# Iron sufficiency in the population of Northern Ireland: estimates from blood measurements

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**Estimates of iron status in a random sample (218 men, 283 women) of the Northern Ireland population (aged 18–64 years) were obtained from blood measurements. Multiple criteria were used to determine Fe deficiency and body Fe stores were calculated as an index of Fe sufficiency. Three distinct groups with respect to Fe stores were identified on the basis of sex and menstrual status. Estimates of body Fe stores, mean (and SD), were 13·4 (SD 5·97), 5·3 (SD 6·09) and 8·5 (SD 6·72) mM for men, women aged 18–44 years and women aged 45–64 years respectively. The prevalence of Fe-deficiency anaemia was low, ranging from 0·5% in men to 6·6% and 4·6% in the younger and older women respectively. The prevalence of Fe deficiency was low in men (1·4%) and older women (5·7%) rising to 11·0% in the younger group of women. The disproportionately elevated serum ferritin relative to transferrin saturation supported the suggestion that chronic inflammation might have influenced Fe status measurements in men and older women.**

**Iron status: Body iron stores: Inflammation**

It is now recognized that the assessment of iron status in a population by using predetermined cut-off points for individual laboratory values can give misleading results. This is due to the degree of overlap in laboratory values between Fe-deficient and Fe-sufficient populations and the influences of inflammatory processes and dietary deficiencies of other nutrients on these values (Cook, 1986). Furthermore, the estimated prevalence of Fe deficiency can vary considerably depending on the laboratory measurement used. Cook *et al.* (1986) have integrated the independent measurements of Fe status to define various degrees of Fe deficiency in the US population. This use of multiple criteria was extended by these workers to include quantitative estimates of body Fe stores for each subject. Thus, information on the levels of body Fe stores of the Fe-sufficient segment of the population was also obtained.

Chronic inflammatory conditions, however, can complicate the assessment of Fe status in a population and this problem has recently been addressed by Yip & Dallman (1988) using data from the first National Health and Nutrition Examination Survey (NHANES I) in the US. Measurement of Fe status in the latter study was based on the serum Fe:Fe-binding capacity ratio and erythrocyte sedimentation rate (ESR) was used as an index of inflammation.

In the present study multiple criteria and body Fe stores were used to estimate the Fe status of a random representative sample of the Northern Ireland population aged 18–64 years. These estimates were then related to indices of inflammatory conditions, i.e. elevated leucocyte count and abnormally high activity of the acute-phase reactant, caeruloplasmin (*EC* 1.16.3.1), in order to evaluate the influence of inflammation on Fe status measurements.

## SUBJECTS AND METHODS

Non-fasted venous blood samples (taken only in the evening) were obtained from a representative sample of the Northern Ireland population (aged 18–64 years). Day-to-day differences in measurements of Fe status in the serum of healthy subjects appear to be largely influenced by diurnal variations (Beaton *et al.* 1989). The sample of subjects was obtained through two stages. At the first stage, a random sample of addresses, stratified by population density of three sub-regions, was selected from the computerized sampling frame based on Household Valuation Rates obtained from the Department of Finance and Personnel, Policy Planning and Research Unit (PPRU; Stormont, Belfast). The second stage sampling involved randomly selecting an individual from each household using the Kish (1965) sampling method which is designed to produce a sample reflecting the age and sex distribution of the population from which the sample is drawn. A total of 522 subjects agreed to give blood, comprising 67% of the effective sample. The sample of respondents who gave blood was compared with those who did not give blood, and it was found that there were no significant differences in the age, sex and socioeconomic group distributions. Further details of subjects and sampling procedures are given in Barker *et al.* (1989).

Blood samples were processed immediately and the serum or plasma was separated. Whole blood and serum were cooled and transported for analysis at the Royal Victoria Hospital (Belfast) laboratories, which subscribe to the UK National External Quality Assessment Scheme. Only those samples analysed within 48 h of blood letting were included in the calculation of Fe status. A full haematological profile was obtained with an automated technique using a Coulter counter. Serum ferritin (SF) was measured by radioimmunoassay using antibodies raised to human spleen ferritin labelled with $^{125}I$ by Bolton & Hunter (1973) reagent (Amersham International plc, Amersham, Bucks). Tests for serum Fe, after reduction and formation of a blue complex with mercuric tripyridyltirazine, and total Fe-binding capacity (TIBC), by calculation from the unbound Fe remaining after the addition of a known amount of excess ferrous Fe, were performed on the American Monitor 'Parallel' system. Transferrin saturation (TS) was calculated by expressing the serum Fe as a percentage of TIBC, and mean corpuscular haemoglobin concentration (MCHC) was the haemoglobin:packed cell volume ratio, expressed as a percentage.

Multiple criteria were used to define Fe deficiency (subjects having abnormally low values of any two of SF, TS or MCHC) and Fe-deficiency anaemia (subjects having abnormally low values of any two of SF, TS or MCHC and an abnormally low haemoglobin). Serum Fe was used instead of TS in those subjects (twenty men, twenty-four women) for whom TIBC values were not available. Cut-off values were those used by Cook *et al.* (1986) and Bindra & Gibson (1986).

Body Fe stores were calculated using the equations of Cook *et al.* (1986) as modified by Ballot *et al.* (1989). In normal subjects with haemoglobin (men $> 130$ g/l, women $> 120$ g/l), SF ($> 12$ $\mu$g/l) and TS ($> 16\%$):

$$\text{Fe stores (mM)} = 7{\cdot}16 \times (\log \text{SF} - \log 12).$$

In subjects with normal haemoglobin values but abnormally low SF:

$$\text{Fe stores (mM)} = -1{\cdot}43 \times \text{index},$$

where the index was calculated by assigning a value of 1 to each of the following: SF $< 9$ g/l and $< 5$ g/l; TS $< 12$, $< 8$ and $< 4\%$.

In subjects with abnormally low haemoglobin, SF and TS:

$$\text{Fe stores (mM)} = -0{\cdot}27 \times (\text{median haemoglobin} - \text{observed haemoglobin}),$$

where the median haemoglobin was taken as 150 g/l for men and 140 g/l for women.

In subjects with abnormally low haemoglobin but normal SF and TS:

$$\text{Fe stores (mM)} = 7{\cdot}16 \times (\log \text{SF} - \log 12) - 0{\cdot}27 \times (\text{median haemoglobin} - \text{observed haemoglobin}),$$

where the median haemoglobin was taken as 150 g/l for men and 140 g/l for women.

Plasma samples were stored frozen at 20° until analysis at this laboratory for caeruloplasmin activity (Strain *et al.* 1989). The upper tenth percentile of the caeruloplasmin distributions (men $> 930$ U/l, women $> 1150$ U/l), a leucocyte count $> 10 \times 10^9$/l (Hercberg *et al.* 1988) and SF $> 50$ $\mu$g/l (Cook *et al.* 1986) were used to assess the influence of inflammatory conditions on abnormally low TS and MCHC measurements. SF distributions were normalized by logarithmic transformation before statistical testing.

Ethical approval for the study was obtained from the Medical Ethical Committee of the Faculty of Medicine, The Queen's University, Belfast.

## RESULTS

The measurements of Fe status in a representative sample, aged 18–64 years, of the Northern Ireland population are given in Table 1. Since SF levels were highly significantly affected by sex and menstrual status (Barker *et al.* 1989), the population was subdivided into three study groups, i.e. men, women aged 18–44 years, and women aged 45–64 years. Apart from MCHC, other measurements of Fe status were much greater in men than in women, and haemoglobin levels, but not TS or serum Fe, reflected the differences in SF with respect to menstrual status.

As expected there was a marked difference in body Fe stores in the three groups with mean and (SD) values of 13·4 (SD 5·97) mM in men, 5·3 (SD 6·09) mM in younger women and 8·5 (SD 6·72) mM in postmenopausal women. These results were very similar to those found by Cook *et al.* (1986) for the US population.

In the current study caeruloplasmin activity and leucocyte counts for the three groups within the population were also measured and are given in Table 1.

When various methods for estimating the prevalence of Fe deficiency were compared (Table 2) it was apparent that the prevalence of Fe-deficiency anaemia, as defined by a single criterion (abnormally low haemoglobin), was much higher than the prevalence defined by multiple criteria or Fe stores ($< -5{\cdot}4$ mM; Cook *et al.* 1986). The estimated prevalence by multiple criteria was low ranging from 0·5% in men to 6·6 and 4·6% in the younger and older women respectively, and was similar to the estimated prevalence by Fe stores for the three groups. The prevalence of Fe deficiency as defined by multiple criteria was also low in men (1·4%) and older women (5·7%), but was appreciably higher at 11·0% in the younger women. Storage estimates ($< 0$ mM or $< -1{\cdot}8$ mM; Cook *et al.* 1986) of Fe deficiency differed slightly from the estimates by multiple criteria.

Estimates of Fe deficiency in men and postmenopausal women were much lower when determined by SF rather than with TS or with MCHC (Table 2). The suggestion that chronic inflammation might have influenced Fe status measurements in men and the older women, resulting in disproportionately elevated SF relative to TS, was supported by the observation that the abnormally low TS values were accompanied by moderately high ($> 50$ g/l) SF in sixteen of twenty-one men and six of twenty-four older women, but in only five of forty-seven of the younger women (Table 3).

Sex differences in caeruloplasmin activity were apparent with men having lower values than women, and separate statistically derived cut-off points (indicating inflammatory disease) for men and women were taken from the 90th percentile of each population

Table 1. *Measurements of iron status and inflammatory indices in subjects aged 18–64 years in Northern Ireland*

| | | Men (18–64 years) | Women (18–44 years) | Women (45–64 years) | SD within groups |
|---|---|---|---|---|---|
| Haemoglobin (g/l) | Mean | 149 | 131 | 134 | 11·3 |
| | *n* | 218 | 192 | 91 | |
| Serum ferritin (μg/l) | Geometric mean | 107 | 35 | 61 | 60·12 |
| | Log mean | 4·4 | 3·24 | 3·8 | |
| | *n* | 218 | 192 | 91 | |
| TS (%) | Mean | 24·7 | 21·4 | 19·5 | 9·18 |
| | *n* | 198 | 176 | 83 | |
| Serum Fe (μM) | Mean | 15·9 | 14·3 | 13·1 | 5·31 |
| | *n* | 217 | 190 | 91 | |
| MCHC (%) | Mean | 33·8 | 33·5 | 33·1 | 2·02 |
| | *n* | 218 | 192 | 91 | |
| Fe stores (mM) | Mean | 13·4 | 5·3 | 8·5 | 6·15 |
| | *n* | 218 | 189 | 91 | |
| Caeruloplasmin (*EC* 1.16.3.1) (Units/l) | Mean | 699 | 816 | 837 | 236·9 |
| | *n* | 203 | 165 | 80 | |
| Leucocyte count ($\times 10^9$/l) | Mean | 8·9 | 8·2 | 7·9 | 3·88 |
| | *n* | 218 | 192 | 91 | |

TS, transferrin saturation; MCHC, mean corpuscular haemoglobin concentration.

Table 2. *Prevalence (%) of iron deficiency in subjects aged 18–64 years in Northern Ireland*

| | Men (18–64 years) | Women (18–44 years) | Women (45–64 years) |
|---|---|---|---|
| Fe-deficiency anaemia | | | |
| Single criterion | 2·3 | 13·5 | 9·8 |
| Multiple criteria | 0·5 | 6·6 | 4·6 |
| Storage estimate (< −5·4 mM) | 0·5 | 5·8 | 3·3 |
| Fe deficiency | | | |
| Single criterion | | | |
| Serum ferritin | 1·8 | 18·2 | 4·3 |
| Transferrin saturation | 10·6 | 26·7 | 31·0 |
| Mean corpuscular haemoglobin concentration | 6·9 | 14·1 | 8·7 |
| Multiple criteria | 1·4 | 11·0 | 5·7 |
| Storage estimate (< 0 mM) | 0·9 | 18·0 | 6·6 |
| (< −1·8 mM) | 0·5 | 13·8 | 6·6 |

distribution. An epidemiologically derived cut-off point for leucocyte count (Hercberg *et al.* 1988) was used as an additional indicator of inflammatory processes. However, when the latter inflammatory indices were investigated in individuals with abnormally low TS values, only three of twenty-one men and one of twenty-two older women had abnormal caeruloplasmin activities while three of twenty-one men and four of twenty-four older women had abnormal leucocyte counts (Table 3). Similarly, abnormal MCHC was accompanied by abnormal caeruloplasmin activities or leucocyte counts in relatively few individuals compared with the number of individuals who had an accompanying moderately high SF.

Table 3. *The relationship between low iron indices and elevated inflammatory indices*
(Ratios with percentages in parentheses)

| Subjects | Inflammatory index | Iron index | |
|---|---|---|---|
| | | TS (< 16%) | MCHC (< 32%) |
| Men (18–64 years) | SF (> 50 g/l) | 16/21 (76·2) | 12/15 (80·0) |
| | Cp (> 930 U/l) | 3/21 (14·3) | 3/21 (14·3) |
| | WCC (> $10 \times 10^9$/l) | 3/21 (14·3) | 2/15 (13·3) |
| Women (18–44 years) | SF (> 50 g/l) | 5/47 (10·6) | 3/27 (11·1) |
| | Cp (> 1150 U/l) | 3/40 (7·5) | 2/26 (7·7) |
| | WCC (> $10 \times 10^9$/l) | 7/47 (14·9) | 1/27 (3·7) |
| Women (45–64 years) | SF (> 50 g/l) | 6/24 (25·0) | 3/7 (42·9) |
| | Cp (> 1150 U/l) | 1/22 (4·5) | 0/7 (0) |
| | WCC (> $10 \times 10^9$/l) | 4/24 (16·7) | 1/7 (14·3) |

SF, serum ferritin; TS, transferrin saturation; Cp, caeruloplasmin (*EC* 1.16.3.1); MCHC, mean corpuscular haemoglobin concentration; WCC, leucocyte count.

## DISCUSSION

The prevalence of Fe deficiency, as defined by multiple criteria, was only slightly higher in the Northern Ireland population at 1·4, 11·0 and 5·7% compared with 0·7, 10·0 and 4·1% for men, premenopausal women and postmenopausal women respectively in the US population (Cook *et al.* 1986).

The prevalence of Fe-deficiency anaemia in the Northern Ireland population using the Fe storage estimate of Cook *et al.* (1986) was low at 0·5, 6·6 and 4·6% in men, premenopausal women and postmenopausal women respectively, yet higher than the respective values for the adult population in the US of 0·2, 2·6 and 1·9% (Cook *et al.* 1986). The prevalence of Fe-deficiency anaemia in Northern Ireland, however, was much lower than in Fe-deficient population subsets described within South Africa (Ballot *et al.* 1989), Algeria (Hercberg *et al.* 1988) or lacto-ovo-vegetarian East-Indian immigrants to Canada (Bindra & Gibson, 1986).

The prevalence of Fe-deficiency anaemia observed in adult males in NHANES II was appreciably lower than the frequency of the Fe-loading gene in the US population (Kushner *et al.* 1984). Cook *et al.* (1986) have argued, therefore, that information should be collected on the Fe-sufficient as well as the Fe-deficient segments in such populations. An important advantage in calculating body Fe stores is that it defines the level of Fe sufficiency in the entire population. The level and distribution of body Fe stores in men, premenopausal women and postmenopausal women are very similar for the Northern Ireland (13·4, 5·3 and 8·5 mM respectively) and US populations (13·9, 5·5 and 10·9 mM respectively). Although information on the frequency of the Fe-loading gene is not available for the Northern Ireland population, it is reasonable to assume that careful monitoring of Fe status is warranted in this population where the high bioavailability of dietary Fe sources and the consumption of Fe-fortified foods, and Fe and ascorbate supplements (Barker *et al.* 1989) could lead to Fe overload, with associated problems (Gordeuk *et al.* 1987), in an appreciable segment of the population.

Measurements of Fe status in a population can be confounded by chronic inflammation which results in low TS values in combination with elevated SF levels. Indeed, up to 80 and 40% of postmenopausal women who had abnormally low TS or MCHC values respectively also had moderately elevated (> 50 g/l) SF levels (Table 3). Although other inflammatory indices, i.e. caeruloplasmin activity and leucocyte count, were less supportive, this suggested that chronic inflammation was an important factor in the relatively high

prevalence of abnormally low TS or MCHC values in men and older women. Inflammation is more common among elderly individuals and in the lower socioeconomic groups. Yip & Dallman (1988) found that in the US both Fe deficiency and inflammatory disease played a major role in the increased prevalence of anaemia among the poor, and that inflammation rather than Fe deficiency was the most common underlying reason for anaemia among elderly individuals. Only serum Fe of the Fe status measurements in the Northern Ireland population was significantly affected by socioeconomic status, being increased in the unemployed (Barker *et al.* 1989). However, 10·9% of men and 17·1% of women had reported taking anti-inflammatory drugs (Barker *et al.* 1989) within 1 week of being surveyed. It is, therefore, probable that mild medical conditions in the general population were responsible for the confounding effects on individual laboratory measures of Fe status observed in the present study. The increase in SF levels with inflammatory conditions could lead to overestimation of calculated body Fe stores. Indeed, in postmenopausal women there was a highly significant ($P < 0{\cdot}001$) positive correlation ($r$ 0·37, Pearson) between activity of the acute-phase reactant, caeruloplasmin, and SF levels. This observation adds weight to the argument that inflammatory conditions are interacting with indices of Fe status, hence confounding the assessment of Fe deficiency and anaemia in the population.

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