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SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for iron[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2,3]

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ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies derived Dietary Reference Values (DRVs) for iron. These include Average Requirement (AR) and Population Reference Intake (PRI). For adults, whole-body iron losses were modelled using data from US adults. Predicted absorption values, at a serum ferritin concentration of 30 µg/L, of 16 % for men and 18 % for women were used to convert physiological requirements to dietary iron intakes. In men, median whole-body iron losses are 0.95 mg/day, and the AR is 6 mg/day. The PRI, calculated as the dietary requirement at the 97.5th percentile, is 11 mg/day. For postmenopausal women, the same DRVs as for men are proposed. In premenopausal women, additional iron is lost through menstruation but, because losses are highly skewed, the Panel set a PRI of 16 mg/day to cover requirements of 95 % of the population. In infants and children, requirements were calculated factorially, taking into consideration the needs for growth, replacement of losses and percentage iron absorption from the diet (10 % up to 11 years and 16 % thereafter). PRIs were estimated using a coefficient of variation of 20 %. They are 11 mg/day in infants (7–11 months), 7 mg/day in children aged 1–6 years and 11 mg/day in children aged 7–11 years and boys aged 12–17 years. For girls aged 12–17 years, the PRI of 13 mg/day is the midpoint of the calculated dietary requirement of 97.5 % of girls and the PRI for premenopausal women; this approach allows for the large uncertainties in the rate and timing of pubertal growth and menarche. For pregnant and lactating women, for whom it was assumed that iron stores and enhanced absorption provide sufficient additional iron, DRVs are the same as for premenopausal women.

KEY WORDS

iron, Average Requirement, Dietary Reference Value, probabilistic modelling, factorial approach

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on Dietary Reference Values (DRVs) for the European population, including iron. These include Average Requirement (AR) and Population Reference Intake (PRI).

Iron is required for oxygen transport, electron transfer, oxidase activities and energy metabolism. The main components of the body that contain iron are erythrocyte haemoglobin and muscle myoglobin, liver ferritin, and haem and non-haem enzymes.

Dietary iron consists of haem (from animal tissues) and non-haem (including ferritin) iron. Foods that contain relatively high concentrations of iron include meat, fish, cereals, beans, nuts, egg yolks, dark green vegetables, potatoes and fortified foods.

Iron is inefficiently and variably absorbed, depending on dietary and host-related factors. Iron absorption occurs primarily in the duodenum. A proportion of non-haem iron in foods is solubilised in the gastrointestinal lumen, reduced by duodenal cytochrome b reductase to Fe^{2+} and transported into the enterocyte by the transmembrane divalent metal transporter 1. There, iron is either stored as ferritin, some of which is subsequently lost when the cells are sloughed, is taken up by mitochondria for the synthesis of haem, or is transported across the basolateral membrane by ferroportin where it is carried in the circulation as diferric-transferrin after oxidation to Fe^{3+} by hephaestin. The mechanisms of absorption of haem iron and ferritin iron are uncertain, but once taken up iron is released from haem iron by haem oxygenase and then follows the same pathways as non-haem iron.

Homeostasis is mediated via the regulation of iron absorption, as there are no active pathways for excreting iron. In healthy individuals, the mucosal uptake and transfer of iron is inversely related to systemic serum ferritin concentrations, and control is exerted via the expression of the hepatic hormone hepcidin.

If the supply of iron is insufficient to meet physiological requirements, iron stores will be mobilised and iron deficiency will develop once the stores are exhausted. Iron deficiency anaemia (a microcytic anaemia with haemoglobin concentrations below normal) is the most common nutritional deficiency disorder, being found in all countries of the world. Subjects at greatest risk are those with high iron requirements owing to growth (infants, children, pregnant women) or high losses (women with high menstrual losses), or those with impaired absorption, e.g. in the presence of infection/inflammation.

The risk of systemic iron overload from dietary sources is negligible with normal intestinal function. Chronic iron overload may occur as a result of specific clinical conditions and genetic mutations, but there is no evidence that heterozygotes for haemochromatosis are at an increased risk of iron overload.

The Panel considers that health outcomes cannot be used to derive DRVs for iron because of the uncertainties in intake measurements, the poor correlation between intake and iron status, and the presence of confounders that prevent the determination of dose–response relationships and the assessment of risks associated with deficiency or excess.

A factorial approach was used to derive dietary iron requirements. Data on iron turnover and total obligatory iron losses from the body (including skin, sweat, urine and faeces) obtained from radioisotope dilution measurements were used to determine iron requirements in men and premenopausal women. Although these data were collected from a North American population group, the Panel agreed to use them as a basis for the estimation and probability modelling of the mean and approximate variability of distribution percentiles for the iron losses of adult men and premenopausal women in the European Union (EU) population. Summary statistics were estimated for the main variables related to iron losses for men and premenopausal women and for associations among the variables which were considered to be explanatory for iron losses. From these, a regression model

equation for iron losses (as mg/day) was fitted to the data using a set of potentially relevant variables. This stage included an assessment of outliers and goodness of fit. The regression model was then used to derive a distribution for iron losses, combining the model equation with parametric distributions fitted to the sampling observations of each of the explanatory variables.

Dietary (haem and non-haem) iron absorption was estimated from a probability model, based on measures of iron intake and status in a representative group of men and women from the UK National Diet and Nutrition Survey. This provides estimates of total iron absorption from a mixed Western-style diet at any level of iron status. The Panel selected a target value of 30 µg/L for serum ferritin concentration. At this level, the predicted iron absorption is 16 % in men and 18 % in premenopausal women. The Panel decided to use 16 % for adults (except premenopausal women) and children aged 12–17 years when converting physiological requirements into dietary intakes, based on the assumption that the relationship between serum ferritin concentration and efficiency of absorption holds for all age groups, as there are no indications that age will affect the relationship.

In men, the 50th percentile of the model-based distribution of obligatory iron losses is 0.95 mg/day. The 90th, 95th and 97.5th percentiles are, respectively, equal to iron losses of 1.48, 1.61 and 1.72 mg/day. Using 16 % iron absorption to convert the physiological requirement into the dietary requirement results in a calculated dietary requirement at the 50th percentile of 5.9 mg/day and of 10.8 mg/day at the 97.5th percentile. After rounding, an AR of 6 mg/day and a PRI of 11 mg/day were set. In the absence of information on the iron requirement for postmenopausal women and despite their lower body weight, the Panel decided to set the same DRVs for postmenopausal women as those set for adult men.

In premenopausal women, the 50th percentile of the model-based distribution of obligatory iron losses is 1.34 mg/day. The 90th, 95th and 97.5th percentiles are, respectively, equal to iron losses of 2.44, 2.80 and 3.13 mg/day. Using 18 % absorption to convert the physiological iron requirement into the dietary requirement results in a calculated dietary requirement at the 50th percentile of 7.4 mg/day. Intakes meeting the dietary iron requirement of approximately 90, 95 and 97.5 % of the premenopausal women are calculated as 13.6, 15.6 and 17.4 mg/day, respectively. After rounding, the Panel derived an AR of 7 mg/day and a PRI of 16 mg/day for premenopausal women. The Panel considers that the PRI meets the dietary requirement of 95 % of women in their reproductive years and is derived from a group of premenopausal women, some of whom used oral contraceptives, as is the case in the EU. The Panel decided that women with very high iron losses should not be included in the premenopausal group, as this would result in unrealistically high DRVs for the majority of this population group.

In infants aged 7–11 months, the requirement for absorbed iron is 0.79 mg/day to replace obligatory losses (0.19 mg/day) and increase haemoglobin mass, tissue iron and storage iron (0.6 mg/day). Assuming 10 % absorption, this gives an AR of 8 mg/day and, based on a coefficient of variation (CV) of 20 %, which allows for high individual variation relating to growth rate, iron losses, absorption and dietary patterns, the PRI is 11 mg/day. In children aged 1–6 years, the AR is 5 mg/day, calculated from the sum of the requirements for growth (0.25 mg/day for ages 1–3 years and 0.27 mg/day for ages 4–6 years) and obligatory losses of 0.022 (1–3 years) and 0.012 (4–6 years) mg/kg body weight per day, and absorption of 10 %. Based on a CV of 20 %, the PRI is 7 mg/day. In children aged 7–11 years, requirements for growth increase to 0.39 mg/day, but losses per kilogram of body weight do not change. Assuming 10 % absorption, the AR (after rounding) is 8 mg/day and, based on a CV of 20 %, the PRI is 11 mg/day.

In boys and girls aged 12–17 years, the requirements for absorbed iron are 1.27 and 1.13 mg/day, respectively, calculated from losses of 0.012 mg/kg body weight per day and menstrual blood losses of 0.25 mg/day in girls, and growth needs of 0.61 mg/day for boys and 0.26 mg/day for girls. Assuming 16 % absorption, the AR (after rounding) is 8 mg/day for boys and 7 mg/day for girls. The PRI for boys is 11 mg/day based on a CV of 20 %. In girls, because of the uncertainties related to the rate and timing of physiological development and the onset of menarche, and because of the skewed distribution of menstrual losses, the Panel decided to set the PRI as the mean of the calculated dietary requirement of 97.5 % of girls aged 12–17 years (9.9 mg/day) and the PRI for premenopausal women (16 mg/day). After rounding, the PRI is 13 mg/day for girls.

In pregnancy, iron intake should cover basal losses during the first trimester, taking into account the cessation of menstruation. The requirements then increase exponentially, and this is associated with a dramatic increase in the efficiency of iron absorption. The total quantity of iron required for a singleton pregnancy is 835 mg. If the serum ferritin concentration is 30 µg/L at conception, around 120 mg of stored iron can be mobilised to support the pregnancy, which means that the total dietary requirement of iron is 715 mg. If the relevant percentage absorption figures determined from a study in pregnant women are applied to the entire pregnancy (7.2 % during weeks 0–23, 36.3 % during weeks 24–35 and 66.1 % during weeks 36–40 for non-haem iron, plus 25 % absorption for haem iron throughout the whole pregnancy), the total quantity of iron absorbed from a diet providing 13 mg iron/day is 866 mg. The Panel notes that using the absorption figures from single-meal studies in fasting mothers may be an overestimate, but, nevertheless, the quantity of iron absorbed is well in excess of the estimated 715 mg calculated by a factorial approach, and the progressive fall in serum ferritin concentration will be accompanied by an increased efficiency of absorption, irrespective of other homeostatic mechanisms. The Panel therefore considers that no additional iron is required in pregnancy.

During lactation, the quantity of iron secreted in breast milk is approximately 0.24 mg/day. When this is added to basal losses of 1.08 mg/day (obtained from data in postmenopausal women), the requirement for absorbed iron during the first months of lactation is calculated to be 1.3 mg/day, assuming that menstruation has not yet resumed. This requirement is slightly less than in non-pregnant, non-lactating women, but, for depleted iron stores to be replenished and to cover losses of iron when menstruation is re-established, the Panel considers that the AR and PRI for lactating women are the same as for non-pregnant women of childbearing age.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and, if necessary, to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on nutrient and energy intakes for the European Community.[4] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context, EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context, EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002,[5] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;
- Protein;

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[4] Scientific Committee for Food, 1993. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food, 31st series. Food – Science and Technique, European Commission, Luxembourg, 248 pp.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

- Dietary fibre.

Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

1. Introduction

In 1993, the Scientific Committee for Food (SCF) adopted an opinion on nutrient and energy intakes for the European Community (SCF, 1993). For iron, the SCF set Population Reference Intakes (PRIs) for infants, boys and non-menstruating girls, adult men, and lactating and postmenopausal women. For menstruating girls and women, intakes at the proposed values were considered to cover the needs of 90 or 95 % of the population. No PRI specific for pregnant women was proposed. For non-pregnant, non-lactating adults, an Average Requirement (AR) and a Lowest Threshold Intake were also proposed.

2. Definition/category

2.1. Chemistry

Iron (atomic mass 55.85 Da, atomic number 26) is the fourth most common element in the Earth’s crust. It has oxidation states from –2 to +6, of which the most biologically relevant are the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states. Biologically, iron complexes with nitrogen, like in the porphyrin ring of haem, and with sulphur forming iron–sulphur clusters, which are thought to have underpinned the evolution of life forms and the release of oxygen into the atmosphere. In higher life forms, iron–sulphur clusters are involved in mitochondrial energy metabolism, the synthesis of the oxygen-binding molecule (haem) and in the regulation of cellular acquisition, homeostasis and the use of iron.

2.2. Function of iron

2.2.1. Biochemical functions

Iron plays a major role in (1) oxygen transport (haemoglobin) and short-term oxygen storage (myoglobin), (2) haem enzymes involved in electron transfer (e.g. cytochromes a, b and c, and cytochrome c oxidase) and oxidase activities (e.g. cytochrome P-450 mixed function oxidases, oxidases and peroxidases) and (3) iron–sulphur clusters in energy transduction and oxido-reductase activities (e.g. succinate, isocitrate and NADPH dehydrogenase, xanthine oxidases). It is also a cofactor in various non-haem-containing enzymes (e.g. phenylalanine, tryptophan and tyrosine hydroxylases, and proline and lysine hydroxylases).

Iron is necessary for most, if not all, pathways for energy and substrate metabolism. Globin-haems are transporters of oxygen, carbon dioxide, carbon monoxide and nitric oxide (e.g. haemoglobin and neuroglobin), stores of oxygen (e.g. myoglobin and neuroglobin) and scavengers of free radicals (Brunori and Vallone, 2006). The cytochrome P-450 oxidase system embraces over 11 000 diverse activities including the metabolism of endogenous substrates such as organic acids, fatty acids, prostaglandins, steroids and sterols including cholesterol and vitamins A, D and K. The citric acid cycle and respiratory chain involves six different haem proteins and six iron–sulphur clusters.

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

The features of iron deficiency are continuously changing. Many have been traditionally attributed to iron deficiency, such as koilonychia (spoon-shaped nails), soft nails, glossitis, cheilitis (dermatitis at the corner of the mouth), mood changes, muscle weakness and impaired immunity, but they can also be secondary features of other nutritional deficiencies. Many studies examining relationships between iron deficiency and adverse sequelae use anaemia as a surrogate indicator of iron deficiency. Iron deficiency anaemia, defined as the combination of iron deficiency and anaemia (low haemoglobin), can be distinguished from that caused by other nutritional deficiencies, such as folate or cobalamin deficiency, by characteristic changes in the shape, density of haem content and size of red blood cells. However, the pathogenesis of iron deficiency may not be dietary. Non-dietary causes of iron deficiency and anaemia include conditions that cause gastrointestinal blood loss or malabsorption, e.g. cancer and inflammatory bowel disease, intestinal infections and parasitism. Blood loss from the genito-urinary and respiratory tracts may also contribute to iron deficiency (Steketee, 2003).

There is evidence that adolescent girls who were anaemic as toddlers have altered memory and spatial awareness. Iron-deficient and anaemic infants and children have delayed attention, poor recognition memory, reduced reward-seeking behaviours and impoverished social interactions. Some studies have shown an association between iron deficiency anaemia in early childhood and long-lasting poor cognitive and behavioural performance. However, much of this research is confounded by socio-economic factors and by the difficulties in standardising the outcome measurements (McCann and Ames, 2007). Existing studies imply that iron-responsive defects occur at haemoglobin concentrations below 80, 95 and 110 g/L. However, in these studies, the degree of anaemia has not been considered as a continuous variable and it is difficult to characterise a specific threshold of anaemia (or even the degree of iron deficiency) for these phenomena. Thus, although the effects of early life deficiencies may persist and be irredeemable by subsequent iron supplementation, the vulnerable periods have not been well characterised.

In women in whom anaemia has been induced by phlebotomy, impaired muscle endurance capacity and energetic efficiency are apparent as haemoglobin concentrations drop below 130 g/L, and the effect becomes greater with every 10 g/L fall in haemoglobin (Gardner et al., 1977). In related studies, iron-responsive impaired muscle endurance capacity has been demonstrated in groups without anaemia but with serum ferritin concentrations < 16 µg/L (Brownlie et al., 2004).

Iron deficiency is a risk factor for increased blood concentrations of cadmium (Olsson et al., 2002; Gallagher et al., 2011) and lead (Zimmermann, 2008; Shah et al., 2011). For cadmium, this is probably due to enhanced intestinal absorption in the presence of raised levels of divalent metal transporter 1 (DMT1) in iron deficiency. For lead, the mechanism is less clear (Bannon et al., 2003), but genotype appears to be a contributory factor; *HFE* variants have been reported to be associated with increased blood lead concentrations (Hopkins et al., 2008).

In animal models, iron deficiency, with or without anaemia, is associated with inefficient energy metabolism, with altered glucose and lactate utilisation. It is also associated with reduced muscle myoglobin content, reducing muscle strength and endurance. Cytochrome c oxidase activity in muscle and the intestinal mucosa may be reduced. Impaired collagen synthesis and osteoporosis may occur, and the latter may be due, in part, to impaired hydroxylation of vitamin D (DeLuca, 1976; Tuderman et al., 1977). Similarly, altered vitamin A and prostaglandin metabolism has been noted (Oliveira et al., 2008). In the brain, dopaminergic and serotonin neurotransmission may be reduced in areas such as the substantia nigra, cerebellar nuclei, globus pallidus and hippocampus, and neuromyelination and synapse and dendrite development may be defective. Membrane fatty acid profiles (e.g. reduced docosahexaenoic acid content) can be altered, thereby affecting neuronal function. Functional impairments include delayed responses to auditory and visual stimuli and impaired memory and spatial navigation. These manifestations provide plausible mechanistic bases for inferring that iron deficiency, with or without anaemia, has similar effects in humans. The risk would be greater during periods of rapid growth (i.e. in infancy, childhood and adolescence and during gestation) and the tissues involved would be those with a rapid turnover, specialised function and high energy dependence, such as immunocytes, enterocytes, brain and muscle. It is important to note that these defects have been associated with severe iron deprivation or deficiency that are not representative of deficiencies customarily encountered in human nutrition, and that there are few data to enable the construction of dose–response curves, relating these outcomes to lesser degrees of iron deficiency.

2.2.2.2. Excess

The risk of systemic iron overload from dietary sources is negligible with normal intestinal function. Acute large intakes of iron (e.g. 20 mg or more elemental iron/kg body weight), particularly without food, cause corrosive haemorrhagic necrosis of the intestinal mucosa, leading to loose stools and blood loss, hypovolaemic shock, damaging failure of systemic organs and death. Early clinical phenomena of this damage (gastritis, nausea, abdominal pain and vomiting) have been used to set exposure levels for health guidance.

Chronic iron overload may occur in individuals affected by haemolytic anaemias, haemoglobinopathies or one of the haemochromatoses and results in increasing sequestration of iron in ferritin and haemosiderin in all tissues throughout the body. Eventually, the haemosiderin degrades releasing iron, which in turn causes oxidative architectural and functional tissue damage resulting in cardiomyopathy, arthropathies, diabetes mellitus and neurological disease. There is no evidence that heterozygotes for haemochromatoses are at an increased risk of iron overload compared with the rest of the population.

African iron overload, previously called Bantu cirrhosis, is an ecogenetic disorder arising from an, as yet, uncharacterised genetic defect combined with increased exposure to iron from food and beer that had been prepared in iron utensils. The increased iron deposition affects the Kupffer reticuloendothelial cells of the liver rather than the hepatocytes, which is the case in the other iron overload syndromes.

No Tolerable Upper Intake Level (UL) has been set for iron by the SCF or EFSA. Adverse gastrointestinal effects have been reported after short-term ingestion of non-haem iron preparations at doses of 50–60 mg/day, particularly if taken without food. EFSA (2004) considered that these adverse gastrointestinal effects are not a suitable basis to establish a UL for iron from all sources. EFSA (2004) also considered that a UL cannot be established for iron based on iron overload, because there were inadequate data to enable the construction of reliable response curves between intake, body burden, homeostatic adaptations and adverse health effects, including increased risk of chronic diseases such as cardiovascular disease, diabetes and cancer. The absence of convincing evidence of a causal relationship between iron intake or stores and chronic diseases was noted (EFSA, 2004).

The Institute of Medicine (IOM, 2001) set a UL based on a Lowest Observed Adverse Effect Level (LOAEL) for gastrointestinal side effects observed in Swedish adults following supplementation with ferrous fumarate (60 mg/day) in addition to an estimated dietary iron intake of 11 mg/day. Using an uncertainty factor of 1.5, the UL was set at 45 mg/day for males and females aged 14 years and older, including pregnant and lactating women. For infants and children, the UL was set at 40 mg/day based on a No Observed Adverse Effect Level (NOAEL) for adverse gastrointestinal effects of 30 mg/day observed in toddlers, taking into account a dietary intake of about 10 mg/day and using an uncertainty factor of 1.

2.3. Physiology and metabolism

The systemic burden and homeostasis of iron is mediated via regulation of iron absorption and the deposition or sequestration of the element into intracellular pools, mainly in the reticuloendothelial system (RES) and liver. A major driver of systemic iron homeostasis is the cellular and mitochondrial need for iron and oxygen (hypoxia).

2.3.1. Intestinal absorption

2.3.1.1. Mechanisms of intestinal uptake and transfer of iron

Iron absorption occurs mainly in the duodenum and proximal small intestine. The contribution by the distal small intestine and the colon is uncertain and is probably very small. Absorption involves the uptake of iron from the intestinal lumen into enterocytes, its transfer within enterocytes and its subsequent translocation across the basolateral membrane to carriers in the plasma of the portal circulation.

The enterocytic carrier mechanisms involved in iron uptake and transfer are responsive to the systemic need for the element. The body has no specific mechanism of excreting iron, and the rigorous control of the uptake and transfer of iron into the body is essential for preventing iron overload.

Iron released by the digestion of food includes non-haem iron, haem iron and ferritin. Solubilisation of non-haem iron occurs in the acidic environment of the stomach and proximal duodenum, and uptake of inorganic iron occurs mainly in the duodenum and proximal jejunum, whereas the alkaline environment of the jejunum reduces the solubility of free, unbound iron. Uptake into enterocytes is initiated by the conversion of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron by duodenal cytochrome b reductase (DcytB/ferric reductase), which is located on the luminal surface of the enterocytes. The iron is then co-transported with protons (possibly provided by gastric hydrochloric acid or by a co-located Na^+/H^+ exchanger) by transmembrane DMT1 across the apical membrane into the cytoplasm (Montalbetti et al., 2013).

The mechanism for haem iron uptake remains unclear. Two main pathways have been proposed: receptor-mediated endocytosis of haem and direct transport into the intestinal enterocyte by haem (and possibly non-haem) iron transporters (West and Oates, 2008). A putative mucosal haem carrier protein 1 (Shayeghi et al., 2005) is now recognised to be principally a folate transporter. A specific haem transporter has been found in macrophages but not as yet in enterocyte apical membranes.

There is controversy over the mechanism of absorption of ferritin. It has been reported to involve a carrier-mediated endocytic pathway into the enterocyte followed by lysosomal dissolution of the ferritin core to release the iron (Kalgaonkar and Lonnerdal, 2008a, 2008b; San Martin et al., 2008), but some (or all) of the iron may be released from the core of the ferritin molecule during gastric digestion and subsequently taken up by DMT1 (Hoppler et al., 2008).

In the enterocyte, iron is released from haem by haem oxygenase, and forms a common exchangeable pool with non-haem iron and, presumably, with any iron that has been released by lysosomal degradation of ferritin. Iron from the enterocyte pool can enter three different pathways: (1) it can be transferred (in the ferrous state) to a transmembrane basal transporter (ferroportin 1) for translocation out of the enterocyte to carrier molecules in the portal plasma; (2) some may be sequestered in ferritin iron depots (and shed into the gut lumen at the end of the enterocyte's lifespan); or (3) a small quantity may be taken into the mitochondria for haem synthesis.

The export of iron across the basolateral membrane by ferroportin requires its oxidation to the ferric state. This is done by hephaestin, which is a copper-dependent ferroxidase bound to the basolateral membrane. The ferric iron is then transferred to apotransferrin for transport to the liver and systemic circulation.

2.3.1.2. Regulation of absorption

The regulation of the intestinal absorption of iron is integrated with that of systemic iron kinetics and distribution. Other tissues, particularly the central nervous system, and macrophages have uptake (DMTs) and export (ferroportins) systems for iron that are analogous to those in the enterocyte, and which respond similarly to iron deficiency, and also to stressors, inflammation and hypoxia (see below). In healthy subjects, the intestinal mucosal uptake and transfer of dietary iron is inversely related to serum ferritin concentrations, particularly at concentrations below 60 µg/L (Ganz, 2013). These reductions in the absorption of iron are mediated by a hepatic hormone, hepcidin, and by control of expression of the iron transport systems in the enterocytes.

Hepcidin is also produced to a lesser extent by monocytes, macrophages and adipocytes (Ganz, 2013). Hepcidin induces the degradation of ferroportin, thereby reducing the enterocytic export of iron that has been taken up from the gut lumen. The iron trapped in the enterocytes is sequestered in ferritin and is subsequently lost into the gut lumen when the cells are shed. It has also been shown in a mouse model that hepcidin reduces DMT1 activity (Chung et al., 2009).

Hepcidin production is decreased when iron depots are low, when iron utilisation, such as erythropoiesis, is increased and when plasma transferrin concentration is reduced. It is increased when tissue, particularly hepatic iron depots and circulating transferrin concentrations, are high. Correlations have been noted between hepcidin mRNA levels and iron content in human liver tissue, and between serum concentrations of ferritin and hepcidin (Ganz, 2013).

The expression of enterocytic carriers involved in the uptake (DMTs) and transfer (ferroportins) of iron is mediated by an interaction between transferrin and transferrin receptor 1 on the basolateral surfaces of the enteroblasts in the mucosal crypts. This crypt programming becomes effective when the enterocytes have matured and migrated to the villi (Montalbetti et al., 2013). Thus, this mechanism takes 1–2 days to modify iron uptake and transfer, whereas responses to increased hepcidin takes about 8 hours (Ganz, 2013). Hepcidin production is also stimulated by cytokines associated with inflammation, such as interleukins 1 and 6. As well as reducing intestinal absorption of iron, it also induces a “shut down” of systemic iron turnover mediated both through the degradation of cellular ferroportins, hence blocking the export of iron, and by reducing the cellular uptake of iron. This response to inflammation overrides adaptation to an inadequate iron supply and sustained inflammation or stress, e.g. frequent infections and chronic inflammatory diseases can induce a functional iron deficiency including anaemia in people with an adequate body iron content. This situation is known as the anaemia of chronic disease (Section 2.4).

Hepcidin production is also down-regulated by hypoxia. Hypoxic conditions, including iron deficiency and anaemia, induce the production of hypoxia-inducible factors and, possibly, a bone marrow factor, both of which depress hepcidin expression and stimulate erythropoiesis, thereby ensuring an iron supply for red blood cell production (Ganz, 2013).

2.3.2. Dietary iron forms and bioavailability

Dietary iron consists of haem iron and non-haem iron; the latter includes ferritin, which is present in some animal and plant foods, particularly liver and legume seeds, but this form of iron makes only a small contribution to total iron intake in European diets. Small amounts of haem iron are present in some plants and fungi. Mixed diets provide about 90 % of the dietary iron as non-haem iron (Milman, 2011; Jakszyn et al., 2013), the remainder being haem iron from animal foods (in non-vegetarian diets). The haem iron content of meat (from haemoglobin and myoglobin) varies considerably (Cross et al., 2012). Balder et al. (2006) undertook a literature search to obtain data for deriving the mean proportion of haem iron relative to total iron for beef, pork, chicken and fish. They selected only those studies that measured total iron directly and, after lipid extraction, haem iron in the same meat sample. The proportion of haem iron from total iron was 69 % for beef; 39 % for pork, ham, bacon, pork-based luncheon meats and veal; 26 % for chicken and fish; and 21 % for liver. Haem iron may be denatured during cooking (Martinez-Torres et al., 1986), and some iron is lost, according to the type of cooking. For example, losses of haem and non-haem iron are greater when lamb meat is boiled than when it is grilled (Pourkhalili et al., 2013).

Fortification iron, commonly added to cereals and infant foods, is usually an iron salt or elemental iron, and percentage absorption varies greatly depending on chemical form and solubility in the gastrointestinal tract and the composition of foods consumed at the same time.

Bioavailability is a measure of the absorption and utilisation (haemoglobin incorporation) of dietary iron, and is expressed as either a percentage or a fraction of the total iron intake. The availability of iron for absorption is dependent on the chemical form of iron in the duodenum and small intestine, and the physiological requirement that determines the quantity of available iron that is taken up into the enterocytes and transported into the blood. It can generally be predicted from measures of body iron stores (serum ferritin concentration). Dietary factors that facilitate or hinder intestinal uptake of iron become increasingly important when systemic needs are increased.

Early studies with radioisotope-labelled foods found that iron from animal foods was better absorbed than that from plant foods (Layrisse et al., 1969). Mean haem iron absorption in eight non-anaemic men given three radioisotopically labelled meals over one day (non-haem iron intake 16.4 mg, haem iron intake 1.0 mg) was 37.3 (standard error (SE) 2.8) % compared with 5.3 (SE 1.8) % for non-haem iron (Bjorn-Rasmussen et al., 1974). When radiolabelled haem iron absorption was measured from six meals given over two days (20–21 mg iron/day) in iron-replete men (geometric mean serum ferritin concentrations ranged from 86 to 110 µg/L) who had been consuming a diet of low or high iron bioavailability for a period of 10 weeks (Hunt and Roughead, 2000), absorption was 22 % from high-bioavailability meals and 21 % from low-bioavailability meals. Absorption values at baseline were not significantly different, and this contrasts with non-haem iron absorption, where adaptation to diets of differing bioavailability results in alterations in the efficiency of iron absorption. Although there is a less marked effect of body iron status on haem than on non-haem iron absorption, the relationship needs to be taken into account when interpreting absorption values. In a study using radioisotopically labelled rabbit haemoglobin to label four meals per day (total iron intake 13 mg/day) for five days, the mean percentage absorption of haem iron was 35 % in 12 male blood donors (serum ferritin concentration 37 ± 16 µg/L), and 23 % in 19 non-blood donors (serum ferritin concentration 91 ± 37 µg/L). From the regression equation describing the relationship between percentage iron absorption and serum ferritin, haem iron absorption was estimated to be 42.3 % when iron stores are close to zero (serum ferritin 15 µg/L) (Hallberg et al., 1997). The Panel considers that absorption of haem iron is approximately 25 %.

In addition to systemic factors that control and/or modulate the efficiency of iron absorption, there are a number of components in food that affect non-haem iron absorption. A number of studies have been undertaken giving single meals labelled with radioisotopes or stable isotopes to subjects after an overnight fast, and have consistently shown an enhancing effect of ascorbic acid and muscle tissue (meat/poultry/fish), and an inhibitory effect of phytate, polyphenols and calcium (Hurrell and Egli, 2010).

Food components classed as inhibitors of non-haem iron absorption generally bind iron in the gastrointestinal tract and prevent its absorption, whereas enhancers of non-haem iron absorption either form complexes that can be taken up by the intestinal iron transport proteins, and thereby prevent the iron from binding to inhibitors, or reduce the more reactive Fe^{3+} iron to its less reactive and more soluble Fe^{2+} state.

Phytate (myo-inositol hexaphosphate) is present at relatively high levels in whole-grain cereals and legume seeds and is the main inhibitor of non-haem iron absorption in vegetarian diets. This effect of phytate is dose-dependent and starts at very low concentrations (Hallberg et al., 1987). At phytate–iron molar ratios of > 6, iron absorption is greatly inhibited from meals containing small amounts of enhancing components, whereas, in cereal or soy meals with no enhancers, non-haem iron absorption is greatly inhibited by a molar ratio > 1 (Hurrell and Egli, 2010). Food processing methods such as milling, germination, fermentation and the addition of phytase enzymes can be used to degrade phytate and improve iron absorption from traditional or processed foods (Hurrell, 2004). Ethylenediaminetetraacetic acid (EDTA) will also overcome phytate inhibition in fortified foods such as wheat flour (Hurrell and Egli, 2010).

Polyphenol compounds from beverages (tea, coffee, cocoa, red wine), vegetables (spinach, aubergine), legumes (coloured beans) and cereals such as sorghum inhibit non-haem iron absorption in a dose-dependent way, depending on the structure of the phenolic compound and extent of polymerisation; the gallate-containing tea polyphenols appear to be most inhibitory (Hurrell et al., 1999).

Calcium reduces both haem and non-haem iron absorption from single meals and, although the mechanism is not fully understood, the reduction in iron uptake and transport into the blood may be effected through temporary internalisation of the apical iron transporter DMT1 (Thompson et al., 2010) and/or changes in expression of the iron transporters (Lonnerdal, 2010). In a small bread meal, the effect was dose-dependent up to 300 mg calcium, with 165 mg calcium causing about 50 %

inhibition whether added as calcium chloride or 150 mL milk (Hallberg et al., 1991). However, the same quantity of milk added to a meal of steak, carrots, French fries, Camembert cheese, apple, bread and water had no effect (Galan et al., 1991).

Muscle tissue from beef, lamb, chicken, pork and fish, as well as liver tissue, enhance iron absorption from inhibitory meals (Lynch et al., 1989). The nature of the meat factor is uncertain, but partially digested cysteine-containing peptides could potentially reduce Fe^{3+} to Fe^{2+} iron and chelate iron in the same way as ascorbic acid (Taylor et al., 1986). Storksdieck genannt Bonsmann and Hurrell (2007) reported that, unlike other food proteins, muscle proteins are rapidly digested by pepsin and the arrival of many small peptides in the jejunum could be responsible for solubilising iron and improving absorption. Conversely, peptides from legume proteins and some milk proteins inhibit iron absorption (Hurrell and Egli, 2010). The inhibitory nature of soy protein is reported to be due to the peptides formed on digestion of the conglycinin fraction (Lynch et al., 1994), whereas the inhibitory nature of casein is thought to be due to non-absorbable complexes formed between iron and casein phosphopeptides (Hurrell et al., 1989).

Ascorbic acid enhances non-haem iron absorption through its ability to reduce Fe^{3+} to Fe^{2+} iron at low pH and also its chelating properties (Conrad and Schade, 1968). The effect is dose-dependent over a wide range (Cook and Monsen, 1977) and is most pronounced with meals containing high levels of inhibitors such as phytate (Hallberg et al., 1989). Ascorbic acid can ameliorate most or all of the inhibitory effects of other food components, as well as enhance the absorption of all iron fortification compounds (Hurrell, 1992) except NaFeEDTA (Troesch et al., 2009).

The relevance of results from single-meal absorption studies to whole diets has been questioned. They appear to exaggerate the effect of dietary enhancers and inhibitors, probably because of the test conditions used for single-meal absorption studies. Absorption efficiency is maximised after an overnight fast; in addition, the effects of enhancers and inhibitors are more pronounced when consumed in a single meal when there is no opportunity for adaptive responses to modulate absorption. The intestinal setting for uptake and transfer of iron, the primary homeostatic mechanism to maintain body iron balance, needs time to respond to changes in diet over longer time periods. Longer term interventions with single enhancers and inhibitors do not support results from single-meal studies, leading to the conclusion that dietary modulators of iron absorption are less important in the context of a Western diet than single-meal studies would suggest (Cook et al., 1991). Either there is a blunted effect, e.g. with ascorbic acid (Cook and Reddy, 2001) and meat (Reddy et al., 2006), or the effect is no longer observed, e.g. with calcium (Reddy and Cook, 1997), and it has been suggested that the association between meat consumption and higher iron status is mainly a result of the intake of haem iron rather than being an enhancing effect on non-haem iron absorption (Reddy et al., 2006).

To compare and contrast results from different absorption studies, the individual data are usually "normalised" with regard to body iron status, as this is the key determinant of efficiency of absorption. One method involves the expression of the results as relative bioavailability by comparing the test substance/food/meal with a reference dose of iron, often 3 mg of well-absorbed iron such as ferrous sulphate or ascorbate (Layrisse et al., 1969). The observed absorption from the test food/meal is corrected to a mean reference value of 40 %, which corresponds to absorption by individuals with borderline low iron stores. This is achieved by multiplying test meal absorption values by 40 / R, where R is the reference dose absorption (Magnusson et al., 1981). Another widely used method is to correct the measured absorption to a serum ferritin concentration corresponding to low levels of iron stores (Cook et al., 1991) by using the following equation:

$$\text{Log } A_c = \text{Log } A_o + \text{Log } F_o - \text{Log } F_r$$

where A_c is corrected dietary absorption, A_o is observed absorption, F_o is the observed serum ferritin concentration and F_r is the reference serum ferritin value selected. Values of 30 and 40 µg/L have been used for F_r (Cook et al., 1991; Reddy et al., 2000). This method does not require administration of a reference dose of iron and is therefore simpler to use.

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) proposed dietary iron bioavailability values for setting Dietary Reference Values (DRVs) of 15, 10 or 5 % depending on the composition of the diet, but the evidence base from which these values were obtained was not provided. The highest bioavailability value is for diversified diets with generous amounts of meat and/or foods rich in ascorbic acid. The lowest bioavailability is for diets based on cereals, tubers and legumes with little or no meat or ascorbic acid-containing fruits and vegetables (Allen et al., 2006).

Collings et al. (2013) undertook a systematic review of studies measuring non-haem iron absorption from whole diets, the aim of which was to derive absorption factors that could be used for setting DRVs. There was a wide range in mean percentage absorption values reported (0.7–22.9 %), with different conversions applied to allow for differences in iron status, so a meta-analysis was not possible. It was, however, clear that diet had a greater effect on absorption when iron status (serum ferritin concentration) was low, and absorption was higher in the presence of one or more enhancers, although single inhibitors did not appear to reduce absorption significantly.

In pregnant women, there are studies demonstrating a higher efficiency of non-haem iron absorption. A longitudinal study reported the geometric mean percentage absorption from a breakfast meal to be 7 % (95 % confidence interval (CI) 5–11 %) at 12 weeks of gestation, 36 % (95 % CI 28–47 %) at 24 weeks of gestation and 66 % (95 % CI 57–76 %) at 36 weeks of gestation (Barrett et al., 1994). There does not appear to be an increase in haem iron absorption; in pregnant women (32–35 weeks of gestation), percentage utilisation (red blood cell incorporation) of haem iron (in pork meat labelled with ^{58}Fe stable isotope) was significantly higher than that of ferrous sulphate (labelled with ^{57}Fe stable isotope), 47.7 (standard deviation (SD) 14.4) % and 40.4 (SD 13.2) %, respectively, whereas, in non-pregnant women, the values were 50.1 (SD 14.8) % and 15.3 (SD 9.7) %, respectively (Young et al., 2010). There are limited data on iron absorption from whole diets in pregnant women. Svanberg et al. (1975) undertook a longitudinal study measuring non-haem iron absorption from a radiolabelled meal given on two consecutive days at 12, 24 and 36 weeks of gestation. Mean absorption was 1.5 (SE 0.4) %, 5.8 (SE 0.8) % and 14.6 (SE 1.3) %, respectively, although there is no means of normalising the data to account for the effect of differences in iron status, as serum ferritin concentration was not measured and a reference dose of iron was not given. However, it is clear that physiological requirements for the products of conception, as with other physiological states associated with increased requirements, such as low body iron status, result in a marked increase in the efficiency of non-haem iron absorption. The Panel notes that percentage absorption values derived from studies in (non-pregnant) adults and algorithms may not be appropriate for pregnant women, particularly in the second and third trimester.

The Panel notes the limited information on the effects of systemic and dietary factors on iron absorption from whole diets in adults and the very limited data in infants and children. One study (Lynch et al., 2007) measured absorption from two consecutive meals in 1- to 4-year-old children and the results appeared to support observations in adults that iron status is a key determinant of efficiency of non-haem iron absorption.

Vegetarians have been reported to have lower iron stores than omnivores, which is attributed to the absence of meat (and fish) in their diet, but they are usually above the cut-off for serum ferritin concentration of 15 µg/L (SACN, 2010). Kristensen et al. (2005) measured the effect of consuming pork meat on radiolabelled non-haem iron absorption over a 5-day period and reported a significantly higher absorption from Danish (7.9, SE 1.1 %) and Polish (6.8, SE 1.0 %) pork meat diets than from a vegetarian diet (5.3, SE 0.6 %). The volunteers had a geometric mean serum ferritin concentration of 19 (range 12–28) µg/L at screening, and when the absorption values were adjusted to a serum ferritin concentration of 30 µg/L (Cook et al., 1991), the corrected absorption fell to 4.2 (SE 0.6) %, 3.6 (SE 0.7) % and 2.5 (SE 0.4) % for the Danish meat, Polish meat and vegetarian diets, respectively. Hunt and Roughead (1999) undertook an intervention study (randomised cross-over design) comparing the effect of a lacto-ovo-vegetarian and omnivorous diet for eight weeks on serum ferritin concentrations of 21 women aged 20–42 years, and reported that the type of diet had no effect on serum ferritin concentrations. The Panel considers that DRVs do not need to be derived for vegetarians as a separate population group because the bioavailability of iron from European vegetarian diets is not substantially different from diets containing meat and other flesh foods.

2.3.3. Metabolism

The body has no mechanism for the excretion of iron, and it is argued that the acquisition and distribution of the element is tightly regulated, in order to avoid excessive accumulation of the element. This control of body iron depends on an effective co-ordination of intestinal uptake and transfer of iron, with the recycling of iron from the red blood cell mass and other tissues, the storage and release of iron from the liver, and integumental (i.e. loss from the epidermis and epithelia) and, in women, menstrual losses. At the functional level, the cells involved are the enterocytes, hepatocytes and macrophages of the RES (i.e. the monocyte–macrophage system). In macrophages, the uptake and export of iron is mediated by DMT1 and ferroportin, respectively, and as with enterocytes these processes are regulated by hepcidin (Ganz, 2013). A schematic diagram of whole-body iron metabolism is shown in Figure 1.

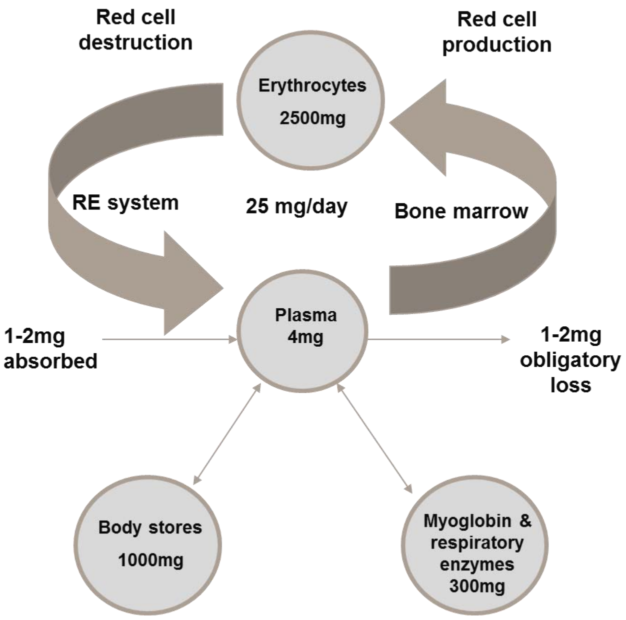

Figure 1: Whole-body iron metabolism. RE, reticuloendothelial

2.3.3.1. Systemic distribution and turnover

The systemic turnover of iron has the liver at its hub. The liver acts as the sensor of systemic requirements for iron and the regulator of intestinal absorption of iron and of its distribution (as di-ferric transferrin) to peripheral organs and tissues, all of which are equipped with cell membrane transferrin receptors that enable the endocytosis of transferrin and the intracellular release of iron. There are two types of transferrin receptor (TfR): TfR1 is ubiquitous and is most abundant in erythroblasts, lymphoid tissues and the neuroepithelium, whereas TfR2 is principally sited on the basolateral membranes of hepatocytes, where it contributes to the sensory system controlling iron metabolism.

The residual apotransferrin is released into the extracellular fluid, whereas the iron is either distributed to cytoplasmic functional sites and depots (ferritin) or transferred into the mitochondria where it is incorporated into the synthesis of iron–sulphur clusters and haem. Degradation of tissues results in the release of iron, which may be redistributed to other organs or recycled to the liver. The largest component to the pool of recycling iron is that produced by the breakdown of senescent red blood cells in the RES including the spleen. The size of the recycling pool is reduced by adventitious losses of iron through blood loss, and epithelial, integumental and urinary losses, and by its use for new tissue synthesis (e.g. growth, pregnancy). The recycling and salvage of endogenous iron is at least 90 % efficient. Any depletion is detected by hepatocytic TfR2, which, in turn via hepcidin, regulates the intestinal uptake and transfer of iron to replenish the recycling pool.

2.3.3.2. Homeostasis of cellular iron

Cellular iron homeostasis is mediated by two iron-responsive proteins (IRP1 and IRP2) which bind to iron-responsive elements (IREs) of mRNAs for proteins involved in iron kinetics. When iron supply is limited, the IRPs repress the production of the apoferritin chains, ferroportin, hypoxaemia-inducible factor 2α and δ-aminolevulinate synthase, the latter being the initial and rate-limiting enzyme in haem synthesis. This conserves cellular iron by reducing the ferroportin export of iron and inhibiting synthesis of erythropoietin and haem. Simultaneously, the IRPs increase induction of TfR1, DMT1 and an organising molecule for the actin cytoskeleton necessary for endocytosis, thereby sustaining production of the cellular apparatus for the uptake of iron (Richardson et al., 2010; Ye and Rouault, 2010).

If cells have an adequate supply of iron, the synthesis of the IRPs is reduced, as is their stability, and the proteins are subjected to proteolysis. This iron-responsive intracellular regulatory complex involves some highly conserved iron–sulphur clusters and proteins, and is disrupted by, amongst other things, hypoxia and inflammation, oxygen and nitrogen radicals, and nitric oxide (Richardson et al., 2010).

2.3.4. Transport in blood

The main carrier of iron in the extracellular space and systemic circulation is transferrin, which is synthesised, mainly in the liver, as a sialylated glycoprotein, apotransferrin. This protein binds one or two ferric iron molecules and delivers them to cell surface TfR1. Approximately 80 % of transferrin-bound iron is used for haemoglobin synthesis, and the half-life of recently absorbed iron in plasma is about 75 minutes.

The degree of sialylation of transferrin affects its function. For example, transferrin is more highly sialylated in pregnancy, which favours binding to placental transferrin receptors and the uptake of iron by the placenta, whereas, with infections and eclampsia, transferrin is less sialylated, which limits its binding to transferrin receptors.

2.3.5. Distribution to tissues

About 25 mg of systemic iron is recycled daily (Figure 1). Much of this turnover represents the salvage and recycling of iron from the 10^{11} senescent erythrocytes daily by the monocyte–macrophage system. Iron is released from the red blood cell haem by haemoxygenase, and it is either exported as ferric iron by the macrophages' ferroportin to apotransferrin, which moves the iron elsewhere, or deposited in the macrophages' intracellular ferritin pool. Iron from the turnover of other tissues is recycled similarly by the monocyte–macrophage system.

Transferrin–TfR complexes on cell membranes are endocytosed. The pH of the endosome is reduced through the activity of a proton pump, which decreases the affinity of transferrin for iron, and iron is released, reduced to the ferrous form by a ferrireductase in the endosomal membrane, and transferred out of the endosome into the cytoplasm by DMT1. In the cytoplasm it forms a chelatable iron pool, which supplies iron for metabolic needs, including iron uptake by the mitochondria for haem and iron–sulphur cluster synthesis (Richardson et al., 2010). The apotransferrin and TfR proteins return to the cell surface and the apotransferrin is recycled into plasma.

The circulation contains a small amount of non-transferrin-bound iron. Some of this is circulating ferritin, which has a high L-chain content, suggesting it is from the RES rather than from the liver. Other circulating ligands include acetate, citrate and albumin. Furthermore, a siderophore-bound form of iron has been found in mammals. The significance of these forms is unknown. However, whereas the transferrin cycle of iron is essential for red blood cell production, other tissues are able to acquire iron from non-transferrin-bound iron (cited in Chen and Paw (2012)).

In pregnant women, similar transport mechanisms exist for the placental transfer of iron. In the developing fetus, iron is accumulated against a concentration gradient and, even with maternal iron deficiency, the placenta can protect the fetus through the increased expression of placental TfR together with a rise in DMT1. Iron released from endosomes is carried across the basolateral membrane by ferroportin and is oxidised from ferrous to ferric iron by zyklopen, prior to incorporation into fetal transferrin. An additional haem transport system has been hypothesised, which may explain why certain gene knockouts are not lethal for the developing fetus (McArdle et al., 2014).

During lactation, the uptake of iron into the mammary gland follows the same process as in other cells, but there is no evidence that DMT1 facilitates iron export from endosomes. Iron in the intracellular chelatable iron pool can be secreted across the luminal membrane into milk. Export of iron from the mammary gland is most likely achieved by ferroportin, which is localised to the endoplasmic reticulum in reticuloendothelial cells, where it is believed to transport iron into intracellular vesicles prior to secretion (Lonnerdal, 2007).

2.3.6. Storage

Whole-body iron is approximately 3.8 g in men and 2.3 g in women, which is equivalent to 50 mg/kg body weight for a 75-kg man (Bothwell et al., 1979; Bothwell, 1995) and 42 mg/kg body weight for a 55-kg woman (Bothwell and Charlton, 1981). More recently, Hunt et al. (2009) assessed obligatory loss of endogenous iron twice yearly for up to three years in 53 free-living subjects using values based on haemoglobin concentrations (3.39 mg iron/g haemoglobin), estimated total blood volumes calculated with formulae based on body weight and height, systemic iron stores calculated from serum concentrations of TfR and ferritin, and the loss of a previously administered radioiron tracer. Whole-body iron was calculated to be 4.4 g in men and 2.8 g in women, and to be 48 mg/kg body weight in males and 38 mg/kg body weight in females (Hunt et al., 2009) (see Section 2.3.7.3).

The main systemic depot for iron is the liver, where it is stored as the soluble protein complex ferritin and, to a lesser extent, ferritin-derived insoluble haemosiderin. Estimates of body iron distribution are as follows: haemoglobin 2.5–3.5 g, myoglobin 0.3–0.4 g, and the haem and non-haem enzymes 100 mg. Ferritin and haemosiderin together comprise 1.0 g of iron (although this is very variable) and the transit pools of extracellular transferrin and intracellular carriers are considered to contain around 3 mg and 7 mg of iron, respectively.

Iron that is not functionally used and that cannot be excreted by cells is deposited in ferritin in the cytosol and mitochondria. Ferritin is a hollow sphere comprising 24 apoferritin subunits. It has channels through which iron can enter and leave the sphere. There are two subunits, heavy and light, and the ratio of these varies between organs (heavy chains predominate in the heart and brain, and light chains in the liver and spleen). Ferritin contains iron in the ferric state; this is enabled by the heavy chain, which has a ferroxidase activity, and the ratio of heavy to light chains influences the mobility of their associated iron. Expression and synthesis of the heavy and light apoferritin chains and that of other proteins mediating iron turnover are controlled by a common intracellular iron-sensing system, and their synthesis is promoted by an adequate iron supply and by inflammation, ionic iron and oxidative stressors. The principal pools of ferritin are the liver and the RES. The former mobilises iron to maintain the systemic pool and is the main repository for excess iron, whereas the latter represents an endogenous recycling pool of iron supporting the erythron.

2.3.7. Losses

As the body has no specific pathway for the excretion of iron, it is only lost from the body adventitiously via turnover and shedding of skin and hair, via the mucosa of the gastrointestinal, respiratory and genito-urinary tracts, and via sweat, intestinal secretions (including bile), urine, semen and menstrual blood.

2.3.7.1. Losses via skin, hair, sweat, urine and faeces

Estimations of dermal and sweat losses of iron are methodologically and analytically challenging. Although some differentiation between the amount of iron in sweat and that in exfoliated skin cells can be achieved when great care is taken (Jacob et al., 1981), these studies demonstrate that dermal iron losses are not directly related to estimated endogenous iron load or to dietary intake, but are closely related to body weight and size. This relates to the greater epithelial surface area of larger people; a similar but non-significant correlation can be detected in women if their data are corrected for menstrual losses (Hunt et al., 2009). The vast majority of iron excreted in the faeces is dietary in origin (unabsorbed iron), but a small quantity of systemic iron is excreted in the intestinal tract, primarily via biliary secretions. Iron losses in urine are very small, totalling approximately 0.08 mg/day (IOM, 2001).

2.3.7.2. Menstrual iron losses

There is a very wide inter-individual variation in menstrual blood loss, but for individuals it is fairly constant between cycles (Hallberg and Nilsson, 1964). Excessive menstrual blood loss (hypermenorrhoea) is a well-established risk factor for iron deficiency anaemia. The classic definition of hypermenorrhoea is a blood loss of 80 mL or more per cycle (Warner et al., 2004), and it is influenced by contraceptive use; losses are reduced with oral contraceptives (Larsson et al., 1992) and increased with intrauterine devices (Milsom et al., 1995). In the 1960s, before widespread use of oral contraceptives, Hallberg et al. (1966a) measured menstrual losses in groups of Swedish females aged 15, 23, 30, 40 and 50 years, and reported a mean value for all 476 females of 43.4 mL. The group of 15-year-olds (n = 95) had the smallest mean value of menstrual blood loss (33.8 mL, 90th percentile 65.1 mL) and the group of 50-year-olds (n = 37) had the highest mean value of menstrual blood loss (62.4 mL, 90th percentile 133.1 mL); the 90th percentile for all ages combined was 83.9 mL. No information on contraceptive use was given. The authors concluded that the upper normal limit of menstrual blood loss is between 60 and 80 mL and that a loss above 80 mL should be considered as pathological. Menstrual iron losses have been estimated to account for 90 % of the variance in the loss of endogenous iron for women (Hunt et al., 2009).

In a small study of 13 premenopausal women, iron losses in menstrual periods ranged from 0.5 to 56 mg per period or, adjusted for the reported number of menstrual periods per year, 0.015 to 1.86 mg/day (Hunt et al., 2009). The geometric mean iron loss from menstruation was 0.28 (0.08 –SD, 1.05 +SD) mg/day when calculated on a daily basis. These values were similar to those derived earlier by Harvey et al. (2005), who undertook measurements in 90 women aged 18–45 years, 35.5 % of whom used oral contraceptives and 5.5 % used an intrauterine device, and reported a mean (SD) iron loss of 0.43 (0.45) mg/day with a median menstrual iron loss of 0.26 mg/day. The data were highly skewed, with 70 % of women losing less than 0.5 mg/day through menses. Hypermenorrhoea was observed in 7 % of the women. There was a significantly lower median blood loss (mL/cycle) in oral contraceptive users than in those using other forms of contraception (excluding intrauterine devices). Percentiles of iron losses in this group of 90 women are shown in Appendix A.

In a cohort of more than 12 000 randomly selected women aged 15–49 years from five European countries (Skouby, 2010), oral contraceptives were reported to be used by 45, 34, 27, 19 and 19 % of women in France, Germany, the UK, Italy and Spain, respectively; the overall mean was 30 %. Information on the use of intrauterine devices (which increase menstrual blood loss) is not provided, but this method of contraception is much less common than oral contraceptives because reversible long-term methods, which include intrauterine devices/systems, implants and injection, were used by only 11 % of the European study population.

According to data collected from Finland in 2007, the median age at natural menopause was 51 years (Pakarinen et al., 2010) and, based on data collected in the period 1979–1986 from 11 different countries for WHO, the median age at natural menopause ranged between 49 and 52 years (Morabia and Costanza, 1998).

2.3.7.3. Whole-body iron losses

In the context of setting DRVs, the most pragmatic approach is to avoid estimating total adventitious iron loss based on data for the individual routes of loss, as this increases variability; it is preferable to use composite data acquired from long-term studies of body iron loss. Iron radioisotopes have been used to label the systemic pool and enable measurement of losses of endogenous iron. Total obligatory losses from the body were measured in white men in the USA (0.95 ± 0.30 mg/day), Mestizo men in Venezuela (0.90 ± 0.31 mg/day) and Indian men in South Africa (1.02 ± 0.22 mg/day) (Green et al., 1968). The average loss was 0.9–1.0 mg/day, which equates to 14 µg/kg body weight per day for a 70-kg man. More recently, Hunt et al. (2009) measured basal losses of iron using a similar method to Green et al. (1968) in 29 men, 19 menstruating women and 5 postmenopausal women. Mean iron loss by men was 1.07 ± 0.47 (range 0.11–2.07, median 1.18) mg/day, which equates to 12 ± 5 µg/kg body weight per day; losses were normally distributed. Losses in the postmenopausal women were similar to those in the men, 1.08 ± 0.28 (range 0.86–1.57, median 0.99) mg/day, which equates to 16 ± 4 µg/kg body weight per day. In contrast, iron losses in the premenopausal women were highly skewed, with a geometric mean of 1.69 (0.98 –SD, 2.92 +SD; range 0.65–4.88) mg/day, which equates to a geometric mean of 23 (13 –SD, 40 +SD) µg/kg body weight per day. When the women using oral contraceptives (n = 4) were excluded from the analysis, the iron loss was higher, with a geometric mean of 1.89 mg/day. The Panel notes the relatively small number of individuals in this study and the wide variability, particularly in the premenopausal women, but considers the data to be the most accurate estimate of whole-body losses for deriving dietary requirements for iron.

2.3.7.4. Breast milk

Regulated transport of iron through the mammary gland epithelium is suggested by the lack of correlation between plasma mineral concentration and milk mineral concentration, and studies in animals have shown that iron is transported by DMT1 through the basolateral membrane into the alveolar cells and is then exported by ferroportin1 in the apical membrane. DMT1 and ferroportin1 concentrations are higher during early lactation and are possibly involved in iron transfer into milk (Leong and Lonnerdal, 2005). Transferrin receptors are also likely to be involved in iron uptake (Sigman and Lonnerdal, 1990). The mammary gland has a capacity to control milk iron concentration by adapting to both maternal deficiency and excess of iron (Lonnerdal, 2007). Thus, the iron concentration of human milk does not correlate with maternal iron intake (Picciano and McDonald, 2005) or status (Celada et al., 1982). No differences in iron concentration of milk from women receiving iron supplements were observed even in women with intakes of at least 30 mg iron/day (Picciano and Guthrie, 1976). This finding is in agreement with other investigators, who have been unsuccessful in attempts to raise the iron concentration in milk with dietary supplementation (Karmarkar and Ramakrishnan, 1960).

A wide range of values has been reported in the literature for iron in human milk at all stages of lactation, partly owing to differences in sampling procedures and timing (e.g. milk iron concentration is lower in the morning than in the afternoon), as well as differences in the stage of lactation. Changes in iron concentration throughout the day were explained by both intra-individual (53 %) and inter-individual (39 %) variation (Picciano and Guthrie, 1976). Milk iron concentration decreases with longer durations of lactation (Feeley et al., 1983); for example, Picciano (2001) reported that the iron concentration of milk in the early stages of lactation was 0.5–1.0 mg/L and that mature milk contained 0.3–0.9 mg/L. IOM evaluated nine studies with small groups of lactating women at various stages of lactation and concluded that the mean iron concentration of human milk is about 0.35 mg/L (IOM, 2001). SCF (2003) considered the iron concentration of mature breast milk to be about 0.3 mg/L on the basis of reported values in European women (Siimes et al., 1979), later confirmed by Domellof et al. (2004). In 30 women of Mexican-American heritage, Hannan et al. (2009) found a mean iron concentration in milk of 0.5 ± 1.0 mg/L on days 30–45 of lactation and 0.4 ± 0.3 mg/L on days 75–90. The Panel considers that the iron concentration of mature human milk in European women is around 0.3 mg/L.

2.3.8. Interactions with other nutrients

The availability of iron for absorption in the duodenum and small intestine is affected by a number of dietary constituents, which act as either inhibitors (e.g. phytate and polyphenols) or enhancers (e.g. ascorbic acid and animal tissue) (see Section 2.3.2). The mechanism of action is the formation of iron complexes in the digestive chyme in the gut lumen, and the strength of binding dictates whether or not the iron can be removed from the complex by DMT1. In addition, ascorbic acid reduces ferric (Fe^{3+}) iron to ferrous (Fe^{2+}), which is the chemical form that is taken up by DMT1 (see Section 2.3.1).

Calcium and zinc have been reported to reduce iron absorption, but the mechanisms are unclear and the effect appears to be short-term. The proposed mechanism for the inhibitory effect of calcium on iron absorption is internalisation of DMT1 (Thompson et al., 2010) and, because this is an acute effect, adaptation will occur with time, which could explain why long-term calcium supplementation studies fail to show an effect on iron status (Lonnerdal, 2010). A recent review of published studies on the effects of zinc on iron absorption concluded that the inhibitory effect of zinc occurs at a Zn–Fe (weight/weight) ratio of 1:1 in aqueous solutions but, importantly, there is no inhibitory effect in food matrices (Olivares et al., 2012). When iron absorption from a hamburger meal, labelled with radioiron, was measured in the presence of additional zinc (15 mg) and manganese (3 mg), there was no effect with added zinc, but manganese had a strong inhibitory effect (Rossander-Hulten et al., 1991). The mechanism is probably via competition for DMT1. Effects of copper and zinc on the regulation of iron transporters have recently been proposed (Scheers, 2013). Although there is no direct competition for DMT1, copper is required for the efflux of ferrous iron through ferroportin. Zinc up-regulates DMT1 expression in Caco-2 cells, thereby increasing iron uptake (Yamaji et al., 2001), and enhances ferroportin transcription by stimulating the binding of metal transcription factor 1 to the ferroportin promoter (Troadec et al., 2010).

Copper–iron interactions are influenced by age and stage of development (Collins et al., 2010). They can affect prenatal development (Gambling et al., 2008). In addition to the well-understood effects of copper deficiency on iron metabolism (leading to anaemia), there is some evidence suggesting that copper deficiency results in lower liver iron concentration, and delivery of iron (as well as copper) to the fetus may be compromised (Andersen et al., 2007).

Vitamin A can affect several stages of iron metabolism, including erythropoiesis and the release of iron from ferritin stores. A number of trials have been undertaken to examine the effect of vitamin A supplementation/fortification on indices of iron status (Michelazzo et al., 2013), and many report an impact of vitamin A on haemoglobin and other parameters. Studies examining the effect of vitamin A on iron absorption have produced conflicting findings and it is not clear whether vitamin A and/or iron status are key determinants of an effect (Hurrell and Egli, 2010).

Riboflavin is involved in erythropoiesis, and deficiency results in disturbances in the production of red blood cells. The mechanism is thought to be impaired mobilisation of iron from ferritin (via reduced flavins). The very limited evidence available suggests that iron absorption is not affected (Fairweather-Tait et al., 1992), and that the effects on iron are through changes in iron economy (Powers, 2003).

The Panel considers that interactions between iron and other minerals, vitamins and certain dietary constituents (see Section 2.3.2), in the context of a mixed European diet, are not relevant for setting DRVs for iron.

2.4. Biomarkers of intake and status

There are no known biomarkers of iron intake, so the information has to be obtained by measuring dietary intake. Accurate measurement of dietary iron intake is hampered by several factors including the quality of food composition data (especially information on haem iron and foods fortified with iron), and use of iron supplements. The approaches that can be used include duplicate diet collection, weighed or estimated (from household measures/portion sizes) dietary records, 24-hour recalls, diet history and (validated) food frequency questionnaires (FFQs) (EFSA NDA Panel, 2010).

A review of methods to assess iron status was published by Zimmermann (2008). They can be categorised according to whether they represent the main functional use of iron (synthesis of haemoglobin), transport and supply of iron to tissues, or iron storage (SACN, 2010), and include:

- Haemoglobin and haematocrit. These markers are widely used but have low specificity and sensitivity, and reference ranges and cut-off criteria differ with ethnicity, age, sex and the laboratory where it is measured. Intra-individual variability of haemoglobin is low (< 3 %). The measurements can be made in fasted or non-fasted blood samples and only small samples are required, so capillary blood can be used. However, this can lead to inaccurate or variable results if the capillary sample is not collected properly.
- Reticulocyte haemoglobin content. Measurement of reticulocyte haemoglobin content in peripheral blood samples is useful for diagnosis of iron deficiency in adults (Mast et al., 2002) and children (Brugnara et al., 1999; Ullrich et al., 2005; Bakr and Sarette, 2006). Reticulocyte haemoglobin content can be used to differentiate iron deficiency from other causes of anaemia.
- Mean cell volume (MCV), mean cell haemoglobin (MCH) and the red cell distribution width are part of the profile obtained from automated cell counter analysers, but are not commonly used in the diagnosis of iron deficiency. MCV is a relatively late indicator of iron deficiency and is affected by thalassaemia.
- Erythrocyte zinc protoporphyrin (ZPP). A lack of iron in the bone marrow during the final stages of haemoglobin synthesis leads to the incorporation of zinc into protoporphyrin instead. This is a common screening tool for field work but is affected by lead poisoning, malaria, chronic infections, inflammation and haemoglobinopathies.
- Serum iron, total iron-binding capacity (TIBC) and transferrin saturation. The serum iron pool comprises Fe^{3+}, bound to transferrin. The percentage transferrin saturation is the ratio of serum iron to TIBC. Although this biomarker can be used to screen for iron deficiency, it is limited by circadian variation and confounding effects of infectious diseases and many other clinical disorders. For these measurements, fasting blood samples must be taken, as serum iron is affected by dietary iron intake. Serum iron is sometimes used to diagnose iron overload (haemochromatosis).
- Bone marrow biopsy. The bone marrow is a major storage site for iron and the absence of stainable iron in the bone marrow is the gold standard for the diagnosis of iron deficiency anaemia, especially in the diagnosis of complicated anaemias in hospital patients. It is, however, an invasive procedure and there may be methodological problems with the aspiration of bone marrow. Therefore, it is not commonly used to measure iron status.
- Serum ferritin. This is probably the most useful laboratory measure of iron status, because the concentration is directly proportional to stainable iron in the bone marrow and thus is indicative of the capacity of hepatic stores to sustain iron levels in the erythron. Estimates from phlebotomy studies indicate that 1 μg/L of serum ferritin corresponds to 8 mg mobilisable iron from systemic stores (Walters et al., 1973). However, because serum ferritin is an acute phase protein, it may not provide an accurate estimate of iron stores in acute or chronic inflammation or infection.
- Soluble serum transferrin receptor (sTfR). This is a useful diagnostic tool for iron deficiency, being less confounded by inflammation than serum ferritin, although its diagnostic value for children in regions where malaria and infection are endemic is less certain.
- Ratio of sTfR (R) to ferritin (F). The ratio has been shown to be more reliable than either parameter alone for the identification of iron deficiency. It is the best predictor of absent bone marrow iron and is the most sensitive indicator of a change in iron status following iron supplementation. It was validated for men using quantitative phlebotomy plus correction for absorbed iron. Body iron can be calculated from the serum transferrin receptor/ferritin ratio

(body iron (mg/kg) = −[log(R/F ratio) − 2.8229] / 0.1207) and is particularly useful for assessing longitudinal changes in iron status, e.g. resulting from an intervention.

The greatest challenge when assessing iron status is to distinguish between iron deficiency anaemia and anaemia of chronic disease, the latter resulting from the enhanced expression of hepcidin (Section 2.3.1.2). Inflammatory biomarkers, such as C-reactive protein or α-1-acid glycoprotein, can be measured to identify the presence of infection or inflammation. Assessing iron status in populations in which infectious diseases are common, as in some developing countries, and in which inflammation is present, as in older adults (Fairweather-Tait et al., 2014), is most problematic. There is also limited information on reference values for infants and young children, and allowances have to be made for blood volume expansion in pregnancy. As most biomarkers of iron status have low sensitivity and specificity, they are sometimes combined in models to define iron deficiency, for example the ferritin model based on low serum ferritin and transferrin saturation and high ZPP. Although this increases specificity, these models tend to underestimate iron deficiency.

A pragmatic approach to identifying iron deficiency or a significant risk thereof is to use, as a threshold, a serum ferritin concentration of 15 μg/L. Where several indices can be measured, the best combination is haemoglobin, serum ferritin, sTfR and/or ZPP (see also Appendix B).

2.5. Effects of genotype

Hereditary haemochromatosis is one of the most common single-gene disorders found in Northern European populations. This disease is due to mutations in the *HFE* gene, and two common variants of the gene, C282Y and H63D, have been identified. The clinical penetrance of homozygosity for C282Y is very variable (ranging from 1 to 25 % depending on the study design and endpoints used) and the majority of individuals with this genotype do not present with iron overload (Beutler et al., 2002). However, in those affected, up to 10–33 % eventually develop haemochromatosis-associated morbidity (Whitlock et al., 2006). The mechanism for the effect is increased iron absorption (Pietrangelo, 2010). Homozygosity for the C282Y mutation has been reported to occur in approximately 0.5 % of the Caucasian population (Allen et al., 2008). The frequency of heterozygotes in Caucasians is estimated to be 13 % (range 9.5–18 %) (Nelson et al., 2001). Iron absorption does not appear to be significantly increased in heterozygotes (Hunt and Zeng, 2004), although the distribution of serum ferritin concentration is shifted to the right, indicating higher body iron levels (Roe et al., 2005). The *HFE* H63D variant is more widespread worldwide but has a less well-defined role in predisposing individuals to iron overload. Other types of genetic haemochromatosis are caused by defects in haemojuvelin, hepcidin, TfR2 and ferroportin, but these are very rare in European populations.

The Panel concludes that carriers of *HFE* mutations have the same dietary requirements for iron as wild-type individuals and that rare polymorphisms should not be taken into consideration when deriving DRVs for iron.

3. Dietary sources and intake data

3.1. Dietary sources

Foods that contain relatively high concentrations of iron include meat, fish, cereals, beans, nuts, egg yolks, dark green vegetables, potatoes and fortified food products; the iron content of dairy products and many fruits and vegetables is much lower.

Currently, ferrous bisglycinate, ferrous carbonate, ferrous citrate, ferric ammonium citrate, ferrous gluconate, ferrous fumarate, ferric sodium diphosphate, ferrous lactate, ferrous sulphate, ferrous ammonium phosphate, ferric sodium EDTA, ferric diphosphate (ferric pyrophosphate), ferric saccharate and elemental iron (carbonyl + electrolytic + hydrogen reduced) may be added to both foods[6] and food supplements,[7] whereas ferrous L-pidolate, ferrous phosphate and iron (II) taurate may be used in food supplements only.[7] The iron content of infant and follow-on formulae[8] and processed cereal-based foods and baby foods for infants and young children[9] is regulated.

3.2. Dietary intake

EFSA estimated dietary intakes of iron from food consumption data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011a), classified according to the food classification and description system FoodEx2 (EFSA, 2011b). Data from 13 dietary surveys from nine EU countries were used. The countries were Finland, France, Germany, Ireland, Italy, Latvia, the Netherlands, Sweden and the UK. The data covered all age groups from infants to adults aged 75 years and older (Appendix C).

Nutrient composition data for iron were derived from the EFSA Nutrient Composition Database (Roe et al., 2013). Food composition information of Finland, France, Germany, Italy, the Netherlands, Sweden and the UK were used to calculate iron intakes in these countries, assuming that the best intake estimate would be obtained when both the consumption data and the composition data are from the same country. For nutrient intake estimates of Ireland and Latvia, food composition data from the UK and Germany, respectively, were used, because no specific composition data from these countries were available. In the case of missing values in a food composition database, data providers had been allowed to make use of values from another country's database. The amount of borrowed iron values in the seven composition databases used varied between 15 and 85 %. Estimates were based on food consumption only (i.e. without dietary supplements). Nutrient intake calculations were performed only on subjects with at least two reporting days.

Data on infants were available from Finland, Germany, the UK and Italy. The contribution of human milk was taken into account if the amounts of human milk consumed (Italian INRAN-SCAI survey and the UK DNSIYC) or the number of breast milk consumption events (German VELS study) were reported. In the case of the Italian INRAN-SCAI survey, human milk consumption had been estimated based on the number of eating occasions using standard portions per eating occasion. In the Finnish DIPP study, only the information "breast-fed infants" was available, but without any indication about the number of breast milk consumption events during one day or the amount of breast milk consumed per event. For the German VELS study, the total amount of breast milk was calculated based on the observations by Paul et al. (1988) on breast milk consumption during one eating occasion at different ages, i.e. the amount of breast milk consumed on one eating occasion was set to 135 g/eating occasion for infants aged 6–7 months and to 100 g/eating occasion for infants aged 8–12 months. The Panel notes the limitations in the methods used for assessing breast milk consumption in infants and related uncertainties in the intake estimates for infants (Appendices D and E).

Average iron intake ranged between 2.6 and 6.0 mg/day (0.9–1.9 mg/MJ) in infants (< 1 year, four surveys), between 5.0 and 7.0 mg/day (1.2–1.6 mg/MJ) in children aged 1 to < 3 years (five surveys), between 7.5 and 11.5 mg/day (1.1–1.7 mg/MJ) in children aged 3 to < 10 years (seven surveys), between 9.2 and 14.7 mg/day (1.1–1.7 mg/MJ) in children aged 10 to < 18 years (seven surveys) and between 9.4 and 17.9 mg/day (1.2–2.1 mg/MJ) in adults (≥ 18 years) (eight surveys). Average daily intakes were in most cases slightly higher in males (Appendix D) than in females (Appendix E), mainly owing to larger quantities of food consumed per day.

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[6] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26.

[7] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

[8] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 1.

[9] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 06.12.2006, p. 16.

The main food group contributing to iron intake was grains and grain products representing more than 20 % and up to 49 % of the iron intake in all population groups except infants. Other main contributing food groups were meat and meat products, vegetable and vegetable products and composite dishes. Differences in the main contributors to iron intakes between sexes were minor (Appendices F and G).

EFSA's iron intake estimates in mg/day were compared with published intake values from the same survey and dataset and the same age class using the German EsKiMo and VELS surveys in children (Kersting and Clausen, 2003; Mensink et al., 2007), the DIPP study in Finnish children (Kyttälä et al., 2008; Kyttälä et al., 2010), the study in Finnish adolescents (Hoppu et al., 2010), the French national INCA2 survey (Afssa, 2009), the Irish National Adult Nutrition Survey (IUNA, 2011), the FINDIET 2012 survey (Helldán et al., 2013), the Italian INRAN-SCAI survey (Sette et al., 2011), the Dutch National Food Consumption Survey (van Rossum et al., 2011), the Swedish national survey Riksmaten (Amcoff et al., 2012), the DNSIYC-2011 study in UK infants and young children (Lennox et al., 2013) and the UK National Diet and Nutrition Survey (Bates et al., 2012) (Table 1).

Table 1: EFSA's average daily iron intake estimates, expressed as percentages of intakes reported in the literature

Country	Percentage of published intake (% range over different age classes in a specific survey)
Finland	83 (DIPP young children, 1 to < 3 years), 104 (DIPP children, 3 to < 10 years), 111–116 (NWSSP), 100–105 (FINDIET 2012)
France	96–115 (INCA2)
Germany	90–99 (VELS infants), 111–122 (VELS children), 101–108 (EsKiMo)
Ireland	104–109 (NANS)
Italy	94–102 (INRAN-SCAI infants and young children, 1 to < 3 years), 98–102 (INRAN-SCAI other age groups)
Netherlands	108–113 (DNFCS)
Sweden	116–121 (Riksmaten)
UK	107–109 (DNSIYC infants and children up to 1.5 years), 95–112 (NDNS Rolling Programme, Years 1–3)

DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

When the EFSA intake estimates were compared with published intake estimates from the same survey and age range, the EFSA estimates differed up to around 15 % from the published values in Finland, France, Ireland, the Netherlands, the UK and Germany, except for German children in the VELS study, for which they were higher by up to 22 % than published values. In Sweden, the EFSA intake estimates were higher by 16–21 % than published values. Overall, several sources of uncertainties may contribute to these differences, including inaccuracies in mapping food consumption data according to food classifications and in nutrient content estimates available from the food composition tables, the use of borrowed iron values from other countries in the food composition database, and replacing missing iron values by values of similar foods or food groups in the iron intake estimation process. It is not possible to conclude which of these intake estimates (i.e. the EFSA intake estimate or the published one) would be closer to the actual iron intake.

Iron intakes in 521 457 individuals aged 35–70 years from 10 European countries were recently calculated as part of the European Prospective Investigation into Cancer and Nutrition study (Jakszyn et al., 2013). Total iron intake was around 12 mg/2 000 kcal with mean (SD) intakes of haem and non-haem iron, expressed as mg/2 000 kcal, of 0.49 (0.26) and 11.51 (2.67), respectively, in tertile 1 of haem iron intake; and 1.91 (0.59) and 11.96 (2.29), respectively, in tertile 3 of haem iron intake. Although haem iron represented only 4 % of the total iron intake in omnivores, it is more bioavailable than non-haem iron; therefore, its potential contribution to total absorbed iron is greater than the intake values indicate.

4. Overview of Dietary Reference Values and recommendations

4.1. Adults

The German-speaking countries (D-A-CH, 2015) considered that iron requirements depend on iron losses through the intestine, the kidneys, the skin (about 1 mg/day) and menses (for menstruating women, about 15 mg/month), although about 20 % of women have substantially higher monthly iron losses (Hallberg et al., 1966b). Dietary iron absorption in the majority of industrial countries was considered to be between 10 and 15 % (FAO/WHO, 1988), or higher by two- or three-fold in the case of iron deficiency. With an absorption of 10–15 %, an iron intake of 15 mg/day was estimated to provide the body with 1.5–2.2 mg of absorbed iron per day and to cover the needs of all women with normal menstrual blood losses. Based on German data (Arab-Kohlmeier et al., 1989), the German-speaking countries considered that postmenopausal women would not have a higher iron requirement than men, for whom the Recommended Intake (RI) was set at 10 mg/day.

The Nordic countries (Nordic Council of Ministers, 2014) considered (1) median basal iron losses of 0.014 mg/kg body weight per day (Green et al., 1968), multiplied by mean body weight for the Nordic population, and (2) for women of childbearing age, menstrual iron losses (median, 90th and 95th percentile) evaluated from the amount of menstrual blood losses (median 30 mL/28 days) (Hallberg et al., 1966b; Hallberg and Rossander-Hultén, 1991), a haemoglobin concentration of 135 g/L and an assumed iron content of 3.34 mg/g of haemoglobin. For women of childbearing age, iron absorption was assumed to be 15 %, although subjects in the top 5th percentile of iron requirement probably have a higher absorption rate. Blood loss during menstruation was considered to be variable among adult women, but fairly constant for a given woman (Hallberg and Rossander-Hultén, 1991). Finally, for women of childbearing age, an AR was set at 10 mg/day and an RI was set at 15 mg/day, which corresponds to the amount of iron required to meet the needs of about 90 % of women. In addition, a lower level of intake of 5 mg/day was set for postmenopausal women, while a value of 7 mg/day was set for men, considering their greater body size. To cover basal iron losses, ARs of 6 mg/day for postmenopausal women and of 7 mg/day for men were derived and RIs were set at 9 mg/day for both population groups.

WHO/FAO (2004) adapted the conclusions from their earlier report (FAO/WHO, 1988), considering more recent calculations on the distribution of iron requirements in menstruating women (Hallberg and Rossander-Hultén, 1991). They considered mean body weights, median basal iron losses and, for women of childbearing age, the median and 95th percentile of menstrual iron losses (without taking into account the normal variation in haemoglobin concentration), in order to calculate the median and the 95th percentile of total requirements for absorbed iron. Total basal iron loss from the skin, the intestine, the urinary tract and the airways was considered to be 0.014 mg/kg body weight per day (Green et al., 1968), and the range of individual variation was estimated to be ±15 % (FAO/WHO, 1988). Median basal iron losses of 1.05 mg/day for adult men and 0.87 mg/day for adult women were estimated. Menstrual blood losses were considered to be constant for a given woman, but variable among women (Hallberg et al., 1966b), and greatly influenced by the choice of contraceptive method; moreover, their distribution was considered to be highly skewed. The median and 95th percentile of menstrual iron losses were estimated to be 0.48 and 1.90 mg/day for women of childbearing age. The median and the 95th percentile of total absorbed iron requirements were estimated to be 1.46 and 2.94 mg/day for women of childbearing age, 1.05 and 1.37 mg/day for men, and 0.87 and 1.13 mg/day for postmenopausal women. WHO/FAO also considered that iron requirements per unit of body weight for postmenopausal women and physically active older adults are the same as for men, but that, when physical activity decreases with advanced age, blood volume and haemoglobin mass decrease, leading to a shift of iron usage from haemoglobin and muscle to iron stores, and therefore a reduction in iron requirements. The main source of variation in iron status in different populations was considered to be variation in iron absorption, and the amount of dietary iron absorbed was considered to be mainly determined by body iron stores and by the properties of the diet, i.e. iron content and bioavailability. WHO/FAO finally based their Recommended Nutrient Intakes on the 95th percentile of the total requirements for absorbed iron, and considered four different bioavailability figures: 15 and 12 % (for Western-type diets, depending mainly on meat intake), and 10 and 5 % (for developing countries). The Recommended Nutrient Intakes for an iron bioavailability of 15 % were set at 9.1 mg/day for adult men, 19.6 mg/day for women of childbearing age and 7.5 mg/day for postmenopausal women.

The SCF (1993) followed a similar approach to that of WHO/FAO (2004), i.e. adapted the data from the earlier report (FAO/WHO, 1988) using more recent data on the distribution of iron requirements in menstruating women (Hallberg and Rossander-Hultén, 1991). It considered the same data for basal iron losses (Green et al., 1968) (2 SD being added to the median basal iron loss to estimate the 95th percentile) and menstrual blood losses (Hallberg et al., 1966b). Assuming a bioavailability of 15 %, the SCF based their PRI on the 95th percentile of total iron requirements and set the same values as WHO/FAO (2004). However, it also proposed rounded figures and, for menstruating women, two PRI values based on the 90th and 95th percentiles of total iron requirements, as the SCF considered that a PRI based on the 95th percentile would be unrealistically high for the great majority of women. The probability of adequacy among menstruating adult women for various amounts of absorbed iron was also provided, as well as the dietary intake necessary to provide these amounts, assuming a bioavailability of 15 %.

The Agence française de sécurité sanitaire des aliments (Afssa, 2001) considered that daily basal iron losses in adults due to desquamation of cells from the surfaces of the body are 0.9–1 mg, i.e. about 14 µg/kg body weight, comprising 0.6 mg for faecal, 0.2–0.3 mg for dermal and 0.1 mg for urinary losses. Iron bioavailability of the usual French diet was considered to be 10 % (Galan et al., 1985; Lynch and Baynes, 1996; Lynch, 1997). The PRI was set at 9 mg/day for adult men and postmenopausal women. For women of childbearing age, menstrual iron losses were considered in addition to basal iron losses (FAO/WHO, 1988; INACG, 1989). Afssa reported median menstrual blood losses between 25 and 30 mL/month, i.e. menstrual iron losses of 12.5–15 mg/month or 0.4–0.5 mg/day, and indicated that 50 % of women would have total iron losses higher than 1.3 mg/day and 10 % would have losses higher than 2.1 mg/day. Factors such as heredity, weight, height, age, parity and particularly choice of contraception method were mentioned to have an impact on the volume of menstrual blood losses. The PRI was set at 16 mg/day for women of childbearing age.

IOM (2001) considered the maximal bioavailability of iron to be 18 % in (non-pregnant, non-lactating) adults, based on a conservative estimate of 10 % for the proportion of haem iron in the diet of adults (Raper et al., 1984) and children (based on data of the Continuing Survey of Food Intakes by Individuals, CSFII, 1994–1996), a conservative estimate of 25 % for overall haem absorption (Hallberg and Rossander-Hultén, 1991), and an estimated bioavailability of non-haem iron in self-selected diets of 16.8 % for individuals with a serum ferritin concentration of 15 µg/L (Cook et al., 1991). IOM only took into account basal losses when estimating the needs for absorbed iron in adult men and postmenopausal women, and did not consider the higher iron stores in men than in women. Basal iron losses in men were assumed to be 0.014 mg/kg body weight per day, based on the study by Green et al. (1968). Owing to the lack of data to estimate the variability of basal losses in adult men, the median and variability for basal losses were calculated using the median body weight recorded in the National Health and Nutrition Examination Survey (NHANES) III and its variability calculated using the square root of the median weight for men. For men, the calculated median and 97.5th percentile for daily iron loss were 1.08 and 1.53 mg/day, respectively. The Estimated Average Requirement (EAR) was calculated by dividing the median daily iron loss by the estimated iron bioavailability and was set at 6 mg/day, and the Recommended Dietary Allowance (RDA) was calculated by dividing the 97.5th percentile of daily iron loss by the bioavailability and was rounded to 8 mg/day. For menstruating women, menstrual iron losses were added to basal iron losses using data from Hallberg et al. (1966b, 1966a); Hallberg and Rossander-Hultén (1991). Percentiles of blood loss were predicted from a log-normal distribution, and the predicted median was 30.9 mL/cycle. Blood losses per menstrual cycle were converted into estimated daily iron losses averaged over the whole menstrual cycle; haemoglobin concentration was taken as a constant (135 g/L) in adult women (Beaton et al., 1989); the iron content of haemoglobin was considered to be 3.39 mg/g (Smith and Rios, 1974); and the duration of the average menstrual cycle was considered to be 28 days (Beaton et al., 1970). Median menstrual iron loss was calculated as 0.51 mg/day and the 97.5th percentile was calculated to be 2.32 mg/day. As there were no direct measurements of basal iron losses (separated from menstrual iron losses) in women, values for women were derived from those used for men (Green et al., 1968) by linear body weight adjustment. The median and variability for basal losses were calculated in the same way as for men. The median and the 97.5th percentile of basal iron losses were therefore 0.896 and 1.42 mg/day, respectively. Distributions of requirement for absorbed iron and dietary iron were calculated by Monte Carlo simulation from the estimated distributions of menstrual and basal iron losses, considering a bioavailability of 18 %. For menstruating women not using oral contraceptives, the median absorbed iron requirement was calculated as 1.41 mg/day and was used to set the EAR at 8 mg/day (rounded value). Moreover, the calculated 97.5th percentile of absorbed iron requirement of 3.15 mg/day was used to set the RDA at 18 mg/day (rounded value). For postmenopausal women, basal iron losses were also taken as 0.014 mg/kg body weight per day (Green et al., 1968) and the median and variability for basal losses were calculated in the same way as for adult men. The calculated median and 97.5th percentile for daily iron loss were estimated at 0.896 and 1.42 mg/day, respectively. The EAR was calculated by dividing the median iron loss by the estimated iron bioavailability of 18 % and was set at 5 mg/day; and the RDA was calculated by dividing the 97.5th percentile of daily iron loss by the bioavailability and was rounded to 8 mg/day. Special considerations were made regarding the use of oral contraceptives and hormone replacement therapy (HRT), vegetarianism, intestinal parasitic infection, blood donation, and increased iron losses in exercise and intense endurance training. Based on a re-analysis of data on decreased menstrual blood losses in women using oral contraceptives (Nilsson and Solvell, 1967), a reduction of about 60 % was estimated, and the requirements at the 50th (EAR) and 97.5th (RDA) percentiles for premenopausal women using oral contraceptives were set at 6.4 and 10.9 mg/day, respectively. Women on HRT and still menstruating were considered to possibly have higher iron requirements than postmenopausal women not on HRT. The iron bioavailability of a vegetarian diet was estimated to be about 10 % (instead of 18 % for a mixed Western diet), and the iron requirement was thus considered to be 1.8 times higher for vegetarians. The EAR for iron was assumed to be 30 % greater in subjects engaged in regular intense exercise (Ehn et al., 1980) and 70 % greater in athletes (Weaver and Rajaram, 1992).

The Netherlands Food and Nutrition Council (1992) estimated average basal iron losses (through faeces, urine and sweat) to be 0.9 mg/day in men and 0.8 mg/day in women, and the average menstrual iron loss to be 0.8 mg/day. The average quantities of absorbed iron to compensate for total losses were 1.1 mg/day for men and 1.7 mg/day for women aged 19–22 years (adding an iron amount for growth to basal iron losses and, for women, menstrual losses); 0.9 mg/day for men and 1.6 mg/day for women aged 22 years and over; and 0.8 mg/day for postmenopausal women. Iron absorption from the Dutch diet was estimated to be 12 %, considering the estimated absorption of haem and non-haem iron (Hallberg, 1981), the average ratio of haem and non-haem iron, the vitamin C content and the quantity of meat in the Dutch diet, as well as studies on complete meals and breakfasts. The minimum requirements were estimated as 9 mg/day (19–22 years) and 8 mg/day (22 years and over) for men, and 14 mg/day (19–22 years), 13 mg/day (22 years and over) and 7 mg/day (postmenopause) for women. A coefficient of variation (CV) of 20 % was applied to cover variation in individual requirements (and a CV of 15 % was applied for growth). Adequate levels of daily intake were derived by adding 2 SD to the average minimum requirements for the different age and sex groups.

The UK Committee on Medical Aspects of Food Policy (COMA) (DH, 1991) considered daily iron losses of 0.14 mg through desquamated gastrointestinal cells, 0.38 mg for haemoglobin, 0.24 mg for bile and 0.1 mg through urine (Green et al., 1968), i.e. a total of 0.86 mg/day with a CV of 15 %, and the amount lost through skin and sweat was considered negligible (Brune et al., 1986). A bioavailability of 15 % was considered typical in industrialised countries (FAO/WHO, 1988). For adults over 50 years of age, the Lower Reference Nutrient Intake (LRNI) was set at 4.7 mg/day, the EAR was set at 6.7 mg/day and the RNI was set at 8.7 mg/day. In women of childbearing age, menstrual iron losses were estimated from Swedish data on menstrual blood loss, showing a highly skewed distribution (Hallberg et al., 1966b). For a 75th percentile of blood loss of 52.4 mL, a haemoglobin concentration of 13 g/100 mL and an iron content of haemoglobin of 0.347 %, the calculated menstrual iron losses were added to basal iron losses, leading to an EAR of 11.4 mg/day, an LRNI of 8.0 mg/day and an RNI of 14.8 mg/day, but this intake was considered to be insufficient for the 10 % of women with the highest menstrual losses. Specific considerations regarding frequent blood donors were also provided. The UK Scientific Advisory Committee on Nutrition (SACN) (2010) considered that these DRVs were derived from limited data but that new data were insufficient to reassess them.

An overview of DRVs for iron for adults is presented in Table 2.

Table 2: Overview of Dietary Reference Values for iron for adults

	D-A-CH (2015)	**NCM (2014)**	**WHO/FAO (2004)** $^{(a)}$	**Afssa (2001)**	**IOM (2001)**	**SCF (1993)**	**NL (1992)** $^{(b)}$	**DH (1991)**
Age (years)	19–50	18–60	≥ 18	≥ 20	19–50	≥ 18	19–22	19–50
PRI men (mg/day)	10	9 $^{(c)}$	9.1 for a bioavailability of 15 % (up to 27.4 for a bioavailability of 5 %)	9	8	9 $^{(c)}$	11	8.7
PRI women (mg/day)	15	15 $^{(d)}$ (postmenopause 9 $^{(c)}$)	19.6 for a bioavailability of 15 % (up to 58.8 for a bioavailability of 5 %)	16	18 (10.9 for women using oral contraceptives)	16 $^{(d)}$ 20 $^{(c)}$	16	14.8
Age (years)	≥ 51	≥ 61			≥ 50		≥ 22	≥ 50
PRI men (mg/day)	10	9 $^{(c)}$	As for younger men		8	As for younger men	9	8.7
PRI women (mg/day)	10	9 $^{(c)}$	Postmenopause: 7.5 for a bioavailability of 15 % (up to 22.6 for a bioavailability of 5 %)		8	Postmenopause 8 $^{(c)}$	15 (22–50 years)/ 8 (≥ 50 years)	8.7

NCM, Nordic Council of Ministers; NL, Netherlands Food and Nutrition Council.
(a): Recommended Nutrient Intake, based on the 95th percentile of iron requirements.
(b): Adequate level of daily intake.
(c): Based on the 95th percentile of iron requirements.
(d): Based on the 90th percentile of iron requirements.

4.2. Infants and children

The German-speaking countries (D-A-CH, 2015) estimated daily iron losses of infants and children to be 0.2–0.4 mg. Requirements for growth were considered to amount to 0.7 mg/day between 6 and 12 months, and 0.3–0.5 mg/day after the age of 1 year (Dallman, 1988; Fairbanks and Bleutler, 1988). The requirement for absorbed iron was estimated to be about 1 mg/day for infants aged 4 to < 12 months; hence, an iron intake of 1 mg/kg body weight per day or 8 mg/day was recommended. For older children, the German-speaking countries took into account iron losses and iron requirements for growth and concluded that about 0.8 mg/day of absorbed iron was needed, also taking into account the increased iron requirement during puberty owing to an increased growth rate and, for girls, the start of menstruation.

For children aged 6 months to 5 years, the Nordic countries (Nordic Council of Ministers, 2014) retained their previous recommendation of 8 mg/day, as no iron deficiency was observed in older infants consuming on average 9 mg/day of iron provided mostly by iron-fortified phytate-rich cereals (Lind et al., 2003), and as a higher recommendation would require a diet much denser in iron for that age group than for older children and adults. For children aged 6–9 years, an intake of 9 mg/day was recommended. For children aged 10–17 years, it was assumed that iron absorption is 15 %, although subjects in the top 5th percentile of iron requirement probably have a higher absorption efficiency. The Nordic countries considered (1) the iron requirements for growth, (2) median basal iron losses estimated to be 0.014 mg/kg body weight per day (Green et al., 1968) multiplied by mean body weight (Andersen et al., 1982), and (3) for menstruating girls, menstrual iron losses evaluated from the amount of menstrual blood losses (median: 28.4 mL/28 days) (Hallberg et al., 1966b; Hallberg et al., 1991; Hallberg and Rossander-Hultén, 1991; Borch-Iohnsen, 1993), a haemoglobin concentration of 135 g/L, an assumed iron content of 3.34 mg/g of haemoglobin and an equation (derived from a fitted log-normal distribution with a Monte Carlo simulation (IOM, 2001)) to calculate the 95th percentile of blood loss. Blood loss during menstruation was mentioned to be less variable among adolescent girls than adult women. The RIs correspond to the amount of iron to meet the needs of about 95 % of children of all age groups, except for girls after menarche, for whom the RIs are assumed to cover the needs of 90 % of the group.

For infants and children, WHO/FAO (2004) adapted the conclusions from their earlier report (FAO/WHO, 1988). They considered mean body weights, the iron requirement for growth, median basal iron losses and, for menstruating girls, the median and 95th percentile of menstrual iron losses (0.48 and 1.90 mg/day), in order to calculate the median and the 95th percentile of total requirements for absorbed iron for children between 0.5 and 17 years. The total basal iron loss was considered to be 0.014 mg/kg body weight per day (Green et al., 1968), and the range of individual variation was estimated to be ±15 % (FAO/WHO, 1988). Iron requirements in term infants were considered to rise markedly in the second half of infancy, as body iron stores almost double between the age of 6 months and 1 year, and then double again between 1 and 6 years of age. WHO/FAO stressed the high iron requirements of adolescents that are the result of rapid growth (Rossander-Hulthén and Hallberg, 1996), and the marked individual variation in growth rate and consequently in iron requirements (Hallberg et al., 1966b; Tanner et al., 1966a, 1966b; Karlberg and Taranger, 1976; Dallman and Siimes, 1979; FAO/WHO, 1988). The same considerations for women of childbearing age (see Section 4.1) are applied to menstruating girls regarding the intra-individual and inter-individual variability of menstrual blood losses (Hallberg et al., 1966b), their statistical distribution and the impact of contraceptive methods, as well as the impact of iron bioavailability. Finally, the Recommended Nutrient Intakes were based on the 95th percentile of the requirements for absorbed iron and the four levels of iron bioavailability already considered for adults (15, 12, 10 and 5 %). Separate values for pre- and post-menarchal girls aged 11–14 years were also provided.

The SCF (1993) followed an approach similar to that of WHO/FAO (2004), i.e. adapted the data from the earlier report (FAO/WHO, 1988) using more recent data on the distribution of iron requirements in menstruating women (Hallberg and Rossander-Hultén, 1991) and considering the same data for basal iron losses (Green et al., 1968) and iron requirements for growth (Karlberg and Taranger, 1976). For infants aged 0.5–1 year, bioavailability of iron from weaning foods was considered to be lower than that of iron from the adult diet because of an often high content of inhibitors of iron absorption such as milk and phytate in infant cereals, and a low content of enhancers of iron absorption such as meat and ascorbic acid. Moreover, the bioavailability of iron used to fortify infant foods was considered unknown. Therefore, bioavailability was assumed to be highly variable and on average lower than for other age groups, i.e. 10 %, and a PRI of 9.3 mg/day was set for older infants. For a bioavailability of 15 %, the SCF based their PRI on the 95th percentile of total iron requirements and set the same values as WHO/FAO (2004), but also proposed rounded figures and two PRI values based on the 90th and 95th percentiles of total iron requirements for menstruating adolescent girls.

Afssa (2001) considered daily basal iron losses of about 14 µg/kg body weight and a bioavailability of 10 % (Galan et al., 1985; Lynch and Baynes, 1996; Lynch, 1997). Afssa reported that iron requirements of infants were very high to cover basal losses, erythrocyte mass expansion and growth of body tissues, and that iron body stores doubled during the first year of life. Total iron requirements at 1 year of age were mentioned to be 8–10 times higher than those of an adult man if expressed per kilogram of body weight. Iron requirements for growth during adolescence and for menstrual losses in adolescent girls were also taken into account.

For infants aged 7–12 months, IOM (2001) modelled the major factorial components of absorbed iron requirements, which were basal (i.e. faecal, urinary and dermal) losses, the increase in haemoglobin mass, the increase in tissue iron and the increase in storage iron. Considering median body weights at 6 and 12 months (Dibley et al., 1987) and reference body weights from NHANES III (1988–1994), a CV for weight of 10 % and an estimated basal iron loss of 0.03 mg/kg body weight per day (Garby et al., 1964), and assuming its variability is proportional to the variability of weight, the mid-range estimate of basal losses for infants aged 6–12 months was calculated to be 0.26 ± 0.03 mg/day. The median weight increment was assessed to be 0.39 kg/month or 13 g/day (Dibley et al., 1987), considering a CV of 50 %. The increase in haemoglobin mass was calculated to be 0.37 ± 0.195 mg/day. This calculation was done by multiplying the median monthly weight increment by a blood volume of 70 mL/kg (Hawkins, 1964), a median haemoglobin concentration of 0.12 mg/mL and an iron content of haemoglobin of 3.39 mg/g (Smith and Rios, 1974), dividing by 30 days and applying the CV accepted for weight gain (50 %). The increase in tissue iron content was calculated as 0.009 ± 0.0045 mg/day. This was done by multiplying the median daily weight increment by the estimated tissue iron content of 0.7 mg/kg body weight at 1 year (Smith and Rios, 1974), assumed to be identical at 7 months of age, and applying the CV accepted for weight gain (50 %). The increase in storage iron was calculated as 0.051 mg/day. This was done by multiplying the sum of the increase in haemoglobin iron and the increase in non-storage iron by the percentage of total tissue iron stored (12 % (Dallman, 1986)), and dividing by the percentage of total iron not stored. The median total requirement for absorbed iron was therefore 0.69 ± 0.145 mg/day, and the 97.5th percentile was 1.07 mg/day. For a moderate bioavailability of 10 % (considering the low bioavailability of iron in fortified infant cereals (Davidsson et al., 2000) and the proportion of infants consuming meat at 1 year (Skinner et al., 1997)), the EAR was set at 6.9 mg/day using the median total requirement, and the RDA was set at 11 mg/day using the 97.5th percentile of the total requirement.

For children aged 1 to 8 years, IOM estimated the median rate of weight gain to be 2.29 kg/year or 6.3 g/day, from the slope of a linear regression of reported median body weights on age (Frisancho, 1990). The midpoints of 2.5 and 6.5 years were used to set the EAR and RDA for the age groups 1–3 years and 4–8 years, respectively. As for infants, the major components of iron requirement modelled by IOM (2001) were basal iron losses and the increase in haemoglobin mass, tissue iron and storage iron. Basal iron losses were derived from total iron losses measured in adult men (Green et al., 1968) adjusted to the child’s estimated body surface area (Haycock et al., 1978) (which is directly related to dermal iron losses (Bothwell and Finch, 1962)). Haemoglobin mass was estimated by multiplying blood volume at specific ages (Hawkins, 1964) by the estimated age- and sex-specific haemoglobin concentration ((Beaton et al., 1989), using 119 ± 1.4 g/L per year in males and 121 ± 1.1 g/L per year in females). The estimated yearly change in haemoglobin mass was multiplied by its assumed iron content (3.39 mg/g). The increase in the tissue iron content was 0.004 mg/day whatever the age, calculated by multiplying the median yearly rate of weight gain by the estimated tissue iron content (0.7 mg/kg body weight (Smith and Rios, 1974)). Up to the age of 3 years, the increase in storage iron was calculated in the same way as for older infants, by multiplying the sum of the increase in haemoglobin mass and the increase in tissue iron by the portion of total tissue iron that is stored. The estimated values fell until 9 years of age (when the value was 0). The median total requirement for absorbed iron was based on the higher estimates for boys and was set at 0.54 mg/day between 1 and 3 years and at 0.74 mg/day between 4 and 8 years. The variability of requirements was estimated, considering the variability of weight velocity (CV of 40 % between 1 and 8 years), which was also assigned to the variability of haemoglobin iron deposition and tissue iron deposition, and an overall CV of basal iron losses of 38 %. Considering the same bioavailability as for adults, i.e. 18 %, EARs and RDAs were calculated based on the median and 97.5th percentile for each year increment between 1.5 and 8.5 years.

For children aged 9–18 years, the major components of iron requirement modelled by IOM were basal iron losses, the increase in haemoglobin mass and the increase in storage iron, like for younger children (but not the increase in tissue non-storage iron), as well as menstrual iron losses for girls aged 14–18 years. Median requirements for absorbed iron were estimated for each year of age, and the variability of these requirements and the 97.5th percentile were assessed at the midpoint of the age ranges 9–13 years and 14–18 years. Median yearly weight gains in boys (aged 9–12, 13–14, 15–17 and 18 years) and girls (aged 9–11, 12–13, 14–17 and 18 years) were estimated from the slopes of linear regressions of median body weights on age (Tanner et al., 1966b), and decreased to 0 at 18 years of age. Basal iron losses for each sex and each year increment between 9 and 18 years were extrapolated from data on adult men (0.014 mg/kg body weight per day) (Green et al., 1968) and multiplied by median body weights recorded in NHANES III. The amount of iron needed for the increase in haemoglobin mass was calculated by adding the estimated yearly rate of change in haemoglobin concentration multiplied by median body weights and the estimated yearly weight gains multiplied by haemoglobin concentration. This sum was then multiplied by blood volume and the iron content of haemoglobin and divided by 365 days. Blood volume was considered to be about 75 mL/kg body weight in boys and 66 mL/kg body weight in girls (Hawkins, 1964), the iron content of haemoglobin was considered to be 3.39 mg/g (Smith and Rios, 1974), and the yearly rates of change in haemoglobin concentration were estimated as the coefficients of linear regressions of haemoglobin concentration on age for boys and girls aged 8–13 and 14–18 years (Beaton et al., 1989). Tissue iron was calculated by multiplying the median yearly weight gains by the iron content in muscle tissue (0.13 mg/kg of total weight gain (Smith and Rios, 1974)) and dividing by 365 days. For the estimation of menstrual losses in adolescent girls, the model assumed that all girls were menstruating at age 14 years and over, and that girls younger than 14 years did not menstruate. As done for menstruating women, a log-normal distribution was fitted to reported menstrual blood losses in Swedish women (Hallberg et al., 1966b, 1966a; Hallberg and Rossander-Hultén, 1991), and the predicted median blood loss was 27.6 mL/cycle. Median menstrual iron loss was calculated as 0.45 mg/day, by multiplying the calculated median blood loss by the haemoglobin concentration estimated according to age (for 14–20 years: 131 g/L + 0.28 × age in years) and the iron content of haemoglobin of 3.39 mg/g (Smith and Rios, 1974). The distributions of the components of the total requirement for absorbed iron were reported to be skewed and the variability of each component was assessed to estimate the variability of the total requirement. The modelled distribution of total iron requirement, combining the several estimated components in a Monte Carlo simulation, was used to set the EAR (based on the median) and the RDA (based on the 97.5th percentile), assuming the same absorption efficiency as for adults, i.e. 18 %. The physiological processes associated with puberty with a major impact on iron requirements were considered to be the growth spurt in both sexes, menarche in girls and the major increase in haemoglobin concentrations in boys. IOM also described how to adjust estimates for requirements for individuals underlying the growth spurt or onset of menstruation. An increased requirement for dietary iron was set at 2.9 mg/day for boys and at 1.1 mg/day for girls identified as currently in the growth spurt, and at 2.5 mg/day for girls under the age of 14 years and starting to menstruate. The estimated percentiles of the distribution of iron requirements in children aged 0.5–1 year, 1–3 years, 4–8 years, 9–13 years and 14–18 years were also provided.

The Netherlands Food and Nutrition Council (1992) calculated basal iron losses in childhood by extrapolation using body weight to the power of 0.75. Menstrual iron losses were estimated to be 0.6 mg/day in girls aged 13–16 years (Schlaphoff and Johnston, 1949). Requirements for growth were calculated from variation in body iron stores (average: 40–50 mg/kg body weight (Fomon and Anderson, 1974)) and their SD was considered to be 15 %. Total average amounts of absorbed iron to compensate for losses (basal, menstrual for adolescent girls) and growth were 0.8 mg/day at 0.5–1 year, and between 0.7 and 1.5 mg/day in boys and 0.7 and 1.8 mg/day in girls aged 1–19 years. Considering an absorption efficiency of 14 % for infants aged 0.5–1 year and girls aged 13–19 years (Hallberg, 1981), and the same absorption efficiency as in adults, i.e. 12 %, for the other age groups of children, the minimum requirements were estimated as 6.5 mg/day at 0.5–1 year and between 6 and 13 mg/day in boys and girls aged 1–19 years. Considering an SD of 15 % for growth and no variation for menstrual losses, adequate levels of daily intakes were set at 7 mg/day for infants aged 0.5–1 year and between 7 mg/day and 15 mg/day (boys) or 14 mg/day (girls) between 1 and 19 years.

For infants and children, the UK COMA (DH, 1991) added to basal losses the amount of iron required for expanding red cell mass and growing body tissues, as well as menstrual iron losses for adolescent girls aged 11–18 years, and considered an iron absorption of 15 %. The LRNI was set at 4.2 mg/day and the EAR was set at 6.0 mg/day for infants aged 7–12 months. The LRNIs ranged between 3.3 and 8.0 mg/day and the EARs ranged between 4.7 and 11.4 mg/day depending on sex and age group between 1 and 18 years. RNIs were 7.8 mg/day for infants aged 7–12 months and ranged between 6.1 and 14.8 mg/day depending on sex and age group between 1 and 18 years.

An overview of DRVs for iron for children is presented in Table 3.

Table 3: Overview of Dietary Reference Values for iron for children

	D-A-CH (2015)	**NCM (2014)**	**WHO/FAO (a) (2004)**	**Afssa (b) (2001)**	**IOM (2001)**	**SCF (1993)**	**NL (1992)**	**DH (1991)**
Age (months)	4–< 12	6–11	6–12	6–12	7–12	6–11	6–12	7–12
PRI (mg/day)	8	8 (c)	6.2 (bioavailability during this period varies greatly)	7	11	6 (c) [9.3] (d)	7	7.8
Age (years)	1–< 7	1–5	1–3	1–3	1–3	1–3	1–4	1–3
PRI (mg/day)	8	8 (c)	3.9	7	7	4 (c)	7	6.9
Age (years)	7–< 10	6–9	4–6	4–6	4–8	4–6	4–7	4–6
PRI (mg/day)	10	9 (c)	4.2	7	10	4 (c)	7	6.1
Age (years)	10–< 19	10–13	7–10	7–9	9–13	7–10	7–10	7–10
PRI (mg/day)	12 (M) 15 (F)	11 (c)	5.9	8	8	6 (c)	8	8.7
Age (years)		14–17	11–14	10–12	14–18	11–14	10–13	11–18
PRI (mg/day)		11 (M) (c) 15 (F) (e)	9.7 (M) 9.3 (f)/21.8 (F)	10	11 (M) 15 (F)	10 (M) (c) 9 (F) (f) 18 (F) (e) 22 (F) (c)	10 (M) 11 (F)	11.3 (M) 14.8 (F)
Age (years)			15–17	13–19		15–17	13–19	
PRI (mg/day)			12.5 (M) 20.7 (F)	13 (M) 16 (F)		13 (M) (c) 17 (F) (e) 21 (F) (c)	15 (M) 12 (F) (g) 14 (F) (g)	

F, females; M, males; NCM, Nordic Council of Ministers; NL, Netherlands Food and Nutrition Council.

(a): Recommended Nutrient Intake, for a bioavailability of dietary iron of 15 %.

(b): Values are from the table on page 507 of the Afssa (2001) report.

(c): Calculations were based on the 95th percentile of iron requirements and absorption was assumed to be 15 %.

(d): Bioavailability during this period varies greatly. The value in square brackets is for a bioavailability of 10 %.

(e): Based on the 90th percentile of iron requirements.

(f): Pre-menarche.

(g): At an absorption efficiency of 14 %.

4.3. Pregnancy

For pregnancy, the German-speaking countries (D-A-CH, 2015) took into account iron requirements of about 300 mg for the fetus, about 50 mg for the placenta and about 450 mg for the increased blood volume of the mother (Hallberg, 1988). D-A-CH considered that the RI of 30 mg/day during pregnancy cannot usually be met with food alone.

The Nordic countries (Nordic Council of Ministers, 2014) did not set RIs for dietary iron for pregnant women, in line with SCF (1993). Iron stores of about 500 mg were reported to be required at the beginning of pregnancy to achieve iron balance during pregnancy. Maternal iron requirements were shown to increase slowly during pregnancy, from the amount needed to cover basal losses in the first trimester to an amount of 10 mg/day in the last six weeks (Barrett et al., 1994), in relation to requirements for growth and maintenance of the fetus and uterus, the increase in red cell mass and the expected iron losses during birth. Total iron requirement during pregnancy was estimated to be 1 040 mg, including 840 mg for the fetus, the rest being lost when giving birth (Hallberg, 1988). Iron absorption was assumed to increase during the last two trimesters. It was noted that, for some pregnant women, the amount of iron in foods is not enough to satisfy the greatly increased iron demand, and iron supplementation starting in the second trimester was therefore recommended.

WHO/FAO (2004) and SCF (1993) did not derive a Recommended Nutrient Intake or a PRI for pregnant women, because the iron balance of pregnant women depends on the properties of the diet and on iron stores. However, iron requirements were reported to be 300 mg for the fetus, 50 mg for the placenta, 450 mg for the expansion of maternal red cell mass and 240 mg for basal iron losses, and thus 1 040 mg in total. Net iron requirement in pregnancy was considered to be 840 mg, assuming sufficient iron stores (i.e. stores of 500 mg available during the last two trimesters). Total daily iron requirements were noted to increase during pregnancy from 0.8 mg to about 10 mg during the last six weeks, and iron absorption was reported to increase during pregnancy. SCF (1993) considered that iron requirements during the second half of pregnancy are huge and cannot be met by diet alone or the body iron stores of the mother. Thus, SCF recommended daily iron supplements during this period, in accordance with DeMaeyer et al. (1989).

Afssa (2001) considered a bioavailability of 10 % like for other age groups (Galan et al., 1985; Lynch and Baynes, 1996; Lynch, 1997) and reported an increased iron requirement during pregnancy (FAO/WHO, 1988; Hercberg et al., 2000) in relation to the increase in red cell mass (about 500 mg of iron) and the synthesis of fetal tissues (about 290 mg of iron) and the placenta (25 mg of iron). Basal iron losses during pregnancy were considered to be 220 mg and total iron requirement was estimated to be over 1 000 mg, i.e. 2.5–5.2 mg/day depending on iron stores at the beginning of pregnancy. Afssa also noted that there was an increase in iron bioavailability during pregnancy (Whittaker et al., 1991; Barrett et al., 1994) related to a gradual decrease in body iron stores. Afssa set a PRI of 30 mg/day during the last trimester of pregnancy and considered that it cannot be met by usual diets.

For pregnant women, IOM (2001) considered basal losses, iron deposited in fetal and related tissues, and iron utilised in the expansion of haemoglobin mass as components for factorial modelling. Basal iron losses of 0.896 mg/day, calculated for non-pregnant, non-lactating women with a body weight of 64 kg and an average basal loss of 0.014 mg/kg body weight per day (Green et al., 1968) were taken into account, i.e. about 250 mg for the whole pregnancy. For iron deposition in the fetus, the umbilicus and the placenta, IOM selected the value of 315 mg (FAO/WHO, 1988), rounded to 320 mg, and provided estimates per trimester (Bothwell and Charlton, 1981). For the expansion of haemoglobin mass, the value of 500 mg (FAO/WHO, 1988) was selected. However, IOM noted that the estimate depends on the haemoglobin concentration and the extent of iron supplementation provided, and referred to the reference curve of the evolution of median haemoglobin concentration by week of gestation in healthy, iron-supplemented pregnant women in industrialised countries (IOM, 1993). In line with FAO/WHO (1988), the expansion of haemoglobin mass was assumed to be zero during the first trimester and equally distributed between the last trimesters (owing to a lack of data on the precise timing), i.e. 250 mg/trimester or 2.7 mg/day. The net cost of pregnancy was estimated to be about 700–800 mg of iron. Bioavailability in the first trimester was estimated to be the same as for non-pregnant women, i.e. 18 %, while the maximal value was estimated to be about 25 % in the last two trimesters (Barrett et al., 1994). The requirement for absorbed iron was finally set at 1.2, 4.7 and 5.6 mg/day, and the dietary iron requirement was set at 6.4, 18.8 and 22.4 mg/day, for the first, second and third trimesters, respectively. For pregnant adolescents, a similar approach was followed, but estimated basal losses and iron deposition in tissue were those calculated for non-pregnant adolescents. The variability of the components of iron requirements was assessed to estimate the variability of the total requirement for absorbed iron. The EARs were established based on estimates for the third trimester to build iron stores during the first trimester of pregnancy and were 23 mg/day for adolescents aged 14–18 years and 22 mg/day for adult women. The RDA was set at 27 mg/day for pregnant women of all ages based on the 97.5th percentile of the requirement for absorbed iron.

The Netherlands Food and Nutrition Council (1992) considered iron absorption to be 12 % during the first trimester of pregnancy and about 16 % in the last two trimesters and during lactation. Basal iron losses during pregnancy were considered the same as those of non-menstruating women (0.8 mg/day). No CV was applied for losses during birth, and a CV of 15 % was considered for the iron requirement for growth of the fetus and the placenta. The iron amount needed during pregnancy for the fetus and the placenta was considered to be about 300–350 mg (Widdowson and Spray, 1951; Bowering and Sanchez, 1976), the distribution being 10, 40 and 60 % in the first, second and third trimesters, respectively. Thus, during the first, second and third trimesters of pregnancy, respectively, the average total amounts of absorbed iron were estimated to be 1.1, 2.2 and 2.9 mg/day, the minimum requirements for dietary iron were estimated to be 9, 14 and 18 mg/day and the adequate levels of daily intake were set at 11, 15 and 19 mg/day.

The UK COMA (DH, 1991) reported an estimated iron requirement for the products of conception of 680 mg (Committee on Iron Deficiency, 1968), but did not set any RNI for iron for pregnant women because of cessation of menstrual losses, mobilisation of maternal iron stores and increased intestinal absorption (Svanberg et al., 1975).

4.4. Lactation

The German-speaking countries (D-A-CH, 2015) recommended an intake of 20 mg/day for both lactating and non-lactating women after birth to compensate for the losses during pregnancy.

For lactating women, the Nordic countries (Nordic Council of Ministers, 2014) considered the frequent absence of menstruation during the first months of lactation (Habicht et al., 1985). However, it was also stated that women in Northern countries breastfeed their infants for prolonged times, so that menstrual losses would occur within the breastfeeding period. The RI set for lactating women was the same as that for non-pregnant, non-lactating women of childbearing age, i.e. 15 mg/day.

For lactating women, WHO/FAO considered a mean body weight of 62 kg, a total basal iron loss of 0.014 mg/kg body weight per day (Green et al., 1968) with an SD of 15 %, a daily iron secretion into milk of about 0.3 mg and, therefore, a median basal iron loss of 1.15 mg/day. Median and 95th percentile of total requirements for absorbed iron were estimated to be 1.15 and 1.50 mg/day, respectively. The Recommended Nutrient Intake was based on the 95th percentile of total iron requirement and the various levels of iron bioavailability already considered for adults and children (15, 12, 10 and 5 %), and was set at 10 mg/day for a bioavailability of 15 % (up to 30 mg/day for a bioavailability of 5 %).

For lactation, SCF (1993) considered an amount of iron secreted with human milk of 0.15–0.3 mg/day, and set a PRI of 10 mg/day assuming a bioavailability of 15 %.

For lactating women, Afssa (2001) recommended an iron intake of 10 mg/day. The iron concentration of human milk was considered to be 0.55 mg/L 2 weeks after birth, 0.4 mg/L 6 to 8 weeks after birth and about 0.3 mg/L 3 to 5 months after birth (Siimes et al., 1979). The iron loss through human milk was thus estimated to be 0.2–0.4 mg/day in the case of exclusive breastfeeding, and the absorption of iron was reported to be increased during lactation.

For lactation, IOM (2001) estimated median iron requirements as the sum of iron secretion in human milk and basal iron losses of non-pregnant, non-lactating women (0.896 mg/day), until the initiation of menstruation after around six months of exclusive breastfeeding. The average iron concentration of human milk was considered to be 0.35 mg/L and the CV was estimated to be 33 %. The average volume of milk secreted during the first six months was estimated to be 0.78 L/day. Iron losses with human milk were thus estimated to be 0.27 ± 0.089 mg/day and the median total requirement for absorbed iron was estimated to be 1.17 mg/day. The approach was similar for lactating adolescents (14–18 years), but provision was also made for the deposition of iron in tissues (0.001 mg/day) and haemoglobin mass (0.14 mg/day), and the median requirement for absorbed iron was estimated as

1.26 mg/day. Like for other age groups, a simulation model was used to derive the 97.5th percentile of this requirement used to set the RDA, and a bioavailability of 18 % was assumed.

For lactating women, the Netherlands Food and Nutrition Council (1992) considered that the amount of iron lost during birth (50–250 mg) represented an increased requirement of about 1.6 mg/day over a lactation period of three months. The average amount of iron secreted with human milk was assumed to be about 0.5 mg/day, and the basal losses were considered to be the same as for non-menstruating women, i.e. 0.8 mg/day. The average total amount of absorbed iron was thus estimated to be 3.0 mg/day. The minimum requirement was set at 19 mg/day and the adequate level of daily intake was set at 20 mg/day.

For lactating women, the UK COMA (DH, 1991) reported iron concentrations in human milk at 6–8 weeks post-partum of 0.4 mg/L and at 17–22 weeks post-partum of 0.29 mg/L (Vuori, 1979), considered a daily volume of milk production of 850 mL, and thus calculated the iron secretion in milk to be 0.25–0.34 mg/day. No PRI was derived for lactating women, as lactational amenorrhoea was considered to compensate for the amount of iron secreted in milk.

An overview of DRVs for iron for pregnant and lactating women is presented in Table 4.

Table 4: Overview of Dietary Reference Values for iron for pregnant and lactating women

	D-A-CH (2015)	**NCM (2014)**	**WHO/FAO (2004)**	**Afssa (2001)**	**IOM (2001)**	**SCF (1993)**	**NL (1992)**	**DH (1991)**
Pregnancy				Third trimester				
PRI (mg/day)	30	No DRV given	No DRV given	30	27	No DRV given	11 (first trimester) 15 (second trimester) 19 (third trimester)	No DRV given
Lactation							3 months	
PRI (mg/day)	20 (also applicable to non-breastfeeding women who gave birth)	15	10 for a bioavailability of 15 % (up to 30 for a bioavailability of 5 %)	10	10 (14–18 years)/ 9 (adult)	10	20	No DRV given

NCM, Nordic Council of Ministers; NL, Netherlands Food and Nutrition Council.

WHO/FAO (2004) and SCF (1993) consider that iron supplements should be given to all pregnant women. NCM (2014) states that the physiological iron requirement of some women cannot be satisfied during the last two-thirds of pregnancy with food only, and supplemental iron might be needed.

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Indicators of iron requirement

Assessments of iron status (see Section 2.4) of individuals show a wide range between the two extremes of iron deficiency and excess, with no good dose–response data to determine thresholds at which adverse or significant adaptive events associated with these two conditions are observed. Adequate iron status implies the presence of normal erythropoiesis and iron-dependent functions, together with a contingency supply of storage iron for physiological requirements. Reference ranges have been developed to indicate iron sufficiency, but values outside the range do not necessarily define deficiency or excess. The Panel notes that the most commonly used biomarkers of iron status are haemoglobin (functional iron) and serum ferritin concentration (storage iron), but these cannot be used to determine iron requirements.

5.1.1. Factorial approach for estimating physiological iron requirement

Obligatory iron losses in all population groups include dermal losses (sweat and skin); epithelial loss from the intestinal, oropharyngeal and respiratory, and genito-urinary tracts; hepatic, pancreatic and intestinal secretions; urine; and menstrual blood loss in women of child-bearing age. To maintain iron balance, the sum of these losses plus the iron required for growth in infants, children and adolescents, and during pregnancy, must be provided by the diet.

5.1.1.1. Adults

From the available data on iron losses (Section 2.3.7), the Panel decided that, instead of combining all of the losses from the different routes (and hence magnifying the uncertainty of the estimate), it would be more accurate to estimate physiological iron requirement using whole-body iron loss data derived from the isotope studies undertaken by Hunt et al. (2009). These authors measured basal losses of iron in 29 men, 19 menstruating women and 5 postmenopausal women.

The Panel used individual data on iron turnover and daily losses of iron from the study of Hunt et al. (2009)[10] as a basis of assessing obligatory losses of iron. It was thought that these data provided an aggregate of overall losses, which was relatively free of the uncertainties inherent in summating basal losses of endogenous iron using, for example, the data of Green et al. (1968). Although these data were collected from a North American population group that is not necessarily representative of the EU healthy adult population, the Panel agreed that it was possible to use these data as a basis for the estimation and probability modelling of the mean and approximate variability of distribution percentiles for the iron losses of adult men and premenopausal women in the EU population. Data on iron losses of the few postmenopausal women included in this study were not further analysed, as the Panel considered this group too small for separate analyses and because the data were different from those of men or premenopausal women (see Appendix H).

Details of the statistical analysis of the data are given in Appendix H. First, summary statistics were estimated for the main variables related to iron losses for adult men and premenopausal women and for associations among the variables which were considered to be potentially explicative for iron losses. From these, a regression model equation for iron losses (as mg/day) was fitted to the data using a set of potentially relevant variables. This stage included an assessment of outliers and goodness of fit. The regression model was then used to derive a distribution for iron losses combining the model equation with parametric distributions fitted to the sampling observations of each of the explanatory variables. The Panel considers that the probabilistic approach is a useful method with which to fill in data gaps as far as major sources of variability are concerned. The Panel also considers that it provides a distribution of iron losses from which percentiles can be estimated as a basis for determining AR and PRI values.

For men, the 50th percentile of the model-based distribution of iron losses is equal to around 0.95 mg/day. The 90th, 95th and 97.5th percentiles are, respectively, equal to iron losses of around 1.48, 1.61 and 1.72 mg/day. For premenopausal women, the 50th percentile of the model-based distribution of iron losses is equal to around 1.34 mg/day. The 90th, 95th and 97.5th percentiles are, respectively, equal to iron losses of around 2.44, 2.80 and 3.13 mg/day.

5.1.1.2. Infants

Newborns have approximately 75 mg iron/kg body weight, corresponding to 260 mg of total iron (Widdowson and Spray, 1951; Oski, 1993), of which approximately 70 % is in haemoglobin, 24 % is in liver stores as ferritin and the remaining 6 % is in myoglobin and iron-containing enzymes (Dallman et al., 1993). A newborn's iron stores can be increased by about 30–35 mg through delayed clamping of the umbilical cord (i.e. two minutes or later after birth) (Hutton and Hassan, 2007), with a calculated difference in serum ferritin concentration of 4 µg/L, resulting from the high haemoglobin

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[10] The very kind provision of the individual data by Gerald Combs and LuAnn Johnson from the USDA Human Nutrition Research Center, Grand Forks, North Dakota, USA, is acknowledged.

content of fetal blood and from placental sources. Owing to redistribution of iron from haemoglobin to iron stores, in healthy, term, normal-birth weight infants, there is sufficient iron for the formation of haemoglobin and myoglobin concomitant with growth until about six months of age in fully breast-fed infants (Chaparro, 2008). Extra iron requirements during this period can be provided by human milk alone (even if its iron concentration is low). Therefore, an additional appreciable requirement for dietary iron does not exist before the sixth month of life (Domellof, 2011). With regard to the dietary iron requirement of infants aged 7–11 months, there is no need to differentiate between their feeding modes, i.e. whether they are breast-fed or formula-fed in addition to complementary feeding.

The main requirements for iron in older infants (7–11 months) are for the replacement of obligatory faecal, urinary and dermal losses (basal losses); the increase in haemoglobin mass (both blood volume and haemoglobin concentration); the increase in tissue (non-storage) iron; and the increase in storage iron to build a reserve. Fomon et al. (2005) used ^{58}Fe as a tracer in 35 normal-weight infants aged 4–168 days, and performed a follow-up study until 26 months of age. They observed endogenous gastrointestinal iron losses of 22 µg/kg body weight per day, i.e. higher than those reported in adult men (12 µg/kg body weight per day). This value is close to that proposed by Oski (1993) (20 µg/kg body weight per day). Based on a loss of 20 µg/kg body weight per day, Oski (1993) estimated a daily requirement of 0.78 mg of absorbed iron for a 10-kg, 12-month-old infant, comprising 0.2 mg to replace losses (0.020 mg/kg body weight × 10 kg) and 0.58 mg required for blood volume increase and tissue growth.

Domellof and Hernell (2002) assumed a requirement of absorbed iron of 0.6 mg/day by the end of the sixth month, made up of 0.5 mg/day for iron in haemoglobin and 0.1 mg/day for iron in muscle and other tissues. The relative proportions of these amounts are similar to those indicated by Oski (1993) for iron in haemoglobin and tissue. Domellof and Hernell (2002) then calculated that there is a need of 0.15 mg for daily obligatory losses according to estimated losses of 20 µg/kg body weight per day (Oski, 1993), resulting in a total requirement of absorbed iron of 0.75 mg for an infant weighing 7.5 kg. Assuming iron losses of 22 µg/kg body weight per day (Fomon et al. (2005), derived from direct isotopic observations) and an average body weight of 8.6 kg for boys and girls aged nine months (WHO Multicentre Growth Reference Study Group, 2006), i.e. the midpoint of the age class 7–11 months, daily losses are 0.19 mg/day. Using the figure derived by Domellof and Hernell (2002) of 0.6 mg/day for iron requirement for growth of infants aged six months leads to a daily requirement of absorbed iron of 0.79 mg/day (Table 5).

Table 5: Calculation of physiological iron requirement of infants aged 7–11 months

	Girls and boys
Median weight of girls and boys (kg) (a)	8.6
Physiological requirement: total losses plus needs for growth (mg/day) (b)	0.79

(a): Average of median weight-for-age of male and female infants aged 9 months according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006).
(b): Algebraic sum of total losses of 0.022 mg/kg body weight per day × body weight (kg) plus growth needs of 0.6 mg/day.

5.1.1.3. Children

The iron requirements of children reflect the synthesis of new tissues involved in their growth rate and losses of body iron per kilogram of body weight. Endogenous losses decrease after the third year of life from 22 to 12 µg/kg body weight per day, as is observed in adult men (Section 2.3.7). From 1 to 7 years of age, dietary iron requirements increase only slightly owing to the small rates of increase in weight. With puberty, higher intakes are needed to compensate for increased requirements for growth and, in girls, for menstrual losses. The mean age of menarche in the EU (with 91.8 % coverage of the EU population) has been estimated to be 12.7 years (van Buuren et al., 2012). However, the age at menarche varies widely and menarche is considered to be normal if occurring between 11 and 15 years of age, and early if occurring at ≤ 10 years (Glueck et al., 2013).

The main compartments containing iron are blood haemoglobin, the liver, the macrophage–monocyte system (i.e. the RES) and myoglobin of muscles (Wang and Pantopoulos, 2011). Using isotopic studies, Fomon et al. (2005) determined that tissue iron contents in 15 boys and 16 girls were 37.6 mg/kg at 6 months, 35.2 mg/kg at 13 months and 34.9 mg/kg at 26 months. Dewey and Chaparro (2007) estimated a body iron content of 420 mg, which is equivalent to a tissue iron content of 42 mg/kg body weight in a 10-kg infant. In adult men and women, tissue iron contents, estimated from isotope dilution, were 48 mg/kg body weight and 38 mg/kg body weight, respectively (Hunt et al., 2009). The iron content per kilogram of body weight is consistent with the value of 45 mg/kg body weight estimated by Oski (1993), i.e. a total amount of body iron of 450 mg in a 10-kg infant subdivided into haemoglobin, tissue iron and iron stores. Considering the possible age-related changes of the average iron content in body compartments and the changes in the distribution of fat mass taking place with puberty, the Panel considers a tissue iron content of 40 mg/kg body weight as a reasonable value for children of both sexes from 1 to 11 years of age, i.e. pre puberty. With early puberty, there is an increase in accretion of fat mass in girls (Laurson et al., 2011) which continues throughout (young) adulthood (Vink et al., 2010). Therefore, from age 12 years onwards, the Panel considers it appropriate to use the tissue iron content estimated in adults (Hunt et al., 2009), i.e. 48 mg iron/kg body weight for boys and 38 mg/kg body weight for girls, for factorial calculations, taking into account the differences in accretion of fat mass taking place in puberty.

Estimated average daily iron requirements for growth between 12 months and 18 years have been derived according to body weights at the 50th percentile for various age classes (1–3, 4–6, 7–11 and 12–17 years), for both sexes combined until 11 years of age and for girls and boys separately from 12 years onwards, as reported in Table 6.

Table 6: Requirements for absorbed iron for growth in boys and girls aged 1 to 17 years

Age group	**1–3 years**		**4–6 years**		**7–11 years**		**12–17 years**			
							Boys		**Girls**	
Age boundary (year)	1	4	4	7	7	12	12	18	12	18
Median weight (kg) of boys and girls at age boundary	9.3 (a)	16.2 (b)	16.7 (c)	24.1 (d)	24.1 (d)	42.1 (e)	41.5 (f)	69.3 (g)	42.6 (h)	57.4 (i)
Weight gain (kg)	6.9 (j)		7.4 (k)		18.0 (l)		27.8 (m)		14.8 (m)	
Body iron (mg/kg)	40		40		40		48		38	
Iron in total weight gained (mg)	276		296		720		1 334		562	
Requirement for absorbed iron for growth per year (mg)	92		99		144		222		94	
Requirement for absorbed iron for growth per day (mg)	0.25		0.27		0.39		0.61		0.26	

To cover the whole age range, it was considered that a child is 3 years of age until his or her 4th birthday, 6 years of age until his or her 7th birthday, 11 years of age until his or her 12th birthday and 17 years of age until his or her 18th birthday. As weight data for the day before the 4th, 7th, 12th and 18th birthdays were not available, median weights for boys and girls aged 4, 7, 12 and 18 years, respectively, were used instead.

(a): Average of median weight-for-age of boys and girls aged 12 months according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006).
(b): Average of median weight-for-age of boys and girls aged 48 months according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006).
(c): Average of median body weight of boys and girls aged 4 years (van Buuren et al., 2012).
(d): Average of median body weight of boys and girls aged 7 years (van Buuren et al., 2012).
(e): Average of median body weight of boys and girls aged 12 years (van Buuren et al., 2012).
(f): Median body weight of boys aged 12 years (van Buuren et al., 2012).
(g): Median body weight of boys aged 18 years (van Buuren et al., 2012).
(h): Median body weight of girls aged 12 years (van Buuren et al., 2012).
(i): Median body weight of girls aged 18 years (van Buuren et al., 2012).
(j): Net weight gain in kilograms between 1 and 4 years.
(k): Net weight gain in kilograms between 4 and 7 years.

(l): Net weight gain in kilograms between 7 and 12 years.
(m): Net weight gain in kilograms from 12 years.

Up to the fourth year of life, losses of iron (resulting from intestinal, renal and dermal losses) have been estimated as 0.022 mg/kg body weight per day (Fomon et al., 2005). Iron requirements for growth are 0.25 mg/day (Table 6) and the requirement for absorbed iron is 0.51 mg/day (Table 7). For children aged 4 years and over, basal iron losses decrease to 0.012 mg/kg body weight per day. For children aged 4–6 years, requirements for growth are stable, in line with the constant yearly gain in body weight, while there is an increase in the daily requirement for absorbed iron for growth of 0.39 mg/day in children aged 7–11 years (see Table 6). The requirements for absorbed iron are 0.50 mg/day for ages 4–6 years and 0.76 mg/day for ages 7–11 years (Table 7).

In adolescence, the need for iron increases in both boys and girls, as it is a period of rapid growth in both sexes and, in females, periodic menstrual blood losses take place after menarche. As the mean age of menarche in the EU is 12.7 years (van Buuren et al., 2012), menstrual blood losses should be considered from 12 years with a geometric mean iron loss of 0.25 mg/day (Harvey et al., 2005).[11] Considering the increased requirement for growth, obligatory losses and menstrual losses in girls after menarche, the requirement for absorbed iron is 1.27 mg/day in boys and 1.13 mg/day in girls (Table 7).

Table 7: Calculation of physiological iron requirement for children aged 1–17 years

Age group	**1–3 years**	**4–6 years**	**7–11 years**	**12–17 years**	
				Boys	**Girls**
Median weight (kg) of girls and boys	11.8 (a)	19.0 (b)	30.3 (c)	52.7 (d)	51.6 (d)
Physiological requirement: total losses plus needs for growth (mg/day)	0.51 (e)	0.50 (f)	0.76 (g)	1.27 (h)	1.13 (i)

To cover the whole age class, it was considered that a child is 3 years of age until his or her 4th birthday, 6 years of age until his or her 7th birthday, 11 years until his or her 12th birthday and 17 years until his or her 18th birthday. As weight data for the day before the 4th, 7th, 12th and 18th birthdays were not available, median weights for boys and girls aged 4, 7, 12 and 18 years, respectively, were used instead.
(a): Average of median weight-for-age of male and female children aged 24 months according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006).
(b): Average of median body weight of boys and girls aged 5 years (van Buuren et al., 2012).
(c): Average of median body weight of boys and girls aged 9 years (van Buuren et al., 2012).
(d): Median body weight of boys or girls aged 14.5 years (van Buuren et al., 2012).
(e): Algebraic sum of total losses of 0.022 mg/kg body weight per day × body weight (kg) plus growth needs of 0.25 mg/day (see Table 6).
(f): Algebraic sum of total losses of 0.012 mg/kg body weight per day × body weight (kg) plus growth needs of 0.27 mg/day (see Table 6).
(g): Algebraic sum of total losses of 0.012 mg/kg body weight per day × body weight (kg) plus growth needs of 0.39 mg/day (see Table 6). In the case of early–normal menarche, menstrual iron losses need to be replaced and the physiological iron requirement increases by 0.25 mg/day.
(h): Algebraic sum of total losses of 0.012 mg/kg body weight per day × body weight (kg) plus growth needs of 0.61 mg/day (see Table 6).
(i): Algebraic sum of total losses of 0.012 mg/kg body weight per day × body weight (kg) plus growth needs of 0.26 mg/day, plus geometric mean menstrual losses of 0.25 mg/day. In the case of late–normal menarche, menstrual iron losses do not need to be replaced and the physiological iron requirement decreases by 0.25 mg/day.

5.1.1.4. Pregnancy

The total quantity of iron required to support a singleton pregnancy of an average adult woman is 835 mg. This is calculated factorially as follows: total obligatory losses (faecal, urinary and dermal) of

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[11] Linda Harvey from the Institute of Food Research, Norwich Research Park, UK, kindly provided individual data on menstrual blood losses. Based on these data, the geometric mean iron loss and percentiles as presented in Appendix A were calculated.

300 mg,[12] 270 mg for the neonate (Bothwell, 2000; Milman, 2006), 90 mg for the placenta and umbilical cord (Bothwell, 2000; Milman, 2006), and 175 mg for blood loss at delivery (mean of values given by Bothwell (2000) and Milman (2006)). Some of this iron can be supplied from maternal liver stores, and the remainder has to be provided by the diet.

Although the need for iron changes throughout the course of pregnancy, in line with the exponential growth of the fetus, it is not possible when setting DRVs to provide values for each stage of gestation; therefore, average daily values are calculated over the 280 days of gestation. Adaptive physiological changes take place to meet the demands of the growing fetus and the other products of conception. Such changes are anticipatory in that they happen before the period of exponential growth of the fetus. They include expansion of the plasma and blood volumes, and of red blood cell mass starting at 6–8 weeks and peaking at 28–34 weeks of gestation. The dilutional effect of this expansion induces a fall in serum ferritin concentration, but its relationship with systemic iron stores is not lost and concentrations approximating 15 µg/L are indicative of depleted liver iron stores (Blackburn, 2012). The increased need for iron is also met by increases in the efficiency of iron absorption (Bothwell et al., 1979; Hallberg and Hultén, 1996). Barrett et al. (1994) determined absorption rates of dietary iron during pregnancy using isotope labels in a group of 12 women consuming a diet supplying daily 9 mg of non-haem iron (see Section 2.3.2). A progressive increase in iron absorption was found in the three trimesters of pregnancy. In parallel, serum ferritin concentrations decreased, reflecting expansion of the plasma volume and the use of maternal iron depots for fetal growth. Accordingly, these increases in iron absorption in healthy women eating a mixed diet may balance the increased requirements in later pregnancy, as indicated in other isotopic studies in pregnant women (Whittaker et al., 1991; Whittaker et al., 2001).

There is a great deal of uncertainty in the estimation of total quantity of iron absorbed during pregnancy. However, the amount of iron absorbed may be predicted using data from an isotopic study (Barrett et al., 1994), and assuming, in a conservative way, that the same percentage iron absorption observed at week 12 of gestation is valid for the period 0–23 weeks of gestation; that the percentage iron absorption observed at week 24 of gestation is valid for the period 24–35 weeks of gestation; and that the percentage iron absorption observed at week 36 of gestation is valid for the period 36–40 weeks of gestation. Percentage iron absorption figures reported in Table 8 are geometric means. The quantity of non-haem iron absorbed (mg/day) has been calculated assuming a dietary non-haem iron intake of 9 mg/day and 4 mg haem iron/day from meat (as given to the women for three days before the absorption study) throughout the entire pregnancy. As there is no evidence for an increase in haem iron absorption during pregnancy (Young et al., 2010), it is assumed to be 25 % at all stages of pregnancy (Section 2.3.2). However, the Panel considers that this may be an underestimate, as insufficient data are available on the efficiency of haem iron absorption throughout pregnancy.

Table 8: Iron absorption during pregnancy calculated based on data from Barrett et al. (1994) on iron absorption from a test meal

		Time of gestation	
	12 weeks (weeks 0–23, days 1–161 = 161 days in total)	**24 weeks (weeks 24–35, gestational days 162–245 = 84 days in total)**	**36 weeks (weeks 36–40, gestational days 246–280 = 35 days in total)**
Geometric mean percentage non-haem iron absorption	7.2	36.3	66.1
Non-haem iron absorbed (mg/day) from a diet supplying 9 mg/day of non-haem iron	0.65	3.27	5.95

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[12] 1.08 mg/day × 280 days. The value of 1.08 mg/day is reported in Hunt et al. (2009) as the mean basal losses in five postmenopausal women. The Panel considers that basal iron losses during pregnancy are the same as those of non-menstruating women.

	Time of gestation 12 weeks (weeks 0–23, days 1–161 = 161 days in total)	24 weeks (weeks 24–35, gestational days 162–245 = 84 days in total)	36 weeks (weeks 36–40, gestational days 246–280 = 35 days in total)
Haem iron absorbed (mg/day) from a diet supplying 4 mg/day of haem iron	1.0	1.0	1.0
Total amount of iron absorbed (mg) in each gestational period	265	358	243
Total iron absorbed (mg) throughout gestation			**866**

According to the study by Barrett et al. (1994), in which the percentage absorption of non-haem iron was measured from a meal containing 3.2 mg of non-haem iron extrinsically labelled with a stable isotope of iron, the total estimated quantity of iron absorbed from a diet providing 13 mg iron/day (9 mg non-haem iron and 4 mg iron from meat daily) would be 866 mg over the entire pregnancy (Table 8). As the quantity of iron required for pregnancy is around 835 mg (see above), if this theoretical calculation is correct, no additional dietary iron will be required. The Panel notes that the percentage absorption measured from the test meal of a white roll, bacon and orange juice may be an overestimate of overall dietary iron absorption. This is supported by the fact that the women in this study had a mean serum ferritin concentration of 43.8 µg/L at week 12 of gestation, which is equivalent to liver iron stores of 350 mg, and a mean serum ferritin concentration of 5.4 µg/L at week 36, indicating that they had mobilised around 300 mg of iron from liver stores. The Panel notes that the quantity cannot be estimated accurately, as the relationship between serum ferritin concentration and liver iron may be confounded by haemodilution (Faupel-Badger et al., 2007).

The calculation above is conservative, as it does not take into account the utilisation of iron stores. The Panel selected a target value of 30 µg/L for serum ferritin in women of child-bearing age, as this reflects an adequate level of iron stores to support a pregnancy. This is also proposed in the UK guidelines of the British Committee for Standards in Haematology, which state that pregnant women with a serum ferritin concentration < 30 µg/L should be offered oral iron supplements (Pavord et al., 2012). The Panel assumed that, at this concentration, in the absence of any other adaptation, a 15 µg/L drop in serum ferritin concentration signifies the release of 120 mg of iron (1 µg/L of serum ferritin equals 8 mg of storage iron in an adult, see Section 2.4) from the liver. Stores would fall to virtually zero by delivery (with a serum ferritin concentration of 15 µg/L, i.e. the level associated with depletion of iron stores). The net cost of pregnancy is therefore 715 mg iron (total cost, 835 mg minus mobilised stores, 120 mg).

The calculations based on the data from the isotope studies can be compared with a different approach using the Dainty et al. (2014) model. Assuming serum ferritin concentrations of 30 µg/L (early pregnancy, up to week 23), which is associated with an efficiency of iron absorption of 18 %, and 15 µg/L (late pregnancy, from week 24 until term), which is associated with an efficiency of iron absorption of 31 % (see Section 5.1.2), the quantity of absorbed iron from a mixed diet can be calculated. With a serum ferritin concentration of 30 µg/L, in order to supply 835 mg of absorbed iron (i.e. the total quantity of iron required for a pregnancy), the total dietary intake needs to be 4 639 mg (835 mg / 0.18), which equates to 16.6 mg/day over 280 days of gestation. With a serum ferritin concentration of 15 µg/L, absorption is 31 % and the total dietary intake needs to be 2 694 mg (835 mg / 0.31), which equates to 9.6 mg/day. In practice, serum ferritin concentration will fall gradually as the pregnancy progresses, and taking the mean value of these two estimates, the average dietary intake to provide the required quantity of iron would be 13.1 mg/day. Assuming a CV of 20 %, to take into account the wide inter-individual variation in iron requirements in pregnant women, this would equate to a theoretical PRI of 18.3 mg/day. If the theoretical calculations are repeated using the net cost of pregnancy of 715 mg iron, the average iron intake required to support a pregnancy would be 11.2 mg/day. Assuming a CV of 20 %, this would equate to a theoretical PRI of 15.7 mg/day. This theoretical calculation is an alternative approach to using percentage iron absorption values derived from the isotope studies and is based solely on the relationship between serum ferritin concentration and efficiency of iron absorption.

The Panel notes that the conclusion from these different approaches is similar in that there is no need for additional dietary iron during pregnancy, provided that there are adequate iron stores at conception. This is a result of the increasing efficiency of iron absorption during pregnancy. However, the Panel notes that the Dainty et al. (2014) model has not been validated for pregnant women. Furthermore, it does not make any allowance for adaptive changes in efficiency of absorption that occur in pregnancy, and hence is likely to be a conservative estimate.

5.1.1.5. Lactation

Based on an iron concentration of mature human milk in European women of around 0.3 mg/L (Section 2.3.7.4) and assuming an average milk volume of 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009), the Panel estimates that the amount of iron secreted in breast milk during the first six months of lactation is 0.24 mg/day. Together with basal iron losses of about 1 mg/day (Hunt et al., 2009) in a non-menstruating woman of normal body weight, the total requirement for absorbed iron during the lactation period amounts to about 1.2–1.3 mg/day. As breastfeeding and its duration may delay the return of menses (lactational amenorrhoea) (Kramer and Kakuma, 2004), the requirement for absorbed iron in most lactating women may be less than in non-lactating premenopausal women. However, taking into account that lactating women might resume menstruation while they are still lactating, the Panel considers that the requirement for absorbed iron in lactating women is similar to that of non-lactating premenopausal women.

5.1.2. Algorithms and models used to estimate iron absorption

Several algorithms have been developed that can be used to predict iron absorption from whole diets in order to derive iron requirements. The first one (Hallberg and Hulthén, 2000) used iron absorption data from single meals labelled with radioiron, adjusted to a reference dose absorption of 40 %. The absorption value was then multiplied by the expected effect of different amounts of dietary factors known to influence iron absorption including phytate, polyphenols, ascorbic acid, meat, fish and seafood, and calcium. For each factor, an equation describing the dose–effect relationship was developed and allowance was made for interactions between individual factors. Estimated absorption, calculated as the sum of iron absorbed from all meals using the algorithm, was not significantly different from measured absorption from radioisotopically labelled meals (four per day for five days) in the haem and non-haem iron extrinsically labelled with radioisotopes. Other algorithms have been developed using absorption data from single meals (Reddy et al., 2000; Rickard et al., 2009).

More recently, there have been attempts to develop complete diet-based algorithms because the single-meal studies overestimate the effect of enhancers and inhibitors. Armah et al. (2013) used data from complete diet studies undertaken in the USA, which were either high or low in meat, tea, calcium or ascorbic acid. They combined 159 observations and used multiple linear regression to quantify the effect of different factors on non-haem iron absorption:

$$\text{Ln absorption (\%)} = 6.294 - 0.709 \ln (SF) + 0.119 \ln (C) + 0.006 \ln (MFP + 0.1) - 0.055 \ln (T + 0.01) - 0.247 \ln (P) - 0.137 \ln (Ca) - 0.083 \ln (NH)$$

where SF is serum ferritin (µg/L), C is ascorbic acid (mg), MFP is meat, fish and poultry (g), T is tea (number of cups), P is phytate (mg), Ca is calcium (mg) and NH is non-haem iron (mg).

Predicted non-haem iron absorption values from the algorithm were compared with measured single-meal and complete diet non-haem iron absorption data, and the R^2 values were 0.57 ($P < 0.001$) and 0.84 ($P < 0.0001$), respectively. The more accurate prediction for whole diets is not surprising, as the algorithm was developed from complete diet datasets. Serum ferritin concentration was the most important explanatory factor with respect to non-haem iron absorption. Dietary factors were relatively unimportant, with phytate being the only significant factor in the model; total phytate was used because data for the hexa- and penta-inositol phosphates (which bind strongly with iron, unlike the lower inositol phosphates) are not generally available, but a better model might have been generated with the use of individual inositol phosphate data.

The systematic review of iron absorption studies from whole diets by Collings et al. (2013) included a detailed analysis of data from studies where there were individual data on iron absorption, iron status and dietary enhancers and inhibitors. Such data were reported in five studies carried out in the USA. Pooled data from 40 individuals undertaking studies of identical design gave a mean percentage absorption from a self-selected diet, a low bioavailability diet (high calcium, low vitamin C, no meat) and a high bioavailability diet (low calcium, high vitamin C, high meat) of 7.09 (SD 6.75) %, 7.17 (SD 5.80) % and 9.92 (SD 8.78) %, respectively. When the Cook et al. (1991) equation was applied to normalise the data to a serum ferritin concentration of 15 µg/L, these values increased to 16.90 (SD 17.3) %, 16.72 (13.37) % and 22.60 (SD 21.76) %, respectively.

Because dietary factors appear to have little effect on absorption in healthy iron-replete individuals consuming Western-style whole diets, a simplified scoring system was used to classify diets and derive a regression equation using data from 58 individuals in order to be able to predict iron absorption from individuals with differing iron status:

$$\text{Log non-haem iron absorption (\%)} = -0.73 \log(\text{ferritin } \mu\text{g/L}) + 0.11 \text{ (modifier)} + 1.82$$

where the modifier is 0 (standard diet), –1 (diets that include at least one inhibitor) or 1 (diets that include at least one enhancer).

Using this equation, non-haem iron absorption from diets with and without enhancers/inhibitors was calculated for different serum ferritin concentrations. With depleted iron stores (serum ferritin concentration ≤ 15 µg/L), non-haem iron absorption from a standard Western diet is 9.2 %; this falls to 7.1 % with a diet containing inhibitors and increases to 11.8 % with a diet containing enhancers.

Armah et al. (2015) applied the complete-diet algorithm developed from absorption studies (Armah et al., 2013) to estimate total iron absorption from the US diet in all population groups participating in NHANES 2001–2002 (≥ 1 year, both sexes), but with the exclusion of pregnant and lactating women and individuals with raised C-reactive protein. Non-haem iron absorption was estimated at the individual level (n = 6 631) using intake data for enhancers and inhibitors of iron absorption (phytate intakes were estimated based on the phytate content of different foods according to Brown et al. (2004) and polyphenol intakes were estimated as black tea equivalents from the intake of tea, coffee and other polyphenol-containing beverages). It was assumed that 90 % of total iron intake was non-haem iron, and that the absorption of the remaining 10 % (haem iron) was 25 %. After correcting individual non-haem iron absorption values to a serum ferritin concentration of 15 µg/L, and adding absorption from haem iron, the percentage total dietary iron absorption was calculated to be 15.5 %.

Most studies on bioavailability have been undertaken in adults, and it is possible that the whole diet absorption figures derived from pooled data and/or algorithms, as described above, may not be appropriate for all population groups. Furthermore, the algorithms predict only non-haem iron absorption and, in order to calculate total iron absorption from the whole diet, an estimate of the quantity of absorbed haem iron has to be added to the value for predicted non-haem iron absorption.

An alternative method to calculate bioavailability factors to be used for deriving DRVs using factorial estimates was developed by Dainty et al. (2014). Data collected for the NDNS, a nationally representative sample of adults living in the UK and consuming a mixed Western-style diet, were used to develop a predictive model. These include serum ferritin concentration and total (haem and non-haem) iron intake determined from a 7-day dietary diary. The acute phase reactant α-1-antichymotrypsin was measured to ensure that the data used were derived from individuals who were free of inflammation. The NDNS sample comprised 495 men and 378 premenopausal women and was an iron-sufficient population. Physiological requirements were calculated from body weight and, in women, menstrual blood loss, following the IOM (2001) procedure for deriving Dietary Reference Intakes. The data were entered into a model to generate values for dietary iron absorption. In the men (mean iron intake 13.5 ± 5.1 mg/day; mean serum ferritin concentration 121.6 ± 112.1 µg/L), the mean calculated (haem and non-haem) iron absorption (50th percentile requirement for 1.08 mg absorbed iron/day) was 8 %. In the women (mean iron intake 9.8 ± 3.8 mg/day; mean serum ferritin concentration 45.5 ± 38.4 µg/L), the mean calculated (haem and non-haem) iron absorption (50th percentile requirement for 1.56 mg absorbed iron/day) was 17 %. The model can be used to predict iron absorption at any level of serum ferritin concentration. For example, at a serum ferritin concentration of 60 µg/L, iron absorption would be 11 % in both men and premenopausal women, whereas, at a serum ferritin concentration of 30 µg/L, iron absorption would be 18 % in women and 16 % in men. Using the well-established ratio method (reference serum ferritin divided by measured serum ferritin concentration) to normalise iron absorption to account for the effect of iron stores (Cook et al., 1991), at serum ferritin concentrations of 60, 45, 30 and 15 µg/L, iron absorption would be 10, 13, 20 and 30 %, respectively.

Although serum ferritin concentrations vary widely in all population groups, the Panel considers that a serum ferritin concentration of 30 µg/L is an appropriate target concentration for premenopausal women, as this reflects iron stores of approximately 120 mg (see Section 2.4). A target serum ferritin concentration of 30 µg/L is supported by observed serum ferritin concentrations in premenopausal women in the EU. Median serum ferritin concentration of premenopausal women in the UK NDNS was 38 µg/L (Dainty et al., 2014), and it was 40 µg/L (2.5th and 97.5th percentile: 4 and 229 µg/L, respectively) in 1 144 women aged 18 to > 65 years in Germany (Kohlmeier, 1995). Geometric mean serum ferritin concentration was 37 µg/L (SD 2.5)[13] in 2 079 women aged 18–65 years in the German Health Interview and Examination Survey (Baune et al., 2010). In Denmark, median serum ferritin concentration in 818 premenopausal women (aged 30–50 years) was 37 µg/L (5th and 95th percentile: 6 and 134 µg/L, respectively) (Milman et al., 1998), and it ranged from 28 to 39 µg/L in 322 Danish females aged 14–23 years, depending on age (Milman et al., 1997).

5.1.2.1. Iron absorption in infants and children

Although there are no data on iron absorption from whole diets in older infants (7–11 months), there is one paper describing two studies in infants aged nine months in which iron absorption was measured from multiple meals labelled with two different forms of stable isotopically enriched iron (Fox et al., 1998). In the first study, 22 infants were fed meals of a vegetable purée weaning food, to which ferrous sulphate or iron glycine was added. Each meal contained 1.6 mg iron in total and they were fed on eight consecutive days. Haemoglobin incorporation of the labelled iron (mean ± SE) was 9.9 ± 0.8 % for ferrous sulphate and 9.0 ± 0.7 % for iron glycine labelled meals. These values were not significantly different. In the second study, 24 infants were fed a high-phytate cereal weaning food (with milk) and iron bioavailability was compared with the same vegetable purée weaning food as in the first study. In the groups fed vegetable purée, haemoglobin incorporation was 9.1 ± 1.3 % from the meal containing added ferrous sulphate and 9.8 ± 1.5 % from the meal containing iron glycine. Iron bioavailability was significantly lower from the high-phytate cereal meals than the vegetable purée weaning food meals ($P < 0.001$), namely 3.8 ± 0.9 % from the high-phytate cereal meal containing ferrous sulphate and 5.2 ± 0.5 % from the meal containing iron glycine. The Panel notes that it is very likely that not all of the absorbed iron is incorporated into haemoglobin, and proposed a value for overall dietary iron absorption of 10 %.

Lynch et al. (2007) measured iron absorption in 28 children aged 1–4 years. After a 7-day home adaptation to a diet representative of their usual daily mineral intake (6.9 mg iron/day), they were given their usual breakfast and lunch, with each meal containing around one-third of the daily iron intake and labelled with a stable isotope of iron as ferrous sulphate. The Panel calculated a mean

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[13] A geometric mean (SD) of 3.6 (0.9) is given in the paper; these figures were back-transformed assuming that they were $\log_e$-transformed data.

absorption of 11.4 % from the reported values for milligrams of iron absorbed (measured from isotopic enrichment of haemoglobin, with the assumption that 90 % of absorbed iron was incorporated into haemoglobin) and iron intake. However, there were nine children with iron deficiency (low serum ferritin concentration) and when these were removed from the calculation the mean absorption of the remaining 19 iron-sufficient children was 9.7 %.

A number of single-meal studies in children have been undertaken using stable isotopes of iron to label iron in a test meal. Absorption has been estimated from isotopic enrichment of haemoglobin, and assuming 90 % of absorbed iron is utilised for haemoglobin synthesis. The Panel notes that absorption values reflect the bioavailability of iron in different foods, but not necessarily the whole diet. Chang et al. (2012) measured iron absorption from traditional Chinese home-made complementary food (millet porridge with wheat flour dumplings filled with cabbage, tofu and pork, containing 0.8 mg iron and fortified with 2 or 4 mg iron) in 29 children aged 24–31 months. Absorption from meals containing 2 mg iron as ferrous sulphate was 8.0 %, and with NaFeEDTA it was 9.2 %. In 21 children aged 3–6 years given a meal of toast, jelly or butter, a portion of non-citrus fruit, and either orange or apple juice (containing 1.2 mg iron) to which 5 mg isotopically enriched iron was added, absorption was 7.8 % from the meal ingested with orange juice and 7.2 % from the meal ingested with apple juice (Shah et al., 2003). Etcheverry et al. (2006) reported 7.6 % absorption of non-haem iron from a beef chilli meal (n = 12) and 3.5 % from a soy chilli meal (n = 14) in children aged 4–8 years. Both meals contained 3.3 mg non-haem iron. Avalos Mishaan et al. (2004) examined the effect of consuming a typical Peruvian breakfast meal (white bread and butter, plus reconstituted evaporated milk with sugar, containing 1.3 mg iron) on iron bioavailability of a micronutrient-fortified beverage (containing 7 mg iron) in 40 children aged 6–9 years. Mean iron absorption was 9.6 % from the meal plus beverage and 11.6 % from the beverage alone.

Fomon et al. (2005) administered a stable isotope of iron to 30 infants aged five months in order to label body pools and thereby measure endogenous iron losses between 13 and 26 months. There was a close relationship between losses and absorption (measured as total iron absorbed over approximately one year), but with very high inter-individual variation. They suggested that greater losses stimulate higher absorption, which would be consistent with the well-established inverse relationship between plasma/serum ferritin concentration and iron absorption, as also observed in their study (data not reported). The Panel assumes that this relationship also applies to older children.

Data from intervention (Appendix I) and observational (Appendix J) studies show that infants with an iron intake ranging from 3.1 to 4.8 mg/day have sufficient iron. Infants consuming an average of 8 mg/day of iron during the second half of infancy (partly through iron-fortified phytate-rich cereals) do not develop iron deficiency (Niinikoski et al., 1997; Lind et al., 2003; Gunnarsson et al., 2004). Diets at this age are rich in cereals and vegetables containing substances that possibly inhibit the absorption of iron (Fomon et al., 2005), but, despite the composition of the diet, it appears to supply sufficient bioavailable iron to infants still consuming breast milk (Domellof et al., 2002a).

5.2. Iron intake and health consequences

For the Nordic Nutrition Recommendations (NNR) 2012, a systematic literature review on health effects of different intakes of iron at different life stages was undertaken to estimate the requirement for adequate growth, development and maintenance of health (Domellof et al., 2013). Two specific research questions were addressed: (1) What is the minimal dose of dietary iron intake that will prevent poor functional or health outcomes in different age groups within the general population including the risk groups for iron deficiency? (2) What is the highest dose of dietary iron intake that is not associated with poor functional or health outcomes in different age groups within the general population including some risk groups for iron overload? A total of 55 articles were identified as relevant and the evidence was graded. Most studies were focused on vulnerable groups, namely young children and women of child-bearing age. There was some evidence that prevention of iron deficiency or iron deficiency anaemia improves cognitive/motor/behavioural development in young children, and treatment of iron deficiency anaemia improves attention and concentration in school children and adult women. There was insufficient evidence to show negative health effects of iron intakes at levels suggested by NNR 2004 (Nordic Council of Ministers, 2004).

A series of systematic reviews were conducted by EURRECA, an EU-funded Network of Excellence (Harvey et al., 2013). The EURRECA standardised systematic review methodology included randomised controlled trials with an adequate control group, as these provide the highest level of evidence. The selected health outcomes included tiredness, physical performance, immune function, impaired thermoregulation, restless leg syndrome and cognitive function. The studies suggested a modest positive effect of iron supplementation on cognition and psychomotor outcomes in anaemic infants and children after supplementation periods of at least two months' duration (Hermoso et al., 2011), but there was no effect on fetal growth (Vucic et al., 2013). A large degree of heterogeneity between study populations, iron doses and outcome measures prevented meta-analyses for most health outcomes, so it was not possible to draw conclusions about the relationships between iron intake and tiredness, physical performance, immune function, thermoregulation and restless leg syndrome. The EURRECA reviews highlight the dearth of health outcome data for setting DRVs for iron.

SACN (2010) undertook a comprehensive literature review of the role of iron in human nutrition, including the potential adverse effects of both iron deficiency and iron excess, in order to inform public health policy makers responsible for developing dietary recommendations for iron. The findings of SACN are summarised as follows. They concluded that, although low haemoglobin concentrations have been associated with impaired physical work capacity, reproductive efficiency and cognitive and psychomotor development, many of the studies had poorly reported outcomes and inadequate characterisation of iron deficiency, making interpretation of the data difficult. Iron supplementation studies indicated that iron deficiency anaemia is a cause of poor motor development in children in the first three years of life and on cognitive development in older children, but there was insufficient evidence to specify thresholds of anaemia or iron deficiency at which these health outcomes might occur. There was some evidence from randomised controlled trials that suggests that iron supplementation may impair physical growth of iron-replete infants and children, but further studies are required to characterise this effect. Intervention studies of iron supplementation during pregnancy have not shown beneficial or adverse effects on pregnancy outcomes. There were insufficient data to demonstrate an association between intakes of total dietary iron or body iron content and colorectal cancer. Observational studies of iron intake and cardiovascular disease did not suggest an association, although high intake of haem iron was associated with increased risk, possibly due to other components of meat or lifestyle factors. There was no evidence that dietary iron is associated with arthritis, diabetes mellitus or neurodegenerative disease.

SACN (2010) also pointed out that a risk assessment of iron and health is complicated by a number of uncertainties. The Panel considers that the following are relevant when attempting to establish DRVs for iron using data on health consequences: inaccurate estimates of iron intake and quantities of haem and non-haem iron in the diet; poor correlation between iron intake and status; difficulties in measuring adaptive and functional responses to variations in iron intake (bioavailability); lack of sensitive and specific markers to assess iron status and confounding by other dietary and lifestyle factors and by responses to infection and inflammation; inadequate characterisation of iron deficiency anaemia and the relative role of iron deficiency and other causes of anaemia in studies investigating the health consequences of iron deficiency. The Panel notes that these uncertainties make it difficult to determine dose–response relationships or to confidently predict the risks associated with iron deficiency or excess.

The Panel concludes that health outcomes cannot be used for the setting of DRVs for iron.

6. Data on which to base Dietary Reference Values

The Panel considers that setting DRVs for iron for adult men and women using modelled obligatory iron losses is appropriate (Section 5.1.1.1 and Appendix H). The 50th and 97.5th percentile losses were used as a basis for calculating an AR and a PRI for men (Section 6.1.1), and these data were also used for postmenopausal women (Section 6.1.3). The skewed distribution of basal losses of iron probably arising from menstrual losses necessitated some careful evaluation of the upper cut-off level for losses and requirements, and of the derivation of a PRI for premenopausal women in general (Section 6.1.2) and during pregnancy (Section 6.3) and lactation (Section 6.4). A factorial approach combined with data on iron turnover, body iron content and the rate of tissue synthesis were used to estimate requirements in infants aged 7–11 months and children up to 17 completed years (Section 6.2).

6.1. Adults

The Panel notes that iron requirements are very different before and after menopause owing to the presence or absence of menstrual iron losses and considers that the occurrence of menopause, rather than age, should define DRVs for women. The Panel also considers that DRVs do not need to be derived for vegetarians as a separate population group, because the bioavailability of iron from European vegetarian diets is not substantially different from diets containing meat (see Section 2.3.2).

6.1.1. Men

The 50th percentile of the model-based distribution of obligatory losses is 0.95 mg/day and the 97.5th percentile is 1.72 mg/day (Section 5.1.1.1 and Appendix H). A representative serum ferritin concentration at the lower end of observed distributions and reference ranges was taken as a serum ferritin concentration of 30 µg/L for men. This is associated with a percentage dietary iron absorption of 16 % (Dainty et al., 2014). Using this figure to convert the physiological requirement into the dietary requirement results in calculated dietary requirements at the 50th percentile of 5.9 mg/day and at the 97.5th percentile of 10.8 mg/day. After rounding, the Panel derives an AR of 6 mg/day and a PRI of 11 mg/day for men.

6.1.2. Premenopausal women

The 50th percentile of the model-based distribution of iron losses for these women who are in their reproductive years (Section 5.1.1.1 and Appendix H) is approximately 1.34 mg/day. The 90th, 95th and 97.5th percentiles are, respectively, 2.44, 2.80 and 3.13 mg/day and reflect the skew resulting from the large menstrual losses of some women (see Section 2.3.7.2). The Panel assumes that this group has a serum ferritin concentration of 30 µg/L, which corresponds to a percentage absorption of 18 % (Dainty et al., 2014). From these data, a dietary requirement at the 50th percentile of 7.4 mg/day can be derived. Intakes meeting the dietary iron requirement of approximately 90, 95 and 97.5 % of the premenopausal women are calculated as 13.6, 15.6 and 17.4 mg/day, respectively. After rounding, the Panel derives an AR of 7 mg/day and a PRI of 16 mg/day for premenopausal women. The Panel considers that the PRI meets the dietary requirement of 95 % of women in their reproductive years and is derived from a group of premenopausal women, some of whom use oral contraceptives, as is the case in the EU (see Section 2.3.7.2). For the remaining 5 % of the women with very high losses, iron absorption is probably up-regulated in accordance with lower serum ferritin concentrations in order to compensate for these losses. However, it is uncertain at which level of absorptive efficiency this up-regulation occurs, and the Panel cannot presume that this does occur. Therefore, it is not possible to derive a dietary requirement for this subgroup of women with very high iron losses. The Panel assumes that these high iron losses are due to high menstrual blood losses. This is supported by the observation in Hunt et al. (2009) that menstrual iron losses accounted for 90 % of the variation in total iron losses for the subset of women who provided complete menstrual collections (n = 13) and accounted for the skewed distribution of iron losses in these women.

6.1.3. Postmenopausal women

In the absence of reliable data on endogenous losses of iron in postmenopausal women, the Panel decided to set the same DRVs for postmenopausal women as those set for adult men, i.e. an AR of 6 mg/day and a PRI of 11 mg/day. The Panel notes that this may be a conservative estimate, as their lower body weight is probably associated with lower endogenous losses of iron.

6.2. Infants aged 7–11 months and children

The dietary iron requirement is estimated from the physiological iron requirement (Sections 5.1.1.2 and 5.1.1.3) considering percentage iron absorption from the diet. For infants aged 7–11 months, the Panel used a value of 10 % absorption based on the results of the two studies in 9-month-old infants carried out by (Fox et al., 1998), described in Section 5.1.2.1. In children aged 1–11 years, there is very limited information on iron absorption from whole diets. Non-haem iron absorption in children aged 1–4 years from a combination of breakfast and lunch, labelled with ^{58}Fe stable isotope, was reported to be 9.7 % in iron-sufficient children (Lynch et al., 2007). Iron absorption from single meals varies according to the type of meal and the form and quantity of added iron, and ranges from 3.5 % (in a soy meal containing inhibitory factors) to 9.6 % (in a simple breakfast meal; Section 5.1.2.1). The Panel considered that 10 % is the best estimate of dietary iron absorption in children up to the age of 11 years. In the absence of any data for dietary iron absorption in children aged 12–17 years, the 16 % absorption value derived from studies in adult men (Section 5.1.2) was used to convert physiological requirements into dietary intakes for this age group. The Panel acknowledges that an assumption has to be made that the relationship between serum ferritin concentration and efficiency of absorption holds for all age groups. There are no data to support this assumption but, from a physiological perspective, there are no indications that age will affect the relationship.

To calculate the PRI, in the absence of knowledge about the variation in requirement, a CV of 20 % is used for infants and children of all ages. The justification for this is the wide variation in rates of growth in children. In addition, a very high inter-individual variation (eight-fold) has been reported in iron losses and iron absorption (three-fold) in children aged 1–2 years (Fomon et al., 2005). The Panel recognises that differences in dietary patterns including the consumption of diets of low iron bioavailability (e.g. little or no meat, high intake of whole-grain cereals, and high intakes of milk), may also contribute to the high variation in requirements.

In infants aged 7–11 months, the requirement for absorbed iron is 0.79 mg/day. Considering that iron absorption is 10 %, the dietary requirement is calculated as 7.9 mg/day, and an AR of 8 mg/day is derived. Based on a CV of 20 %, the PRI is 11 mg/day.

In children aged 1–3 years, the requirement for absorbed iron is 0.51 mg/day (Table 7). Assuming 10 % absorption, the dietary requirement is calculated as 5.1 mg/day, and an AR of 5 mg/day is derived. Based on this and using a CV of 20 %, the PRI is 7 mg/day.

For children aged 4–6 years, the physiological requirement is estimated as 0.50 mg/day (Table 7). Assuming 10 % absorption, the dietary requirement is calculated as 5.0 mg/day, and an AR of 5 mg/day is derived. Based on this and using a CV of 20 %, the PRI is 7 mg/day.

In children aged 7–11 years, the requirement for absorbed iron is 0.76 mg (Table 7). Assuming 10 % absorption, the dietary requirement is calculated as 7.6 mg/day. After rounding, an AR of 8 mg/day is derived. Based on this and using a CV of 20 % and rounding, the PRI is 11 mg/day.

In children aged 12–17 years, the requirement for absorbed iron is 1.27 mg/day in boys and 1.13 mg/day in girls (Table 7). Assuming 16 % absorption, the dietary requirement is calculated as 7.9 mg/day for boys and 7.1 mg/day for girls. After rounding, an AR of 8 mg/day for boys and of 7 mg/day for girls aged 12–17 years is derived. In the absence of knowledge about the variation in requirement, the PRI for boys aged 12–17 years is estimated based on a CV of 20 % and, after rounding, is set at 11 mg/day.

In setting a PRI for girls aged 12–17 years, the Panel considers that there are uncertainties related to the great variability in the rate and timing of physiological development and maturation, the onset of menarche, and the extent of and the skewed distribution of menstrual iron losses. The factorially calculated AR for girls aged 12–17 years is slightly lower than that derived for premenopausal women based on probabilistic modelling. It is probable that the 16 % absorption used to calculate the dietary requirement of approximately half of adolescent girls underestimates that of adolescents in general, and there is evidence to support this possibility, but it is not enough to inform the setting of a PRI. Using a CV of 20 % to set a PRI would result in a value of 9.9 mg/day for the dietary requirement of about 97–98 % of adolescent girls. However, once growth has ceased in adolescent girls, their physiological and dietary requirements for iron can be expected to match those of premenopausal women. Thus, to take into account the uncertainties described above, in the transition to adulthood, the Panel has elected to set the PRI for adolescent girls as the mean of the calculated dietary requirement of 97–98 % of adolescent girls (9.9 mg/day) and the PRI for premenopausal women (16 mg/day). After rounding, a PRI of 13 mg/day is derived for girls aged 12–17 years.

6.3. Pregnancy

In the first trimester of pregnancy, iron intake should cover basal losses of about 1.08 mg/day. The requirements for absorbed iron then increase exponentially, up to about 10 mg/day during the last six weeks of pregnancy, but at the same time there is a progressive increase in the efficiency of iron absorption (Section 5.1.1.4). This can compensate for the higher needs, provided adequate iron stores are present at conception. The Panel therefore considers that ARs and PRIs for pregnant women are the same as for non-pregnant women of childbearing age (Section 6.1.2), with the important caveat that women enter pregnancy with an adequate iron status (serum ferritin concentration ≥ 30 µg/L).

6.4. Lactation

The Panel notes that the amount of iron secreted in breast milk during the first six months of lactation is 0.24 mg/day. Together with basal losses of 1.08 mg/day, the total requirement for absorbed iron during the first months of lactation is calculated to be 1.3 mg/day, assuming that menstruation has not yet resumed. The requirement for absorbed iron is slightly less than in non-pregnant, non-lactating women, but, for depleted iron stores to be replenished, the Panel considers that the AR and PRI for lactating women are the same as for non-pregnant women of childbearing age (Section 6.1.2).

CONCLUSIONS

The Panel concludes that ARs and PRIs for iron can be derived factorially. ARs for men and premenopausal women were estimated based on modelled whole-body iron losses using data from North American adults and a percentage dietary iron absorption that relates to a serum ferritin concentration of 30 µg/L. In men, obligatory losses at the 50th percentile are 0.95 mg/day and the AR was calculated taking into account 16 % absorption. The PRI was calculated as the requirement at the 97.5th percentile of whole-body iron losses and was rounded. For postmenopausal women, the same DRVs as for men are set. In premenopausal women, the 50th percentile of the model-based distribution of iron losses is equal to 1.34 mg/day, and the AR was calculated, taking into account 18 % absorption. The Panel decided to set a PRI covering the needs of 95 % of premenopausal women, and this is based on the 95th percentile of whole-body iron losses in this population group. For the remaining 5 % of the women with very high losses, iron requirements are higher, but there may be a compensatory up-regulation in the efficiency of absorption. However, it is uncertain to which level of absorptive efficiency this up-regulation occurs, so it is not possible to derive a dietary requirement for this subgroup of women with very high losses. In infants aged 7–11 months and children, requirements were calculated factorially, considering needs for growth and replacement of iron losses, and assuming 10 % dietary iron absorption for ages 7 months to 11 years and 16 % dietary iron absorption thereafter. In the absence of knowledge about the variation in requirement, PRIs for infants and children were estimated using a CV of 20 %. In girls aged 12–17 years, the PRI was set at the midpoint of the calculated dietary requirement of 97–98 % of adolescent girls and the PRI for premenopausal women. For pregnant and lactating women, for whom it was assumed that iron stores and enhanced absorption provide sufficient additional iron, DRVs are the same as for premenopausal women.

Table 9: Summary of Dietary Reference Values for iron

Age	**Average Requirement (mg/day)**	**Population Reference Intake (mg/day)**
7–11 months	8	11
1–6 years	5	7
7–11 years	8	11
12–17 years (M)	8	11
12–17 years (F)	7	13
≥ 18 years (M)	6	11
≥ 18 years (F)		
Premenopausal	7	16 (a)
Postmenopausal	6	11
Pregnancy	As for non-pregnant premenopausal women	As for non-pregnant premenopausal women
Lactation	As for non-lactating premenopausal women	As for non-lactating premenopausal women

F, females; M, males.
(a): The PRI covers the requirement of approximately 95 % of premenopausal women.

RECOMMENDATIONS FOR RESEARCH

The Panel recommends that:

- Iron homeostasis be better characterised to enable the development and validation of markers indicating adaptation to insufficient iron supply.
- Dose–response data be generated for iron intake/status and functional outcomes/health endpoints, e.g. growth and development in children, pregnancy outcome, dementia.
- Iron absorption and metabolism in pregnancy be investigated, including causes of iron deficiency and its effect on fetal development and consequences for later life. The Panel also recommends that longitudinal data on serum ferritin concentration and other appropriate markers of iron status in pregnancy be generated in order to predict the risk of developing iron deficiency anaemia.
- Effects of different physiological states on iron requirements be investigated, e.g. overweight, obesity, low-grade inflammation, pregnancy, ageing.
- Iron absorption from whole diets in all age groups, effects of different dietary patterns on bioavailability, and haem iron content of cooked and processed meat, meat products and other flesh foods be investigated.
- Data on whole-body iron losses in all population groups be generated, especially in menstruating women. The Panel also recommends that the relationship between iron losses and absorption efficiency be investigated, especially in women with high menstrual losses.
- The bioavailability of iron fortificants be investigated, as well as their contribution to total dietary iron intake.

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APPENDICES

Appendix A. Percentiles of daily iron loss with menstruation based on individual data from Harvey et al. (2005)

Percentile	Menstrual iron loss (mg/day)
5	0.03
10	0.07
15	0.09
20	0.11
25	0.13
30	0.17
35	0.19
40	0.21
45	0.23
50	0.26
55	0.29
60	0.36
65	0.41
70	0.48
75	0.59
80	0.69
85	0.82
90	0.91
95	1.32
97	1.51
98	1.92

Menstrual iron losses were quantified by the direct measurement of menstrual blood loss per menstrual cycle. Menstrual iron loss was subsequently calculated by Harvey et al. (2005) from the total menstrual blood loss of each participant based on the following equation:

$$\text{MIL (mg/day)} = \frac{\text{MBL (mL)} \times \text{Hb (mg/mL)} \times 0.00334}{\text{cycle length}}$$

Where MIL is menstrual iron loss, MBL is menstrual blood loss and 0.00334 is equivalent to the fraction of iron in haemoglobin (Hb) at a concentration of 1 mg/mL.

Appendix B. Cut-off values for biochemical indicators of iron deficiency proposed in the literature

Table 10: Cut-off values for haemoglobin concentration (UNICEF/UNU/WHO, 2001) and other biomarkers of iron status that indicate the presence of anaemia (at altitudes < 1 000 m) (Zimmermann, 2008)

Population group	Hb (g/L)	Haematocrit (%)	ZPP (µmol/mol haem)	MCV (fL)	Serum iron (µg/L)	TSAT (%)
6–59 months	< 110	0.33			< 40–50	
5–11 years	< 115	0.34	> 40		< 40–50	
12–14 years	< 120	0.36	> 40	< 82	< 40–50	< 15 %
Women	< 120	0.36	> 40	< 82	< 40–50	< 15 %
Pregnant women	< 110	0.33				
Men > 15 years	< 130	0.39	> 40	< 82	< 40–50	< 15 %

Hb, haemoglobin; MCV, mean corpuscular volume; TSAT, transferrin saturation; ZPP, erythrocyte zinc protoporphyrin.

Table 11: Definition of anaemia according to the UK guidelines on the management of iron deficiency in pregnancy (Pavord et al., 2012)

Timepoint	Haemoglobin (g/L)
1st trimester	< 110
2nd trimester	< 105
3rd trimester	< 105
Post-partum	< 100

The guidelines also state that non-anaemic women identified to be at increased risk of iron deficiency should have their serum ferritin concentration checked early in pregnancy and be offered oral iron supplements if serum ferritin is < 30 µg/L.

Table 12: Cut-off values for serum ferritin concentration (UNICEF/UNU/WHO, 2001)

	Serum ferritin (µg/L)	
	< 5 years of age	≥ 5 years of age
Severe risk of iron overload	No cut-off	> 200 (adult male) > 150 (adult female)
Depleted iron stores in the presence of infection	< 30	No cut-off
Depleted iron stores	< 12 (a)	< 15

(a): < 9 µg/L at 6 months and < 5 µg/L at 9 months (Domellof et al., 2002b).

Appendix C. Dietary surveys in the EFSA Comprehensive European Food Consumption Database included in the nutrient intake calculation and number of subjects in the different age classes

Country	Dietary survey	Year	Method	Days	Age (years)	Number of subjects						
						Infants < 1 year	Children 1–< 3 years	Children 3–< 10 years	Children 10–< 18 years	Adults 18–< 65 years	Adults 65–< 75 years	Adults ≥ 75 years
Finland/1	DIPP	2000–2010	Dietary record	3	0.5–6	499	500	750				
Finland/2	NWSSP	2007–2008	48-hour dietary recall (a)	2 × 2 (a)	13–15				306			
Finland/3	FINDIET2012	2012	48-hour dietary recall (a)	2 (a)	25–74					1 295	413	
France	INCA2	2006–2007	Dietary record	7	3–79			482	973	2 276	264	84
Germany/1	EsKiMo	2006	Dietary record	3	6–11			835	393			
Germany/2	VELS	2001–2002	Dietary record	6	< 1–4	158	347	299				
Ireland	NANS	2008–2010	Dietary record	4	18–90					1 274	149	77
Italy	INRAN-SCAI	2005–2006	Dietary record	3	< 1–98	16 (b)	36 (b)	193	247	2 313	290	228
Latvia	FC_PREGNANTWOMEN	2011	24-hour dietary recall	2	15–45				12 (b)	991 (c)		
Netherlands	DNFCS	2007–2010	24-hour dietary recall	2	7–69			447	1 142	2 057	173	
Sweden	Riksmaten	2010–2011	Dietary record (web) (d)	4	18–80					1 430	295	72
United Kingdom/1	DNSIYC	2011	Dietary record	4	0.3–1.5	1 369	1 314					
United Kingdom/2	NDNS Rolling Programme (Years 1–3)	2008–2011	Dietary record	4	1–94		185	651	666	1 266	166	139

DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): A 48-hour dietary recall comprises two consecutive days.

(b): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation, as the results may not be statistically robust (EFSA, 2011a) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates will not be presented in the intake results.

(c): One subject with only one 24-hour dietary recall day was excluded from the dataset, i.e. final n = 990.

(d): The Swedish dietary records were introduced through the internet.

Appendix D. Iron intake in males in different surveys according to age classes and country

Age class	Country	Survey	Intake expressed in mg/day					Intake expressed in mg/MJ				
			n [(a)]	Average	Median	P5	P95	n	Average	Median	P5	P95
Infants [(b)]	Germany	VELS	84	6.0	5.9	3.2	9.4	84	1.9	1.9	1.0	3.0
	Finland	DIPP_2001_2009	247	3.0	3.2	0.4	5.7	245	1.5	1.5	0.8	2.2
	United Kingdom	DNSIYC_2011	699	5.9	5.8	2.7	9.5	699	1.7	1.7	0.9	2.5
	Italy	INRAN_SCAI_2005_06	9	2.6	1.9	(c)	(c)	9	0.9	0.5	(c)	(c)
1 to < 3	Germany	VELS	174	7.0	6.5	3.6	11.4	174	1.5	1.4	1.0	2.2
	Finland	DIPP_2001_2009	245	5.4	5.2	2.8	7.9	245	1.5	1.5	1.0	2.1
	United Kingdom	NDNS Rolling Programme Years 1–3	107	6.3	6.0	4.2	10.0	107	1.3	1.3	0.9	1.9
	United Kingdom	DNSIYC_2011	663	5.9	5.7	3.1	9.2	663	1.4	1.4	0.8	2.2
	Italy	INRAN_SCAI_2005_06	20	6.0	6.2	(c)	(c)	20	1.2	1.1	(c)	(c)
3 to < 10	Germany	EsKiMo	426	11.5	11.2	7.2	17.0	426	1.5	1.5	1.1	2.1
	Germany	VELS	146	8.7	7.7	5.3	14.0	146	1.5	1.4	1.1	2.4
	Finland	DIPP_2001_2009	381	8.3	8.0	5.5	12.3	381	1.4	1.4	1.0	1.9
	France	INCA2	239	10.7	10.2	5.7	17.3	239	1.7	1.6	1.1	2.4
	United Kingdom	NDNS Rolling Programme Years 1–3	326	8.6	8.3	5.1	12.6	326	1.4	1.3	1.0	1.9
	Italy	INRAN_SCAI_2005_06	94	9.9	9.6	5.6	16.3	94	1.3	1.3	1.0	2.1
	Netherlands	DNFCS 2007–2010	231	9.2	9.0	5.6	13.3	231	1.1	1.0	0.8	1.5
10 to < 18	Germany	EsKiMo	197	11.8	11.3	7.2	18.7	197	1.5	1.4	1.0	2.1
	Finland	NWSSP07_08	136	11.6	11.2	6.9	18.1	136	1.4	1.4	1.0	2.1
	France	INCA2	449	13.6	12.8	7.5	22.2	449	1.7	1.7	1.2	2.6
	United Kingdom	NDNS Rolling Programme Years 1–3	340	11.2	10.8	6.7	17.8	340	1.4	1.3	1.0	2.0
	Italy	INRAN_SCAI_2005_06	108	12.3	11.8	7.4	18.2	108	1.3	1.2	1.0	1.9
	Netherlands	DNFCS 2007–2010	566	11.2	10.9	6.7	17.6	566	1.1	1.0	0.7	1.5
18 to < 65	Finland	FINDIET2012	585	13.2	12.5	7.4	21.2	585	1.4	1.4	1.0	2.1
	France	INCA2	936	14.4	13.7	7.5	23.1	936	1.7	1.6	1.1	2.6
	United Kingdom	NDNS Rolling Programme Years 1–3	560	12.8	12.3	6.6	20.1	560	1.5	1.4	0.9	2.1
	Ireland	NANS_2012	634	14.7	14.3	8.3	22.2	634	1.5	1.5	1.0	2.1
	Italy	INRAN_SCAI_2005_06	1 068	12.6	12.2	7.1	19.8	1 068	1.4	1.4	1.0	1.9
	Netherlands	DNFCS 2007–2010	1 023	13.1	12.7	7.7	19.4	1 023	1.2	1.1	0.8	1.7
	Sweden	Riksmaten 2010	623	14.1	13.4	7.8	22.3	623	1.4	1.4	1.0	2.0

Age class	**Country**	**Survey**	**Intake expressed in mg/day**					**Intake expressed in mg/MJ**				
			n (a)	**Average**	**Median**	**P5**	**P95**	**n**	**Average**	**Median**	**P5**	**P95**
65 to < 75	Finland	FINDIET2012	210	11.9	11.4	6.6	18.8	210	1.5	1.4	0.9	2.1
	France	INCA2	111	15.0	14.3	7.6	24.5	111	1.8	1.6	1.2	2.7
	United Kingdom	NDNS Rolling Programme Years 1–3	75	12.9	12.2	6.2	19.8	75	1.5	1.5	0.9	2.2
	Ireland	NANS_2012	72	13.3	13.4	6.9	19.0	72	1.5	1.5	1.1	2.1
	Italy	INRAN_SCAI_2005_06	133	13.3	12.8	7.0	19.4	133	1.5	1.5	1.1	2.1
	Netherlands	DNFCS 2007–2010	91	12.1	11.8	6.2	18.3	91	1.3	1.3	1.0	1.7
	Sweden	Riksmaten 2010	127	13.0	12.9	7.5	19.8	127	1.5	1.5	1.1	2.0
≥ 75	France	INCA2	40	12.6	11.4	(c)	(c)	40	1.6	1.5	(c)	(c)
	United Kingdom	NDNS Rolling Programme Years 1–3	56	10.8	9.7	(c)	(c)	56	1.5	1.5	(c)	(c)
	Ireland	NANS_2012	34	11.4	10.1	(c)	(c)	34	1.5	1.5	(c)	(c)
	Italy	INRAN_SCAI_2005_06	69	12.6	12.0	7.8	18.6	69	1.4	1.4	1.0	2.0
	Sweden	Riksmaten 2010	42	12.1	12.1	(c)	(c)	42	1.4	1.4	(c)	(c)

DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale des Consommations Alimentaires; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; P5, 5th percentile; P95, 95th percentile; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Number of individuals in the population group.

(b): The proportions of breast-fed infants were 58 % in the Finnish survey, 40 % in the German survey, 44 % in the Italian survey and 21 % in the UK survey. Most infants were partly breast-fed. For the Italian and German surveys, breast milk intake estimates were derived from the number of breastfeeding events recorded per day multiplied by standard breast milk amounts consumed on an eating occasion at different ages. For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(c): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation, as the results may not be statistically robust (EFSA, 2011a) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates will not be presented in the intake results.

Appendix E. Iron intake in females in different surveys according to age classes and country

Age class	Country	Survey	Intake expressed in mg/day					Intake expressed in mg/MJ				
			n (a)	Average	Median	P5	P95	n	Average	Median	P5	P95
Infants (b)	Germany	VELS	75	5.5	5.7	2.0	9.0	75	1.9	1.9	0.9	3.1
	Finland	DIPP_2001_2009	252	2.8	2.5	0.4	5.7	251	1.6	1.5	0.9	2.6
	United Kingdom	DNSIYC_2011	670	5.2	5.0	2.0	8.3	670	1.7	1.7	0.8	2.5
	Italy	INRAN_SCAI_2005_06	7	3.5	4.1	(c)	(c)	7	1.2	1.1	(c)	(c)
1 to < 3	Germany	VELS	174	6.6	6.4	3.8	10.6	174	1.6	1.5	1.1	2.4
	Finland	DIPP_2001_2009	255	5.0	5.0	2.8	7.6	255	1.5	1.4	0.9	2.0
	United Kingdom	NDNS Rolling Programme Years 1–3	78	6.1	5.8	2.9	10.0	78	1.3	1.3	0.7	1.8
	United Kingdom	DNSIYC_2011	651	5.7	5.4	2.8	9.6	651	1.4	1.4	0.9	2.2
	Italy	INRAN_SCAI_2005_06	16	6.0	5.4	(c)	(c)	16	1.3	1.2	(c)	(c)
3 to < 10	Germany	EsKiMo	409	10.6	10.3	6.5	16.3	409	1.6	1.5	1.1	2.1
	Germany	VELS	147	7.8	7.4	4.7	12.9	147	1.5	1.4	1.0	2.5
	Finland	DIPP_2001_2009	369	7.5	7.3	4.7	11.0	369	1.4	1.4	1.0	2.0
	France	INCA2	243	9.5	8.9	5.7	15.1	243	1.7	1.6	1.2	2.4
	United Kingdom	NDNS Rolling Programme Years 1–3	325	8.5	7.9	4.7	13.7	325	1.4	1.3	0.9	2.1
	Italy	INRAN_SCAI_2005_06	99	9.1	9.2	5.1	13.4	99	1.2	1.2	0.9	1.7
	Netherlands	DNFCS 2007–2010	216	8.8	8.4	5.5	13.1	216	1.1	1.1	0.8	1.4
10 to < 18	Germany	EsKiMo	196	11.6	11.2	7.5	17.3	196	1.6	1.5	1.1	2.1
	Finland	NWSSP07_08	170	9.9	9.4	5.7	16.1	170	1.5	1.5	1.1	2.1
	France	INCA2	524	10.9	10.3	5.8	17.2	524	1.7	1.7	1.2	2.6
	United Kingdom	NDNS Rolling Programme Years 1–3	326	9.2	8.9	5.0	13.6	326	1.4	1.3	0.9	2.0
	Italy	INRAN_SCAI_2005_06	139	10.5	10.2	6.2	16.9	139	1.3	1.2	0.9	2.1
	Latvia (d)	FC_PREGNANTWOMEN_2011	12	14.7	15.3	(c)	(c)	12	1.5	1.5	(c)	(c)
	Netherlands	DNFCS 2007–2010	576	9.6	9.2	6.0	14.6	576	1.1	1.1	0.7	1.6
18 to < 65	Finland	FINDIET2012	710	10.5	10.3	6.0	16.0	710	1.5	1.4	1.0	2.1
	France	INCA2	1 340	11.1	10.5	5.7	18.3	1 340	1.7	1.6	1.1	2.6
	United Kingdom	NDNS Rolling Programme Years 1–3	706	10.5	10.2	5.4	16.1	706	1.6	1.5	1.0	2.4
	Ireland	NANS_2012	640	11.0	10.7	6.1	17.6	640	1.5	1.5	1.0	2.1
	Italy	INRAN_SCAI_2005_06	1 245	10.2	9.9	5.7	15.8	1 245	1.4	1.3	1.0	2.0
	Latvia (d)	FC_PREGNANTWOMEN_2011	990	17.9	15.2	8.8	34.9	990	2.1	1.8	1.1	4.1
	Netherlands	DNFCS 2007–2010	1 034	11.0	10.4	6.6	16.7	1 034	1.3	1.3	0.9	2.0
	Sweden	Riksmaten 2010	807	11.6	11.1	6.2	18.6	807	1.5	1.5	1.0	2.2

Age class	Country	Survey	Intake expressed in mg/day					Intake expressed in mg/MJ				
			n [(a)]	Average	Median	P5	P95	n	Average	Median	P5	P95
65 to < 75	Finland	FINDIET2012	203	9.4	9.0	5.4	14.7	203	1.5	1.5	1.1	2.3
	France	INCA2	153	10.6	10.1	6.2	16.9	153	1.7	1.6	1.2	2.5
	United Kingdom	NDNS Rolling Programme Years 1–3	91	10.7	10.7	6.3	17.5	91	1.8	1.6	1.2	2.8
	Ireland	NANS_2012	77	11.0	11.2	6.7	16.7	77	1.6	1.6	1.1	2.5
	Italy	INRAN_SCAI_2005_06	157	10.1	10.0	5.7	16.7	157	1.5	1.4	1.0	2.1
	Netherlands	DNFCS 2007–2010	82	10.7	10.6	6.2	16.2	82	1.5	1.4	1.1	2.0
	Sweden	Riksmaten 2010	168	11.1	10.7	6.4	17.6	168	1.6	1.6	1.1	2.3
≥ 75	France	INCA2	44	9.9	9.7	(c)	(c)	44	1.6	1.6	(c)	(c)
	United Kingdom	NDNS Rolling Programme Years 1–3	83	10.5	9.8	6.3	16.0	83	1.7	1.6	1.2	2.4
	Ireland	NANS_2012	43	10.5	10.5	(c)	(c)	43	1.7	1.6	(c)	(c)
	Italy	INRAN_SCAI_2005_06	159	9.6	9.3	5.8	14.1	159	1.4	1.4	1.0	2.0
	Sweden	Riksmaten 2010	30	10.3	9.7	(c)	(c)	30	1.5	1.4	(c)	(c)

DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; P5, 5th percentile; P95, 95th percentile; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Number of individuals in the population group.

(b): The proportions of breast-fed infants were 58 % in the Finnish survey, 40 % in the German survey, 44 % in the Italian survey and 21 % in the UK survey. Most infants were partially breast-fed. For the Italian and German surveys, breast milk intake estimates were derived from the number of breastfeeding events recorded per day multiplied by standard breast milk amounts consumed on an eating occasion at different ages. For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(c): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation, as the results may not be statistically robust (EFSA, 2011a) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates will not be presented in the intake results.

(d): Pregnant women only.

Appendix F. Minimum and maximum percentage contribution of different food groups (FoodEx2 level 1) to iron intake in males

Food groups	**Age**						
	< 1 year	**1 to < 3 years**	**3 to < 10 years**	**10 to < 18 years**	**18 to < 65 years**	**65 to < 75 years**	**≥ 75 years**
Additives, flavours, baking and processing aids	< 1	< 1	0	< 1–1	< 1	< 1	0
Alcoholic beverages	< 1	< 1	< 1	< 1	2–9	2–13	3–13
Animal and vegetable fats and oils	< 1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1
Coffee, cocoa, tea and infusions	< 1–1	< 1–8	2–14	3–8	1–9	1–11	1–7
Composite dishes	< 1–3	< 1–11	< 1–11	1–14	1–14	1–12	< 1–14
Eggs and egg products	< 1–1	1–2	1–4	1–4	1–3	1–3	1–3
Fish, seafood, amphibians, reptiles and invertebrates	< 1	< 1–6	< 1–6	1–5	1–6	2–6	2–5
Food products for young population	44–67	4–22	< 1–1	< 1	< 1	–	–
Fruit and fruit products	3–9	5–9	2–5	1–4	1–5	3–6	2–6
Fruit and vegetable juices and nectars	< 1–2	1–5	1–8	1–6	1–4	< 1–4	< 1–3
Grains and grain-based products	10–18	32–38	31–42	31–40	25–42	21–43	20–49
Human milk	< 1–15	< 1–1	–	–	–	–	–
Legumes, nuts, oilseeds and spices	1–3	1–7	1–7	1–6	2–7	2–7	2–5
Meat and meat products	< 1–7	5–14	6–19	9–24	11–27	11–27	11–21
Milk and dairy products	1–4	4–8	3–7	2–6	1–4	1–4	1–3
Products for non-standard diets, food imitates and food supplements or fortifying agents	0	0	0–1	< 1–1	< 1–1	< 1	0
Seasoning, sauces and condiments	< 1–1	< 1–4	< 1–2	< 1–2	< 1–2	< 1–2	< 1–1
Starchy roots or tubers and products thereof, sugar plants	< 1–10	2–10	3–8	4–10	3–8	3–10	4–9
Sugar, confectionery and water-based sweet desserts	< 1	1–6	2–8	2–9	1–4	1–3	< 1–3
Vegetables and vegetable products	1–7	4–7	4–9	4–12	3–14	3–15	4–13
Water and water-based beverages	< 1–1	< 1–9	< 1–10	< 1–9	< 1–4	< 1–2	< 1–2

"–" means that there was no consumption event of the food group for the age and sex group considered, whereas "0" means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.

Appendix G. Minimum and maximum percentage contribution of different food groups (FoodEx2 level 1) to iron intake in females

Food groups	**Age**						
	< 1 year	**1 to < 3 years**	**3 to < 10 years**	**10 to < 18 years**	**18 to < 65 years**	**65 to < 75 years**	**≥ 75 years**
Additives, flavours, baking and processing aids	< 1	0	0	< 1–1	< 1	0	0
Alcoholic beverages	< 1	< 1	< 1	< 1	< 1–6	1–6	2–5
Animal and vegetable fats and oils	< 1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1
Coffee, cocoa, tea and infusions	< 1–1	< 1–10	1–13	2–11	2–10	1–11	2–11
Composite dishes	< 1–2	< 1–11	< 1–11	< 1–15	1–14	1–12	1–13
Eggs and egg products	< 1–1	1–2	1–4	1–3	1–3	1–3	1–3
Fish, seafood, amphibians, reptiles and invertebrates	< 1–1	< 1–5	< 1–4	< 1–8	1–6	2–5	1–4
Food products for young population	45–72	4–22	< 1–1	< 1	< 1	–	< 1
Fruit and fruit products	3–8	5–6	2–5	2–6	2–6	4–8	3–8
Fruit and vegetable juices and nectars	< 1–2	1–4	2–7	2–6	1–4	1–3	1–3
Grains and grain-based products	9–19	31–42	31–39	31–42	26–48	20–43	19–47
Human milk	< 1–5	< 1	–	–	–	–	–
Legumes, nuts, oilseeds and spices	< 1–7	1–7	1–6	1–5	3–7	3–6	2–4
Meat and meat products	1–7	5–14	6–19	8–20	9–24	10–26	8–23
Milk and dairy products	1–5	4–8	2–8	1–6	1–5	2–4	2–4
Products for non-standard diets, food imitates and food supplements or fortifying agents	0	0	0–1	0–1	< 1–2	< 1–1	0–2
Seasoning, sauces and condiments	< 1–1	< 1–1	1	< 1–2	< 1–2	< 1–1	1
Starchy roots or tubers and products thereof, sugar plants	2–9	4–9	3–8	3–10	3–7	3–7	3–8
Sugar, confectionery and water-based sweet desserts	< 1–2	< 1–5	2–8	2–12	1–13	< 1–3	1–2
Vegetables and vegetable products	4–8	4–6	4–9	4–11	4–16	4–17	5–16
Water and water-based beverages	< 1–1	< 1–7	< 1–11	< 1–8	< 1–5	< 1–4	< 1–3

"–" means that there was no consumption event of the food group for the age and sex group considered, whereas "0" means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.

Appendix H. Re-analysis of data on endogenous iron losses from Hunt et al. (2009)

SOURCE OF INFORMATION

H1. Data sources

The current analysis is based on individual data provided by the US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center and the University of North Dakota, Grand Forks, USA. The individual data are the property of these institutions and, therefore, they cannot be disclosed by EFSA. The study and the corresponding set of data were identified in the literature and selected by the NDA Panel.

The original research was aimed at measuring total endogenous iron losses in men and women. The study recruited men and women who had participated at least one year earlier in studies of healthy subjects who were administered iron radioisotope (^{55}Fe). All subjects meeting this criterion were enrolled in a 3-year study that involved semi-annual blood sampling. Subjects completed a questionnaire on general health and factors that might affect body iron excretion at the beginning and at the end of the study. The list of questions and the outcomes of the questionnaire were not made available to EFSA. Throughout the study the subjects had to update information about health, iron supplement use or blood losses due to medical conditions or care, pregnancy, use of chemical forms of birth control or hormone replacements and dates of menstruation.

Subjects were considered eligible for the final analysis according to the following criteria:

- provision of semi-annual blood samples for at least one year;
- no use of iron supplements;
- no surgery;
- no blood donation;
- if women, no occurrence of pregnancy or menopause during the study.

Based on the weak X-rays emitted by the radioisotope, the biological half-life of iron was determined for each subject from blood samples collected semi-annually. Body iron was determined as the sum of circulating haemoglobin iron plus body iron stores, based on measurements from samples collected on two separate days at the beginning and again at the end of each subject’s participation.

The metabolic body weight (body weight to the power of 0.75) (EFSA NDA Panel, 2010), not available from the original dataset, was computed for the current analysis in order to better investigate the potential effect of body weight on iron losses. Since fat mass does not contribute significantly to iron losses, the transformation of body weight into metabolic body weight was assumed to be able to better highlight the association between iron losses and lean body mass.

The variable named “turnover rate” in the dataset, expressing the percentage of iron losses per year, was transformed into a rate, dividing it by 100, in order to get values between 0 and 1. However, the same name was maintained for the variable. The transformation was carried out because, for variables bounded by values 0 and 1, it may be easier to find a parametric distribution to represent variability (typically a beta distribution).

While 53 subjects entered the analysis performed by Hunt et al. (2009), 55 were included in the dataset provided to EFSA; the difference being the inclusion of two women for whom the menstruating status was not specified.

It is not clear from the paper by Hunt et al. (2009) how repeated measurements on blood samples collected twice per year for 1–3 years have been summarised in the dataset provided to EFSA. The latter includes only one value per subject. Therefore, it was not possible to estimate intra-subject variability and increase precision of the estimate.

The composition of the sample in terms of sex/menstruating status subgroup is reported in Table 13.

Table 13: Frequency of the four subgroups

Group	Number	Frequency (%)
Men	29	52.7
Women—menstruating	19	34.6
Women—postmenopausal	5	9.1
Women—unknown menstruating status	2	3.6
All subgroups	55	100

H2. Eligibility criteria for subject selection and data preprocessing

The same eligibility criteria established by Hunt et al. (2009) were maintained in the analysis, except for exclusion of postmenopausal women. The summary statistics of age at the beginning of the study, body weight, BMI, metabolic body weight, serum ferritin concentration, iron losses, biological half-life and turnover rate are reported in Table 14 (by sex) and Table 15 (by subgroups).

Table 14: Summary statistics by sex

	Mean	Standard deviation	Median	Minimum	Maximum
Age at start (years)					
Female	42.07	7.09	41.79	30.19	57.62
Male	42.96	8.03	42.54	30.42	58.30
Body weight (kg)					
Female	71.87	11.58	72.95	52.00	89.20
Male	91.65	14.89	90.40	61.80	130.90
BMI (kg/m^2)					
Female	27.11	4.49	27.39	18.65	36.14
Male	28.78	3.69	28.27	21.77	35.32
Metabolic body weight (actual body weight to the power of 0.75, kg)					
Female	24.62	3.00	24.96	19.36	29.03
Male	29.55	3.59	29.32	22.04	38.70
Iron losses (mg/day)					
Female	1.73	1.12	1.53	0.57	4.88
Male	1.07	0.47	1.18	0.11	2.07
Iron biological half-life (years)					
Female	3.83	1.72	3.92	0.72	7.46
Male	8.99	6.20	7.24	4.30	31.61
Iron turnover rate (rate/year) (a)					
Female	0.25	0.20	0.18	0.09	0.96
Male	0.10	0.04	0.10	0.02	0.16
Serum ferritin (µg/L)					
Female	58.65	60.33	36.61	6.58	284.75
Male	164.19	87.41	138.50	50.70	356.75

(a): Percentage of iron losses per year, transformed into a rate, i.e. dividing by 100, in order to get values between 0 and 1.

Table 15: Summary statistics by subgroups by sex/menstruating status

	Mean	Standard deviation	Median	Minimum	Maximum
Age at start (years)					
Women—menstruating	39.86	4.72	38.72	31.60	46.63
Women—postmenopausal	49.92	4.92	48.40	45.53	57.62
Women—unknown menstruating status	43.49	18.82	43.49	30.19	56.80
Men	42.96	8.03	42.54	30.42	58.30
Body weight (kg)					
Women—menstruating	73.48	10.21	73.60	56.00	87.60
Women—postmenopausal	67.56	14.36	64.80	53.00	89.20
Women—unknown menstruating status	67.25	21.57	67.25	52.00	82.50
Men	91.65	14.89	90.40	61.80	130.90
BMI (kg/m^2)					
Women—menstruating	27.89	2.63	25.13	20.47	36.14
Women—postmenopausal	25.49	3.72	22.84	19.64	30.68
Women—unknown menstruating status	23.77	5.66	23.37	19.36	28.89
Men	28.78	3.59	29.32	22.04	35.32
Metabolic body weight (body weight to the power of 0.75, kg)					
Women—menstruating	25.05	4.33	28.04	19.38	28.63
Women—postmenopausal	23.49	4.08	23.80	20.70	29.03
Women—unknown menstruating status	23.37	7.24	23.77	18.65	27.37
Men	29.55	3.69	28.27	21.77	38.70
Iron losses (mg/day)					
Women—menstruating	1.97	1.22	1.58	0.65	4.88
Women—postmenopausal	1.08	0.28	0.99	0.86	1.57
Women—unknown menstruating status	1.11	0.77	1.11	0.57	1.66
Men	1.07	0.47	1.18	0.11	2.07
Iron biological half-life (years)					
Women—menstruating	3.46	1.78	3.67	0.72	7.46
Women—postmenopausal	4.69	1.01	4.24	3.78	5.92
Women—unknown menstruating status	5.16	1.69	5.16	3.96	6.36
Men	8.99	6.20	7.24	4.30	31.61
Iron turnover rate (rate/year) (a)					
Women—menstruating	0.29	0.22	0.19	0.09	0.96
Women—postmenopausal	0.15	0.03	0.16	0.12	0.18
Women—unknown menstruating status	0.14	0.05	0.14	0.11	0.17
Men	0.10	0.04	0.10	0.02	0.16
Serum ferritin (µg/L)					
Women—menstruating	47.82	41.40	32.48	6.575	148.75
Women—postmenopausal	96.88	111.55	39.42	21.93	284.75
Women—unknown menstruating status	65.96	27.03	65.96	46.85	85.075
Men	164.19	87.41	138.50	50.70	356.75

(a): Percentage of iron losses per year, transformed into a rate, i.e. dividing by 100, in order to get values between 0 and 1.

For the two women with unknown menstruating status the Panel considered it reasonable to allocate them into one of the two groups: menstruating women or postmenopausal women based on the assessment of age and the use of birth control measures (if any). Owing to the limited size of the group, the postmenopausal women could not be analysed independently. Therefore, it was decided to test whether these women could be merged with either the men or menstruating women groups.

H2.1. Allocation of women with unknown menstruating status

The dataset included two females for which menstruating status was unknown. In order to avoid their exclusion from the dataset, the size of which was already limited, the two individuals were included in one of the two female subgroups on the basis of age and use of birth control measures.

According to this criterion the following attribution was performed:

Subject code	Age	Birth control measure	Subgroup
25	30	Yes	Menstruating women
26	57	Unknown	Postmenopausal women

H2.2. Allocation of the subgroup of postmenopausal women

The limited number of observations available for postmenopausal women did not allow any analysis on this group independently. The option of merging these women with either the men or menstruating women groups was investigated. The criterion of the similarity with respect to the variables iron losses, iron turnover rate, iron half-life and metabolic body weight was considered appropriate for this purpose. The boxplot of iron losses in the four subgroups is presented in Figure 2. The *t*-test with unequal variance (Ramsey, 1980) was used for this scope.

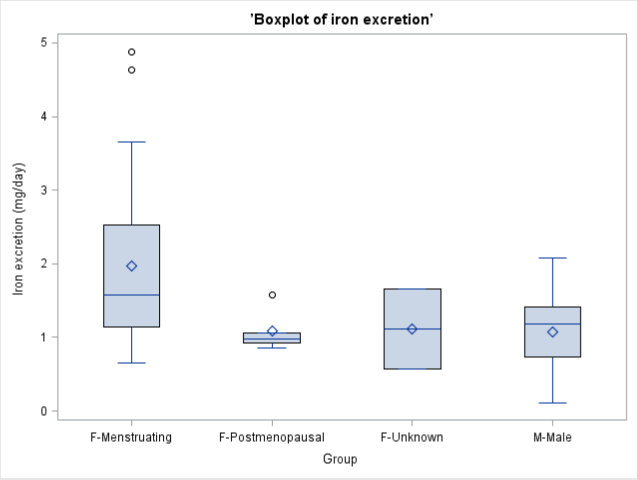


Figure 2: Boxplot of iron losses by group

The results of the comparison between postmenopausal women and men are presented in Table 16.

Table 16: Comparison of postmenopausal women and men

	Mean difference	Lower CI	Upper CI	P-value
Iron losses	–0.1075	–0.4806	0.2657	0.5321
Iron turnover rate	–0.0593	–0.0904	–0.0281	0.0021
Iron biological half-life	4.4219	1.9481	6.8957	0.0009
Metabolic body weight	5.4162	1.5365	9.2959	0.0130

CI, confidence interval.

A significant difference in the iron turnover rate, iron half-life and metabolic body weight is observed between the two groups. The distribution of the variables in the two groups is presented in Figures 3–6. In the figures, number 1 is the group of men and number 3 is the group of postmenopausal women.

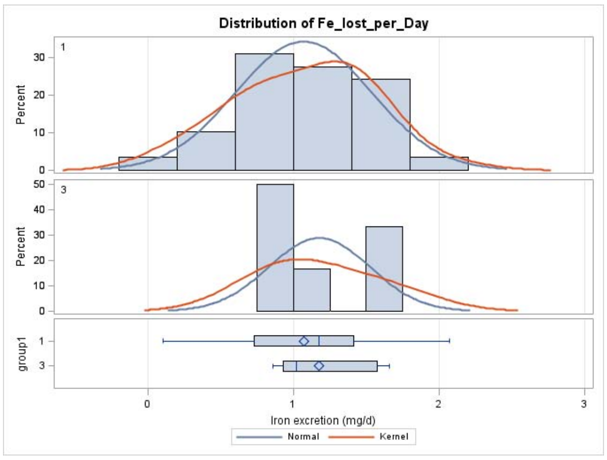


Figure 3: Distribution of iron losses in men (top) and postmenopausal women (bottom)

Figure 4: Distribution of turnover rate in men (top) and postmenopausal women (bottom)

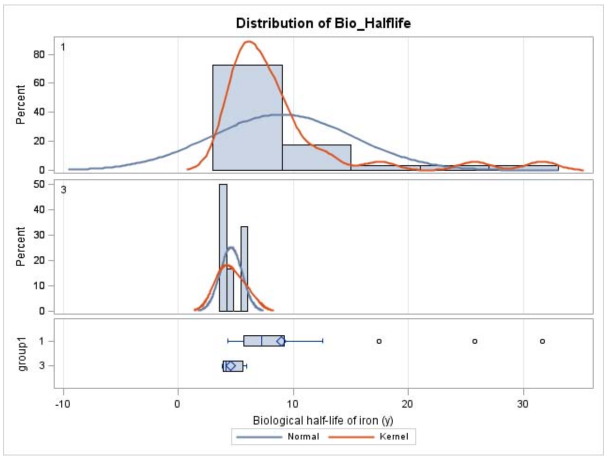


Figure 5: Distribution of biological half-life in men (top) and postmenopausal women (bottom)

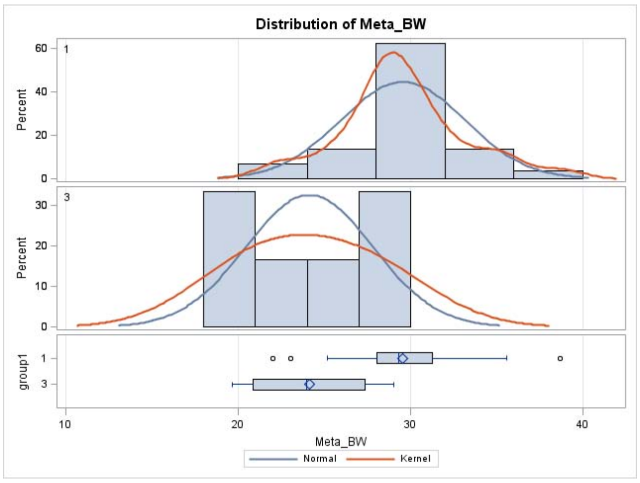


Figure 6: Distribution of metabolic body weight in men (top) and postmenopausal women (bottom)

The results of the comparison of postmenopausal and menstruating women are reported in Table 17.

Table 17: Comparison of postmenopausal and menstruating women

	Mean difference	Lower CI	Upper CI	P-value
Iron losses	0.7181	0.0824	1.3538	0.0285
Iron turnover rate	0.1201	0.0170	0.2232	0.0246
Iron biological half-life	–0.9589	–2.1539	0.2360	0.1087
Metabolic body weight	0.6346	–3.2414	4.5106	0.7096

CI, confidence interval.

A significant difference in iron losses and turnover rate is observed between the two groups, which is also evident from the comparison of the distribution of variables given in Figures 7–10. In the figures, number 2 is the group of menstruating women and number 3 is the group of postmenopausal women.

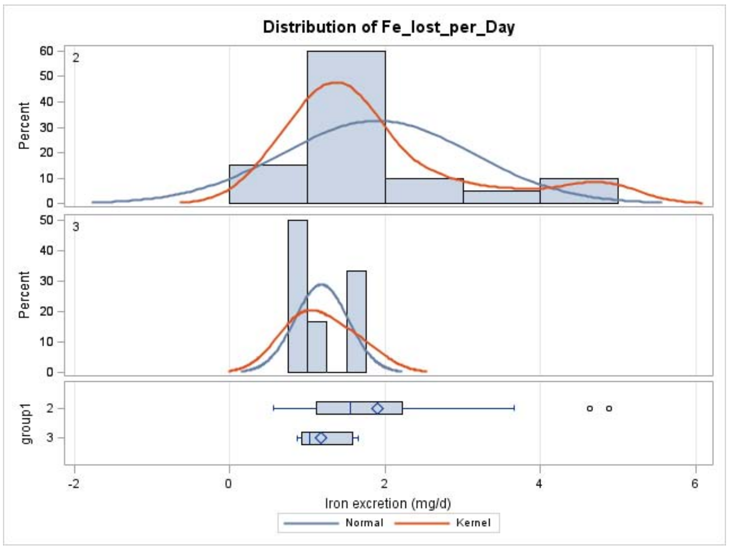


Figure 7: Distribution of iron losses in postmenopausal (bottom) and menstruating women (top)

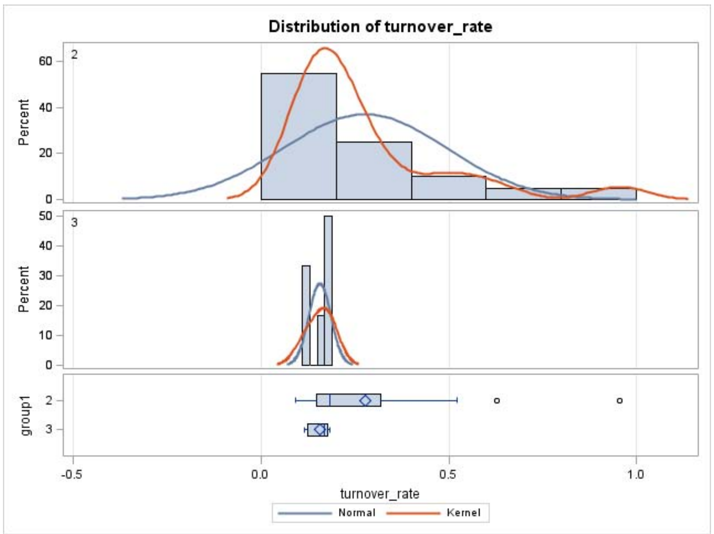


Figure 8: Distribution of turnover rate in postmenopausal (bottom) and menstruating women (top)

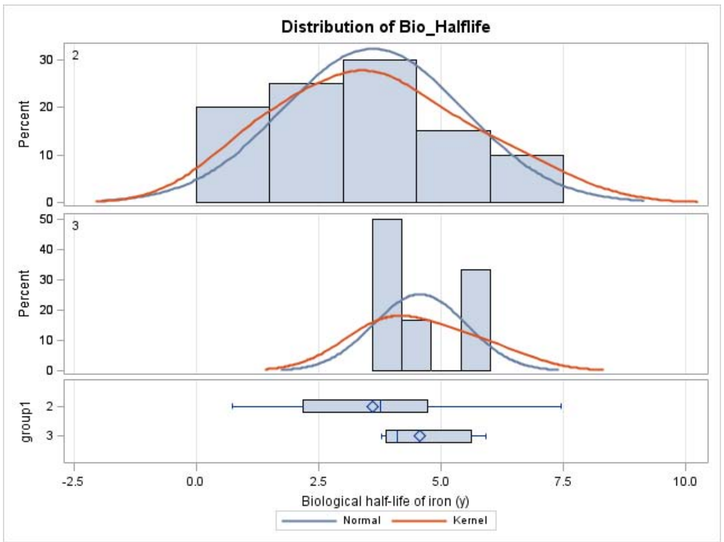


Figure 9: Distribution of biological half-life in postmenopausal (bottom) and menstruating women (top)

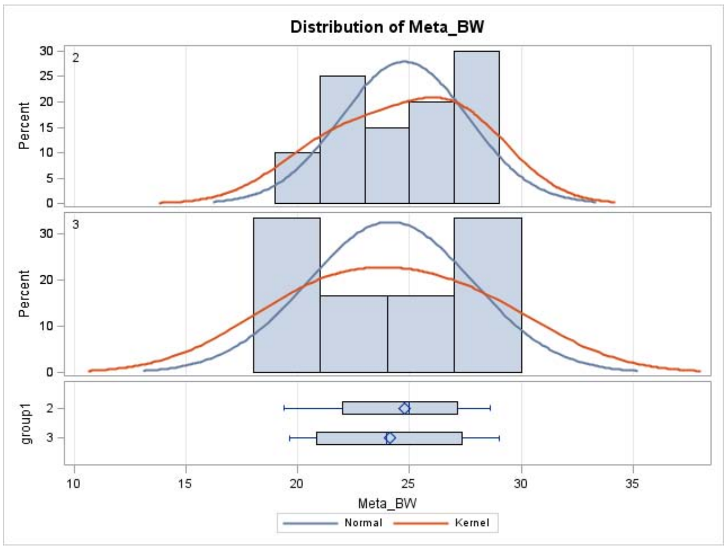


Figure 10: Distribution of metabolic body weight in postmenopausal (bottom) and menstruating women (top)

Owing to the significant difference observed in the means of several variables when comparing postmenopausal women to either men or menstruating women, it was decided to exclude postmenopausal women from the analysis.

DATA QUALITY

Information about the setting of the studies and the methodology used to collect the data (including laboratory techniques) can be found in the references provided by Hunt et al. (2009).

One of the major strengths of the data is represented by the effort carried out by the researchers to control for potential confounding deriving from blood loss that could have occurred for reasons other than elimination via usual routes. Strict eligibility criteria were set up in this respect. Some variables related to dietary consumption habits and lifestyle were measured in the study using a questionnaire. Such data were not made available to EFSA. These aspects could represent potential confounding factors that influence iron losses and that cannot be accounted for in the current analysis because of lack of data. It is assumed that the dietary consumption habits and lifestyle of subjects in the sample are representative of those of the North American healthy adult population. Blood samples were collected every six months. The processing of these data in order to provide a summary measure per subject, as in the dataset provided to EFSA, was performed by Hunt et al. (2009) and could not be investigated further in the present analysis because of lack of information.

The subjects in the sample received a different dose of iron supplements from their participation in a previous study, and from which they were recruited. Eleven subjects received a single intravenous dose of 5 µCi Fe mixed with each subject’s own plasma. One to two years before the present study, 42 subjects had received two oral doses separated by several weeks, with a total dose of 1–2 µCi Fe as haemoglobin iron. For the two subjects with unknown menstruating status, the dose of iron administered in the previous study is not reported since they were not included in the final analysis. In principle, differences in the dose of iron administered in the previous study could represent a confounding factor in the assessment of iron losses, but the Panel considers that sufficient time had elapsed to enable the physical decay of this isotope with a half-life of 44.5 days.

METHODS OF ANALYSIS

In order to provide a basis for the estimate of various percentiles of iron losses for the healthy EU adult population, a model was developed according to the following steps:

- summary statistics were estimated for the main variables related to iron losses for the two subgroups as resulting from preprocessing (men and menstruating women);
- possible association among variables indicated by the Panel as potentially explanatory variables for iron losses was investigated in order to reduce the risk of introducing autocorrelated variables into the regression model;
- a regression model for iron losses (in mg/day) was fitted to the data provided by Hunt et al. (2009) selecting among the set of potentially exploratory variables those with limited correlation. This step also included analysis of outliers and assessment of goodness of fit;
- the equation estimated via the regression model was used to derive a distribution for iron losses combining the latter equation with parametric distributions fitted on sample data for each of the input factors.

Owing to the significant differences in the distribution of iron losses between men and menstruating women, the Panel decided to perform separate analyses for the two subgroups. Postmenopausal women were excluded from the analysis since their numbers were too limited and the similarity with one of the other two groups did not appear sufficient to merge them.

H3. Statistical analysis—men

H3.1. Summary statistics

A description of the main characteristics of the sample of male subjects is provided in Table 18.

Table 18: Summary statistics for men

Variable	Number	Mean	Standard deviation	Median	Minimum	Maximum
Initial age (years)	29	42.96	8.03	42.54	30.42	58.30
Body weight (kg)	29	91.65	14.89	90.4	61.8	130.9
BMI (kg/m^2)	29	28.78	3.59	29.32	22.04	35.32
Metabolic body weight (kg)	29	29.55	3.59	29.32	22.04	38.70
Iron losses (mg/day)	29	1.07	0.47	1.18	0.11	2.07
Iron losses (µg/kg actual body weight per day)	29	11.63	4.80	11.82	1.38	20.84
Biological half-life of iron (years)	29	8.99	6.20	7.24	4.30	31.61
Iron turnover rate (rate/year)	29	0.10	0.04	0.10	0.02	0.16
Serum ferritin (µg/L)	29	164.19	87.41	138.50	50.70	356.75

The median body weight, about 90 kg, and the median BMI, about 29 kg/m^2, of this sample of North American healthy adult men are larger than the corresponding values in the EU adult male population (measured median body weight in 16 580 men aged 18–79 years is 80.8 kg; median BMI is 26.1 kg/m^2) (EFSA NDA Panel, 2013). This difference could introduce a bias in estimating the population mean of iron losses with a regression model. As a mitigation action it was decided to use the metabolic body weight instead. In addition, it was considered appropriate to perform a sensitivity analysis at the end of the process in order to assess the influence of this input variable on the estimate of iron losses.

The values of 31.6 for biological half-life of iron (subject 49) and 0.16 for iron turnover rate (subject 46) appear extreme with respect to the mean of the sample (8.99 and 0.10, respectively). An investigation of the possibility that these subjects represent outliers was performed (see Section H3.4).

H3.2. Assessing association among variables

A Pearson correlation coefficient was estimated in order to assess the linear correlation among iron losses (mg/day) and potential explanatory factors; metabolic body weight, iron biological half-life, iron turnover rate, serum ferritin concentration. The variables with the highest level of association are the iron turnover rate and biological half-life, which are also highly correlated (–0.84). The iron turnover rate was retained because it had the highest level of correlation. Metabolic body weight was also significantly correlated with iron losses and was retained for setting up the regression model.

Table 19: Pearson correlation coefficients (Prob > |r| under H0: Rho = 0)

	Body weight (kg)	**Metabolic body weight (kg)**	**Iron losses (mg/day)**	**Biological half-life of iron (years)**	**Iron turnover rate (rate/year)**	**Serum ferritin (µg/L)**
Body weight (kg)	1	0.99954 (< 0.0001)	0.40809 (0.0280)	–0.16678 (0.3872)	0.04941 (0.7991)	0.41500 (0.0252)
Metabolic body weight (kg)	0.99954 (< 0.0001)	1	0.41197 (0.0264)	–0.16739 (0.3854)	0.05343 (0.7831)	0.40939 (0.0274)
Iron losses (mg/day)	0.40809 (0.0280)	0.41197 (0.0264)	1	–0.79348 (< 0.0001)	0.91898 (< 0.0001)	0.17266 (0.3704)
Biological half-life of iron (years)	–0.16678 (0.3872)	–0.16739 (0.3854)	–0.79348 (< 0.0001)	1	–0.83988 (< 0.0001)	0.1833 (0.3412)
Iron turnover rate (rate/year)	0.04941 (0.7991)	0.05343 (0.7831)	0.91898 (< 0.0001)	–0.83988 (< 0.0001)	1	–0.0664 (0.7322)
Serum ferritin (µg/L)	0.41500 (0.0252)	0.40939 (0.0274)	0.17266 (0.3704)	0.1833 (0.3412)	–0.0664 (0.7322)	1

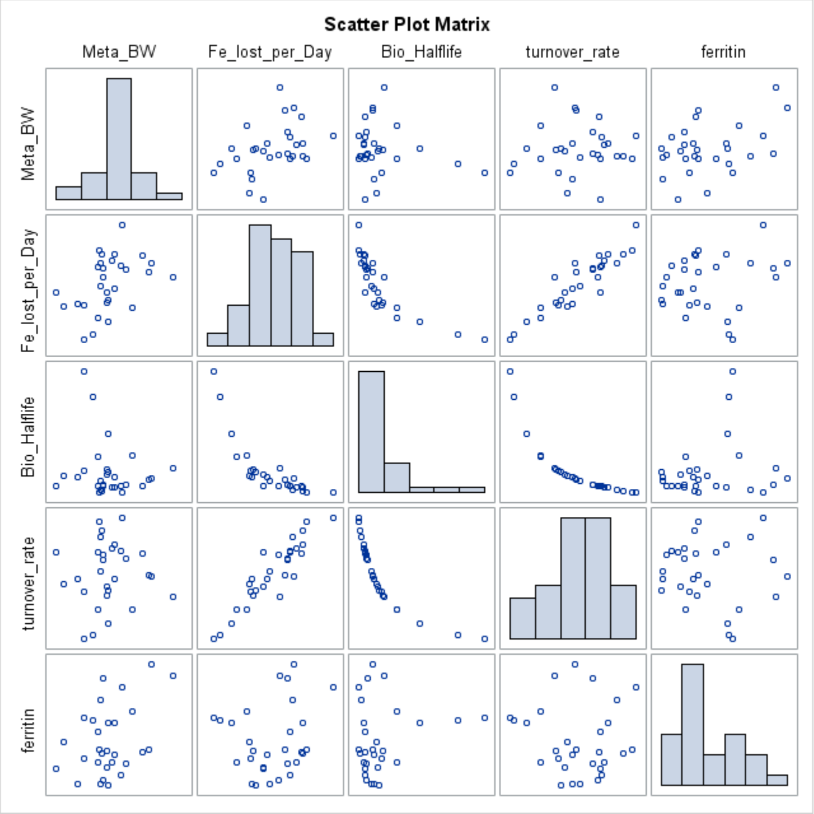


Figure 11: Scatter plot and frequency distribution

Table 19 shows that iron turnover rate and biological half-life are highly correlated. Iron turnover rate has a stronger linear association with iron losses. In addition, its relationship with iron losses is linear while that with half-life is not. Therefore, in order to use a simpler and more parsimonious structure for the model, iron turnover rate was kept in the analysis. Metabolic body weight is preferred over body weight based on the reasoning above.

H3.3. Setting up a regression model

A linear regression model was used to explain iron losses. Based on previous correlation analysis, metabolic body weight and iron turnover rate were considered as potential covariates that might have an effect on the output and have limited autocorrelation.

The form of the model is given in equation [1]:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon_i \qquad [1]$$

where:

Y_i is iron losses (in mg/day)

β_j are regression coefficients for the explanatory factors

X_1 is metabolic body weight

X_2 is iron turnover rate

ε_i is the random error term on individual i-th with $\varepsilon \propto N\left(0, \sigma^2\right)$.

The goodness of fit of the model was assessed using as indicators the adjusted R squared and the Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Normality of the residuals was assessed graphically.

The output of model fitting is reported in Tables 20–22.

Table 20: Analysis of variance

Source	Degrees of freedom	Sum of squares	Mean square	F value	Pr > F
Model	2	5.93161	2.96581	541.79	< 0.0001
Error	26	0.14233	0.00547		
Corrected total	28	6.07394			

Table 21: Indicators for goodness of fit

Root mean-square error	0.07399	**R squared**	0.9766
Dependent mean	1.07059	**Adjusted R squared**	0.9748
Coefficient of variation	6.91085	**Akaike (AIC)**	–148.2
		Bayesian (BIC)	–144.1

Table 22: Parameter estimates

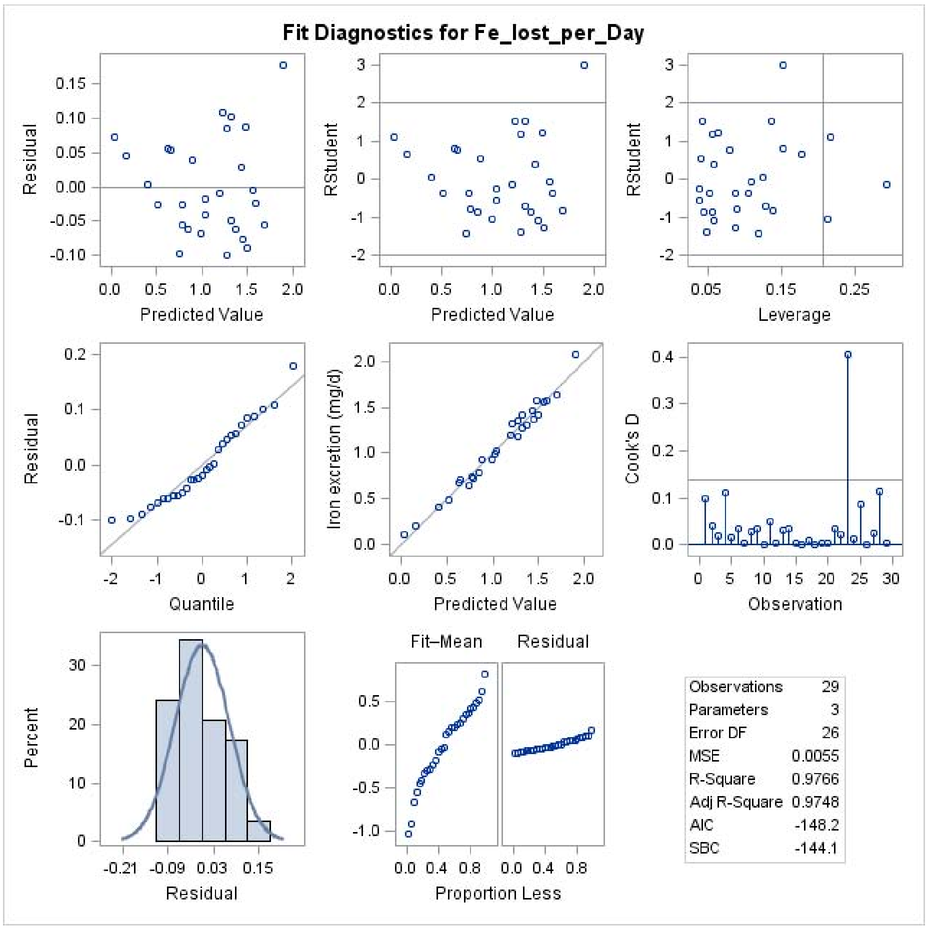
Variable	Parameter estimate	Standard error	Lower 95 % CI	Upper 95 % CI	Pr > |t|
Intercept	–1.44460	0.12000	–1.69126	–1.19794	< 0.0001
Metabolic body weight (kg)	0.04718	0.00390	0.03917	0.05520	< 0.0001
Iron turnover rate (rate/year)	11.504	0.384	10.713	12.294	< 0.0001

CI, confidence interval.

Both variables are able to explain a significant component of the variability of iron losses in men and are retained in the model.

H3.4. Outlier analysis

Graphical diagnostics for detection of outliers are reported in Figure 12. No individual had externally studentised residuals outside the range (–3; +3). However, subject 46 was borderline (iron turnover rate 0.16, iron losses 2.07 mg/day – Cook's D influence statistic = 0.4, externally studentised residual = 2.99). The Panel considered it appropriate to exclude the subject from the analysis.



Observations	29
Parameters	3
Error DF	26
MSE	0.0055
R-Square	0.9766
Adj R-Square	0.9748
AIC	-148.2
SBC	-144.1

Figure 12: Diagnostics for detection of outliers

Summary statistics of the main factors in men after removal of outliers are reported in Table 23.

Table 23: Summary statistics for men after removal of outliers

Variable	Number	Mean	Standard deviation	Median	Minimum	Maximum
Initial age (years)	28	42.90	8.18	41.61	30.42	58.30
Body weight (kg)	28	91.37	15.09	89.40	61.80	130.90
BMI (kg/m^2)	28	28.65	3.69	28.05	21.77	35.32
Metabolic body weight (kg)	28	29.48	3.64	29.07	22.04	38.70
Iron losses (mg/day)	28	1.03	0.43	1.10	0.11	1.63
Iron losses (µg/kg actual body weight per day)	28	11.30	4.54	11.40	1.38	19.08
Biological half-life of iron (years)	28	9.16	6.25	7.31	4.41	31.61
Iron turnover rate (rate/year)	28	0.10	0.03	0.09	0.02	0.16
Serum ferritin (µg/L)	28	159.39	85.02	136.92	50.70	356.75

After exclusion of the outlier, the change in indicators for goodness of fit was negligible. The revised parameter estimates are reported in Table 24.

Table 24: Parameter estimates after exclusion of one outlier

Variable	**Parameter estimate**	**Standard error**	**Lower 95 % CI**	**Upper 95 % CI**	**Pr > |t|**
Intercept	–1.38942	0.10668	–1.60912	–1.16971	< 0.0001
Metabolic body weight (kg)	0.04624	0.00343	0.03918	0.05330	< 0.0001
Iron turnover rate (rate/year)	11.14889	0.35698	10.41367	11.88410	< 0.0001

CI, confidence interval.

H3.5. Estimate the distribution of endogenous iron losses via a probabilistic model

The knowledge of the probability distribution of iron losses representing its variation in the target population is an information of paramount importance when setting DRVs. Data collected on a reduced sample are unlikely to represent the overall distribution of the EU healthy adults, especially for the tails of the distribution.

The probabilistic approach provides a useful methodological support to fill in gaps in the data as far as major sources of variability and uncertainty are concerned. Variation in iron losses can be modelled by fitting a parametrical distribution to the observed measurements of the input factors and using them to derive a probability distribution for the mineral losses with the aid of the model estimated via the regression analysis. The same approach can be used to account for important sources of uncertainty in the model inputs.

In real life, the explanatory factors of the regression model (metabolic body weight and iron turnover rate) represent quantities whose value varies across the target population. Parametric modelling uses parametric distributions that are based on the observed data but generate additional values below, between and above the observed values. This has the advantage of being able to represent the full range of potential values for the factors of interest, but requires assumptions to be made about the shape of the distribution. If unbounded distributions are used, they will certainly generate a small proportion of unrealistically high values, even if they fit the data well. Truncations have been used in this analysis to avoid this issue. The model fitting accounts for the inter-individual variability of the factors in the population. In practice, the distribution of these factors is also somehow uncertain because of the limited size of the datasets (sampling uncertainty) and the potential limitation in the representativeness of the sample towards the target population. These considerations could affect the choice of the shape of the distribution, especially in the lower and upper tails. In this analysis the potential sources of uncertainty are not assessed quantitatively. Their impact on the distribution of iron losses and final conclusions are described in Sections H3.1 and H7.2.

A different approach was taken for the regression coefficient parametric modelling. These inputs are assumed to be deterministic (not variable in the population) but uncertain because estimated on a sample. The uncertainty for these parameters was addressed modelling the 95 % interval estimates with appropriate distributions.

Monte Carlo simulation techniques were used to generate the parametric distributions and combine them into the equation model estimated by the regression analysis. Monte Carlo simulations are numerical sampling techniques that are the most robust and least restrictive with respect to model design and model input specification (Frey and Rhodes, 1999). One advantage of using Monte Carlo sampling is that, with a sufficient sample size, it provides an excellent approximation of the output distribution. Also, since it is a random sampling technique, the resulting distribution of values can be analysed using standard statistical methods (Burmaster and Anderson, 1994). In a Monte Carlo simulation the model combining the input distributions is recalculated many times with random samples of each distribution to produce numerous scenarios or iterations. Each set of model results or outputs represents a scenario that could occur and the joint distribution of output parameters is a representation of the variability and/or uncertainty in the outputs.

In this analysis, Monte Carlo sampling techniques have been used to propagate probabilistic factor inputs through the equation estimated via the regression analysis to generate a probability distribution for iron losses. The issue of correlation among variables whose distributions are combined is not addressed in the following since explanatory variables with limited association were selected for the regression analysis.

This approach foresees the performance of the following steps:

- a parametric probability distribution is fitted to the observed data for each input factor included in the regression model. Since regression parameters are affected by sampling uncertainty, a distribution is used to account for it;
- the fitted distributions are combined in the equation model estimated via the regression analysis using Monte Carlo sampling techniques;
- a distribution for iron losses is estimated;
- estimates of the percentiles of the distribution are provided as a basis for computing the AR and PRI.

H3.6. Probability distribution for the explanatory variables

The probabilistic distributions for the explanatory variables metabolic body weight and iron turnover rate have been fitted on the data from Hunt et al. (2009).

A normal distribution was used for modelling variability in metabolic body weight. Visual analysis of the data confirmed that this is a reasonable choice. The median and standard deviation of the observed data after removal of the outlier were taken as mean and standard deviation of the normal distribution. The median was preferred over the mean since it is more robust with respect to extreme values of the distribution. Truncation was applied (22, 39) in order to avoid unrealistic values.

The beta distribution is used for fitting iron turnover rate. In fact, the beta distribution, bounded by the interval between 0 and 1, is useful for representing variability in a fraction that cannot exceed 1. Because the beta distribution can take on a wide variety of shapes, such as negatively skewed, symmetric and positively skewed, it can represent a large range of empirical data. The sampling median and standard deviation obtained after removal of the outlier were assumed to be the true mean and standard deviation of the distribution. The shape parameters of the beta distribution were derived from them using the method of matching moments (Frey and Rhodes, 1999):

$$\hat{\alpha} = \overline{X}\left[\frac{\overline{X}\left(1-\overline{X}\right)}{s^2}-1\right]$$

$$\hat{\beta} = \left(1-\overline{X}\right)\left[\frac{\overline{X}\left(1-\overline{X}\right)}{s^2}-1\right]$$

where:

$\overline{X}$ and s^2 are the sampling mean and variance, respectively; and

$\hat{\alpha}$ and $\hat{\beta}$ are the estimates of the parameters of the beta distribution.

It was assumed that the uncertainty in the regression coefficients β_0, β_1, β_2 could be well represented using a Pert distribution assigning the largest probability to the central value of the estimated CIs and decreasing probabilities to the other values included between the lower and upper bound of the CI.

A description of the distributions used for the input factors and the specification of whether they model variability or uncertainty is provided in Table 25.

Table 25: Fitted distributions for the explanatory variables and regression coefficients

Input factor	V/U (a)	Distribution	Unit
Distribution of metabolic body weight (X_1)	V	~*normal*(29, 3.6) truncated (22, 39)	kg
Distribution of iron turnover rate (X_2)	V	~*beta*(6.661, 63.642) truncated (0.02, 0.16)	
Intercept (β_0)	U	~*Pert*(–1.61, –1.39, –1.17)	mg/day
Metabolic body weight regression coefficient (β_1)	U	~*Pert*(0.039, 0.046, 0.053)	mg/day per kg
Iron turnover rate regression coefficient (β_2)	U	~*Pert*(10.41, 11.15, 11.88)	mg/day per rate

(a): V, variability; U, uncertainty.

The distributions of metabolic body weight and iron turnover rate are provided in Figures 13–16 (in couples, frequency distribution based on data and fitted distribution obtained via simulation). Fitted distributions for the regression coefficients are shown in Figures 17–19.

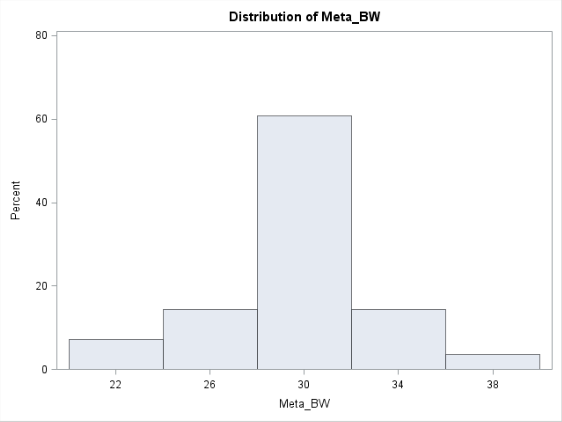


Figure 13: Frequency distribution of metabolic body weight in the sample of men

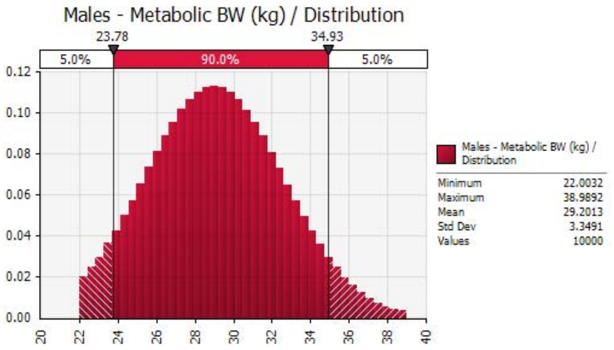


Figure 14: Probability distribution of metabolic body weight

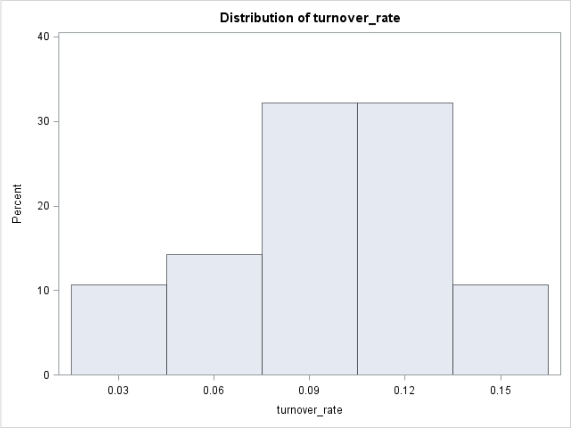


Figure 15: Frequency distribution of iron turnover rate in the sample of men

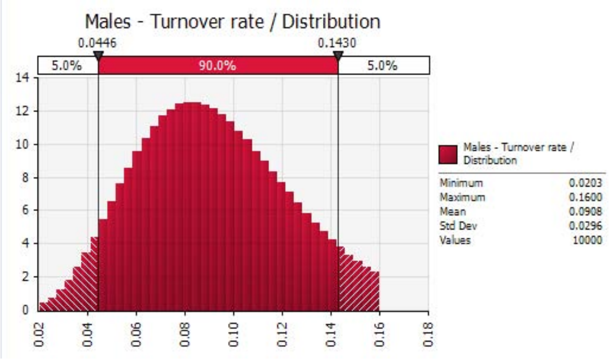


Figure 16: Probability distribution of iron turnover rate

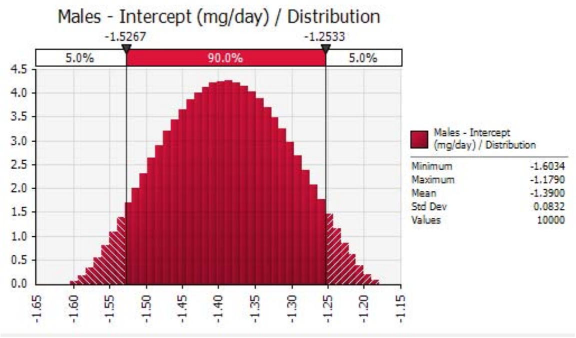


Figure 17: Probability distribution of intercept

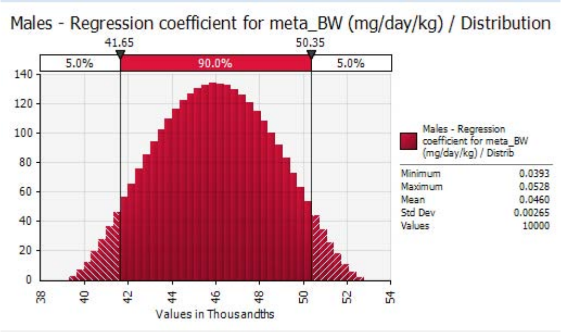


Figure 18: Probability distribution of regression coefficient for metabolic body weight

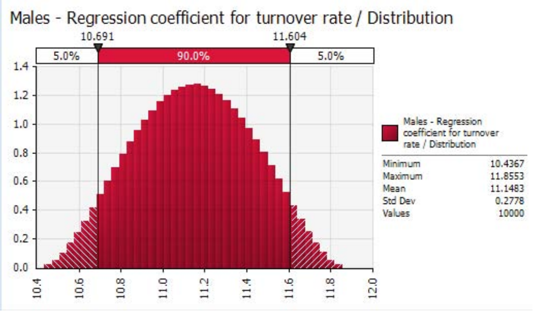


Figure 19: Probability distribution of regression coefficient for iron turnover rate

H4. Results—men

A distribution of daily iron losses is obtained by combining the probability distributions for the explanatory variables and regression coefficients into equation [1]. From the distribution it is possible to derive percentiles of interest.

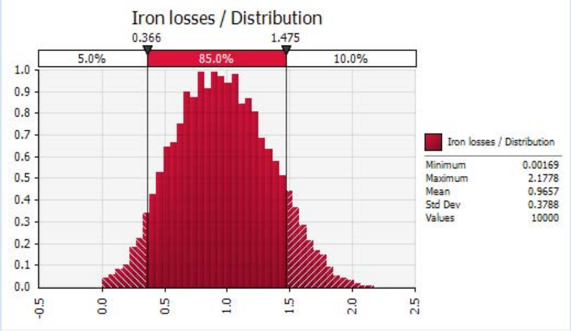


Figure 20: Distribution of iron losses—90th percentile

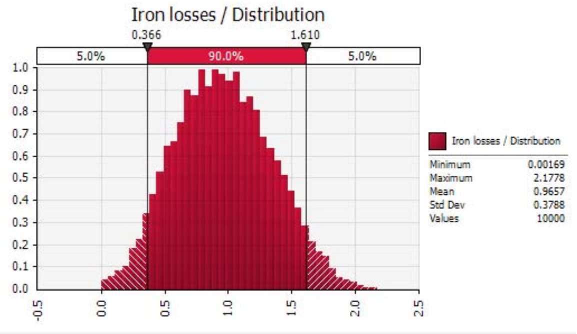


Figure 21: Distribution of iron losses—95th percentile

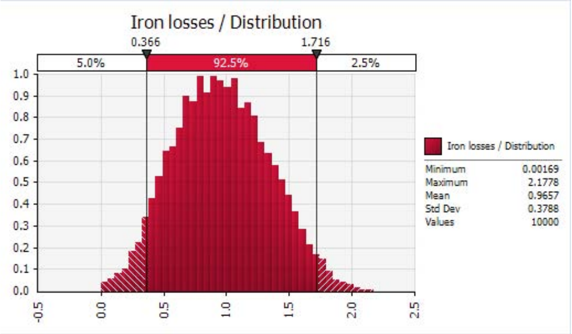


Figure 22: Distribution of iron losses—97.5th percentile

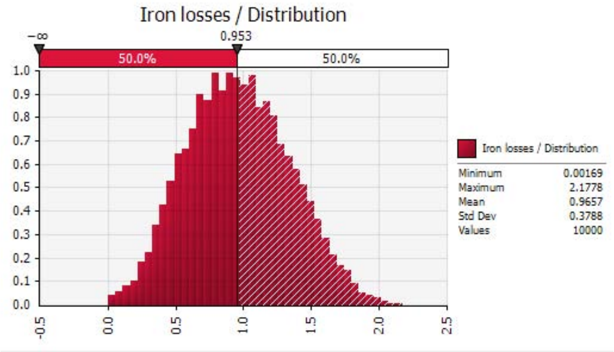


Figure 23: Distribution of iron losses—50th percentile

The 90th, 95th and 97.5th percentiles of iron losses (Figure 20–22) are, respectively, equal to around 1.48, 1.61 and 1.72 mg/day. The 50th percentile of the distribution is equal to around 0.95 mg/day (Figure 23).

H5. Statistical analysis—menstruating women

H5.1. Summary statistics

Summary statistics for the group of menstruating women are provided in Table 26.

Table 26: Summary statistics for menstruating women

Variable	Number	Mean	Standard deviation	Median	Minimum	Maximum
Initial age (years)	20	39.37	5.08	38.55	30.19	46.63
Body weight (kg)	20	72.41	11.04	73.05	52.00	87.60
BMI (kg/m^2)	20	27.43	4.70	27.39	18.65	36.16
Metabolic body weight (kg)	20	24.77	2.86	24.99	19.36	28.63
Iron losses (mg/day)	20	1.90	1.22	1.55	0.57	4.88
Iron losses (µg/kg actual body weight per day)	20	26.36	17.54	20.58	9.03	75.17
Biological half-life of iron (years)	20	3.61	1.85	3.76	0.72	7.46
Iron turnover rate (rate/year)	20	0.28	0.22	0.18	0.09	0.96
Serum ferritin (µg/L)	20	47.77	40.30	33.38	6.58	148.75

The median body weight, about 72 kg, and the median BMI, about 27 kg/m^2, of this sample of North American healthy adult menstruating women are larger than the corresponding values in the EU adult female population (measured median body weight in 19 998 women aged 18–79 years is 65.1 kg;

median BMI is 24.5 kg/m^2) (EFSA NDA Panel, 2013). This difference could introduce a bias in estimating the population mean of iron losses with a regression model. As a mitigation action it was decided to use the metabolic body weight instead. In addition, it was considered appropriate to perform a sensitivity analysis at the end of the process in order to assess the influence of this input variable on the estimate of iron losses.

The values of 0.7 years for iron biological half-life (subject 14) and 0.96 for iron turnover rate (same subject) appear extreme with respect to the mean of the sample (3.6 and 0.28, respectively). An investigation of the possibility that this subject represents an outlier was performed (Section H5.4).

The same summary statistics have also been computed for the group of menstruating women taking hormonal birth control measures to investigate whether they differ in some respect from the rest of the group, and are reported in Table 27.

Table 27: Summary statistics for menstruating women taking hormonal birth control measures

Variable	Number	Mean	Standard deviation	Median	Minimum	Maximum
Age (years at start)	5	35.25	3.23	36.42	30.19	38.72
Body weight (kg)	5	71.88	15.03	77.30	52.00	87.60
Metabolic body weight (kg)	5	24.60	3.92	26.07	19.36	28.63
Iron losses (mg/day)	5	1.01	0.25	1.09	0.57	1.15
Iron losses (µg/kg actual body weight per day)	5	14.06	3.03	13.30	10.89	18.81
Biological half-life of iron (year)	5	5.16	1.12	5.72	3.96	6.36
Iron turnover rate (rate/year)	5	0.14	0.03	0.12	0.11	0.18
Serum ferritin (µg/L)	5	66.60	56.26	46.85	10.90	148.75

H5.2. Assessing association among variables

A Pearson correlation coefficient was estimated in order to assess the linear correlation among iron losses (mg/day) and potential explanatory factors; metabolic body weight, iron biological half-life, iron turnover rate, serum ferritin concentration. As for men, the variables with the highest level of association are iron turnover rate and biological half-life, which are also highly correlated (–0.81). The iron turnover rate was retained because it had the highest level of linear correlation. Metabolic body weight was not significantly correlated with iron losses but was retained for setting up the model in order to more thoroughly investigate any potential influence on the variability of iron losses. Serum ferritin was significantly correlated with iron losses but also with iron turnover rate (–0.52). It was also retained for further analysis.

Table 28: Pearson correlation coefficients (Prob > |r| under H0: Rho = 0)

	Body weight (kg)	**Metabolic body weight (kg)**	**Iron losses (mg/day)**	**Biological half-life of iron (years)**	**Iron turnover rate (rate/year)**	**Serum ferritin (µg/L)**
Body weight (kg)	1	0.99986 (< 0.0001)	0.13992 (0.5563)	–0.07419 (0.7559)	–0.03843 (0.8722)	0.08033 (0.7364)
Metabolic body weight (kg)	0.99986 (< 0.0001)	1	0.14358 (0.5459)	–0.07777 (0.7445)	–0.03518 (0.8829)	0.07979 (0.7381)
Iron losses (mg/day)	0.13992 (0.5563)	0.14358 (0.5459)	1	–0.85037 (< 0.0001)	0.94545 (< 0.0001)	–0.48441 (0.0304)
Biological half-life of iron (years)	–0.07419 (0.7559)	–0.07777 (0.7445)	–0.85037 (< 0.0001)	1	–0.80864 (< 0.0001)	0.60698 (0.0045)
Iron turnover rate (rate/year)	–0.03843 (0.8722)	–0.03518 (0.8829)	0.94545 (< 0.0001)	–0.80864 (< 0.0001)	1	–0.52045 (0.0186)
Serum ferritin (µg/L)	0.08033 (0.7364)	0.07979 (0.7381)	–0.48441 (0.0304)	0.60698 (0.0045)	–0.52045 (0.0186)	1

With respect to the preference of iron turnover rate over biological half-life, similar considerations as for men apply (see Section H3.2).

No significant correlation between metabolic body weight and iron losses was observed, but it was decided to nevertheless keep metabolic body weight in the model. This was carried out as metabolic body weight may still explain a small part of the variability, since it is not correlated with any other variable.

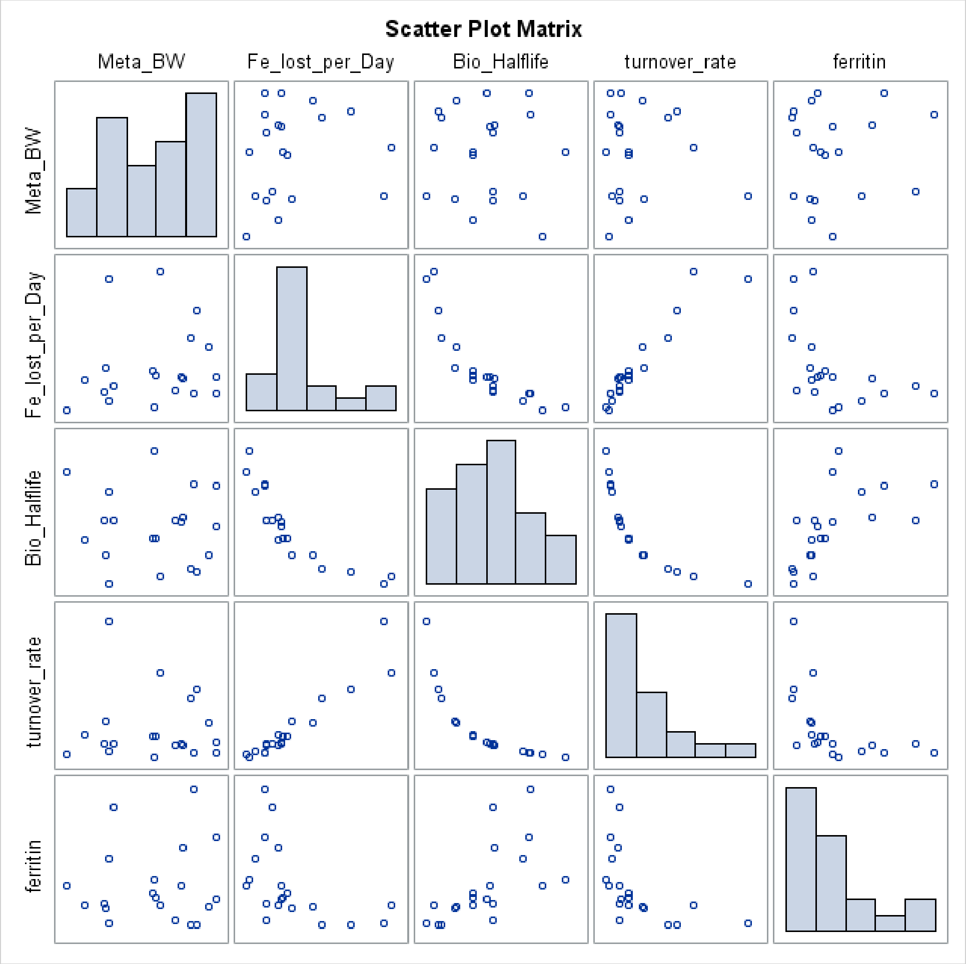


Figure 24: Scatter plot and frequency distribution

H5.3. Setting up a regression model

As for men, a linear regression model was used in order to explain iron losses in menstruating women. Based on previous correlation analysis, metabolic body weight, iron turnover rate and serum ferritin concentration were considered as potential covariates that might have an effect on the output and have limited autocorrelation among them.

The form of the model is given in equation [2]:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon_i \qquad [2]$$

where:

Y_i is iron losses (in mg/day)

β_j are regression coefficients for the explanatory factors

X_1 is metabolic body weight

X_2 is iron turnover rate

X_3 is serum ferritin concentration

ε_i is the random error term on individual i-th with $\varepsilon \propto N(0, \sigma^2)$.

The goodness of fit of the model was assessed using as indicators the adjusted R squared and AIC and BIC. Normality of the residuals was assessed graphically.

The output of model fitting is reported in Tables 29–31.

Table 29: Analysis of variance

Source	Degree of freedom	Sum of squares	Mean square	F value	Pr > F
Model	3	26.34716	8.78239	65.97	< 0.0001
Error	16	2.12998	0.13312		
Corrected total	19	28.47713	19		

Table 30: Indicators for goodness of fit

Root mean-square error	0.36486	**R squared**	0.9252
Dependent mean	1.89619	**Adjusted R squared**	0.9112
Coefficient of variation	19.24176	**Akaike (AIC)**	–38.79
		Bayesian (BIC)	–35.8

Table 31: Parameter estimates

Variable	Parameter estimate	Standard error	Lower 95 % CI	Upper 95 % CI	Pr > |t|
Intercept	–1.47222	0.75311	–3.06874	0.12431	0.0683
Metabolic body weight (kg)	0.07594	0.02937	0.01367	0.13821	0.0199
Iron turnover rate (rate/year)	5.39667	0.45518	4.43173	6.36160	< 0.0001
Serum ferritin (µg/L)	–0.00013562	0.00244	–0.00531	0.00503	0.9563

CI, confidence interval.

Metabolic body weight and iron turnover rate significantly explained the variance of iron losses, the intercept was marginally insignificant and was kept in the model. Serum ferritin concentration is not significant when the other variables are in the model.

H5.4. Outlier analysis

Graphical diagnostics for detection of outliers are reported in Figure 25. Two individuals had externally studentised residuals well outside the range (–3; +3). These are subjects 14 and 16.

Table 32: Outlier analysis for menstruating women

Subject	Iron losses	Iron turnover rate	Biological half-life	Metabolic body weight	Serum ferritin	Cook's D	Externally studentised residuals
14	4.64	0.96	0.72	22	8.30	5	–5.7
16	4.88	0.63	1.10	25	26.7	0.65	5.4

Cook's D, Cook's distance.

The Panel considered it appropriate to exclude the subjects from the analysis.

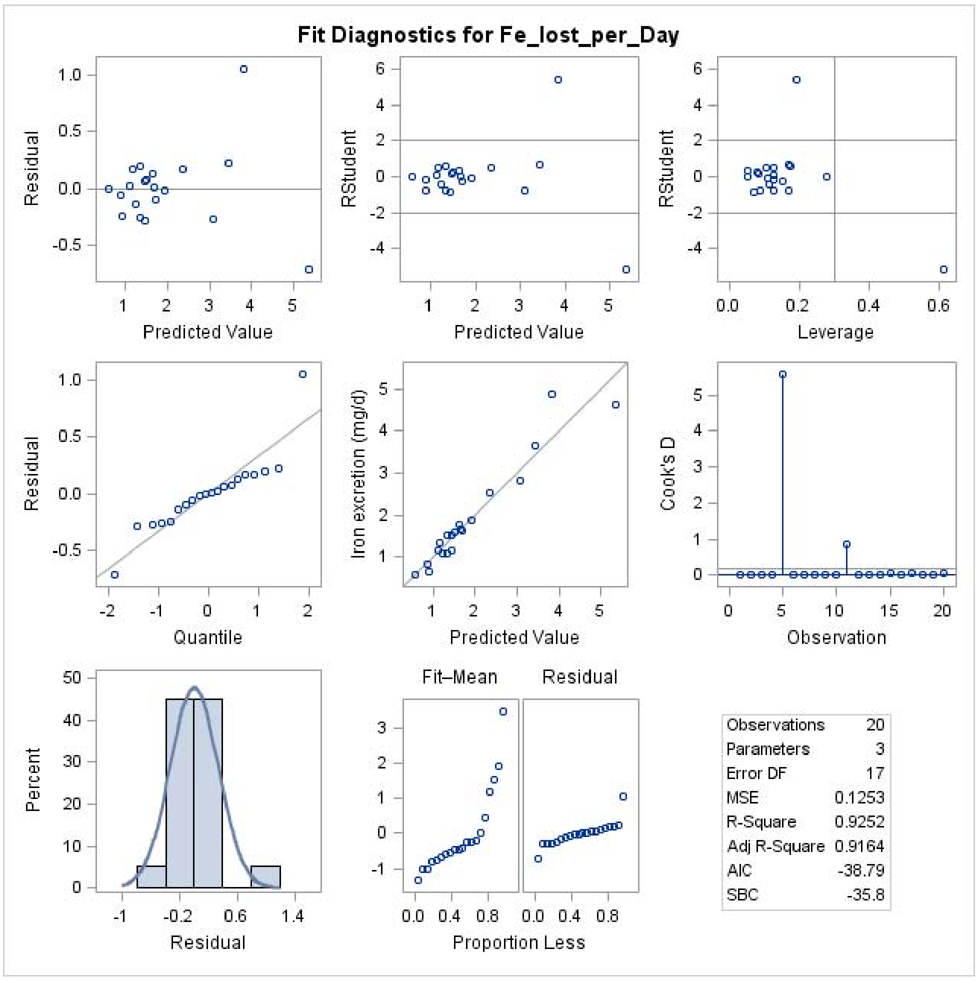


Figure 25: Diagnostics for detection of outliers

Summary statistics of the main factors in menstruating women after removal of outliers are reported in Table 33.

Table 33: Summary statistics after removal of outliers—menstruating women

Variable	Number	Mean	Standard deviation	Median	Minimum	Maximum
Initial age (years)	18	38.67	4.85	37.72	30.19	46.63
Body weight (kg)	18	72.94	11.36	74.90	52.00	87.60
BMI (kg/m^2)	18	27.57	4.90	27.79	18.65	36.14
Metabolic body weight (kg)	18	24.90	2.94	25.46	19.36	28.63
Iron losses (mg/day)	18	1.58	0.78	1.53	0.57	3.67
Iron losses (µg/kg actual body weight per day)	18	21.43	9.20	19.61	9.03	44.16
Biological half-life of iron (years)	18	3.91	1.69	3.90	1.32	7.46
Iron turnover rate (rate/year)	18	0.22	0.12	0.18	0.09	0.52
Serum ferritin (µg/L)	18	51.13	41.05	36.61	6.58	148.75

H5.5. Model estimates without outliers

After exclusion of the outliers, the change in goodness of fit indicators was negligible. The revised parameter estimates are reported in Table 34.

Table 34: Parameter estimates after exclusion of two outliers

Variable	Parameter estimate	Standard error	Lower 95 % CI	Upper 95 % CI	Pr > |t|
Intercept	–1.08987	0.33011	–1.79349	–0.38624	0.0048
Metabolic body weight (kg)	0.05460	0.01359	0.02564	0.08356	0.0011
Iron turnover rate (rate/year)	5.95745	0.33714	5.23885	6.67605	< 0.0001

The revised model [2a] includes only two explanatory variables significantly explaining the variability of iron losses:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon_i \qquad [2a]$$

The other assumptions remain fixed.

H5.6. Estimate the distribution of iron losses via a probabilistic model

Following the same approach as for men, the following steps have been performed:

- a parametric probability distribution is fitted to the observed data for each input factor included in the regression model. Since regression parameters are affected by sampling uncertainty, a distribution is used to account for it;
- the fitted distributions are combined in the equation model estimated via the regression analysis using Monte Carlo sampling techniques;
- a distribution for iron losses is estimated;
- estimates of the percentiles of the distribution are provided as a basis for computing the AR and PRI.

H5.7. Probability distribution for the explanatory variables

The probabilistic distributions for the explanatory variables metabolic body weight and iron turnover rate have been fitted on the data from Hunt et al. (2009).

In the group of menstruating women the distribution of metabolic body weight is bimodal. This is probably because a large proportion of women in the sample had a high body weight, which could raise doubts on the representativeness of the sample with respect to the target population. A mixture of two normal distributions with means of 22, 28 and both with a standard deviation of 2 was used in order to fit the observed data after exclusion of outliers. The sampling median and standard deviation were taken as mean and standard deviation of the combined normal distribution. Truncation was applied in order to avoid unrealistic values (20,26) and (24,29).

The beta distribution was used to fit the iron turnover rate. The same reason as for men applies here. Sampling median and standard deviation obtained after removal of the outliers were assumed to be mean and standard deviation of the population distribution.

It was assumed that the uncertainty in the regression coefficients β_0, β_1, β_2 could be well represented using a Pert distribution assigning the largest probability to the central value of the estimated CIs and decreasing probabilities to the other values included in the lower and upper bound of the CI.

A description of the distributions used for the input factors and the specification of whether they model variability or uncertainty is provided in Table 35.

Table 35: Fitted distributions for the explanatory variables and regression coefficients

Input Factor	V/U [(a)]	Distribution	Unit
Distribution of metabolic body weight (X_1)	V	~*bimodal*(0.5*Normal(22,2) truncated (20,26), 0.5*Normal(28,2) truncated (24,29))	kg
Distribution of iron turnover rate (X_2)	V	~*beta*(1.845,8.540) truncated (0.04,0.6)	
Equation intercept (β_0)	U	~*Pert*(–1.79, –1.090, –0.386)	mg/day
Metabolic body weight regression coefficient (β_1)	U	~*Pert*(0.026, 0.055, 0.084)	mg/day per kg
Iron turnover rate regression coefficient (β_2)	U	~*Pert*(5.239, 5.957, 6.676)	mg/day per rate

(a): V, variability; U, uncertainty.

The same methodology as for men was applied to generate the distributions for metabolic body weight, iron turnover rate and regression coefficients.

The distributions of metabolic body weight and iron turnover rate are provided in Figures 26–29 (in couples, frequency distribution based on data and fitted distribution obtained via simulation). Fitted distributions for the regression coefficients are shown in Figures 30–32.

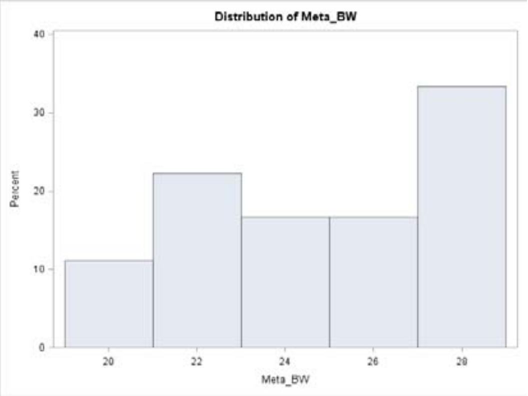


Figure 26: Frequency distribution of metabolic body weight in the sample of menstruating women

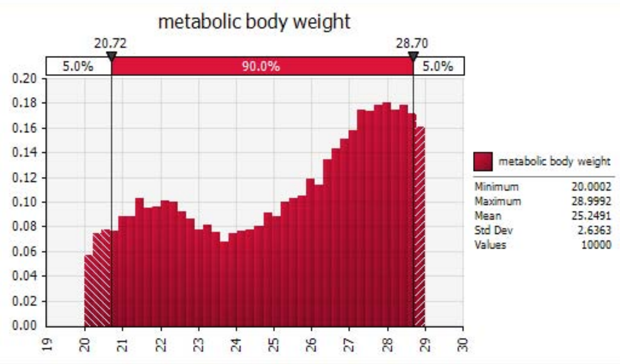


Figure 27: Probability distribution of metabolic body weight

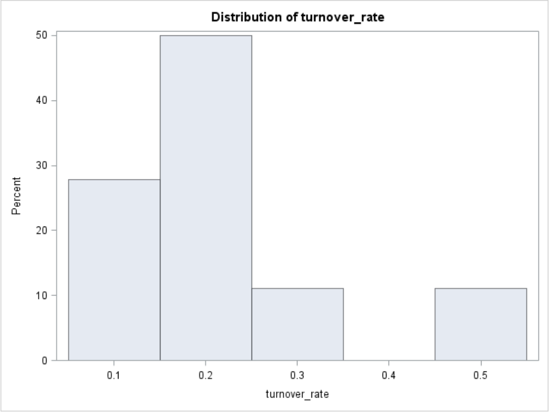


Figure 28: Frequency distribution of iron turnover rate in the sample of menstruating women

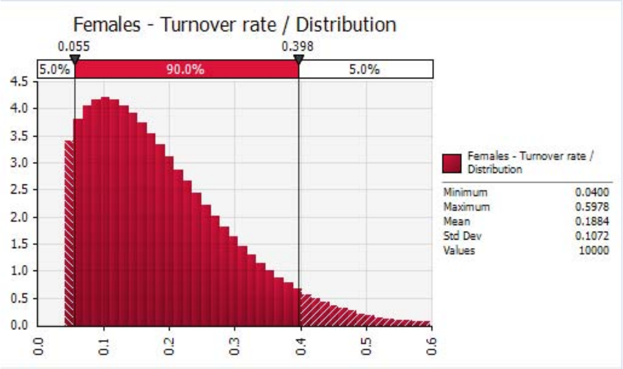


Figure 29: Probability distribution of iron turnover rate

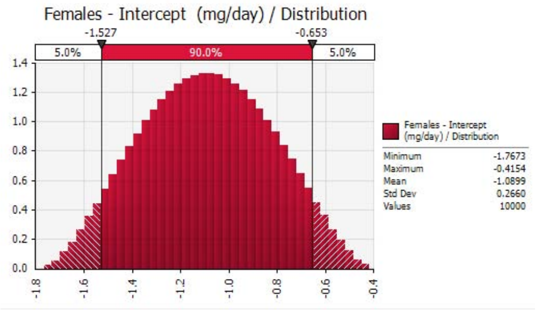


Figure 30: Probability distribution of intercept

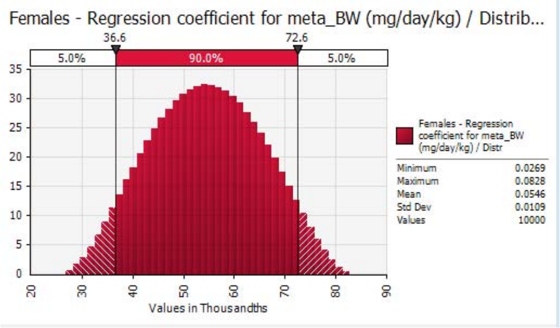


Figure 31: Probability distribution of regression coefficient for metabolic body weight

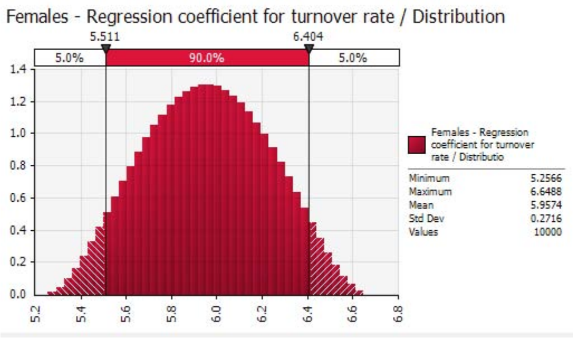


Figure 32: Probability distribution of regression coefficient for iron turnover rate

H6. Results—menstruating women

A distribution of daily iron losses is obtained by combining the probability distributions for the explanatory variables and regression coefficients into equation [2a]. From the distribution it is possible to derive percentiles of interest.

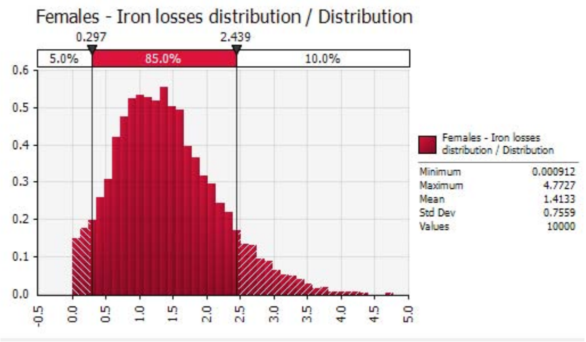


Figure 33: Distribution of iron losses—90th percentile

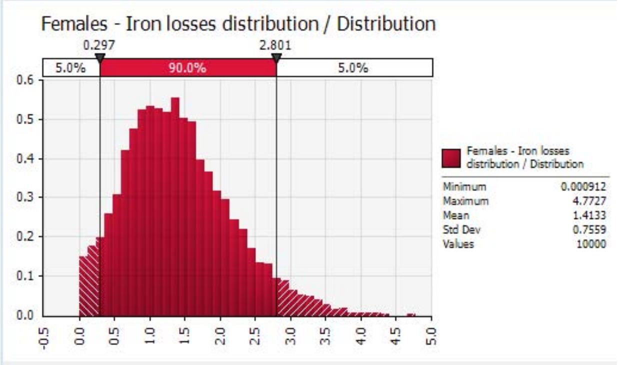


Figure 34: Distribution of iron losses—95th percentile

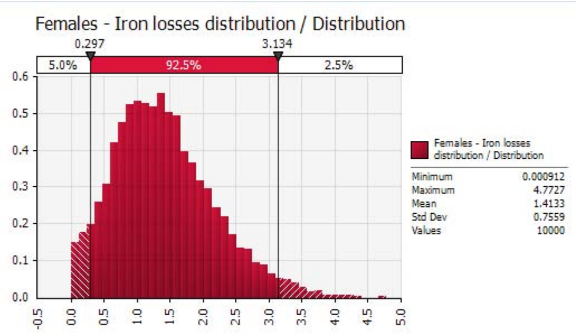


Figure 35: Distribution of iron losses—97.5th percentile

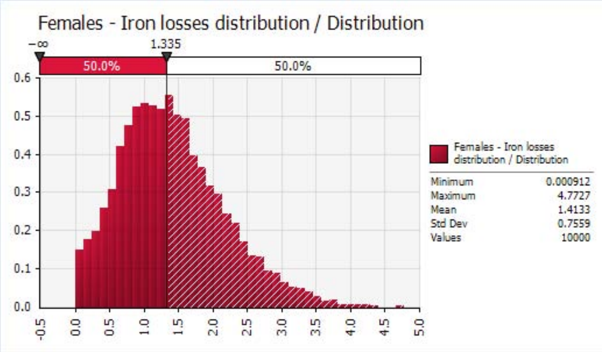


Figure 36: Distribution of iron losses—50th percentile

The 90th, 95th and 97.5th percentiles of iron losses (Figures 33–35) are, respectively, equal to around 2.44, 2.80 and 3.13 mg/day. The 50th percentile of the distribution is equal to around 1.34 mg/day (Figure 36).

INTERPRETATION OF THE RESULTS

H7. Sources of uncertainty and their potential impact on the final estimates

H7.1. Definitions and general concepts

In the EFSA context the term uncertainty is intended to cover “all types of limitations in the knowledge available to assessors at the time an assessment is conducted and within the time and resources agreed for the assessment” (EFSA Scientific Committee draft Guidance on Uncertainty in Risk Assessment, unpublished). The need to address uncertainty is expressed in the Codex Working Principles for Risk Analysis. These state that “constraints, uncertainties and assumptions having an impact on the risk assessment should be explicitly considered at each step in the risk assessment and documented in a transparent manner” (Codex Alimentarius Commission, 2015). The Scientific Committee of EFSA explicitly endorsed this principle in its Guidance on Transparency in Risk Assessment (EFSA, 2009).

In the risk assessment process it is important to characterise, document and explain all types of uncertainty arising in the process to allow risk managers to properly interpret the results.

Ideally, analysis of the uncertainty would require the following steps:

1. identifying uncertainties;
2. describing uncertainties;
3. assessing individual sources of uncertainty;

4. assessing the overall impact of all identified uncertainties on the assessment output, taking account of dependencies;
5. assessing the relative contribution of individual uncertainties to overall uncertainty;
6. documenting and reporting the uncertainty analysis.

Uncertainty can be expressed adopting six main approaches: descriptive expression, ordinal scales, sets, bounds, ranges and distributions. The first and second of these are qualitative, while the other four quantify uncertainty to an increasing extent.

An EFSA Working Group is currently working on the provision of guidelines on how the uncertainty analysis should be performed in a harmonised and structured way. Since the activity is ongoing, in the current assessment, only the first two steps (i.e. identification and description) will be considered in analysing the uncertainty. This will include stating which assumptions have been made in the various steps of the assessment, if any.

The Panel aimed to assess, in a qualitative way, the potential impact of the individual sources of uncertainty on the final outcome and, possibly, on the combined impact of the multiple uncertainties.

H7.2. Identification and description of the sources of uncertainty

The model used to set up the estimates that served as a basis for the AR and PRI relies on some assumptions about the structure of the regression model (i.e. explanatory variables and linearity of the relationship). These assumptions have an influence on the final results in the sense that they determine the equation used as a basis for further probabilistic modelling. In addition, the structure of the regression model determines the size of the CIs for the regression parameters and, consequently, their lower and upper bounds that are used as reference for the PERT distributions fitted to them. Different choices may lead to different results. The Panel considers that the fitting of the regression model is quite good for both groups (men and menstruating women), which is reassuring.

Some limitations in the data represent a potential source of uncertainty that could introduce a bias in the final estimates. Observations were taken on North American healthy adult subjects. The assumption of their representativeness for the EU healthy adult population may not be completely met, especially as far as the distribution of body weights is concerned. The small size of the sample is an additional source of uncertainty that could affect the true shape and variability of the distribution of the variables involved in the assessment. Further research is needed to collect more data of this kind. Sources of uncertainty and their potential impact are described in Table 36.

Table 36: Sources of uncertainty and their potential impact on the estimates

Outcome	**Source of uncertainty**	**Direction of the effect on the outcome**
Estimates of the body weight, BMI and metabolic body weight, iron losses and various serum parameters	Lack of information about: • how repeated measures on the same individual (2–6 observations per subject taken during the study) have been summarised; • aspects related to dietary consumption and life-style (i.e. not measured)	It is difficult to evaluate the impact of this on the estimate of the distribution of iron losses
Representativeness of the healthy European adult population	Individuals were North American subjects with body weight, on average, larger than that of the EU population. The representativeness of the sample in terms of aspects that might impact on iron losses is difficult to assess	The percentiles of the body weight distribution for both men and menstruating women are larger than those of the corresponding EU population. Owing to the linear positive relationship assumed between body weight and iron losses, possible direction of the impact of this source of uncertainty would be to overestimate the percentiles of the distribution of iron losses. As a mitigation action a sensitivity analysis is performed to evaluate how much of the variability in iron losses is attributable to variations in metabolic body weight. Since information is lacking on other aspects characterising the sample, it is not possible to predict the impact of potential differences

Appendix I. Data derived from intervention studies in Europe on iron intake and markers of iron deficiency and/or iron deficiency anaemia in children

Reference	Design	Number of individuals (number of males/number of females)	Age group	Iron intake (mg/day) Mean or mean ± SD			Indices of iron status: Hb (g/L), serum ferritin (µg/L), serum transferrin (g/L), serum iron (µmol/L), ZPP (µmol/mol haem), TSAT (%) Mean or mean ± SD			Discussion
Dube et al. (2010a)	Healthy term infants at the age of 4–10 months were studied. Dietary intake was recorded with a daily diet record. The high meat group received commercial baby jars with a meat content of 12 % by weight, and the low meat group received 8 % by weight. Intervention was from 4 to 10 months	High meat: 48 (24 M/24 F)	Infants	5–7 months: 3.86	8–10 months: 5.84		7 months (baseline): Hb: 118 Ferritin: 33.3 Serum Fe: 56.5 ZPP: 39.9	10 months (after intervention): Hb: 121 Ferritin: 28.8 Serum Fe: 54.1 ZPP: 48.7		
		Low meat: 49 (25 M/24 F)		3.72	5.74		Hb: 116 Ferritin: 35.5 Serum Fe: 58.2 ZPP: 39.2	Hb: 119 Ferritin: 25.5 Serum Fe: 70.2 ZPP: 45.0		
Dube et al. (2010b)	Retrospective analysis of data from a randomised controlled trial. Dietary iron and indicators of iron status were analysed at the age of 4 (exclusively milk-fed period), 7 and 10 months (complementary feeding period)	Breast-fed: 53 (27 M/26 F)	Infants	3–4 months: 0.46	5–7 months: 1.55	8–10 months: 4.81	4 months: Hb: 118 Ferritin: 75.2 Serum Fe: 57.4 ZPP: 37.1	7 months: Hb: 114 Ferritin: 32.5 Serum Fe: 53.5 ZPP:38.8	10 months: Hb: 119 Ferritin: 23.5 Serum Fe: 54.7 ZPP: 48.4	
		Iron-fortified formula: 23 (8 M/15 F)		6.14	6.99	6.96	Hb: 120 Ferritin: 63.4 Serum Fe: 69.7 ZPP: 48.6	Hb: 121 Ferritin: 36.4 Serum Fe: 66.1 ZPP: 40.8	Hb: 123 Ferritin: 35.6 Serum Fe: 76.5 ZPP: 47.2	

Reference	Design	Number of individuals (number of males/number of females)	Age group	Iron intake (mg/day) Mean or mean ± SD		Indices of iron status: Hb (g/L), serum ferritin (µg/L), serum transferrin (g/L), serum iron (µmol/L), ZPP (µmol/mol haem), TSAT (%) Mean or mean ± SD			Discussion
Engelmann et al. (1998)	Parallel intervention study (blinded). The low meat group received a diet with a meat content aimed at the average found in an observational study of infants from the same area and the high meat group received a diet aimed at a meat content about three times higher than the low meat group	High meat: 21 (14 M/7 F)	8 months	3.1		Hb: 119.1 Ferritin: 15.5 [(a)] Transferrin receptor: 8			The results suggest that an increase in meat intake can prevent a decrease in Hb in late infancy. However, there was no effect on iron stores or on cellular iron deficiency, evaluated by serum ferritin and TfR levels, respectively
		Low meat: 20 (15 M/5 F)	8 months	3.4		Hb: 113.7 Ferritin: 17.3 [(a)] Transferrin receptor: 7.4			
Haschke et al. (1993)	The Fe-fortified whey predominant formula contained 3 mg Fe/L, whereas infants in the higher Fe level group received formula containing 6 mg Fe/L. Dietary intake was assessed at 183 and 274 days		Infants	183 days:	274 days:	90 days:	183 days:	274 days:	
		Breast-fed infants until 274 days: 30		Not reported	Not reported	Hb: 118 Ferritin: 136	Hb: 123 Ferritin:49	Hb: 121 Ferritin: 16	
		Fe-fortified whey predominant formula: 27		2.7	2.4	Hb: 121 Ferritin: 86	Hb: 124 Ferritin: 41	Hb: 125 Ferritin: 21	
		Higher Fe level: 24		4.9	4.3	Hb: 118 Ferritin: 102	Hb: 124 Ferritin: 42	Hb: 126 Ferritin: 29	

Reference	Design	Number of individuals (number of males/number of females)	Age group	Iron intake (mg/day) Mean or mean ± SD		Indices of iron status: Hb (g/L), serum ferritin (µg/L), serum transferrin (g/L), serum iron (µmol/L), ZPP (µmol/mol haem), TSAT (%) Mean or mean ± SD		Discussion
Ilich-Ernst et al. (1998)	Girls in pubertal stage 2 who were premenarcheal at baseline. 7-year, randomised, double-blind, placebo-controlled trial to assess the effects of calcium supplementation on bone mass acquisition. Intervention group treated with 1 000 mg Ca/day as calcium citrate malate. The follow-up period was 4 years and the girls were seen every 6 months	354 girls (baseline)	10.8 years	13.2		Ferritin: 29.2		Serum ferritin concentrations at 0, 1, 2, 3, and 4 years were not significantly different between groups. In addition, there was no significant difference between groups in any of the red blood cell indices. In summary, growth spurt and menstrual status had adverse effects on iron stores in adolescent girls with low iron intake (< 9 mg/day), whereas long-term supplementation with calcium (total intake: < 1 500 mg/day) did not affect iron status
		354 girls (1 year)	11.8 years	12.1		Ferritin: 33.4		
		354 girls (2 years)	12.9 years	12.7		Ferritin: 31		
		354 girls (3 years)	13.9 years	14.3		Ferritin: 30.8		
		354 girls (4 years)	14.9 years	14.0		Ferritin: 29.6	Hb (placebo): 134 Hb (supplemented): 132	
Lind et al. (2003)	Double-blind parallel intervention trial in infants lasting for 2 months	Commercial milk-based cereal drink and porridge: 94 (50 M/44 F)	6–12 months	6–8 months: 7.5	9–10 months: 9.9	6 months: Hb: 116 Ferritin: 48.5	12 months: Hb: 119 Ferritin: 25.3	Extensive production in the phytate content of weaning cereals had little long-term effect on the iron and zinc status of Swedish infants
		Phytate-reduced commercial milk-based cereal drink and phytate-reduced porridge: 90 (44 M/46 F)		7.6	10.3	Hb: 115 Ferritin: 40.9	Hb: 120 Ferritin: 21.3	
		Milk-based infant formula and porridge with the usual phytate content: 83 (39 M/44 F)		4.7	6.2	Hb: 115 Ferritin: 44.1	Hb: 117 Ferritin: 25.2	

Reference	**Design**	**Number of individuals (number of males/number of females)**	**Age group**	**Iron intake (mg/day) Mean or mean ± SD**		**Indices of iron status: Hb (g/L), serum ferritin (µg/L), serum transferrin (g/L), serum iron (µmol/L), ZPP (µmol/mol haem), TSAT (%) Mean or mean ± SD**		**Discussion**
Makrides et al. (1998)	Dietary intake was assessed with a food frequency questionnaire	Control: 26 (12 M/14 F)	6 months, breast-fed infants	6 months: 1.5 ± 1.7	12 months: 5.2 ± 3.4	6 months: Hb: 120 ± 8 Ferritin: 53 ± 61 Serum Fe: 7 ± 3 Serum transferrin: 2.6 ± 0.4 TSAT: 12 ± 4	12 months: Hb: 115 ± 9 Ferritin: 35 ± 37 Serum Fe: 8 ± 3 Serum transferrin: 2.8 ± 0.4 TSAT: 11 ± 5	
		High iron weaning diet: 36 (19 M/17 F)		1.9 ± 1.9	8.2 ± 2.9	Hb: 122 ± 10 Ferritin: 53 ± 49 Serum Fe: 8 ± 3 Serum transferrin: 2.7 ± 0.3 TSAT: 13 ± 6	Hb: 120 ± 7 Ferritin: 26 ± 18 Serum Fe: 9 ± 5 Serum transferrin: 2.7 ± 0.3 TSAT: 13 ± 7	
Niinikoski et al. (1997)	Dietary intake assessed with a 4-day food record	Control group: 39	3–4 years	8.6 ± 2.8		Hb: 122 ± 7 Serum transferrin: 2.85 ± 0.29 Ferritin: 19.2 ± 12.4 Iron: 14.8 ± 5.0		The children in the intervention group consumed less saturated fat than those in the control group and had higher ratios of dietary polyunsaturated to saturated fatty acids. Long-term supervised use of a diet low in saturated fat and cholesterol did not influence intake or serum indicators of iron in children
		Intervention group: 40		8.8 ± 4.2		Hb: 123 ± 8 Serum transferrin: 2.90 ± 0.30 Ferritin: 21.8 ± 11.6 Iron: 15.2 ± 5.3		

F, females; Fe, iron; Hb, haemoglobin; M, males; TSAT, plasma transferrin saturation (%); ZPP, zinc protoporphyrin.

(a): Geometric mean.

Appendix J. Data reported in observational studies in Europe on iron intake and markers of iron deficiency and/or iron deficiency anaemia in children

Reference	Design	Number of individuals		Age (years)	Iron intake (mg/day) Mean ± SD	Indices of iron status: Hb (g/L), serum ferritin (µg/L), transferrin saturation (%), ZPP (µmol/mol haem) Mean ± SD			Discussion
Gibson (1999)	Data of the UK National Diet and Nutrition Survey (NDNS). Dietary intakes assessed with 4-day weighed records		904	1.5–4.5	5.45 ± 0.06 (a)	Hb: 122 ± 0 (a) Ferritin: 23.4 ± 0.6 (a) ZPP: 54 ± 0.7 (a)			Despite the difference in total iron intake between the cereal consumption groups, there was no significant difference in iron status as measured by ferritin, Hb or ZPP
Gunnarsson et al. (2004)	3-day weighed food records		71	2	7.5 ± 4.2	Hb: 121.8 ± 8.5 Ferritin: 17.6 ± 9.8			
Thane et al. (2003)	7-day weighed dietary records	Boys	167	4–6	% RNI 131 (RNI: 6.1 mg/day) → 8 mg/day (b)	Hb: 125 ± 9	Ferritin (b): 30	TSAT: 20 ± 10	Adequacy of dietary iron intake (as % RNI) was significantly higher in boys than in girls for each age group. Poor iron status was generally more prevalent in adolescent girls of non-Caucasian ethnic origin or in those who were vegetarians
			228	7–10	% RNI 109 (RNI: 8.7 mg/day) → 9.5 mg/day (b)	130 ± 8	31	23 ± 9	
			212	11–14	% RNI 94 (RNI: 11.3 mg/day) → 10.6 mg/day (b)	134 ± 10	30	22 ± 8	
			163	15–18	% RNI 105 (RNI: 11.3 mg/day) → 11.9 mg/day (b)	149 ± 9	45	26 ± 11	
		Girls	151	4–6	% RNI 118 (RNI: 6.1 mg/day) → 7.2 mg/day (b)	125 ± 9	24	21 ± 8	
			207	7–10	% RNI 96 (RNI: 8.7 mg/day) → 8.4 mg/day (b)	128 ± 9	33	22 ± 8	
			209	11–14	% RNI 59 (RNI: 14.8 mg/day) → 8.7 mg/day (b)	133 ± 9	29	22 ± 8	
			183	15–18	% RNI 56 (RNI: 14.8 mg/day) → 8.3 mg/day (b) Values after the arrow were calculated based on intakes given as % RNI and RNIs in the paper	131 ± 10	25	22 ± 10	

Reference	Design	Number of individuals	Age (years)	Iron intake (mg/day) Mean ± SD		Indices of iron status: Hb (g/L), serum ferritin (µg/L), transferrin saturation (%), ZPP (µmol/mol haem) Mean ± SD	Discussion
Thorisdottir et al. (2011)	Iron status, dietary intake and anthropometry were prospectively assessed in a randomly selected infant population	141 (73 boys) 141 (61 girls)	Infants	At 9 months: 6.28 ± 3.19 6.27 ± 2.73	At 12 months: 6.82 ± 3.97 5.77 (1.97) (c)	At 12 months: Hb: 120.96 ± 8.19 Hb: 120.28 ± 8.28	

Hb, haemoglobin; RNI, reference nutrient intake; TSAT, plasma transferrin saturation (%); ZPP, zinc protoporphyrin.

(a): Mean ± SE.

(b): Geometric mean.

(c): Median (interquartile range).

ABBREVIATIONS

Afssa	Agence française de sécurité sanitaire des aliments
AR	Average Requirement
CI	confidence interval
COMA	Committee on Medical Aspects of Food Policy
CV	coefficient of variation
D-A-CH	Deutschland–Austria–Confoederatio Helvetica
DH	UK Department of Health
DIPP	type 1 Diabetes Prediction and Prevention
DMT	divalent metal transporter
DNFCS	Dutch National Food Consumption Survey
DNSIYC	Diet and Nutrition Survey of Infants and Young Children
DRV	Dietary Reference Value
EAR	Estimated Average Requirement
EDTA	ethylenediaminetetraacetic acid
EsKiMo	Ernährungsstudie als KIGGS-Modul
FAO	Food and Agriculture Organization of the United Nations
FC_PREGNANTWOMEN	food consumption of pregnant women in Latvia
FFQ	food frequency questionnaire
FINDIET	national dietary survey of Finland
Hb	haemoglobin
HRT	hormone replacement therapy
INCA	étude individuelle nationale des consommations alimentaires
INRAN-SCAI	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia
IOM	US Institute of Medicine of the National Academy of Sciences
IRE	iron-responsive element
IRP	iron-responsive protein

LOAEL	Lowest Observed Adverse Effect Level
LRNI	Lower Reference Nutrient Intake
MCH	mean cell haemoglobin
MCV	mean corpuscular volume
mRNA	messenger ribonucleic acid
NANS	National Adult Nutrition Survey
NCM	Nordic Council of Ministers
NDNS	National Diet and Nutrition Survey
NHANES	National Health and Nutrition Examination Survey
NL	Netherlands Food and Nutrition Council
NNR	Nordic Nutrition Recommendations
NOAEL	No Observed Adverse Effect Level
NWSSP	Nutrition and Wellbeing of Secondary School Pupils
PRI	Population Reference Intake
RDA	Recommended Dietary Allowance
RES	reticuloendothelial system
RI	Recommended Intake
RNI	Reference Nutrient Intake
SACN	UK Scientific Advisory Committee on Nutrition
SCF	Scientific Committee for Food
SD	standard deviation
SE	standard error
sTfR	soluble serum transferrin receptor
TfR	transferrin receptor
TIBC	total iron-binding capacity
TSAT	transferrin saturation
UL	Tolerable Upper Intake Level

VELS	Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln
WHO	World Health Organization
ZPP	erythrocyte zinc protoporphyrin