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Effects of Folic Acid Supplements on Zinc-65 Absorption and Retention

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The effect of high dietary folic acid on ^{65}Zn absorption from a breakfast meal was studied in 7 men and 6 women who were eating self-selected diets. An 800 μg folic acid supplement, fed either with the labeled meal or daily for 2 weeks before the meal, significantly impaired zinc absorption when the control absorption without added folic acid was greater than 30% ($P < 0.05$), but apparently not when the control absorption was less than 30%. Individuals whose absorption was affected by folic acid supplements tended to have lower zinc intakes (10.8 vs. 12.3 mg/d), lower plasma zinc (77 vs. 81 μg/dl) and higher red blood cell folate (254 vs. 192 ng/ml) than those whose absorption was not affected by folic acid. These results indicate an interaction between zinc and folic acid that seems to be manifested only in conditions of elevated zinc need or low zinc intake.

Key words: zinc metabolism, zinc status, folate, folacin

INTRODUCTION

Both zinc and folic acid are essential for the survival and well-being of all animals. However, there is mounting evidence, despite some controversy, of metabolic interactions between these two nutrients. In an earlier study, we found decreased urinary zinc and increased fecal zinc in men fed a diet marginal in zinc when supplemented with 400 μg of folic acid per day [1]. This suggested that folic acid supplements impaired zinc absorption in these men. In a study with pregnant women, an increased incidence of complications at delivery and fetal complications were associated with high serum folate and low serum zinc concentrations [2]. Subsequently, Simmer et al. [3] demonstrated that folic acid and iron-folic acid supplements impaired the uptake in the blood of a 25 mg dose of zinc, and concluded that folic acid, along with iron, impaired zinc absorption. Similar results were reported by Wilson et al. [4] and Ghishan et al. [5], who showed decreased zinc absorption in rats, as measured by single-pass intestinal perfusion, when folic acid was added to the perfusion fluid, but not when folic acid was administered intraperitoneally. More recently, however, Keating et al. [6] were unable to find any inhibition of zinc utilization by folic acid in either a human study or in rats fed adequate levels of zinc.

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Some questions have been raised as to the validity of zinc tolerance tests that use unphysiologically high levels of oral zinc to produce a response [7]. In addition to a zinc dose that is much higher than normally consumed, the initial rate of zinc uptake from the proximal intestine may not reflect absorption from the entire length of the intestine, and the clearance of recently absorbed zinc into the tissues is not considered. The rate of gastric emptying can also influence the area and shape of the tolerance curve. This prompted further study on the effect of folic acid on the absorption of zinc using techniques that were more physiological, that is the absorption and retention of a tracer dose of ^{65}Zn from a breakfast meal.

MATERIALS AND METHODS

The effects of folic acid supplements on zinc absorption and retention were studied in seven men and six women between the ages of 20 and 46. During the study, they lived in the community and followed their usual dietary practices except that they were not taking any medications or dietary supplements except as provided by the study. The subjects entered the study after they had been informed in detail about the nature of the research, and clinical and nutritional evaluations had established that they had no underlying disease. This study was approved by the Institutional Review Boards of the University of North Dakota and the United States Department of Agriculture and followed the guidelines of the Department of Human Health and Human Services and the Helsinki Doctrine regarding human subjects.

The study was divided into two 8-week periods and one 10-week period. A breakfast meal, containing 0.1 μCi of ^{65}Zn, as $^{65}ZnCl_2$, in apple juice and 2.7 mg of zinc (Table I) was fed at the beginning of each of the first two periods and at the end of the second week of the third period. The subjects were then counted the

TABLE I. Test Breakfast Meal*

	Amounts in grams
Apple juice	80
(0.1 μCi ^{65}Zn as $ZnCl_2$)	
Milk, 2%	200
French toast	
Margarine	5
Eggs	60
Milk, 2%	30
Vanilla	2
Sugar	5
Cinnamon	0.2
Bread, white	40
Maple syrup	25
Pork sausage	
Pork	40
Bouillon	0.4
Deionized water	200 (rinse bottle)

*Contains 2.75 mg Zn (by analysis), and 67 μg folacin (by calculation).

same day as each meal and at weekly intervals for the next 8 weeks in a whole-body counter to determine the absorption and retention of the ^{65}Zn by a method described by Lykken [8]. The whole-body counts plotted against time typically gave a biphasic decay curve. A rapid drop in the first 7 days was primarily a result of passage of unabsorbed zinc through the intestinal tract. The slope of the slower component, between 1 week and 8 weeks after the labeled meal, was fed represented the turnover (or biological half-life) of absorbed zinc. Calculations of the ratio of the intercept of this slower component and the counts obtained shortly after the labeled meal gave the estimate of zinc absorption from the labeled meal.

An 800 μg folic acid supplement was consumed by each subject along with one of the first two labeled meals, but not with the other in a cross-over design. During the last 10 weeks of the study, which was period 3, the subjects ingested a daily supplement of 800 μg of folic acid, given as two 400 μg tablets. They were fed a labeled breakfast after 2 weeks of supplementation. Five of the subjects took their folate supplement during this meal, while eight did not, to see if the effect due to folate was a meal effect or related to folate concentrations in the tissues. Dietary intakes were estimated from 3-day dietary diaries, maintained by each subject at some point during the first period and reviewed by a dietitian upon completion.

Blood was drawn after a 10 hour fast prior to each meal to determine indices related to zinc and folate nutriture. Plasma zinc was determined by atomic absorption spectroscopy after dilution with deionized water [8]. Serum and erythrocyte folacin concentrations were determined by radioimmunoassay [9]. The significance of folate supplement effects was determined by repeated measures of analysis of variance, followed by Scheffé contrasts for differences between means [10].

RESULTS

Absorption of a tracer dose of ^{65}Zn from the labeled meal during the control phase, with no folate supplement, was between 10.8 and 61.4% (Table II). Overall, folate supplements did not seem to have an effect on zinc absorption. However, the effects of the folic acid supplements were directly related to the control absorption of zinc (Fig. 1). Subjects who absorbed higher percentages of zinc during control were affected by the folate supplement, while those with a lower control zinc absorption were not affected. Folic acid impaired zinc absorption in those individuals whose control zinc absorption was greater than 30% ($P < 0.05$) when 800 μg of folate was fed with the meal or supplemented daily for two weeks prior to the labeled meal. Folate did not significantly affect, or slightly enhance, zinc absorption in those individuals whose control absorption was less than 30%. Both acute and chronic folate supplements affected zinc absorption to a similar degree in the individuals who responded to the supplements. During chronic supplementation, subjects fed folate at the labeled meal responded similarly to those who did not receive folate with the meal. No differences were noted in the turnover (biological half-life) of zinc between control and folate supplemented periods.

In order to ascertain differences between those subjects whose zinc absorption seemed to be affected by folate supplements and those who were not, subjects

TABLE II. Effect of Folic Acid Supplements on the Absorption and Retention of Zinc†

Subject	Sex	Control	800 μg Folate with meal	Chronic folate supplement
% Zn^{65} absorbed from labeled meal				
A[a] 1	F	61.4	29.4	44.2[b]
2	M	53.7	47.2	41.3
3	F	42.2	26.3	23.5
4	F	37.7	20.8	25.0[b]
5	M	37.7	24.3	21.3[b]
6	M	30.7	26.9	33.2
7	F	30.6	43.6	21.7
8	M	30.2	20.1	24.6[b]
B 9	M	26.8	28.6	32.8
10	M	22.6	25.7	36.2[b]
11	F	22.2	9.8	22.9
12	F	14.9	26.2	19.5
13	M	10.8	20.9	16.3
Mean ± SD		32.4 ± 14.3	26.9 ± 9.7	27.9 ± 8.7
Mean A (8)		40.5 ± 11.5*	29.8 ± 10.1**	29.4 ± 9.1**
Mean B (5)		19.5 ± 6.5	22.2 ± 7.5	25.5 ± 8.6
Zn^{65} turnover (biological ½ life, days)				
Mean A		188 ± 52	—	209 ± 59
Mean B		212 ± 89	—	197 ± 59

†800 μg folate given daily for 10 weeks, labeled meal fed after two weeks of supplements.
[a]A, control absorption >30%; B, control absorption <30%.
[b]Subjects received folic acid supplement with labeled meal in the chronic folate supplement period.
*,**Means with different superscripts significantly different, $P < 0.05$.

were placed into two groups, based upon efficiency of zinc absorption during the control phase and their response to folate supplements: those who had a control zinc absorption above 30% and depressed zinc absorption caused by the folate supplements, and those whose control zinc absorption was below 30%. There was a nonsignificant ($P > 0.05$) tendency towards a lower dietary intake of zinc (10.8 ± 4.3 vs. 12.3 ± 2.0 mg/day) as estimated by 3-day diaries (Table III), in those who were affected by the supplements vs those who did not seem to respond to folate supplements. Plasma zinc seemed to be slightly lower in the responders than in nonresponders (Table IV, $P > 0.05$), whereas both serum and red cell folate concentrations tended to be slightly higher in the responders. Plasma zinc was not significantly ($P > 0.05$) affected by the chronic folate supplement. Both serum and red cell folate increased when the folate supplements were fed.

DISCUSSION

Results of this study may explain why an interactive effect of folate on zinc balance or absorption was observed in some studies [1–5,11,12], but not in others [6,11,13,14]. In this study, folic acid seemed to have an effect on zinc absorption in some of the subjects, but not in others. That the subjects with the highest control zinc absorption and a tendency towards lower dietary zinc intakes and

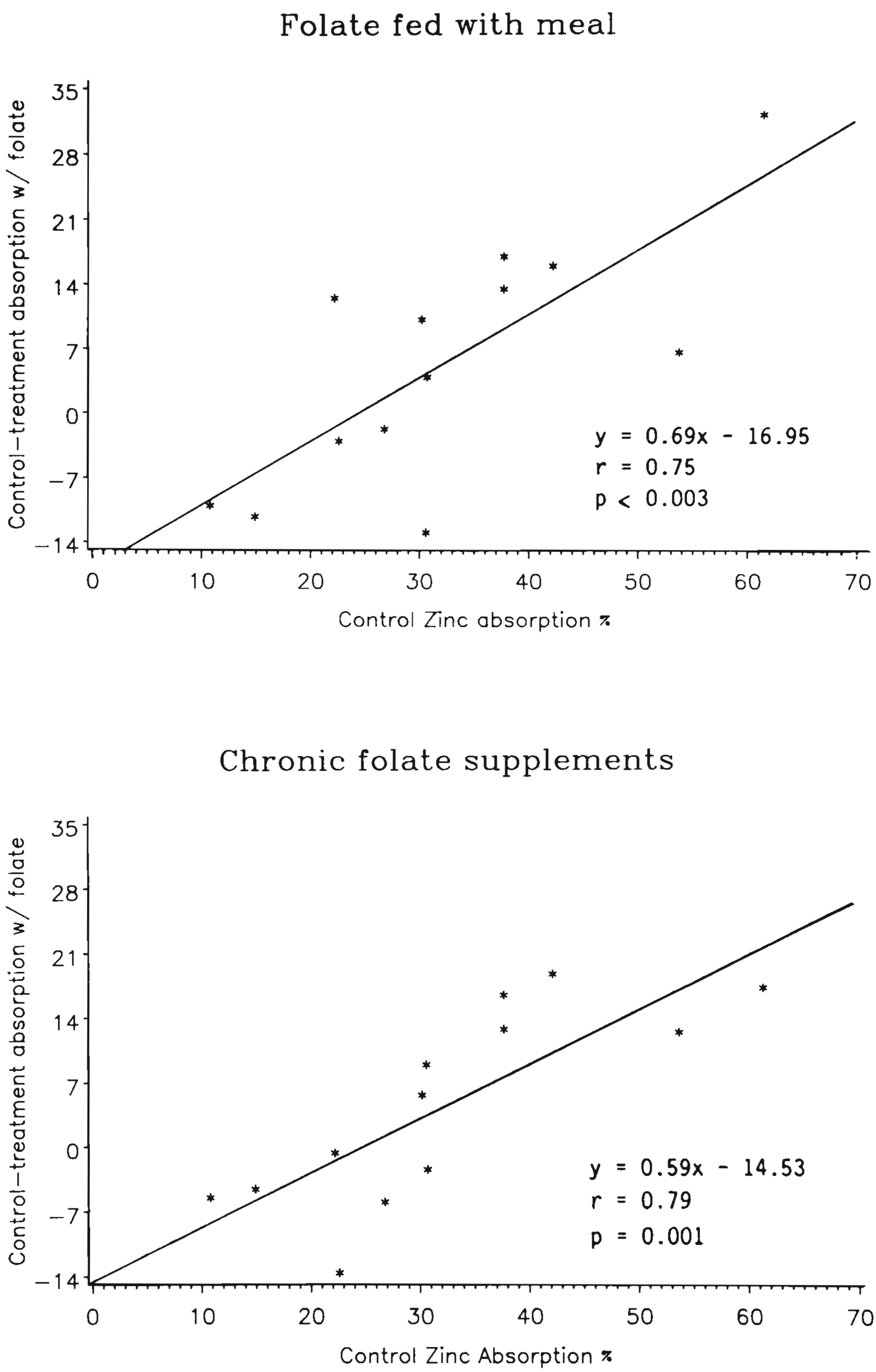

Fig. 1. Relationships between Zn-65 absorption during the control period and changes in zinc absorption caused by folic acid supplements.

TABLE III. Zinc Intake Estimated From 3-Day Food Diaries*

	Zn mg/day
A (8)	10.8 ± 4.2
B (5)	12.3 ± 2.0

*A, 8 subjects control ^{65}Zn absorption >30%; B, 5 subjects control ^{65}Zn absorption < 30%.

plasma zinc were affected by folate supplements, while those with the lowest control absorption and tendency towards higher zinc intakes and plasma zinc were not affected by the supplements suggests that this phenomenon is manifested primarily in conditions of greater zinc need. However, the possible effect that genetics may have on zinc absorption and on the effects of folic acid on zinc absorption and metabolism can not be ruled out. Gene effects on the requirements and metabolism of several nutrients are recognized, but poorly understood [15].

Folate effects on zinc metabolism have been noted in studies where marginal zinc was fed or in people who may have an increased need for zinc. Men fed a diet marginal in zinc (3.6 mg/day) had a reduction in urinary zinc and an increase in fecal zinc when fed supplements of 400 μg of folate every other day [1]. During pregnancy, there is an increased need for zinc to support the growing fetus [16]. Simmer et al. [3] demonstrated that a 350 μg/day folate supplement impaired zinc absorption in pregnant women. Mukherjee et al. [2] found that complications at delivery were associated with low plasma zinc and high plasma folate. Sandstead et al. [12] noted that folate supplementation of low income adolescents during pregnancy was associated with a decrease in plasma zinc that continued throughout pregnancy. Plasma zinc did not decrease after the twenty-fifth week of pregnancy if additional zinc was given. Healthy young men were unable to mobilize zinc into the plasma during strenuous exercise, a condition associated with depletion of zinc stores [17], after eating supplements of 400 or 800 μg of folic acid per day [11].

On the other hand, no effects of folic acid supplements were seen in people of adequate zinc status and who had adequate intakes of zinc. Keating et al. [6] were unable to find an effect of a 10 mg dose of folic acid on the serum uptake of a 25 mg dose of zinc in healthy college aged men. Butterworth et al. [13] reported no changes in erythrocyte zinc in patients who were undergoing long-term folic acid

TABLE IV. Blood Nutrient Levels*

	Zn (μg/dl)		Serum folate (ng/ml)		Erythrocyte folate (ng/ml)	
	Plasma control	Chronic folate[a]	Control	Chronic folate	Control	Chronic folate
A	77.3 ± 5.9	80.5 ± 7.3	4.8 ± 3.4	10.4 ± 2.1	254 ± 74	284 ± 123
B	80.8 ± 9.9	83.4 ± 6.9	3.7 ± 0.8	9.5 ± 1.4	192 ± 86	268 ± 15

*A, 8 subjects control ^{65}Zn absorption > 30%; B, 5 subjects control ^{65}Zn absorption < 30%.
[a]Blood values obtained after 10 weeks of 800 μg folic acid per day supplementation.

therapy and concluded that folic acid had no effect on zinc status. No other zinc status indices were measured in this study. In a companion study, we noted that supplements of 400 or 800 μg of folic acid had no effect on zinc balance or on the usual measures of zinc status in men fed 12.6 mg of zinc per day [11]. However, as noted above, these men had an impaired ability to mobilize zinc during heavy exercise.

Studies of a zinc-folate acid interaction using rat models have been inconclusive. Wilson et al. [4] and Ghishan et al. [5] demonstrated decreased zinc absorption in rats, as measured by single-pass intestinal perfusion, when folic acid was added to the perfusion fluid. However, no effect of folic acid on zinc absorption or utilization could be demonstrated in intact rats by others [6,14] (DB Milne and PE Johnson, unpublished observations). It is possible that a rat is better able to counteract an interlumen interaction between zinc and folic acid than humans [14], and thus may not appropriate model for evaluating interactions that are to be extrapolated to humans.

A sixfold range of zinc absorption from identical meals by the subjects during the control phase suggested differences in zinc status or need, even though there were no overt differences in plasma zinc or in estimated zinc intakes. No differences in absorption were noted between men or women. Weigand and Kirchgessner [18] demonstrated that zinc absorption is increased in states of zinc deficiency and conversely, is decreased when zinc status is elevated above normal levels. More recent studies [19–21] have indicated that zinc absorption is directly related to the dose, and inversely related to recent or usual intakes of zinc. In this study, the zinc content of the labeled meal was constant for all subjects and meals, and there were only subtle differences in reported usual intakes.

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