

Relationship between dietary iron intake, corrected for diet reporting error, and serum ferritin in Danish women aged 35–65 years

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Several studies have failed to demonstrate an association between Fe status and intake of dietary Fe. However, in the long term, it seems logical to presume that body Fe reserves are, fundamentally, dependent on the intake of bioavailable dietary Fe. This discrepancy may depend on several factors: (1) interindividual variation in biological availability of dietary Fe (differences in intestinal absorption), (2) interactions between dietary Fe and absorption enhancers and inhibitors, (3) variations in physiological (menstruation, childbirth) or unphysiological (blood donation) Fe losses, (4) the failure to adjust dietary intake data for Fe supplements, (5) uncertain food composition data (discrepancies between calculated and chemically measured Fe content in the diet), and (6) diet reporting error (reported intake of dietary Fe may deviate considerably from the true intake). The present study examined associations between dietary intake of Fe (assessed by diet history interview) and Fe status (assessed by ferritin status) among 167 Danish women aged 35–65 years, who were not blood donors, by taking into account diet reporting error (assessed from *p*-amino benzoic acid-validated urinary N), physiological blood losses (menstruation, childbirth, abortions), and Fe supplementation. Our results indicate that the lack of a general association between Fe status and dietary Fe intake may, in part, be caused by selective diet reporting error.

Dietary iron: Serum ferritin: Diet reporting error

Fe deficiency is considered to be one of the most common deficiency disorders in the Western world, primarily affecting premenopausal women, due to blood losses at menstruation and pregnancy. Accordingly, of premenopausal Danish women aged 30–50 years, 23% have reduced, and 18% have low Fe reserves, judged by the serum ferritin level (Milman *et al.* 1992). Among Danes, prophylactic measures against Fe deficiency are relevant for premenopausal women only (Milman *et al.* 1983, 1992). Using intake of dietary Fe to identify women at risk has not proved valid, as several studies have failed to demonstrate an association between Fe status and intake of dietary Fe (Hallberg, 1982; Milman *et al.* 1990; Milman & Kirchoff, 1991, 1992). This discrepancy depends on a number of factors such as differences in bioavailability of dietary Fe (for instance haem *v.* non-haem Fe), interactions between dietary Fe and absorption inhibitors and enhancers (for instance phytic and ascorbic acids, tannins, polyphenols), variations in physiological (menstrual losses, childbirth, abortions) or unphysiological (blood donation) blood losses, or the failure to adjust dietary intake data for Fe supplements. However, diet reporting

errors in nutritional surveys may also play a role. Thus, subjects with an apparently low Fe intake due to dietary underreporting may in fact have a sufficient Fe intake.

The purpose of the present study was to examine the relationship between dietary Fe intake, corrected for diet reporting error, and Fe status in a sample of Danish women aged 35–65 years.

SUBJECTS AND EXPERIMENTAL METHODS

The present study was part of the Danish MONICA project (an international study conducted under the auspices of the World Health Organization to monitor trends in and determinants of mortality from cardiovascular disease), and was performed from December 1987 to November 1988 (Heitmann, 1991). The study population included 276 Danish women in age cohorts of 35, 45, 55, and 65 years, selected at random from a larger sample of 1725 subjects (Kirchhoff *et al.* 1983), together with twenty-eight women from the same population, who had developed gallstones in the past 5 years. The women were invited to a health examination, and agreed to give a diet history interview and complete a 24-h urine collection.

The project was approved by the Ethical Committee for Copenhagen County.

Diet

The same dietician interviewed all the participants, using the diet history method. The diet was assessed, based on information from the preceding month, and average daily intakes were calculated. Meal pattern, dishes and foods were explored using a pre-coded interview form. Quantities were explored using food models, photo series, cups and measures. Nutrient calculations were performed with the DANKOST-programme (Møller, 1986, 1989). Fe from supplements was not included in the dietary nutrient calculations.

Clinical examination

A medical history was obtained from all participants, and included menopausal status, number of deliveries and abortions, and intake of Fe supplements, being classified into two categories: regular use (daily/weekly) and no intake (none of the women reported that they took supplements occasionally, e.g. monthly). The amount of supplemental ferrous Fe consumed by Danes in vitamin–mineral tablets, recorded in another study, was median 18 (5–95% percentile 10–51) mg Fe/d (Milman *et al.* 1995). Premenopausal women yielded information about the present duration of menstruation (classified into four categories: 1–3 d, 4–6 d, 7–9 d or > 10 d), the intensity (weak, normal, strong), as well as regularity of menstruation (regular, irregular).

Serum ferritin

Blood samples were drawn from women in the fasting state. Serum ferritin was analysed with a radioimmunoassay (Ferritin RIA Amersham, Amersham International plc, Cardiff, South Glamorgan) (Milman *et al.* 1993a). The assay was calibrated against the international human liver ferritin standard, WHO 80/602 (Milman *et al.* 1994). Fe stores were considered small or absent at serum ferritin values < 20 µg/l (Milman *et al.* 1993a).

Urine analysis

The participants were instructed to collect 24 h urine, as described earlier (Heitmann, 1993). In order to monitor collection completeness, each participant was given 240 mg *p*-amino benzoic acid (PABA) to be taken during the day of collection (Bingham & Cummings, 1983). Urinary N was analysed by a flash combustion technique in a Carlo

Erba NA 1500 nitrogen analyser (Carlo Erba, Milan, Italy). PABA was analysed using spectrophotometric analysis (Gilson Stasar II, Gilson Medical Electronics Inc.). Urine volume was calculated from the dilution of added Li, measured by flame photometry (Corning Clinical Flame Photometer, 410C, Ciba Corning). Calculation of protein intake (Prot_u) from 24 h urine N was made using the formula (Isaksson, 1980):

$$\text{Prot}_u \text{ (g)} = (\text{N}_u + 2 \text{ g}) \times 6.25,$$

where N_u is N output (g) in 24 h urine.

Diet reporting error

There was a positive association (r 0.58, $P < 0.0001$) between reported dietary Fe intake (without supplements) and reported protein intake, indicating that Fe intake follows protein intake. Due to this association, we considered it acceptable to adjust reported dietary Fe intake according to 'true' protein intake calculated from urine analysis.

Recommended daily allowance

The recommended daily allowance (RDA) for Fe is 15 mg for premenopausal and 10 mg for postmenopausal women (National Research Council, 1989). An Fe intake below 2 SD of the RDA (Beaton, 1985), i.e. below 5.6 mg for postmenopausal and 8.1 mg for premenopausal women, was considered as a low intake.

Statistical methods

Statistical analyses were performed with the SPSS/PC V2.0 program (Statistical Package for the Social Sciences, Chicago, IL, USA). Multiple regression analyses, corrected for covariates (menstrual losses, and number of pregnancies and abortions), were used to examine associations between dietary Fe intake and serum ferritin levels. A log-transformation of serum-ferritin and dietary Fe-intake values was performed to achieve normality. Estimated underreporting error was calculated as the log transformation of the ratio between protein from urinary N and reported protein. Mean serum ferritin levels adjusted for covariates, by dietary Fe intake in quintiles, were calculated using ANOVA.

RESULTS

A group of 217 women agreed to participate in this study, together with the twenty-eight women who developed gallstones in the preceding 5 years; of these, 225 completed both the urine collection and the dietary interview. In thirty-four women (15%) the collected urine contained less than 85% of the administered PABA (Bingham & Cummings, 1983) and they were excluded from further analyses. Of the remaining 191 women, twenty-four were excluded because they had donated blood. Thus, the final series comprised 167 women. No systematic bias of PABA recovery was found between age-groups (Heitmann, 1993). There were no significant differences in reported energy, protein or Fe intakes, or serum ferritin levels between participants (n 167) and non-participants (n 58) (all P values > 0.60), but participants were older (51 (SD 11) years) than non-participants (48 (SD 11) years; $P = 0.05$). Characteristics of the subjects are given in Table 1. The women were divided according to menopausal status (sixty-three premenopausal, 104 postmenopausal); 97% (32/33) of the 35-year-old women, 69% (29/42) of the 45-year-old women and 2% (2/92) of the 55–65-year-old women reported that they had not reached menopause. Of the premenopausal women, 17% (9/63) had small or absent Fe stores ($< 20 \mu\text{g/l}$), v. none of the postmenopausal women. Out of all 167 women, only one premenopausal woman had Fe stores below $12 \mu\text{g/l}$ serum ferritin.

Table 1. *BMI, energy intake, reported dietary protein intake, protein calculated from urinary nitrogen output and serum ferritin level in 167 Danish women according to age and menopausal status**

(Mean or median values and standard deviation or range)

Age (years)	Menopause	n	BMI (kg/m ²)		Energy intake (MJ/d)		Protein (diet) (g/d)		Protein (urine) (g/d)		Serum ferritin (μg/l)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Median	Range
35		33	23.5	4.2	7.8	2.1	66.8	15.8	79.2	18.5	42	11-176
45		42	23.7	2.9	7.4	1.7	64.2	13.7	82.3	25.1	47	14-331
55		47	25.4	4.2	7.2	1.7	62.2	15.5	74.7	14.7	87	35-233
65		45	25.5	3.7	7.0	2.0	61.9	20.6	73.5	21.1	103	30-321
	Post	104	25.2	3.9	7.2	1.9	63.1	17.7	75.4	17.7	89	26-331
	Pre	63	23.7	3.6	7.4	1.9	64.2	14.8	80.1	23.9	43	11-256
	All	167	24.6	3.8	7.3	1.9	63.5	16.6	77.2	20.3	67	11-331

* For details of subjects and procedures, see pp. 906-907.

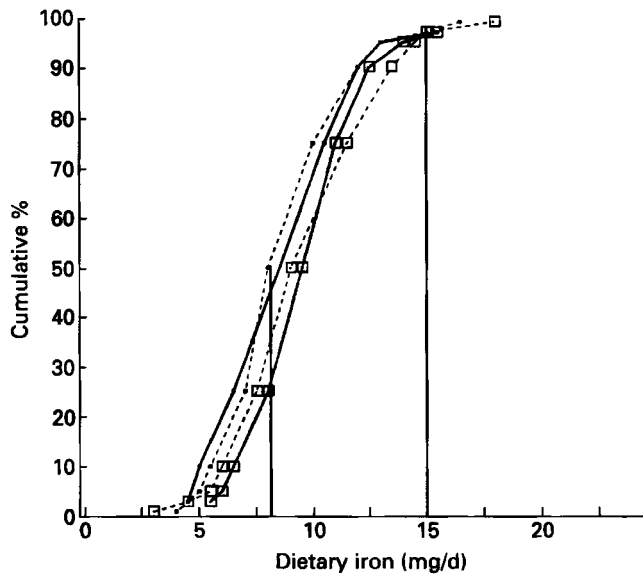


Fig. 1. Cumulative distribution curves for reported (●) and adjusted (□) intakes of dietary Fe in a sample of premenopausal (—) and postmenopausal (---) women. The recommended daily allowance (15 mg) and 2 SD (8.1 mg) for premenopausal women are indicated.

None of the women was pregnant. Among premenopausal women, nine had taken oral contraceptives, three had had abortions and eight had been lactating within the past 5 years, ten used an intrauterine device (IUD), and none had had a hysterectomy or an oophorectomy. Of the postmenopausal women, one had used an IUD, two had taken oral contraceptives and none had been lactating within the last 5 years. Twenty-three of the postmenopausal women had undergone gynaecological surgery.

Table 2. Regression coefficients (β), standard errors (SE) and significance level (P) of associations between dietary Fe intake (mg/d; log values) and serum ferritin levels ($\mu\text{g/l}$; log values) in pre- and postmenopausal women, with or without Fe supplementation and before and after adjustment for diet reporting error

	Unadjusted for diet reporting error			Adjusted for diet reporting error		
	β	SE	P	β	SE	P
Premenopausal*						
Unsupplemented (n 25)	1.03	0.37	0.01	1.34	0.39	0.003
Supplemented (n 38)	-0.16	0.41	0.70	-0.56	0.47	0.25
Postmenopausal†						
Unsupplemented (n 42)	-0.27	0.34	0.43	-0.21	0.32	0.51
Supplemented (n 62)	-0.15	0.25	0.56	-0.19	0.33	0.57

* Adjustment made for length, intensity and regularity of menstruation and number of abortions and births within the past 5 years.

† Adjustment made for number of abortions.

Dietary underreporting increased as estimated protein intake increased. Fig. 1 shows cumulative distribution curves for reported and adjusted intake of dietary Fe for premenopausal and postmenopausal women. After adjustment of Fe intake, based on the reporting bias of protein, the curves shifted to the right, indicating that adjusted Fe intake was on average 14% higher than reported Fe intake.

Of the premenopausal women, 48% (30/63) reported an Fe intake below 2 SD of RDA, i.e. below 8.1 mg (a low Fe intake). After adjustment for diet reporting error, 32% (20/63) still had a low Fe intake. Before adjustment 9% (9/104), and after adjustment 4% (4/104) of the postmenopausal women had a low intake of Fe (below 5.4 mg). There was no association between dietary Fe intake and serum ferritin levels in the 167 women ($\beta = -0.05$, $P = 0.80$), nor were there any associations in pre- ($\beta = -0.14$, $P = 0.50$) or postmenopausal women ($\beta = 0.09$, $P = 0.76$), before adjustment for dietary underreporting error, or before correction for co-variables (length, intensity and regularity of menstruation, as well as number of abortions and births within the past 5 years; results not shown).

Multiple-regression coefficients for associations between dietary Fe intake and serum ferritin, with and without adjustment for underreporting error, are shown in Table 2. In the premenopausal women, length, intensity and regularity of menstruation, as well as number of abortions and births within the past 5 years, were included as co-variables. In the postmenopausal women, only the number of abortions was included as a co-variate. When analyses were performed without the adjustment for dietary underreporting error, associations between Fe intake and stores were not significant except in premenopausal women who did not take any supplements. After adjustment for diet reporting error the association in the premenopausal women became stronger. Adjustment for diet reporting error did not change associations in premenopausal women who took supplements or in any of the groups of postmenopausal women.

The association between serum ferritin and adjusted dietary Fe intake in premenopausal women, with and without supplementation, is shown in Fig. 2. With increasing adjusted dietary Fe intake, serum ferritin levels increased in women not taking Fe supplements ($P = 0.11$ for mean differences), whereas serum ferritin decreased in women taking regular supplements ($P = 0.82$).

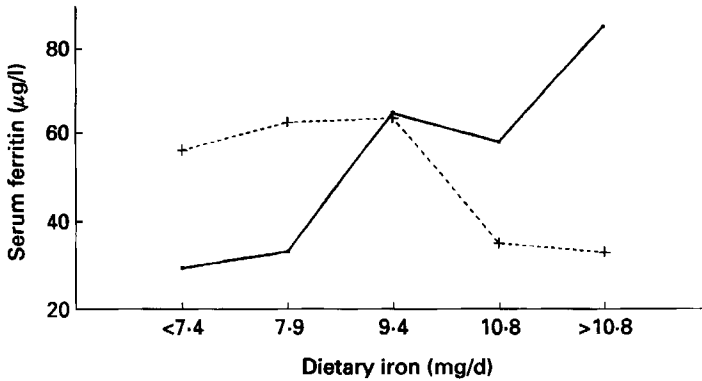


Fig. 2. Mean levels of serum ferritin ($\mu\text{g/l}$), by levels of adjusted Fe intake (mg/d) (figures indicate mid-interval values of quintiles) among premenopausal women with (---) or without (—) Fe supplementation. Adjustment was made for number of deliveries and abortions, duration, intensity and regularity of menstruation.

DISCUSSION

The use of protein values as a standard for diet reporting bias of Fe is probably justified, as both protein and Fe intake seem to be related to energy intake (in the present study, energy intake displayed a positive association with both protein ($r\ 0.75$, $P < 0.0001$) and Fe intakes ($r\ 0.71$, $P < 0.0001$). Furthermore, most of the dietary Fe in Danish food is associated with protein-rich food (e.g. in 1985 70% of dietary Fe came from meat, bread and cereals (Haraldsdóttir *et al.* 1986)). Data on sources of Fe intake in the Danish diet after the cessation of mandatory fortification of flour in 1987 are not yet available. However, presumably the proportion of Fe intake from meat would have increased. Finally, considering the high variability in dietary interview data, the correlation coefficient of 0.58 between reported intake of protein and Fe is high (Willett, 1990).

The analytical variance concerning serum ferritin is small compared with that for dietary interviews, which have a pronounced variation and poor reproducibility (Black *et al.* 1991). In the long term, it seems logical to presume that body Fe reserves are, fundamentally, dependent on the intake of dietary Fe. However, it has been stated that a straightforward correlation between dietary Fe intake and Fe status seems most unlikely, due to variations in Fe availability and Fe requirements (Southon *et al.* 1988). This is in accordance with the present and previous studies, in which there was no correlation between reported dietary Fe intake and serum ferritin (Galán *et al.* 1985; Milman *et al.* 1990; Milman & Kirchoff, 1991, 1992). This lack of association may be the result of several factors. First, the interindividual variation in biological availability of dietary Fe, which, among other things, is determined by the degree of intestinal absorption, also depends on the composition of meals, i.e. the absorption from two different meals with similar Fe content may vary considerably due to interaction between dietary Fe and absorption enhancers and inhibitors (Monsen, 1988; Skikne & Baynes, 1994). Second, the calculation of reported dietary intake is performed using food composition tables. However, it may be assumed that a discrepancy exists between calculated and chemically measured nutrient content in the diet. Third, physiological (menstruation, childbirth) or unphysiological (blood donation) Fe losses tend to obscure the relationship between dietary Fe intake and Fe status, and fourth, the widespread use of Fe supplements, consumed by 50% of Danish women (Milman *et al.* 1993b), is another confounder in this context. Last, but not least, the reported intake of dietary Fe may deviate considerably from the true intake, due to diet reporting error. In the present study we have attempted to correct for some of these factors,

Table 3. Median, 5th and 95th percentiles of Fe intake (mg/d) in women, calculated without and with Fe fortification of flour, unadjusted and adjusted for underreporting compared with Fe intake data from the Danish National Dietary Survey in 1985 (Haraldsdóttir et al. 1986)

Age (years)	Adjusted	Fortified	Percentiles		
			5	50	95
35-45	-	-	4.9	8.5	12.9
	+	-	5.7	9.7	14.8
	+	+	7.4	12.5	17.1
	National Survey 1985		7.7	13.0	20.8
	-	-	5.0	7.9	13.3
55-65	+	-	5.3	8.9	14.1
	+	+	6.0	11.2	16.6
	National Survey 1985		6.9	12.1	18.3
	-	-	5.0	8.1	12.9
	+	-	5.7	9.2	14.2
35-65	+	+	7.2	11.6	16.9
	National Survey 1985		7.4	12.6	20.3

i.e. diet reporting error, physiological blood losses, and Fe supplementation. Furthermore, analyses were restricted to women who were not blood donors. Finally, we attempted to adjust analyses for physiological blood losses by including self-reported information about intensity of menstruation. This may have introduced a bias in the analysis. However, although at present reports from other literature on associations between reported and measured menstruation intensity are sparse, the present study would seem to indicate that self-reported intensity of menstruation was strongly associated with serum ferritin levels, particularly in premenopausal women without Fe supplementation ($P < 0.003$), suggesting that self-reported menstruation intensity may indeed reflect actual losses. In this group of premenopausal women, those reporting a weak menstruation intensity had serum ferritin levels four times higher than those with strong intensity (median values: 24 and 108 $\mu\text{g/l}$ respectively, $P = 0.03$).

The common use of Fe supplements contributes to the disagreement between Fe status and intake of dietary Fe. Indeed, premenopausal women who did not take regular Fe supplements displayed a positive association between reported dietary Fe intake and serum ferritin, which became stronger once adjustment for diet reporting error had been performed. In premenopausal women taking Fe supplements a similar association could not be demonstrated.

The regulation of Fe absorption is dependent on a number of factors, of which Fe status is of major importance (Hallberg, 1982; Hallberg & Rossander-Hultén, 1991). In subjects with replete and stable Fe stores, who are in a steady state concerning Fe balance, with only obligatory Fe losses, and an adequate Fe intake, no association would be expected between dietary Fe intake and serum ferritin. In the present series, nearly all postmenopausal women reported an adequate dietary Fe intake above the RDA, and none had Fe deficiency. Indeed, in this group there was no association between dietary Fe and Fe reserves. For these reasons an association between dietary Fe intake and Fe status should be anticipated only in premenopausal women not taking Fe supplements, whereas in Fe-supplemented premenopausal women and in postmenopausal women such an association would not be expected.

In 1987 mandatory fortification of flour in Denmark became optional. Therefore, dietary surveys of Fe intake conducted before and after 1987 are not directly comparable. However, independent of sex and age, estimated dietary Fe intake was reduced by approximately 20% when food tables that did not include Fe fortification were used (Møller, 1989) (Table 3). After correction for diet reporting error and for Fe fortification, the Fe intake in this series was similar to that reported in the *National Dietary Survey* in 1985 (Haraldsdóttir *et al.* 1986).

In conclusion, the lack of general agreement between reported intake of dietary Fe and Fe status in Danish women may, in part, be caused by selective diet reporting error. A prediction of low Fe reserves from the intake of dietary Fe seems possible only in premenopausal women taking no Fe supplements. Preventive interventions against Fe deficiency should be directed towards this group in particular.

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