The absorption of iron from whole diets: a systematic review[1–4]

Rachel Collings, Linda J Harvey, Lee Hooper, Rachel Hurst, Tracey J Brown, Jennifer Ansett, Maria King, and Susan J Fairweather-Tait

ABSTRACT

Background: Absorption factors are required to convert physiologic requirements for iron into Dietary Reference Values, but the absorption from single meals cannot be used to estimate dietary iron absorption.

Objective: The objective was to conduct a systematic review of iron absorption from whole diets.

Design: A structured search was completed by using the Medline, EMBASE, and Cochrane CENTRAL databases from inception to November 2011. Formal inclusion and exclusion criteria were applied, and data extraction, validity assessment, and meta-analyses were undertaken.

Results: Nineteen studies from the United States, Europe, and Mexico were included. Absorption from diets was higher with an enhancer (standard mean difference: 0.53; 95% CI: 0.21, 0.85; P = 0.001) and was also higher when compared with low-bioavailability diets (standard mean difference: 0.96; 95% CI: 0.51, 1.41; $P <$ 0.0001); however, single inhibitors did not reduce absorption (possibly because of the limited number of studies and participants and their heterogeneity). A regression equation to calculate iron absorption was derived by pooling data for iron status (serum and plasma ferritin) and dietary enhancers and inhibitors from 58 individuals (all from US studies): log[nonheme-iron absorption, %] = −0.73 log[ferritin, μg/L] + 0.11 [modifier] + 1.82. In individuals with serum ferritin concentrations from 6 to 80 μg/L, predicted absorption ranged from 2.1% to 23.0%.

Conclusions: Large variations were observed in mean nonheme-iron absorption (0.7–22.9%) between studies, which depended on iron status (diet had a greater effect at low serum and plasma ferritin concentrations) and dietary enhancers and inhibitors. Iron absorption was predicted from serum ferritin concentrations and dietary modifiers by using a regression equation. Extrapolation of these findings to developing countries and to men and women of different ages will require additional high-quality controlled trials. *Am J Clin Nutr* 2013;98:65–81.

INTRODUCTION

Anemia affects nearly 25% of the world's population—an estimated 1.62 billion people (1). Although data on its etiology are limited (2), iron deficiency resulting from low intakes or low absorption of iron is the most common cause, particularly when coupled with high physiologic requirements. In healthy individuals, ∼80% of absorbed iron is used for hemoglobin synthesis (3), and iron absorption is used as a surrogate measure of bioavailability. The amount of iron absorbed from a food or meal by an individual is determined by physiologic variables such as body iron status in combination with the modulating effect of dietary inhibitors and enhancers (4, 5), and iron absorption from whole diets is often different from that predicted from single-meal experiments (6, 7). The key dietary enhancers of iron absorption include vitamin C (ascorbic acid), meat, poultry, fish, and alcohol, and inhibitors include tannins (found in tea and coffee), calcium and dairy products, polyphenols, phytate, animal proteins (milk and eggs), and other micronutrients, eg, zinc and copper (5, 8). Several approaches have been used to estimate dietary iron bioavailability. The earliest involved a semiquantitative model, based on data obtained from single-meal radioisotope studies that incorporated the effect of both ascorbic acid and animal flesh on nonheme-iron absorption (9, 10). Later, algorithms were developed by using data from single-meal absorption studies investigating the influence of both dietary enhancers and inhibitors (11). Unfortunately, to date, none of these approaches have generated definitive data for predicting dietary iron bioavailability.

The composition of a diet is accepted as having an effect on iron bioavailability, as illustrated by the global recommendations made by the WHO/FAO, which cover diets with different bioavailabilities (15%, 12%, 10%, and 5%) (12). However, no transparent justification is provided for the selection of these values, and there is also disagreement about the importance of the effect of diet on bioavailability. The UK Scientific Advisory Committee on Nutrition recently concluded that individual effects of dietary enhancers and inhibitors on iron absorption are diminished when consumed as part of a whole diet. Additionally, effects may only be detected in individuals with a higher absorptive capacity as a result of increased iron requirements (13). The primary aim of this systematic review was to collate data on iron absorption from whole diets to analyze the effect of various

[1] From Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, United Kingdom.

[2] This manuscript does not necessarily reflect the views of the Commission of the European Communities and in no way anticipates the future policy in this area.

[3] Supported in part by the Commission of the European Communities, specific RTD Programme "Quality of Life and Management of Living Resources," within the 6th Framework Programme (contract no. FP6-036196-2 European Micronutrient Recommendations Aligned).

[4] Address correspondence to SJ Fairweather-Tait, Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom. E-mail: s.fairweather-tait@uea.ac.uk.

Received September 6, 2012. Accepted for publication March 27, 2013.
First published online May 29, 2013; doi: 10.3945/ajcn.112.050609.

Supplemental Material can be found at:
http://ajcn.nutrition.org/content/suppl/2013/06/17/ajcn.112.050609.DCSupplemental.html

dietary enhancers, inhibitors, and host-related factors on absorption and to generate an evidence base from which bioavailability values could be derived for setting dietary reference values.

METHODS

Study selection

This review was limited to studies that met the following inclusion criteria: *1*) conducted within an apparently healthy adult or elderly human population group (≥18 y of age); *2*) included an appropriate control group or dietary phase (not a historic control); *3*) had a randomized or nonrandomized controlled trial design (parallel or crossover); *4*) reported an iron-absorption outcome, measured and calculated by using an appropriate method (radioisotope or stable isotope) from whole diets; and *5*) reported one or more of the following iron-status biomarkers at baseline: serum or plasma ferritin, soluble transferrin receptor, hemoglobin, or body iron (14). Studies that included participants with unspecified acute illnesses or active disease were excluded, although subjects with iron deficiency or iron deficiency anemia were included (other causes of anemia were excluded). Exceptions with regard to health status were made for studies reporting absorption in relation to genotype, which may be associated with disease or health conditions (eg, hemochromatosis). No limits were imposed on population size because it was predicted that most of the studies would include relatively few participants. Whole-diet assessments were defined as a minimum of 1 d of 3 labeled meals or 2 d of 2 labeled meals and could be either self-selected or standardized to be representative of a typical whole diet. No other duration limitations were set.

Search strategy

Searches were conducted by using the Cochrane Library CENTRAL (http://www.thecochranelibrary.com), Medline (http://www.ncbi.nlm.nih.gov/pubmed), and EMBASE (both OvidSP; http://gateway-di.ovid.com/) databases from inception to November 2011 for iron-absorption studies by using both free text terms and appropriate indexing terms. The basic search structure was [iron or isotope terms] AND [absorption or bioavailability terms] AND [diet or meal] AND [limited to human studies]. The OvidSP Medline search is given as an example in Supplementary File 1 (*see* "Supplemental data" in the online issue). The reference lists of relevant review articles and included studies were searched for further potential references, and an expert within the field was consulted to identify additional studies. A clinical trials database (www.clinicaltrials.gov) was also searched to identify any unpublished studies. The included studies were limited to those published in a European language in which the research team was competent, ie, English, Spanish, French, or German.

Data collection and extraction

Titles and abstracts of references identified through the database searches were screened for inclusion by 3 reviewers, each responsible for 30% of the references. Initially, a 10% portion of the references was screened by all 3 reviewers, and the results were compared. In the case of any disagreements, the results were discussed and a decision reached by consensus. The full texts of potentially relevant articles were collected and assessed by using an inclusion or exclusion form based on the criteria listed earlier in this section. The assessment was carried out by 2 reviewers with 10% duplication. When agreement could not be reached, the articles were discussed, and a third reviewer was consulted if necessary. During the full text assessment, articles were classified as single-meal, multiple-meal, or whole-diet studies, although only the latter were extracted and included in the meta-analysis.

Data from included whole-diet studies were extracted onto paper forms by a single reviewer with a minimum duplication of 10% of studies by a second reviewer. A third reviewer randomly selected the studies to be duplicated. The extraction form was created and trialed by the team before the data extraction stage, and designed to assimilate the same information from each study. Publication details, study design, population characteristics, study objectives, and principal outcomes relating to iron absorption were recorded for each study. Potential confounders, such as iron status, sex, age, inhibitors and enhancers of absorption, and recent iron intake, were recorded when data and information were provided.

Quality and risk of bias

The quality of the included studies and potential sources of bias were assessed and explored. Specific data fields were included in the extraction forms to collect information on methods of randomization (if applicable), methods of analysis, numbers of dropouts and incomplete data or selective outcome reporting. No formal grading of the studies was possible because of the variation in study design and methods that can be used for measuring iron absorption. A table summarizing the validity assessment criteria is presented elsewhere (*see* Supplementary File 2 under "Supplemental data" in the online issue).

Data synthesis and analysis

To maximize the number of studies included in each analysis, standard mean differences (SMDs) were used in the forest plots to account for presentational differences in terms of units of the primary outcome—iron absorption. Typically, this was presented as a percentage of the nonheme-iron dose or intake, although it was not always explicitly clear or it was sometimes presented as an absolute amount or as a percentage of total iron absorption. Arithmetic means and SDs were used in the forest plots, except where stated, and authors were contacted for missing data or when data were presented in a manner that prevented them being pooled. Random-effects models were used throughout, and the I^2 test for heterogeneity was conducted and reported. When high levels of heterogeneity were observed, subgrouping was conducted (where sufficient data were available) to assess the effects of potential confounders such as study design, age, sex, and iron status. Sensitivity analyses were conducted to check whether certain studies overly influenced the pooled results. Logarithms are all natural logarithms. Forest plot analyses were all conducted by using Review Manager 5 software (version 5.0; The Nordic Cochrane Centre, The Cochrane Collaboration).

The limited number of included studies prevented any informative subgroup analysis and meta-regression, so we decided

post hoc to use the individual participant data provided by some studies. Because of the skewed distributions of percentage nonheme-iron absorption and serum ferritin, both were log transformed and the individual data from the standard diet phases were plotted as a scatter plot. The ferritin method developed by Cook et al (6) was used to correct the standard diet phase data of all studies with individual data, plus all dietary phases of 3 similar studies, to a range of ferritin concentrations (15–60 μg/L). The corrected data were plotted to give absorption curves at a range of ferritin concentrations, and the uncorrected data were analyzed for within-subject effects by using a repeated-measures ANOVA. Because log transformation of percentage nonheme-iron absorption and serum ferritin suggested a straight line relation, we carried out linear regression in a model that initially included ferritin (log serum ferritin), presence of enhancer or inhibitor, age, and sex as independent variables for predicting (log) percentage nonheme-iron absorption. The presence of enhancer or inhibitor (modifier) was coded as 0 (for a standard or self-selected diet), −1 for a diet with inhibitors, and +1 for a diet with enhancers. For each individual, data from 2 arms were included, but for studies with >2 arms the most extreme arms (−1 and +1) were selected. Additional analyses, including linear regression, on individual data were carried out by using SPSS software (PASW Statistics 18, release 18.0.0; IBM Corporation).

RESULTS

The flow diagram for the review is shown in **Figure 1**. The search identified 2636 potentially relevant titles and abstracts, 437 of which were assessed as full texts as part of this review. Of these, 19 studies (20 articles; 6, 7, 16–33) met the full list of inclusion criteria and reported the absorption of iron from a whole diet. Articles were excluded for a variety of reasons, including use of an inappropriate study design, unhealthy population group, and lack of baseline iron-status markers. Studies that met all other inclusion criteria, but measured absorption from single or multiple meals, were also excluded from the meta-analysis. The latter were defined as meals that did not represent a typical habitual whole diet. Details of the included studies are given in **Table 1**.

Of the 19 included studies, 9 were undertaken in Europe (3 in Sweden, 3 in Denmark, 2 in the United Kingdom, and 1 in the Netherlands), 9 in the United States, and 1 in Mexico. Five of the studies were in mixed-sex populations, 10 were in women, and 4 were in men. Most of the studies did not explicitly characterize the status of the population other than as "healthy" or "non-anemic" or selected volunteers with a normal ferritin concentration range for sex or age (11 in total). Six studies were conducted in individuals selected for low iron status—typically premenopausal women. In one study, the individuals were selected by genotype and in another by blood donation. Fourteen of the studies measured iron absorption by using radioisotopes, and 5 used stable-isotope techniques. Many of the studies involved diets that were described as "typical" for their region, as low bioavailability, or were self-selected during the control period. Most of the studies were classed as manipulating one principal factor (eg, the effect of an enhancer or inhibitor on absorption) or multiple factors to create low- and high-bioavailability diets.

The included studies were selected for high methodologic quality (ie, used isotopes to measure absorption). Most of the studies had small numbers of subjects (range: 8–45)—the largest comprising 45 participants assigned to 3 groups of 15 in a parallel design. Dropouts were often not explicitly reported; however, in most cases it appeared that there were none. Exclusions from the data analyses were generally well reported, with adequate justification. Blinding was often not possible because of

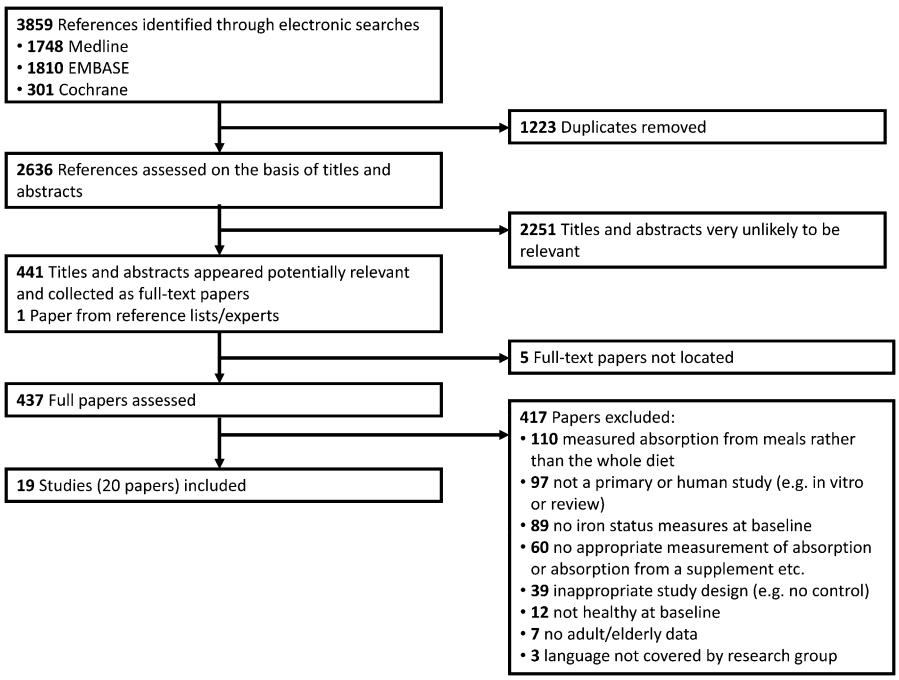

FIGURE 1. Flow diagram of search and selection process; modified from Quality of Reporting of Meta-analyses (15).

TABLE 1
Characteristics of the included studies[1]

Author, year, reference	Population characteristics	Study design	Absorption method	Baseline biomarkers reported	Diets and main confounders tested	Main outcome measures
Cook et al, 1991 (6)	Description: mixed sex, healthy, nonanemic n = 45 (15 per group) Age: 21–40 y Country: USA	Parallel, nonrandomized, 3 groups each receiving 4 interventions	Radiolabeling, RBC incorporation of ^{55}Fe and ^{59}Fe	Ferritin value given for each parallel (diet) group	Basal diet: self-selected diet Confounders: enhancing and inhibiting diets by modulation of meat, vitamin C, tannins (tea/coffee), and phytate	Absorption from diet uncorrected (%) Absorption from diet corrected (%) Absorption of reference dose (%)
Cook and Reddy, 2001 (7)	Description: mixed sex, healthy, nonanemic n = 12 Age: 20–38 y (mean: 25 y) Country: USA	Crossover, partly randomized; first a self-selected diet and then randomization to order of high/low vitamin C diets	Radiolabeling, RBC incorporation of ^{55}Fe and ^{59}Fe	Ferritin for whole population	Basal diet: self-selected diet Confounder: vitamin C	Nonheme-iron absorption, not corrected (%)
Diaz et al, 2003 (16)	Description: nonpregnant, nonlactating women; iron deficient (ferritin $<$12 μg/L) n = 15, absorption data for n = 11 Age: 28.3 $\pm$ 7.7 y (mean $\pm$ SD) Country: Mexico	Crossover, no randomization	Stable-isotope labeling with ^{57}Fe, ^{58}Fe isotopes	Hb and ferritin for each group	Basal diet: typical rural Mexican diet Confounder: ascorbic acid	Iron absorption (%) Absorption of reference dose (%)
Gleerup et al, 1995 (17)	Description: healthy females of fertile age n = 21 (20 for second intervention period) Age: 21–44 y (mean $\pm$ SE: 29.2 $\pm$ 1.8 y) Country: Sweden	Crossover, no randomization	Radioisotope ^{59}Fe, whole-body counter measurement	Hb and ferritin	Basal diet: standard diet Confounders: calcium (milk intake)	Nonheme-iron absorption (%) Heme-iron absorption (mg) Total iron absorption (mg)
Grinder-Pedersen et al, 2004 (18)	Description: young women with low iron stores (Hb $\geq$ 110 g/L, ferritin $\leq$40 μg/L) n = 14 Age: 24.2 $\pm$ 3.0 y (mean $\pm$ SD) Country: Denmark	Crossover with randomization	Radioisotope ^{59}Fe absorption, whole-body retention	Hb and ferritin	Basal diet: basic diet with low calcium content Confounder: sources of calcium	Nonheme-iron absorption, unadjusted (%) Nonheme-iron absorption, adjusted for ferritin (%)

(Continued)

TABLE 1 (*Continued*)

Author, year, reference	Population characteristics	Study design	Absorption method	Baseline biomarkers reported	Diets and main confounders tested	Main outcome measures
Hallberg et al, 1997 (19)	Description: healthy men; a subset were blood donors with lower ferritin than nondonors *n* = 31 (19 nondonors, 12 donors) Age: 20–59 y (mean ± SD: 29.4 ± 10.8 y) Country: Sweden	Parallel groups, no randomization	Radioisotope iron tracers (^{59}Fe, ^{55}Fe), incorporation into blood and whole-body counter measurement (^{59}Fe)	Hb and ferritin for each group (nondonors and donors)	Basal diet: varied and realistic high-bioavailability diet Confounder: blood donation (iron status)	Nonheme-iron absorption (%, mg) Heme-iron absorption (mg) Total iron absorption (mg) Absorption of reference dose (%)
Hulten et al, 1995 (20)	Description: healthy women of fertile age *n* = 21 Age: 21–36 y (mean: 27.1 y) Country: Sweden	Crossover, randomization unclear	Radioisotope iron tracers (^{59}Fe, ^{55}Fe), incorporation into blood and whole-body counter measurement	Hb and ferritin	Basal diet: high-bioavailability diet with high meat intake and low phytate content Confounders: meat and phytate intakes	Nonheme-iron absorption (mg) Heme-iron absorption (mg) Total iron absorption (mg)
Hunt et al, 2003 (21), 2004 (25)	Description: healthy, nonanemic, premenopausal women *n* = 36 (35 included in data analysis) Age: 20–44 y (mean ± SD: 32 ± 7 y) Country: USA	Parallel groups with randomization	Radioisotope labeling (^{55}Fe, ^{59}Fe), whole-body scintillation counting	Hb, ferritin, and transferrin receptor for each group	Basal diet: high- and low-bioavailability diets Confounder: adaptation to high- and low-bioavailability diets	Nonheme-iron absorption (%, mg) Heme-iron absorption (%, mg) Total iron absorption (mg)
Hunt et al, 1994 (22)	Description: healthy, nonpregnant women with low serum ferritin (3.5–17.7 μg/L) *n* = 25 Age: 20–45 y Country: USA	Crossover/parallel, randomization unclear	Whole body scintillation counting (^{59}Fe) and fecal monitoring	Hb and ferritin for each group	Basal diet: low-bioavailability diet and typical Western diet Confounder: ascorbic acid	Apparent iron absorption (mg/d)
Hunt et al, 1999 (23)	Description: healthy women (with ferritin between 6 and 149 μg/L) *n* = 21, absorption measured in 10 Age: 20–42 y (mean ± SD: 33.2 ± 7.0 y) Country: USA	Crossover with randomization	Whole-body scintillation counting (^{59}Fe) and fecal monitoring	Hb and ferritin	Basal diet: lactoovovegetarian (no meat) and nonvegetarian Confounders: meat, phytic acid, ascorbic acid, and fiber intakes	Nonheme-iron absorption (%, mg) Total iron absorption (mg)

(Continued)

TABLE 1 (*Continued*)

Author, year, reference	Population characteristics	Study design	Absorption method	Baseline biomarkers reported	Diets and main confounders tested	Main outcome measures
Hunt, 2000 (24), 2004 (25)	Description: healthy men with ferritin ≥20 and <450 μg/L *n* = 31 Age: 32–56 y (mean ± SD: 44 ± 7 y) Country: USA	Parallel with randomization	Radioisotope labeling (^{55}Fe, ^{59}Fe), whole-body scintillation counting	Hb, ferritin, and transferrin receptor for each group	Basal diet: high-bioavailability controlled weighed diet Confounders: meat, phytic acid (cereal content), vitamin C, tannins (tea and coffee)	Nonheme-iron absorption (%, mg) Heme-iron absorption (%, mg) Total iron absorption (mg)
Kristensen et al, 2005 (26)	Description: healthy young omnivorous women with low iron stores (ferritin 12–30 μg/L) *n* = 22 total, 19 analyzed Age: 18–40 y (mean ± SE: 25 ± 5.2 y) Country: Denmark	Crossover with randomization to treatment order	Whole-body counting of ^{59}Fe	Hb and ferritin for whole population	Basal diet: standard low-iron-availability diet (low vitamin C and high phytic acid) Confounder: pork meat	Nonheme-iron absorption (%, mg) Total iron absorption (mg)
Minihane et al, 1998 (27)	Description: mixed-sex vegetarian volunteers, healthy and nonanemic *n* = 14 Age: 40 ± 4 y (mean ± SD) Country: UK	Crossover, no randomization	Stable-isotope labeling (^{57}Fe ^{54}Fe, ^{58}Fe) and fecal collection	Hb and ferritin	Basal diet: standard diets Confounder: calcium	Nonheme-iron absorption, corrected for reference dose (%) Nonheme-iron absorption, corrected for ferritin (%)
Reddy and Cook, 1997 (28)	Description: mixed sex, healthy, nonanemic *n* = 14 Age: 19–37 y (mean: 25 y) Country: USA	Crossover, partly randomized; first self-selected diet then randomization to order of high/low calcium diets	Radiolabeling, RBC incorporation of ^{55}Fe and ^{59}Fe	Serum ferritin for whole population	Basal diet: self-selected diet Confounder: calcium	Iron absorption, not corrected (%)
Reddy et al, 2006 (29)	Description: mixed sex, healthy *n* = 14 Age: 19–38 y (mean: 27 y) Country: USA	Crossover, randomization unclear, but similar in design to others (7, 28); self-selected diet then allocation to either high-meat or low-meat diet	Radiolabeling, RBC incorporation of ^{55}Fe and ^{59}Fe	Serum ferritin for whole population	Basal diet: self-selected diet Confounder: meat	Iron absorption, not corrected (%)

(*Continued*)

TABLE 1 (*Continued*)

Author, year, reference	Population characteristics	Study design	Absorption method	Baseline biomarkers reported	Diets and main confounders tested	Main outcome measures
Roe et al, 2005 (30)	Description: men n = 35 (15 wild/wild, 15 C282Y/wild, and 5 C282Y/H63D) Age: ≥40 y Country: UK	Parallel (divided by genotype groups) with treatments in identical order	Stable-isotope labeling (^{54}Fe and ^{57}Fe) and RBC incorporation	Hb, serum ferritin, and plasma transferrin receptor for each genotype group	Basal diet: high-bioavailability and cereal diets Confounder: genotype	Isotope dose absorbed (%, mg) Nonheme-iron absorption (mg)
Tetens et al, 2005 (31)	Description: healthy women with low ferritin (12–30 μg/L) but no IDA (Hb > 110 g/L) n = 32 Age: 21–29 y Country: Denmark	Crossover with randomization to treatment order (3 studies each with 2 arms)	Whole-body counter measurement of ^{59}Fe	Hb and serum ferritin for whole population	Basal diet: typical Danish foods Confounders: vitamin C, meat, phytic acid	Reference dose absorption (%) Nonheme-iron absorption (%, mg) Heme-iron absorption (%, mg) Corrected values for nonheme iron
Turnlund et al, 1990 (32)	Description: healthy young women, Hb ≤ 150 g/L, and serum ferritin ≤50 μg/L n = 9 total, 8 analyzed Age: 28 ± 4 y (mean ± SD) Country: USA	Crossover with randomization to treatment order	Stable isotope (^{54}Fe) labeling and fecal collection	Hb and serum ferritin for whole population	Basal diet: cereal-based Confounder: milk	Iron absorption with or without milk (%)
van den Heuvel et al, 1998 (33)	Description: healthy male subjects n = 12 Age: 20–30 y Country: Netherlands	Crossover with randomization (control group and 3 groups received different nondigestible oligosaccharides)	Stable-isotope labeling (^{57}Fe and ^{58}Fe) and RBC incorporation	Hb and ferritin for each group	Basal diet: controlled basal diet Confounders: oligosaccharides, inulin, fructooligosaccharide, galactooligosaccharide	Iron absorption, uncorrected data presented, but corrected for serum ferritin during analysis (%)

[1] Hb, hemoglobin; IDA, iron deficiency anemia; RBC, red blood cell.

TABLE 2
Summary of iron absorption from all included studies[1]

Author, year, reference	Sex	Baseline ferritin	Diet description	Iron absorption		Labeling period
				Mean	SE, SD, or 95% CI	
		μg/L		%	%	
Cook et al, 1991 (6)	M/F	34 (range: 8–242)	Self-selected diet	NH: 7.4 (G)	5.6–9.9 (± 1 SE)	14 d (2 meals/d)
				corr F: 6.4 (G)	5.5–7.3 (± 1 SE)	
		48 (range: 6–252)	Self-selected, enhancing diet	NH: 6.6 (G)	5.4–8.0 (± 1 SE)	
				corr F: 8.0 (G)	7.0–9.2 (± 1 SE)	
		37 (range: 12–236)	Self-selected, inhibiting diet	NH: 3.4 (G)	2.6–4.6 (± 1 SE)	
				corr F: 3.2 (G)	2.8–3.6 (± 1 SE)	
Cook et al, 2001 (7)	M/F	31, 26–37 (±1 SE)	Self-selected diet	NH: 4.57 (G)	3.39–6.17 (± 1 SE)	5 d (3 meals/d)
			Self-selected diet, high vitamin C	NH: 7.69 (G)	5.93–9.99 (± 1 SE)	
			Self-selected diet, low vitamin C	NH: 5.69 (G)	4.76–6.80 (± 1 SE)	
Diaz et al, 2003 (16)	F	6.3 ± 3.3 (SD)	Typical Mexican diet	NH: 6.6	3.0 (SD)	14 d (2 meals/d)
		6.4 ± 2.3 (SD)	Typical Mexican diet + ascorbic acid	NH: 22.9	12.6 (SD)	
Gleerup et al, 1995 (17)	F	25.4 ± 3.9 (SD)	Standard diet + milk with lunch and dinner	NH: 12.1	4.76 (SE)	4 × 5 d (4 meals/d)
			Standard diet + milk with breakfast and evening meal	NH: 15.9	2.20 (SE)	
Grinder-Pedersen et al, 2004 (18)	F	15 (range: 6–44)	Basal diet (low calcium)	NH: 7.4 (G)	5.3, 10.5 (95% CI)	4 d (3 meals/d)
				corr F: 2.6 (G)	1.5, 4.4 (95% CI)	
			Basal diet (low calcium) + milk	NH: 5.2 (G)	3.5, 7.9 (95% CI)	
				corr F: 1.9 (G)	1.1, 3.4 (95% CI)	
			Basal diet (low calcium) + calcium lactate	NH: 6.7 (G)	5.0, 8.9 (95% CI)	
				corr F: 2.3 (G)	1.6, 3.3 (95% CI)	
			Basal diet (low calcium) + milk mineral	NH: 5.1 (G)	3.2, 7.9 (95% CI)	
				corr F: 2.1 (G)	1.4, 3.3 (95% CI)	
Hallberg et al, 1997 (19)	M	91 ± 36.9 (SD)	Standard diet, nondonors	NH: 4.5	1.96 (SD)	5 d (4 meals/d)
				H: 23.2	7.0 (SD)	
		36.8 ± 15.8 (SD)	Standard diet, blood donors	NH: 17.4	8.4 (SD)	
				H: 34.9	8.1 (SD)	
Hulten et al, 1995 (20)	F	30.4 (range: 10–70)	High-bioavailability diet with high meat intake and lower phytate	NH: 1.91 mg/d	0.26 (SE)	2 × 5 d (4 meals/d)
				T: 2.31 mg/d	0.27 (SE)	
			Medium-bioavailability diet with low meat intake and 2-fold higher phytate	NH: 1.22 mg/d	0.21 (SE)	
				T: 1.32 mg/d	0.21 (SE)	
Hunt, 2003 (21), Hunt and Zeng, 2004 (25)	F	21 (range: 4–73)	High-bioavailability diet + high-bioavailability test diet	NH: 11.8 (G)	7.9, 17.4 (pooled SD)	2 × 2 d (3 meals/d)
				H: 40 (G)	33, 48 (pooled SD)	
			High-bioavailability diet + low-bioavailability test diet	NH: 2.4 (G)	1.6, 3.6 (pooled SD)	
				H: 38 (G)	31, 46 (pooled SD)	
			Low-bioavailability diet + high-bioavailability test diet	NH: 9.0 (G)	6.1, 13.3 (pooled SD)	
				H: 29 (G)	24, 35 (pooled SD)	
			Low-bioavailability diet + low-bioavailability test diet	NH: 2.3 (G)	1.5, 3.4 (pooled SD)	
				H: 33 (G)	27, 40 (pooled SD)	
Hunt et al, 1994 (22)	F	12.2 ± 2.5 (SD)	Low-bioavailability diet	4.3 mg/d	3.1 (pooled SD)	Balance study 9 d
			Low-bioavailability diet + ascorbic acid	5.8 mg/d	3.1 (pooled SD)	
		10.2 ± 1.9 (SD)	Western diet	6.4 mg/d	2.3 (pooled SD)	
			Western diet + ascorbic acid	6 mg/d	2.3 (pooled SD)	

(Continued)

TABLE 2 (*Continued*)

Author, year, reference	Sex	Baseline ferritin	Diet description	Iron absorption		Labeling period
				Mean	SE, SD, or 95% CI	
Hunt and Roughead, 1999 (23)	F	38.3 (range: 6–149) 43.6 (SD)	Omnivorous diet Lactoovovegetarian	NH: 3.8 (G) NH: 1.1 (G)	2.1–6.7 (± 1 SD) 0.6–2.1 (± 1 SD)	2 d (3 meals/d)
Hunt and Roughead, 2000 (24), Hunt and Zeng, 2004 (25)	M	118, 102–139 (± 1 SE)	High-bioavailability diet	NH: 3.4 (G) H: 26 (G)	2.4–4.8 (± 1 SD) 22–31 (± 1 SD)	2 × 2 d (3 meals/d)
		100, 86–118 (±1 SE)	Low-bioavailability diet	NH: 0.7 (G) H: 22 (G)	0.5–1.0 (± 1 SD) 18–26 (± 1 SD)	
Kristensen et al, 2005 (26)	F	19 (range: 12–28)	Low bioavailability diet Diet + Danish pork meat Diet + Polish pork meat	NH: 5.3 NH: 7.9 NH: 6.8	0.6 (SE) 1.1 (SE) 1.0 (SE)	5 d (3 meals/d)
Minihane et al, 1998 (27)	M/F	39 ± 9 (SD)	Basal diet Basal diet + calcium 	NH: 15.8 corr F: 11.1 corr R: 16.7 NH: 4.7 corr F: 3.8 corr R: 4.3	2.1 (SE) 3.0 (SE) 3.1 (SE) 1.4 (SE) 1.5 (SE) 1.0 (SE)	1 d (3 meals/d)
Reddy et al, 1997 (28)	M/F	50, 42–60 (±1 SE)	Self-selected diet Self-selected diet, high calcium Self-selected diet, low calcium	NH: 5.01 (G) NH: 4.71 (G) NH: 5.83 (G)	4.08–6.17 (± 1 SE) 3.80–5.85 (± 1 SE) 4.65–7.30 (±1 SE)	5 d (2 meals/d)
Reddy et al, 2006 (29)	M/F	34, 26–46 (±1 SE)	Self-selected diet Self-selected diet + high meat Self-selected diet (no meat)	NH: 4.81 (G) NH: 6.47 (G) NH: 5.08 (G)	3.72–6.22 (± 1 SE) 4.76–8.81 (± 1 SE) 3.76–6.87 (± 1 SE)	5 d (3 meals/d)
Roe et al, 2005 (30) (wildtype only)	M	62 ± 50 (SD)	Cereal diet High-bioavailability diet	NH: 4.9 NH: 6.8	2.0 (SD) 6.8 (SD)	3 d (2 meals/d) 2 d (3 meals/d)
Tetens et al, 2005 (31)	F	20.2 ± 1.4 (SE)	Typical Danish foods with low meat, low vitamin C, and high phytic acid Typical Danish foods with low meat, high vitamin C, and high phytic acid Typical Danish foods with low meat, low vitamin C, and high phytic acid Typical Danish foods with high meat, low vitamin C, and high phytic acid Typical Danish foods with high meat, high vitamin C, and low phytic acid Typical Danish foods with high meat, high vitamin C, and high phytic acid	NH: 1.5 corr R: 1.9 NH: 2.7 corr R: 3.4 NH: 2.9 corr R: 3.5 NH: 3.4 corr R: 3.5 NH: 4.9 corr R: 4.9 NH: 3.7 corr R: 3.8	0.57 (SE) 0.5 (SE) 0.60 (SE) 0.4 (SE) 0.59 (SE) 0.6 (SE) 0.5 (SE) 0.6 (SE) 0.93 (SE) 0.9 (SE) 0.9 (SE) 0.7 (SE)	2 × 5 d (3 meals/d)
Turnlund et al, 1990 (32)	F	Day 1: 31.9 ± 16.1 (SD)	Cereal based diet (no milk) Cereal based diet + milk	NH: 8.04 NH: 8.97	2.56 (SD, calculated from chart) 2.18 (SD, calculated from chart)	2 d (3 meals/d)
van den Heuvel et al, 1998 (33)	M	84 ± 48 (SD) 84 ± 58 (SD) 86 ± 53 (SD) 82 ± 49 (SD)	Control diet Control diet + inulin Control diet + fructooligosaccharide Control diet + galactooligosaccharide	NH: 5.1 NH: 5.5 NH: 6.1 NH: 5.3	1.5 (SE) 1.6 (SE) 1.9 (SE) 1.9 (SE)	7 d (3 meals/d)

[1] corr F, corrected/adjusted for ferritin data; corr R, corrected/adjusted for reference dose; G, geometric mean; H, heme-iron absorption; NH, nonheme-iron absorption; T, total iron absorption.

obvious differences in diet composition. Most of the studies were crossover in design, which allowed a direct comparison between intraindividual absorption values. When a parallel design was used, most of the studies corrected the absorption data either by using the ferritin method [to 40 μg/L (6)] or to a reference dose. There was considerable variation in the assessment of intake of iron and other nutrients from the diets, and dose verification was seldom reported. The diets were normally analyzed either by duplicate diet analysis or by using nutritional software and food-composition databases.

The mean iron-absorption data in each study from all relevant diets, groups, or intervention phases are shown in **Table 2**. Data corrected by using ferritin values or reference dose absorption were also presented. The lowest reported fractional nonheme-iron absorption was 0.7%—in men with a mean ferritin concentration of 100 μg/L who consumed a low-bioavailability diet (24). The highest absorption, 22.9%, was observed in women with iron deficiency (mean ferritin: 6.4 μg/L) who consumed a basic Mexican diet (low bioavailability) but with added limeade (high in vitamin C) at each meal (16). Absorption typically appeared to be ~5–8%, and it should be noted that many of the diets were specifically designed to be of low bioavailability.

Meta-analyses were undertaken to assess the effect of enhancers and inhibitors of iron absorption. Studies were initially grouped into 3 categories: manipulation of one enhancing factor, manipulation of one inhibitory factor, or manipulation of multiple factors (ie, high- compared with low-bioavailability diet). The studies in each forest plot, or subgroup of a forest plot, were ordered by the mean baseline ferritin concentration of the study population (low to high). SMDs were translated into percentage absorption units by using the SD of the Kristensen et al study (26); the translated units are given in the text for each figure and are presented in **Table 3** and **Table 4** for the sensitivity and subgroup analyses, respectively. The Kristensen et al study (26) was selected for the translation of SMDs because it was one of the largest included studies and reported results in the most common format (% iron absorption). Studies in which one enhancing or inhibitory factor was altered are shown in **Figure 2**; these included studies, where a diet rich in an inhibitor or enhancer was compared with one low in the inhibitor or enhancer and those that gave the diet alone or with the inhibitor or enhancer, usually as a supplement or in a drink. Enhancers (meat and ascorbic acid) significantly increased iron absorption from whole diets (SMD: 0.53; 95% CI: 0.21, 0.85; $P = 0.001$; $n = 102$ control participants; P-heterogeneity = 0.26; $I^2 = 21\%$); 8 studies were included in the plot. Reconverting these data into units of absorption [by using the SD in the Kristensen et al study (26)] suggested an increase in iron absorption of 2.0% (95% CI: 0.8%, 3.2%) when enhancers were added to the diet or were naturally present in high quantities in the diet. The study by Diaz et al (16) showed a far larger effect of the enhancer on iron absorption from the standard diet, but this study was conducted in women with very low iron stores (mean ferritin concentration: 6.3 μg/L). However, removal of this study from the plot did not change the overall result, which remained significant. All of the sensitivity analyses conducted and the effect on the overall pooled results for each analysis are summarized in Table 3. A trend for inhibitory factors (milk, calcium, and phytate) to reduce iron absorption from whole diets which did not quite reach statistical significance (SMD: −0.44; 95% CI: −0.90, 0.02; $P = 0.06$; $n = 81$ control participants; P-heterogeneity = 0.07, $I^2 = 52\%$) or a 1.7% reduction in iron absorption (−3.4%, 0.1%) is shown in Figure 2. Removal of the Minihane et al study (27), in which absorption was measured over only 1 d, eliminated the observed heterogeneity and showed a smaller but nearly significant reduction in iron absorption (Table 3).

The single inhibitory and enhancing effects pooled in Figure 2 are shown by subgroup of individual dietary enhancers and inhibitors in **Figure 3** and **Figure 4**. The effect of ascorbic acid on iron absorption from whole diets was assessed in 4 studies and increased iron absorption significantly (SMD: 0.63; 95% CI: 0.10, 1.16; $P = 0.02$; $n = 58$ control participants; P-heterogeneity = 0.10, $I^2 = 48\%$)—an increase in iron absorption of 2.4% (95% CI: 0.4%, 4.4%). The effect remained significant with the removal of the Diaz et al (16) study ($P = 0.05$; Table 3). The effect of meat on nonheme-iron absorption reached only borderline significance (SMD: 0.43; 95% CI: 0.01, 0.86; $P = 0.05$; $n = 44$ control participants; 3 studies; P-heterogeneity = 0.67, $I^2 = 0\%$)—an increase in iron absorption of 1.6% (95% CI: 0.0%, 3.2%). Three inhibitory factors were assessed in the included studies; calcium, milk, and phytate. Milk contains significant amounts of calcium but was analyzed separately because it also contains casein, which is also thought to be an inhibitor (5). Although a significant inhibitory effect was not observed for any of the 3 dietary components (Figure 4; $P = 0.36$ for milk, $P = 0.12$ for calcium, and $P = 0.38$ for phytic acid), there were very limited data, particularly for phytate (only one study).

Six included studies manipulated multiple dietary components to maximize and minimize bioavailability. Iron absorption was significantly higher in diets specifically selected to be high in bioavailability (ie, low in inhibitors and high in enhancers) compared with those of low bioavailability (SMD: 0.96; 95% CI: 0.51, 1.41; $P < 0.0001$; $n = 96$ control participants; P-heterogeneity < 0.0001, $I^2 = 82\%$), which suggested an increase of 3.6% in iron absorption (1.9%, 4.3%). A sensitivity analysis was performed to remove the study by Hulten et al (20), because this was the only study to report results in milligrams rather than as a percentage. The effect remained highly statistically significant ($P < 0.0001$, Table 3).

Because of the different aims of the included studies, subgroup analyses could only be completed by using subsets of the data. The analyses were conducted in the studies presenting data for single enhancing factors and high- or low-bioavailability diets separately, because these included the greatest number of studies. The results are summarized in Table 4. Because of the limited number of studies in each subset, it was not possible to complete all the subgroup analyses as originally planned (sex, age, iron status, and study design). The effect of parallel and crossover methodologic designs in studies comparing high- with low-bioavailability diets is shown in **Figure 5**. Both study designs showed a significantly higher absorption from high-bioavailability diets, although the effect was more pronounced in studies of parallel design (P for subgroups = 0.04). Because of inconsistencies in the presentation of results in these studies, it was not possible to use corrected absorption data in the forest plots; correction to the reference dose absorption or to a mean iron status could help to eliminate or reduce these differences. The other subgroup analyses were limited by the number of studies available in each subgroup, and no other significant

TABLE 3
Sensitivity analyses[1]

Analysis	Modified analysis	SMD (95% CI)	*P*-overall effect	Heterogeneity (I^2)	Results altered after modification?	SMD translation into percentage (95% CI)
		%		%		%
Single enhancing factors (Figure 2)	Removal of 2 arms from Hunt et al (22); only study that measured "apparent absorption" and reported in mg/d rather than as %; analyzed as MD because all units were comparable	2.84 (0.52, 5.15)[2]	0.02	72	No; remains a significant effect, though heterogeneity now significant	NA
	Removal of Diaz et al (16); effect size considerably greater in this study	0.42 (0.12, 0.72)	0.005	0	No; remains a significant effect	1.6 (0.5, 2.7)
Single inhibiting factors (Figure 2)	Removal of Gleerup et al (17); study administered milk with different meals (breakfast/evening meal or lunch/dinner)	−0.50 (−1.08, 0.09)	0.10	59	No; remains a nonsignificant effect	−1.9 (−4.1, 0.3)
	Removal of Minihane et al (27); effect size considerably greater than that of other studies; only study to measure absorption over only 1 d	−0.25 (−0.59, 0.09)	0.16	0	No; remains a nonsignificant effect, but removes all heterogeneity	−0.9 (−2.2, 0.3)
Ascorbic acid (Figure 3)	Removal of Diaz et al (16); effect size considerably greater in this study	0.41 (−0.00, 0.82)	0.05	0	Borderline significance with this study removed, no heterogeneity	1.5 (0.0, 3.1)
Calcium (Figure 4)	Removal of Minihane et al (27); effect size considerably greater than that of other studies; only study to measure absorption over only 1 d	−0.25 (−0.78, 0.28)	0.36	0	Only 2 studies now included	−0.9 (−2.9, 1.1)
Milk (Figure 4)	Removal of Gleerup et al (17). Study administered milk with different meals (breakfast/evening meal or lunch/dinner)	−0.13 (−1.01, 0.75)	0.77	50	Only 2 studies now included	−0.5 (−3.8, 2.8)
Multiple enhancing and inhibiting factors (Figure 5)	Removal of Cook et al (6); data presented as geometric means only	1.03 (0.48, 1.57)	0.0002	60	No; remains a significant effect, does not reduce heterogeneity	3.9 (1.8, 5.9)
	Removal of Hunt et al (24); effect size considerably greater in this study	0.77 (0.45, 1.10)	<0.00001	0	No; remains a significant effect, removes all heterogeneity	2.9 (1.7, 4.1)
	Removal of Hulten et al (20); only study to report data only in mg/d; analyzed as MD because all units were comparable	3.62 (1.83, 5.42)[2]	<0.0001	53	No; remains a significant effect, but has no effect on heterogeneity.	NA

[1] MD, mean differemce; NA, not applicable; SMD, standard mean difference.
[2] MD.

TABLE 4
Subgroup analyses[1]

Subgrouping and analysis	Subgroup analysis (no. of studies; no. control group)	SMD (95% CI)	SMD translation into % absorption (95% CI)	*P*-overall effect	Heterogeneity (I^2)	Subgroup differences	
						P	I^2
Study design		%	%		%		%
Multiple enhancing and inhibiting factors (Figure 5)	Parallel (3; 50)	1.31 (0.60, 2.03)	4.9 (2.3, 7.6)	0.0003	60	0.04	77.5
	Crossover (3; 46)	0.61 (0.19, 1.03)	2.3 (0.7, 3.9)	0.004	0		
Single enhancing factors	All studies crossover	NA	NA	NA	NA	NA	
Sex							
Multiple enhancing and inhibiting factors	Females only (3; 49)	0.94 (0.50, 1.58)	3.5 (1.9, 5.9)	<0.0001	6	0.78	0
	Males only (2; 32)	1.17 (−0.44, 2.77)	4.4 (−1.7, 2.77)	0.15	87		
	Mixed sex (1; 15)	NA	NA	NA	NA		
Single enhancing factors	Females only (6; 76)	0.57 (0.14, 1.01)	2.1 (0.5, 3.8)	0.009	40	0.69	0
	Males only (0; 0)	NA	NA	NA	NA		
	Mixed sex (2; 26)	0.43 (−0.13, 0.98)	1.6 (−0.5, 3.7)	0.13	0		
Mean age						0.67	0
Multiple enhancing and inhibiting factors	Age < 40 y (4; 64)	0.88 (0.51, 1.24)	3.3 (1.9, 4.7)	<0.00001	0		
	Age ≥ 40 y (2; 32)	1.17 (−0.44, 2.77)	4.4 (−1.7, 2.77)	0.15	87		
Single enhancing factors	All populations <40 y of age	NA	NA	NA	NA		
Iron status (mean ferritin)							
Multiple enhancing and inhibiting factors	Ferritin < 60 μg/L (4; 64)	0.88 (0.51, 1.24)	3.3 (1.9, 4.7)	<0.00001	0	0.67	0
	Ferritin ≥ 60 μg/L (2; 32)	1.17 (−0.44, 2.77)	4.4 (−1.7, 2.77)	0.15	87		
Single enhancing factors	Ferritin < 15 μg/L (3; 36)	0.68 (−0.30, 1.65)	2.6 (−1.1, 6.2)	0.17	74	0.77	0
	Ferritin ≥ 15 μg/L (5; 66)	0.49 (0.14, 0.84)	1.8 (0.5, 3.2)	0.005	0		

[1] NA, not applicable; SMD, standard mean difference.

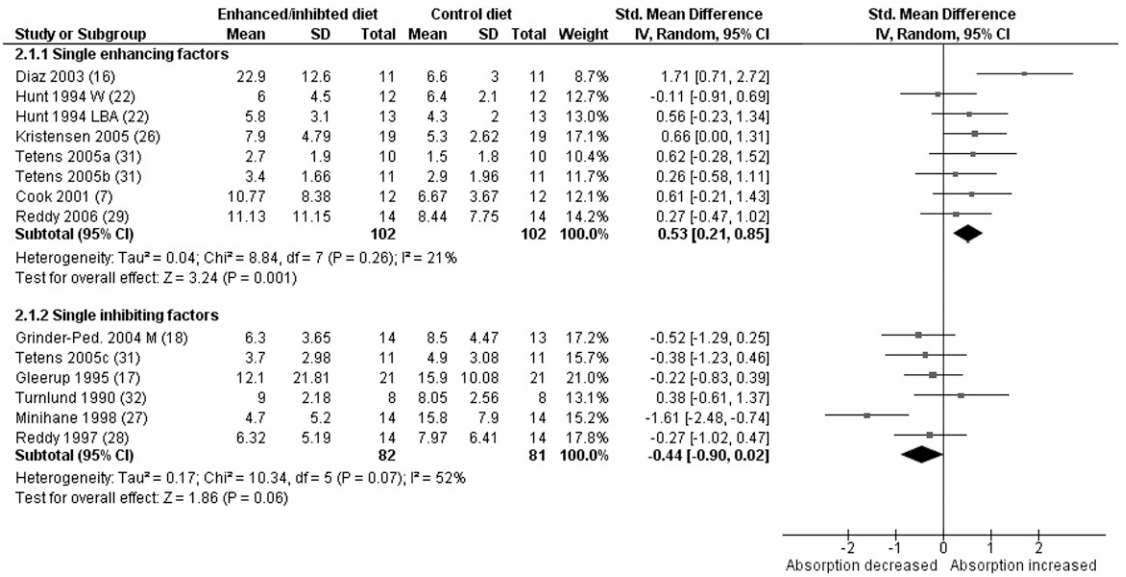

FIGURE 2. Forest plot showing the effect of single enhancing and inhibiting factors on iron absorption from a whole diet. IV, inverse variance; LBA, low bioavailability; M, milk; std., standard; W, Western diet.

differences were identified for subgroups based on sex, age, and iron status (Table 4).

Five of the included articles (all from studies in the United States) presented data for each individual in the study (7, 23, 28, 29, 32). A scatter plot of log(% nonheme absorption) plotted against log(serum ferritin) for the standard or control dietary phases of the interventions for each individual (n = 58) is shown in **Figure 6**. Uncorrected values were used for absorption and ferritin, but because both variables had a skewed distribution when tested for normality using the Shapiro-Wilk test, they were subsequently log transformed, which resulted in a normal distribution. The individual data from the standard diet phases were corrected to a range of ferritin concentrations (15–60 μg/L) by using the ferritin method developed by Cook et al (6). Absorption was plotted against serum ferritin, as shown in **Figure 7**A. Three of the 5 studies with individual data had an identical design structure; they compared a self-selected dietary phase with phases in which calcium (28), vitamin C (7), or meat (29) consumption were high or low. The intervention phases were grouped as self-selected, low bioavailability (high calcium, low vitamin C, no meat), and high bioavailability (low calcium, high vitamin C, high meat), and the absorption was corrected to the same range of ferritin concentrations (15–60 μg/L) at an individual level. The means are plotted in Figure 7B. The uncorrected absorption values from the 3 intervention phases across the 3 Cook and Reddy studies were significantly different within subjects when tested with a repeated-measures ANOVA (test for within-subject effects: F = 5.878, P = 0.004). Pairwise comparisons indicated that absorption from the high-bioavailability phases was significantly higher than that from both the self-selected and low-bioavailability diets. The individual data analyses are summarized in **Table 5**.

The absorption data from these 58 individuals were further analyzed by using a linear regression model that initially included

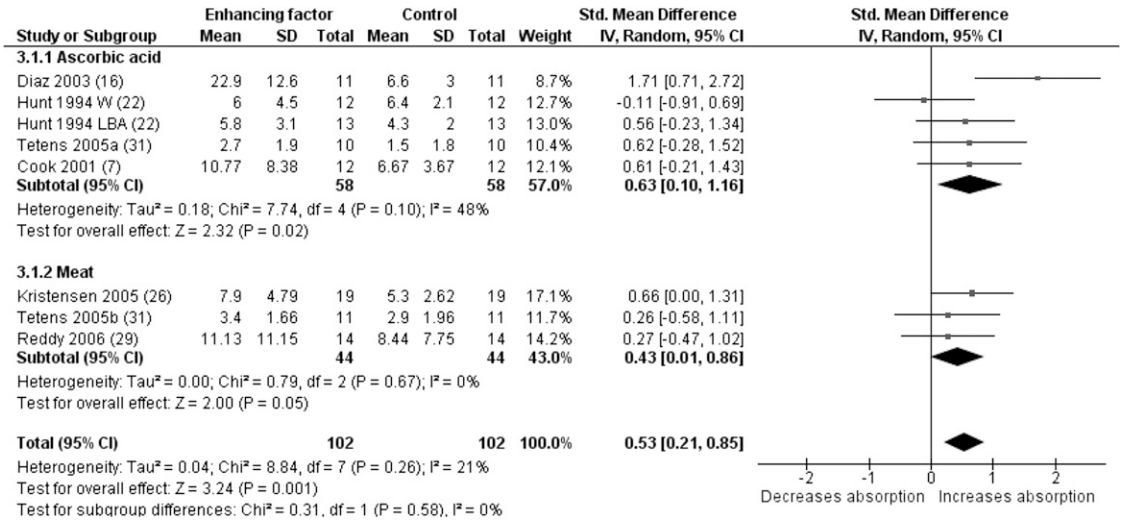

FIGURE 3. Forest plot showing the effect of enhancing factors (ascorbic acid and meat) on iron absorption from a whole diet. IV, inverse variance; LBA, low bioavailability; std., standard; W, Western diet.

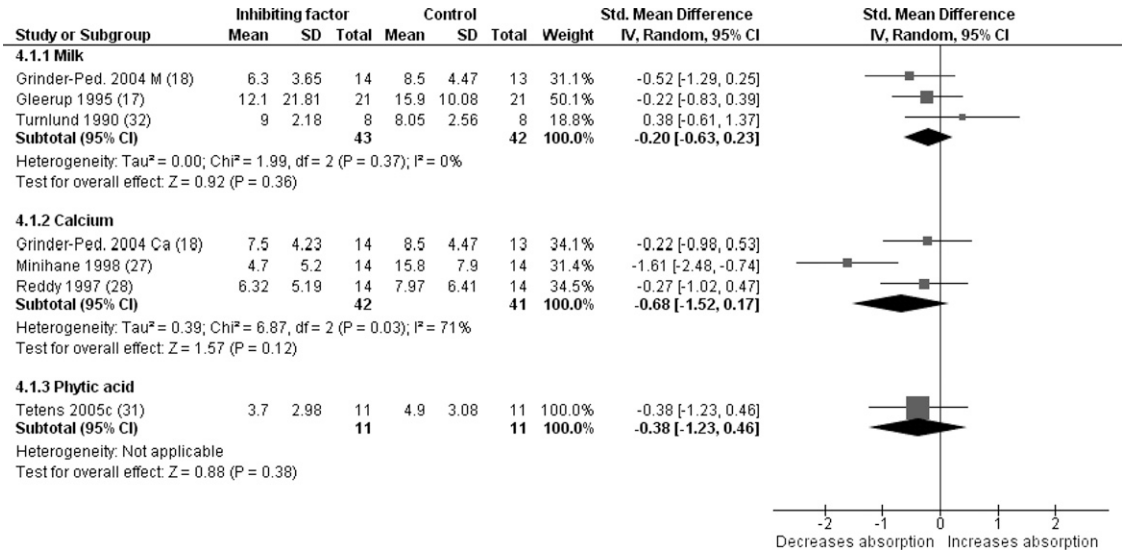

FIGURE 4. Forest plot showing the effect of individual inhibiting factors on iron absorption from a whole diet. Two comparisons from the Grinder-Pedersen study are included; as a result, no pooled total is presented. Ca, calcium lactate; IV, inverse variance; M, milk; std., standard.

ferritin, presence of enhancer or inhibitor, age, and sex as independent variables; however, because age ($P = 0.44$) and sex ($P = 0.63$) were found not to be influential, the regression was rerun with ferritin and dietary modifiers (enhancers/inhibitors) as the only variables ($R^2 = 0.384$, ANOVA $F = 35.193$, $P < 0.0001$). This generated the following equation:

$$\begin{aligned}\text{Log[nonheme} - \text{ironabsorption, \%]} = &-0.73\log[\text{ferritin, } \mu\text{g/L}] \\ &+ 0.11[\text{modifier}] \\ &+ 1.82\end{aligned} \qquad (1)$$

where [modifier] is 0 for standard diets, −1 for diets that include an inhibitor, and 1 for diets that include an enhancer. The equation was used to predict the effect of enhancers and inhibitors on percentage absorption in individuals with low to high serum ferritin concentrations (6–80 μg/L). These are shown in **Table 6** and range from 2.1% to 23.0%, depending on iron status and type of diet. In individuals with a serum ferritin concentration of 12 μg/L, absorption is predicted to be between 8.4% and 13.9%—values that are considerably lower than those corrected to a ferritin concentration of 15 μg/L (Table 5) with the Cook et al equation (6), namely 16.7–22.6%.

DISCUSSION

Most of the published iron-absorption studies have been undertaken by using the single-meal approach, which tends to exaggerate the effect of inhibitors and enhancers. Whereas confirmation of the effects of dietary enhancers and inhibitors of iron absorption underpinned the subsequent analyses, our primary aim was to collate data on bioavailability factors that could be applied when setting dietary reference values for population groups. Our systematic approach showed the potential utilization of individual absorption data corrected for iron status to calculate iron bioavailability from whole diets.

The studies included in our meta-analysis (all from industrialized countries) showed a wide range in mean iron absorption (0.7–22.9%) in the volunteers, which is likely to reflect

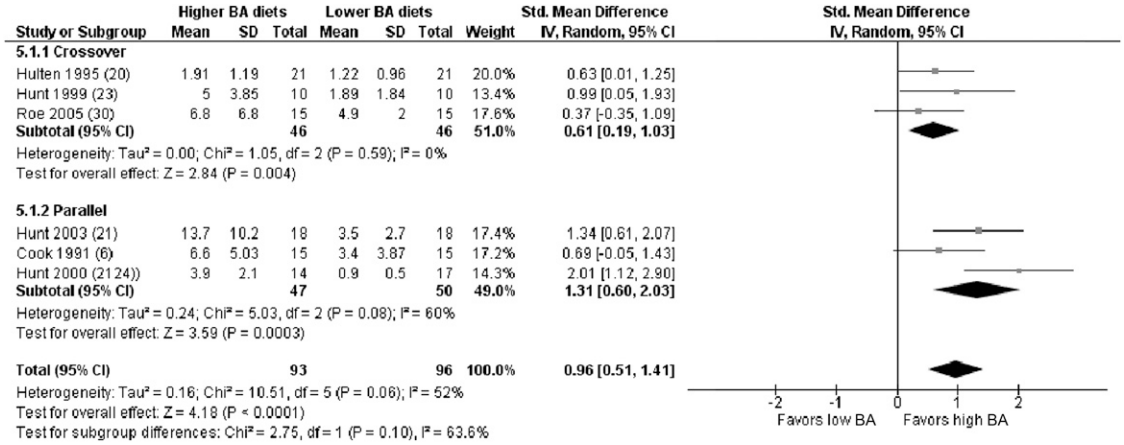

FIGURE 5. Forest plot showing the effect of manipulating multiple dietary factors on iron absorption from a whole diet, including subgrouping by study design. BA, bioavailability; IV, inverse variance; std., standard.

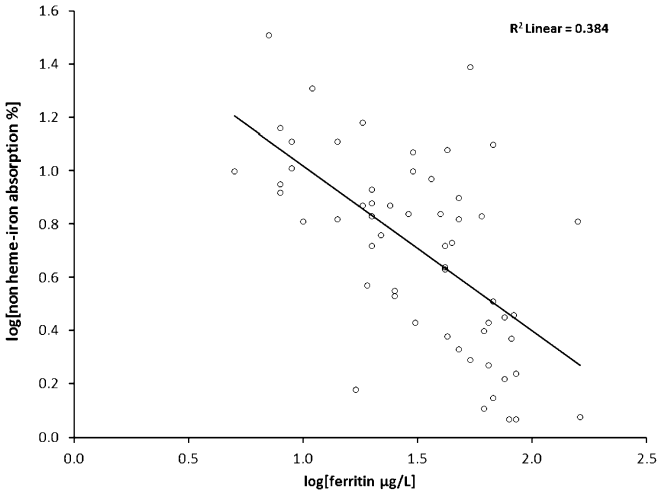

FIGURE 6. Scatter plot of individual data (n = 58) from 5 studies (7, 23, 28, 29, 32) showing the relation between percentage iron absorption (from standard or control diets/phases) and serum ferritin. Both variables were log transformed.

the real-life situation in populations from industrialized countries. Further data are required from the developing world in order for our findings to have worldwide application. Iron status is currently considered to be the principal factor that determines the efficiency of iron absorption, and our results indicate that dietary composition also affects iron absorption from whole diets. The results of the meta-analysis of iron-absorption and iron-status relations underpinned the subsequent regression analyses. Although the studies in each forest plot were ordered by mean baseline ferritin status (low to high), this did not appear to show any consistent patterns of decreasing absorption with increasing iron status, and subsequent subgrouping potential was restricted because of the lack of similar studies (Table 4). Initially, it was planned to subgroup according to predetermined cutoffs: $<$15 μg/L indicating depleted iron stores, 15–60 μg/L suggested suboptimal iron status, and $>$60 μg/L indicated iron repletion. In the 2 data subsets used for subgrouping, however, it was only possible to use one of these cutoffs in each analysis. Variations in study design, isotope methods, and presentation of the absorption data may have masked the effects of iron status.

The subgroup analyses for sex were split into female, all male, and mixed-sex populations. The effects of enhancers or high-bioavailability diets remained significant only in the all-female studies; however, because there were limited data for the other sex groups, it was not possible to say whether the absence of an effect was real or whether it was the result of insufficient power. Similarly, for age, most of the populations were young healthy adults, which limited the potential for subgrouping. Two studies that measured the effects of manipulating multiple dietary factors were conducted in slightly older adults; therefore, a cutoff of 40 y of age was used, but the number of studies was too few to draw any firm conclusions. Ultimately, many of the subject-related confounders explored in the subgroup analysis were not distinct. Age and sex are accepted predictors of iron status, so, ideally, had sufficient studies been identified, a meta-regression would have been conducted in which each factor would be entered as a variable.

Despite the potential benefits offered by a combined systematic review/mathematical modeling approach, it is essential to be aware of potential caveats. Whereas we did not include studies of single meals, many of the included studies were still of very short duration. Two of the included studies (21, 24) investigated the effect of adaptation on iron absorption (over 10 wk) and concluded that individuals tend to absorb less iron over time when consuming a high-bioavailability diet compared with a low-bioavailability diet. The potential importance of such an effect must not be overlooked when interpreting the results of this review. Caution should also be applied when using a systematic approach, because included studies are often not designed to address the same specific question as the overall review, as exemplified by the Hallberg et al study (19). That study could not be included in any meta-analyses because of the different primary aim of the original study (comparing absorption between blood donors and nondonors), but it serves as an important example of the importance of iron status in determining absorption (Table 2).

The comparison of studies was impeded by the combination of studies of parallel and crossover design, and the inconsistencies in correcting absorption data. The forest plot meta-analysis method underestimates the effect of a crossover design by considering the control and intervention groups as distinct individuals. When parallel groups were used, there was no standard method of correcting and reporting the absorption data presented. General data presentation was also an issue for the absorption data, which varied greatly between studies. Both geometric and arithmetic means and various units of absorption were presented,

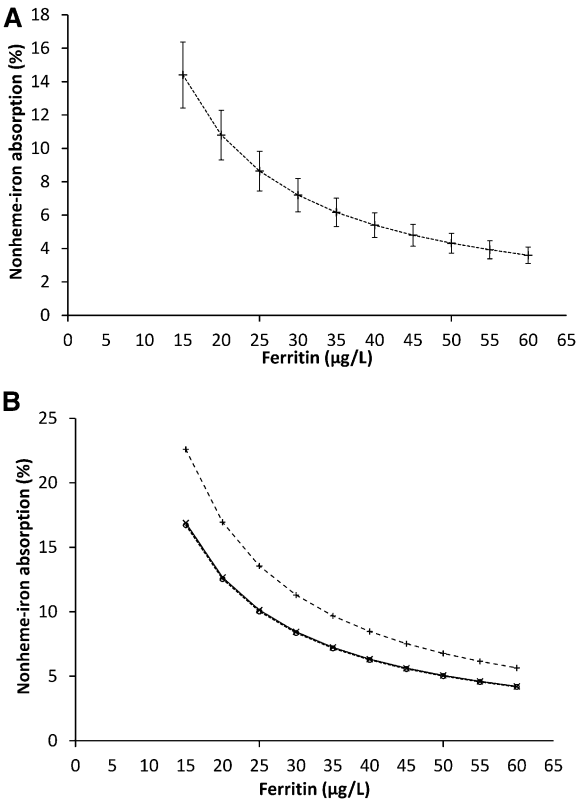

FIGURE 7. Mean corrected percentage nonheme-iron absorption from whole diets. Individual data were corrected by using the ferritin method (6) at a range of ferritin concentrations (15–60 μg/L). A: Data from the standard or control phase (n = 58) for 5 studies (7, 23, 28, 29, 32); error bars indicate SEs. B: Means of individual corrected data (n = 40) from 3 studies of similar design (7, 28, 29); the diets were categorized into 3 types: self-selected (×), low (high calcium, low vitamin C, no meat) (–), or high (+; low calcium, high vitamin C, high meat).

TABLE 5
Summary of analyses using individual absorption and ferritin data[1]

Diet	Nonheme-iron absorption		
	Uncorrected	Corrected to a ferritin concentration of 15 μg/L (6)	Corrected to the mean ferritin concentration of the study population (6)
	%	%	%
Self selected	7.09 ± 6.75[a]	16.89 ± 17.30	5.11 ± 5.74
Low bioavailability	7.17 ± 5.80[a]	16.72 ± 13.37	5.03 ± 3.72
High bioavailability	9.92 ± 8.78[b]	22.60 ± 21.76	6.87 ± 6.40

[1] All values are means ± SDs; n = 3 studies, n = 40 participants. Values with different superscript letters are significantly different, $P < 0.05$.

and, in some instances, it was difficult to determine what fraction of absorption was presented (heme, nonheme, or total).

The results of this review indicate that iron absorption, even under test conditions, can vary greatly between individuals and across relatively similar diets. A bioavailability factor of ~15% is typically used in setting iron Dietary Reference Values, and this is normally set based on the absorption of people with no iron stores (serum ferritin concentration ≤15 μg/L). The data collated in this review (Table 2) suggest that the vast majority of populations, even those with low iron stores, may not absorb iron to this extent, particularly if consuming a diet low in bioavailable iron. Hunt et al (24) measured nonheme-iron absorption from whole diets (by using ^{59}Fe labeling) in men with high ferritin concentrations (~100 μg/L) and observed absorption values of 2.1% to 3.4% from a high-bioavailable diet and of 0.7% to 0.9% from a low-bioavailable diet. Absorption was measured by both blood and fecal isotope monitoring and the data were in agreement. Calculated absorption from whole diets with the use of our regression equation (Table 6) ranged from 1.8% to 3.0% in people with a ferritin concentration of 100 μg/L, which is similar to the results of Hunt et al (24). Average absorption appeared to be ~5–8% across all studies, although many studies used a low-bioavailability diet within their study design.

The results of this systematic review confirm the effect of known enhancers of iron absorption on whole diets, but the effect of inhibitors is less clear. With the use of the newly developed regression equation, nonheme-iron absorption was predicted to be 10.8% in individuals with low iron stores (serum ferritin: 12 μg/L) consuming a standard diet, rising to 13.9% in a higher-bioavailability diet and falling to 8.4% in a lower-bioavailability diet. In addition, it is important when estimating Dietary Reference Values to include an allowance for the intake of heme iron from meat, poultry, and fish in the population. Although the contribution of heme iron to total iron intake is low, recently estimated to be 4–6% in the United Kingdom, it is generally more efficiently absorbed and less influenced by iron status than is nonheme iron (13).

One of the key outputs of this review is the equation derived from the regression analysis of the individual absorption data, pooled from several studies conducted in the United States, because this can be used to predict absorption from whole diets in relation to iron status in similar populations. The combined effect of iron status and dietary enhancers and inhibitors included in the regression model showed a large effect of diet on percentage absorption when iron stores are low, with absorption values ranging from 13.9% to 23.0% when serum ferritin concentrations were 6 μg/L (Table 6). However, with higher iron status, absorption was very much reduced, ranging from 1.8% to 3.0% with a serum ferritin concentration of 100 μg/L. The use of this equation permits a more transparent process to be followed for estimating dietary iron absorption and only requires information on the iron status of the population together with an assumption about the type of diet in relation to the presence of enhancers and inhibitors. Because different bioavailability factors need to be applied to very different types of diets, extrapolation of the equation will require data on iron absorption from diets that deviate markedly from the ones described in the included studies in industrialized countries. It is hoped that the combination of a rigorous systematic review and appropriate mathematical modeling will provide a useful approach for selecting bioavailability factors for the future derivation of dietary reference values for iron.

TABLE 6
Predicted nonheme-iron absorption from diets containing enhancers and inhibitors in individuals with different iron statuses

Serum ferritin	Absorption		
	With inhibitor	Standard diet	With enhancer
	%	%	%
6 μg/L	13.9	17.9	23.0
12 μg/L	8.4	10.8	13.9
15 μg/L	7.1	9.2	11.8
40 μg/L	3.5	4.5	5.8
60 μg/L	2.6	3.3	4.3
80 μg/L	2.1	2.7	3.5
100 μg/L	1.8	2.3	3.0

The authors' responsibilities were as follows—RC, LJH, LH, and SJF-T: developed the study design and methods; LJH, RC, and LH: wrote the review protocol; RC: conducted the electronic searches; LJH, JA, RC, TJB, MK, and RH: assessed the studies, extracted data, and assessed the study validity; RC and LH: conducted the meta-analyses and regressions; RC, TJB, MK, and RH: tabulated the data; and RC, LH, and SJF-T: wrote the first draft of the manuscript. All authors contributed to the final version of the manuscript. Initial screening of the search results on the basis of titles and abstracts was completed in collaboration with other partners of the European Micronutrient Recommendations Aligned Network of Excellence (University of Central Lancashire and University of Milan). None of the authors had a conflict of interest.

REFERENCES

1. World Health Organization. Worldwide prevalence of anaemia 1993-2005: WHO Global Database on Anaemia. Geneva, Switzerland: World Health Organization, 2008.
2. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr 2009;12:444–54.

3. Finch CA, Deubelheiss K, Cook JD, Eschbach JW, Harker LA, Funk DD, Marsaglia G, Hillman RS, Slichter S, Adamson JW, et al. Ferrokinetics in man. Medicine (Baltimore) 1970;49:17–53.
4. Beshara S, Sörensson J, Lubberink M, Tolmachev V, Långström B, Antoni G, Danielson B, Lundqvist H. Pharmacokinetics and red cell utilization of 52Fe/59Fe-labelled iron polymaltose in anaemic patients using positron emission tomography. Br J Haematol 2003;120:853–9.
5. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr 2010;91:1461S–7S.
6. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme iron bioavailability in iron balance. Am J Clin Nutr 1991;54:717–22.
7. Cook JD, Reddy MB. Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 2001;73:93–8.
8. Fairweather-Tait SJ. Iron nutrition in the UK: getting the balance right. Proc Nutr Soc 2004;63:519–28.
9. Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W, Finch CA. Estimation of available dietary iron. Am J Clin Nutr 1978;31:134–41.
10. Monsen ER, Balintfy JL. Calculating dietary iron bioavailability: refinement and computerization. J Am Diet Assoc 1982;80:307–11.
11. Reddy MB. Algorithms to assess non-heme iron bioavailability. Int J Vitam Nutr Res 2005;75:405–12.
12. World Health Organization, Food and Agricultural Organization of the United Nations. Vitamin and mineral requirements in human nutrition. 2nd ed. Geneva, Switzerland: WHO: 2004.
13. Scientific Advisory Committee on Nutrition. Iron and health. London, United Kingdom: The Stationery Office, 2010.
14. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359–64.
15. Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup D, Stroup DF. Improving the quality of reports of meta-analyses of randomized controlled trials: the QUOROM statement. Lancet 1999;354:1896–900.
16. Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP. The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. Am J Clin Nutr 2003;78:436–40.
17. Gleerup A, Rossander-Hulthen L, Gramatkovski E, Hallberg L. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. Am J Clin Nutr 1995;61:97–104.
18. Grinder-Pedersen L, Bukhave K, Jensen M, Højgaard L, Hansen M. Calcium from milk or calcium-fortified foods does not inhibit nonheme-iron absorption from a whole diet consumed over a 4-d period. Am J Clin Nutr 2004;80:404–9.
19. Hallberg L, Hulten L, Gramatkovski E. Iron absorption from the whole diet in men: How effective is the regulation of iron absorption? Am J Clin Nutr 1997;66:347–56.
20. Hultén L, Gramatkovski E, Gleerup A, Hallberg L. Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. Eur J Clin Nutr 1995;49:794–808.
21. Hunt JR. High-, but not low-bioavailability diets enable substantial control of women's iron absorption in relation to body iron stores, with minimal adaptation within several weeks. Am J Clin Nutr 2003;78:1168–77.
22. Hunt JR, Gallagher SK, Johnson LK. Effect of ascorbic acid on apparent iron absorption by women with low iron stores. Am J Clin Nutr 1994;59:1381–5.
23. Hunt JR, Roughead ZK. Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. Am J Clin Nutr 1999;69:944–52.
24. Hunt JR, Roughead ZK. Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. Am J Clin Nutr 2000;71:94–102.
25. Hunt JR, Zeng H. Iron absorption by heterozygous carriers of the HFE C282Y mutation associated with hemochromatosis. Am J Clin Nutr 2004;80:924–31.
26. Bach Kristensen M, Hels O, Morberg C, Marving J, Bügel S, Tetens I. Pork meat increases iron absorption from a 5-day fully controlled diet when compared to a vegetarian diet with similar vitamin C and phytic acid content. Br J Nutr 2005;94:78–83.
27. Minihane AM, Fairweather-Tait SJ. Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status. Am J Clin Nutr 1998;68:96–102.
28. Reddy MB, Cook JD. Effect of calcium intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 1997;65:1820–5.
29. Reddy MB, Hurrell RF, Cook JD. Meat consumption in a varied diet marginally influences nonheme iron absorption in normal individuals. J Nutr 2006;136:576–81.
30. Roe MA, Heath ALM, Oyston SL, Mackrow C, Hoogewerff JA, Foxall R, Dainty JR, Majsak-Newman G, Willis G, Fairweather-Tait SJ. Iron absorption in male C282Y heterozygotes. Am J Clin Nutr 2005;81:814–21.
31. Tetens I, Larsen TM, Kristensen MB, Hels O, Jensen M, Morberg CM, Thomsen AD, Højgaard L, Henriksen M. The importance of dietary composition for efficacy of iron absorption measured in a whole diet that includes rye bread fortified with ferrous fumerate: a radioisotope study in young women. Br J Nutr 2005;94:720–6.
32. Turnlund JR, Smith RG, Kretsch MJ, Keyes WR, Shah AG. Milk's effect on the bioavailability of iron from cereal-based diets in young women by use of in vitro and in vivo methods. Am J Clin Nutr 1990;52:373–8.
33. van den Heuvel EG, Schaafsma G, Muys T, van Dokkum W. Nondigestible oligosaccharides do not interfere with calcium and nonheme-iron absorption in young, healthy men. Am J Clin Nutr 1998;67:445–51.

The American Journal of Clinical Nutrition