No enhancing effect of vitamin A on iron absorption in humans[1,2]

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ABSTRACT

Background: Vitamin A and β-carotene were recently reported to enhance iron absorption by counteracting the inhibitory effect of phytic acid in cereal-based meals and of polyphenol-containing beverages on nonheme-iron absorption in humans.

Objective: Our objective was to further evaluate the influence of vitamin A on iron absorption.

Design: Iron absorption from corn bread with or without added vitamin A (retinyl palmitate) was determined in 5 studies in young adult human subjects by using either a stable-isotope method (2 studies) or a radioisotope technique (3 studies). Iron absorption was measured by erythrocyte incorporation of the isotopic labels and by whole-body retention of ^{59}Fe. Corn bread was served with water (studies 1 and 3) or coffee (studies 2, 4, and 5). The studies differed in the amounts and chemical forms of added tracer and fortification iron. The possibility of methodologic artifacts in earlier investigations was evaluated.

Results: No effect of vitamin A on iron absorption from the test meals was identified in the individual studies by using paired Student's *t* test. A slightly negative effect of vitamin A on iron absorption was found with the use of analysis of variance.

Conclusions: The previously reported findings of a positive effect of vitamin A on nonheme-iron absorption in humans was not confirmed. Incomplete isotopic equilibration of the tracer with native iron in the meal or with fortification iron cannot explain the previous findings. However, the present study does not exclude the possibility that suboptimal vitamin A status influences the effect of dietary vitamin A on iron absorption. *Am J Clin Nutr* 2003;77:144–9.

KEY WORDS Vitamin A, retinyl palmitate, iron absorption, stable isotopes, radioisotopes

INTRODUCTION

Vitamin A is needed for erythropoeisis, and hemoglobin is not incorporated into red blood cells in a normal way in persons who suffer from vitamin A deficiency. An interaction between vitamin A and iron metabolism was first reported in 1978 by Hodges et al (1). These investigators fed vitamin A–deficient diets to adult volunteers for ≤450 d, and as the serum retinol concentrations of the volunteers progressively decreased, so did their hemoglobin concentrations, even though the diet contained adequate amounts of iron. The study subjects did not respond well to supplemental iron until their vitamin A deficiency was corrected.

Several cross-sectional studies have since confirmed the interaction between iron and vitamin A. A positive correlation between serum retinol and hemoglobin values was reported in children in Guatemala (2), India (3), Thailand (4), Indonesia (5), Ethiopia (6), Bangladesh (7, 8), and South Africa (9), as well as in pregnant women in Indonesia (10). Supplementation with vitamin A alone significantly increases hemoglobin, hematocrit, or plasma ferritin in children (3, 11) and pregnant women (12, 13). In some populations, simultaneous supplementation with iron and vitamin A increases hemoglobin concentrations to a greater extent in anemic pregnant women than does iron supplementation alone (12, 13, 14).

Several possible mechanisms were suggested to explain this interaction. It was suggested that vitamin A deficiency decreases transferrin synthesis and thus reduces iron transport to the bone marrow (15); reduces the uptake of iron by the bone marrow (9, 10); impairs the differentiation of blood cells due to lack of retinoic acid (16, 17); results in ineffective erythropoeisis (18); and impairs mobilization of iron from ferritin stores (19). Alternatively, it was suggested that the high prevalence of infections, which are frequently reported during vitamin A deficiency, is indirectly responsible for decreasing hemoglobin concentrations because the body sequesters iron during infections (20).

Vitamin A added to corn flour was reported to increase iron absorption ≤4-fold in adult Venezuelan subjects who consumed a meal of iron-fortified corn bread served with coffee or tea (21–23). It was proposed that vitamin A has an enhancing effect on iron absorption because of the formation of soluble iron complexes in the intestine. The aim of the present study was to further investigate the influence of vitamin A on iron absorption in young adult subjects who consumed test meals of corn bread with and without vitamin A that were served with water or coffee. Iron absorption was measured by stable- and radioactive-isotope techniques.

SUBJECTS AND METHODS

Two iron absorption studies using stable-isotope techniques (studies 1 and 2) were conducted at the Swiss Federal Institute of Technology, Zürich, Switzerland, and 3 iron absorption studies

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TABLE 1
Composition of the test meals[1]

	Stable-isotope studies		Radioisotope studies		
	Study 1	Study 2	Study 3	Study 4	Study 5
Test meal A					
Composition	Corn bread + water	Corn bread + coffee	Corn bread + water	Corn bread + coffee	Corn bread + coffee
Isotopic label	^{58}Fe	^{58}Fe	^{55}Fe[2]	^{55}Fe[2]	^{55}Fe[2]
Fortification iron	Ferrous sulfate	Ferrous sulfate	None	None	Ferrous fumarate
Iron as isotopic label (mg)	1.75	1.75	Trace	Trace	Trace
Iron as natural iron compound (mg)	0.75	0.75	0.5	0.5	5
Test meal B					
Composition[3]	Corn bread + vitamin A + water	Corn bread + vitamin A + coffee	Corn bread + vitamin A + water	Corn bread + vitamin A + coffee	Corn bread + vitamin A + coffee
Isotopic label	^{57}Fe	^{57}Fe	^{59}Fe[2]	^{59}Fe[2]	^{59}Fe[2]
Fortification iron	Ferrous sulfate	Ferrous sulfate	None	None	Ferrous fumarate
Iron as isotopic label (mg)	2	2	Trace	Trace	Trace
Iron as natural iron compound (mg)	0.5	0.5	0.5	0.5	5

[1] The corn bread was made from 50 g corn flour and was served with 200 mL water or coffee.
[2] Added as ferric chloride, which contributed only trace amounts of iron to the test meals.
[3] Vitamin A (1000 μg retinol) was added as retinyl palmitate.

using radioisotope techniques (studies 3–5) were conducted at the University of Göteborg, Göteborg, Sweden. In the stable-isotope studies, iron absorption was determined on the basis of the fraction of stable-isotopic labels present in the erythrocytes 14 d after administration of the test meal. In the radioisotope experiments, iron absorption was determined by whole-body counting and erythrocyte incorporation of the isotopic labels.

Subjects

Forty-nine free-living subjects (22 men and 27 women) aged 20–35 y were recruited from the student population and scientific staff at the Swiss Federal Institute of Technology and from the medical student population at the University of Göteborg. All subjects were apparently healthy and denied having a history of gastrointestinal disorders or metabolic diseases. No pregnant or lactating women participated in the studies. No medication, except for oral contraceptives, or iron supplementation was allowed for 2 wk before the study and until the last blood sample was drawn. Venous blood samples for analysis of hemoglobin, serum ferritin, and serum retinol were drawn before administration of the first test meal.

Subjects were informed about the study orally and in writing. In addition, the women were asked about their last menstruation and their use of contraceptives. Written informed consent was obtained from all volunteers. The study protocol involving stable-isotope techniques was approved by the ethical committee at the Swiss Federal Institute of Technology, and the protocol for use of radioisotope techniques was approved by the ethical committee of the Medical Faculty, University of Göteborg.

Test meals

Corn bread was prepared from corn flour (polenta) and low-extraction wheat flour. The same batches of corn flour and wheat flour were used in all 5 studies. Minor ingredients included sugar, baking powder, salt, skim milk, eggs, and corn oil. Each serving corresponded to 50 g corn flour. The bread was prepared in bulk under standardized conditions and stored frozen until served. The compositions of the test meals are shown in **Table 1**. Stable-isotope techniques were used to measure iron absorption in studies 1 and 2. Radioisotope techniques were used to measure iron absorption in studies 3–5. In each study, the test meal was fed either without (meal A) or with added vitamin A (meal B; 1000 μg retinol as water-soluble retinyl palmitate; Hoffman-LaRoche, Basel, Switzerland).

In study 1, the corn bread was fortified with ferrous sulfate (2.5 mg Fe/50 g corn flour) and served with water; in study 2, ferrous sulfate–fortified corn bread (2.5 mg Fe/50 g corn flour) was served with coffee; in study 3, unfortified corn bread was served with water; in study 4, unfortified corn bread was served with coffee; and in study 5, ferrous fumarate–fortified corn bread (5 mg Fe/50 g corn flour) was served with coffee. The test meals served in study 5 most closely resembled those used by Layrisse et al (21).

In all studies, an aqueous vitamin A solution was prepared from water-soluble retinyl palmitate immediately before addition to the test meal. An aliquot was pipetted onto the corn bread before serving. The isotopic labels and ferrous sulfate were added as aqueous solutions to the corn bread immediately before serving. Ferrous fumarate was added to the dough during bread making.

Test meals were served with either water or coffee. Coffee was prepared from a single batch of instant coffee (Nescafe; Nestlé SA, Vevey, Switzerland). In studies 2, 4, and 5, 4 g instant coffee/serving was dissolved in 200 mL hot, ultrapure (18 MΩ) water. Ultrapure water (200 mL) was served as the drink in studies 1 and 3.

Experimental design

Within each study, all subjects consumed 2 test meals (A and B). Both test meals were consumed twice during 4 consecutive days in the order of ABAB or BABA (studies 1 and 2) and ABBA or BAAB (studies 3–5). The iron in test meals A and B was labeled diffferently by using stable isotopes (^{58}Fe and ^{57}Fe, respectively) or radioisotopes (^{55}Fe and ^{59}Fe, respectively). Subjects were randomly assigned to start with test meal A or B. Sample size was calculated to detect a nutritionally relevant difference in iron absorption of 50% between test meals A and B in the same subject by using paired Student's *t* test. Estimates were based on previous data on interindividual and intraindividual variations in iron absorption. All test meals were administered at breakfast under the supervision of the investigators after the subjects had

fasted overnight. Body weight and height were recorded before administration of the first test meal. No food or drink was allowed for 3 h after administration of the test meal. Before intake of the first test meal, 5 mL venous blood was drawn into EDTA-treated, evacuated tubes for measurements of iron status (studies 1–5) and serum retinol (studies 3–5). The second blood sample was drawn 14 d after intake of the last test meal (day 18) for analysis of hemoglobin and iron isotopes. Whole-blood hemoglobin concentrations were measured by using the cyanomethemoglobin technique (Sigma, St Louis), and plasma ferritin concentrations were measured by using enzyme-linked immunosorbent assay kits (Ramco Laboratories, Houston). Commercial-quality control materials (DiaMed, Cressier sur Morat, Switzerland, and Ramco Laboratories) were analyzed with all samples, and the assays were calibrated against World Health Organization 1st International Standard IS 80/602 (National Institute for Biological Standards and Control, London). Serum retinol concentrations were measured by using HPLC (24).

Stable-isotope studies

Stable-isotopic labels

The stable-isotope solutions, $^{57}FeSO_4$ and $^{58}FeSO_4$, were gravimetrically prepared from isotopically enriched elemental iron (^{57}Fe at 95.48% enrichment or ^{58}Fe at 93.11% enrichment; Isotec, St Quentin, France) by dissolution in a 0.1-mol H_2SO_4/L solution. The solutions were diluted by mass with a 0.1-mol H_2SO_4/L solution to a suitable concentration for individual dosing. Solutions of $^{57}FeSO_4$ and $^{58}FeSO_4$ were stored in polytetrafluoroethylene containers that were flushed with argon to keep iron in the +2 oxidation state. The isotopic composition of the iron in solution was determined by using negative thermal ionization mass spectrometry. Iron concentrations were measured by isotope dilution mass spectrometry against an iron standard prepared gravimetrically from an iron isotope reference material (IRM-014; EU Institute of Reference Material, Geel, Belgium).

The total doses of stable-isotopic labels (4.0 mg ^{57}Fe and 3.5 mg ^{58}Fe) to be administered were calculated on the basis of the estimated total amount of circulating iron in the subjects, the expected range of fractional iron absorption, and the attainable reproducibility of the isotopic analysis. The administered dose was determined by weighing the test meal before and after the addition of the labeling solution. Stable-isotopic labels replaced fortification iron in the test meals. To adjust all test meals to a total added iron content of 2.5 mg/serving, iron of natural isotopic composition was added as an aqueous solution of food-grade ferrous sulfate (Merck, Darmstadt, Germany).

Isotopic analysis of blood samples

Each isotopically enriched blood sample was analyzed in duplicate under chemical blank monitoring by using previously described techniques (25). Sample handling was done under clean laboratory conditions to reduce the risk of sample contamination during analysis. Whole blood samples were mineralized by microwave digestion (MLS 1200; Milestone, Leutkirch, Germany), and iron was separated from the matrix by anion-exchange chromatography. Sample iron was further purified by a solvent-solvent extraction step into diethylether. All isotopic analyses were performed by using negative thermal ionization mass spectrometry with a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT, Bremen, Germany) equipped with a multicollector system for simultaneous ion beam detection. Sample iron was analyzed isotopically by generation of negatively charged FeF_4^- ions (26). Because of the high enrichment of the isotopic labels and the low amounts of isotopic labels present in the red blood cells, the ^{54}Fe/^{56}Fe isotopic ratio in the blood remained unchanged within the reproducibility of isotopic analysis after incorporation of the isotopic labels. It was therefore possible to normalize measured data sets internally for the natural ^{54}Fe/^{56}Fe isotopic ratio (0.06370; 27) to correct for mass-dependent isotopic fractionation effects in the ion source. Relative differences in isotopic ratios between samples analyzed in duplicate were on the order of 0.005% for the ^{57}Fe/^{56}Fe isotopic ratio and 0.02% for the ^{58}Fe/^{56}Fe isotopic ratio. Normalized iron isotopic ratios of blood samples taken before test meal administration were identical to the natural iron isotopic ratios.

Calculation of iron absorption

The amounts of ^{57}Fe label and ^{58}Fe label in the blood of the subjects after erythrocyte incorporation were calculated on the basis of the shift of the iron isotopic ratios in the blood samples and of the amount of iron present in the blood of the subjects. Calculations were made by following the principles of isotope dilution and by considering that the iron labels used were not monoisotopic (25). Circulating iron was calculated on the basis of blood volume and hemoglobin concentration (28). Blood volume calculations were made on the basis of height, weight, and sex by following empirically derived formulas (29, 30), and 80% incorporation of the absorbed iron into red blood cells was assumed (31). Fractional iron absorption of the isotopic label was calculated relative to the amount of label administered.

Radioisotope studies

Radioisotopic labels

Isotope solutions of ^{55}Fe (Amersham, Buckinghamshire, United Kingdom) as ferric chloride in a 0.1-mol HCl/L solution and of ^{59}Fe (Amersham) as ferric chloride in a 0.1-mol HCl/L solution were prepared at the start of the study. Before use, the specific activities of the solutions were determined at the Radiation Physics Department, Sahlgrenska University Hospital, Göteborg, Sweden. The isotope solutions were added to the liquid during preparation of the dough. Each subject received a total activity of 111 MBq ^{55}Fe and 111 MBq ^{59}Fe.

Measurement of iron absorption

Two weeks after the last meal was served, a blood sample was drawn for analysis of ^{55}Fe and ^{59}Fe. At the same time, ^{59}Fe retention was measured by whole-body counting. Analysis of ^{55}Fe and ^{59}Fe in whole blood was performed by using a liquid-scintillation counter (Tri-Carb; Packard Instruments, Houston) and following a modified version of the method described by Eakins and Brown (32). The absorption of ^{55}Fe was calculated from the absorption of ^{59}Fe via the retention of ^{59}Fe, as measured by whole-body counting, and the relative incorporation of the 2 tracers in the erythrocytes. The methodology was described previously by Björn-Rasmussen et al (33) and Hallberg (34).

Food analysis

Corn bread was analyzed for its native amounts of iron, calcium, and phytic acid. Results are given as the mean of 2 independent analyses, which differed by < 3% in all cases. Vitamin A, β-carotene, and ascorbic acid in corn bread were not meas-

TABLE 2
Subject characteristics and fractional iron absorption from test meals with or without added vitamin A (1000 μg retinol as retinyl palmitate)

Study	Age[1]	Serum ferritin[2]	Iron absorption[3]		
			Test meal A (no added vitamin A)[4]	Test meal B (added vitamin A)[4]	Iron absorption ratio (test meal B:test meal A)[5]
	y	*μg/L*	*%*	*%*	
1 (*n* = 7 M, 5 F)	22 (19–23)	28 (7–180)	5.0 (1.9, 12.1)	4.3 (1.1, 10.8)	0.91 ± 0.10
2 (*n* = 1 M, 7 F)	22 (19–23)	13 (5–44)	4.0 (1.1, 14.4)	3.5 (1.0, 11.7)	0.91 ± 0.07
3 (*n* = 6 M, 4 F)	27 (19–35)	36 (9–132)	3.1 (1.4, 7.1)	3.0 (1.3, 7.3)	0.99 ± 0.06
4 (*n* = 4 M, 5 F)	26 (20–29)	22 (6–188)	2.1 (1.0, 4.6)	2.0 (0.9, 4.2)	0.96 ± 0.07
5 (*n* = 4 M, 6 F)	27 (20–36)	19 (5–80)	3.1 (1.0, 9.2)	2.9 (1.0, 8.0)	0.94 ± 0.06

[1] Arithmetic $\bar{x}$; range in parentheses.
[2] Geometric $\bar{x}$; range in parentheses.
[3] There were no significant differences between the test meals in any of the 5 studies (paired Student's *t* test).
[4] Geometric $\bar{x}$; −1 SD and +1 SD values in parentheses.
[5] $\bar{x}$ ± SEM.

ured but would not be expected to be present in significant amounts. Iron and calcium contents were determined by using flame and electrothermal atomic absorption spectrometry (SpectrAA 400; Varian, Mulgrave, Australia) after mineralization by microwave digestion (MLS 1200; Milestone) with concentrated HNO_3:H_2O_2 (7:3, vol:vol). To minimize matrix effects, analysis was carried out with a standard addition technique by using commercially available aqueous standards (Titrisol; Merck). A certified reference material (SRM 1567a; National Institute of Standards and Technology, Gaithersburg, MD) was processed in parallel. Total phytic acid was determined after extraction of the different inositol phosphates with a 0.5-mol HCl/L solution and purification by anion-exchange chromatography. Quantification was performed with HPLC and refraction index detection (35, 36). Aqueous standards were prepared from commercially available sodium inositolhexaphosphate (Sigma-Aldrich, Steinheim, Germany).

Statistical analysis

All calculations and statistical analyses were made by using commercially available spreadsheet software (EXCEL 97 for WINDOWS). Values for fractional iron absorption were logarithmically transformed before statistical analysis to adjust for the skewed distribution of the data. Absorption data are given as geometric means with −1 SD and +1 SD values in parentheses. Individual studies were evaluated by pairwise comparison (paired Student's *t* test) of iron absorption in the same subject, with each subject acting as his or her own control. Comparisons between studies were made by using two-factor analysis of variance (ANOVA) with logarithmically transformed absorption values as the dependent variable and subject, study, and the presence of vitamin A as independent variables. To test the effect of coffee on iron absorption, studies 1 and 3 (water) were compared with studies 2, 4, and 5 (coffee). The effect of serum ferritin concentrations on iron absorption was examined by introducing ferritin as a covariate in a mixed model analysis (dependent variable: logarithmically transformed iron absorption values; fixed factors: study and the presence of vitamin A; random factor: subject). Differences were considered statistically significant at $P < 0.05$.

RESULTS

The corn bread test meals that were served with water contained 0.62 mg native iron and 93 mg calcium. The native iron and calcium contents of the test meals that were served with coffee were 0.69 and 99 mg, respectively. The total phytic acid content of the corn bread was 109 mg/serving. The content of chlorogenic acid (as the major polyphenolic compound in coffee) was estimated to be 240 mg/serving on the basis of previous analyses (37).

None of the study subjects was anemic. Serum ferritin concentrations varied from 5 to 188 μg/L. Twelve subjects had a serum ferritin concentration < 12 μg/L (studies 1–5). The mean serum retinol concentrations in studies 3, 4, and 5 were 622 (range: 516–689), 665 (487–917), and 533 (401–659) mg/L, respectively. The serum retinol concentrations of these subjects were in the normal range. Serum retinol concentrations were not measured in studies 1 and 2 for practical reasons.

The addition of vitamin A did not significantly alter iron absorption in any of the individual studies (paired Student's *t* test) (**Table 2**). However, an ANOVA of studies 1–5 showed that iron absorption from all test meals to which vitamin A was added (test meal B) was significantly ($P = 0.01$) lower than that from the test meals without vitamin A. Iron absorption from the test meals served with water (studies 1 and 3) was also found to be significantly higher ($P < 0.001$) than that from the test meals served with coffee (studies 2, 4, and 5). Serum ferritin concentrations and iron absorption were significantly correlated ($P = 0.02$) as previously shown (37, 38).

DISCUSSION

The individual studies were designed to answer the question whether vitamin A has a nutritionally relevant effect on iron absorption. No such effect was observed in the individual studies on the basis of paired comparisons. ANOVA of the combined data, however, showed that the addition of vitamin A led to a small but significant (5%) decrease in iron absorption. Such a small effect would be expected to have a very limited effect on iron nutrition. This effect may even have resulted from systematic errors, eg, in the preparation, characterization, and administration of the isotopic labels, or isotopic fractionation effects at the absorptive stage (39). Using ANOVA, we also found that iron absorption was 20–30% lower when coffee was given with the meal than when water was given instead. This confirms previous observations (37, 40). We conclude, therefore, that vitamin A has no enhancing effect on iron absorption.

These findings contradict those of previous reports from Venezuela (21, 22), in which a 2–4-fold increase in iron absorption

from ferrous fumarate–fortified corn bread was found after addition of vitamin A (retinyl palmitate). Layrisse et al (21) suggested that the observed enhancing effect of vitamin A on iron absorption was due to a physicochemical interaction between iron and vitamin A, resulting in a complex from which iron was readily available for absorption. The formation of such a complex would prevent iron from binding with dietary inhibitors of iron absorption such as phytic acid in cereals or phenolic compounds in coffee and tea. The molar ratios of vitamin A to iron in the Venezuelan corn bread were very low, ≈0.01:1–0.04:1 before processing and 0.004:1–0.008:1 after bread baking and storage. Therefore, it is unlikely that the formation of vitamin A–iron complexes can explain the enhancing effect of vitamin A on iron absorption that was reported in these previous studies. In addition, Garcia-Casal et al (22) did not find a dose-response effect of vitamin A on iron absorption. Such an effect has been reported for inhibitors and enhancers of iron absorption, such as ascorbic acid (41, 42), phytic acid (42), and phenolic compounds (37). These compounds are believed to influence iron absorption primarily (phytic acid and phenolics) or partly (ascorbic acid) through complex formation.

Using an extrinsic tag to determine iron absorption from a test meal in human subjects requires that both the isotopic label and the native iron in the test meal enter a common pool in the gastrointestinal tract for isotopic exchange. In the present stable-isotope studies (studies 1 and 2), fortification iron was in the form of ferrous sulfate (labeled ferrous sulfate plus ferrous sulfate of natural isotopic composition). In studies 3 and 4, only trace amounts of iron were added as the radioactive tag (ferric chloride). Both ferrous sulfate and ferric chloride are readily soluble in the gastric juice and can be expected to enter the common pool. In study 5, as well as in the Venezuelan studies, labeled test meals were prepared by the addition of an extrinsic tag of radiolabeled ferric chloride to meals fortified with ferrous fumarate. Studies with intrinsically labeled ferrous fumarate have shown that this iron compound has the same relative bioavailability in adults as does ferrous sulfate (43–45). However, there are indications that ferrous fumarate does not completely enter the common nonheme-iron pool (46). If ferrous fumarate failed to enter or only partly entered the common pool in the studies in Venezuela, the molar ratio of vitamin A to iron would have been greatly increased. This explanation seems unlikely, however, because even at a significantly increased molar ratio of vitamin A to iron in the test meals (studies 3 and 4), no beneficial effect of vitamin A on iron absorption was observed in our studies. Furthermore, a methodologic artifact in the previous studies due to incomplete isotopic equilibration is not supported by the results of our study 5, in which we found no influence of vitamin A on iron absorption as measured by a $^{59}FeCl_3$ tag added to ferrous fumarate–fortified corn bread. The conditions in study 5 closely resembled those in the previous experiments, in which most of the iron was added as ferrous fumarate and negligible amounts of ferric chloride were added as the radioisotope tag.

The study design and methodology used in Venezuela were similar but not identical to those of the present studies conducted in Zürich and Göteborg. For example, there were differences in the time at which vitamin A was added to the corn bread. In the present study, vitamin A was added to the test meal immediately before consumption, whereas in most of the Venezuelan studies, vitamin A was added with the iron before baking the bread. The heat treatment resulted in significant degradation and alteration of the vitamin (21), and the degradation products themselves could have been responsible for the observed effects. However, Garcia-Casal et al (22) also added vitamin A immediately before consumption and also found a significant enhancing effect on iron absorption. There were also minor differences in test meal composition and size. Both studies used corn bread as the major component in the test meal, but in the Venezuelan studies the meal size was larger (100 g corn flour) and the corn bread was served with cheese and margarine. These differences are presumably irrelevant when considering direct physicochemical interactions between iron and vitamin A in the gut or the process of isotopic equilibration during digestion.

When comparing the present studies with those from Venezuela, it is important to realize that differences in the socioeconomic status of the study populations may have resulted in differences in nutritional status. The Venezuelan studies were conducted with adult subjects from lower socioeconomic strata, whereas the subjects in the present study were young, healthy adults living in Zürich and Göteborg. Thus, differences in vitamin A status may explain the contradictory findings. The Venezuelan findings may be explained either by a physiologic effect of vitamin A or β-carotene on the iron absorption process or by the influence of vitamin A or β-carotene on iron utilization for hemoglobin synthesis in subjects with marginal vitamin A status. Many studies indicated that vitamin A influences erythrocyte incorporation of iron by influencing iron transport to the bone marrow (15), iron uptake by the bone marrow (9, 10), or erythropoeisis (16–18). In iron absorption studies of erythrocyte incorporation of isotopic labels, the fraction of isotopic label that is incorporated into the erythrocytes is used as the basis for calculation. It is commonly assumed that 80% of absorbed iron is incorporated into erythrocytes after 14 d (31, 47, 48). In studies 1 and 2, as well as in the Venezuelan studies, it was assumed that the incorporation rate was constant at 80%. It is possible, however, that the incorporation rate varies with vitamin A status and that even a small amount of vitamin A can trigger erythropoeisis in subjects with suboptimal vitamin A status.

In conclusion, the present study of healthy human subjects did not confirm earlier reports of an enhancing effect of vitamin A on iron absorption. Methodologic artifacts such as incomplete equilibration of the isotopic label with the fortification iron and native meal iron do not explain these conflicting results. It is possible that vitamin A enhances the absorption of iron or its subsequent incorporation into red blood cells only in subjects with impaired vitamin A status. However, this remains to be investigated.

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