

## Iron nutrition in the UK: getting the balance right

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Fe homeostasis is considered in the context of the UK diet, using information on Fe intake and status from the National Diet and Nutrition Surveys. The importance of assessing Fe availability rather than total Fe intake is discussed. Dietary and host-related factors that determine Fe bioavailability (Fe utilised for Hb production) are reviewed using information from single-meal studies. When adaptive responses are taken into consideration, foods associated with higher Fe status include meat (haem-Fe and the 'meat factor') and fruits and fruit juice (vitamin C). Foods that may have a negative impact include dairy products (Ca), high-fibre foods (phytate) and tea and coffee (polyphenols), but the effects are more apparent in groups with marginal Fe deficiency, such as women of childbearing age. Analysis of dietary intake data on a meal-by-meal basis is needed to predict the influence of changing dietary patterns on Fe nutrition in the UK. Current information suggests that in the UK Fe deficiency is a greater problem than Fe overload.

### Fe intake and status: Bioavailability of Fe: Fe-deficiency risk: Dietary patterns

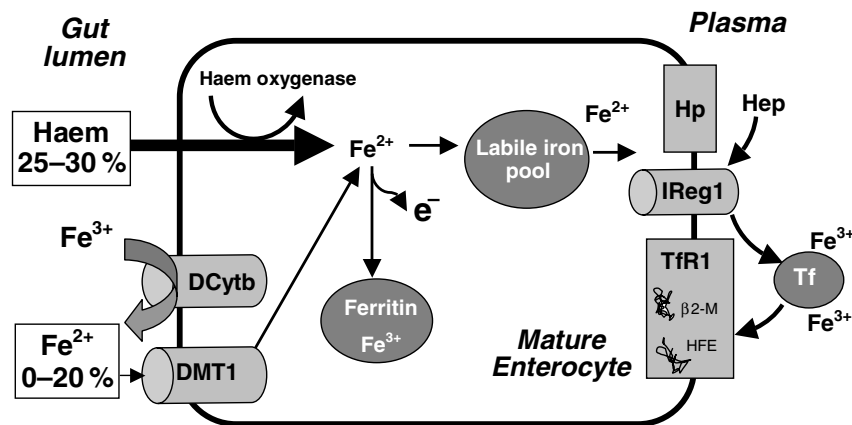
Fe plays an essential role in many biochemical reactions, and its ability to accept or donate electrons is central to the redox reactions of oxidative phosphorylation in the respiratory chain. Fe provides a specific binding site for O<sub>2</sub> in the haem moiety of Hb in erythrocytes and myoglobin in muscle. There is also a mobilisable Fe store of varying size in ferritin, mainly present in the liver. Free Fe can catalyse the formation of free radicals, which may cause damage to proteins and DNA; therefore, the body has evolved complex systems to ensure free Fe cannot exist *in vivo*. It is transported between different compartments in the body by transferrin, and is supplied to cells by three mechanisms: (1) continuous recycling of Fe from catabolised erythrocytes; (2) newly-absorbed dietary Fe; (3) release of Fe from ferritin stores in the liver (Bothwell *et al.* 1979). Dietary Fe is absorbed in the mucosal cells of the duodenum and jejunum (for review, see Miret *et al.* 2003). Haem- and non-haem-Fe are taken up by two independent pathways, but once inside the cell all Fe enters a common pool (Fig. 1). Uptake of Fe into cells is regulated by the synthesis of transferrin receptors on the cell surface that control the rate of flow of Fe to different tissues according to need.

### Iron deficiency and excess

Fe balance is maintained through changes in the efficiency of absorption whereby absorption is up regulated to redress Fe deficit. When the functional Fe compartment is replenished and mobilisable Fe has accumulated in ferritin stores (represented by a serum ferritin concentration >60–70 µg/l), the quantity of haem- and non-haem-Fe absorbed falls to a level just sufficient to cover basal losses in order to avoid excessive deposition of Fe (Hallberg *et al.* 1997). Nutritional Fe deficiency arises when the supply of Fe is insufficient to cover physiological requirements, whereas Fe overload is either the result of chronic exposure to high levels of Fe, as found in some dietary supplements, or occurs in individuals who lack the normal homeostatic mechanisms that regulate Fe absorption. Health implications of Fe overload and the role of diet and genotype have been reviewed by Heath & Fairweather-Tait (2003). Hallberg & Hulthen (2002) argue that the strong relationships observed between Fe requirements, bioavailability of dietary Fe and quantity of stored Fe demonstrate the presence of very effective control mechanisms that prevent the development of Fe overload in healthy individuals, even if the diet is fortified with Fe

**Abbreviations:** NDNS, National Diet and Nutrition Survey; SF, serum ferritin; TS, transferrin saturation.

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**Fig. 1.** Schematic diagram to illustrate how iron is absorbed in the mucosal cells of the small intestine. Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter; IReg1, iron-regulated protein 1 (also known as ferroportin 1); Hp, hephaestin; Hep, hepcidin; Tf, transferrin; TfR1, transferrin receptor 1; HFE, haemochromatosis protein;  $\beta$ 2-M,  $\beta$ 2-microglobulin.

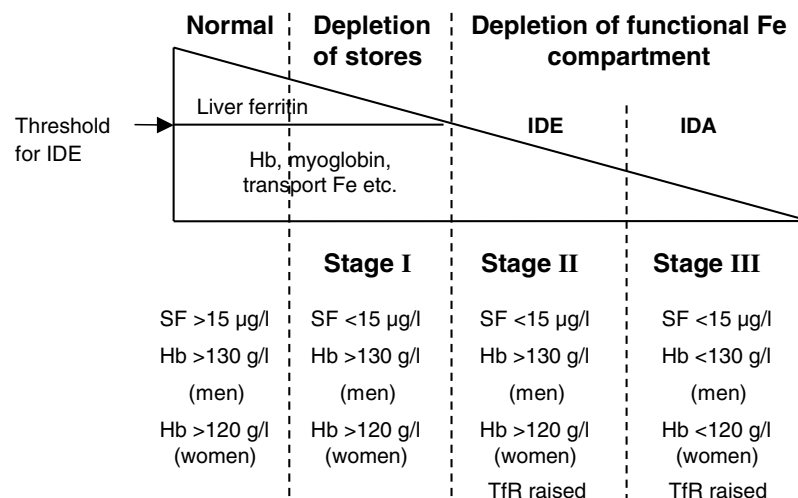
or the meat intake is high. The genetic basis and treatment of Fe-storage diseases, the clinical penetrance of haemochromatosis and the diagnosis and treatment of Fe deficiency have been reviewed by Beutler *et al.* (2003).

#### Measurement of iron status

A range of biochemical and haematological indices are used for the detection of Fe deficiency and the assessment of Fe status. The extent of Fe deficiency or overload is directly proportional to the size of the Fe compartments in the body. When demand outstrips supply, stores of Fe in ferritin will be mobilised and the body will become progressively Fe depleted (Fig. 2). Once the storage Fe compartment is exhausted Fe-deficient erythropoiesis will take place (stage I). Recycling of the functional Fe compartment (Hb, myoglobin, cytochromes, transport

Fe, etc.) supplies Fe to the erythrocyte precursors of the bone marrow (stage II) and with a continuing negative Fe balance, the deficiency will progress to Fe-deficiency anaemia, characterised by the appearance of microcytic hypochromic erythrocytes (stage III).

In population studies, including the UK National Diet and Nutrition Surveys (NDNS), the most-widely-used indices of Fe status are Hb concentration, transferrin saturation (TS) and serum ferritin (SF). Cut-off values for Fe deficiency or Fe overload in different groups are given in Table 1. Detection of early Fe-deficiency anaemia, when the Hb concentration is only just beginning to fall, is not easy because the cut-off point differs between individuals; normal Hb distributions vary with gender (Wintrobe, 1981), age (Yip *et al.* 1984), pregnancy (Yip, 2000), altitude (Tufts *et al.* 1985), smoking (Van Tiel *et al.* 2002), season (Lee *et al.* 1987) and ethnic origin (Pan & Habicht, 1991).



**Fig. 2.** Stages in the development of iron deficiency. IDE, iron-deficient erythropoiesis; IDA, iron-deficient anaemia; SF, serum ferritin; TfR, transferrin receptor. (Adapted from Suominen *et al.* 1998.)

**Table 1.** Cut-off values for iron deficiency or overload in groups of the UK population studied in National Diet and Nutrition Surveys (NDNS)

(Values used to evaluate NDNS data (Fig. 3) are shown in parentheses; other values are referenced)

	Children	Men (≥15 years)	Women (≥15 years)
Fe deficiency			
Hb (g/l)	110–120*†	130‡ (125; adult survey)	120‡ (125; adult survey)
TS (%)	10* (15)	15§	15§
SF (µg/l)	12* (15)	15† (13; adult survey)	12† (13; adult; 15, elderly)
Fe overload			
Hb (g/l)		>180	>165
SF (µg/l)		>200†	>150†
TS (%)		>50¶	>45¶

TS, transferrin saturation; SF, serum ferritin.

\*Osaki (1993).

†World Health Organization/UNICEF/United Nations University (2001).

‡World Health Organization (1972).

§Bates *et al.* (1997).

||Dacie &amp; Lewis (1995).

¶Velati *et al.* (2003).

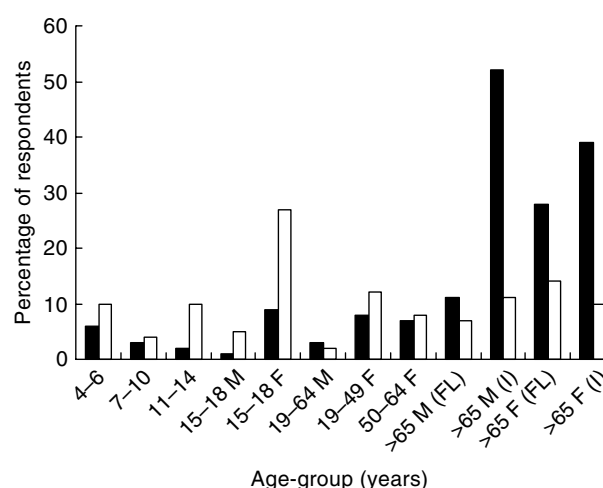
Fe deficiency without anaemia is generally determined from SF concentration. Phlebotomy studies indicate that 1 µg SF/l corresponds to about 8 mg storage Fe (Walters *et al.* 1973), which makes SF the most informative laboratory measure of body Fe. However, because apoferritin is an acute-phase reactant protein, irrespective of changes in Fe stores, SF concentration will increase in response to infection and inflammatory processes and can remain elevated for several weeks post infection (Hulthen *et al.* 1998). It is common practice to measure plasma C-reactive protein or, in the case of NDNS (Finch *et al.* 1998),  $\alpha_1$ -antichymotrypsin (Calvin *et al.* 1988) as markers of infection or inflammation. SF measurements should be calibrated against a reference standard (National Institute for Biological Standards and Control, Potters Bar, Herts.) (World Health Organization/UNICEF/United Nations University, 2001) and at least three independent measurements are required to allow for day-to-day variation (Borel *et al.* 1992). TS (serum Fe/total Fe-binding capacity) measurements are subject to diurnal variation (Guillygomarc'h *et al.* 2003), but are considered useful as a first screening step for the inherited Fe-overload disorder, hereditary haemochromatosis.

The detection of Fe-deficient erythropoiesis is a key measure, as it identifies marginal Fe deficiency and is characterised by elevated levels of Zn protoporphyrin. The final reaction in haem biosynthesis is the chelation of Fe with protoporphyrin, but when Fe is not available Zn is used as an alternative metal substrate for ferrochetalase. The measurement and diagnostic use of Zn protoporphyrin has been reviewed in detail by Labbe *et al.* (1999). Serum transferrin receptors also increase in the early stages of Fe deficiency and are not affected by infection or inflammation, and do not vary with age, gender or pregnancy

(Beutler *et al.* 2003). However, the diagnostic usefulness of the serum transferrin receptors is limited because of the divergent values obtained from different assays and the lack of an international standard. The serum transferrin receptors:log SF index has been proposed for identifying subclinical Fe deficiency (Punnonen *et al.* 1997). More recently, Cook *et al.* (2003) used National Health and Nutrition Examination Survey III data to calculate body Fe based on serum transferrin receptors:SF; the use of this ratio was validated by means of an Fe-supplementation trial in Jamaica and an Fe-fortification study in Vietnam. Quantitative estimates of body Fe are useful for epidemiological studies examining Fe nutrition and for assessing the efficacy of intervention trials to improve Fe status. However, as discussed earlier, methods that rely on SF may be confounded by infection or inflammation. The limitations of each index in terms of specificity and sensitivity have led to the adoption of multiple indices for the detection of Fe deficiency in populations, e.g. National Health and Nutrition Examination Survey, although this approach underestimates Fe deficiency. A useful combination would be Hb, serum transferrin receptors and SF to reflect functional impairment, tissue depletion and storage Fe respectively.

### Iron status of subgroups in the UK population

Data from NDNS provide information on the Fe status of the UK population, including the elderly (age ≥65 years; Finch *et al.* 1998), the young (age 4–18 years; Gregory *et al.* 2000) and adults (Ruston *et al.* 2004). Fe deficiency is more common in women than men (Fig. 3), particularly in the 15–18 years age-range. In adults Fe-deficiency anaemia is observed in 3% of men and 8% of women, and low Fe stores in 2% of men and 11% of women. In the survey of the elderly the large number of men and women with a low Hb concentration is not consistent with the SF



**Fig. 3.** Iron deficiency in the UK: percentage of individuals participating in the National Diet and Nutrition Surveys who fall below the suggested cut-off value (see Table 1). (■), Low Hb; (□), low serum ferritin. M, male; F, female; FL, free-living; I, institutionalised.

**Table 2.** Upper 2.5 percentiles for serum ferritin and transferrin saturation in the National Diet and Nutrition Survey of adults in 2000–1 and of the elderly (free-living) in 1994–5

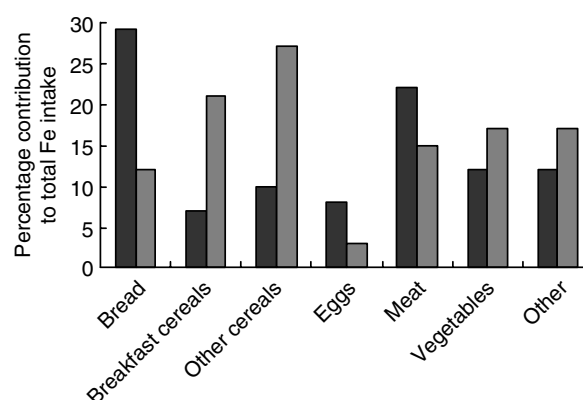
Age-group (years)	Serum ferritin ( $\mu\text{g/l}$ )	Transferrin saturation (%)
<b>Men</b>		
25–34	201	50.3
35–49	512	50.8
50–64	505	51.3
65–74	384	50.8
75–84	408	55.6
$\geq 85$	651	52.5
<b>Women</b>		
50–64	232	52.3
65–74	258	48.4
75–84	352	43.2
$\geq 85$	295	43.7

data, which probably means that the cut-off values used to define deficiency are inappropriate.

It is not possible to quantify Fe overload from the SF and TS data, but according to the upper 2.5 percentile values for SF and percentage TS for adults and the elderly (Table 2) Fe stores are higher in men than in women. The upper 2.5 percentile is above the normal range for SF in both men and women, but the TS values are very close to the upper limit, with the exception of 50–64-year-old women. The lack of consistency between the two measures raises questions about the appropriateness of the cut-off values. Further analysis of the data and more definitive tests of body Fe content are required to assess the extent of Fe overload.

### Iron intake

According to data from the National Food Survey (<http://statistics.defra.gov.uk/esg/publications/nfs/default.asp>) Fe intake in the UK has fallen steadily from a mean of approximately 14 mg/d in 1965 to 10 mg/d in the 1990s, at which level it has remained relatively constant. Fe intake mirrors energy intake, indicating that the Fe density of the UK diet has not changed but the sources of Fe have changed. Prynne *et al.* (1999) compared the food and nutrient intake of a national sample of 4-year-old children in 1950 with that in the 1990s. In 1950 bread and red meat were the most important sources of Fe, whereas in 1992–3, bread consumption had fallen dramatically and much of the Fe was provided by breakfast cereals and other cereal-based products such as biscuits; also, the contribution made by meat and eggs had fallen (Fig. 4). There are no marked differences in total Fe intake in the 1986–7 and 2000–1 NDNS, which endorses the findings of the National Food Survey. However, total Fe intake is a poor predictor of Fe status, as exemplified by results from a recent study on women of childbearing age in the UK, in which Fe intakes were analysed from a 7 d duplicate diet (Harvey LJ, Armah C, Dainty J, Foxall R, Lewis J, Langford N and Fairweather-Tait SJ, unpublished results). Fig. 5 illustrates the lack of relationship between mean daily Fe intake and Fe stores assessed from SF concentration.



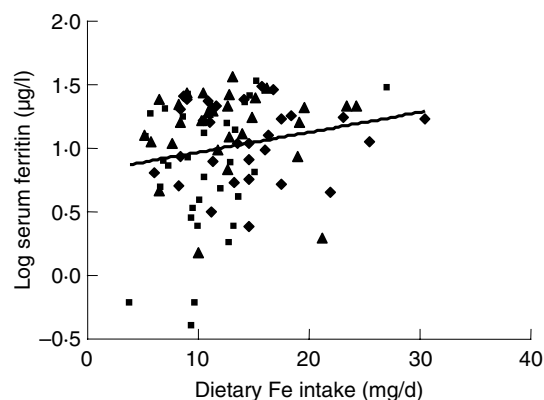
**Fig. 4.** Contribution of food groups to the iron intake of children aged 4 years in national studies in 1950 ( $n$  4599) and 1992–3 ( $n$  493). (■), 1950; (□), 1992–3. (From Prynne *et al.* 1999.)

The reason is that Fe absorption depends on both the availability for absorption and the absorptive capacity of the intestinal cells.

### Iron bioavailability

The proportion of an ingested nutrient that is utilised for normal body function is a measure of its bioavailability. In normal individuals approximately 80% of absorbed Fe is used for Hb synthesis (Finch *et al.* 1970), thus Fe absorption can be used to assess bioavailability. Fe-deficiency anaemia increases Fe utilisation (Beshara *et al.* 2003), therefore it is important to characterise Fe status when assessing Fe bioavailability. There are a number of dietary and physiological factors that are known to influence Fe absorption (Table 3).

The primary role of host-related variables is the maintenance of Fe homeostasis by ensuring an appropriate response to increased or decreased requirements for Fe. Thus, absorption is up regulated with Fe depletion (Baynes *et al.* 1987), until the body has accumulated sufficient



**Fig. 5.** Relationship between mean iron intake (mg/d) and iron stores (serum ferritin  $\mu\text{g/l}$ ) in UK women of childbearing age consuming different diets. (■), Red meat; (▲), poultry and fish; (◆), vegetarian; (—), all data.  $R^2$  0.0384. (From Harvey LJ, Armah C, Dainty J, Foxall R, Lewis J, Langford N and Fairweather-Tait SJ, unpublished results.)



**Table 3.** Factors that affect iron absorption

Dietary	Host-related
Enhancing	Enhancing
Meat, poultry and fish	Fe-deficiency anaemia
Vitamin C	Low body stores
Alcohol	Gastric acid
Inhibitory	Bile and pancreatic secretions
Phytate (IP <sub>6</sub> ) and inositol phosphates IP <sub>3</sub> –IP <sub>5</sub>	Hypoxia (high altitude)
Polyphenols and other flavonoids	Pregnancy
Tea and coffee	Increased erythropoiesis (e.g. after blood loss)
Eggs	Homozygosity for C282Y mutation of HFE gene
Ca and dairy products	Inhibitory
Other transition metals (Zn, Cu)	High body stores
	Previous high intake of iron
	Rapid gastric emptying

IP<sub>6</sub>, IP<sub>3</sub>, IP<sub>5</sub>, inositol hexa-, tri- and pentaphosphates respectively.

storage Fe (Hallberg *et al.* 1997), and with increased erythropoietic activity (Skikne & Cook, 1992) resulting from blood loss or tissue hypoxia (Skikne & Baynes, 1994). Conversely, absorption is down regulated when stores are high or following a large dose of Fe, sometimes referred to as the 'mucosal block' (O'Neil-Cutting & Crosby, 1987). Pregnancy is associated with a progressively higher efficiency of Fe absorption (Barrett *et al.* 1994), and factorial estimates indicate that absorption increases during periods of rapid growth in children (Taylor *et al.* 1988).

Regulation of Fe absorption is affected by mutations in the haemochromatosis protein gene; C282Y homozygotes are at risk of developing hereditary haemochromatosis, although clinical penetrance is low (Waalén *et al.* 2002). The C282Y mutation is relatively common in Caucasian populations, particularly in those of Celtic origin, with a reported frequency as high as 17% in Northern Ireland (Murphy *et al.* 1998). C282Y heterozygous men and women have slightly elevated TS, but SF concentration does not appear to be raised (Burt *et al.* 1998; Jackson *et al.* 2001). Relatives of patients with haemochromatosis who carry the C282Y mutation and wild-type controls have been reported to absorb similar quantities of Fe from a hamburger meal, suggesting that heterozygotes have similar regulatory mechanisms (Lynch *et al.* 1989), but when given 20 mg Fe (as ferrous sulphate) plus 100 mg vitamin C, absorption is markedly higher. However, a recent study has shown no difference between male heterozygotes and wild-type controls in the absorption of Fe from Fe-fortified cereals or from a diet high in available Fe (Roe MA, Heath A-L, Oyston SL, Macron C, Hoogewer J, Foxall R, Dainty J, Majsate-Newman G, Willis G and Fairweather-Tait SJ, unpublished results).

Digestion plays a key role in determining how much Fe is released from the food matrix and subsequently made available for absorption. Gastric acid and the rate of stomach emptying affect Fe solubilisation (Bezwdoda *et al.* 1978), and amino acids, peptides and mucoproteins produced by pancreatic enzymes and bile are believed to act as absorption-promoting ligands (Jacobs & Miles, 1970). Day-to-day variability in the efficiency of Fe absorption

(Kuhn *et al.* 1968) has not been widely reported, as it is technically difficult to measure. Variation presumably occurs because of fluctuations in physiological factors that determine Fe availability, and/or short-term mechanisms that maintain Fe homeostasis emanating from the mucosal cells of the small intestine (Frazer *et al.* 2003) as opposed to the systemic longer-term control associated with body Fe status (Leong & Lonnerdal, 2004). Multiple-dosing protocols are generally adopted to compensate for meal-to-meal variations in absorption (Fox *et al.* 1998) or to enable absorption from a whole diet to be measured (Minihane & Fairweather-Tait, 1998).

Dietary components affect Fe availability by means of chemical reactions in the stomach and lumen of the small intestine. For example, ascorbic acid reduces Fe<sup>3+</sup> to the more soluble Fe<sup>2+</sup>, which is the form required for transport into mucosal cells (Fig. 1). It also binds Fe, thus preventing it forming a complex with phytate or tannin that renders the Fe unavailable to divalent metal transporter 1. The relative binding affinity for Fe of different dietary constituents and divalent metal transporter 1 will determine whether the Fe is transported into the cell or remains in the gut lumen. Recent research has shown that duodenal cytochrome b is responsible for reducing Fe<sup>3+</sup> to Fe<sup>2+</sup> (McKie *et al.* 2001); the main role of ascorbic acid is therefore to promote Fe solubility. Binding constants of dietary components and gastrointestinal conditions will determine how much Fe is released from chyme and the compounds to which it is bound. Any free Fe released from food by gastric acid will precipitate as insoluble oxide or hydroxide when the pH rises above 4–5 in the duodenum or stomach of individuals with achlorhydria.

The absorption of Fe from meat is higher than that from plant foods (Bjorn-Rasmussen *et al.* 1974) because haem-Fe is absorbed more efficiently than non-haem-Fe (Hallberg *et al.* 1997) and non-milk animal proteins enhance non-haem-Fe absorption (Cook & Monsen, 1976; Glahn *et al.* 1996). The 'meat factor' has yet to be identified, but candidates include protein, cysteine-containing peptides or oligo-saccharides (Layrisse *et al.* 1984; Swain *et al.* 2002; Huh *et al.* 2004). However, the observation that meat solubilises non-haem-Fe independent of proteolytic digestion (Carpenter & Mahoney, 1989) suggests that peptides are not the active component.

Ascorbic acid increases Fe absorption from meals; the increase in Fe absorption is directly proportional to the amount of ascorbic acid added over a range of 25–1000 mg (Cook & Monsen, 1977). The reducing and chelating properties of ascorbic acid are one reason why fruits and fruit juices (Hallberg & Rossander, 1984; Ballot *et al.* 1987) promote Fe absorption from meals. Other organic acids such as lactic acid (Gillooly *et al.* 1983) enhance Fe absorption, but citric acid has been reported to have a limited (Ballot *et al.* 1987) or even an inhibitory effect (Hallberg & Rossander, 1984). Ascorbic acid supplements (500 mg) given three times daily with meals for 5.5 weeks improve the Fe status of Fe-depleted premenopausal women (Hunt *et al.* 1990).

Polyphenols inhibit Fe absorption by binding with Fe in the gut lumen. The active principal is the galloyl group found in tannic and gallic acids, but not present in chlorogenic

acid (Brune *et al.* 1989). Fe absorption is reduced by foods containing these compounds, including tea (Hurrell *et al.* 1999), coffee (Disler *et al.* 1975; Morck *et al.* 1983) and nuts (Macfarlane *et al.* 1988). Phytate (*myo*-inositol hexaphosphate) also has a powerful inhibitory effect on Fe absorption (Brune *et al.* 1992). This effect is counteracted by ascorbic acid but not greatly affected by the consumption of meat (Hallberg *et al.* 1989). When foods are processed by soaking, malting or fermenting the effect of phytate is reduced (Hurrell *et al.* 2003), although extensive degradation is necessary before Fe bioavailability is improved (Hurrell *et al.* 2002). The inhibitory effect of phytate can only be predicted by measuring individual inositol phosphates, as inositol hexaphosphate to inositol tetraphosphate, but not inositol triphosphate to inositol monophosphate, have Fe-binding activity (Sandberg *et al.* 1999). The inhibitory effect of soyabean (Derman *et al.* 1987) and other legumes (Lynch *et al.* 1984) is almost certainly a result of their phytate content.

Ca (threshold 50 mg) has been shown to reduce non-haem-Fe absorption in a dose-dependent manner (Hallberg & Hulthen, 2000). Since it reduces haem-Fe absorption (Hallberg *et al.* 1993), and haem-Fe is transported independently into the mucosal cell, it is likely that Ca affects basolateral transport of Fe.

Predictive algorithms have also been developed to calculate Fe bioavailability from different diets, taking into account the major dietary factors that modulate Fe absorption (Hallberg & Hulthen, 2000; Reddy *et al.* 2000).

### Interactions and adaptive responses

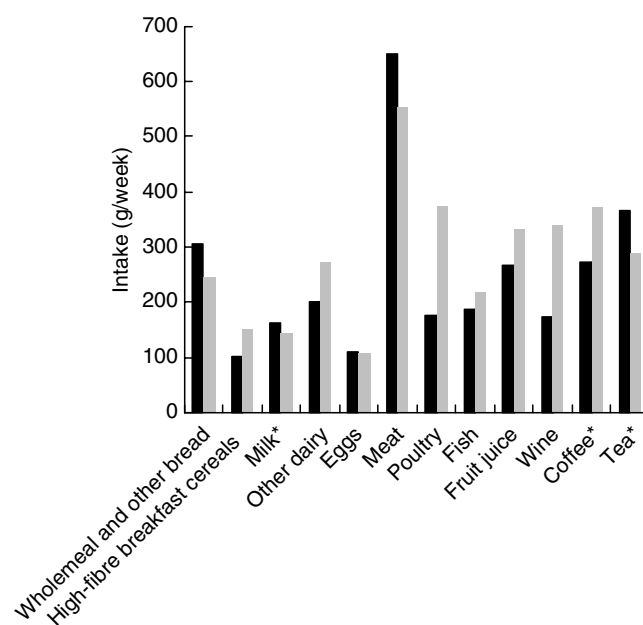
Single-meal studies have highlighted the very real and often dramatic effects that certain dietary constituents have on Fe absorption. However, findings from longer-term intervention, cross-sectional and prospective studies do not always support findings from single-meal studies. The reason for this inconsistency is that adaptive responses in absorption take place to maintain Fe homeostasis. Thus, when exposed to a meal of low Fe bioavailability, the efficiency of absorption will be subsequently up regulated to compensate for the reduced supply of Fe, and absorption of Fe from the next meal will be enhanced. This adaptation results in an apparent exaggeration of the effect of inhibitors and enhancers in single-meal studies, as illustrated by Cook *et al.* (1991). However, single-meal studies do not, in fact, influence the effect of dietary factors *per se*, although prolonged fasting is probably unusual given today's dietary patterns, and may result in higher Fe absorption values as a result of the absence of chyme and reduced exposure to Fe in the mucosal absorptive cells. Single-meal protocols cannot measure compensatory adaptation (a function of both the diet and the host) but multiple-dosing protocols more closely mirror the situation in free-living individuals, with a blunted response to dietary enhancers and inhibitors (Tidehag *et al.* 1996).

### Dietary patterns and iron nutrition

Epidemiological studies investigating the effects of diet on Fe status have produced inconsistent findings. As already

discussed, there may be difficulties and/or inaccuracies in the determination of Fe status. The next challenge is the measurement of habitual Fe intake, because Fe status is a function of Fe supplied (and lost) over the past months or years. Total Fe intake is of limited value; indeed, an estimate of available Fe is required, and this measure can only be calculated from meal-based data, such as the validated questionnaire developed by Heath *et al.* (2000) to measure intake of Fe and key enhancers and inhibitors of absorption in meals (Matthys *et al.* 2004). Such an approach makes it possible to relate diet to measures of Fe status. The NDNS report Fe intakes on a daily basis, determined from a specially-adapted and regularly-updated nutrient databank based on McCance & Widdowson's *The Composition of Foods* (Holland *et al.* 1991). Available Fe cannot be estimated from the NDNS reports, although the most recent survey of adults has collected meal-based data that could be analysed appropriately. However, more data on the phytate, polyphenol and haem-Fe contents of foods are required, and the calculated values will only be an approximation because of variance from food composition data, e.g. losses of ascorbic acid on storage and cooking, hydrolysis of phytate, etc. Another problem exists with Fe-fortified foods that can generate large errors in calculated Fe intake because of overages in products.

Despite all the problems associated with estimating available Fe intake, a number of observations on Fe status and dietary patterns can be deduced from cross-sectional epidemiological studies that have been conducted in other countries. The US Framingham Heart Study of 634 healthy free-living elderly subjects (Fleming *et al.* 1998) has reported that haem-Fe, dietary vitamin C, alcohol and Fe supplements are all positively associated with SF, whereas



**Fig. 6.** National Diet and Nutrition Survey of adults 1986–7 (■; Gregory *et al.* 1990) and 2000–1 (□; Henderson *et al.* 2002); a comparison of intakes of foods (g/week) that may have an impact on iron nutrition. \* Values are shown as intake  $\times 10^{-1}$ .

coffee intake has a negative association. In 1108 French subjects (aged 6 months–97 years) SF has been found to be positively correlated with haem- and non-haem-Fe intakes, and negatively correlated with Ca and P intakes (Preziosi *et al.* 1994). A larger French study (6648 women aged 35–60 years, and 3283 men aged 45–60 years) has reported that SF is positively correlated with meat, fish and total Fe intake, and negatively correlated with dairy products, Ca and fibre intake (Galan *et al.* 1998).

Further analysis of the NDNS of the elderly has found that Fe status is positively associated with meat, poultry and fish, but negatively associated with dairy foods, Ca and tea (Doyle *et al.* 1999). In contrast, a systematic review of sixteen studies on tea and Fe status, with adjustment for confounding factors, has concluded that tea consumption does not influence Fe status in Fe-replete Western populations, but there may be a negative association in populations with marginal Fe status (Temme & Van Hooijdonck, 2002). The impact of Ca on Fe status is both important and controversial. Dairy foods are a key food group for bone health, as well as providing protein for lacto-ovo-vegetarians, thus public health messages to reduce milk and dairy food intake in order to reduce the risk of Fe deficiency (Hallberg *et al.* 1992) may have a detrimental effect on other health outcomes. A cross-sectional study in six European countries has demonstrated a weak but consistent inverse association between Ca intake and SF (van de Vijver *et al.* 1999), but the relationship was not found to be dependent on simultaneous ingestion of Ca and Fe, nor was a dose–response effect observed. Most intervention studies have shown no effect of Ca on Fe nutrition. Reddy & Cook (1997) have found that Ca intake has no effect on non-haem-Fe absorption from a varied diet over 5 d, and other dietary interventions with Ca do not generally confirm the results from single-meal studies (Sokoll & Dawson-Hughes, 1992; Minihane & Fairweather-Tait, 1998).

Trends in dietary patterns in the UK from the National Food Survey include a fall in the intakes of red meat and tea and an increase in fruit juice consumption (Heath & Fairweather-Tait, 2002). Studies in vegetarians confirm that meat is a useful source of available Fe, as vegetarians generally have a lower SF concentration (Worthington-Roberts *et al.* 1988; Alexander *et al.* 1994; Ball & Bartlett, 1999), but Fe stores in many women of childbearing age, whether they are vegetarian or not (Donovan & Gibson, 1995), appear to be low. In the NDNS of adults (Gregory *et al.* 1990) 14% of all women had a SF concentration of <13 µg/l, the highest percentage (21) being found in the 35–49 years age-group. Intakes of foods that may impact on Fe nutrition are compared for the 1986–7 and 2000–1 NDNS in Fig. 6. The fall in wholemeal and other breads is offset by the increase in high-fibre breakfast cereals, some of which are fortified with Fe, but they are consumed by only 48% of the respondents (Henderson *et al.* 2002). Meat (beef, lamb, pork, ham and bacon) intake has fallen and poultry intake has increased; 5% of respondents in the 2000–1 survey reported that they were vegetarian. Tea consumption is lower but coffee intake has risen, so the net effect on Fe availability is probably zero. Wine consumption has doubled, but the enhancing effect of alcohol is

offset by the inhibitory effect of wine phenolics. There has been a slight increase in Ca intake and a more marked increase in vitamin C intake, but the latter only increases Fe absorption when consumed with main Fe-containing meals.

In order to evaluate the effect of diet on Fe status, further analysis of NDNS data is required, on a meal-by-meal basis, focused on the groups at risk of Fe deficiency (which might include infants and children, women of childbearing age, pregnant and lactating women and ethnic groups) or overload (men aged ≥40 years). Dietary effects can be ascertained from measures of Fe status, including Hb, SF (excluding high values resulting from infection or inflammation) and TS. In order to examine the relationship between diet and Fe status (perhaps concentrating on the upper and lower quintiles of Fe status), the nutrient databank requires expanding to incorporate information on the phytate, polyphenol, and haem-Fe contents of the UK diet. Dietary recommendations should be developed for vulnerable groups, taking into consideration the falling energy requirement, and thus the need to increase Fe density and availability in the diet. A simple self-screening method for high menstrual blood loss is required to identify women at risk of Fe-deficiency anaemia, both for use in the NDNS and for preventative medicine in primary care. Until UK dietary patterns change substantially, the main public health concern lies with Fe deficiency rather than Fe excess.

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### References

- Alexander D, Ball MJ & Mann J (1994) Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. *European Journal of Clinical Nutrition* **48**, 538–546.
- Ball MJ & Bartlett MA (1999) Dietary intake and iron status of Australian vegetarian women. *American Journal of Clinical Nutrition* **70**, 353–358.
- Ballot D, Baynes RD, Bothwell TH, Gillooly M, MacFarlane BJ, MacPhail AP *et al.* (1987) The effects of fruit juices and fruits on the absorption of iron from a rice meal. *British Journal of Nutrition* **57**, 331–343.
- Barrett JFR, Whittaker PG, Williams JG & Lind T (1994) Absorption of non-haem iron from food during normal pregnancy. *British Medical Journal* **309**, 79–82.
- Bates CJ, Thurnham DJ, Bingham SA, Margetts BM & Nelson M (1997) Biochemical markers of nutrient intake. In *Design Concepts in Nutritional Epidemiology*, 2nd ed., pp. 70–240 [BM Margetts and M Nelson, editors]. Oxford: Oxford University Press.
- Baynes RD, Bothwell TH, Bezwoda WR, MacPhail AP & Derman DP (1987) Relationship between absorption of inorganic and food iron in field studies. *Annals of Nutrition and Metabolism* **31**, 109–116.



- Beshara S, Sörensen J, Lubberink M, Tolmachev V, Långström B, Antoni G, Danielson B & Lundqvist H (2003) Pharmacokinetics and red cell utilization of  $^{52}\text{Fe}/^{59}\text{Fe}$ -labelled iron polymaltose in anaemic patients using positron emission tomography. *British Journal of Haematology* **120**, 853–859.
- Beutler E, Hoffbrand AV & Cook JD (2003) *Hematology (American Society of Hematology)* **1**, 40–61.
- Bezowda W, Charlton R, Bothwell T, Torrance J & Mayet F (1978) Gastric hydrochloric acid and iron absorption. *Journal of Laboratory and Clinical Medicine* **92**, 108–116.
- Bjorn-Rasmussen E, Hallberg L, Isaksson B & Arridsson B (1974) Application of the two-pool extrinsic tag method to measure haem and non-haem iron absorption from the whole diet. *Journal of Clinical Investigation* **53**, 247–255.
- Borel MJ, Smith SM, Derr J & Beard J (1992) Day-to-day variation in iron-status indices in healthy men and women. *American Journal of Clinical Nutrition* **55**, 729–735.
- Bothwell TH, Charlton RW, Cook JD & Finch CA (1979) *Iron Metabolism in Man*. London: Blackwell Scientific Publications.
- Brune M, Rossander L & Hallberg L (1989) Iron absorption and phenolic compounds: importance of different phenolic structures. *European Journal of Clinical Nutrition* **43**, 547–558.
- Brune M, Rossander-Hulthen L, Hallberg L, Gleerup A & Sandberg AS (1992) Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *Journal of Nutrition* **122**, 442–449.
- Burt MJ, George PM, Upton JD, Collett JA, Frampton CM, Chapman TM, Walmsley TA & Chapman BA (1998) The significance of haemochromatosis gene mutations in the general population: implications for screening. *Gut* **43**, 830–836.
- Calvin J, Neale G, Fotherby KJ & Price CP (1988) The relative merits of acute phase proteins in the recognition of inflammatory conditions. *Annals of Clinical Biochemistry* **25**, 60–66.
- Carpenter CE & Mahoney AW (1989) Proteolytic digestion of meat is not necessary for iron solubilization. *Journal of Nutrition* **119**, 1418–1422.
- Cook JD, Dassenko SA & Lynch SR (1991) Assessment of the role of nonheme-iron availability in iron balance. *American Journal of Clinical Nutrition* **54**, 717–722.
- Cook JD, Flowers CH & Skikne BS (2003) The quantitative assessment of body iron. *Blood* **101**, 3359–3364.
- Cook JD & Monsen ER (1976) Food iron absorption. I. Use of semisynthetic diet to study absorption of nonheme iron. *American Journal of Clinical Nutrition* **28**, 1289–1295.
- Cook JD & Monsen ER (1977) Vitamin C, the common cold, and iron absorption. *American Journal of Clinical Nutrition* **30**, 235–241.
- Dacie JV & Lewis SM (1995) *Practical Haematology*, 8th ed. Edinburgh: Churchill Livingstone.
- Derman DP, Ballot D, Bothwell TH, Macfarlane BJ, Baynes RD, Macphail AP, Gillooly M, Bothwell JE, Bezowda WR & Mayet F (1987) Factors influencing the absorption of iron from soya-bean protein products. *British Journal of Nutrition* **57**, 345–353.
- Disler PB, Lynch SR, Charlton RW, Torrance JD, Bothwell TH, Walker RB & Mayet F (1975) The effect of tea on iron absorption. *Gut* **16**, 193–200.
- Donovan UM & Gibson RS (1995) Iron and zinc status of young women aged 14 to 19 years consuming vegetarian and omnivorous diets. *Journal of the American College of Nutrition* **14**, 463–472.
- Doyle W, Crawley H, Robert H & Bates CJ (1999) Iron deficiency in older people: interactions between food and nutrient intakes with biochemical measures of iron; further analysis of the National Diet and Nutrition Survey of people aged 65 years and over. *European Journal of Clinical Nutrition* **53**, 552–559.
- Finch CA, Deubelbeiss K, Cook JD, Eschbach JW, Harker LA, Funk DD, Marsaglia G, Hillman RS, Slichter S, Adamson JW, Ganzoni A & Biblett ER (1970) Ferrokinetics in man. *Medicine (Baltimore)* **49**, 17–53.
- Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC (1998) *National Diet and Nutrition Survey: People Aged 65 Years and Over*. London: The Stationery Office.
- Fleming DJ, Jacques PF, Dallal GE, Tucker KL, Wilson PWF & Wood RJ (1998) Dietary determinants of iron stores in a free-living elderly population: The Framingham Heart Study. *American Journal of Clinical Nutrition* **67**, 722–733.
- Fox TE, Eagles J & Fairweather-Tait SJ (1998) Bioavailability of iron glycine as a fortificant in infant foods. *American Journal of Clinical Nutrition* **67**, 664–668.
- Frazer DM, Wilkins SJ, Becker EM, Murphy TL, Vulpe CD, McKie AT & Anderson GJ (2003) A rapid decrease in the expression of DMT1 and Dcytb but not Iregl or hephaestin explains the mucosal block phenomenon of iron absorption. *Gut* **52**, 340–346.
- Galan P, Yoon HC, Preziosi P, Viteri F, Valeix P, Fieux B, Briancon S, Malvy D, Roussel AM, Favier A & Hercberg S (1998) Determining factors in the iron status of adult women in the SU.VI.MAX study. Supplementation en Vitamines et Mineraux Antioxydants. *European Journal of Clinical Nutrition* **52**, 383–388.
- Gillooly M, Bothwell TH, Torrance JD, Macphail AP, Derman DP, Bezowda WR, Mills W, Charlton RW & Mayet F (1983) The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *British Journal of Nutrition* **49**, 331–342.
- Glahn RP, Wien EM, Van Campen DR & Miller DD (1996) Caco-2 cell iron uptake from meat and casein digests parallels in vivo studies: use of a novel in vitro method for rapid estimation of iron bioavailability. *Journal of Nutrition* **126**, 332–339.
- Gregory J, Foster K, Tyler H & Wiseman M (1990) *The Dietary and Nutritional Survey of British Adults*. London: H. M. Stationery Office.
- Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey: Young People Aged 4 to 18 Years*. London: The Stationery Office.
- Guillygomarc'h A, Christian J, Romain M, Vincnet Q, Veronique D & Deugnier Y (2003) Circadian variations of transferrin saturation levels in iron-overloaded patients: implications for the screening of C282Y-linked haemochromatosis. *British Journal of Haematology* **120**, 359–363.
- Hallberg L, Brune M & Rossander L (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *American Journal of Clinical Nutrition* **49**, 140–144.
- Hallberg L & Hulthen L (2000) Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *American Journal of Clinical Nutrition* **71**, 1147–1160.
- Hallberg L & Hulthen L (2002) Perspectives on iron absorption. *Blood Cells, Molecules and Diseases* **29**, 562–573.
- Hallberg L, Hulthen L & Gramatkovski E (1997) Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *American Journal of Clinical Nutrition* **66**, 347–356.
- Hallberg L & Rossander L (1984) Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal. *American Journal of Clinical Nutrition* **39**, 577–583.



- Hallberg L, Rossander-Hulthen L, Brune M & Gleerup A (1992) Calcium and iron absorption: mechanism of action and nutritional importance. *European Journal of Clinical Nutrition* **46**, 317–327.
- Hallberg L, Rossander-Hulthen L, Brune M & Gleerup A (1993) Inhibition of haem-iron absorption in man by calcium. *British Journal of Nutrition* **69**, 533–540.
- Heath ALM & Fairweather-Tait SJ (2002) Clinical implications of changes in the modern diet: iron intake, absorption and status. *Best Practice and Research Clinical Haematology* **15**, 225–241.
- Heath AL & Fairweather-Tait SJ (2003) Health implications of iron overload: the role of diet and genotype. *Nutrition Reviews* **61**, 45–62.
- Heath A-LM, Skeaff CM & Gibson RS (2000) The relative validity of a computerized food frequency questionnaire for estimating intake of dietary iron and its absorption modifiers. *European Journal of Clinical Nutrition* **54**, 592–599.
- Henderson L, Gregory J & Swan G (2002) *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years. Types and Quantities of Foods Consumed*. London: The Stationery Office.
- Holland B, Welch AA, Unwin ID, Buss DH, Paul AA & Southgate DAT (1991) *McCance and Widdowson's The Composition of Foods*. 5th ed. Cambridge: Royal Society of Chemistry and MAFF.
- Huh EC, Hotchkiss A, Brouillette J & Glahn RP (2004) Carbohydrate fractions from cooked fish promote iron uptake by Caco-2 cells. *Journal of Nutrition* **134**, 1681–1689.
- Hulthen L, Lindstedt G, Lundberg P-A & Hallberg L (1998) Effect of mild infection on serum ferritin concentration – clinical and epidemiological implications. *European Journal of Clinical Nutrition* **52**, 376–379.
- Hunt JR, Mullen LM, Lykken GI, Gallagher SK & Nielsen FH (1990) Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. *American Journal of Clinical Nutrition* **51**, 649–655.
- Hurrell RF, Reddy MB, Burri J & Cook JD (2002) Phytate degradation determines the effect of industrial processing and home cooking on iron absorption from cereal-based foods. *British Journal of Nutrition* **88**, 117–123.
- Hurrell RF, Reddy M & Cook JD (1999) Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition* **81**, 289–295.
- Hurrell RF, Reddy MB, Juillerat M-A & Cook JD (2003) Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *American Journal of Clinical Nutrition* **77**, 1213–1219.
- Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, Napier JA & Worwood M (2001) HFE mutations, iron deficiency and overload in 10,500 blood donors. *British Journal of Haematology* **114**, 474–484.
- Jacobs A & Miles PM (1970) The formation of iron complexes with bile and bile constituents. *Gut* **11**, 732–734.
- Kuhn IN, Monsen ER, Cook JD & Finch CA (1968) Iron absorption in man. *Journal of Laboratory and Clinical Medicine* **71**, 715–721.
- Labbe RF, Vreman HJ & Stevenson DK (1999) Zinc protoporphyrin: a metabolite with a mission. *Clinical Chemistry* **45**, 2060–2072.
- Layrisse M, Martinez-Torres C, Leets I, Taylor P & Ramirez J (1984) Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. *Journal of Nutrition* **114**, 217–223.
- Lee CJ, Lawler GS & Panemangalore M (1987) Nutritional status of middle-aged and elderly females in Kentucky in two seasons: Part 2. Hematological parameters. *Journal of the American College of Nutrition* **6**, 217–222.
- Leong W-I & Lonnerdal B (2004) Hepcidin, the recently identified peptide that appears to regulate iron absorption. *Journal of Nutrition* **134**, 1–4.
- Lynch SR, Beard JL, Dassenko SA & Cook JD (1984) Iron absorption from legumes in humans. *American Journal of Clinical Nutrition* **40**, 42–47.
- Lynch SR, Skikne BS & Cook JD (1989) Food iron absorption in idiopathic hemochromatosis. *Blood* **74**, 2187–2193.
- McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E *et al.* (2001) An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* **291**, 1755–1759.
- Macfarlane BJ, Bezwoda WR, Bothwell TH, Baynes RD, Bothwell JE, MacPhail AP, Lamparelli RD & Mayet F (1988) Inhibitory effect of nuts on iron absorption. *American Journal of Clinical Nutrition* **47**, 270–274.
- Mattys C, Pynaert I, Roe M, Fairweather-Tait SJ, Heath A-LM & De Heanauw S (2004) Validity and reproducibility of a computerised tool for assessing the iron, calcium and vitamin C intake of Belgian women. *European Journal of Clinical Nutrition* (advance online publication).
- Minihane AM & Fairweather-Tait SJ (1998) Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status. *American Journal of Clinical Nutrition* **68**, 96–102.
- Miret S, Simpson RJ & McKie AT (2003) Physiology and molecular biology of dietary iron absorption. *Annual Reviews of Nutrition* **23**, 283–301.
- Morck TA, Lynch SR & Cook JD (1983) Inhibition of food iron absorption by coffee. *American Journal of Clinical Nutrition* **37**, 416–420.
- Murphy S, Curran MD, McDougall N, Callender ME, O'Brien CJ & Middleton D (1998) High incidence of the Cys 282 Tyr mutation in the HFE gene in the Irish population – implications for haemochromatosis. *Tissue Antigens* **52**, 484–488.
- O'Neil-Cutting MA & Crosby WH (1987) Blocking of iron absorption by a preliminary oral dose of iron. *Archives of Internal Medicine* **147**, 489–491.
- Oski FA (1993) Iron deficiency in infancy and childhood. *New England Journal of Medicine* **329**, 190–193.
- Pan WH & Habicht JP (1991) The non-iron-deficiency-related difference in haemoglobin concentration distribution between blacks and whites and between men and women. *American Journal of Epidemiology* **134**, 1410–1416.
- Preziosi P, Hercberg S, Galan P, Devanlay M, Cherouvrier F & Dupin H (1994) Iron status of a healthy French population: factors determining biochemical markers. *Annals of Nutrition and Metabolism* **38**, 192–202.
- Prynne CJ, Paul AA, Price GM, Day KC, Hilder WS & Wadsworth MEJ (1999) Food and nutrient intake of a national sample of 4-year-old children in 1950: comparison with the 1990's. *Public Health Nutrition* **2**, 537–547.
- Punnonen K, Irjala K & Rajamaki A (1997) Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood* **89**, 1052–1057.
- Reddy MB & Cook JD (1997) Effect of calcium intake on nonheme-iron absorption from a complete diet. *American Journal of Clinical Nutrition* **65**, 1820–1825.
- Reddy MB, Hurrell RF & Cook JD (2000) Estimation of nonheme-iron bioavailability from meal composition. *American Journal of Clinical Nutrition* **71**, 937–943.
- Ruston D, Hoare J, Henderson L, Gregory J, Bates CJ, Prentice A, Birch M, Swan G & Farron M (2004) *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years*. London: The Stationery Office.
- Sandberg A-S, Brune M, Carlsson N-G, Hallberg L, Skoglund E & Rossander-Hulthen L (1999) Inositol phosphates with

- different numbers of phosphate groups influence iron absorption in humans. *American Journal of Clinical Nutrition* **70**, 240–246.
- Skikne B & Baynes RD (1994) Iron absorption. In *Iron Metabolism in Health and Disease*, p. 177 [JH Brock, JW Halliday, MJ Pippard and LW Powell, editors]. London: WB Saunders.
- Skikne BS & Cook JD (1992) Effect of enhanced erythropoiesis on iron absorption. *Journal of Laboratory and Clinical Medicine* **20**, 746–751.
- Sokoll LJ & Dawson-Hughes B (1992) Calcium supplementation and plasma ferritin concentrations in premenopausal women. *American Journal of Clinical Nutrition* **56**, 1045–1048.
- Suominen P, Punnonen K, Rajamaki A & Irjala K (1998) Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. *Blood* **92**, 2934–2939.
- Swain JH, Tabatabai LB & Reddy MB (2002) Histidine content of low-molecular-weight beef proteins influences nonheme iron bioavailability in Caco-2 cells. *Journal of Nutrition* **132**, 245–251.
- Taylor PG, Mendez-Castellano H, Lopez-Blanco M, Landaeta-Jiminez M, Hernandez-Valera Y, Arenas O, Martinez-Torres C & Layrisse M (1988) Daily physiological iron requirements in children. *Journal of the American Dietetic Association* **88**, 454–458.
- Temme EHM & Van Hoydonck PGA (2002) Tea consumption and iron status. *European Journal of Clinical Nutrition* **56**, 379–386.
- Tidehag P, Hallmans G, Wing K, Sjostrom R, Agren G, Lundin E & Zhang J-X (1996) A comparison of iron absorption from single meals and daily diets using radioFe ( $^{55}\text{Fe}$ ,  $^{59}\text{Fe}$ ). *British Journal of Nutrition* **75**, 281–289.
- Tufts DA, Haas JD, Beard JL & Spielvogel H (1985) Distribution of haemoglobin and functional consequences of anemia in adult males at high altitude. *American Journal of Clinical Nutrition* **42**, 1–11.
- van de Vijver LPL, Kardinaal AFM, Charzewska J, Rotily M, Charles P, Maggiolini M *et al.* (1999) Calcium intake is weakly but consistently negatively associated with iron status in girls and women in six European countries. *Journal of Nutrition* **129**, 963–968.
- Van Tiel ED, Peeter PHM, Smit HA, Nagelkerke NJD, Van Loon AJM, Grobbee DE & Bueno-de-Mesquita HB (2002) Quitting smoking may restore haematological characteristics within five years. *Annals of Epidemiology* **12**, 378–388.
- Velati C, Marlianici E, Rigamonti D, Barillari G, Chiavilli F, Fugiani P *et al.* (2003) Mutations of the haemochromatosis gene in Italian candidate blood donors with increased transferrin saturation. *Hematology Journal* **4**, 436–440.
- Waalén J, Felitti V, Gelbart T, Ho NJ & Beutler E (2002) Penetrance of haemochromatosis. *Blood Cells, Molecules and Diseases* **29**, 418–432.
- Walters GO, Miller FM & Worwood M (1973) Serum ferritin concentration and iron stores in normal subjects. *Journal of Clinical Pathology* **26**, 770–772.
- Wintrobe MM (1981) *Clinical Hematology*, 8th ed. Philadelphia, PA: Lea & Febiger.
- World Health Organization/UNICEF/United Nations University (2001) Iron deficiency anaemia. Assessment, prevention and control. A guide for programme managers. WHO/UNICEF/UNU [http://www.who.int/nut/documents/ida\\_assessment\\_prevention\\_control.pdf](http://www.who.int/nut/documents/ida_assessment_prevention_control.pdf)
- World Health Organization (1972) *Nutritional Anaemias*. WHO Technical Report Series no. 503. Geneva: WHO.
- Worthington-Roberts BS, Breskin MW & Monsen ER (1988) Iron status of premenopausal women in a university community and its relationship to habitual dietary sources of protein. *American Journal of Clinical Nutrition* **47**, 275–279.
- Yip R (2000) Significance of an abnormally low or high haemoglobin concentration during pregnancy: special consideration of iron nutrition. *American Journal of Clinical Nutrition* **72**, 272S–279S.
- Yip R, Johnson C & Dallman PR (1984) Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. *American Journal of Clinical Nutrition* **39**, 427–436.