorus levels on trace mineral utilization in humans have been demonstrated only when inorganic zinc (21) or when inorganic iron and ascorbic acid (13) were administered with a phosphorus supplement in a solution. There seems to be little evidence that a high level of dietary phosphorus, by itself, adversely, affects trace mineral utilization from meals by human subjects. Data from animal studies have been less consistent, some investigators have found that high levels of dietary phosphorus decreased iron (1, 9, 32, 33), and zinc (7) utilization, while other investigators have not demonstrated an effect (15, 16, 18).

High levels of dietary phosphorus fed in combination with high levels of dietary calcium appear to have more of an effect on iron utilization than just high dietary levels of phosphorus (20, 33, 34). In this study, average apparent retention of iron was negative when subjects consumed the HCaHP diet while average apparent retention of iron was positive when subjects consumed the other two diets, Monsen and Cook (20) also observed that human subjects retained less $^{56}$Fe when fed a single semi-synthetic meal which contained high levels of dietary calcium and phosphorus than when fed diets which contained high levels of calcium or phosphorus alone.

The difference Monsen and Cook observed, however, was statistically significant. The less dramatic reduction of iron absorption by subjects observed in this study may be attributed to the differences in the type and solubility of the calcium and phosphorus supplements fed the subjects. Monsen and Cook (20) fed calcium chloride and dipotassium phosphate. We fed our subjects calcium gluconate in solution and glycerol phosphate in gelatin capsules because in many high protein foods, calcium and phosphorus are partially present as organic complexes (35). The inorganic calcium and phosphorus salts fed with a semi-synthetic meal in Monsen and Cook's study would have been immediately available to form a complex with iron in the gut upon ingestion of the diet. Calcium gluconate and glycerol phosphate would have been hydrolyzed quickly in the gut, but perhaps some absorption of iron could have occurred before the calcium and phosphorus exerted their effect. Moreover, Ellinger (3) has stated that many phosphate salts, particularly the calcium-phosphate salts, are insoluble in water, while many of the potassium- and sodium-phosphate salts are readily soluble in water. Chapman, et al. (2) has noted that the more insoluble the calcium salts are, the more they depressed hemoglobin regeneration in anemic rats.

Monsen and Cook fed subjects one semi-synthetic meal and measured retention of $^{56}$Fe in blood; we fed subjects a mixed diet for 12 days and measured dietary fecal and urinary levels of iron. Some investigators believe that the isotope retention technique is a more sensitive measure of iron absorption than chemical balance (36). Other differences, however, in the diets could also be important: a) The protein source in the semisynthetic meal was egg albumin (20) which contains no heme iron nor “meat factor” (37). In our study, subjects received 35% of their protein from lean ground beef. The beef contained 1.5 mg heme iron and the unidentified “meat factor” which has been demonstrated to enhance non-heme iron absorption (37). b) The level of ascorbic acid, a factor that enhances iron absorption (37), in our diet on a per meal basis was 45 mg. Monsen and Cook (20) did not state the level of ascorbic acid contained in their semi-synthetic meal (20, 38).

Serum transferrin, plasma iron, and especially serum ferritin levels are all considered to be indicators of nutritional status in regard to iron (36). None of the factors were statistically correlated to iron retention by the subjects, but their apparent absorption of iron and serum ferritin levels tended to be lower when fed the HCaHP diet rather than the other two diets.

Although some investigators have noted that the combination of high dietary levels of calcium and high dietary levels of phosphorus had a greater effect on the zinc utilization of animals than just high dietary levels of phosphorus (6, 7, 39), this effect was not observed among human subjects in this study. The effect of dietary phosphorus on
zinc utilization may be moderated by other dietary factors. Previous work from our laboratory has indicated that the apparent retention of zinc in human subjects was affected by the interaction of dietary protein and phosphorus levels (12).

In this study the addition of organic calcium and phosphorus supplements to a mixed diet, containing ascorbic acid, heme iron, an unidentified meat factor, and low levels of dietary phytate, did not affect significantly the trace element utilization of male subjects. The addition of inorganic calcium and phosphorus salts to a diet low in factors, which enhance mineral bioavailability, could have a more pronounced effect on the utilization of trace elements.

The effects of both organic and inorganic calcium and phosphorus supplements on trace element utilization in humans deserve further study because the use of these supplements are widespread. Inorganic phosphate salts are presently being added to processed foods to improve their organoleptic properties (3). Manufacturers, who now want to reduce the sodium content of processed foods, may consider the use of calcium phosphates rather than sodium phosphates. In addition, inorganic phosphorus and calcium supplements are readily available to consumers in health food stores.

LITERATURE CITED