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Effects of Mineral Separation by Time and Enteric Coating Mechanism for Calcium and Iron Absorption in Mammalia

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Abstracts: ***It is best-known that in rats and humans, the metallic element markedly inhibits the absorption of iron once each mineral is present within the little intestine. The interaction between iron and Ca was studied in two species, rats and dogs. feminine rats were orally treated with six mg calcium as metallic element citrate-malate followed by seventy ug iron as metal sulfate labeled with [59]Fe. Doses were separated by zero to a hundred and eighty min. Iron absorption was measured by the victimization of the whole-body atom retention technique. we have a tendency to determined that iron and calcium should be separated by a minimum of one hour to forestall a negative impact of calcium on iron absorption. This info was wont to style tablets containing iron (radiolabeled with [59]Fe) and Ca. Iron tablets were created by enteric coating iron with or while not ascorbic acid (attention of non-heme iron absorption). The enteric coat delayed the discharge of iron into the little internal organ by preventing iron dissolution within the abdomen for a minimum of one hour. Iron absorption from these tablets and uncoated tablets was measured in dogs victimization an assay of [59]Fe incorporation into hemoproteins. Calcium inhibited the absorption of uncoated iron-ascorbic acid by concerning 50~. However, once the enteric-coated iron-ascorbic acid tablets were dosed with metallic element tablets, iron absorption wasn't inhibited. We conclude that Ca inhibits iron absorption in rats and dogs and that this inhibition may be overcome once the 2 minerals square measure separated by a minimum of one hour, which can be achieved by enteric coating the iron supply.***

Keywords: Calcium, Iron, Absorption, Enteric Coating, Mineral, Time Separation

1. Introduction

From a biological process perspective, iron and Ca are 2 minerals of concern within the Indian diet. each is typically consumed in but suggested amounts (i), particularly by immature and adult ladies. Deficiency of iron will lead to easily recognizable symptoms, like fatigue and vertigo, which are generally corrected by supplementation with iron salts. Ca deficiency, in contrast to iron deficiency, is difficult to diagnose, and there is very little agreement on the number necessary within the diet to prevent deficiency. The Recommended Dietary Allowance (RDA) for Ca of 800 mg per day has been proposed to satisfy the wants of most healthy adults. Since many people have difficulty overwhelming this abundant Ca daily, and since Ca deficiency has been connected to pathology, Ca supplement usage continues to extend. supplements exist when the two much consumed at the same time since calcium inhibits iron absorption once each mineral is present in the tiny intestine. The iron-calcium interaction was documented over thirty years past once Chapman and Campbell (2) clearly incontestable that Ca caused attenuated iron absorption in rats fed mixed diets. ulterior reports have confirmed that in rats, increasing dietary calcium in a very form of form ends up in diminished iron absorption (3-6). An effect of the metallic element on iron absorption has additionally been incontestable in humans. Monsen and Cook (7) reportable that nonheme iron absorption was significantly reduced by 50-70~ once Ca and phosphorus was other to check meals. However, iron absorption was reduced by roughly 30~ once either calcium or phosphorus was other to check meals, suggesting that metallic element and phosphorus every affected iron absorption which these restrictive effects were additive. The authors speculated regarding the attainable formation of an iron calcium-phosphate complicated.

An iron-calcium interaction is also particularly vital in pregnant women since most prenatal supplements contain giant amounts of iron and calcium, and it's not unreasonable to suspect that iron bioavailability from such supplements is low. Indeed, in nonpregnant ladies, iron absorption from a variety of prenatal supplements containing ferrous fumarate and Ca (as sulfate or carbonate) was significantly reduced compared to iron bioavailability from ferrous fumarate alone (8). Babior et al. (9) reportable that pregnant ladies absorbed considerably additional iron from prenatal supplements containing reduced amounts of Ca and metal compared to supplements containing higher amounts of Ca and Mg. However, this study has an important limitation therein iron absorption within the absence of metallic element and magnesium wasn't measured. Two subgroups within which the use of metallic element supplements is common and growing are hypertensives and biological time ladies. In such folks, it's not uncommon to see metallic element intakes larger than I000 mg/day. 2 reports recommend that this level of Ca supplementation compromises iron standing (i0,II). for instance, after solely eight weeks of metallic element supplementation, each normotensive and hypertensive subjects had considerably reduced humor protein concentrations, indicating reduced iron stores (I0). The negative result of metallic element supplementation on iron absorption and status has therefore been well documented. One obvious answer is to instruct people to ingest metallic elements and iron supplements at separate times, though this is far from ideal. there's a transparent would like for a mix iron-calcium supplement that delivers bioavailable iron. the aim of this series of experiments was to document that metallic element inhibits iron absorption in associate degree animal model then to see an effective means of separating the 2 minerals such that the interaction would be alleviated.

2. Methods and Materials

2.1 Rat Study

The purpose of this experiment was to document that calcium inhibited iron absorption in a rat model and to determine the time delay between administration of calcium and iron which would eliminate the interaction. The form of calcium used in these experiments, calcium citrate-malate, has been shown to provide more absorbed calcium in humans than calcium carbonate (12,13), a commonly used calcium supplement. Ferrous sulfate was used as the iron source.

221 Thirty-six female rats (C/D strain, Charles River Breeding Laboratories, Portage, MI) weighing approximately 200 g each have fasted for 18 h. On the morning following the fast, rats were orally gavaged (French catheter) with 6 mg calcium as calcium citrate-malate (molar ratios 6:2:3, The Procter & Gamble Company, Cincinnati, OH) in 4 mL deionized water. The calcium dose was followed with a dose of 70 ug iron as ferrous sulfate in 4 mL deionized water containing I uCi [59]Fe as ferric chloride (specific activity 13.3 uCi/ug iron, Amersham Radiochemicals, Arlington Heights, IL). The separation times between the doses of calcium and iron were 0, 30, 60, 90, 120 and 180 min. The doses of calcium and iron (86:1 wt:wt) were chosen to represent an excess of calcium relative to iron-based on U.S. Recommended Dietary Allowances of I000 mg calcium and 18 mg iron (56:1 wt:wt). Iron bioavailability was measured with the whole-body isotope retention technique, which has been described elsewhere (6). Initial whole animal radioactivity (100P) was measured within one hour of dosing. The final whole animal radioactivity was measured seven days post-dosing. All counts were corrected for decay, and percent radioactivity retained was calculated as final counts divided by initial counts multiplied by i00.

2.2 Dog Studies

Experimental Designs

In order to work with solid dose forms, which would be preferred for human consumption, dogs were used as test animals. The same eighteen adult female beagle dogs (age 2 yrs) were used for three experiments. Dogs were housed in individual runs and were fed once daily. One week prior to each experiment dogs were fed a low-iron semi-purified pelleted diet (#D78701, Research Diets, New Brunswick, NJ) to maximize iron absorption from the tablets. Analysis of each batch of diet showed mean iron content to be 10-15 ppm, which is below the National Research Council's recommended dietary iron level for the dog of 60 ppm (14). A weighed portion of food (350-400 g) was made available to the dogs for one hour each day. Each experiment lasted three weeks. One week after initiation of the low-iron diet, dogs were dosed with tablets as shown below.

	Treatment	Number of Dogs
Experiment One	Uncoated Iron	6
	Enteric- Coated Iron	6
	Enteric- Coated Iron-ascorbic acid	6
Experiment Two	Uncoated Iron-ascorbic acid	6
	Uncoated Iron-ascorbic acid, Calcium	6
Experiment Three	Enteric- Coated Iron- ascorbic acid	9
	Enteric- Coated Iron ascorbic acid, Calcium	9

The purpose of Experiment One was to compare iron absorption from uncoated and enteric-coated tablets and to determine if adding ascorbic acid to the iron tablet would increase iron absorption. In Experiment Two, we tested the hypothesis that calcium inhibited iron absorption in the dog. The purpose of Experiment Three was to demonstrate that enteric-coated iron-ascorbic acid was bioavailable when consumed with calcium.

mg ascorbic acid, which was chosen to provide an I: I molar ratio of iron to ascorbic acid. In Experiments Two and Three, the levels of iron and ascorbic acid were reduced to 4.5 mg and 14.2 mg, respectively (explained below). The dose level of calcium was 250 mg (two tablets), which provided calcium: iron weight ratio of 56:1, identical to the U.S. RDA weight ratio of calcium: iron.

Tablets

Five different types of tablets were used in these studies: uncoated iron (with or without ascorbic acid), enteric-coated iron (with or without ascorbic acid), calcium. The iron source was ferric chloride hexahydrate which was intrinsically labeled with [59]FeCI 3 (specific activity 13.3 uCi/ug iron, Amersham Radiochemicals, Arlington Heights, IL). In Experiment One, each tablet contained i0 mg of elemental iron with or without 31.5 mg ascorbic acid and 20 uCi radioactivity. Because of high blood radioactivity levels, the amounts of iron, ascorbic acid, and radioactivity were reduced in Experiments Two and Three to 4.5 mg, 14.2 mg and i0 use, respectively. The calcium source was calcium citrate-malate (molar ratios 6:2:3, The Procter & Gamble Company, Cincinnati, OH) and tablets contained 125 mg elemental calcium. All tablets contained excipients typical to a tablet dosage form.

In order to deliver iron to the small intestine and separate it from calcium, some of the iron tablets were enteric-coated. The desirable property of an enteric coat is to resist dissolution at low pH, thus keeping the tablet intact in the stomach, then dissolve and quickly release material in the higher pH environment of the upper intestine. The polymer used for the enteric coat was hydroxypropyl methylcellulose phthalate NF, HP-50 (Shin-Etsu Chemical Company, Tokyo, Japan) and is designed to dissolve at a pH of 5 or higher. Tablets were tested for in vitro disintegration properties in a basket-rack assembly with no disks (15). This test, carried out in 800 mL of 0.I N HCI at 37~ showed that the enteric-coated tablets disintegrated in an average time of 85.8 minutes, compared to 5.4 minutes for the uncoated tablets, thus indicating an effective enteric coat for these experiments.

Dosing and Blood Sampling

Dogs were dosed approximately 24 hours after they had last eaten. Tablets were placed in the back of the dog's mouth and were washed down the esophagus with deionized water. When both iron and calcium were dosed, iron was dosed immediately prior to calcium. Dogs were observed for one hour after dosing to check for vomiting of the test material. Food was withheld until at least 3 hours after dosing. Blood (15-20 mL) was drawn from the jugular vein into Vacutainer tubes containing heparin. In Experiment One, blood was sampled one and two weeks post-dosing. In Experiments Two and Three, a blood sample was taken before dosing (approximately one week) in order to calculate a correction factor for background radioactivity from the previous experiment. Blood was also sampled two weeks post-dosing. Blood was analyzed for hemoglobin by the cyanomethemoglobin method (16), hematocrit by microcentrifugation, and amount of radioactivity by gamma counting. Iron absorption Percent iron absorption was calculated by measuring the incorporation of [59]Fe into hemoglobin (17). Counts were corrected for background radioactivity and pre-dosing radioactivity (Experiments Two and Three). Blood volume was calculated as 90 mL/kg body weight (18).

Statistics

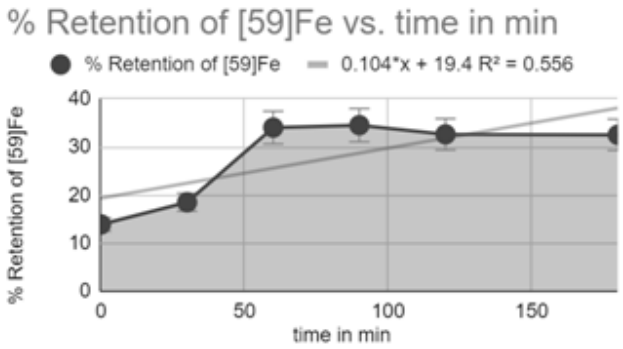
In Experiment One, means were compared by using ANOVA, normal distribution methods. In Experiments Two and Three, means were compared by using nonparametric and parametric Student's t-tests, respectively.

3. Results and Discussion

Rat Study

The results of the rat study are presented in Table I. Based on the whole-body isotope retention assay, it was concluded that calcium inhibited iron absorption if calcium was given within one hour of iron. This information was used to design a solid dose form of iron for the dog experiments which would remain undissolved in the stomach for at least one hour.

Time Delay of Iron Dose	% Retention of [59] Fe
0 min	13.9± 2.4*a
30	18.5 ± 1.9 a
60	34.0± 3.6 b
90	34.5 ± 2.7 b
120	32.6 ± 4.0 b
180	32.5 ± 6.0 b



*Mean • SE for 6 rats per group. Means with different letters are significantly different, P < 0.05, based on ANOVA and LSD tests.

Dog Studies

All dogs adapted to the semi-purified diet very easily. There were no episodes of vomiting or diarrhea. To assess the overall growth of the animals, we calculated the mean body weight of the dogs after each experiment. Body weights remained unchanged during the three experiments (Table 2). This was not unexpected, since the dogs were adults prior to Experiment One, and we fed them an amount of food calculated to maintain weight (14).

We also monitored the iron status of the dogs to see if the low-iron diet resulted in the depletion of liver iron stores. Other than dietary iron, the dogs received no exogenous iron from either their water supply or environment (concrete floors in the runs, stainless-steel food dishes).

Hemoglobin and hematocrit did not change during the experiments (Table 2). Therefore, it is likely that the small amount of iron in the dogs' diet was well absorbed and as a result, iron stores were not significantly compromised.

Experiment One

In this experiment, the objective was to study a solid dose form of iron designed to remain undissolved in the stomach for at least one hour and still provide bioavailable iron when it dissolved. The disintegration properties of the tablets indicated that the enteric coat successfully prevented the release of iron in an acidic environment for at least one hour. We compared the bioavailability of enteric-coated iron tablets (with or without ascorbic acid) to that from uncoated iron tablets. The results are presented in Table 3. Enteric-coated iron was absorbed slightly less well than uncoated iron, although these two treatment groups were not significantly different. When both iron and ascorbic acid were enteric-coated, iron was very well absorbed. We concluded that enteric-coated iron-ascorbic acid was an acceptable iron dose form. One might predict that even better absorption would be obtained if the iron source was ferrous, i.e. ferrous sulfate, since this form of iron is better absorbed in humans than ferric iron (17). This hypothesis was not tested in these experiments since ferric and ferrous iron are reportedly equally well absorbed in dogs (20).

Table 2: Body Weights and Iron Status of Dogs

Treatment	Experiment One	Experiment Two	Experiment Three
Body Weight*, kg	9.6± 0.3+	9.7± 0.3	9.7± 0.4
Hemoglobin, g/dL	16.2 ± 0.4	17.3 ± 0.3	16.1 ± 0.3
Hematocrit, %	46.3 ± 1.0	47.9 ± 0.7	44.3 ± 0.9

*Body weights on the last day of each experiment (14 days post-dosing).
+Mean • SE, n=18 in Experiments One and Three and n=12 in Experiment Two.

Table 3: Bioavailability of Enteric –Coated Iron in Dogs

Treatment	% Iron Absorbed
Uncoated Iron	13.6± 4.4*a,b
Enteric- Coated Iron	9.0 ± 3.1 a
Enteric- Coated Iron ascorbic acid	20.2± 3.5 b

*Mean • SE, n=6 dogs/group.
Means with different letters are significantly different,

P < 0.05, as determined by ANOVA, normal distribution methods

Experiments Two and Three

The objectives in these experiments were to demonstrate the iron-calcium interaction in dogs and to determine if the enteric-coated iron-ascorbic acid dose form would eliminate this interaction. Including ascorbic acid in the iron tablet-optimized conditions for iron absorption. In the case of uncoated iron (Experiment Two), we found that calcium inhibited iron absorption by more than 50% (6.3 • 2.4% vs. 15.6 • 5.1% for iron-ascorbic acid with and without calcium, respectively, mean • SE). Although this inhibition was not statistically significant, the very low percentage of iron absorbed in the presence of calcium indicated a biologically significant inhibition of iron absorption. When calcium was dosed with enteric-coated iron-ascorbic acid (Experiment Three), no inhibitory effect of calcium on iron absorption was observed.

Iron was absorbed equally well with or without calcium (18.1 i 2.1% vs 19.5 i 1.9%, respectively, mean i SE). We concluded that the enteric coat effectively separated iron-ascorbic acid from calcium and delivered bioavailable iron.

In summary, these experiments in rats and dogs demonstrate that calcium inhibits iron absorption when the two minerals are present together in the small intestine. The negative effect of calcium on iron absorption can be overcome by enteric coating iron and ascorbic acid such that the release of iron-ascorbic acid into the small intestine is delayed by at least one hour relative to the release of calcium. Although enteric-coated iron is bioavailable with or without the addition of ascorbic acid, the presence of ascorbic acid is desirable, since it enhances iron absorption.

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