MEAL PROTEIN AND ZINC LEVELS INTERACT TO INFLUENCE ZINC RETENTION BY THE RAT

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ABSTRACT

The relationship between the effect of egg white protein and zinc in a meal on zinc retention was examined by feeding test meals labeled with Zn-65 to rats and monitoring retention of radioactivity with a whole body counter. Corrected retention (an estimate of absorption) was determined by extrapolating to zero time the semilogarithmic plot of Zn-65 retention between 5 and 26 days after the meal. Test meals contained fixed amounts of sucrose, cornstarch, corn oil, and variable amounts of egg white and zinc chloride. Fractional zinc retention was reduced by increasing the usual dietary zinc intake or zinc in the meal, and was enhanced by increasing protein in the meal. Meal zinc and protein interacted, such that with more zinc in the meal, more protein was needed to observe enhanced zinc retention. Enhancement of zinc retention by protein was not linear with the amount of protein and was not related to the protein to zinc ratio in the meal. The interaction was similar whether rats were accustomed to diets containing 14 or 37 mg zinc/kg. These data indicate that zinc absorption is affected by an interaction between the protein and zinc content of a meal, and the dose-response relationship between the amount of zinc consumed and the amount retained may be amplified by a moderate amount of protein.

Key Words: zinc retention, diet protein, diet zinc, zinc bioavailability, zinc absorption

INTRODUCTION

Both dietary protein (1-4) and zinc (5, 6) levels have been observed to influence the retention of ingested zinc. In humans (1, 2) and rats (4), retention of zinc from meals containing a variety of protein sources was

proportional to the protein content of the meal. Ingestion of larger single doses of zinc was associated with greater total zinc retention, but a lower percent retention (5, 6). In rats fed single doses between 0.1 and 26 μmol zinc and dietary concentrations between 12 and 150 mg zinc/kg, percent zinc retention from a single dose was proportional to the natural logarithm of the dose and to the reciprocal of the usual dietary zinc concentration (5). In humans, small amounts of supplemental zinc substantially lowered percent zinc retention when added to a meal (1, 7), but had very little effect when similar increments were administered as zinc salts without food (8, 9).

The purpose of the present investigation was to determine if zinc retention is affected by an interaction between the zinc concentration of the usual diet and protein and zinc levels in a meal. Zinc retention was evaluated by whole body counting after feeding meals labelled with Zn-65.

METHODS

Animals: Male Long-Evans rats 30 days old were obtained from an on-site breeding colony (experiment 1) or commercially (Harlan Sprague-Dawley, Indianapolis, IN) (experiments 2 and 3). A nonpurified diet (laboratory chow #5001, Ralston-Purina, St. Louis, MO) containing 65 mg Zn/kg was provided ad libitum to commercially obtained rats for 2 days or to breeding colony rats until 1 1/2 weeks post-weaning, at which time the rats weighed (mean ± SD) 115 ± 22 g. Five to six rats were randomly assigned to experimental treatments from similar body weight groupings. Rats were maintained in individual, suspended, wire-bottom, stainless steel cages in a controlled environment with a 12 hour alternating dark/light cycle (lights on at 2000 hours). Deionized water was provided ad libitum.

Diets: The previously described diets (5) were based on the diet proposed by a committee of the American Institute of Nutrition (10) but modified in zinc content. Rats were meal-trained for 20 days by making food available only during the first four hours of the dark cycle. The Zn-65 labeled test meal was fed the 21st day between 0800 and 1200 hours. The diet fed before the test meal was reintroduced at 1600 hours the day of the test meal, and fed ad libitum during the remainder of the experiment.

Test meals: All test meals contained 1.5 g sucrose, 0.45 g corn starch, 0.15 g corn oil, and 1 μCi carrier-free 65ZnCl₂ (Dupont New England Nuclear, Boston, MA). The meals differed in the amount of protein from egg white (Teklad Test Diets, Madison, WI) (amounts listed do not correct for the non-protein portion of this product, which is approximately 5%) and zinc from zinc chloride (MCB Manufacturing Chemists, Inc., Gibbstown, NJ). Zinc chloride was mixed with finely ground sucrose, and added to the meal in substitution for part of the total sucrose. Egg white protein was added without substitution for other ingredients. Test meals for each treatment group were mixed with a mortar and pestle, then weighed as individual portions. During the four hour test meal period, spilled food was returned to the animal's food cup. Animals

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not consuming at least 70% of the meal in four hours (2 of 20th animals) were omitted from the study. The remaining animals consumed (mean ± SD) 98 ± 3% of the test meals.

Experimental design: The three experiments, similar in general protocol, differed in the amount of zinc in the diets which the rats regularly consumed, and the amounts of zinc and egg white protein in a Zn-65 labeled test meal. Experiment 1 investigated the interaction between zinc and protein in a meal, by employing test meals with 0.25 or 1.5 μmol zinc and 0, 0.6, or 1.2 g dry egg white, given to rats consuming 38 mg zinc/kg of diet (by analysis). Experiment 2 tested the hypothesis that the interaction between zinc and protein in a meal could be explained by the meal protein-to-zinc ratio. Rats in this experiment consumed a diet containing 13 mg zinc per kg. The test meals again contained 0.25 or 1.5 μmol zinc, and amounts of egg white were varied to yield egg white:zinc ratios (g:μmol) of 0, 0.4, 0.6, 0.8, and 1.2. Two more comparisons (one at each zinc dose) were made to determine whether addition of minerals in amounts contributed by egg white would affect zinc retention, as follows: the egg white contained (mg/g by analysis) calcium, 0.81; potassium, 14.95; magnesium, 0.99; sodium, 13.44; and phosphorus, 10.71. These were added to the test meals in a mineral mix prepared to contain, per 100 g, 0.413 g calcium phosphate dibasic, 7.51 g potassium chloride, 0.246 g magnesium oxide, 5.12 g sodium chloride, 0.293 g potassium monobasic phosphate, and 86.4 g sucrose. This mineral mix was substituted for sucrose in quantities of 0.2 g and 1.2 g per meal to provide the mineral equivalent of 0.3 and 1.8 g egg white to meals containing 0.25 and 1.5 μmol zinc, respectively. Experiment 3 investigated interactions between all three dietary factors by using a fully-crossed 2 X 2 X 4 factorial design with two levels of zinc in the usual diet (14 and 37 mg/kg), two levels of zinc in the test meals (0.25 and 1.5 μmol) and 4 levels of egg white in the test meals (0, 0.6, 1.2, and 1.8 g).

Retention measurements: Retention measurements were adapted from the method of Heth and Hoekstra (11), as discussed previously (5). Briefly, radioactivity in each rat was measured within 2 1/2 hours after the test meal and 3 times weekly for 3 weeks by whole body counting. Counting measurements were corrected for background, radiolotope decay and daily fluctuations in the measurement of a Zn-65 standard, then expressed as a percentage of the initial count. Retention plots of the natural logarithms of percent retention versus time after 5 days were linear. Percent corrected retention was determined by extrapolating the linear portion of the curve to zero time, and transforming this intercept from the logarithmic to a linear scale. Retention was not corrected for the retention of intramuscularly injected Zn-65, as described by Heth and Hoekstra (11).

Measurements of zinc and other elements: Zinc was determined in diets as follows: Organic matter in the dry sample was destroyed by alternately heating for two days at 450 °C and adding concentrated nitric acid (Baker Instra Analyzed, Phillipsburg, NJ) which was evaporated to dryness on a hot plate. The resulting ash was diluted with 2% nitric acid to a concentration between 0.1 and 1.0 mg Zn/ml, and measured by using an inductively-coupled argon plasma spectrometer (Perkin-Elmer Model ICP/6500, Norwalk, CT). Analytical accuracy was monitored intermittently by assaying zinc in bovine liver samples (Standard Reference Material 1577a) from the National Bureau of Standards. Mean (± SD) zinc measurements were 98.8 ± 4.4 percent (n = 3) of certified values.
Statistics: Diet effects on percent zinc retention were evaluated by analysis of variance, with Bonferroni contrasts to compare the effects of protein or added minerals with similar zinc levels in the diet and test meal (12).

RESULTS

The levels of protein and of zinc in a meal interacted to affect zinc retention (Experiment 1, Table 1). More egg white protein was required to enhance retention from a 1.5 μmol zinc dose than was required to enhance retention from a 0.25 μmol zinc dose.

TABLE 1

<table>
<thead>
<tr>
<th>Meal Zn μmol</th>
<th>Egg White g</th>
<th>Protein/Zn g/μmol</th>
<th>Zn Retention %</th>
<th>Zn Retained μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0</td>
<td>0</td>
<td>60 ± 6</td>
<td>0.15</td>
</tr>
<tr>
<td>0.25</td>
<td>0.6</td>
<td>2.4</td>
<td>76 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.25</td>
<td>1.2</td>
<td>4.8</td>
<td>78 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>44 ± 5</td>
<td>0.66</td>
</tr>
<tr>
<td>1.5</td>
<td>0.6</td>
<td>0.4</td>
<td>41 ± 3</td>
<td>0.62</td>
</tr>
<tr>
<td>1.5</td>
<td>1.2</td>
<td>0.8</td>
<td>56 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly different, p < 0.05, from similar meal without protein.

Because the enhancing effects of protein were observed in experiment 1 only when the ratio of protein to zinc in the meal was greater than 0.4 (g/μmol), experiment 2 was planned to test the effect of protein to zinc ratios between 0 and 1.2 (g/μmol). The interaction between protein and zinc could not be explained by the protein to zinc ratio; a ratio of 1.2 g protein to 1 μmol zinc in the meal improved retention of a 1.5 μmol zinc dose, without affecting a 0.25 μmol zinc dose (Experiment 2, Table 2). Addition of calcium, potassium, magnesium, sodium and phosphorus in amounts found in egg white had little effect on retention compared to retention from meals with no egg white, and resulted in significantly less zinc retention than with the meals containing equivalent amounts of egg white (Experiment 2, Table 2).

Although similar interactions between protein and zinc meal content were observed in experiments 1 and 2, the enhancement of zinc retention from a 1.5 μmol zinc dose that was observed with 1.2 g egg white in experiment 1, did not occur in experiment 2. Because the zinc concentration of the usual diet differed in these experiments, a third experiment investigated a possible interaction with the usual dietary zinc concentration. When all 3 dietary factors were examined in the same experiment (Experiment 3, Figure 1), highly
significant effects were observed for the diet zinc concentration, meal zinc level, meal protein level, and the interaction between meal zinc and meal protein, without any further interactions involving the usual dietary zinc concentration.

**TABLE 2**

Effect of Zinc and Protein in a Meal on Zinc Retention by Rats Usually Consuming Diets Containing 13 mg Zn/kg (Experiment 2).

<table>
<thead>
<tr>
<th>Meal Zn μmol</th>
<th>Egg White g</th>
<th>Protein/Zn g/μmol</th>
<th>Zn Retention %</th>
<th>Zn Retained μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0</td>
<td>0</td>
<td>88 ± 6</td>
<td>0.22</td>
</tr>
<tr>
<td>0.25</td>
<td>0.10</td>
<td>0.4</td>
<td>89 ± 3</td>
<td>0.22</td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td>0.6</td>
<td>92 ± 4</td>
<td>0.23</td>
</tr>
<tr>
<td>0.25</td>
<td>0.20</td>
<td>0.8</td>
<td>91 ± 3</td>
<td>0.23</td>
</tr>
<tr>
<td>0.25</td>
<td>0.30</td>
<td>1.2</td>
<td>93 ± 3</td>
<td>0.23</td>
</tr>
<tr>
<td>0.25</td>
<td>(minerals equivalent to 0.3 g egg white&lt;sup&gt;a&lt;/sup&gt;)</td>
<td></td>
<td>83 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>68 ± 9</td>
<td>1.02</td>
</tr>
<tr>
<td>1.5</td>
<td>0.6</td>
<td>0.4</td>
<td>68 ± 11</td>
<td>1.02</td>
</tr>
<tr>
<td>1.5</td>
<td>0.9</td>
<td>0.6</td>
<td>75 ± 6</td>
<td>1.13</td>
</tr>
<tr>
<td>1.5</td>
<td>1.2</td>
<td>0.8</td>
<td>75 ± 8</td>
<td>1.14</td>
</tr>
<tr>
<td>1.5</td>
<td>1.8</td>
<td>1.2</td>
<td>88 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5</td>
<td>(minerals equivalent to 1.8 g egg white&lt;sup&gt;a&lt;/sup&gt;)</td>
<td></td>
<td>72 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Results for meals supplemented with Ca, K, Mg, Na, and P were significantly less than results for meals containing equivalent minerals from egg white.

<sup>b</sup>Significantly different, p <0.05, from similar meal without protein.

**DISCUSSION**

The observed enhancement of zinc retention by dietary protein is consistent with previous results. The meal content of protein from a variety of sources has been found to correlate with zinc retention in humans (1, 2) and rats (4). Growing rats fed a diet with 45% (as compared with 15%) lactalbumin retained more zinc as indicated by higher bone zinc concentrations (3). Certain amino acids and dipeptides have been reported to enhance zinc absorption by the perfused rat ileum (13).
The present investigation demonstrates that, although protein enhances zinc retention, the relationship is not linear with the protein content of the meal (Figure 1). Furthermore, the protein content of the meal altered the dose-response relationship between the zinc content of a meal and zinc retention. The reduction in percent zinc retention with an increased zinc dose was much more substantial when meals contained 0.6 g egg white protein (38% reduction), as compared to protein-free meals (13% reduction) (Figure 1). This observation may help explain why, in human studies, small increments in the zinc dose have a much more substantial effect on zinc retention if given as part of a meal. When zinc salts were used in human studies, the percent zinc absorption was not substantially affected by the zinc dose until doses exceeded 10 mg (8, 9). However, when zinc came from foods, small additions of zinc salts within the range of 0.4 to 8.5 mg zinc resulted in substantial reductions in percent zinc absorption (1, 7). For instance, percent zinc absorption from white bread with butter (5.2 g total protein) was reduced from 38 to 13 when zinc chloride was added to increase the zinc content of the meal from 0.4 to 3.6 mg (1). The small amount of protein in the meal may have been sufficient to enhance zinc absorption from the meal with low zinc content, but not from the meal with more zinc.
The mechanism for the interaction between meal zinc and meal protein contents is unknown. It is not a simple matter of the protein to zinc ratio in the meal, as seen from the enhanced retention of 1.5 μmole zinc by 1.8 g egg white protein, without similar enhancement when feeding 1/6 these amounts (Table 2). Though speculative, the following hypothesized mechanism is consistent with the experimental data: The interaction between the meal zinc and protein contents may be caused by differences in the intraluminal concentrations of zinc and protein digestion products at various sites in the intestine postprandially. Although the proximal intestine is the major site of both protein and zinc absorption when low amounts of these nutrients are ingested (14, 15), the effect of ingesting increasing amounts of these nutrients on the site of absorption has received little investigation. It seems reasonable to expect that ingestion of larger amounts would result in absorption at both proximal and more distal sites. The ingestion of greater amounts of protein may therefore result in peptides present farther into the small intestine. Low doses of zinc, absorbed principally in the proximal intestine, would be equally affected by low or high amounts of protein, because either would result in an abundance of peptides at the site of zinc absorption. Absorption of high doses of zinc, presumably extending through a much greater length of the intestine, would be minimally affected by ingestion of low amounts of protein, because peptides would occur mainly in the proximal intestine, influencing only a fraction of the area of zinc absorption. Absorption of high doses of zinc would only be enhanced by ingestion of enough protein to cause peptides to extend distally through a similar length of intestine as the extended area of zinc absorption.

Alternatively, the mechanism for the observed interaction between meal zinc and protein contents may require attainment of a minimum ratio of protein digestion products to total intraluminal zinc. Pancreatic secretions contain substantial amounts of zinc (16, 17) that may contribute considerably to postprandial intraluminal zinc concentrations (16). Increasing the amount of protein ingested also increases pancreatic protein secretion (18), and presumably, zinc secretion in the form of carboxypeptidase (17). Such a mechanism could explain the data from the present investigation, depending on the amount of endogenous zinc secreted, and the extent to which endogenous zinc and dietary zinc form a common pool for absorption. While the data is consistent with both of the above mechanisms, it is not sufficient to distinguish between them or other possible mechanisms. In addition, the two mechanisms discussed are not mutually exclusive, and may be useful together in explaining the observed interaction between meal zinc and protein.

In conclusion, increasing amounts of egg white protein in the same meal enhanced retention of dietary zinc by rats, in a nonlinear fashion. Zinc retention from a meal was significantly affected by the meal protein content, meal zinc content, and zinc concentration in the usual diet. In addition, zinc retention was affected by an interaction between the meal protein and meal zinc contents, such that more protein was required to enhance retention of a larger zinc dose. Thus, the dose-response relationship between zinc ingested and zinc retained was altered by the amount of protein ingested. The enhancing effect of protein on zinc retention was not influenced by the animal's usual zinc intake, between dietary zinc concentrations of 14 and 37 mg/kg, which meet or exceed the zinc requirement for growth (19, 20).
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REFERENCES


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