

IDPAS # 140

Food iron absorption in man II. The effect of EDTA on absorption of dietary non-heme iron^{1, 2}

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ABSTRACT One hundred and eighty iron absorption tests were performed in 45 normal men to determine the effect of EDTA on the absorption of dietary non-heme iron. The addition of 50 mg EDTA to test meals containing 4.1 mg iron reduced absorption by approximately one-half from meals of both high (standard meal) and low (semisynthetic meal) iron availability. Studies employing dual radioiron labels demonstrated complete isotopic exchange of ferric EDTA with dietary non-heme iron. Further studies were carried out to determine the decrease in food iron absorption at varying levels of EDTA. At a 1:1 molar ratio of EDTA to iron, absorption of non-heme iron was reduced to 72% and at a 2:1 molar ratio, to 50% of absorption without EDTA. These levels of EDTA are within the range believed to be present in the United States diet. *Am J. Clin. Nutr.* 29: 614-620, 1976.

Chelates in the diet may have a major effect on the assimilation of polyvalent transitional cations such as iron. They can either enhance or reduce iron absorption depending on the stability constant, solubility at the intraluminal pH of the intestinal tract, and the ability of the iron-complex to penetrate the mucosal barrier. Certain chelates such as ascorbic acid enhance iron absorption by forming an iron ascorbate complex at low pH which remains soluble at the higher pH of the duodenum and donates its iron to the mucosal cell (1). The enhancing effect on iron absorption of certain amino acids such as cysteine and histidine (2, 3) and reducing sugars such as fructose (4, 5) has been similarly explained. Other chelates can reduce iron absorption either by forming complexes which are insoluble at the neutral pH of the small intestine, e.g., ferric phytate (6) or which, although stable, are very poorly absorbed, e.g., ferrioxamine (7, 8).

EDTA is the most widely used synthetic chelate of polyvalent cations incorporated into the diet in the United States. The purpose is to prevent oxidative damage to food by free metals. EDTA is allowed in certain foods at levels ranging from 25 to 800 mg/kg (9). For food items such as dressing, mayonnaise, sandwich spreads, and sauces, the amount of added EDTA is stated presently on the label but with other items such as

canned foods and vegetables, no statement of EDTA content is usually made. Perhaps the largest quantity of EDTA is employed in the manufacture of carbonated soft drinks, beer, and liquor although here in particular, the quantity employed is considered a trade secret by industry. In view of the widespread use of a chelate known to react with iron, the present study was undertaken to determine its effects on the absorption of food iron.

Methods

Absorption studies were performed in 45 healthy male subjects ranging in age from 18 to 33 years. Informed consent was obtained from all subjects prior to study. None of the subjects was anemic or iron deficient. Mean values on blood drawn from fasting subjects were as follows: hematocrit 46.6%, transferrin saturation 39%, and serum ferritin 61 ng/ml.

Iron absorption studies were performed with two basic types of test meals which have been described fully in

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²Supported by Public Health Service Research Grant HL-06242, National Institutes of Health, Bethesda, Maryland, and the Department of Agriculture Grant [12-14-1001-27 and 12-14-100-0018 (61)].

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previous report (10). The first of these was a standard (STD) meal which was considered to represent a typical American dinner. This meal, which contained lean beef, cornmeal, potatoes, bread, margarine, peaches, and ice milk, was prepared and served as it would normally be eaten. The total caloric and iron content of the meal was 700 cal and 4.1 mg iron, respectively.

The second basic test meal was a semisynthetic (SS) meal prepared from purified dietary components in amounts equal to the total contained in the STD meal. Carbohydrate, fat, and protein were supplied as dextrimaltose, corn oil, and egg albumin, respectively. Calcium and phosphorus were added as inorganic salts (dibasic calcium phosphate and dibasic potassium phosphate). Iron as ferric chloride was added to bring the total iron content to 4.1 mg. In certain studies, iron absorption was also measured from a standard reference (REF) dose of inorganic iron containing 3 mg iron as ferrous sulphate and 2 moles of freshly prepared ascorbic acid per mole of iron dissolved in 50 ml iron-free water. By expressing iron absorption from the various test meals as a ratio of absorption from the REF, the effect of differences in iron status of the subjects can be largely eliminated (11).

The study was divided into 2 parts. In study 1 (19 subjects) the effect of EDTA⁵ on the absorption of non-heme iron from the STD and SS meal was determined. In study 2 (26 subjects) the effect of adding varying amounts of EDTA to the STD meal was examined. In both studies, four separate radioiron absorption tests were performed in each subject. The following general protocol was employed for these studies. Blood was initially obtained on the first morning of the study for measurement of background ⁵⁵Fe and ⁵⁹Fe. A test meal containing radioiron was then fed to fasting subjects between 8:00 and 9:00 AM and nothing further by mouth was allowed for 3 hr. In study 1, this meal was doubly labeled with ⁵⁵Fe and ⁵⁹Fe whereas in study 2, test meals separately labeled with ⁵⁵Fe and ⁵⁹Fe were administered on 2 successive days. Iron absorption was determined from the radioiron content of blood drawn 4 days later. Two additional test meals tagged with either ⁵⁵Fe or ⁵⁹Fe were administered on day 15 and 16 of the study. Iron absorption from these meals was determined from the rise in ⁵⁵Fe and ⁵⁹Fe activity in blood obtained 14 days later.

Measurements of ⁵⁵Fe and ⁵⁹Fe radioactivity were performed on duplicate 5 ml blood samples and measured portions of the test meals. Samples were prepared by liquid scintillation counting using a modification of the method of Eakins and Brown (12). Sufficient counts were obtained on each sample to reduce the net counting error for each isotope to less than $\pm 2\%$ in subjects absorbing more than 1% of the test dose. Blood volume was estimated from the height and weight of the subject (13) and red cell incorporation of absorbed iron was assumed to be 80% in all subjects (14). Because of the skewed distribution of iron absorption data, statistical analysis was performed on the logarithmic scale. Statistical comparison of iron absorption from any two test meals was performed by the Student's *t* test applied to logarithms of absorption ratios. Serum iron and iron-binding capacity were measured by established methods (15, 16) and serum ferritin by a radioimmunochemical technique (17).

Results

Study 1 was designed to measure the effect of 50 mg EDTA on food iron absorption and to determine the extent to which iron in the form of an EDTA complex exchanges with the non-heme iron of the test meal. In the first group of 9 subjects, studies were performed with the STD meal as follows. For the first test meal which contained double radioiron tags, the potatoes were divided into two portions. The first portion was tagged extrinsically by adding 0.1 mg iron as ⁵⁹FeCl₃. To the second portion 50 mg EDTA were added which had been first mixed with 0.1 mg iron as ⁵⁵FeCl₃. The two portions of the potatoes were then mixed together and fed with the remainder of the STD meal. The third absorption measurement was performed 2 weeks later using the STD meal without EDTA tagged extrinsically by mixing 0.1 mg iron as ⁵⁹FeCl₃ with the potatoes. On the following day the REF dose tagged with ⁵⁵Fe was administered without food.

The results of this study are listed in Table 1. Geometric mean absorption from the STD meal containing no EDTA was 4.4% as compared with the mean absorption of 11.6% from the REF dose. The geometric mean ratio of the STD/REF absorption was 0.38. Absorption from the STD meal containing 50 mg EDTA and tagged extrinsically with ⁵⁹FeCl₃ averaged 2.1%. This reduction in absorption due to added EDTA (mean absorption ratio of 0.48 for the STD meal with/without EDTA) was highly significant ($t = 3.64, P < 0.001$).

This same study was performed in a second group of 10 subjects using the SS meal in place of the STD meal. For the first pair of iron absorption measurements, the SS meal was tagged extrinsically by adding 3.7 mg iron as ⁵⁹FeCl₃. After thorough mixing, 50 mg EDTA tagged with ⁵⁵Fe as described above was mixed with the meal just prior to administration. Two weeks later, absorption from the SS meal without EDTA and from the REF dose was measured as described above.

The results observed with the SS meal are listed in Table 2. Iron absorption from the SS meal containing no chelate averaged 1.3% as compared with a mean of 17.6% absorption

⁵ Disodium EDTA dihydrate was used in all studies.

TABLE 1
The effect of 50 mg EDTA on non-heme iron absorption from the STD meal

No.	Sex age	Packed cell vol.	Transferrin saturation	Serum ferritin	Fe absorption				Absorption ratio	
					STD meal + EDTA		STD meal	REF	⁵⁹ FeCl ₃ : ⁵⁵ FeEDTA	STD meal + EDTA: STD meal
					⁵⁹ FeCl ₃	⁵⁵ FeEDTA				
		%	%	ng/ml	% of dose					
1	M28	45.4	39	212	1.1	1.0	2.9	7.2	1.09	0.38
2	M33	41.7	49	137	1.2	1.0	3.4	16.8	1.19	0.35
3	M22	47.8	38	89	1.4	1.5	4.2	4.5	0.95	0.33
4	M22	45.9	37	70	1.4	1.3	4.8	3.8	1.07	0.30
5	M23	48.6	46	136	1.5	1.5	4.7	10.4	0.99	0.30
6	M21	45.9	31	46	2.4	2.4	6.8	22.9	0.99	0.30
7	M21	45.4	53	94	3.6	3.4	2.1	13.5	1.06	0.34
8	M19	46.3	31	37	4.6	4.3	7.2	21.6	1.06	1.73
9	M23	45.5	54	52	5.8	5.8	6.7	26.0	0.99	0.64
Mean ^a	24	45.8	42	84	2.1	2.0	4.4	11.6	1.05	0.86
										.48

^a Geometric means have been calculated for iron absorption and serum ferritin values.

TABLE 2
The effect of 50 mg EDTA on non-heme iron absorption from the SS meal

No.	Sex age	Packed cell vol.	Transferrin saturation	Serum ferritin	Fe absorption				Absorption ratio	
					SS meal + EDTA		SS meal	REF	⁵⁹ FeCl ₃ : ⁵⁵ FeEDTA	SS meal + EDTA: SS meal
					⁵⁹ FeCl ₃	⁵⁵ FeEDTA				
		%	%	ng/ml	% of dose					
1	M24	46.0	37	132	.3	.4	.7	17.4	0.60	0.38
2	M27	46.1	52	147	.5	.8	.4	2.3	0.67	1.20
3	M26	45.8	37	57	.6	.8	4.1	31.0	0.67	0.13
4	M27	44.8	32	101	.7	.7	4.8	28.5	1.04	0.14
5	M25	44.5	73	85	.7	.7	1.3	21.6	0.98	0.49
6	M22	49.0	41	48	.8	1.0	1.1	22.5	0.81	0.69
7	M23	47.9	39	176	.9	1.0	0.3	42.5	0.91	2.75
8	M23	45.3	27	47	1.0	1.1	1.0	30.1	0.86	0.94
9	M22	48.4	25	40	1.0	1.2	2.3	7.2	0.85	0.43
10	M18	48.4	34	104	1.2	1.2	2.6	13.9	0.97	0.45
Mean	24	46.6	40	83	0.7	0.9	1.3	17.2	0.83	0.53

from the REF dose. The mean absorption ratio for the SS/REF was 0.08. Mean absorption from the SS meal containing 50 mg EDTA was 0.7%. This decrease in absorption due to EDTA (mean absorption ratio of 0.53 for SS meal with/without EDTA) was similar to that obtained with the STD meal and was also highly significant ($t = 2.22, P < 0.005$).

The degree of isotopic exchange of ferric EDTA with non-heme iron of the STD and SS meals can be determined from the ratio of ⁵⁹Fe:⁵⁵Fe listed in the last two columns of Tables 1 and 2. With the STD meal, absorption of the extrinsic tag was slightly higher than iron EDTA (mean ratio 1.05) whereas the converse was observed with the SS meal (mean ratio 0.83). Although the overall mean

ratio of 0.93 indicated a slightly higher absorption of ferric EDTA ($t = 1.95, P < 0.05$), the data were taken to indicate complete or nearly complete mixing of ferric EDTA with non-heme dietary iron.

The purpose of study 2 was to determine the effect of adding increasing amounts of EDTA on the absorption of non-heme iron from the STD meal. All test meals were tagged extrinsically by adding 0.1 mg iron labeled FeCl₃ to the potatoes. Unlabeled EDTA was dissolved in a small volume of water and mixed with cornmeal immediately prior to administration of the test meal. The first group of 14 subjects was given four STD meals containing 0, 6, 25, and 100 mg EDTA (Table 3). A second group of 12 subjects was

TABLE 4
The effect of va

No.	Sex age
1	M30
2	M23
3	M29
4	M21
5	M27
6	M27
7	M26
8	M18
9	M18
10	M22
11	M23
12	M19
Mean	24

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TABLE 3
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given four STD meals containing 0, 10, 50, and 500 mg EDTA (Table 4). The effect of EDTA is indicated by the ratios of iron absorption with/without added EDTA which are listed in the last three columns of Tables 3 and 4 and shown graphically in Figure 1. Although mean ratios of 1.10, 0.98, and 0.72 observed with 6, 10, and 25 mg EDTA, respectively, suggested inhibition in iron absorption, the difference was not statistically significant at these levels of EDTA. The mean

ratios of 0.47, 0.31, and 0.23 observed with 50, 100, and 500 mg EDTA indicated a highly significant depression in absorption by EDTA ($P < 0.001$).

Discussion

Prior studies have established that ferric EDTA is an effective source of iron for both plants (18) and animals (19). Administration of therapeutic amounts of iron as an EDTA salt has been used successfully for the treat-

TABLE 3
The effect of varying amounts of EDTA on iron absorption from STD meal

No.	Sex age	Packed cell vol.	Transferrin saturation	Serum ferritin	Fe absorption				Absorption ratio + EDTA: 0 EDTA		
					0 mg EDTA	6 mg EDTA	25 mg EDTA	100 mg EDTA	6 mg EDTA	25 mg EDTA	100 mg EDTA
		%	%	ng/ml	% of dose						
1	M18	44.9	51	71	2.3	3.7	3.1	2.6	1.60	1.34	1.13
2	M22	45.9	47	57	3.1	4.3	3.3	2.1	1.38	1.06	0.65
3	M20	46.2	34	38	3.3	3.0	3.0	1.0	0.90	0.92	0.31
4	M20	48.5	20	29	3.5	4.7	5.2	1.9	1.35	1.49	0.54
5	M20	47.2	39	49	3.7	3.0	3.3	1.1	0.79	0.87	0.28
6	M19	41.3	34	31	3.7	4.8	1.1	1.1	1.50	0.33	0.34
7	M18	47.2	42	35	4.6	7.7	5.6	0.9	1.68	1.23	0.20
8	M18	46.8	41	44	4.7	7.8	4.9	1.1	1.68	1.05	0.23
9	M20	47.6	38	63	5.6	4.5	3.2	1.3	0.79	0.57	0.22
10	M22	48.0	44	12	7.5	9.1	3.1	2.9	1.21	0.41	0.38
11	M26	48.9	37	149	8.6	4.2	0.7	0.9	0.48	0.08	0.10
12	M20	50.2	43	34	9.3	7.9	13.7	1.1	0.84	1.48	0.11
13	M22	44.8	38	21	15.1	17.0	12.8	6.4	1.12	0.84	0.42
14	M18	46.1	70	9	28.0	24.4	13.9	7.8	0.87	0.49	0.27
Mean	19	46.7	41	36.5	5.6	6.1	4.0	1.7	1.10	0.72	0.31

TABLE 4
The effect of varying amounts of EDTA on iron absorption from STD meal

No.	Sex age	Packed cell vol.	Transferrin saturation	Serum ferritin	Fe absorption				Absorption ratio + EDTA: 0 EDTA		
					0 mg EDTA	10 mg EDTA	50 mg EDTA	500 mg EDTA	10 mg EDTA	50 mg EDTA	500 mg EDTA
		%	%	ng/ml	% of dose						
1	M30	43.2	32	119	0.9	1.1	0.6	0.5	1.21	0.68	0.52
2	M23	47.5	34	83	1.8	2.9	1.2	0.6	1.63	0.65	0.34
3	M29	47.1	46	74	2.0	2.6	0.8	0.4	1.29	0.37	0.20
4	M21	49.7	36	58	2.4	2.6	2.0	0.6	1.07	0.83	0.25
5	M27	43.5	33	57	2.6	1.6	1.7	1.7	0.62	0.65	0.65
6	M27	47.1	18	117	2.8	1.1	0.8	0.8	0.39	0.27	0.28
7	M26	49.0	50	68	2.9	2.9	0.9	0.7	1.01	0.30	0.22
8	M18	48.1	44	26	3.4	3.8	1.7	0.8	1.11	0.51	0.22
9	M18	50.0	28	56	3.5	7.6	3.7	0.6	2.17	1.05	0.16
10	M22	46.3	33	120	4.9	5.2	1.9	0.8	1.06	0.38	0.15
11	M23	40.6	24	19	11.0	5.5	2.3	0.8	0.50	0.20	0.07
12	M19	47.8	46	10	11.0	10.1	3.5	1.4	0.91	0.31	0.12
Mean	24	46.7	35	54	3.2	3.2	1.5	0.7	0.98	0.47	0.23

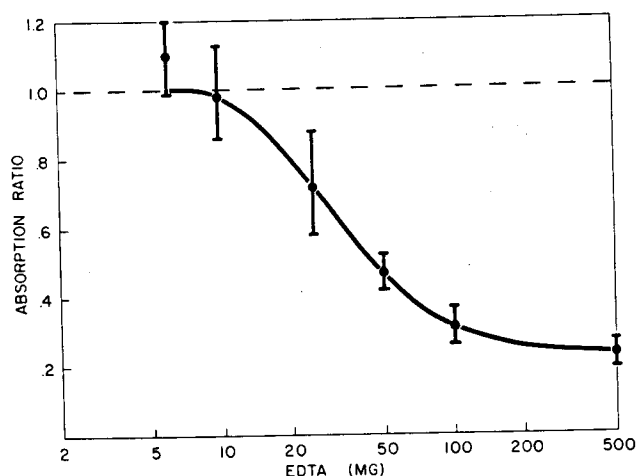


FIG. 1. Effect of EDTA on iron absorption from the STD meal. The ratio of iron absorption with and without EDTA is plotted against the amount added. The vertical bars represent the limits of ± 1 SE.

ment of iron deficiency in man (20, 21). However, studies employing precise radioactive measurements have shown that the absorption of ferric EDTA is only about 20% that of a highly available iron salt such as ferrous sulphate (22). The effect of EDTA on food iron absorption may be quite different from its effect on inorganic iron because food alone substantially reduces the availability of non-heme iron (23). In the present study, clear evidence was obtained that EDTA substantially reduces the absorption of food iron. Thus, when 50 mg EDTA were added to a meal containing 4.1 mg iron (molar ratio of EDTA to iron approximately 2:1) iron absorption was decreased to about one-half of the level observed without added EDTA.

There are two methodological aspects of the present study which deserve comment. As in virtually all studies of food iron assimilation in recent years, iron absorption was estimated only indirectly from red cell incorporation of radioiron. However, by performing multiple tests in the same subject, systematic errors with this approach are largely avoided. It should also be noted that it was whole body retention of radioiron that was actually measured rather than iron absorption. For example, it is possible that larger amounts of iron were absorbed but then rapidly excreted as an iron EDTA complex. However, it is known that parenteral EDTA is excreted almost exclusively in the urine (24) and previous workers have shown that less

than 1% of the radioactivity following an oral dose of ^{59}Fe EDTA is excreted in the urine during the first 48 hr (5). Finally, from a nutritional standpoint, the important parameter is whole body retention of iron rather than gastrointestinal absorption.


Other observations in this study related to the nature of the EDTA effect on iron absorption. Recent studies have shown that a common pool of non-heme iron is formed by foods ingested in the same meal and that iron absorption from this pool can be measured by adding an extrinsic tag of $^{59}\text{FeCl}_3$ to the meal just prior to administration (8, 25). It is possible that EDTA could form a complex with iron which is assimilated independently of the remaining food iron. This would be analogous to the absorption of heme iron which is not influenced by dietary factors such as ascorbic acid which have a marked effect on the absorption of non-heme iron (26). On the other hand, EDTA might exert an effect on the total pool of non-heme iron. These possibilities were investigated by comparing in the same meal, the absorption of ferric EDTA with non-heme dietary iron labeled with an extrinsic tag of ferric chloride. If complete mixing of ferric EDTA with non-heme iron did occur, the ratio of the extrinsic tag to iron EDTA would differ from unity. The ratios observed of 0.83 with the SS meal and 1.05 with the STD meal were consistent with complete or nearly complete mixing.

These findings could also be obtained if the EDTA was able to chelate all of the non-heme iron of the meal and the absorption of this iron became independent of the usual effect of dietary iron. This possibility was examined by comparing the effect of EDTA on meals of both low (SS) and high (STD) iron availability. In a previous study in 32 female subjects (10), mean absorption from the SS and STD meal was 1.8 and 10.0%, respectively, compared with means of 1.3 and 4.4% in the present study. The lower absorption observed in the present study are explained by the higher iron stores in men. The difference between the two studies can be corrected by relating absorption to the REF dose of inorganic iron. In the previous study in females these ratios were 0.08 and 0.42 for the SS and the STD meals, respectively, as compared with ratios of 0.05 and 0.16 in the present study. The 6-fold difference in iron from these ratios is due to the decrease in iron absorption of 53% for the STD meal. The effect of EDTA on the diet as a whole with the non-heme iron. Studies with 50 mg EDTA showed an effect on iron absorption. However, the increase in iron absorption was only at meals with a ratio of iron greater than 1. It is possible that the effect is because of the consumption of EDTA (90 mg) of this study (J. Fritz, personal communication) intake of 50 mg EDTA may be 50 mg of peculiar level of iron absorption. Ferric iron or iron labeled iron appeared in the study for fortification guidelines. In this study by EDTA, iron availability, iron absorption was reduced. EDTA iron absorption reduction is related to diet and iron absorption. In an iron and 50

with ratios of 0.08 and 0.38 obtained in the present study. There was, therefore, a 5- to 6-fold difference in absorption of non-heme iron from the STD and SS meals. With both of these meals, EDTA produced a similar decrease in iron absorption which averaged 53% for the SS meal and 47% for the STD meal. These data indicate that the effect of EDTA on iron absorption is superimposed on the diet as a whole through an interaction with the non-heme iron pool.

Studies involving the addition of 6 to 500 mg EDTA suggest that there is a depressing effect on iron absorption at any concentration. However, a statistically significant decrease in absorption could be demonstrated only at molar ratios of EDTA to iron greater than 1. It is difficult to extrapolate these data to the effect of EDTA on a normal diet because of uncertainty regarding EDTA consumption. In 1975, the estimated production of EDTA in the United States is in excess of 90 million pounds although the proportion of this used for food production is not known (J. Fritz, personal communication). With an intake of 2,000 g of food daily in which 5 servings of 100 g portions of food contain 100 ppm EDTA, daily intake of EDTA would be 50 mg. Certain segments of the population may have even greater intakes because of peculiarities in their food habits. This level of EDTA would reduce non-heme iron absorption by roughly 50%.

Ferric EDTA has been proposed as a salt for iron fortification (27) because this chelated iron does not adversely affect the appearance or the taste of food. The present study does not define its potential usefulness for fortification but does impose certain guidelines for its use. Under the conditions of this study, iron availability was not improved by EDTA, and in diets with low iron availability, iron absorption may be further reduced. It is unlikely that molar ratios of EDTA to iron below 0.5 would reduce absorption and a considerable degree of fortification might be possible within this limit. For example, if 5 mg iron as EDTA were added to a diet containing 15 mg iron per day to give a molar ratio of 0.25, no decrease in iron absorption should occur. On the other hand, in an American diet containing 10 mg iron and 50 mg EDTA, the addition of 5 mg iron

as ferric EDTA might reduce the overall absorption of iron by 25% and thereby contribute little to iron nutrition. 

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The effect of iron deficiency on the skeletal development during the first 6 months of life

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ABSTRACT
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Almost all of the effects of malnutrition has been done in a variety of significant research, the study of several problems in human subjects. First, it is a difficult and impossible task to study the effects of a child's malnourished condition. In fact, the majority of investigators the nutritional history of nutritionally generated problems is complicated by other diseases and a lack of constant observation might further obscure the data. Finally, the problems facing the researcher are the inspectional and the data were mainly from children in urban areas. Cultural variations that are genetically generated in "normal," healthy Danish children 6 months for children. The balance is even greater between Ohioans and children found:

"... A racial difference in the cortical bone that indicates a difference in calcium r