A stable isotope study of copper absorption in young men: effect of phytate and α-cellulose¹-³

Judith R Turnlund, PhD, Janet C King, PhD, Bonnie Gong, BS, William R Keyes, MA, and Maynard C Michel, PhD

ABSTRACT A sixty-three day study was conducted with young men confined to a metabolic unit to study the effects of α-cellulose and phytate on copper absorption. Copper absorption was determined with $^{65}$Cu, a stable isotope of copper, during each of 3 dietary treatments (basal diet, basal diet + α-cellulose, or basal diet + phytate). The addition of α-cellulose or phytate to the basal diet did not affect copper absorption. Average copper absorption was 35.0% from the basal diet, 34.1% from the diet with 0.5 g α-cellulose per kg body weight added, and 31.4% from the diet with 2.34 g of phytate as sodium phytate added to the diet. Copper absorption was significantly different between subjects and averaged 44.1%, 26.8%, 33.4%, and 29.5% in individual subjects. The results suggest that high levels of either α-cellulose or phytate do not have marked effects on copper absorption, but copper absorption differs between individuals. Am J Clin Nutr 1985; 42:18-23.

KEY WORDS Copper, bioavailability, absorption, stable isotopes, phytate, fiber

Introduction

A number of studies have been conducted to evaluate the effect of dietary patterns and/or components on trace element absorption in man (1). Little is known, however, of the effect of dietary components on copper absorption. This is due at least in part to the lack of suitable isotopes for use in human absorption studies. Radioisotopes of copper are short-lived: the two with the longest half lives are $^{67}$Cu, with a half life of 61.9 h, and $^{64}$Cu, with a half life of 12.7 h (2). The least abundant stable isotope of copper, $^{65}$Cu, has an abundance of 30.2% (3). Thus it is a valuable tool for study of copper absorption in man only when a precise and accurate analytical method for determination of $^{65}$Cu is used. Few analytical approaches are capable of the required precision and accuracy.

Two components of the diet, phytate and fiber, may affect mineral absorption in humans (4, 5), but very little research has been conducted on the effect of phytate and fiber on copper absorption in humans. Only balance studies have been reported. Results of one balance study suggested that addition of fiber sources to the diets low in fiber increased the requirement for copper by 18% with a diet containing 8% protein. The copper requirement increased even more when both protein and fiber contents of the diets were increased (6). The investigators suggested that either the fiber or phytate which accompanied the fiber may have affected the copper requirements. However, another study by the same group demonstrated that copper balance improved when wheat bran and corn bran were added to the diet (7).

Rat studies have been conducted to evaluate the effects of phytate on copper bioavailability. Results of the studies do not agree. One experiment demonstrated de-

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increased copper absorption and retention in rats with phytate added to the diet (8). Two others concluded bioavailability of copper to rats was enhanced by phytate, based on improvements in indicators of copper status (9, 10).

We conducted a study in which 5 young women were each fed two diets (11). The protein of one diet was primarily from animal sources with 19.8 g of neutral detergent fiber, 0.9 g phytate, and 1.44 mg copper. The protein of the other diet was primarily from plant sources with 34.1 g of neutral detergent fiber, 1.9 g phytate, and 2.53 mg of copper. The fractional copper absorption was lower from the vegetable protein diet. However, the copper content of the vegetable protein diet was higher so the amount of copper absorbed was higher. It was not possible to distinguish the effect of the level of dietary copper from the effects of dietary components on copper absorption.

The study described here was conducted to determine whether high intake of the dietary fiber, \( \alpha \)-cellulose, or phytate affect dietary copper absorption. The dietary copper level was held constant and was within the recommended safe and adequate range of copper intake (12). The stable isotope, \( \text{Cu}^{65} \), was fed to a group of young men to assess copper absorption.

**Methods**

Four healthy young men were confined to a metabolic unit for 63 days to assess the effects of \( \alpha \)-cellulose and phytate on mineral absorption and balance. The men were between 25 and 32 years of age. The semipurified liquid formula diets and experimental design have been described (4). The experimental protocol was approved by the University of California, Berkeley, Committee for the Protection of Human Subjects. The diets contained 2.32 mg of copper per day. The study was divided into 5 periods. During the 1st, 3rd and 5th periods, subjects were fed a basal diet. Copper absorption was determined three times during the study in each subject, with the basal diet only (period 1), when \( \alpha \)-cellulose (ICN Pharmaceuticals, Cleveland, OH) at a level of 0.5 g/kg body weight was added to the diet (period 2 or 4), and when 2.34 g of phytic acid as sodium phytate (Sigma Chemical Co, St Louis, MO) was added to the diet (period 2 or 4). Periods 1, 2, and 4 were each 15 days long. On day 7 of these periods a stable isotope solution containing 2.03 mg \( \text{Cu}^{65} \) replaced 1.74 mg of copper in the regular copper intake was 2.61 mg on the days of the isotope feedings.

Complete fecal collections were made throughout the study. Fecal samples were prepared for analysis as previously described (4). Following each isotope feeding, aliquots of 3-day fecal pool homogenates were combined into 12-day composites and prepared for \( \text{Cu}^{65} \) determination by a modification of our previously reported procedure (13). Organic material was eliminated from the samples and a 6 N HCl solution of minerals was applied to an 7 mm ID anion exchange column. Copper was then separated from the minerals in the samples. The ion exchange columns were washed with 6 N HCl and copper was eluted with 2.5 N HCl. Copper could usually be seen as a light yellow band as it eluted from the column. Ultrex HCl (Baker Company, Phillipsburg, NJ) was added to the copper collection to achieve a strength of 6 N. The solution was applied to a 3 mm ID column and washed with 5 or 6 column volumes of 6 N HCl. Copper was then eluted with 2.5 N HCl in about 6 drops of solution. The 3 mm column procedure was repeated for each copper sample to improve purity.

Prior to loading the purified copper onto a rhenium filament for analyses by thermal ionization mass spectrometry (TIMS), a silica gel suspension was applied to the filament and dried at 1.0 amp (4). A solution containing about 30 \( \mu \)g of copper was applied to the filament and dried at 1 amp for 10 min. Ultrapure phosphoric acid was then applied to the filament (4), the sample was dried at 1.5 amp, and the filament was inserted into the mass spectrometer (MS6).

When pressure was \( 10^{-7} \) torr, the filament current was set at 1.50 amp for 1 h, then raised to 1.85 amp for 1 h, and to 2.00 amp for 1 h. If the ion beam was stable and the voltage was of sufficient intensity that background noise did not interfere, ratio data were taken in 10 sweeps of the mass range. If the beam was not stable or of sufficient intensity, the filament current was increased to between 2.20 and 2.40 amps before data were taken. The use of silica gel and improved sample purity due to additional ion exchange columns improved analytical precision.

The \( \text{Cu}^{65}/\text{Cu}^{64} \) isotopic ratio and the amount of copper in the 12-day fecal pools, determined by atomic absorption spectrophotometry (Instrumentation Laboratories, Wilmington, MA, model 951), were used to calculate the amount of \( \text{Cu}^{65} \) spike eliminated, as follows:

\[
M^* = \frac{M^*}{M^* + 1}
\]

**References**

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\[
M^* = \frac{M^*}{M^* + 1}
\]
TABLE 1
Apparent copper absorption in young men

<table>
<thead>
<tr>
<th>Subject</th>
<th>Basal diet</th>
<th>Basal diet + α-cellulose</th>
<th>Basal diet + phytate</th>
<th>Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>45.3</td>
<td>42.5</td>
<td>44.6</td>
<td>44.1</td>
</tr>
<tr>
<td>B</td>
<td>28.5</td>
<td>30.0</td>
<td>22.0</td>
<td>26.8</td>
</tr>
<tr>
<td>C</td>
<td>40.9</td>
<td>31.1</td>
<td>28.3</td>
<td>33.4</td>
</tr>
<tr>
<td>D</td>
<td>25.3</td>
<td>32.8</td>
<td>30.5</td>
<td>29.3</td>
</tr>
<tr>
<td>Mean†</td>
<td>35.0</td>
<td>34.1</td>
<td>31.4</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of subject means = 2.67. Effect of subject on copper absorption, p < 0.05.
† Standard error of diet means = 2.31.

Results

Apparent copper absorption (Table 1) averaged 35.0% with a range of 25.3 to 45.3% from the basal diet, 34.1% with a range of 30.0 to 42.5% when 0.5 g/kg body weight of α-cellulose was added to the diet, and 31.4% with a range of 22.0 to 44.6% when 2.34 g of phytate was added to the diet. The standard error of diet means was 2.31%. The effects of α-cellulose and phytate on copper absorption by subject are shown in Figure 1. There was no significant difference in copper absorption due to α-cellulose or phytate (p = 0.55). Copper absorption differed significantly between subjects (p < 0.02), due pri-
TABLE 2
Average daily fecal copper

<table>
<thead>
<tr>
<th>Subject</th>
<th>Basal diet*</th>
<th>Basal + α-cellulose</th>
<th>Basal + phytate</th>
<th>Mean†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.82</td>
<td>1.97</td>
<td>2.15</td>
<td>1.98</td>
</tr>
<tr>
<td>B</td>
<td>1.93</td>
<td>1.89</td>
<td>2.04</td>
<td>1.95</td>
</tr>
<tr>
<td>C</td>
<td>2.00</td>
<td>1.80</td>
<td>2.32</td>
<td>2.04</td>
</tr>
<tr>
<td>D</td>
<td>2.07</td>
<td>1.74</td>
<td>2.05</td>
<td>1.95</td>
</tr>
<tr>
<td>Mean‡</td>
<td>1.96</td>
<td>1.85</td>
<td>2.14</td>
<td></td>
</tr>
</tbody>
</table>

* Metabolic period 1 only.
† Standard error of subject means 0.067.
‡ Standard error of diet means 0.058.

Phytate and Copper Absorption in Men

Serum copper levels, shown in Table 3, averaged 99 µg/dl on admission to the metabolic unit, 78 µg/dl during the α-cellulose diet, 76 µg/dl during the phytate diet, and 96 µg/dl on the last day of the study at the end of the final basal diet period. The standard error of diet means was 3.82 µg/dl. Serum copper was significantly lower (p < 0.01) when either α-cellulose or phytate was added to the diet. Serum copper also differed significantly between subjects (p < 0.01).

The addition of α-cellulose or phytate to the diet did not change fecal copper significantly, but fecal copper was higher with phytate in the diet than with α-cellulose (p < 0.05). Fecal copper did not differ significantly between subjects.

TABLE 3
Serum copper

<table>
<thead>
<tr>
<th>Subject</th>
<th>Entry to study</th>
<th>Basal diet</th>
<th>Basal + α-cellulose</th>
<th>Basal + phytate</th>
<th>Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>93</td>
<td>91</td>
<td>84</td>
<td>105</td>
<td>93‡</td>
</tr>
<tr>
<td>B</td>
<td>81</td>
<td>60</td>
<td>55</td>
<td>86</td>
<td>70b</td>
</tr>
<tr>
<td>C</td>
<td>125</td>
<td>89</td>
<td>94</td>
<td>109</td>
<td>104a</td>
</tr>
<tr>
<td>D</td>
<td>95</td>
<td>70</td>
<td>71</td>
<td>82</td>
<td>80b</td>
</tr>
<tr>
<td>Mean†</td>
<td>99c</td>
<td>78c</td>
<td>76d</td>
<td>96d</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of subject means 3.82. Effect of subjects (p < 0.01).
† Standard error of diet means 3.82. Effect of diet (p < 0.01).
‡ Subjects and diets with different superscripts are significantly different.
dietary components or the level of dietary copper affected copper absorption. The levels of α-cellulose and phytate used in this study would only be found in very high fiber and phytate diets.

The results of this study combined with our previous studies suggest that the level of dietary copper may affect fractional copper absorption. However, even high levels of the dietary components α-cellulose and phytate do not markedly affect copper absorption. The levels of α-cellulose and phytate used in this study would only be found in very high fiber and phytate diets.

Differences in copper absorption were noted between subjects, due primarily to subject A, who consistently absorbed more copper than the other young men. We were unable to establish any reason for the higher absorption. His serum copper, the only other indicator of copper status measured in this study, was 82 μg/dl, within the reported normal range of 80 to 120 μg/dl (15), and within the range of serum copper concentration for the other subjects in this study who absorbed less copper. However, serum copper may not be a good indicator of copper status. Fecal copper excretion, in contrast to copper absorption, did not differ between subjects. Fecal copper of subject A was similar to that of the other young men, and even though he absorbed more copper he tended to excrete more endogenous copper, an average of 0.68 mg per day, via the gastrointestinal tract. Endogenous copper was calculated by subtracting the amount of unabsorbed dietary copper, e.g., 55.9% of 2.32 mg from total daily fecal copper. The other subjects excreted 0.25 to 0.49 mg of endogenous copper daily. Since subject A absorbed more copper, he maintained copper balance despite higher endogenous copper losses.

While serum copper for subjects B and D fell to below the normal range of 80 to 120 μg/dl when either α-cellulose or phytate was added to the diet, there is no apparent explanation for this phenomenon. However, serum copper ranges vary widely between laboratories and the nutritional significance of serum copper concentrations below the “normal range” has not been established (17). When α-cellulose or phytate were added to the basal diet, neither fecal copper nor copper absorption changed significantly. While urinary copper was not determined in this study, urinary copper losses are so low, about 10–20 μg per day, they should not affect serum copper or copper status.

While addition of phytate to the diet did not impair copper absorption, it impaired zinc absorption (4). Phytate reduced zinc absorption from 34.0 to 17.5% in these subjects and resulted in increased loss of fecal zinc. The different effects of phytate on bioavailability of zinc and copper demonstrate that the effects of dietary components on mineral bioavailability must be evaluated for each mineral and that generalizations cannot be made.

In vitro experiments have been conducted which may help explain the results of our experiment. A study of phytic-acid metal complexes (18) demonstrated that sodium phytate binds copper more strongly than zinc. However, the zinc-phytate complex precipitates in an insoluble complex at the pH of the gastrointestinal tract, while the copper-phytate complex is soluble at that pH (18, 19). A recent study demonstrated that phytate in the phytate-copper complex can easily be replaced by other chelators, including EDTA and amino acids (19). Phytate can also be replaced by other chelators in phytate-zinc complexes, but at the pH of the gastrointestinal tract the phytate zinc complex is an insoluble precipitate. This could explain why phytate inhibits zinc, but not copper absorption.

References

PHYTATE AND COPPER ABSORPTION IN MEN