Bioavailability of Iron, Magnesium, Zinc, and Calcium in Commercially Produced Citrate Phosphate Complexes[1,2]

G. S. RANHOTRA, C. LEE, J. A. GELROTH, G. L. WINTERRINGER, and F. A. TORRENCE, Nutrition Research, American Institute of Baking, Manhattan, KS 66502

ABSTRACT

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The bioavailability of four minerals (Fe, Mg, Zn, and Ca) in commercially produced mineral complexes was determined by animal experimentation. For Fe, the determination was based on the standard hemoglobin depletion-repletion technique. The retention of minerals in bone (femur) was used as the test criterion to assess the bioavailability of the other three minerals. Compared to reference compounds recognized as high in "available" mineral content, the relative bioavailabilities of the four mineral complexes were found to be 101% for Fe (as Bio-Plex Fe), 88% for Mg (as Bio-Plex Mg), 84% for Zn (as Bio-Plex Zn), and 62% for Ca (as Bio-Plex Ca).

In choosing mineral complexes as supplements for fortification of food products, cost and product compatability are the obvious major considerations (Austin 1979). However, nutritional efficacy (mineral bioavailability, for example) of the supplements is also becoming an important consideration.

The processing technology involved in commercially producing mineral supplements and the necessity to include (in the complex) certain functional ingredients may affect the bioavailability of minerals even if compounds with established high bioavailabilities are initially used. In the present studies, we examined the bioavailability (relative to a reference standard) of certain minerals in commercially produced mineral complexes intended for use in cereal-based and other food products.

MATERIALS AND METHODS

The major constituents of four mineral complexes (R. G. B. Laboratories, Kansas City, MO) are presented in Table I. These are mineral-containing water-soluble polymeric complexes composed of monopotassium citrate and phosphate anions bound by ionic types of attractions to the relevent elements. Four basal diets (Table II) were formulated to assess the bioavailability of minerals in these complexes. The sucrose in the basal diets was replaced with appropriate amounts of complexes (and reference compounds) to make test diets (Tables III–VI).

Bioavailability was studied in male weanling rats (Sprague-Dawley) initially weighing an average of 50 g. The animals were housed individually in mesh-bottomed stainless steel cages under a controlled environment. Diet and deionized water were offered (8–10 rats per diet) ad libitum for two weeks for the Fe experiment

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TABLE I
Composition of Mineral Complexes

Major Constituents (%)	Complexes[a] Bio-Plex Fe	Bio-Plex Mg	Bio-Plex Zn	Bio-Plex Ca
Mineral				
Fe, Mg, Zn, or Ca	16.51	5.49	8.41	11.19
K	17.25	12.17	22.16	13.19
P	11.57	4.36	2.28	5.83
Citrate	39.26	33.78	51.25	28.40
Carbohydrates	···	20.25	···	21.31
Others (unspecified)	15.41	23.95	15.90	20.08

[a]R.G.B. Laboratories, Kansas City, MO. (U.S. Patent: 4,214,996).

TABLE II
Composition of Basal Diets

Ingredients (g)	Experiment Iron[a]	Magnesium	Zinc	Calcium
Egg albumin	···	···	20	···
Casein	20	20	···	20
Vitamins[b]	2.2	2.2	2.2	2.2
Non-nutritive fiber	···	2.0	2.0	2.0
Trace minerals[c]	1.0	1.0	1.0	1.0
Corn oil	5.0	4.0	4.0	4.0
NaCl	0.5	1.0	1.0	1.0
KCl	0.5	0.36	0.36	0.36
$NaH_2PO_4 \cdot H_2O$	2.0	1.09	1.67	1.10
Ca carbonate	2.0	···	···	···
Ca sulfate	···	2.12	2.09	···
dl-Methionine	0.1	···	···	···
Sucrose	66.70	66.23	65.68	68.34
Test mineral[d] (mg/100 g)	0.2	0.6	0.06	5.6

[a]Standard AOAC (1975) diet except that cornmeal and gelatin were not added.
[b]Vitamin diet fortification mixture from ICN Pharmaceuticals. Extra biotin (0.4 mg) added to Zn diet.
[c]To meet requirements (NAS/NRC 1978) but excluding the test mineral. Minerals included (sucrose base) are: Fe, Zn, Cu, Mn, Mg, and I.
[d]By analysis.

or four weeks for the Mg, Zn, and Ca experiments.

For the Fe experiment, rats were first made anemic (AOAC 1975) on the low-iron diet. When their hemoglobin (Hb) levels dropped to an average of 6 g/dl, Hb repletion studies (Table III) were initiated. Bioavailability was assessed, based on the extent of repletion of Hb. Hb was measured (on tail blood) by the cyanmethemoglobin method (Crosby et al 1954). For the other experiments, the concentration of minerals in bone (femur) was used as the test criterion to assess bioavailability. Minerals in diets, femur, and blood serum (blood collected by heart puncture) were determined by atomic absorption spectrophotometry as described earlier (Ranhotra et al 1976, 1977, 1979, 1980a, 1980b). Femurs were ether-extracted (Ca experiment only) and vacuum-dried (all experiments) before being weighed. Some statistical analysis of the data was also undertaken.

RESULTS AND DISCUSSION

The bioavailability of potential Fe sources differs appreciably (Ranhotra et al 1971). Such does not appear to be the case for

TABLE III
Bioavailability of Iron in Bio-Plex Fe[a,b]

	Fe Source and Diet						
	None	Ferrous Sulfate[c]			Bio-Plex Fe		
	A	B	C	D	E	F	G
Dietary Fe (ppm)	···	6	12	24	6	12	24
Body weight gain (g)	51 ± 18	79 ± 9	82 ± 11	91 ± 24	65 ± 20	79 ± 8	89 ± 11
Diet intake (g)	171 ± 30	200 ± 13	201 ± 17	215 ± 18	186 ± 24	194 ± 22	210 ± 11
Hemoglobin[d] (g/dl)	5.16 ± 0.60	7.19 ± 0.88	8.79 ± 0.68	11.64 ± 1.14	7.14 ± 0.67	8.85 ± 0.76	11.50 ± 0.98
Relative bioavailability[e]	···	←	100	→	←	101	→

[a]Two-week experiment.
[b]All values represent average of eight rats ± SD.
[c]Chemical grade (Fe, 29.9%).
[d]At day 14 (end of repletion phase).
[e]Method of AOAC (1975).

TABLE IV
Bioavailability of Magnesium in Bio-Plex Mg[a,b]

	Mg Source and Diet						
	None	Carbonate[c]			Bio-Plex Mg		
	A	B	C	D	E	F	G
Dietary Mg (mg/100 g)	···	5	10	20	5	10	20
Body weight gain (g)	75 ± 3	123 ± 12	143 ± 9	142 ± 14	104 ± 10	119 ± 15	136 ± 13
Diet intake (g)	246 ± 17	327 ± 20	353 ± 19	333 ± 26	292 ± 21	336 ± 18	338 ± 37
Mg intake (mg)	···	16.4 ± 1.0	35.3 ± 1.9	66.6 ± 5.3	14.6 ± 1.1	33.6 ± 1.8	67.7 ± 7.3
Serum Mg (mg/dl)	···	0.68 ± 0.11	1.03 ± 0.19	0.93 ± 0.04	0.63 ± 0.07	0.56 ± 0.10	0.81 ± 0.24
Femur weight[d] (mg)	277 ± 3	339 ± 25	329 ± 37	348 ± 32	303 ± 22	326 ± 22	341 ± 14
Femur Mg (total, mg)	0.258 ± 0.010	0.401 ± 0.078	0.477 ± 0.108	0.596 ± 0.073	0.319 ± 0.028	0.413 ± 0.083	0.513 ± 0.107
Relative bioavailability[e]	···	100[f]	100[f]	100[f]	89.4[g]	91.0[g]	84.7[g]

[a]Four-week experiment.
[b]All values represent average of seven to nine rats ± SD; for diet A, average of three rats.
[c]Chemical grade (Mg, 23.7%).
[d]Moisture-free basis.
[e]For comparable diets: availability = [total femur Mg (mg)/Mg (mg) intake] × 100.
[f]Standard, average of diets B–D.
[g]Average of diets E–G was 88.4.

TABLE V
Bioavailability of Zinc in Bio-Plex Zn[a,b]

	Zn Source and Diet						
	None	Carbonate[c]			Bio-Plex Zn		
	A	B	C	D	E	F	G
Dietary Zn (ppm)	···	3	6	9	3	6	9
Body weight gain (g)	55 ± 18	87 ± 16	102 ± 8	102 ± 12	86 ± 12	96 ± 11	109 ± 19
Diet intake (g)	132 ± 34	144 ± 29	161 ± 18	207 ± 18	169 ± 26	204 ± 19	220 ± 30
Zn intake (mg)	···	0.431 ± 0.088	0.963 ± 0.109	1.863 ± 0.162	0.508 ± 0.078	1.225 ± 0.144	1.977 ± 0.270
Serum Zn (μg/dl)	83 ± 18	98 ± 37	127 ± 24	151 ± 13	95 ± 18	129 ± 19	114 ± 22
Femur weight[d] (mg)	226 ± 20	290 ± 36	276 ± 19	289 ± 36	267 ± 19	284 ± 23	276 ± 31
Femur Zn (total, μg)	18.5 ± 3.2	31.9 ± 6.4	36.8 ± 5.6	40.4 ± 4.8	25.4 ± 3.5	37.9 ± 7.6	38.4 ± 8.1
Log of femur Zn	···	1.504	1.566	1.606	1.405	1.579	1.584
Relative bioavailability[e]	···	100[f]	100[f]	100[f]	79.3[g]	79.3[g]	92.9[g]

[a]Four-week experiment.
[b]All values represent average of seven to nine rats ± SD.
[c]Chemical grade (Zn, 51.9%).
[d]Moisture-free basis.
[e]For comparable diets: availability = [Log μg Zn per femur/Zn (mg) intake] × 100; Atwal et al 1980.
[f]Standard, average of diets B–D.
[g]Average of diets E–G was 83.8.

TABLE VI
Bioavailability of Calcium in Bio-Plex Ca[a,b]

	Ca Source and Diet						
	None	Sulfate[c]			Bio-Plex Ca		
	A	B	C	D	E	F	G
Dietary Ca (mg/100 g)	···	100	200	300	100	200	300
Body weight gain (g)	121±6	133±8	139±18	139±9	138±14	150±14	156±10
Diet intake (g)	306±22	302±18	312±29	304±20	305±24	300±23	322±15
Ca intake (mg)	···	302±18	623±58	913±61	305±24	600±46	966±45
Serum Ca (mg/dl)	8.8±0.7	9.5±0.6	9.7±0.6	10.2±0.6	9.7±0.4	10.2±0.5	10.2±0.5
Femur weight[d] (mg)	158±9	205±14	217±18	228±14	189±15	197±17	209±15
Femur ash (%)	35.0±1.1	45.1±1.9	47.3±1.5	50.4±1.6	43.5±1.1	43.8±0.7	44.7±1.9
Femur Ca (total, mg)	17.7±1.3	30.7±2.9	34.4±3.6	39.6±3.5	26.7±2.1	28.1±2.6	30.0±3.2
Relative bioavailability[e]	···	100[f]	100[f]	100[f]	68.5[g]	64.7[g]	53.1[g]

[a] Four-week experiment.
[b] All values represent average of seven to nine rats ± SD.
[c] Chemical grade (Ca, 22.9%).
[d] Fat-free, moisture-free basis.
[e] For comparable diets, based on increase in total femur Ca over diet A: availability = [total femur Ca (mg) increase/Ca (mg) intake] × 100.
[f] Standard, average of diets B–D.
[g] Average of diets E–G was 62.1.

potential Mg (Ranhotra et al 1976), Zn (Ranhotra et al 1977), and Ca (Ranhotra et al 1980b) sources, however. Thus, except for ferrous sulfate, the specified (AOAC 1975) reference compound for the Fe experiment, reference compounds for the other three experiments (Tables IV–VI) were chosen arbitrarily. Also, all test diets were formulated to contain submarginal (NAS/NRC 1978) levels of these minerals to accentuate detection of differences, if any, in bioavailability.

Bioavailability of Fe in Bio-Plex Fe

During the repletion phase (Table III), while rats on the basal diet (diet A) became even more anemic (Hb below 6 g/dl), those on test diets showed a rapid regeneration of Hb; on comparable diets (B and E, C and F, D and G), the Hb levels were almost identical. Because diet, and hence Fe, intake on comparable diets did not differ significantly, similarity of the Hb values suggests that Fe in Bio-Plex Fe was as available as that in ferrous sulfate. Calculated by the standard method (AOAC 1975), using ferrous sulfate as 100, relative bioavailability of Fe in Bio-Plex Fe was 101%.

Bioavailability of Mg in Bio-Plex Mg

Compared to the basal diet (Table IV, diet A), the addition of Mg (diets B–G) significantly ($P < 0.01$) improved the responses (weight gain, femur weight, femur Mg content) of rats during the four-week experiment. In addition, it effectively controlled the high incidence of mortality observed on the basal diet. The responses, however, suggest that Mg in Bio-Plex Mg was somewhat less available than that in the reference compound (Mg carbonate). Growth response and serum Mg levels have been used as indicators of bioavailability, but they appear less suited because of the nonlinearity of response to increasing dietary Mg levels (Lo et al 1980). On the contrary, the degree of correlation between femur Mg and dietary Mg is quite high (Lo et al 1980, Ranhotra et al 1980a). Thus, based on femur Mg content (adjusted for differences in Mg intake on comparable diets), the relative (Mg carbonate = 100) bioavailability of Mg in Bio-Plex Mg was 88% (average of values for the three Mg-supplement diets).

Bioavailability of Zn in Bio-Plex Zn

The addition of Zn (Table V, diets B–G) significantly ($P < 0.01$) improved the responses (weight gain, serum Zn level, femur weight, femur Zn level) of the rats compared to their performance on the basal diet (diet A). Comparable diets (B and E, C and F, D and G) suggest that Zn in Bio-Plex Zn was quite available. Femur Zn level is most sensitive to dietary Zn intakes (Ranhotra et al 1977) and has come to be recognized as the method of choice by which to assess bioavailability. Based on femur Zn values as described by Atwal et al (1980), the relative (Zn carbonate = 100) bioavailability of Zn in Bio-Plex Zn was 84% (average of values for the three Zn-supplement diets).

Bioavailability of Ca in Bio-Plex Ca

The responses (femur weight, femur ash, and Ca contents) of rats significantly ($P < 0.01$) improved as a result of the addition of Ca to the basal diet (Table VI). Such improvement was, however, significantly ($P < 0.05$) higher for the reference (Ca sulfate) than for the test Ca source. Serum Ca level is little affected (Clark 1969) by dietary Ca inadequacy; on the contrary, femur ash and Ca contents are most affected. Because over 99% of the Ca absorbed is deposited in the bone mass, bioavailability of Ca in test diets was calculated from the increase in femoral Ca content over that found in rats on the control diet. Such calculations revealed the relative (Ca sulfate = 100) bioavailability of Ca in Bio-Plex Ca to be 62% (average of values for the three Ca-supplement diets).

Thus, the data suggest that three of the four mineral complexes rate quite high in the content of "available" mineral while the "availability" of Ca in Bio-Plex Ca appears only fair to good.

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