A modelling tool for calculating dietary iron bioavailability in iron sufficient adults

Susan J Fairweather-Tait, Amy Jennings, Linda J Harvey, Rachel Berry**,** Janette Walton, and Jack R Dainty

Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, Norfolk, UK (SFT, AJ)

Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, Norfolk, UK (LJH, RB, JRD)

School of Food and Nutritional Sciences, University College Cork, Ireland (JW)

Last names: Fairweather-Tait, Jennings, Harvey, Berry, Walton, Dainty

Changed affiliations: Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, Norfolk, UK (JRD)

Corresponding author: Susan Fairweather-Tait, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, Norfolk, UK

Telephone number: +44 1603 591304

E-mail: s.fairweather-tait@uea.ac.uk

Sources of support: the work was supported by the University of East Anglia (SFT, AJ, JRD), the UK Biotechnology and Biological Sciences Research Council (LJH, RB, JRD), the EURRECA Network of Excellence, European Union Sixth Framework Programme, contract 036196 (SFT, LJH, RB), and the NU-AGE (New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe) project, European Union Seventh

Framework Programme, grant number 266486 (SFT, AJ). The Irish National Adult Nutrition Survey was funded by the Irish Department of Agriculture, Food and the Marine. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Short running head: Model to calculate dietary iron bioavailability

Abbreviations:

SF serum ferritin

NDNS National Diet and Nutrition Survey

NANS National Adult Nutrition Survey

NU-AGE New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe

PRI Population Reference Intake

Clinical Trial Registry number (ClinicalTrials.gov): NCT01754012 (NU-AGE)

Abstract

Background: Values for dietary iron bioavailability are required for setting dietary reference values. Different approaches have been adopted to produce these values, including predictive algorithms, measurements of non-heme iron absorption from meals, and a combined model of iron intake, serum ferritin concentration and estimates of physiological iron requirements.

Objective: To provide a new interactive tool to predict dietary iron bioavailability in populations where iron intakes and serum ferritin concentrations have been measured.

Design: Data for iron intake and serum ferritin (a quantitative marker of body iron stores) from three studies, two of which were nationally representative surveys of adults in the UK and Ireland, and one a study in elderly men and women, were used to develop a model for the prediction of dietary iron absorption at each level of serum ferritin concentration. Individuals with raised inflammatory markers or taking supplements that contained iron were excluded.

Results: Mean iron intakes (mg/d) were 13.6 (SD 5.2), 10.3 (SD 4.1) and 10.9 (SD 3.5), and mean serum ferritin concentrations (µg/L) were 140.7 (SD 113.6), 49.4 µg/L (SD 45.8) and 96.7 µg/L (SD 72.8) in men, pre-menopausal and post-menopausal women, respectively. The model predicts that at serum ferritin concentrations of 15, 30 and 60 µg/L respectively, mean dietary iron absorption would be 22.3%, 16.3% and 11.6% in men, 27.2%, 17.2% and 10.6% in pre-menopausal women, and 18.4%, 12.7% and 10.5% in post-menopausal women.

Conclusions: An interactive program for calculating dietary iron absorption at any level of serum ferritin concentration is presented. Differences in iron status were partly explained by age but also by diet, with meat being a key determinant of serum ferritin concentration. The effect of diet was more marked at lower serum ferritin concentrations. The model can be applied to any adult population where representative, good quality data on iron intake and iron status have been collected. Furthermore, dietary iron bioavailability values can be derived for any target level of serum ferritin, thus giving risk managers and public health

professionals a flexible and transparent basis upon which to base their dietary recommendations.

Keywords: iron bioavailability, dietary iron absorption, dietary reference values, serum ferritin, iron intake

Introduction

The bioavailability of dietary iron can be defined as the proportion (or %) of ingested iron that is absorbed and utilised within the body. A value for dietary iron bioavailability (sometimes referred to as the bioavailability factor) is required to transform physiological requirements (i.e. absorbed iron) into dietary intakes, and hence to derive dietary reference values (DRVs), and to develop dietary recommendations and public health policies. Initially, bioavailability factors were derived from predictive algorithms based on the intake of heme iron and enhancers of non-heme iron absorption (1). This was followed by more complex algorithms which included inhibitors as well as enhancers of non-heme iron absorption (2, 3) where the magnitude of effect of modifiers of non-heme iron absorption was determined from single meal studies. In view of the fact that the effect of enhancers and inhibitors may be exaggerated in single meal studies (4), average absorption of non-heme iron from more than one meal was used to reflect more closely the whole diet (5, 6). However, these do not reflect the diet that is consumed over time, and also an adjustment has to be made to take into account the heme content of the diet, with an assumed absorption value.

We recently developed a novel predictive model for estimating dietary iron bioavailability based on measurements of total iron intake (heme and non-heme iron), serum ferritin (SF) concentration and factorial calculations of iron requirements (7). The latter were derived using the National Academy of Medicine approach for estimating iron losses (8). Individual data for 495 men and 378 pre-menopausal women were used for a model that estimated the prevalence of dietary intakes that were assumed to be insufficient to meet the needs of men and women (separately) based on their daily iron intake and a series of absorption values. The prevalence of SF concentrations below selected cut-off values was derived and an estimate of dietary iron absorption required to maintain specific SF values was then calculated by matching the observed prevalence of insufficiency with the prevalence predicted for the series of absorption estimates. It was therefore possible to estimate dietary iron absorption (bioavailability) at a population level from the individual measurements of total iron intake and SF concentration. In this article, we describe the results of applying the model to other studies, and present a refined interactive model that can be used as a tool to predict dietary iron bioavailability in populations where iron intakes and serum ferritin concentrations have been measured.

Subjects and Methods

Data were used from three studies, the National Diet and Nutrition Survey (NDNS), the National Adult Nutrition Survey (NANS) and the New Dietary Strategies Addressing the Specific Needs of the Elderly Population for Healthy Ageing in Europe study (NU-AGE). Briefly, NDNS (9) and NANS (10) were nationally representative samples of adults (excluding pregnant and breast-feeding women) in the UK (19-64 years) and Republic of Ireland (19 years and older), respectively. The NU-AGE study was a randomised controlled multicentre trial of healthy, independent older people (without frailty, heart failure or serious chronic illness) aged 65–79 years with the aim of assessing the effects of a one year dietary intervention on markers of inflammation and health (11, 12). We used baseline data from the UK participants only, as their dietary patterns were likely to be similar to the other UK surveys; the data were collected between September 2012 and January 2014. The detailed methods for data collection have been previously published (9, 10, 11, 12), but the information pertinent to this article (dietary assessment and analytical methods) are summarised below.

Dietary intake was assessed using seven-day food diaries in NDNS and NU-AGE and four-day semi-weighed food records in NANS. Participants were asked to record detailed information on the amount and type of all foods and drinks consumed over consecutive days. To ensure accuracy of recording, participants were interviewed or a researcher visited participants in their homes to review the food records and clarify any inconsistencies.

Height was measured to the nearest 0.1 cm using the Leicester height measure in all three studies and weight was measured to the nearest 100g using calibrated scales (Soehnle Quantratronic scales, NDNS; Tanita body composition analyzer BC-420MA (NANS): and Seca electronic column scales, NU-AGE).

Blood samples reached laboratories within five hours of collection and were processed and stored at −80°C until required for further analysis. Serum ferritin (SF) was measured either using a microparticle enzyme immunoassay assay (IMx, Abbott Laboratories, NDNS), automated analyser (RX Daytona, Randox, NANS) or an electrochemiluminescence immunoassay (Cobas 6000, Roche Diagnostics, NU-AGE). Hemoglobin concentrations were determined using either a Bayer H3 automated analyzer (NDNS), Coulter LH700 series analyser (NANS) or Sysmex XN (NU-AGE).

SF is an acute phase reactant, therefore in the presence of infection or inflammation, the concentration does not accurately reflect iron stores. C-reactive protein (CRP) and $_{\alpha}$-1-antichymotrypsin (ACT) are two of the biomarkers used to detect the presence of infection or inflammation and hence enable the exclusion of individuals with artificially high SF values

(13). Serum CRP (hs-CRP) concentrations were measured using an automated analyser, RX Daytona, Randox (NANS) or ProcartaPlex kits (Affimetrix) (NU-AGE) and any participants with a raised hs-CRP (>5 mg/L) were excluded. For the NDNS the acute phase reactant ACT was measured.

In NANS, 1500 individuals were recruited to the study and hsCRP was measured in 849 subjects. Those with a CRP < 5 mg/L (n=719) who were not taking supplements containing iron (n=656) and in whom SF had been measured (n=650) were included in the analysis. In the UK arm of the NU-AGE study 272 participants were recruited. Complete data on all relevant parameters were available for 246 participants, but 13 participants (5%) with raised hs-CRP levels (>5 mg/L) were subsequently excluded and 37participants were excluded because they were taking supplements containing iron; this left 196 subjects whose data were included in the current analysis. In the NDNS data we used the same exclusion criteria as for the other two studies (i.e. excluded if taking supplements containing iron and/or having raised inflammatory markers). This has been described previously (7).

Iron absorption was estimated from the measured iron intakes along a scale of assumed iron absorption values (1-40%). Requirements for absorbed iron were predicted using the Institute of Medicine’s distribution of dietary intake requirements, with values interpolated to derive iron absorption requirements for each 0.5th percentile (9). These values were compared to each individual’s absorbed iron estimate at each point on the 1-40% scale and the average absorption for the population was calculated. Subtracting these values from 100 gave the estimated percentage of the population who require a higher iron absorption to meet their requirements (i.e. the estimated prevalence of inadequate iron intakes). A model was created for the prediction of dietary iron absorption at each level of SF concentration using the assumption that the estimated prevalence of inadequate intakes would be equivalent to the observed prevalence of iron insufficiency, as defined by SF concentrations.

Ethics

Ethical approval for NDNS was granted by The South Thames Multi-Centre Research Ethics Committee (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/216484/dh_128550.pdf), for NANS by University College Cork Clinical Research Ethics Committee of the Cork Teaching Hospitals (ECM 3 (p) 04/11/08), and for NU-AGE by the National Research Ethics Committee East of England (12/EE/0109). Written informed consent was obtained from all participants.

Statistics

In view of the effects of sex and menstrual blood loss in women on iron status, all analyses were stratified by sex and by menopausal status. Differences in the characteristics of the participants in the three study cohorts were compared using one-way ANOVA. The distribution of SF was calculated individually for each sex and menopausal group and the cumulative frequencies calculated.

We examined associations between estimated iron intake from meat and SF concentrations. Quintiles of intake were calculated and ANCOVA was used to calculate adjusted means and evaluate statistical trends with adjustment for age, BMI, total iron intake and study cohort.

Statistical analysis was performed using Stata version 14 (StataCorp, College Station, Texas, USA) and R version 3.2.3 (14).

Results

A flow chart (**Supplemental Figure 1**) showing the numbers of participants recruited and excluded at different stages of the 3 studies is available as Online Supplemental Material. Details of the three studies, including study subjects, exclusion criteria, analytical methods and dietary assessment are summarised in **Supplemental Table 1.** The characteristics of the participants from the three studies are presented in **Table 1** and individual data are given in **Supplemental File 1**. The % of individuals with acute phase reactant values indicative of inflammation or infection (hsCRP >5 mg/L or α-1-ACT >0.65 g/L) were 0% in NDNS, 15% in NANS, and 5% in NU-AGE. These individuals were excluded from the analysis as their SF concentration may have been elevated and therefore not reflect iron stores accurately.

The combined mean iron intakes were 13.6 (SD 5.2), 10.3 (SD 4.1) and 10.9 (SD 3.5) mg/d in men, pre- and post-menopausal women, respectively. For post-menopausal women, the mean intake was very close to the Population Reference Intake (PRI) of 11 mg/d, and for men it was higher than the PRI of 11 mg/d, but for pre-menopausal women the intake was lower than the PRI of 16 mg/d (15). However, all groups had intakes above the Average Requirement (6, 7, and 6 mg/d for men, pre- and post-menopausal women respectively).

The majority of the participants (95%) were iron sufficient (SF >15 µg/L). Mean SF values were 140.7 µg/L (± 113.6), 49.4 µg/L (± 45.8) and 96.7 µg/L (± 72.8) in men, pre-menopausal women and post-menopausal women, respectively; the cumulative distributions of SF concentrations are shown in **Figure 1**. There was a significant difference in mean SF concentrations between the three cohorts, with higher values reported in the NANS across all sex and menopausal status groups. Despite higher SF levels, iron intake was not higher in the NANS compared to the other two cohorts although iron intake from meat was significantly higher.

Figure 2 shows the predicted prevalence of inadequate iron intakes at different levels of estimated iron absorption, using combined data from the three cohorts. When iron absorption was 18% the predicted prevalence of inadequate iron intakes were 5%, 35% and 3% in men, pre-menopausal women and post-menopausal women, respectively. These data reflect the capacity of the diet to meet iron requirements and when combined with SF values allow prediction of the dietary absorption required to maintain a specific iron status (see **Supplemental File 2**). For example, at SF concentrations below 15 µg/L the mean dietary iron absorption ranges from 19% in post-menopausal women to 27% in pre-menopausal women, compared to 11-12 % for SF concentrations of 60 µg/L (**Figure 3**).

In both men and women there was a positive association between iron intake from meat and SF after adjustment for total iron intake, age and BMI (**Figure 4**). There was a difference in iron intake from meat between extreme quintiles of intake of 4.3 mg for men and 3.0 mg for women. SF was 32.0 µg/L (± 11.8) higher in quintile 5 compared to quintile 1 of intake for men (P-trend = 0.02) and 14.9 µg/L (± 6.1) higher for women (P-trend = 0.01).

The program for calculating dietary iron absorption at any level of SF concentration can be found in Supplemental File 2.

Discussion

In our model, the differences in iron status between the 3 study population groups were partly explained by age (post-menopausal women have a lower iron status than pre-menopausal women due to their lower iron requirements) but also by diet i.e. the higher intake of meat in the NANS groups was associated with higher SF concentration. When adequate body iron stores are present at a SF concentration of 60 µg/L, the efficiency of iron absorption is no longer upregulated (16), and the computed differences in dietary iron absorption were minimal, but with a lower SF, the effect of diet became more marked, illustrating the importance of applying iron intake and SF data collected in populations with different dietary patterns. In particular, it appears that meat consumption is a key determinant of body iron status.

Although iron requirements for individuals can be estimated reasonably accurately (15) the dietary intake needed to supply this quantity of absorbed iron is notoriously difficult to estimate because of the uncertainty about dietary iron absorption. In healthy individuals, the key determinants of fractional iron absorption are dietary factors (17) and body iron status (18), plus short-term regulation related to previous exposure of the mucosal cells to iron (19). However, when reliable measures of total iron intake and body iron status exist, the unknown variable (dietary iron absorption) can be computed by taking into account calculated physiological requirements. A strength of our study is the use of high quality data for iron intake and iron status. Furthermore, individuals with raised inflammatory markers were removed from the dataset used to derive the model as they may have had an artificially high SF concentration that did not reflect body iron stores accurately. We also excluded individuals who had been taking supplements containing iron as it impossible to quantify their contribution to total iron intake.

There are some limitations that should be considered when using the model. Although the three datasets used for this study were obtained from 4/7-day dietary intakes (see Supplemental Table 1), participant burden should be considered, particularly for large-scale epidemiology studies or surveys. Data collected using other dietary assessment methods, such as 24 hr recall or Food Frequency Questionnaire (FFQ) may still be applied to the model, but the limitations of these intake methods should be acknowledged in the conclusions. Although we were able to exclude users of supplements containing iron and also individuals with elevated inflammatory markers from the datasets, there was insufficient information available to assess whether any individuals were taking prescribed or over-the-counter medicines, or had particular medical conditions, that could affect iron absorption or body iron status. Individuals with chronic conditions were generally excluded from participation in the studies, although the aim was to select a cohort that was representative of the population group. Furthermore, evidence for the effect of specific medical conditions and medicines on iron absorption and/or status is limited, and a large proportion of the general population routinely take some form of medication, therefore excluding these individuals is not practical and would result in a very limited dataset. However, it remains important to consider all of these potential issues when collecting data for the model and interpreting the results.

Although it is not possible to measure iron requirements accurately in large numbers of individuals, particularly women of child-bearing age whose requirements are largely dictated by the magnitude of menstrual blood loss, population means can be computed, and these are what are needed to set DRVs, and to develop dietary guidelines and public health policies. When setting DRVs for adults, the National Academy of Medicine (2001) used an iron bioavailability value of 18%. This was computed by assuming 10% of dietary iron was heme iron, with an absorption of 25%, and that the absorption of the remaining 90% of iron (non-heme) was 16.8% in individuals with a SF of 15 µg/L (4). WHO/FAO took variations in the properties of the diet into account when proposing bioavailability figures, and set DRVs based on 4 different values: 15% and 12% for Western-type diets, depending mainly on the level of meat intake, and 10% and 5% for developing countries (20). In Europe, the European Food Safety Authority (EFSA) (15) applied the probability model developed by Dainty et al (7) to derive values of 16% for men and 18% for pre-menopausal women with a population mean SF concentration of 30 µg/L. The UK Committee on Medical Aspects of Food Policy

(21) selected 15% absorption as typical in industrialised countries, and the Nordic countries (22) also applied an iron absorption value of 15% when setting DRVs.

The lack of consensus in values for dietary iron absorption reflect, in part, differences in the type of diet that is considered representative for the adult population in the country (or group of countries) under consideration, but also illustrates differences in the selection and interpretation of evidence upon which to base the value. We have further evaluated the model developed by Dainty et al (7) using survey data from populations consuming Western-diets and the interactive model is provided in Supplemental File 2. Use of this model would facilitate harmonisation in deriving values for dietary iron absorption, and thereby reduce uncertainty. It can be applied to any adult population where representative, good quality data on iron intake and iron status have been collected. Furthermore, dietary iron bioavailability values can be derived for any target level of SF, thus giving risk managers and public health professionals a flexible and transparent basis upon which to base their dietary recommendations.

Acknowledgements

None of the authors has a conflict of interest.

SFT, JRD, and LJH were responsible for the conception and design; JRD, LJH, RB, JW, and AJ conducted research; JRD and AJ analysed data; SFT wrote paper and had primary responsibility for final content; all authors read and approved the final manuscript.

References

1. 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.
2. Hallberg L, Hulthen L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. Am J Clin Nutr 2000;71:1147-60.
3. Reddy MB, Hurrell RF, Cook JD. Estimation of nonheme-iron bioavailability from meal composition. Am J Clin Nutr 2000;71:937-43.
4. Cook JD1, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr. 1991;54:717-22.
5. Armah SM, Carriquiry A, Sullivan D, Cook JD, Reddy MB. A complete diet-based algorithm for predicting nonheme iron absorption in adults. J Nutr 2013;143:1136-40.
6. Collings R, Harvey LJ, Hooper L, Hurst R, Brown TJ, Ansett J, King M, Fairweather-Tait SJ. The absorption of iron from whole diets: a systematic review. Am J Clin Nutr 2013;98:65-81.
7. Dainty JR, Berry R, Lynch SR, Harvey LJ, Fairweather-Tait SJ. Estimation of dietary iron bioavailability from food iron intake and iron status. PLoS ONE 9(10): e111824. doi:10.1371/journal.pone.0111824.
8. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC: National Academy Press, 2001.
9. Hoare J HL, Bates CJ, Prentice A, Birch M, Swan G, Farron M. National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 5: Summary report. London: TSO, 2004.

10. Irish Universities Nutrition Alliance (2011). National Adult Nutrition Survey Main Report. Accessed online at: www.iuna.net September 2016.

11. Berendsen A, Santoro A, Pini E, Cevenini E, Ostan R, Pietruszka B, Rolf K, Cano N, Caille A, Lyon-Belgy N et al. A parallel randomized trial on the effect of a healthful diet on inflammageing and its consequences in European elderly people: design of the NU-AGE dietary intervention study. Mech Ageing Dev 2014;136-137:14-21.

12. Santoro A, Pini E, Scurti M, Palmas G, Berendsen A, Brzozowska A, Pietruszka B, Szczecinska A, Cano N, Meunier N, et al. Combating inflammaging through a Mediterranean whole diet approach: the NU-AGE project's conceptual framework and design. Mech Ageing Dev 2014;136-137:3-13.

13. C-reactive protein concentrations as a marker of inflammation or infection for interpreting biomarkers of micronutrient status. Vitamin and Mineral Nutrition Information System, Geneva: World Health Organization; 2014 (WHO/NMH/NHD/EPG/14.7; http://apps.who.int/iris/bitstream/10665/133708/1/WHO_NMH_NHD_EPG 14.7 eng.pdf?ua=1, accessed [05 Dec 2016].

14. R Development Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

15. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015.Scientific Opinion on Dietary Reference Values for iron. EFSA Journal 2015;13 (10):4254, 115 pp. doi:10.2903/j.efsa.2015.4254.

16. Hallberg L, Hultén 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.

17. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr. 2010;91:1461S-7S.

18. Ganz T. Systemic iron homeostasis. Physiol Rev. 2013;93:1721-41.

19. Viteri FE, Xunian L, Tolomei K, Martin A. True absorption and retention of supplemental iron is more efficient when iron is administered every three days rather than daily to iron-normal and iron-deficient rats. J Nutr 1995;125:82-91.

20. WHO/FAO (World Health Organization/Food and Agriculture Organization of the United Nations). Vitamin and mineral requirements in human nutrition: report of a Joint FAO/WHO Expert Consultation. Geneva: World Health Organization, 2004.

21. Department of Health. Dietary Reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. London: HMSO, 1991.

22. Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. Copenhagen: Narayana Press, 2012.

Table 1: Characteristics, iron status and dietary intake of participants, stratified by study, sex and menopausal status[1]

	Males				Pre-menopausal females			Post-menopausal females			
	NANS	**NDNS**	**NU-AGE**	***P***	**NANS**	**NDNS**	***P***	**NANS**	**NDNS**	**NU-AGE**	***P***
n	336	494	77		197	363		117	158	119	
Age (y)	42.8 (16.4)	42.4 (12.0)	70.2 (3.8)	<0.001	34.8 (9.4)	34.9 (7.4)	0.80	62.4 (8.9)	57.5 (4.1)	69.8 (3.9)	<0.001
Weight (kg)[2]	87.1 (13.6)	83.7 (14.1)	82.4 (12.2)	0.001	69.7 (12.1)	67.8 (14.3)	0.13	72.0 (12.7)	71.2 (13.2)	68.7 (10.9)	0.09
BMI (kg/m^2)[2]	28.0 (4.0)	27.1 (4.3)	27.0 (3.7)	0.01	25.9 (4.4)	25.9 (5.5)	0.97	28.0 (4.7)	27.7 (5.1)	26.4 (3.7)	0.02
Hemoglobin (g/dL)[3]	15.2 (1.1)	15.1 (1.1)	14.7 (0.9)	<0.001	13.3 (1.1)	13.4 (1.0)	0.69	13.4 (1.0)	13.5 (1.1)	12.9 (3.6)	0.07
Serum ferritin (ug/L)	172 (135)	119 (92.5)	146 (102)	<0.001	57.9 (57.8)	44.7 (37.0)	0.001	116 (90.8)	77.0 (55.3)	104 (67.6)	<0.001
Iron (mg/d)	13.8 (5.7)	13.4 (5.1)	14.3 (3.4)	0.37	11.1 (4.6)	9.8 (3.8)	0.001	10.2 (3.3)	10.9 (3.8)	11.6 (3.1)	0.01
Iron from meat (mg/d)	2.8 (1.7)	2.6 (1.6)	1.3 (0.8)	<0.001	1.8 (1.4)	1.5 (1.1)	0.002	1.7 (1.2)	1.5 (1.1)	1.0 (0.7)	<0.001

[1]Values are mean (SD), n=1861. NANS= National Adult Nutrition Survey; NDNS=National Diet and Nutrition Survey; NU-AGE= New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe. [2,3]Missing data for [2]n=21 and [3]n=20

The American Journal of Clinical Nutrition AJCN/2016/147389 Version 4

Legends for Figures

Figure 1: The cumulative distribution of serum ferritin concentrations for men, pre- and post-menopausal women by study.

Values are the percentage of participants in each group. The number of participants were; men ▬ n=336, ▬ n=494 and ▭ n=77, pre-menopausal women ▬ n=197 and ▬ n=363 and post-menopausal women ▬ n=117, ▬ n=158 and ▭ n=119. Mean (±SD) serum ferritin values were 140.7 µg/L (± 113.6), 49.4 µg/L (± 45.8) and 96.7 µg/L (± 72.8) in men, pre-menopausal women and post-menopausal women, respectively. NANS= National Adult Nutrition Survey; NDNS=National Diet and Nutrition Survey; NU-AGE= New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe.

Figure 2: The predicted prevalence of inadequate iron intakes at different levels of iron absorption in men, pre- and post-menopausal women.

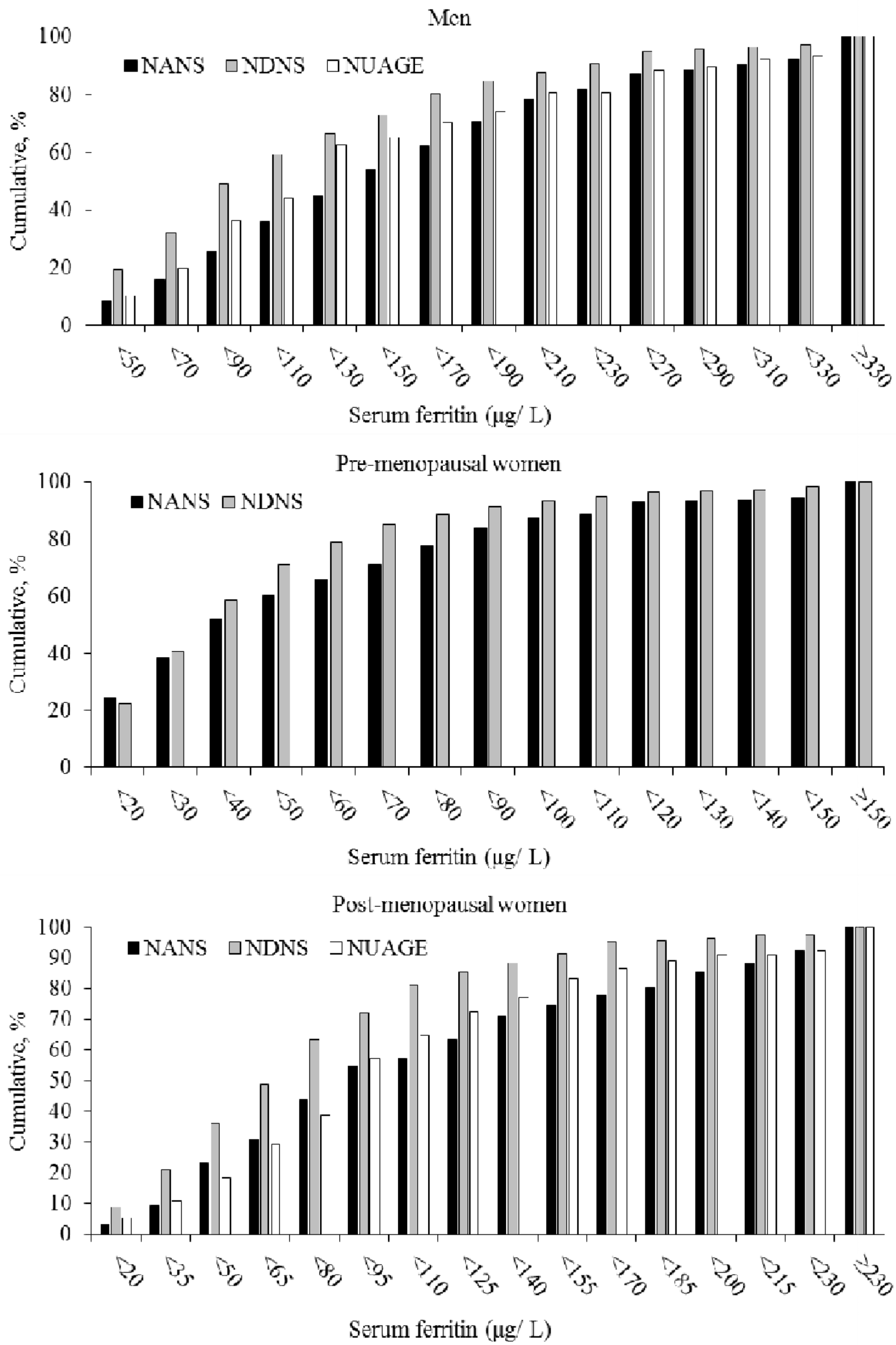
Values for predicted prevalence of inadequate iron intake for dietary absorption values ranging from 0 to 40%.

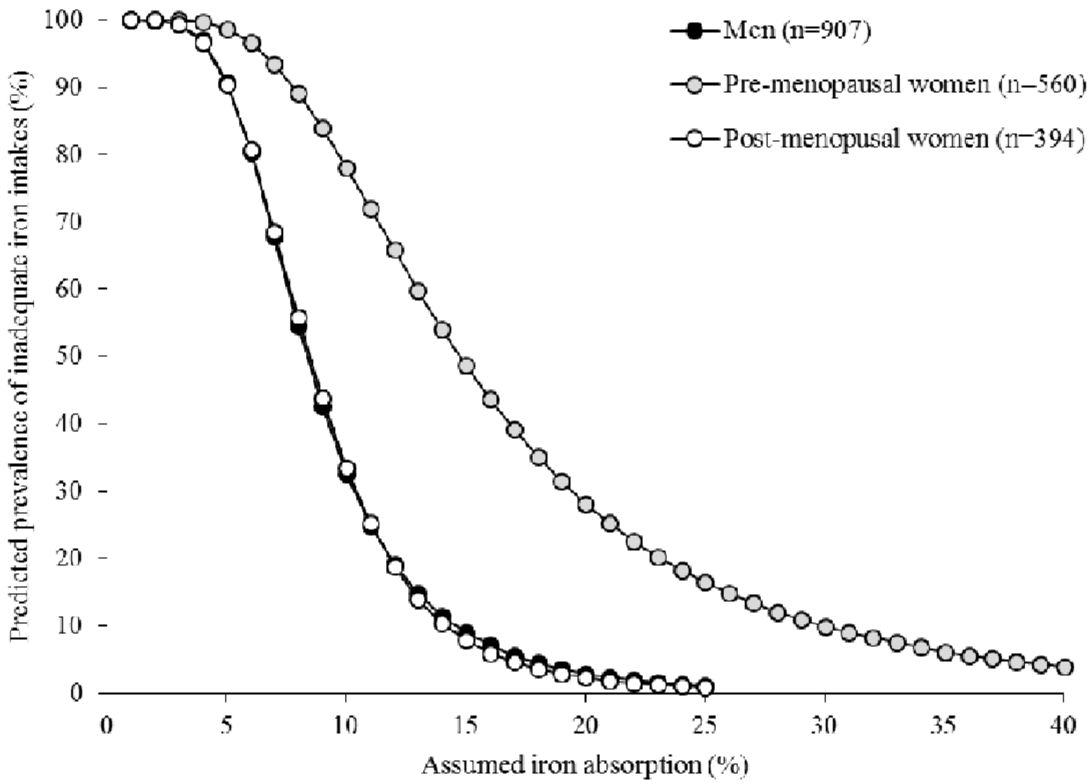
Figure 3: Estimated dietary iron absorption for selected serum ferritin values for men, and pre- and post-menopausal women.

Predicted dietary iron absorption (%) for serum ferritin concentrations ranging from <15 to 100 µg/L.

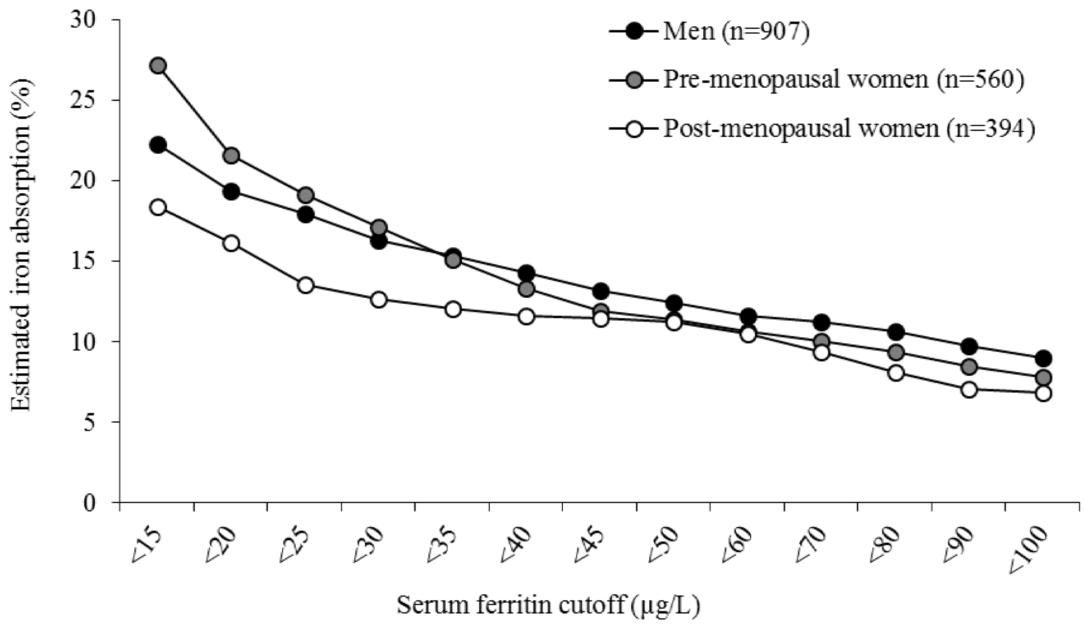
Figure 4: Adjusted serum ferritin values by quintile of iron intake from meat, stratified by sex.

Values are adjusted means (SE), means are adjusted for age (y), BMI (kg/m^2), total iron intake (mg/d) and study cohort. Mean ± SD values for iron intake from meat in each quintile were as follows; females Q1 = 0.2 ± 0.2, Q2 = 0.8 ± 0.1, Q3 = 1.3 ± 0.1, Q4 = 1.9 ± 0.2, Q5 = 3.2 ± 1.2; males Q1 = 0.6 ± 0.3, Q2 1.5 ± 0.2, Q3 2.3 ± 0.2, Q4 3.1 ± 0.3, Q5 5.0 ± 1.5. *P*-trend calculated using ANCOVA.

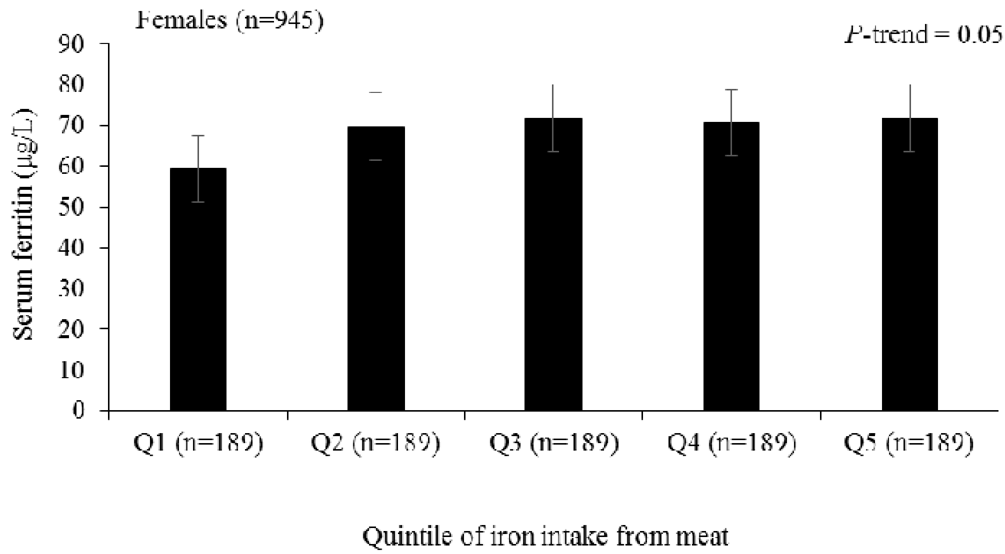

Men
NANS
NDNS
NUAGE
Cumulative, %
0
20
40
60
80
100
≤50
≤70
≤90
≤110
≤130
≤150
≤170
≤190
≤210
≤230
≤270
≤290
≤310
≤330
≥330
Serum ferritin (µg/ L)
Pre-menopausal women
NANS
NDNS
Cumulative, %
0
20
40
60
80
100
≤20
≤30
≤40
≤50
≤60
≤70
≤80
≤90
≤100
≤110
≤120
≤130
≤140
≤150
≥150
Serum ferritin (µg/ L)
Post-menopausal women
NANS
NDNS
NUAGE
Cumulative, %
0
10
20
30
40
50
60
70
80
90
100
≤20
≤35
≤50
≤65
≤80
≤95
≤110
≤125
≤140
≤155
≤170
≤185
≤200
≤215
≤230
≥230
Serum ferritin (µg/ L)

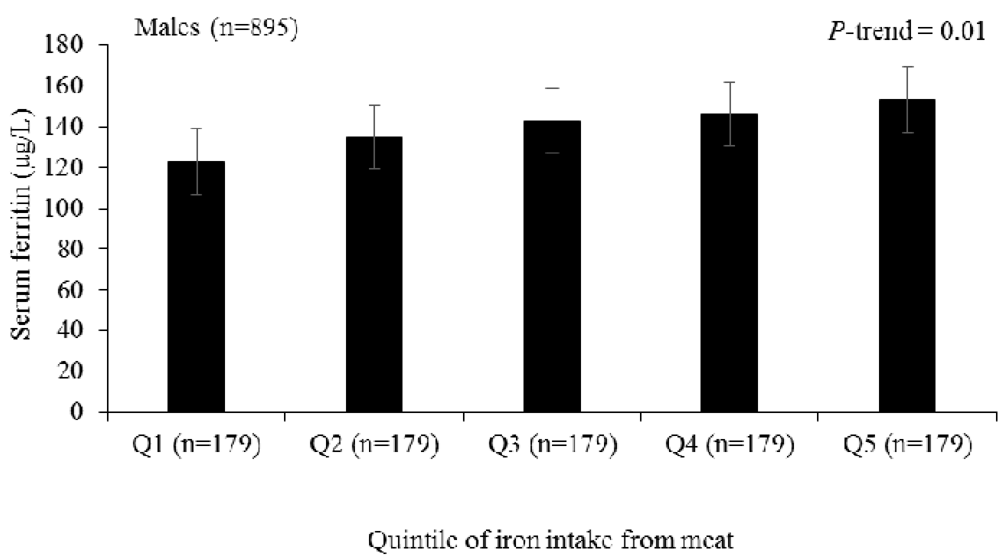


Men (n=907)
Pre-menopausal women (n=560)
Post-menopusal women (n=394)
Predicted prevalence of inadequate iron intakes (%)
Assumed iron absorption (%)
0
10
20
30
40
50
60
70
80
90
100
5
15
25
35



Men (n=907)
Pre-menopausal women (n=560)
Post-menopausal women (n=394)
Estimated iron absorption (%)
30
25
20
15
10
5
0
<15
<20
<25
<30
<35
<40
<45
<50
<60
<70
<80
<90
<100
Serum ferritin cutoff (µg/L)


Females (n=945)
P-trend = 0.05
Serum ferritin (μg/L)
0
10
20
30
40
50
60
70
80
90
Q1 (n=189)
Q2 (n=189)
Q3 (n=189)
Q4 (n=189)
Q5 (n=189)
Quintile of iron intake from meat


Males (n=895)
P-trend = 0.01
Serum ferritin (μg/L)
0
20
40
60
80
100
120
140
160
180
Q1 (n=179)
Q2 (n=179)
Q3 (n=179)
Q4 (n=179)
Q5 (n=179)
Quintile of iron intake from meat