INTERACTION AND BIOAVAILABILITY OF IRON, ZINC, AND MAGNESIUM[1]

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ABSTRACT

Interaction and bioavailability of iron, zinc, and magnesium was assessed using young rats fed diets containing the required levels (iron, 25 ppm; zinc, 12 ppm; magnesium, 400 ppm) and half the required levels of each of these minerals. Growth appeared to be maximum on all diets, and although diet efficiency reflected slightly on mineral bioavailability and interaction, concentration of minerals in sensitive tissues and their absorption furnished more convincing evidence of bioavailability and interaction. Bioavailability of test minerals, especially of iron and zinc, increased appreciably with raised dietary levels. Although some interaction between minerals was also observed and in spite of complexity of interpretation, the magnitude of mineral interaction appears to be of little physiologic significance when sources of high bioavailability are used.

Evidence suggesting prevalence of iron (Fe), zinc (Zn), and magnesium (Mg) deficiency in the diet of a sizable segment of the U.S. population has led to the suggestion (1) that cereal grain products be fortified with additional Fe and with Zn and Mg using sources of high bioavailability (2–4). Even when using Fe, Zn, and Mg sources showing high bioavailability, however, possible interaction between these and other minerals might occur, affecting their bioavailability. Such a possibility was examined in these studies.

MATERIALS AND METHODS

Sprague-Dawley (weanling, male) rats averaging about 45 g of body weight initially were housed individually in mesh-bottom stainless steel cages and offered diets (premixed with water to minimize spillage) and deionized water ad libitum for four weeks (eight rats per diet). Gain in body weight and diet consumption records were maintained.

Table I lists the composition of basal (deficient in Fe, Zn, and Mg, diet A) and eight test diets (B–I). Test diets were formulated to contain Fe, Zn, and Mg at the required (5) and half the required levels each. All diets contained 500 mg each of calcium (Ca) and phosphorous (P).

Assessment of bioavailability was based on growth response, diet efficiency, apparent absorption (feces collected quantitatively throughout studies), and retention of minerals in sensitive tissues. Fe, Zn, Mg, and Ca in diet, dried and pulverized feces, blood serum (blood collected by heart puncture), bone (femur), and liver were determined by atomic absorption spectrophotometry (6) using an IL model 251 spectrophotometer (Instrumentation Laboratory Inc.). Sample preparations and bone ash determinations were performed as described earlier (3,4,7). Inorganic P in the serum was determined by Fiske and Subbarow's (8) method. Total P in the diet and in femur and liver was determined by the standard AOAC method (9). Hemoglobin (Hb) was determined by the cyanomethemoglobin method using tail blood (10).

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RESULTS AND DISCUSSION

A number of dietary and nondietary factors affect the bioavailability of minerals. In these studies, however, only the effect of possible interaction between certain minerals was studied. These minerals were provided at the required levels (Fe, 25 ppm; Zn, 12 ppm; Mg, 400 ppm) and half the required levels of each (5). These widely different levels were chosen to enable detection of possible mineral interaction.

Animals on the basal diet (A) did not show any visible symptoms of mineral deficiency in four weeks. The Fe, Zn, and Mg levels in diet A, however, were still above the levels needed (2–4) to induce mineral deficiency. Biochemical (Tables II–IV) and other (Table V) parameters on diet A, however, were sufficiently affected in four weeks, suggesting gross inadequacy of minerals in the basal diet. In rats fed mineral-supplemented diets (diets B–I) as compared with diet A, growth rate and diet efficiency (Table V) improved strikingly and significantly ($P < 0.01$).

Although differences in diet/gain ratios observed with mineral-supplemented diets suggested possible mineral interaction, tissue concentration (Tables II–IV) and absorption (Table V) of minerals furnished more convincing evidence of mineral interaction. For example, inadequacy of dietary Fe resulted in significantly ($P < 0.01$) low Hb (percent and total) levels (diets F–I versus B–E). Wide differences in Hb concentration (Table II) and Fe absorption (Table V), especially on low-Fe diets (diets F–I), also became apparent, suggesting possible interaction of Fe with other minerals. Although the extent of involvement of other minerals in this interaction is difficult to assess, simultaneous administration of Fe and Zn has been reported (11) to impair the use of minerals in children.

Like Hb, serum Fe levels also were significantly ($P < 0.01$) low on low-Fe diets (Table II). Wide variations in serum Fe levels again suggested possible mineral interaction, but complexity of interaction involving more than two minerals simultaneously precludes a simple interpretation. Liver Fe levels tended to be low on low-Fe diets, but no consistent pattern emerged (Table IV). Hb levels are

TABLE I
Composition of Test Diets[a,b]

	Diet								
	A	B	C	D	E	F	G	H	I
Fe (mg/100 g)	0.616	2.5	2.5	2.5	2.5	1.25	1.25	1.25	1.25
Zn (mg/100 g)	0.060	1.2	1.2	0.6	0.6	1.2	1.2	0.6	0.6
Mg (mg/100 g)	18.45	40.0	20.0	40.0	20.0	40.0	20.0	40.0	20.0

[a] All diets contained 20 g of egg albumin, 2 g of vitamin diet fortification mixture (ICN Pharmaceuticals) (0.044 mg of biotin), 2 g of alphacel (nonnutritive fiber from ICN Pharmaceuticals), 4 g of corn oil, 1 g of NaCl, 0.3432 g of KCl, 1.2314 g of $CaCO_3$, 1.8819 g of $NaH_2PO_4 \cdot H_2O$, trace mineral mixture (in starch base and containing 0.5 mg of Cu, 5 mg of Mn, 0.015 mg of I), and wheat starch, to make a total of 100 g. All diets also contained 0.5 g% each of Ca (intrinsic, 0.0069 g%) and P (intrinsic, 0.0775 g%).

[b] All diets, except diet A (basal) contained additional Fe ($FeSO_4$), Zn (ZnO), and Mg (MgO) to obtain desired levels as indicated.

TABLE II
Hemoglobin Concentration and Serum Minerals (Four-Week Experiment)[a]

	Diet								
	A	B	C	D	E	F	G	H	I
Hb[b] (g%)	13.68 ± 1.36	14.13 ± 0.80	13.98 ± 0.63	13.99 ± 0.76	14.57 ± 0.86	11.08 ± 0.36	10.35 ± 0.54	10.31 ± 0.71	9.50 ± 0.40
Hb-total[c] (g)	0.47 ± 0.04	1.30 ± 0.13	1.36 ± 0.20	1.20 ± 0.12	1.25 ± 0.14	1.02 ± 0.11	0.93 ± 0.12	0.88 ± 0.09	0.83 ± 0.11
Fe (μg/dl)	...	212 ± 34	238 ± 12	191 ± 63	222 ± 40	129 ± 26	80 ± 22	85 ± 19	135 ± 33
Zn[d] (μg/dl)	87 ± 4	149 ±17	168 ±20	151 ±28	155 ±25	170 ±17	168 ±20	115 ±12	118 ±22
Mg[d] (mg/dl)	1.45 ± 0.23	1.44 ± 0.19	1.37 ± 0.35	1.50 ± 0.21	1.23 ± 0.10	1.22 ± 0.29	1.31 ± 0.36	1.30 ± 0.20	1.32 ± 0.27
Ca[d] (mg/dl)	9.8 ± 0.5	10.1 ± 0.8	11.3 ± 1.8	11.1 ± 1.9	9.8 ± 0.7	10.7 ± 1.0	9.5 ± 1.2	9.9 ± 0.9	9.9 ± 0.8
P (mg/dl)	...	7.6 ± 0.8	8.2 ± 0.9	8.0 ± 0.6	7.6 ± 0.7	9.3 ± 1.0	8.6 ± 0.8	7.6 ± 0.3	9.1 ± 1.4

[a]Values represent mean (eight rats per diet) ± SD.
[b]Initial (0-day) hemoglobin value: 13.29 ± 0.83.
[c]Calculated based on blood volume of rats and their Hb concentration.
[d]Initial (0-day) serum values: Zn, 111 ± 7; Mg, 1.68 ± 0.17; Ca, 10.3 ± 0.7.

TABLE III
Femur Weight and Minerals (Four-Week Experiment)[a,b]

	Diet								
	A	B	C	D	E	F	G	H	I
Weight (mg)	103 ± 6	214 ±18	221 ±23	204 ±19	198 ±15	211 ±21	212 ±14	199 ±22	204 ±15
Ash (%)	52.4 ± 1.4	57.1 ± 1.0	58.7 ± 0.6	58.3 ± 1.0	58.9 ± 0.9	58.1 ± 0.9	58.5 ± 0.7	58.4 ± 1.1	58.7 ± 0.9
Zn (μg)	7.30 ± 0.96	45.73 ± 3.37	47.27 ± 5.98	23.20 ± 1.73	22.40 ± 2.13	43.90 ± 4.70	46.97 ± 3.51	22.60 ± 3.39	29.57 ± 2.91
Mg (mg)	0.46 ± 0.05	0.82 ± 0.05	0.74 ± 0.13	0.77 ± 0.08	0.61 ± 0.08	0.69 ± 0.13	0.68 ± 0.09	0.72 ± 0.09	0.66 ± 0.04
Ca (mg)	22.1 ± 1.6	45.9 ± 5.5	48.4 ± 5.9	44.6 ± 4.1	43.4 ± 3.5	44.6 ± 5.1	46.1 ± 3.1	39.8 ± 5.4	43.7 ± 2.9
P (mg)	10.4 ± 0.8	23.1 ± 2.4	25.5 ± 3.0	23.2 ± 2.1	22.0 ± 2.1	23.0 ± 3.0	23.3 ± 1.7	23.0 ± 2.6	22.3 ± 1.8

[a]Values represent mean (eight rats per diet) ± SD.
[b]Initial (0-day) femur values: Weight, 42 ± 3; ash, 41.6 ± 0.8; Zn, 9.47 ± 1.03; Mg, 0.24 ± 0.06; Ca, 6.8 ± 0.5; P, 3.8 ± 0.4.

TABLE IV
Liver Weight and Minerals (Four-Week Experiment)[a,b]

	Diet								
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Weight (g)	1.68 ± 0.14	5.15 ± 0.54	5.19 ± 0.66	5.57 ± 0.97	5.27 ± 0.48	5.21 ± 0.52	4.99 ± 0.74	4.98 ± 0.33	5.55 ± 0.57
Fe (μg)	116 ±31	382 ±60	393 ±59	395 ±56	432 ±76	393 ±84	330 ±54	313 ±52	334 ±74
Zn (μg)	32 ± 4	107 ± 7	113 ± 9	108 ±17	98 ± 7	107 ± 8	113 ±13	94 ± 6	109 ±11
Mg (μg)	353 ±36	982 ±94	1023 ±139	963 ±119	1033 ±71	1042 ±87	1011 ±101	1006 ±74	1023 ±163
Ca (μg)	139 ±29	241 ±45	427 ±85	339 ±77	325 ±41	259 ±54	242 ±51	463 ±71	331 ±123
P (mg)	5.89 ± 0.31	16.52 ± 1.80	16.47 ± 1.22	17.13 ± 2.87	15.70 ± 1.30	16.16 ± 1.35	15.40 ± 1.74	14.67 ± 1.35	15.23 ± 1.68

[a]Values represent mean (eight rats per diet) ± SD.
[b]Initial (0-day) liver values: Weight, 1.02 ± 0.09; Fe, 108 ± 14; Zn, 46 ± 5; Mg, 221 ± 22; Ca, 59 ± 12; P, 3.72 ± 0.33.

TABLE V
Apparent Absorption of Minerals (Four-Week Period)

	Diet								
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Weight gain (g)	14 ± 3	99 ±10	107 ±18	91 ±13	91 ± 9	99 ±11	95 ±12	90 ± 8	92 ±13
Diet/gain, ratio	8.72 ± 1.71	2.48 ± 0.18	2.39 ± 0.24	2.52 ± 0.20	2.49 ± 0.14	2.48 ± 0.15	2.57 ± 0.25	2.58 ± 0.08	2.67 ± 0.34
Fe absorbed (μg)	361 ±55	3783 ±307	4182 ±397	3772 ±339	3802 ±350	2024 ±90	2073 ±129	1914 ±386	1837 ±330
Fe absorbed (%)	49.0 ± 5.4	63.3 ± 5.7	65.9 ± 3.3	66.7 ± 4.7	67.9 ± 5.0	68.1 ± 6.5	68.2 ± 4.2	65.3 ±10.7	60.8 ±10.8
Zn absorbed (μg)	23 ± 8	2414 ±179	2486 ±227	1232 ±95	1202 ±82	2422 ±203	2371 ±180	1241 ±95	1216 ±100
Zn absorbed (%)	32.1 ±11.0	82.8 ± 2.9	81.6 ± 3.9	90.7 ± 3.1	89.5 ± 2.2	84.3 ± 2.5	81.2 ± 5.6	89.3 ± 2.1	83.8 ± 6.0
Mg absorbed (mg)	15.73 ± 1.70	83.15 ± 7.35	42.73 ± 3.24	79.73 ± 6.30	36.41 ± 2.02	79.43 ± 8.29	40.17 ± 2.41	78.03 ± 8.07	38.99 ± 1.79
Mg absorbed (%)	71.5 ± 6.7	85.5 ± 4.1	84.2 ± 3.0	88.0 ± 3.9	81.4 ± 3.7	82.8 ± 3.6	82.5 ± 4.5	84.1 ± 4.2	80.6 ± 3.7
Ca absorbed (mg)	445 ±42	1053 ±69	1089 ±87	999 ±77	963 ±56	1015 ±97	1034 ±60	981 ±92	1016 ±55
Ca absorbed (%)	76.2 ± 5.6	86.7 ± 2.1	85.8 ± 3.3	87.3 ± 3.5	86.2 ± 2.6	84.8 ± 2.2	85.0 ± 4.6	84.7 ± 3.6	84.0 ± 2.3
P absorbed (mg)	520 ±31	1143 ±75	1191 ±102	1061 ±83	1041 ±61	1102 ±99	1136 ±51	1072 ±89	1131 ±52
P absorbed (%)	87.2 ± 3.1	94.2 ± 1.3	94.0 ± 2.5	93.7 ± 2.4	93.1 ± 2.5	92.0 ± 1.1	93.3 ± 2.4	92.6 ± 1.5	93.5 ± 1.1

routinely used to assess Fe bioavailability (2,12). Thus, based on Hb levels, one may conclude that in spite of possible interaction with other minerals, additional Fe (diets B–E versus F–I) became quite available.

As results in Table III seem to suggest, total femur Zn is reported (13) as the best indicator of Zn status and hence most suitable for Zn bioassay. High-Zn diets (B, C, F, G) caused a significant ($P < 0.01$) increase, about twofold, in femur Zn as compared with the low-Zn diets (D, E, H, I). Among the low-Zn diets, Zn deposition in the femur was significantly ($P < 0.01$) higher when dietary Fe and Mg levels were low, also (diet I) suggesting possible interaction between these minerals. Serum Zn levels (Table II) were significantly ($P < 0.01$) low on two low-Zn diets (diets H and I). Perhaps some interaction is involved, but its elucidation is difficult based on the present data. In agreement with the results of Murthy and Petering (14), a direct relationship between serum Hb and Zn levels was observed on two low-Zn diets (H and I, Table II). No relationship between liver, femur, or serum Zn levels was found (Tables II–IV). Since skeletal Zn (as femur levels suggest) represents the major concentration of Zn in the body, one may infer, based on this, that additional dietary Zn became greatly available in spite of some interaction occurring.

As was observed earlier (4), femur Mg level also appears to be a better index of Mg bioavailability than does serum or liver Mg levels (Table III). Mg concentration in the femur was high on all high-Mg diets (B, D, F, H), being significantly ($P < 0.01$) so for three of these as compared with low-Mg diets (C, E, G, I). For two high-Mg diets (B and D), serum Mg levels were also significantly ($P < 0.01$) higher (Table II). As for liver Fe and Zn, no consistent pattern emerged for liver Mg levels (Table IV).

Although all diets contained the same level of Ca and P, significant ($P < 0.01$) differences were observed in the serum levels of these minerals (Table II). For Ca but not for P, significant ($P < 0.01$) differences in the femur and liver levels also were found (Tables III and IV). The significance of this, because of the greatly involved nature of mineral interaction, is difficult to ascertain.

Table V shows the apparent absorption of minerals. Urinary losses were not measured, and hence true absorption could not be calculated. Because of the rapid growth of animals and limited availability of test minerals in their diet, mineral absorption tended to be quite high. Except for Fe, more than three-fourths of the dietary amounts of minerals were absorbed. Percentage absorption of Zn, but not of Fe or Mg, increased significantly ($P < 0.01$) when the diet was low in Zn as reported also by Evans et al (15).

Although arriving at a definite conclusion is difficult because of the complexity of many nutrient interactions involved, results (Table II–V) indicate the magnitude of mineral interaction to be of limited physiologic consequence.

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