

Nonheme-iron absorption from a phytate-rich meal is increased by the addition of small amounts of pork meat¹⁻⁴

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ABSTRACT

Background: Muscle tissue from various sources is known to promote nonheme-iron absorption. However, systematic studies of the dose dependency of this effect of meat on iron absorption from an inhibitory meal with low amounts of meat are lacking.

Objective: We investigated the dose-response effect of small amounts of meat on nonheme-iron absorption from a meal presumed to have low iron bioavailability.

Design: Forty-five healthy women with a mean (\pm SD) age of 24 \pm 3 y were randomly assigned to 1 of 3 groups, each of which was served (A) a basic meal (rice, tomato sauce, pea purée, and a wheat roll) and (B) the basic meal with either 25, 50, or 75 g pork (longissimus muscle). Meal A contained 2.3 mg nonheme iron, 7.4 mg vitamin C, and 220 mg (358 μ mol) phytate. Each meal was served twice, and the order of the meals was ABBA or BAAB. The meals were extrinsically labeled with ⁵⁵Fe or ⁵⁹Fe. Iron absorption was determined from measurements of ⁵⁹Fe whole-body retention and the activity of ⁵⁵Fe and ⁵⁹Fe in blood samples.

Results: Twenty-five grams meat did not increase nonheme-iron absorption significantly ($P = 0.13$), whereas absorption increased 44% ($P < 0.001$) and 57% ($P < 0.001$), respectively, when 50 and 75 g meat were added to the basic meal. In absolute values, this corresponds to an absorption that was 2.6% and 3.4% higher, respectively, than that with the basic meal after adjustment of the data to a level of 40% absorption from a reference dose.

Conclusion: Small amounts of meat (≥ 50 g) significantly increase nonheme-iron absorption from a phytate-rich meal low in vitamin C. *Am J Clin Nutr* 2003;77:173–9.

KEY WORDS Nonheme-iron absorption, iron absorption, meat, pork, phytate, phytic acid, bioavailability, radioisotopes, ⁵⁵Fe, ⁵⁹Fe, dose response, women, Denmark

INTRODUCTION

In Western countries and developing countries, iron deficiency and low iron stores are prevalent in infants, teenagers, and women of childbearing age (1–4). This may be due to a diet that provides insufficient amounts of available iron to cover the extra needs for growth and menstrual losses. Muscle protein (beef, veal, pork, lamb, chicken, and fish) has long been known to enhance absorption of nonheme (5, 6) and heme iron (5, 7). Because nonheme iron accounts for 85–90% of the iron content in a typical Western diet (8), enhancement of nonheme-iron absorption from the diet is of particular importance. The enhancing effect of meat on iron

absorption, known as the “meat factor” or “meat effect,” may be related to the potential ability of sulfhydryl-containing amino acids or peptides to chelate nonheme iron and thereby facilitate intestinal absorption. In vitro studies suggested that the sulfhydryl content of meat proteins may play a key role in the reduction of Fe³⁺ to the more bioavailable Fe²⁺ (9), and in vivo studies in man showed enhanced nonheme-iron absorption in the presence of cysteine (10, 11).

The effect of meat on iron absorption in adults has mostly been documented with the use of single-meal radioisotope studies that tested a single meat dose ranging from 40 to 100 g (6, 12–17). In general, a 1.5–4-fold increase in fractional nonheme-iron absorption over that from a reference meal without meat was observed in these studies. The effect was also observed in an infant study in which the addition of 25 g meat to a vegetable meal increased fractional nonheme-iron absorption 1.5 fold (18). Algorithms developed to predict iron bioavailability from various diets are based on such isotope studies with only a single dose of meat, and these algorithms assume a linear relation between iron absorption and the amount of meat in a meal (19–22). The dose-response effect of meat was explored in only a few studies (23, 24), and the lowest amount of meat required for a significant enhancing effect has not yet been fully elucidated. Because iron deficiency is a major health problem in many developing countries in which the populations subsist on diets with a low content of iron-absorption promoters and a high content of inhibitors, knowledge of the enhancing effect of meat on iron absorption at relatively low meat intakes and in diets high in phytate would be

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useful. However, improvement in iron status through improved iron availability is also needed in subgroups of the European population, eg, women of fertile age in the Nordic countries, among whom the high prevalence of iron deficiency is ascribed to low intakes of meat and vegetables (25).

In the present study the effects of 25, 50, and 75 g lean pork on nonheme-iron absorption were evaluated by adding the pork to a basic meal presumed to have low iron bioavailability. Pork is the major meat source in Denmark (26), where the study was carried out.

SUBJECTS AND METHODS

Subjects

Forty-five healthy women with a mean (\pm SD) age of 24 ± 3 y, mean weight of 64 ± 8 kg, and mean body mass index (in kg/m^2) of 22.6 ± 2.7 volunteered for the study. All subjects were non-smokers and were not pregnant or lactating, and none of the subjects took any vitamin or mineral supplements during the study or for ≥ 2 mo before the study. Twenty-four subjects used oral contraceptives, but none of the subjects were routinely taking any other medication. Blood donation was not allowed during the study or during the 2 mo before the study. Each participant was informed orally and in writing about the study before written consent was obtained. The research protocol was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg [(KF) 01-100/97] and the National Institute of Radiation Hygiene, Denmark.

Experimental design

Each subject was given 2 test meals: a basic meal (A) and the basic meal with added meat (B). The meat meals contained either 25, 50 or 75 g pork. The subjects were randomly assigned to receive 1 of the 3 meat meals. Each test meal was served twice, and the meals were served in the order of ABBA (for one-half of the subjects) or BAAB (for the other half of the subjects) on 4 consecutive mornings to minimize potential effects of day-to-day variation. Iron absorption was measured with the dual-label extrinsic-tag method (27), and all meals were labeled extrinsically with ^{55}Fe or ^{59}Fe . The basic meal and the meat meal were labeled with ^{55}Fe and ^{59}Fe , respectively, for one-half of the subjects in each group and were labeled vice versa for the other half of the subjects. ^{59}Fe retention was measured in a whole-body counter at baseline and 17 d after intake of the last meal. On the day after the whole-body counting, a blood sample was drawn, and the activity of ^{55}Fe and ^{59}Fe was determined for the estimation of ^{55}Fe whole-body retention. To standardize the results in relation to iron status, a reference dose of ^{59}Fe was given to each subject on 2 consecutive mornings after the blood sample was drawn, and ^{59}Fe retention was measured by whole-body counting 14 d later. The whole-body counter measurements of ^{59}Fe absorption from the reference dose were corrected for residual ^{59}Fe from the original dosing. For this purpose, iron excretion from the body between the 2 whole-body countings was assumed to be zero.

Composition of test meals and serving procedure

On the evening before each test morning in which a test meal or reference dose was to be given, subjects consumed a standard meal in their homes that was prepared at the Research Department of Human Nutrition. The subjects had a choice of either turkey or

minced beef with pasta and vegetables, and each participant was given the same type of meat each time. All subjects completed a questionnaire in connection with each test meal to ensure adherence to all procedures.

The basic meal consisted of 60 g boiled, polished rice (≈ 160 g cooked rice), tomato sauce (100 g peeled tomatoes cooked with 5 g salt), pea purée (50 g minced green peas with 10 g rapeseed oil and 2.5 g ultrapure water), and a 50-g wheat roll (25 g wheat flour, 17 g water, 13 g whole-wheat flour, 1 g yeast, 1 g salt, and 1 g rapeseed oil). All the basic meals were prepared in one batch, stored frozen, and reheated in an oven (150°C) for 17 min before serving. For preparation of meat patties, 14 loins (pork longissimus dorsi) were obtained from a local slaughterhouse and trimmed of visible fat and connective tissue. The meat was coarsely minced with stainless steel knives in a meat grinder, vacuum-packed in 1-kg portions, and stored at -20°C . Before use, the meat was thawed at 4°C . Meat patties weighing 50 ± 0.5 g and having a diameter of ≈ 9 cm were formed, vacuum-packed, and heat-treated in a circulating water bath at 70°C for 20 min. Immediately after being cooked, the meat patties were served on top of the test meals in 25- (one-half patty), 50- (one patty), and 75-g (one and one-half patties) portions. All test meals were served with 200 mL ultrapure water. The nonheme-iron content of the meals was adjusted to that present in the meal with 75 g meat by adding 0.32, 0.21, and 0.10 mg Fe as ferrous sulfate (Struers KEBO Lab A/S, Albertslund, Denmark) in ultrapure water to the basic meal and the meals with 25 and 50 g added meat, respectively.

The test meals were thawed overnight before serving and were extrinsically labeled by adding 1 mL radioisotope solution (FeCl_3 in 0.1 mol HCl/L) directly on top of each frozen meal ≥ 16 h before serving. Each dose contained 55 kBq $^{55}\text{FeCl}_3$ or 38 kBq $^{59}\text{FeCl}_3$ (Amersham, Buckinghamshire, United Kingdom). The meals were served in the morning after 12 h of fasting. The subjects were allowed to consume a maximum of 0.5 L water overnight. Neither moderate or hard physical activity nor the intake of any alcohol or medication was allowed during the 24 h before the intake of the test meal. The subjects were instructed to eat and drink alternately and to wipe off the plate with the last part of the roll to achieve complete intake of the isotope dose. A staff member ensured that everything was eaten. After consuming the test meals, the subjects were not allowed to eat or drink for 4 h, and the intake of alcohol was prohibited for the next 48 h.

Duplicate portions of the test meals were homogenized, freeze-dried, and analyzed in duplicate for total, nonheme, and heme iron; ascorbic acid; phytic acid; calcium; zinc; and nitrogen. The energy content was calculated from a food-composition computer program (Danish Tables of Food Composition, DANKOST 2000, version 1.20; Danish Catering Center, Herlev, Denmark). Total iron, calcium, and zinc were determined by atomic absorption spectrometry (Spektr-AA 200; Varian, Zug, Switzerland) after wet-ashing in a MES 1000 Microwave Solvent Extraction system with 65% (by wt) suprapure nitric acid (CEM Corp, Matthews, NC). A typical diet containing standard reference material 1548a (National Institute of Standards and Technology, Gaithersburg, MD) was used as the reference for iron [$\bar{x} \pm U$ (expanded uncertainty): 35.3 ± 3.8 $\mu\text{g}/\text{g}$], calcium (1.97 ± 0.11 mg/g), and zinc (24.6 ± 1.97 $\mu\text{g}/\text{g}$), and the mean (\pm SD) analyzed values were 37.6 ± 2.7 $\mu\text{g}/\text{g}$ ($n = 4$), 2.0 ± 0.06 mg/g ($n = 4$), and 28.3 ± 0.9 $\mu\text{g}/\text{g}$ ($n = 4$), respectively. Phytic acid analysis was performed by HPLC as described previously (28). Nitrogen analysis

was carried out on an NA 1500 Automatic Nitrogen Analyzer (Carlo Erba Strumentazione, Milan, Italy) (29). An internal diet standard was used as the nitrogen reference material (reference value: $26.5 \pm 0.3 \mu\text{g}$), and the analyzed value was $26.6 \pm 0.5 \mu\text{g}$. A conversion factor for nitrogen to protein of 6.25 was used. Non-heme iron was determined spectrophotometrically by using the Ferrozine method (30) with iron standard number 109972 (Merck, Darmstadt, Germany) as a reference material. Heme iron was analyzed by using a modified protocol (31) of the acidified acetone extraction method originally described by Hornsey (32). Vitamin C analysis was performed by HPLC under the conditions reported previously (33).

Determination of iron status

The subjects were asked not to drink alcohol or take any kind of medication for 36 h before collection of the blood sample and were asked to fast for 12 h before collection of the blood sample. Blood samples were drawn from the cubital vein after the subject had rested for 10 min in a supine position. Serum ferritin analysis was performed by using a 2-site fluoroimmunoassay with a Delfia fluorometer 1232 (Wallac Oy, Turku, Finland) and a Delfia Ferritin kit (kit B069-101; Wallac Oy). The analysis was performed on venous blood (3.0 mL) collected in plain tubes (Vacutainer system; Becton Dickinson, Franklin Lakes, NJ), and appropriate reference sera were also analyzed (World Health Organization NIBSC-ferritin; Blanche Lane, South Mimms, United Kingdom). Intraassay and interassay variations were 2.1% ($n = 12$) and 5.0% ($n = 17$), respectively. Hemoglobin analysis was carried out on venous blood (4.5 mL) collected in tubes containing dissolved EDTA (Vacutainer system; Becton Dickinson) by using a Cobas Minos-ST ABX automatic cell counter (F Hoffmann-La Roche, Basel, Switzerland) and appropriate controls (Hematology Reference Control; F Hoffmann-La Roche). Intraassay and interassay variations were 0.7% ($n = 20$) and 1.3% ($n = 16$), respectively.

Determination of nonheme-iron absorption

Iron absorption was estimated by using the dual-label extrinsic-tag method (27). Approximately 50 mL blood was drawn from each subject for analysis of ^{55}Fe and ^{59}Fe , and heparin was used as an anticoagulant. Simultaneous determination of ^{55}Fe and ^{59}Fe in blood was performed by dry ashing followed by recrystallization of the remaining iron and a single washing step before counting ^{55}Fe and ^{59}Fe in a Tricarb 2100TR Liquid Scintillation Analyzer (Packard Instruments, Meriden, CT) with automatic quench correction (34). ^{59}Fe whole-body retention was measured in a lead-lined steel chamber with 4 NE110 plastic scintillator blocks (Nuclear Enterprises Limited, Edinburgh) connected to conventional nuclear electronic modules and a multichannel analyzer system. The counting efficiency and energy window settings were established through measurements of water-filled phantoms whose outlines and weights are approximately equal to those of humans. The phantoms were filled with known concentrations of each isotope. In the actual setup and with the energy window used, the overall counting efficiency for ^{59}Fe evenly distributed in a 77-kg phantom was $\approx 20\%$. To minimize the contamination by atmospheric background activity, all subjects had a shower, washed their hair, and were dressed in hospital clothing before each measurement. The counting time was 10 min, and the results were corrected for chamber background radiation and for the individual background radiation level of the subjects, which was determined

1 wk before intake of the test meals. All results from determination of radioactivity were corrected for the physical decay of the isotope.

To correct for interindividual differences in iron status, iron absorption from a standardized reference dose was measured on 2 consecutive mornings after an overnight fast. No food or drink was allowed for 4 h after intake of the reference doses. Each reference dose contained 3 mg Fe as ferrous sulfate (Struers KEBO Lab A/S) and 30 mg L(+)-ascorbic acid (Merck) in 10 mL of a 0.01-mol HCl/L solution labeled with 38 kBq ^{59}Fe . After intake of the reference dose, the vial was rinsed twice with ultrapure water, which was also consumed. The same restriction protocol concerning physical activity, alcohol and medication intake, and evening meals as stated above for the test meals was used. Each subject received a total of 110 kBq ^{55}Fe and 152 kBq ^{59}Fe from the 4 meals and 2 reference doses and thus was calculated to receive a maximal radiation dose of 0.7 mSv.

Expression of iron absorption

To compare the results with other data, iron absorption was expressed in 3 different ways that are commonly used to express such data: 1) as unadjusted absorption, 2) as absorption adjusted to 40% absorption from the reference dose, and 3) as absorption adjusted to a serum ferritin concentration of 40 $\mu\text{g/L}$. The unadjusted iron absorption was expressed as ^{59}Fe whole-body retention measured directly from whole-body counting and as ^{55}Fe whole-body retention determined by using the following equation, which is based on the assumption that the fractions of ^{55}Fe and ^{59}Fe in blood are similar (27):

$$^{55}\text{Fe} \text{ absorption (\%)} = \frac{(^{55}\text{Fe} \text{ activity in blood}) / ^{59}\text{Fe} \text{ activity in blood} \times ^{59}\text{Fe} \text{ whole-body retention (\%)}}{\text{whole-body retention (\%)}} \quad (1)$$

The absorption data were adjusted to a level of absorption corresponding to 40% absorption from the reference dose, because this was previously determined to be the mean percentage of absorption from a reference dose containing 3 mg nonheme Fe and 30 mg ascorbic acid when iron stores are depleted (35). In this way the reference-adjusted data can be compared with other researchers' data also adjusted to 40% absorption from the reference dose.

$$A \text{ adjusted to 40\% } A \text{ from reference dose} \\ = A \text{ from meal (\%)} \times [40 (\%)/A \text{ from reference dose (\%)}] \quad (2)$$

where A is nonheme-iron absorption. Iron absorption adjusted to a serum ferritin concentration of 40 $\mu\text{g/L}$ was calculated from (36):

$$\text{Log } A_{\text{adjusted}} = \text{log } A_{\text{observed}} + \text{log } F - \text{log } 40 \quad (3)$$

where F is the serum ferritin concentration taken as the mean of 2 samples. Finally the ratio of iron absorption from the meat meals to that from the basic meals was calculated as an expression of the effect of meat on iron absorption.

Statistical analyses

Serum ferritin and absorption data were converted to logarithms before statistical analyses, and the results were reconverted to antilogarithms. All data used for statistical analysis were normally distributed, with variance homogeneity tested by plots and histograms of residuals. Shapiro-Wilk's test for normal distribution was performed. Data are presented as estimates of least-squares means with 95% CIs (37). Nonheme-iron absorption from control

TABLE 1
Iron status and nonheme-iron absorption data¹

	Meat content of meal			
	0 g (n = 45)	25 g (n = 15)	50 g (n = 15)	75 g (n = 15)
Serum ferritin (μg/L)	—	16.9 (14.4, 19.7)	20.7 (17.8, 24.2)	21.0 (18.0, 24.5)
Hemoglobin (g/L)	—	125.1 (121.7, 128.6)	126.8 (123.3, 130.2)	125.9 (122.4, 129.4)
Absorption from reference dose ² (%)	—	36.4 ^a (29.2, 45.4)	27.1 ^{a,b} (21.8, 33.8)	25.0 ^b (20.0, 31.1)
Absorption from meals (%)				
Unadjusted data	4.3 ^c (3.5, 5.4)	5.1 ^{c,d} (4.0, 6.5)	6.3 ^e (4.9, 8.0)	6.7 ^{e,f} (5.3, 8.5)
Data adjusted to 40 μg serum ferritin/L	2.1 ^c (1.7, 2.6)	2.4 ^{c,d} (1.9, 3.1)	3.1 ^e (2.4, 3.8)	3.3 ^{e,f} (2.6, 4.1)
Data adjusted to 40% absorption from reference dose	5.9 ^c (4.9, 7.2)	7.1 ^{c,d} (5.7, 8.8)	8.6 ^e (6.9, 10.7)	9.2 ^{e,f} (7.4, 11.5)
Meat meal:basic meal	—	1.15 ^g (1.02, 1.34)	1.44 ^{g,h} (1.26, 1.65)	1.57 ^h (1.34, 1.77)

¹Least-squares means; 95% CI in parentheses. Values in a row with different superscript letters are significantly different (linear mixed models with post hoc Tukey-Kramer tests): ^{ab}*P* < 0.05, ^{ce}*P* < 0.001, ^{cf}*P* < 0.001, ^{df}*P* < 0.04, ^{gh}*P* < 0.05.

²Three milligrams Fe as ferrous sulfate and 30 mg ascorbic acid in 10 mL of a 0.01-mol HCl/L solution.

meals was compared with that from meat meals by using linear mixed models with log (nonheme-iron absorption) as the response variable:

$$\text{Log (nonheme-iron absorption)}_{ijk} = \mu + \alpha_i + \beta_j + C_k + \epsilon_{ijk} \quad (4)$$

where μ is the grand average; α_i is the effect of meat in the meal (0, 25, 50, or 75 g), with $i = 1-4$; β_j is the effect of the order in which the meals were served (ABBA or BAAB), with $j = 1$ or 2 ; C_k is the random variation of subject, with $k = 1-45$; and ϵ_{ijk} is the residual random variation. Differences between least-squares means were compared, and post hoc Tukey-Kramer adjustments were performed. Pearson's correlation test was performed to test the correlation between the iron absorption ratio and the meat content of the meat meal. Mixed linear models were performed with the SAS statistical software package, version 8.1 (SAS Institute Inc, Cary, NC). Pearson's correlation test was performed with SPSS 9.0 for WINDOWS (SPSS Inc, Chicago).

RESULTS

No significant differences in iron status (serum ferritin and hemoglobin) were observed between the 3 groups (**Table 1**). All subjects had low serum ferritin concentrations (8–35 μg/L) and normal hemoglobin concentrations (> 120 g/L). The nonheme-iron content of the basic meal was 2.3 mg, and the addition of meat had only a marginal effect on the total iron content (**Table 2**). The phytate content of the meals was 358 μmol, resulting in a molar ratio of phytic acid to iron of 8.7.

TABLE 2
Composition of the test meals¹

	Basic meal	Meat meals		
		25 g meat	50 g meat	75 g meat
Energy (MJ)	2.2	2.3	2.4	2.5
Total protein (g)	10.6	18.4	22.2	27.3
Nonheme iron (mg)	2.3	2.5	2.5	2.3
Heme iron (mg)	—	0.06	0.12	0.19
Phytic acid				
(mg)	220	220	220	220
(μmol)	358	358	358	358
Ascorbic acid (mg)	7.4	7.4	7.4	7.4
Calcium (mg)	50.5	58.4	58.8	64.3
Zinc (mg)	2.6	2.9	3.0	3.2

¹The basic meal contained 0 g meat.

There were significant differences in nonheme-iron absorption from the reference dose between the 25- and 75-g meat groups (*P* < 0.05) (**Table 1**). The absorption of nonheme iron from the basic meal did not differ between the groups either in unadjusted values or when adjusted to 40% absorption from the reference dose or to ferritin concentrations (40 μg/L). In the group given 25 g meat with the basic meal, iron absorption from the meat meal was not significantly higher than that from the basic meal (*P* = 0.13), whereas 50 and 75 g added meat resulted in 44% (2.6% in absolute values when adjusted to 40% absorption from the reference dose) and 57% (3.4%) higher absorption (*P* < 0.001). There was a significant effect of meat on nonheme-iron absorption when the ratios of absorption with the meat meal to absorption with the basic meal were compared between the 3 groups (*P* = 0.04). The correlation between the ratio of nonheme-iron absorption with meat to that without meat and the meat content of the meal may be expressed as a linear function (*r* = 0.35, *P* = 0.018) within the range of meat doses given in the study:

$$\begin{aligned} \text{Nonheme-iron absorption ratio} &= 0.008 \\ &\times \text{meat dose (g)} + 1.046 \end{aligned} \quad (5)$$

DISCUSSION

This study showed that small amounts (≥ 50 g) of pork meat significantly increased nonheme-iron absorption in a dose-dependent manner from a meal with a high content of inhibitors and a low content of promoters for iron absorption. The absorption from the meal without meat was low, ≈6% when adjusted to 40% absorption from the reference dose.

To our knowledge, only one similar dose-response study investigating low amounts of meat has been published (23). It also showed a dose-dependent effect of meat on nonheme-iron absorption when egg albumin was substituted with 25, 50, 75, and 100 g beef in a semisynthetic meal including dextrimaltose, corn oil, CaHPO₄, KHPO₄, and 4 mg Fe as FeCl₃. Iron absorption increased 14% (NS), 88%, 111%, and 255%, respectively (*P* < 0.02). In another dose-response study, larger doses of beef (50, 100, 200, and 300 g) were added to a corn meal containing 2 mg Fe, and iron absorption increased 43% (NS), 83%, 102%, and 143%, respectively (*P* < 0.001) (24). Thus, the results from the present study are comparable to those of other laboratories investigating beef. The differences in the absolute effects of meat can probably be ascribed to the varying amounts of phytate, ascorbic acid, and calcium in the meals.

The results from this study agree with estimates obtained by using 2 recently published algorithms for the calculation of the meat effect on nonheme-iron absorption. The first algorithm is as follows:

$$\ln[A_{\text{adjusted}} (\%)] = 1.9786 + (0.0123 \times \text{AT}) - (0.0034 \times \text{PA}) + (0.0065 \times \text{AA}) \quad (6)$$

where A_{adjusted} is nonheme-iron absorption adjusted to a serum ferritin concentration of 30 $\mu\text{g/L}$, AT is g animal tissue (meat or fish, cooked weight), PA is mg phytic acid, and AA is mg ascorbic acid (21). This algorithm was derived from results with 25 different meals including 18–70 g beef, pork, turkey, chicken, or seafood (cod, tuna, and shrimp) given to 86 subjects in 9 studies. The second algorithm is as follows:

$$\begin{aligned} \text{Ratio of absorption with and without meat} \\ = 1 + (0.00628 \times M) \times [1 + (0.006 \\ \times P)] \end{aligned} \quad (7)$$


where M is g raw meat, poultry, or fish, and P is mg phytate-phosphor (22). This algorithm was derived from pooled results from studies investigating single amounts of beef (mainly 75–100 g) in single-meal radioisotope studies and was validated with 24 meals including beef, pork, cod, and shrimp in 4 different studies (38–41). The estimated absorption ratios with 25, 50, and 75 g raw meat from the first algorithm were 1.26, 1.58, and 1.99, respectively, and the ratios from the second algorithm were 1.22, 1.43, and 1.65, respectively.

Compared with the inhibiting effect of phytate on nonheme-iron absorption, the enhancing effect of meat is less pronounced. The inhibiting effect of the phytate in the basic meal (62 mg phytate phosphorus, 220 mg phytic acid) can be estimated at 71% by using one of the above-mentioned algorithms (22), corresponding to a ratio of absorption of 0.29 with and without phytate in the meal. To overcome this phytate effect with meat, the ratio of iron absorption from the basic meal with and without meat must be ≈ 3.5 , which is equivalent to the effect of 290 g meat when calculated with the second algorithm (22). Within more realistic amounts of meat, the phytate effect of the basic meal is not completely overcome. In comparison, ascorbic acid seems to be a stronger enhancer of nonheme-iron absorption than is meat within realistic dietary amounts. In a single-meal study, 30 mg ascorbic acid was enough to overcome the effect of 58 mg phytate phosphorus (205 mg phytic acid) in maize bran, and with the addition of 50 and 150 mg ascorbic acid, the absorption ratio increased further to 2.97 and 3.21, respectively (42). Because the effect of ascorbic acid is known to be stronger the more phytate is present in the meal, it can be estimated from the second algorithm (22) that ≈ 120 mg ascorbic acid is required to overcome the phytate effect of the basic meal. This is a high but not unrealistic amount of ascorbic acid in a meal containing fruit and vegetables.

In addition to the increased nonheme-iron absorption due to the meat effect, heme iron contributes substantially to the total amount of iron absorbed from meals containing meat. In the present study, the amount of heme iron absorbed from the basic meal with 75 g added meat was calculated to be 0.07 mg [estimated heme-iron absorption: 38% (43)], whereas 0.10 mg nonheme iron was absorbed. Thus, given this combined effect of meat on iron absorption, an intake of small-to-moderate doses of meat could contribute to improved iron status in populations or population subgroups with vegetable-based diets high in phytate. In Denmark, 25% of women aged 25–44 y have a meat intake < 70 g/d (44). The prevalence of low iron stores (serum ferritin concentra-

tion < 30 $\mu\text{g/L}$) among Danish women aged 30–40 y was 40% in 1982–1984, and 4% of these women had a low hemoglobin concentration (< 121 g/L) (3). Thus, the addition of ≈ 50 g meat to the main meals in this population group would be expected to have a positive effect on iron status.

In the present study pork was selected as a meat source because of its prevalent consumption in Denmark. However, pork meat is also the major animal source in developing countries, and its consumption is expected to increase within the near future (45). Although the test meal containing rice and vegetables that was used in the present study was created primarily to be acceptable to subjects with Danish food habits, the meal had a phytate content corresponding to approximately one-half of the total daily phytate intake of children in Papua New Guinea and Ghana (46, 47) and to one-third of the daily phytate intake of children in Guatemala (48). Thus, in the light of the present results, increasing the intake of pork meat by populations in developing countries may be a useful dietary approach to improve their iron status. To fully explore the potential of meat, studies of the dose response of alternative meat sources and of the addition of meat to meals based on other phytate-rich foods are warranted.

The long-term effect of an increased intake of meat on iron status has been assessed in only a few studies. Cross-sectional studies of premenopausal women showed lower ferritin concentrations in vegetarians than in omnivores (49, 50); however, the results from intervention studies are inconsistent. In infants, those who consumed 27 g meat/d for 2 mo had higher hemoglobin concentrations than did those who consumed 10 g meat/d during the same period ($P = 0.008$) (51). In iron-replete subjects, no effect of meat on iron status was observed in short-term intervention studies (7–8 wk) or in longer intervention studies (6 mo) (52–54). However, the time needed to observe significant alterations in iron status because of changes in iron bioavailability can be estimated (55) and is relatively long, especially in iron-replete subjects. In conclusion, the present study showed that small amounts of pork meat (≥ 50 g) added to a phytate-rich meal low in vitamin C significantly increased nonheme-iron absorption. 

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