Iron and Health

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Scientific Advisory Committee on Nutrition

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Preface

In 1998, the Committee on Medical Aspects of Food and Nutrition Policy (COMA), in their report, *Nutritional Aspects of the Development of Cancer,* recommended that high consumers of red and processed meat should consider reducing their intakes because of possible links with a risk of colorectal cancer. However, since red and processed meat are sources of iron in the UK diet, COMA recommended that the possible adverse nutritional implications of a reduction in red and processed meat intakes should be assessed. The present report has been prepared in response to COMA's recommendation.

The report provides a comprehensive review of the role of iron in human nutrition which was drawn from an extensive body of literature. The scope of the report was wide-ranging and included consideration of potential adverse effects both of iron deficiency and of iron excess. The Scientific Advisory Committee on Nutrition (SACN) also assessed the adequacy of iron intakes and status of the general and low income populations in the UK. There are difficulties in making these assessments because of uncertainties around the dietary recommendations for iron intake, which are based on limited data, and because the thresholds of biological markers used to define iron status are not related to functional consequences. The potential implications of a recommendation to reduce consumption of red and processed meat on intakes of iron were assessed by modelling data from the National Diet and Nutrition Survey (2000/01).

For the general population, SACN is recommending a public health approach to achieving adequate iron status based on a healthy balanced diet that includes a variety of foods containing iron. This is a change to current dietary advice that iron-rich foods should be consumed at the same time as foods/drinks which enhance iron absorption (e.g., fruit, meat) but should not be consumed with those that inhibit iron absorption (e.g., tea, coffee, milk).

Groups identified as being at risk of iron deficiency anaemia include toddlers, girls and women of reproductive age, and some adult groups aged over 65 years. The report recommends that health professionals need to be aware of increased risk of iron deficiency anaemia in these groups and those with evidence suggestive of iron deficiency anaemia should receive appropriate clinical assessment and advice, including dietary advice and the use of iron supplements if required.

After detailed consideration of the epidemiological evidence on the relationship between red and processed meat intake and colorectal cancer risk, the report concludes that red and processed meat is probably associated with an increased risk of colorectal cancer. Since the evidence does not allow quantification of the amount of red and processed meat that may be linked with increased colorectal cancer risk, SACN is advising high consumers of red and processed meat to consider reducing their intakes. The modelling exercise suggests that reducing red and processed meat intake to the population average for adult consumers (estimated to be about 70 g/day cooked weight in 2000/01) would have little effect on the proportion of the population with iron intakes below the lower limit of recommended intake for iron.

Completion of this report has been a lengthy process and has taken longer than originally envisaged. In part, this is because work on the report (which commenced in 2002) was suspended from 2006 to 2008 due to other SACN priorities, but also because of the innate complexity of the topic.

The draft report was made available for comment and I would like to thank all those who responded. All the comments were carefully considered before the report was finalised. I would also like to thank the members of the Iron Working Group for their commitment and continued work on the report over the years, particularly the Chair, Professor Peter Aggett, and the scientific secretariat.

Professor Alan Jackson
Chair of the Scientific Advisory Committee on Nutrition

Contents

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Summary

Background

1. In their report, *Nutritional Aspects of the Development of Cancer* (Department of Health, 1998), the Committee on Medical Aspects of Food and Nutrition Policy (COMA) highlighted possible links between red and processed meat and colorectal cancer. The report recommended that "higher consumers should consider a reduction" in red and processed meat consumption. However since red meat is a source of iron in the UK diet, the report also recommended that "the possible associated adverse implications of a reduction in meat consumption on other aspects of health, particularly iron status" should be reviewed. This report was prepared in response to this recommendation.

Terms of reference

2. The terms of reference of the Iron Working Group were: *To review the dietary intakes of iron in its various forms and the impact of different dietary patterns on the nutritional and health status of the population and to make proposals*.

3. It was agreed that it was necessary to consider both beneficial and adverse effects of increasing iron intakes, including the:

   - effect of dietary components on iron absorption and utilisation in the body;
   - interaction of infections and inflammation with iron metabolism and the possibility that this may affect the apparent incidence of iron deficiency;
   - effect of iron deficiency on health and well-being, for example mental and physical development;
   - potential adverse effects of excess iron, including free radical damage and the risk of cardiovascular disease (CVD) and cancer.

4. The associations between consumption of red and processed meat and cancer risk were also considered since these foods contain high levels of iron.

Function and metabolism

5. Iron is an essential nutrient. It is a component of haemoglobin in red blood cells and of myoglobin which respectively distribute oxygen around the body and store oxygen in muscles and tissues. Iron is also a component of enzymes that are integral for energy metabolism, the metabolism of proteins and nucleotides, and the synthesis of proteins, tissues, some hormones and neurotransmitters.

6. Iron is potentially toxic because it reacts readily with oxygen. Consequently, organisms, including humans, have evolved mechanisms to limit the amount of iron that enters the body and to limit and control the chemical reactivity of the element in the body.

A series of organic molecules control iron uptake from the diet and transfer into the body, bind free iron and distribute it in the circulation to functional sites or to tissue depots for storage as ferritin.

7. The body cannot excrete iron, so the amount in the body is controlled by matching the intestinal uptake and transfer of iron to the amount needed to replace adventitious losses of iron (e.g., through blood loss including menstruation, shed skin, hair, sweat, urine) and the amount needed for growth and reproduction. Therefore, the principal determinant of the amount of iron that enters the body from the diet is the body's need for iron to meet these requirements.

Physiological requirements

8. The Dietary Reference Values (DRVs) for iron intake estimate the amount of dietary iron that needs to be consumed to meet the systemic physiological needs for iron. There is a lack of good-quality data on body losses, systemic iron stores, the efficiency of iron uptake from the diet and the effect of dietary components on iron uptake, and measuring the body's adaptation and functional use of iron to intakes (i.e., dose-response data) to enable good estimations. It is probable that the current DRVs for iron are too high, particularly for girls and women of reproductive age, because they are based on cautious assumptions about the bioavailability of dietary iron and metabolic adaptation. The necessity of DRVs for infants (aged 0–6 months) is questionable because infants born at term have sufficient systemic iron to meet their needs for the first six months of life. A delay in clamping the umbilical cord at delivery is associated with higher systemic iron depots in the first six months of life; however, it might also increase the risk of jaundice requiring phototherapy.

Measuring iron status

9. Iron status describes whether an individual has too little, enough or too much iron in their body to meet their needs as well as indicating the possible risk of deficiency or excess. Currently this assessment depends on the interpretation of a battery of markers, all of which have limitations. Most assessments combine ferritin and haemoglobin concentrations as markers of the deposition of iron in tissues and iron utilisation. The reference ranges for markers of iron status are based on values observed in a population that is presumed to be healthy. The reference ranges are not based on functional correlates and defects. Therefore, the limits of values define iron sufficiency but they do not define iron deficiency or excess. This is an important consideration in public health risk assessments and in population surveys.

Iron in the diet

10. Dietary iron exists in two forms: haem (found almost exclusively in meat) and non-haem. The richest sources of non-haem iron are cereals, vegetables, nuts, eggs, fish and meat. Iron is also added to food as a fortificant and is available as supplements. The main contributors to iron intakes in the UK are iron fortified cereals (including bread), meat/meat products and vegetables.

11. The most important determinant of dietary iron absorption is systemic iron need: more is absorbed in a state of iron deficiency and less is absorbed when iron depots are replete. In circumstances of marked iron need, however, the influence of dietary factors on iron absorption may become limiting. Haem iron is 2-6 times more available for absorption from the diet than non-haem iron. Calcium, phytates in cereals and legumes, and phenolic compounds found in tea, coffee and other beverages bind iron and restricts its availability for absorption, while meat and vitamin C found in fruit and vegetables enhance the potential availability of iron for mucosal uptake. However, these effects have been predominantly determined in studies using single meals; the effects of enhancers and inhibitors of iron absorption are attenuated in longer term studies and with the consumption of whole diets. Current evidence suggests that, in populations representative of those in the UK, dietary inhibitors and enhancers of iron absorption do not substantially affect iron status.

12. Iron fortification of foods has been the main approach used to improve the iron intakes of the UK population. Addition of iron to white and brown wheat flour and to breast milk substitutes is mandatory in the UK and a number of other foods are fortified on a voluntary basis. Elemental iron powders are widely used to fortify foods because they have a longer shelf-life than other iron fortificants; however, evidence suggests that foods fortified with iron are of little practical use in improving iron status in the UK.

13. Although iron depots are lower in vegetarians compared to non-vegetarians, haemoglobin concentrations are similar in both diet groups.

Health consequences of iron deficiency

14. There are difficulties in relating the functional effects seen with iron deficiency to iron deficiency *per se* or to anaemia from other accompanying causes. As well as an inadequate dietary intake or reduced dietary availability of iron, other causes of iron deficiency in the UK include impaired absorption or increased blood losses (due to menstruation or gastrointestinal losses).

15. Haemoglobin values below 80 g/L have been associated with impaired physical work capacity, reproductive efficiency and cognitive and psychomotor development. Early phenomena of functional defects have been discerned at haemoglobin concentrations at or below 110–120 g/L and ferritin concentrations at or below 16–20 µg/L. However, many of these associations have derived from studies in which it is not possible to determine thresholds for impairments because of difficulties in measuring the outcomes, poor characterisation of iron deficiency, and assumptions that anaemia is solely caused by iron deficiency. Additionally, many studies were performed in developing economic communities where there are multiple nutritional deficiencies and social and economic deprivations that could also affect physical work capacity and cognitive and psychomotor development.

16. Studies using iron supplementation suggest that iron responsive 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 the long term implications of these findings are

unknown. It is unclear if iron deficiency or iron deficiency anaemia affects cognitive or language development in children aged 3 years or under. There is insufficient evidence to determine the effect of iron treatment on school achievement. Based on current evidence, it is not possible to specify thresholds of anaemia or iron deficiency at which cognitive, motor and behavioural development might be at risk; however, early adverse effects do not appear to be present at haemoglobin concentrations above 110 g/L but have been observed at levels below this value (irrespective of the cause of the anaemia).

17. Epidemiological studies suggest that maternal haemoglobin concentrations at either the low or high end of the distribution during pregnancy (usually in the first or second trimester) are markers of increased risks of low birth weight and perinatal mortality. However, a causal relationship between these and iron supply or nutrition is not established. There are a number of physiological changes during pregnancy which make it difficult to interpret markers of iron metabolism at this time. Intervention studies of iron supplementation during pregnancy have not shown beneficial or adverse effects on pregnancy outcomes.

Health consequences of high iron intake and high iron burden

18. Acute high doses of iron can damage the intestinal mucosa and cause systemic shock and death. Continuous exposure to lower amounts may interfere with the metabolism of copper and zinc. High systemic iron burden, which is never attributable to nutritional causes, is associated with free radical tissue damage caused by iron released from degradation of tissue ferritin. In the UK, the Guidance Level (GL[1]) for supplemental intake of iron (i.e., additional to intakes from foods) is 17 mg/day for adults, which is based on adverse gastrointestinal effects.

19. Most studies on iron and cancer risk have examined the relationship between iron and colorectal cancer. This is because most dietary iron is not absorbed and luminal exposure to excessive intakes could cause direct oxidative damage to the colorectal lumen. Overall, there are insufficient data on the association between intakes of total dietary iron or body iron burden and colorectal cancer risk to reach clear conclusions. Meat, especially red and processed meat, is almost exclusively the source of haem iron. A substantial body of epidemiological evidence suggests that red and processed meat intake is probably associated with increased colorectal cancer risk. It is not possible to discern a clear dose-response relationship, or a threshold level of intakes of red or processed meat associated with increased colorectal cancer risk because of inconsistencies in categorisation and quantification of red and processed meat intake.

20. Observational studies of total iron intake/body iron burden and CVD do not suggest an association. Although evidence from a small number of prospective studies suggests that high intakes of haem iron are associated with increased CVD risk, this

1 The GL is based on limited data and represents an approximate indication of intakes that would not be expected to produce adverse effects.

could be due to other components of meat (the main source of haem iron), such as saturated fats, or dietary and lifestyle factors associated with meat intake. Similarly, there is no substantive evidence that dietary intakes of iron are associated with arthritis or with diabetes mellitus. There is no evidence that dietary iron is associated with neurodegenerative disease.

21. Some evidence from randomised controlled trials suggests that iron supplementation may impair physical growth of iron replete infants and children (haemoglobin above 110 g/L and serum ferritin above 12 µg/L); however, further studies are required to characterise this effect.

Infection and immunity

22. Iron deficiency anaemia (typically haemoglobin <100 g/L plus one or more markers of iron deficiency) and iron overload (usually from multiple blood transfusions) impair some aspects of immune function; however, the functional consequences of these impairments on morbidity are unclear.

23. It has been suggested that iron supplementation may favour infectious pathogens by providing them with a supply of iron for their growth and replication. The effect of iron supplementation on morbidity and mortality from infections is uncertain. The evidence suggests that iron supplementation does not increase the risk of non-diarrhoeal or respiratory tract infections in children but may increase diarrhoea risk. It is not clear if iron supplementation increases malaria risk. There is currently insufficient evidence to draw conclusions on the relationship between iron supplementation and HIV or TB.

24. In the UK, there is little evidence to suggest that iron supplementation of children would have any adverse effects on infectious disease incidence or morbidity. However, some evidence suggests that iron supplementation might have adverse effects on individuals with HIV and children at risk of diarrhoea.

Dietary iron intakes and iron status of the UK population

25. In the UK, groups in the general population with substantial proportions below the Lower Reference Nutrient Intake (LRNI[2]) for iron are children aged 1½–3½ years, girls aged 11–18 years and women aged 19–49 years. The highest proportions with intakes below the LRNI in low income populations are females aged 11–49 years.

26. The prevalence of iron deficiency anaemia (both haemoglobin and serum ferritin concentrations below the World Health Organization's thresholds for adequacy)[3] in the general population ranges between 0 and 6% according to age and sex. In the

2 The LRNI represents the amount of a nutrient that is likely to meet the requirements of 2.5% of the population.

3 The World Health Organization's (WHO) criteria for identification of anaemia, irrespective of cause, are haemoglobin concentrations of: children under 5 years, 110 g/L; children 5-11.99 years, 115 g/L; children 12-14.99 years and non-pregnant females over 15 years, 120 g/L; males over 15 years, 130 g/L. The criteria used to define depleted storage iron are serum ferritin concentrations of: children under 5 years, <12 µg/L; males and females over 5 years, <15 µg/L

general population, groups with the highest prevalence of iron deficiency anaemia are children aged 1½–2½ years, girls aged 15–18 years, women aged 35–49 years, institutionalised men aged 65 years and over, and free-living adults aged 85 years and over. In low income groups, the prevalence of iron deficiency anaemia is highest for women aged 19–39 years and 65 years and over.

27. Overall, data from national surveys broadly show that men aged under 65 years in the UK are at minimal risk of iron deficiency anaemia while women aged 15–50 years are at risk (this is consistent with increased iron losses in this age group due to menstrual blood loss). Iron deficiency anaemia observed in some adults aged 65 years and over is consistent with blood loss due to gastrointestinal disease or medication in older age groups.

28. The high proportions of the UK population with intakes below the LRNI and the relatively low prevalence of iron deficiency anaemia suggest that the DRVs for iron may be too high.

29. Limited data suggest that iron intakes of minority ethnic groups (aged 16 years and over) are not below those of the general population. Data from the Health Survey for England (HSE) 2004 indicate that the prevalence of anaemia[4] (irrespective of cause) is low in men from minority ethnic groups (0-4%) compared to women (ranging from 6-7% for Irish and Chinese women to 16% in Black Caribbean women and 29% in Indian women). The prevalence of iron deficiency anaemia[5] was not assessed in the HSE. The prevalence of iron deficiency anaemia in infants and children from minority ethnic communities is unclear because there are limited representative data on biochemical markers of iron status in these groups.

The potential impact of reducing red and processed meat consumption on intakes of iron and zinc

30. Results from a modelling exercise to explore possible effects of a recommendation for adults to lower their consumption of red and processed meat suggest that red and processed meat makes a greater contribution to total zinc intake (32% for men; 27% for women) than to total iron intake (12% for men; 9% for women). The average red and processed meat consumption of adult consumers is approximately 70 g/day (88 g/day, men; 52 g/day, women). The modelling exercise indicates that reducing total red meat consumption of consumers in the upper range of the distribution of intakes, down to 70 g/day, would have little effect on the proportion of adults with iron intakes below the LRNI.

4 Defined as haemoglobin concentrations below 120 g/L for men and women. This differs from the WHO criteria for identifying anaemia in men (see footnote 3).

5 Both haemoglobin and serum ferritin below thresholds used to define anaemia and iron deficiency.

Limitations in the evidence base

31. A risk assessment of iron and health is complicated by several uncertainties. These include: lack of definitive data on amounts of haem and non-haem iron in the diet; inaccurate assessments of iron intakes; poor correlation between iron intakes and status; difficulty in measuring adaptive and functional responses to variations in iron intake; uncertain and possibly conservatively high estimates of DRVs; lack of sensitive and specific markers to assess iron status; lack of consistent quality control and reference values in measurement of customary markers of iron status; inadequate characterisation of the role 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; small sample sizes; and confounding by other dietary and lifestyle factors and by alterations in iron metabolism in response to infection. All these uncertainties make it difficult to determine dose-response relationships or confidently characterise the risks associated with iron deficiency or iron excess.

Recommendations

32. It is important to ensure that the UK population has a safe and adequate supply of iron to meet physiological requirements. It is recommended that a public health approach to achieving adequate iron status should emphasise the importance of a healthy balanced diet that includes a variety of foods containing iron. Such an approach is more important than focusing on particular inhibitors or enhancers of the bioavailability of iron from diets.

33. While substantial proportions of the UK population appear to have iron intakes below dietary recommendations for iron, this is not clearly consistent with the low prevalence of poor iron status (see paragraph 34). This might be because there are important uncertainties in the DRVs for iron intake which may be too high, particularly for girls and women of reproductive age. It is recommended that the DRVs for iron should be reviewed when more data become available (see research recommendations, paragraph 38).

34. Although there are many uncertainties in the data, about 95% of the UK population is iron replete.[6] However, some population groups may be at risk of iron deficiency anaemia.[7] These include toddlers, girls and women of reproductive age (particularly those from low income groups) and some adult groups aged over 65 years.[8] It is recommended that health professionals be alert to the increased risk of iron deficiency anaemia in these groups. Those with signs and symptoms suggestive of iron deficiency anaemia should receive appropriate clinical assessment and advice, including dietary advice on how to increase their iron intakes and to consider use of iron supplements if required.

6 Haemoglobin and serum ferritin concentrations above the WHO thresholds used to define iron deficiency and anaemia (see footnote 3).

7 Haemoglobin and serum ferritin concentrations below the WHO thresholds used to define iron deficiency and anaemia (see footnote 3).

8 Men aged 65 years and over living in institutions; free-living men and women aged 85 years and over.

35. Current evidence does not support routine iron supplementation of pregnant women but this should be kept under review. The recommendation by NICE (2008) is therefore supported, that iron supplementation should not be offered routinely to all pregnant women but should be considered for women identified with haemoglobin concentrations below 110 g/L in the first trimester and 105 g/L at 28 weeks.

36. Red and processed meat is a source of iron in the diet of the UK population. COMA reported possible links between red and processed meat consumption and colorectal cancer risk in 1998 and the evidence that has accumulated since then generally supports this association. However, it is not possible to quantify the amount of red and processed meat that may be associated with increased colorectal cancer risk because of limitations and inconsistencies in the data. It may be advisable for adults with relatively high intakes of red and processed meat (e.g., it is estimated that those above the 75th percentile consume over 90 g/day) to consider reducing their intakes. Evidence from a theoretical modelling exercise indicates that a reduction in the red and processed meat intakes of high consumers, to the population average for adult consumers (about 70 g/day cooked weight in 2000/01), would have little impact on the proportion of the adult population with iron intakes below the LRNI. However, this estimate is based on data from 2000/01 and will need to be kept under review.

Research recommendations

37. A more coordinated approach to research on iron in the UK and elsewhere is required to characterise iron status, involving harmonisation of reference ranges and analytical quality control for markers of iron metabolism. Consistent study designs and protocols will enable better characterisation of functional thresholds in relation to iron sufficiency, deficiency, or excess. This would improve the cost effectiveness of the research and enable research findings to be more relevant to public health needs.

38. Good quality dose-response data are required to enable a reassessment of the DRVs for iron. Knowledge of the systemic regulation and mediation of iron homeostasis should be applied to characterise better the responses to increased and reduced systemic needs for iron and the development, or better validation, of existing markers used to assess the adequacy of iron status in populations and individuals.

39. Future studies assessing the relationship between iron excess and chronic disease should employ a standardised approach to measure iron exposure and categorisation of red and processed meat and other sources of organic and inorganic iron. This, together with the maintenance and expansion of food composition databases, with particular reference to iron content, would improve the quality of dietary assessments of iron intake for studies relating to iron and chronic disease. Assessments of systemic iron depots in such studies should be based on measurement of serum ferritin concentration.

40. Iron intakes and iron status of vulnerable groups, particularly minority ethnic groups and infants aged up to 18 months, need to be better characterised.

41. An improved understanding is required of the factors underlying differences in the risk of iron deficiency anaemia between women of reproductive age from low income populations and those in the general population.

42. The extent to which foods fortified with iron contribute to the supply of absorbed iron and to achieving adequate iron status, particularly in vulnerable groups, should be assessed.

43. An improved understanding of the possible adverse effects of iron supplements on iron replete children is required.

44. Further randomised controlled trials with adequate power and sufficient duration are required to examine the effect of iron supplementation on mental development in children under 3 years old with iron deficiency anaemia.

45. Further studies are required on the benefits, risks and long term effects associated with a delay in clamping the umbilical cord after birth until it has stopped pulsing.

1 Introduction

Background

1.1 In their report, *Nutritional Aspects of the Development of Cancer* (Department of Health [DH], 1998), the Committee on Medical Aspects of Food and Nutrition Policy (COMA) highlighted possible links between red and processed meat and colorectal cancer and recommended that "higher consumers should consider a reduction" in red and processed meat consumption.[9]

1.2 Red meat is a dietary source of iron (100 g of cooked red meat contains amounts ranging from 0.5 to 3.0 mg[10]). The government currently advises that meat can be consumed as part of a healthy, balanced diet; that it is a good source of iron, zinc, B vitamins and protein; however, due to its high saturated fat content, it should be eaten in moderation.

1.3 COMA was aware that a recommendation to reduce meat consumption might compromise dietary sources of iron, as well as other micronutrients, and recommended that "the possible associated adverse implications of a reduction in meat consumption on other aspects of health, particularly iron status" should be the subject of review.

1.4 Public reactions to the risk of human exposure to bovine spongiform encephalopathy (BSE[11]) in cattle have also raised concerns regarding the possibility that any reduction in consumption of red meat (beef, lamb, pork and meat products) in the past 20 years might be associated with low iron intakes and, consequently, an increased risk of iron deficiency, even though findings from the National Diet and Nutrition Surveys indicate that similar amounts of meat were consumed in 1987 and 2000 (Gregory *et al*, 1990; Hoare *et al*, 2004).

1.5 Progressive iron deficiency leads to anaemia, for which there are reported associations with a number of adverse effects including impairments of physical performance, cognitive and psychomotor development, immune function, and reproductive efficiency. The severity and thresholds of iron deficiency at which these adverse effects might develop have not been well characterised and their prevalence in the UK population is not known.

1.6 UK national surveys have consistently shown that a proportion of the population, particularly young women and children, have low dietary intakes of iron relative to reference intakes (Finch *et al*, 1998; Gregory *et al*, 1990; Gregory *et al*, 1995; Gregory *et al*, 2000; Henderson *et al*, 2003a; Nelson *et al*, 2007a), which has raised concerns

9 See paragraph 9.3.9.2, page 197 of COMA report.

10 Food Standards Agency, 2002.

11 BSE, commonly known as mad cow disease, is a fatal progressive neurological degeneration in cattle.

that they may be at risk of iron deficiency. Additionally, some smaller studies have suggested that children from minority ethnic groups may also be at risk of iron deficiency (Grindulis *et al*, 1986; Lawson *et al*, 1998).

1.7 The possibility that some subgroups of the UK population might be iron deficient, or be at risk of iron deficiency, together with concerns about the potential consequences of iron deficiency, is a public health issue that requires critical exploration and assessment.

1.8 Concerns about the possible prevalence and severity of iron deficiency in populations have prompted iron fortification of food products and the use of iron supplements. However, as there are also concerns about possible adverse effects of increased intakes of iron, consideration of the extent and consequences of inadequate iron nutrition in the UK population needs to be sensitive to the balance between iron deficiency and iron excess.

Terms of reference

1.9 The Scientific Advisory Committee on Nutrition (SACN) Working Group on Iron was established in 2001 in response to the recommendation in the COMA report on *Nutritional Aspects of the Development of Cancer* (DH, 1998) for a review of the implications of a reduction in meat consumption on the adequacy of iron status in the UK population.

1.10 The terms of reference of the Iron Working Group were: To review the dietary intakes of iron in its various forms and the impact of different dietary patterns on the nutritional and health status of the population and to make proposals.

1.11 It was agreed that it was necessary to consider both beneficial and adverse effects of increasing iron intakes, including the:

- effect of dietary components on iron absorption and utilisation in the body;
- interaction of infections and inflammation with iron metabolism and the possibility that this may affect the apparent incidence of iron deficiency;
- effect of iron deficiency on health and well-being, for example mental and physical development;
- potential adverse effects of excess iron, including free radical damage and the risk of cardiovascular disease (CVD) and cancer.

1.12 The associations between consumption of red and processed meat and cancer risk were also considered since these foods contain high levels of iron.

Methodology

1.13 The SACN *Framework for the Evaluation of Evidence* (SACN, 2002) was used as the basis for identifying and assessing the available evidence. Consideration of the evidence, which included studies published up to March 2010,[12] was mainly restricted to cohort studies and randomised controlled trials in humans, but cross-sectional studies, case reports and experimental cell and animal studies were also considered where these informed the interpretation of data.

1.14 The key issues considered were: iron in the diet; the health consequences of iron deficiency and iron excess; adequacy of iron intakes and body iron content of the UK population; and the possible impact of reducing intakes of red and processed meat on the risk of iron and zinc deficiency in the UK.

1.15 In the cited literature on iron and health outcomes, various and inconsistent biological markers and thresholds have been used to define iron deficiency and iron deficiency anaemia. Even when studies have used similar markers and thresholds, the variability in laboratory measurements may mean that they would still not be comparable. It was not possible to compensate for this uncertainty; therefore, in this report, the values used to define iron deficiency and iron deficiency anaemia in the original publication are provided.

1.16 The draft report was made available for public consultation and the comments received from interested parties, which included the Committee on Toxicity and Committee on Carcinogenicity, were taken into consideration before the report was finalised. The working procedures for the preparation and finalisation of the report are described in Annex 1.

12 No literature searches were carried out after November 2009.

2 Biochemistry and metabolism

2.1 Iron[13] (Fe) is a transition metal which exists in two biologically relevant oxidation or valency states: the reduced ferrous form (Fe^{2+}) and the oxidised ferric form (Fe^{3+}). The ability of iron to readily accept or donate single electrons means it is an efficient catalyst for electron transfer and free-radical reactions. Although these properties lend themselves to fundamental metabolic processes, the reactivity of iron also means that "free iron" (i.e., when it is not bound to protein or other organic molecules) is potentially toxic and that organisms need to reduce the risk of this damage by minimising their exposure to free iron. The role of iron in free radical and oxidative reactions is considered in section 7.

2.2 Organisms have evolved mechanisms to exploit and control the chemical reactivity of elemental iron, using it to support their metabolism whilst limiting the risk of tissue architectural and functional damage from free iron. This control depends on proteins which are specifically involved in its uptake from the diet and transfer into the systemic circulation, its transport around the body and storage in tissues, as well as its delivery to functional sites.

Function

2.3 Iron, as a component of haemoglobin in erythrocytes (red blood cells), is required for transporting oxygen around the body and, in the form of myoglobin, for the storage and use of oxygen in muscles. The iron in the haem complexes of haemoglobin and myoglobin is stabilised in the ferrous state and interaction with the adjacent globin protein enables it to bind reversibly to oxygen. This enables oxygen, itself a potentially toxic element, to be safely distributed and stored around the body. In the chemical environment of the lungs, where oxygen concentration and activity is high, haemoglobin binds oxygen while the low concentrations of oxygen in the tissues facilitate its release from haemoglobin. Simultaneously, haemoglobin binds carbon dioxide in the tissues and carries it to the lungs where it is exhaled. The oxygen released in the tissues from haemoglobin is used in oxidative metabolism or stored, for example, in myoglobin.

2.4 Iron is also present as a component of haem and iron-sulphur complexes in enzymes that are responsible for electron transport and energy generation in mitochondrial respiration and the citric acid cycle, and for ribonucleotide reductase, which is essential for DNA[14] synthesis. Examples of functional iron-containing enzymes are provided in Annex 2 (Table A1).

13 Atomic number, 26; atomic weight, 55.85.

14 Deoxyribonucleic acid.

2.5 Body iron content is approximately 4.0 and 3.5 g in men and women respectively[15] (Bothwell *et al,* 1979). In adults, most body iron is present in haemoglobin (60–70%) in circulating erythrocytes where it is essential for oxygen transport, and in muscle myoglobin (10%). The remaining body iron (20–30%) is found primarily in storage pools located in the liver and reticuloendothelial (macrophage) system as ferritin and haemosiderin, which is a degraded form of ferritin. Only about 1% of body iron is incorporated in the range of iron-containing enzymes and less than 0.2% of body iron is in the plasma transport pool where most of it is bound to transferrin.

2.6 Iron in the body is constantly recycled between the functional and non-functional pools (see paragraph 2.9).

Metabolism

2.7 Finely tuned mechanisms maintain iron homeostasis by balancing the body's need for iron with minimising the risk of iron toxicity. Absorption of iron from the gastrointestinal tract is regulated by the systemic need for iron. The risk of tissue damage by free reactive iron is limited by a series of organic molecules which have specific roles in binding free iron, carrying it in the circulation and delivering it to functional sites or to depots in which iron that is not immediately needed is deposited in a safe form. This is the basis for the systemic recycling of iron released during tissue and enzyme turnover. A key element of this salvage system is the deposition of iron in ferritin. The principal pool of ferritin is in the liver which serves as a buffer pool for any iron excess to immediate requirements. Approximately 25% of body iron is found in the liver: two-thirds as ferritin and up to one-third as insoluble haemosiderin.

2.8 Since the body has no means of excreting excess iron, control of the body iron burden is by regulation of iron absorption. The only way in which iron is lost from the body is adventitiously in desquamated skin cells and sweat (0.2–0.3 mg/day), urine (<0.1 mg/day), gastrointestinal secretions, and hair. In premenopausal women, iron is also lost in menses (see paragraphs 3.28–3.33).

2.9 Iron turnover is driven by the formation and destruction of haemoglobin present in erythrocytes, which have a life span of approximately 120 days; senescent erythrocytes are then engulfed and destroyed by the macrophages of the reticuloendothelial system. Their haemoglobin is broken down in the lysosomes, where iron is released from haem by haem oxygenase and transferred to the protein, apotransferrin, to form transferrin which is the iron carrier molecule in the plasma (see paragraph 2.26). Transferrin-bound iron is transported to the erythroblasts in the bone marrow for incorporation into haem for new erythrocytes, or delivered to cells in tissues undergoing growth and development or to tissue ferritin depots. The macrophages of the reticuloendothelial system recycle approximately 30 mg/day of iron from senescent erythrocytes. This demonstrates the efficiency of the cyclic conservation and reutilisation of systemic iron.

15 In men, this represents about 4.3×10^{22} atoms of iron (calculated using Avogadro's number, which is the number of atoms in the gram molecular weight of an element).

Iron losses

2.10 In healthy individuals, obligatory iron losses from the skin and gastrointestinal mucosa are thought to be approximately 1 mg/day in males (Green *et al*, 1968) and slightly more in women of child-bearing age because of additional losses due to menstruation, pregnancy and lactation.

Absorption

2.11 The process of absorption comprises the uptake of a nutrient into the intestinal mucosa and its subsequent transfer into the body. Although some nutrients can enter the body by passing between gut mucosal cells, iron uptake and transfer depends on specific cellular carrier mechanisms. The principal, and probably the only, physiological and primary determinant of how much iron is absorbed is the systemic need for iron; this would be to compensate the adventitious losses (see paragraph 2.8) and for new tissue synthesis (e.g., growth in children and reproduction in women). Secondary dietary factors affecting iron absorption are considered in section 5.

Molecular control of iron absorption

2.12 Iron absorption occurs mainly in the proximal small intestine[16] and involves the uptake and transfer of iron across the enterocyte into the systemic circulation. The enterocytes are equipped with iron uptake carrier proteins on their apical surface, which is in contact with the intestinal lumen and its contents, and at their basal surface which is in contact with the portal circulation. Additionally, enterocytes have mechanisms that sense and are responsive to the hepatic mediators of the systemic response to the need for iron and which control the delivery of iron to the basal transporter.

2.13 There are at least two separate mechanisms for the uptake of haem and non-haem iron into the enterocyte.

2.14 The divalent metal transporter 1 (DMT1) transports inorganic iron, i.e., iron that is not part of the haem molecule, and is specific for ferrous iron. Non-haem iron uptake requires an acid pH, which is provided by gastric hydrochloric acid, to make it more soluble and to produce the protons that are required for its co-transport by DMT1. A haem enzyme, duodenal cytochrome B reductase (DcytB), located on the luminal surface of the enterocytes, converts dietary ferric iron to the ferrous state (ferrous compounds are more soluble than ferric salts in physiological conditions and alterations in the oxidation state, between ferric and ferrous, is an additional means of regulating the distribution and use of iron).

16 There may also be some uptake and transfer of iron in the distal intestine. Some studies in rat pups (Frazer *et al*, 2007) and pigs (Blachier *et al*, 2007) have found that colonic mucosal cells in vivo and in culture can express these molecules and transfer iron. However, their capacity for iron uptake and transfer is very much less than in the proximal intestine.

2.15 In the enterocyte, ferrous iron enters a labile or "exchangeable" iron pool from which it can enter three different pathways, depending on the requirements of the body. It may be taken into the local mitochondria for haem synthesis, sequestered into ferritin iron depots (and shed into the gut lumen at the end of the enterocyte's lifespan), or transferred (still in the ferrous state) to the basal transporter (ferroportin 1) for translocation into the body.

2.16 The mechanism of haem iron absorption remains unclear. The intestinal haem transporter described by Shayeghi *et al* (2005), named haem carrier protein 1 (HCP 1), has now been identified as a folate transporter and its role in haem transport is uncertain (Qiu *et al*, 2006). However, once it has been taken up by the enterocyte, the haem molecule is degraded by haem oxygenase to release ferric iron, which is thought to join the enterocytic labile or exchangeable iron pool (Uzel and Conrad, 1998).

2.17 Studies have also demonstrated that an efficient pathway exists for the intestinal uptake of ferritin, which may be derived from plant and meat based dietary sources. This involves enterocyte uptake via an endocytic pathway followed by lysosomal dissolution of the ferritin core to release the iron (Kalgaonkar and Lonnerdal, 2008; San Martin *et al*, 2008; Kalgaonkar and Lonnerdal, 2009).

2.18 Hephaestin, a ferroxidase found mostly in the basal membrane of enterocytes, is thought to facilitate basolateral iron export from the intestinal epithelial cells by oxidising the ferrous iron back to its ferric form. It is not known whether hephaestin works independently of ferroportin 1, or if the two proteins interact to cause the oxidation (Miret *et al*, 2003). Ceruloplasmin, which is found in plasma, is also a ferroxidase and may be involved in the oxidation of ferrous iron to ferric iron during binding to transferrin.

Regulation of iron absorption

2.19 Regulation of intestinal iron absorption occurs both at the stage of mucosal uptake and at the stage of its transfer to the blood.

2.20 Adaptation in intestinal uptake and transfer of iron in response to systemic needs is mediated in part by changes induced in the maturing enterocytes in the mucosal crypts and does not become effective until the newly matured enterocytes have moved to the villi. This means there is a time lag of 1–2 days between changes in systemic iron need and in the corresponding mucosal setting for iron uptake and transfer. This has implications for interpreting data from absorption studies and their relevance to practice and for understanding the potential toxicity of acute exposure to iron (see paragraphs 7.5–7.6). However, a large intake of dietary iron, in excess of that required to meet systemic needs, can itself induce the enterocytes autonomously to develop a "mucosal block" (Granick, 1946) through ferritin induction which prevents excessive absorption by reducing the intestinal transfer of iron for several days (Frazer *et al,* 2003), even in the presence of systemic iron deficiency.

2.21 The principal regulator of iron absorption is hepcidin (Ganz, 2004), a small peptide of 20–25 amino acids encoded by the HAMP[17] gene, which is predominantly expressed in the liver. Transgenic mouse models have shown that hepcidin is the principal down regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages and hepatocytes (Nicolas *et al*, 2002). *In vitro* studies in macrophage cells have shown that hepcidin exerts its effects by directly binding to and degrading the iron exporter molecule, ferroportin, on the cellular membrane; as a consequence, iron is prevented from leaving the cell (Nemeth *et al*, 2004). In fact, the mechanism of hepcidin action may be twofold and cell-type specific. Studies using *in vitro* and *in vivo* models have indicated that hepcidin inhibits iron export from macrophages by causing degradation of ferroportin; however, in enterocytes it may also down regulate iron uptake by inhibiting DMT1 transcription (Mena *et al*, 2008; Chung *et al*, 2009).

2.22 Hepatic hepcidin production is increased when iron stores are adequate or high and during inflammation. The released hepcidin, through its effect on ferroportin, then prevents the transfer of iron from the enterocyte to plasma transferrin. The iron that is not transferred is sequestered within the enterocytes and is eventually lost in the gut lumen when the enterocytes are shed and lost in the faeces. Similarly, during inflammation or when the systemic iron content is adequate, hepcidin blocks the release of iron from macrophages. However, when systemic iron requirements are increased or iron stores are low, or both, hepcidin production is decreased, allowing intestinal iron transfer and the release of iron from depots in the macrophages. In addition, hepcidin production is reduced by systemic hypoxia, which also stimulates the production of erythropoietin which, in turn, stimulates the production of red blood cells (erythropoiesis). The coincident depression of hepcidin therefore ensures a supply of iron needed for the synthesis of haemoglobin as part of the erythropoietic response.

2.23 Defective regulation of hepcidin, or of its receptor ferroportin, causes a range of iron overload disorders known as the haemochromatoses (Ganz, 2005) (see paragraphs 2.42–2.45 and Table 1). These are characterised by increased iron absorption leading to excessive systemic iron accumulation and overload. The most common form is associated with hepcidin deficiency.

2.24 Hepcidin deficiency is a characteristic of mutations in the HAMP gene but most patients with genetic haemochromatosis have alterations in the HFE[18] gene or, rarely, in the transferrin receptor 2 (TFR2) or hemojuvelin (HJV) genes, suggesting that these proteins are involved in the regulation of hepcidin synthesis (Nemeth and Ganz, 2006). Juvenile haemochromatosis (JH), the most severe form of haemochromatosis, is caused by mutation of the HJV gene (Papanikolaou *et al*, 2004) or the HAMP gene (Roetto *et al*, 2003), indicating that both genes function in the same pathway (Papanikolaou *et al*, 2004). Haemochromatosis caused by mutation of the TFR2 gene also results in severe iron overload (Nemeth *et al*, 2005). Hepcidin deficiency is not as severe in HFE-linked haemochromatosis compared to HJV- or TFR2-linked

17 Hepcidin antimicrobial peptide.

18 High iron Fe.

haemochromatosis (Nemeth *et al*, 2004), suggesting that HFE modulates the signal from the iron sensor to hepcidin but is not essential for the function of this pathway (Nemeth and Ganz, 2006). Since concentrations of TFR2 are regulated by transferrin saturation (Johnson and Enns, 2004), it has been proposed that TFR2 may influence hepcidin expression by acting as a sensor of circulating iron (Fleming and Bacon, 2005).

2.25 Mutations of the ferroportin gene either cause the protein to be non-functional (i.e., does not export any iron) or unresponsive to hepcidin (leading to excessive iron export from cells) (De Domenico *et al*, 2005). This leads to either iron accumulation in phagocytic cells or in hepatic parenchymal cells and, unlike the other forms of genetic haemochromatosis, there is autosomal dominant inheritance.[19]

Plasma iron transport

2.26 Iron is distributed systemically in the circulation as transferrin. Transferrin comprises a core carrier glycoprotein, apotransferrin, which can bind one or two atoms of ferric iron to form holotransferrin, which is usually called transferrin. This is an efficient carrier system; however, non transferrin-bound iron has been detected in the plasma of patients with iron overload conditions (Grootveld *et al,* 1989).

2.27 The subsequent major pathways of iron exchange and whole-body iron economy have been delineated from tissue uptake studies using ^{59}Fe bound to transferrin as an intravenous tracer (Finch *et al*, 1970; Cavill and Ricketts, 1980).

2.28 The uptake of iron by cells is mediated by the binding of holotransferrin (Tf) to transferrin receptors (TfR) on cell membranes which is then internalised by endocytosis. The resulting endosome contains the Tf-TfR complex. Ferrous iron atoms are released and transferred out of the endosome to the cytoplasm by a local DMT1. The iron is then either stored as ferritin or used within the cell, e.g., for haemoglobin synthesis in erythroid precursors. The apotransferrin and the TfR return to the cell surface and the apotransferrin is recycled into the plasma.

2.29 Transferrin receptors have a greater affinity for fully saturated, diferric transferrin than for monoferric transferrin (Huebers *et al*, 1981, 1985), and do not bind apotransferrin at the neutral pH of plasma. A second transferrin receptor (TFR2) is thought to be involved in the regulation of iron absorption by influencing hepcidin expression (see paragraph 2.24).

2.30 Mammalian cells may also acquire iron through transferrin-independent pathways. The transmembrane protein, "stimulator of Fe transport", facilitates the uptake of both ferrous and ferric iron independently of transferrin and may also have a role in intracellular iron transport (Gutierrez *et al*, 1997; Yu and Wessling-Resnick, 1998; Yu *et al*, 1998). Its significance in iron metabolism is presently unclear.

19 Mutation needs to be present on just one chromosome to cause disease.

Hepatocyte iron uptake

2.31 The liver is a major systemic depot of iron. Hepatocytes take up iron from transferrin by the receptor-mediated endocytosis described previously (see paragraph 2.28), and by the route taken by non-transferrin bound iron (Baker and Morgan, 1994). Iron is released from the hepatocytes in times of increased need subject to regulation by hepcidin.

2.32 In diseases which cause increased transferrin iron saturation and iron overload (see Table 1), the liver continues to accumulate iron, even when iron stores are high, and is therefore vulnerable to developing damage secondary to iron overload.

Iron storage and deposition

2.33 All cells have the ability to sequester iron either in the soluble complex ferritin or, as its insoluble derivative, haemosiderin. Ferritin is the major intracellular storage protein found in all cells with the highest concentrations in the liver, spleen and bone marrow.

2.34 Ferritin binds iron as a ferric oxo-hydroxide (ferrihydrite) (Pan *et al*, 2009) phosphate complex within a protein shell of molecular mass 480 kD. Each molecule can theoretically store up to 4500 atoms of ferric iron but, in practice, it is typically less than 2000 atoms. The protein shell surrounding the iron core is penetrated by six channels through which ferrous iron enters to interact with a ferroxidase at the centre of the molecule (Harrison and Arosio, 1996). Iron is able to exit after it has been reduced. This iron depot is readily accessible for haemoglobin synthesis.

2.35 Serum ferritin concentrations are normally within the range 15–300 µg/L. They are lower in children than in adults; from puberty to middle age, mean concentrations are higher in men than in women (Worwood, 1982). Good correlations have been found between serum ferritin concentrations and storage iron mobilised by quantitative phlebotomy, stainable iron in bone marrow biopsies, and the concentration of both non-haem iron and ferritin in the bone marrow. This suggests a close relationship between the total amount of storage iron and serum ferritin concentration in normal individuals (Walters , 1973). Phlebotomy studies have demonstrated that a serum ferritin concentration of 1 µg/L is equivalent to approximately 8 mg stored iron.

2.36 Haemosiderin is produced by lysosomal denaturation of ferritin, in which the protein shells degrade and the iron cores aggregate. Haemosiderin iron is found in lysosomes and cytosol and, as it is less soluble than ferritin iron, it is less easily mobilised.

Cellular iron homeostasis

2.37 Synthesis of several of the proteins involved in iron metabolism is regulated at the level of RNA[20] translation by two cytoplasmic iron regulatory proteins: IRP1 and IRP2 (Cairo and Pietrangelo, 2000). These proteins are capable of binding to messenger

20 Ribonucleic acid.

RNAs that contain stem-loop structures, known as iron-responsive elements (IREs). IRP1 contains an iron-sulphur (4Fe-4S) cluster and has a low affinity for the IRE when intracellular iron is abundant. When iron is scarce, however, the iron-sulphur cluster is no longer present and IRP1 binds to the IRE with high affinity. Activation of IRP2 requires accumulation of the protein after new synthesis. Degradation takes place in the presence of iron. Studies in cell lines have shown that IRP1 is the major contributor to iron regulating activity but at physiological tissue oxygen concentrations, IRP2 is the dominant regulator (Meyron-Holtz *et al*, 2004).

Response to increased systemic iron needs

2.38 Increased needs for iron are met initially by increased release of iron from ferritin. Both haem and non-haem iron absorption show an inverse relationship to serum ferritin concentrations which reflect iron reserves (Lynch *et al*, 1989) (see section 4): absorption of dietary iron increases as ferritin depots decrease.

2.39 The suggested threshold below which intestinal uptake and transfer responds to iron depletion in humans is at serum ferritin concentrations of approximately 60 µg/L (Hallberg *et al*, 1997). If absorption is not adequate, tissue iron stores are slowly depleted and the amount available for recycling and redistribution to tissues is decreased; this results in less iron bound to circulating transferrin (reduction in "transferrin saturation"). As a result, the delivery of iron to functional sites decreases and iron dependent functions, such as erythropoiesis, become impaired, leading to a decrease in haemoglobin concentration and the development of anaemia (see paragraphs 6.2–6.5). At a cellular level, ferritin synthesis is inhibited and transferrin receptor synthesis is increased in an effort to enhance cellular iron uptake. Apotransferrin synthesis by the liver is also increased by iron depletion. Concentrations of other iron-containing proteins such as myoglobin, cytochromes and iron-sulphur proteins are decreased (Dallman *et al*, 1982).

Inborn errors of iron metabolism

2.40 Characterisation of mutations affecting the genes coding for proteins involved in the metabolism of iron has improved understanding of iron metabolism.

2.41 While some of these genetic changes need be present on just one chromosome to cause disease (autosomal dominant or X-linked), the majority need to be present in two corresponding chromosomes (autosomal recessive). In the autosomal recessive diseases, although heterozygotes (i.e., individuals with one normal and one aberrant gene) have altered iron metabolism, this does not appear to affect their iron requirements or predispose them to excessively accumulate iron (see paragraph 2.44).

Gene mutations affecting proteins involved in iron absorption

HFE (type 1/hereditary/genetic haemochromatosis)

2.42 Hereditary or genetic haemochromatosis is one of the most common single gene disorders found in populations of north European origin. It is an autosomal recessive disease caused by mutation of the gene coding for the HFE protein (Feder *et al*, 1996). It results in excessive absorption of dietary iron, causing high levels of iron to accumulate in the body. This can cause organ damage, leading to clinical manifestations including diabetes, arthritis and cirrhosis of the liver (Bothwell and MacPhail, 1998).

2.43 Two common variants of this gene, C282Y[21] and H63D,[22] have been identified. In the UK, over 90% of patients with hereditary haemochromatosis are homozygous for C282Y. In Europe, the highest allele frequency of C282Y (10%) is found in Ireland, followed by the UK, Brittany and Scandinavia (around 8%); and the lowest is in Italy (0.5%) (Merryweather-Clarke *et al*, 1997; 2000). The variant is virtually absent in populations of non-European origin. The clinical penetrance of homozygosity for C282Y is very variable; the majority of people with this genotype never become ill as a result of iron overload (Beutler *et al*, 2002; Asberg *et al*, 2002; McCune *et al*, 2006). The H63D variant is more widespread in the general population worldwide and has a less defined role in predisposing towards iron loading. Compound heterozygotes account for 4% of patients (UK Haemochromatosis Consortium, 1997), though most individuals with this genotype do not develop iron overload (Jackson *et al*, 2001).

2.44 Less than a quarter of heterozygotes for the C282Y mutation show mild increases in serum ferritin concentration or transferrin saturation but do not have clinical features of iron overload (Bulaj *et al*, 1996). Although it has been suggested that heterozygotes may also have poorer control of iron absorption (Lynch *et al*, 1989), two studies reported no differences in iron absorption between heterozygotes and wild-type controls (Hunt and Zeng, 2004; Roe *et al*, 2005).

2.45 A third variant, S65C, is less frequent than C282Y and H63D but may be associated with mild forms of haemochromatosis (Mura *et al*, 1999; Wallace *et al*, 2002).

2.46 Other types of genetic haemochromatosis are outlined in Table 1.

21 Cysteine282tryosine: the amino acid cysteine is replaced by tyrosine at position 282 in the HFE protein.

22 Histidine63aspartic acid: the amino acid histidine is replaced by aspartate at position 63 in the HFE protein.

Table 1: Classification of genetic haemochromatoses

Type	Mutated protein	Mode of transmission	Phenotype	Mechanism	Severity	Relative incidence in populations of European origin
1	HFE	Recessive	Parenchymal iron overload	Hepcidin deficiency	Highly variable	Common (1 in 100 to 1 in 1000)
2A Juvenile haemochromatosis	Hemojuvelin	Recessive	Parenchymal iron overload. Early onset (2nd or 3rd decades)	Hepcidin deficiency	Severe	Rare
2B Juvenile haemochromatosis	Hepcidin	Recessive	Parenchymal iron overload. Early onset (2nd or 3rd decades)	Hepcidin deficiency	Severe	Rare
3	Transferrin receptor 2	Recessive	Parenchymal iron overload	Hepcidin deficiency	Severe	Rare
4A (Ferroportin disease)	Ferroportin 1	Dominant	Reticuloendothelial iron overload	Functional deficiency of ferroportin	Variable	Rare
4B (Ferroportin disease)	Ferroportin 1	Dominant	Parenchymal iron overload	Ferroportin shows defective binding of hepcidin	Variable	Rare

2.47 "African iron overload" is a disorder caused by an unidentified genetic defect in iron metabolism combined with increased exposure to iron. The condition is associated with a propensity to accumulate iron by a different mechanism to those found in the haemochromatoses. The source of iron is contamination from drinks (e.g., beer) or food prepared or stored in ungalvanised steel containers or iron cooking pots. In contrast to the haemochromatoses, the excess iron is in both the hepatocytes and Kupffer cells, and both heterozygotes and homozygotes appear to be affected (Andrews, 1999). Its prevalence amongst those of sub-Saharan heritage in the UK is unknown.

Molecular mechanisms causing iron overload in genetic haemochromatosis

2.48 In type 1 haemochromatosis, HFE modulates iron transport through reduction of hepcidin synthesis in the liver (Beutler, 2006). Hepcidin is directly responsible for regulating both iron release from intestinal epithelial cells and from macrophages through binding to the iron export protein, ferroportin 1. In types 2 and 3 haemochromatosis, mutations in HAMP (hepcidin), HJV (hemojuvelin) and the TFR2 gene also lead, through changes in signal induction cascades, to reduced synthesis of hepcidin in the liver and increased activity of ferroportin 1. In all three types, this results in enhanced iron transfer from the small intestine and enhanced release of iron from phagocytes breaking down senescent red cells. The consequence is an increase in systemic iron load which is manifested by elevated plasma iron and ferritin concentrations and increased iron in liver parenchymal cells.

2.49 In type 4A (the more frequent form of ferroportin disease), iron release from macrophages by the mutated protein is decreased and iron accumulates in these cells. In type 4B, the mutated protein is resistant to the action of hepcidin. Consequently,

iron release from intestinal epithelial cells and macrophages is increased (as in types 1, 2 and 3) which leads to iron accumulation in hepatic parenchymal cells (Brissot *et al*, 2008).

Other inborn errors of iron metabolism

2.50 Other genetic disorders of iron metabolism are relatively rare (Worwood, 1999) and include: congenital atransferrinaemia, which is characterised by absence of transferrin in the plasma; and inherited sideroblastic anaemias, characterised by hypochromic anaemia, with progressive iron accumulation in bone marrow erythroblasts. Aceruloplasminemia, a rare autosomal recessive disorder of iron metabolism, is caused by mutations in the gene encoding ceruloplasmin; six mutations have been characterised. It results in iron accumulation in the parenchymal cells (as seen in hereditary haemochromatosis) but the predominant clinical features are neurological and retinal degeneration accompanied by iron deposition in the brain (Gitlin, 1998).

The effect of infection and inflammation on iron metabolism

2.51 Acute and chronic inflammation affects the systemic distribution and turnover of iron: deposition of iron in tissue ferritin is increased and availability of iron for distribution to functional sites, as well as gastrointestinal iron absorption, is reduced; concentrations of circulating iron are decreased and those of ferritin increased. This paradoxical situation, of red cell and systemic functional iron deficiency accompanied by increased systemic and macrophage iron deposits, can become sustained with chronic inflammatory conditions and is known as the anaemia of chronic disease.

2.52 Infection and inflammation are accompanied by an acute phase response which involves the hepatic synthesis and release of a series of proteins known as acute phase reactants. Hepcidin, the key regulator of iron absorption and of its release from macrophages and hepatocytes, is part of this response and its production is increased as part of the acute phase reaction (Nemeth *et al*, 2003). Increased amounts of hepcidin may contribute to the development of the anaemia of inflammation by reducing iron absorption and preventing the release of iron from macrophages. Ferritin also is an acute phase reactant.

2.53 The rapid drop in serum iron concentration following the induction of inflammation is accompanied by an increase in apoferritin synthesis which sequesters iron and inhibits its release into the plasma (Konijn and Hershko, 1977). Interleukin-1 (IL-1), which is released from macrophages and monocytes, is the primary mediator of the acute-phase response (Dinarello, 1984). Studies of cultured human hepatoma cells have shown that IL-1ß directly enhances the rate of apoferritin synthesis by translational control of its mRNA (Rogers *et al*, 1990).

2.54 In the anaemia of chronic disease, serum ferritin concentrations are higher than those of individuals with similar levels of tissue iron deposits but without infection and inflammation. This condition is observed frequently in clinical practice and

chronic disease and is likely to be a significant confounder in population studies. Such transient disturbances of iron metabolism in response to intercurrent infections need to be considered when interpreting the standard markers of iron metabolism (see section 4). In developing countries, poverty, malnutrition and infection are associated with the acute phase response and a correspondingly high prevalence of the anaemia of chronic disease.

3 Physiological requirements

Current recommendations for iron intake in the UK

3.1 Dietary Reference Values (DRVs) for food energy and nutrients in the UK were revised by COMA in 1991 (DH, 1991). DRVs provide benchmark levels of nutrient requirements which can be used to compare mean values for population intakes. Although information is usually inadequate to calculate precisely and accurately the range of requirements for a nutrient in a group of individuals, it has been assumed to be normally distributed. This gives a notional mean requirement or Estimated Average Requirement (EAR) with the Reference Nutrient Intake (RNI) defined as two notional standard deviations above the EAR. Intakes above the RNI will almost certainly be adequate to meet the needs of 97.5% of the population. The Lower Reference Nutrient Intake (LRNI), which is two notional standard deviations below the EAR, represents the lowest intakes which will meet the needs of approximately 2.5% of individuals in the group. Intakes below this level are almost certainly inadequate for most individuals.

3.2 Although the DRVs are used as benchmarks for requirements of nutrients for population groups, there are a number of uncertainties in their establishment. This is because there was a limited amount of data for most nutrients as well as inherent errors in the data, such as inaccuracies in assessment of food intakes, day-to-day variation in nutrient intakes, limitations in food composition data, as well as uncertainties about the appropriate biological marker to assess an individual's "status" for a particular nutrient. These considerations are relevant to iron and it is important to recognise that, as a result, reference values are usually translated conservatively from the data. As a consequence, they may be more than populations or individuals actually need. The values therefore represent thresholds of concern rather than diagnostic thresholds for clinical and public health problems.

3.3 Since the COMA DRV report (DH, 1991), iron requirements for some or all population groups have been considered by a number of expert committees including the IOM[23] (2001) in the USA, the SCF[24] (1993) in the European Union (EU), and by the Food and Agriculture Organization and World Health Organization (FAO/WHO, 2002).

3.4 The universal approach taken to assess iron requirements has been to use a factorial method based on estimates of obligatory losses, menstrual losses and accretion of iron in synthesised tissues. Requirements for dietary iron are then estimated using an average figure for the absorption and functional systemic use (i.e., bioavailability) of iron from a typical diet. In the case of iron, this can be problematic, as these estimates are based on short term studies that are usually carried out in iron replete individuals. Iron absorption will be down regulated in these individuals and might therefore not accurately reflect the potential bioavailability of iron from

23 Institute of Medicine.

24 Scientific Committee on Food.

the study diets (see section 5). Other uncertainties such as the paucity of data for some population groups, difficulties in comparing long and short term studies, problems measuring menstrual blood loss, as well as variability in individuals, limit the confidence with which requirements can be defined.

3.5 The DRVs for iron recommended by COMA (DH, 1991) are provided in Table 2. In their considerations, COMA assumed the following daily losses of endogenous iron: desquamated gastrointestinal cells (0.14 mg), haemoglobin (0.38 mg), bile (0.24 mg) and urine (0.1 mg) (Green *et al*, 1968); negligible amounts lost through skin and sweat (Brune *et al*, 1986). Much of these data are derived from studies on adult males. Basal iron losses among normal healthy individuals were assumed to have a coefficient of variation of 15%. For infants, children and adolescents, iron required for expanding red cell mass and growing body tissues was added to basal losses. In women of reproductive age, menstrual losses (average of 20 mg/28 day cycle[25]) were added to basal losses. It was assumed that only 15% of dietary iron is absorbed, which is considered typical for most population groups in industrialised countries (FAO, 1988); for infants up to the age of 3 months, iron absorption was assumed to be 10% from breast milk substitutes (Flanagan, 1989). The limitations of these assumptions were previously described (paragraphs 3.2) and are further considered in section 4 (*Measuring iron status*) and section 5 (*Iron in the diet*). The necessity for a DRV for exogenous iron for healthy babies in the first six months of life is questionable (see paragraph 3.16). This is recognised by some international published reference values (e.g., EU and FAO) which do not provide reference values for infants aged 0–6 months.

3.6 An important limitation with iron DRVs for women of reproductive age is that the DRVs assume a normal distribution; however, the distribution of iron requirements in women of reproductive age is highly skewed because menstrual blood losses have a right-skewed distribution (Hallberg *et al,* 1966). To take account of the higher iron requirements of women with high menstrual blood losses, the EAR for women of reproductive age was based on the 75th percentile[26] of menstrual blood losses. However, COMA recognised that the RNI might not meet the requirements of approximately 10% of the women with the highest menstrual blood losses and suggested that the most practical way of meeting their iron requirements would be to take iron supplements. The effects of menstruation on iron requirements are considered in more detail in paragraphs 3.28–3.33.

3.7 Other international dietary reference values are provided in Annex 3 (Table A2). Although they are derived from similar sets of data, the differences in the reference values between committees are primarily due to differences in assumptions regarding the efficiency of iron absorption and utilisation in different population groups.

25 Based on study in Sweden (Hallberg *et al*, 1966).

26 The EAR is usually based on the 50th centile.

Table 2: Dietary reference values for iron – mg/day (µmol/day*) (DH, 1991)

Age	Lower reference nutrient intake (LRNI)	Estimated average requirement (EAR)	Reference nutrient intake (RNI)
0–3 months	0.9 (15)	1.3 (20)	1.7 (30)
4–6 months	2.3 (40)	3.3 (60)	4.3 (80)
7–9 months	4.2 (75)	6.0 (110)	7.8 (140)
10–12 months	4.2 (75)	6.0 (110)	7.8 (140)
1–3 years	3.7 (65)	5.3 (95)	6.9 (120)
4–6 years	3.3 (60)	4.7 (80)	6.1 (110)
7–10 years	4.7 (80)	6.7 (120)	8.7 (160)
11–14 years (males)	6.1 (110)	8.7 (160)	11.3 (200)
11–14 years (females)	8.0 (140)	11.4 (200)	14.8 (260)
15–18 years (males)	6.1 (110)	8.7 (160)	11.3 (200)
15–18 years(females)	8.0 (140)	11.4 (200)	14.8 (260)
19–50 years (males)	4.7 (80)	6.7 (120)	8.7 (160)
19–50 years (females)	8.0 (140)	11.4 (200)	14.8 (260)
50+ years	4.7 (80)	6.7 (120)	8.7 (160)

*1µmol = 55.9µg

3.8 Existing public health measures to improve iron nutrition in the UK include mandatory addition of iron to white and brown wheat flour, mandatory fortification of breast milk substitutes, and dietary advice aimed specifically at infants, young children and pregnant women, as well as the general population. Dietary advice for the general population regarding maximising iron absorption includes consuming iron-rich foods at the same time as foods or drinks high in vitamin C, such as fruit or fruit juice (which have been shown to enhance iron absorption), and not consuming foods containing iron with tea, coffee, or foods or drinks containing calcium (which have been shown to inhibit iron absorption). The effect of inhibitors and enhancers of iron absorption from the diet are considered in section 5. Further details of existing health measures to optimise iron nutrition in the UK are provided in Annex 4.

Population groups

The neonate and infant

3.9 At 20 and 40 weeks gestation, the fetus contains about 58 and 94 µg of iron respectively per gram of lean tissue (Dallman *et al*, 1988). During the last third of pregnancy, the fetus accumulates about 2 mg of iron daily and the mature term neonate contains 150–250 mg of iron. Almost 80% of this iron is in haemoglobin (1 g of haemoglobin contains 3.47 mg iron). Much of the remainder is in the reticuloendothelial and hepatic tissue iron depots.

3.10 The fetus has two main adaptations to compensate for the low availability of oxygen *in utero*: firstly, it has a distinct form of haemoglobin, fetal haemoglobin, which has a greater affinity than adult haemoglobin for oxygen at low concentrations; and secondly, it has a high haemoglobin concentration in the circulation. After delivery, when the neonate starts breathing, it adapts to the higher ambient concentration of oxygen and the better oxygenation of its tissues by reducing its number of red cells and the level of haemoglobin by about 30%, and by changing the type of

haemoglobin to the adult form. The latter change occurs gradually throughout the first two years of life. The reduction in haemoglobin concentration occurs more rapidly. At birth, haemoglobin concentration of neonates is about 160–180 g/L; by 2 months of age, this has decreased and stabilises at 90–110 g/L. This change is effected by the reticuloendothelial system. The iron released from the degraded haemoglobin is retained as ferritin in the reticuloendothelial system and is subsequently redistributed peripherally for tissue synthesis. This is a major source of iron during early infancy; for example, in a term infant, a fall in haemoglobin of 60 g/L would recycle up to 60 mg of iron. This is then available to support lean tissue synthesis, which requires about 35 mg of iron per kg. The concomitant changes in iron deposition in ferritin are reflected by an initial increase in serum ferritin concentrations which may reach 400 µg/L at 1 month of age, followed by a decline, as iron is used in synthesis of new tissue, to about 30 µg/L at 6 months of age (Siimes *et al*, 1974). These postnatal changes in haemoglobin synthesis and iron stores occur irrespective of gestational age.

3.11 After birth, a delay in clamping the umbilical cord, until it has stopped pulsing, is associated with, on average, 32% higher neonatal blood volume (Nelle *et al*, 1995) and a correspondingly increased transfer of iron (30–50 mg) to the neonate (Pisacane, 1996). A randomised controlled trial in Mexico (Chaparro *et al*, 2006) reported that, at 6 months of age, infants (n=358) with delayed clamping (2 minutes after delivery) had similar haemoglobin values to infants who had their cords clamped around 10 seconds after delivery, but their systemic iron depots, reflected by serum ferritin concentrations, were significantly higher ($p<0.0002$). Although the WHO (2007) considered the evidence to be of "low quality", they recommend that the umbilical cord should not be clamped "earlier than is necessary" (estimated as approximately 3 minutes).

3.12 A Cochrane review (McDonald and Middleton, 2008) reported that, compared to early clamping, delayed cord clamping was associated with significantly higher newborn haemoglobin concentrations (mean difference, 21.7 g/L; 95% CI,[27] 2.8–40.6; 3 trials, n=671); however, heterogeneity between trials was very high. Although this effect on haemoglobin was not evident at 6 months (2 trials), serum ferritin concentrations were significantly higher at 3 months (mean difference, 17.9 µg/L; 95% CI, 16.59–19.21; 1 trial, n=107) and 6 months (mean difference, 11.8 µg/L; 95% CI, 4.07–19.53; 1 trial, n=358), suggesting higher systemic iron depots. However, significantly fewer infants in the early cord clamping group received phototherapy for jaundice compared with those in the late cord clamping group (relative risk: 0.59; 95% CI, 0.38–0.92; 5 trials). The authors of the review concluded that long term follow-up studies were needed to confirm the long term benefits of late cord clamping.

3.13 Therefore, healthy term infants probably have sufficient endogenous iron to cover their iron needs for about the first six months of life and do not have a large, if any, dependence on iron from breast milk or other dietary sources (Aggett *et al*, 2002; Griffin and Abrams, 2001), particularly if clamping of the umbilical cord at delivery is delayed until it has stopped pulsing. However, after six months, the demands of

27 Confidence interval.

growth and haemoglobin synthesis result in a requirement for exogenous dietary iron by which time intestinal absorption, previously down regulated because of high systemic depots of iron, would be expected to increase (see paragraph 3.20) and acquire iron from the diversified diet.

3.14 Since the fetal accretion of iron is greatest in the last trimester of pregnancy, preterm infants are born with a lower total body content of iron and consequently have higher needs for iron to support their growth after birth (Oski, 1985). The iron requirements for preterm and low birth weight infants need special consideration and are not within the remit of this report.

3.15 Iron concentrations in human breast milk (0.2–0.4 mg/L) (Domellof *et al*, 2002a) decline during lactation, decreasing from 0.97 mg/L in early transitional milk (Vuori, 1979) to 0.3 mg/L after 5 months (Siimes *et al*, 1979). Iron deficiency in the mother has no or little effect on the iron content of breast milk, and iron supplementation during lactation does not increase the iron content of breast milk (Zavaleta *et al*, 1995).

3.16 Stable isotope studies have reported fractional iron absorption (assessed by measuring incorporation of iron into erythrocytes) from breast milk of approximately 12–21% at 6 months of age (Davidsson *et al*, 1994; Abrams *et al*, 1997; Domellof *et al*, 2002a). This means that an infant receiving 800 ml/day of breast milk (assuming an iron content of 0.3 mg/L and absorption efficiency of 20%) will only absorb about 0.05 mg/day of iron. Such low absorption reflects the ability of systemic iron reserves, accumulated *in utero,* to meet an infant's functional needs for iron in the first six months of life. This implies that infants have no need for exogenous iron in the first six months of life irrespective of whether they are breast fed or fed breast milk substitute. Therefore iron DRVs for infants aged 0–6 months appear to be redundant.

3.17 There are conflicting data on the risk of term infants exclusively breast fed for six months developing iron deficiency. A trial in Honduras (n=139) reported that mean haemoglobin and plasma ferritin concentrations were significantly lower at six months in infants who had been exclusively breast fed compared to those who had been introduced to complementary foods at four months (Dewey *et al*, 1998). However, birth weight was the factor most strongly associated with haemoglobin and ferritin concentrations: infants with birth weights <2500 g were at greater risk of low haemoglobin (<103 g/L) and low plasma ferritin (<12 µg/L) concentrations compared to infants with birth weights >3000 g, but values were within reference ranges. However, a study in Italy (Pisacane *et al*, 1995) of infants with birth weight >2500 g (n=30) reported that mean haemoglobin concentrations were significantly higher in infants aged 12 months who had been exclusively breast fed (i.e., received only human milk without any other types of milk, medicinal iron, or iron fortified formula or cereals) for seven months or longer compared to infants who received other foods at an earlier age. There was no difference in mean serum ferritin concentration between the two groups.

3.18 Cows' milk contains about 0.5–0.6 mg/L of iron (DH, 1994), which is more than that contained in human breast milk; however, the iron in cows' milk is poorly absorbed because it is complexed with ligands, which limits its release for mucosal

uptake from the intestinal lumen. The principal ligand is phosphate, which binds iron as independent phosphate groups or in a much larger pool of phosphorylated protein complexes in the casein proteins. Although the iron content of human milk is relatively lower (see paragraph 3.15), it is more bioavailable than that in cows' milk (Fomon *et al,* 1993; Lonnerdal *et al*, 1981). However, in usual circumstances, the bioavailability of iron from human breast milk is likely to be less than its potential availability because mucosal uptake and transfer of iron in infants is down regulated (see paragraph 3.16).

3.19 Introduction of cows' milk to infants aged 6 months has also been associated with small losses of blood from the intestinal tract (Ziegler *et al,* 1990). Several studies have reported that consumption of cows' milk, especially in the first six months of life (Sullivan, 1993; Robson, 1993) but also in the second six months (Michaelsen *et al,* 1995; Zlotkin, 1993), is associated with lower haemoglobin and ferritin concentrations.

Children aged 6 months to 3 years

3.20 Iron requirements of infants are highest during this period of rapid growth. After 6 months of age, dietary requirements for iron are increased as iron stores diminish and the amount of iron provided from breast milk is no longer sufficient, even with up regulation of intestinal absorptive mechanisms, to meet the increasing demands for growth and blood volume expansion.

3.21 Domellof *et al* (2002a) found no difference in iron absorption from breast milk between iron supplemented and unsupplemented infants at 6 months of age (n=25) but by 9 months of age, absorption from breast milk was significantly higher (p=0.01) in the infants not receiving iron supplements (37%) compared to supplemented infants (17%). No correlation was found between iron absorption and plasma ferritin concentration at both 6 and 9 months.

3.22 There are difficulties in setting reference standards to inform assessments of iron nutriture during the rapid transition of early childhood. This is demonstrated in the study by Domellof *et al* (2002a) (see paragraph 3.21) and further illustrated by a study of infants (n=59) in the first two years of life which reported wide variation in haemoglobin concentrations among infants with similar iron intakes (Beal and Meyers, 1970). Consequently, reference values are inclined to be conservative and cautious, assuming, for example, an estimated 10% absorption of iron from breast milk.

Children aged over 3 years

3.23 For children aged over 3 years, as for all children, iron is required to meet the needs for an expanding red cell mass, for growth, and to replace basal losses.

Adolescents

3.24 Increased iron requirements for this age group are driven by the pubertal growth spurt and increased blood volume, haemoglobin and lean tissue synthesis. Compared with boys, these changes are less for girls due to their lower growth velocity and differences in body composition. Whereas lean tissue, as a percentage of body mass,

increases during puberty in boys, there is actually a small decrease in girls (Mølgaard *et al*, 1998). The peripheral use of iron due to increased growth rate means that serum ferritin concentrations may fall to levels at or below the usual reference range. This phenomenon does not necessarily indicate iron deficiency *per se* but reflects the use of endogenous iron depots and the probable preferential distribution of recently absorbed iron to functional sites rather than to ferritin depots. However, the decline indicates an increased risk of deficiency in adolescents.

3.25 For adolescent girls, iron requirements also increase to cover additional losses due to menstruation. However, the growth spurt precedes menarche and as a consequence the decline in serum ferritin concentrations in pubertal girls also precedes menarche. Although menstrual blood losses are initially small, the menstrual blood loss of adolescent girls aged 15 years, is only marginally lower than in older women and it is generally assumed that adolescent menstrual losses are at the same level as those for adults (Hallberg and Rossander-Hulten, 1991).

Women in reproductive years

3.26 Menstruation, pregnancy, lactation and growth during adolescence all influence the systemic metabolism of iron and dietary requirements.

3.27 There are concerns that women in their reproductive years are at increased risk of iron deficiency due to changes in dietary habits and lifestyle that have led to lower energy expenditure and lower dietary energy intakes (Eaton and Konner, 1985; Hallberg and Rossander-Hulten, 1991). The iron nutriture of women of reproductive age in the UK is considered in section 9.

Menstruation

3.28 Menstrual blood loss is an important determinant of iron depots in women of reproductive age. A number of studies have observed an association between serum ferritin concentration and length of the menstrual period (Galan *et al*, 1985; Soustre *et al*, 1986; Milman *et al*, 1993). Harvey *et al* (2005) reported a strong inverse correlation between menstrual blood loss and serum ferritin concentration with higher menstrual iron losses resulting in lower serum ferritin concentrations ($p<0.001$); on average, an increase in menstrual iron loss of 1 mg/day resulted in a decrease in serum ferritin of 7 µg/L.

3.29 There is little intra-individual variation between menstrual cycles (Hallberg and Nilsson, 1964) but considerable inter-individual variation which follows a right-skewed distribution in a population (see paragraph 3.30) (Hallberg *et al*, 1966). Calculation of DRVs for women in their reproductive years takes account of this distribution (see paragraph 3.6). Menstrual losses are affected by method of contraception, being significantly increased with intrauterine devices (Milsom *et al*, 1995) and reduced with oral contraceptives (Larsson *et al*, 1992).

3.30 Menstrual blood loss, and the associated iron loss, is difficult to measure accurately. Self-reported menstrual blood loss is inaccurate (Hallberg *et al,* 1966) and conclusions from qualitative or semi-quantitative studies are not considered reliable. A study in Sweden (Hallberg *et al*, 1966) reported that menstrual blood losses in women

had a skewed distribution: 95% per cent of women lost 118 ml or less of blood per cycle; the median and mean menstrual blood loss was 30 ml and 44 ml respectively, equating to a median iron loss of 0.49 mg/day and a mean iron loss of 0.7 mg/day. These data were obtained before the widespread use of oral contraceptives.

3.31 A later study in the UK (Harvey *et al*, 2005) (see paragraph 3.28), which determined menstrual iron losses by direct measurement of menstrual blood loss during one menstrual cycle (n=90), reported that median blood loss was 18 ml per cycle, which corresponds to a median iron loss of 0.26 mg/day (mean blood loss was 26 ml/cycle, corresponding to a mean iron loss of 0.43 mg/day) and 70% of the women lost less than 0.5 mg/day of iron through menstruation. In this study, the median blood loss of oral contraceptive users was significantly lower ($p<0.001$) than for those using other forms of contraceptives (excluding IUD users as there were too few to draw any conclusions).

3.32 The DRVs for women of reproductive age may have been set too high and could be considered conservative and cautious since they do not take account of adaptive responses to compensate for blood losses through menstruation, such as increasing the amount of iron absorbed from the diet; however, the extent of this compensation is not clear and would be difficult to measure.

3.33 The long term implications of menstrual iron losses over 20–30 years in the development of iron deficiency anaemia in women of reproductive age and the volume of menstrual blood loss which should be regarded as abnormal and needing clinical management to treat iron deficiency anaemia are not well characterised.

Pregnancy and lactation

3.34 Physiological adaptations during pregnancy and lactation ensure an adequate supply of iron to the fetus and developing infant, even in the presence of iron deficiency. However, severe iron deficiency and anaemia can affect reproductive efficiency (see section 6).

The effect of pregnancy on iron metabolism

3.35 Adaptations during pregnancy ensure the supply of nutrients to the fetus and other products of conception and sustain the additional metabolic burden of pregnancy. Maternal plasma volume and red cell mass increase early in pregnancy in advance of the systemic metabolic changes and significant growth that occurs in the fetus during the latter half of gestation. Iron supply to the conceptus is favoured by changes in the structural and compositional characteristics of transferrin which facilitate preferential delivery of iron to placental rather than systemic transferrin receptors.

3.36 Plasma volume, which increases steadily until 32–34 weeks of gestation, is related to the size and health of the fetus. In a non-pregnant woman (55–60 kg), the plasma volume is approximately 2.6 L. This increases by 1.3 L in a singleton pregnancy and by about 1.96 L and 2.4 L with twins and triplets respectively. The increase in plasma volume is not related to the preceding non-pregnant plasma volume. Consequently, since increments in plasma volume in small women are as large as those in bigger

women, the dilutional effects on standard blood parameters of iron metabolism and haemoglobin are greater in small than in large women (Letsky, 1991). This is an important consideration in the interpretation and use in population studies of the customary parameters of iron status in pregnant women. Therefore, despite an increase in red cell mass and haemoglobin during pregnancy, the dilutional effect of the increased plasma volume produces lower values of serum iron, ferritin and transferrin, which are not necessarily indicative of anaemia or of iron deficiency. The amount of iron in the circulation actually increases during pregnancy.

3.37 Red cell mass increases linearly from the end of the first trimester and may be related to the size of the fetus, but this is not established. The increase in red cell mass is associated with a 2–4-fold increase in erythropoietin which is most evident in the first 16 weeks of gestation. In non-pregnant women there is an inverse relationship between erythropoietin levels and haemoglobin which is lost during the first and second trimesters of pregnancy and becomes evident again in the third trimester, but only in women with haemoglobin less than 90 g/L. The effects of iron supplementation, maternal age and fetal size on red cell mass are uncertain. The fetus is unaffected by maternal erythropoietin and there is no evidence that fetal erythropoietin influences the mother (Schneider and Malek, 1995; Reisenberger *et al*, 1997).

3.38 The increase in plasma volume, concomitant with the rise in red cell mass, causes a reduction in peripheral resistance to blood flow which offsets any increase in blood viscosity caused by the increased red cell volume.

3.39 Since the relative balance between plasma volume and red cell mass changes throughout pregnancy, different cut-off points have been established for the diagnosis of anaemia during this period. In healthy, iron supplemented women, haemoglobin concentrations decrease until the 25–30th week of gestation and then increase towards term.

3.40 The WHO (2001) recommends a cut-off of 110 g/L for anaemia throughout pregnancy. In the UK, the National Institute for Clinical Excellence (NICE) recommends that iron supplementation should be considered for women with haemoglobin concentrations below 110 g/L in the first trimester and 105 g/L at 28 weeks (NICE, 2008). In the USA, the Centers for Disease Control and Prevention (CDC) recommends routine iron supplementation (30 mg/day) for all women during pregnancy and screening for iron deficiency anaemia at the first prenatal care visit (CDC, 1998). A series of cut-offs for anaemia during pregnancy are set at the 5th percentile of haemoglobin, for four-weekly intervals, from 12 weeks gestation onwards[28] (CDC, 1989). A dose of 60–120 mg/day of iron is recommended to treat anaemia, which is lowered to 30 mg/day when haemoglobin normalises. The CDC recommendations are based on Institute of Medicine guidelines (IOM, 1993). In contrast, the US Preventive Services Task Force (USPSTF), like NICE, recommends routine screening for iron deficiency anaemia during pregnancy but does not recommend routine supplementation (USPSTF, 2006).

28 12 weeks, 110 g/L; 16 weeks, 106 g/L; 20 and 24 weeks, 105 g/L; 28 weeks, 107 g/L; 32 weeks, 110 g/L; 34 weeks, 114 g/L; 40 weeks, 119 g/L.

3.41 In a study of pregnant women (n=501) in the UK (Foulkes and Goldie, 1982), 12% (n=59) developed haemoglobin concentrations below 105 g/L at 36 weeks; out of these, 20% (n=12) were taking iron supplements (100 mg/day) and 80% (n=47) were not taking iron supplements. Eighty-seven per cent of the women not taking iron supplements had serum ferritin concentrations below 50 µg/L at 12–16 weeks gestation. This suggests the use of selective iron supplementation of pregnant women with serum ferritin concentration <50 µg/L in the first trimester.

3.42 The relationship between maternal iron status and pregnancy outcomes and the effect of iron supplementation in pregnancy on haematological parameters and pregnancy outcomes is considered in section 6 (paragraphs 6.39–6.47).

3.43 Reference intakes for iron during pregnancy are the same as those of non-pregnant women in the UK (DH, 1991) and in the EU (SCF, 1993). The FAO/WHO (2002) recommendation for iron intake during pregnancy also does not differ from that for non-pregnant women. In the USA and Canada (IOM, 2001), recommended intakes during pregnancy are 1.5 times more than that for non-pregnant women.

Intestinal absorption of iron during pregnancy

3.44 Intestinal uptake and transfer of iron increases during pregnancy (Whittaker *et al,* 2001). Svanburg *et al* (1975) reported that iron absorption from a standard meal was 3.1% in non-pregnant women, 0.8% in early pregnancy, 4.5% at 24 weeks gestation and 13.5% at 36 weeks gestation. A longitudinal study in healthy pregnant women (n=12) (Barrett *et al*, 1994) found that the mean absorption of iron from a standardised meal, extrinsically labelled with a stable isotope of iron, increased from 7% at 12 weeks to 36% and 66% at 24 and 36 weeks gestation respectively; at 16–24 weeks postpartum, absorption decreased to 11%.

Effect of birth spacing on maternal iron status

3.45 It has been suggested that women with short interpregnancy intervals (<18 months) may not have sufficient time to replace nutrients used during the previous pregnancy and that this may compromise their nutritional status at conception and their ability to support fetal development (King, 2003). Studies on the association between interpregnancy interval and maternal iron status have important methodological limitations. These include differences in the definition of interpregnancy birth interval and inadequate control for potential confounding factors such as nutritional adequacy, socioeconomic status, education, prenatal care (e.g., iron supplementation), parity, breast feeding and maternal morbidity.

3.46 A review which considered the effect of birth spacing on maternal iron status (Dewey and Cohen, 2007) included three cross-sectional studies (Singh *et al*, 1998; Conde-Agudelo and Belizán 2000; Pathak *et al*, 2004) and one retrospective cohort study measuring haemoglobin change between the first and second pregnancy (Khan *et al*, 1998). Three studies measured maternal haemoglobin (Conde-Agudelo and Belizán, 2000; Singh *et al*, 1998; Khan *et al*, 1998) and one measured serum ferritin concentration (Pathak *et al*, 2004). One study included data (n=456,889) from 18 countries in Latin America and the Caribbean (Conde-Agudelo and Belizán, 2000); the other three studies were conducted in India (n=283) (Pathak *et al*, 2004), Pakistan (n=278) (Khan *et al,* 1998) and Singapore (n=3738) (Singh *et al*, 1998). Only one of the

cross-sectional studies (Conde-Agudelo and Belizán, 2000) reported an increased risk of anaemia (haemoglobin <110 g/L) associated with an interpregnancy interval of less than 6 months compared to that of 18–23 months (relative risk, 1.3; 95% CI, 1.18–1.43) but not for any other interpregnancy intervals (12–17, 24–59 or ≥60 months); however, the analysis did not control for iron supplementation during pregnancy, breast feeding or for pregnancy complications that could increase blood loss.

Lactation

3.47 Assuming an average iron content of 0.4 mg/L and milk production of 800 ml/day, the average daily iron loss in breast milk is estimated to be 0.32 mg, which represents about half the average iron loss with menstruation (DH, 1991). This means that a breast feeding woman with lactational amenorrhoea has a more positive iron balance than during pregnancy or menstruation. Long periods of lactation accompanied by amenorrhoea may therefore have a positive effect on the iron stores of fertile women, especially if they have multiple pregnancies, but this is not known.

3.48 For lactating women, reference intakes for iron differ between countries (see Annex 3, Table A2). In the UK, the recommendation for iron is the same as that of non-pregnant women (DH, 1991), while the FAO/WHO recommendation (2002) is about half that of non-pregnant, menstruating women. The EU (1993), US and Canadian recommendations (2001) are similar to those of the FAO/WHO.

Older people

3.49 Physiological requirements for iron are not increased in older people and iron depots usually increase with age (Lynch, 1982). This increase is greater in women than men because, compared to younger premenopausal women, non-menstruating women no longer lose endogenous iron. Iron requirements for older people are therefore the same as those of adults aged 19–50 years and requirements for women after the menopause are the same as those of men (DH, 1991). The most common cause of iron deficiency in older people is gastrointestinal blood loss which can be caused by a number of factors including chronic disease (such as colorectal cancer), anti-inflammatory drugs (e.g., aspirin), diseases of the genitourinary system, or frequent blood drawings (Lipschitz, 1991). Another risk factor for iron deficiency in some older people is a reduction in their energy intakes which results in lower intakes of micronutrients, including iron.

Summary and conclusions

3.50 The DRVs for iron are based on limited data. Insufficient new good quality systematic dose-response data, to inform a reassessment of the DRVs for iron, have emerged since the DRVs were considered by COMA in 1991. However, since then, improved understanding of iron metabolism and its regulation has provided new markers and further insights that could enable a reappraisal of existing data and revision of the DRVs.

3.51 The paucity of data clearly relating iron intakes and body iron with markers of adaptation and functional use of iron (i.e., dose-response data) makes it difficult to estimate accurately requirements for different population groups, particularly for infants, children and women in their reproductive years.

3.52 It is probable that the current DRVs for iron are too high, particularly for girls and women of reproductive age, because they are based on cautious assumptions about the bioavailability of dietary iron and metabolic adaptation.

3.53 Iron DRVs for infants aged 0–6 months appear to be redundant since infants have no requirement for exogenous iron in the first six months of life.

3.54 The DRVs for iron intake in the UK take account of the increased absorption of dietary iron during pregnancy and lactation arising from maternal adaptations which facilitate the effective delivery of nutrients, including iron, to the conceptus.

3.55 Evidence from randomised controlled trials suggests that delayed clamping of the umbilical cord after birth, until it has stopped pulsing, is associated with higher systemic iron depots in the first six months of life but it might also increase the risk of jaundice requiring phototherapy. It is not known if there are long term benefits associated with late cord clamping.

4 Measuring iron status: markers of depletion, deficiency, sufficiency and excess

Iron status

4.1 The term "iron status" is used to describe whether an individual has too little, enough, or too much iron in their body for their needs as well as to indicate the possible risk of deficiency or excess. It is of limited descriptional value unless it is more specifically qualified by reference to whether or not it relates to possible deficiency, adequacy or excess.

4.2 The spectrum of iron status (see Table 3) extends between the extremes of iron excess and iron deficiency, at both of which homeostasis has failed and functional and structural defects develop. In between these extremes, when the body needs iron, it is first mobilised from ferritin depots. When these fall to a certain level, hepatic production of hepcidin decreases further, which facilitates increased intestinal uptake and transfer of iron from the diet into the body. Simultaneously, peripheral needs for iron are manifested by increased expression of transferrin receptors and by changes in the amount of iron being transported by transferrin. If the body does not need iron, tissue ferritin depots increase, hepcidin concentrations increase, and intestinal uptake and transfer of iron is down regulated. Usually, in healthy individuals, when exposure to iron is solely from the diet, this adaptation is enough to prevent excessive systemic accumulation of iron. However, if iron is provided parenterally (e.g., in repeated blood transfusions) or if intestinal regulation of iron absorption is defective (as in the haemochromatoses), the body accumulates excess iron which is sequestered in ferritin, and there is an increased risk of systemic iron toxicity.

4.3 Assessment of an individual's iron status depends, therefore, on being able to determine where they lie on the above spectrum. This assessment depends on measurement, interpretation and synthesis of various markers of iron metabolism. Adequate iron status (iron sufficiency) implies presence of normal erythropoiesis and iron dependent functions that are not limited by iron supply, as well as a small contingency reserve of "storage iron" to supply other physiological functions. Determination of "adequacy" or "sufficiency" is dependent on measurement of more than one marker. It is important to recognise that there is no single reliable marker of iron status, except at the extremes of deficiency and excess.

Table 3: A conceptual spectrum of iron status

Iron excess	Cellular and tissue architectural and functional damage Increased tissue haemosiderin from degradation of ferritin Increased ferritin depots Reduced expression of transferrin receptors
Iron adequacy	Reduced intestinal uptake and transfer of iron Increased hepcidin
Iron deficiency	Reduced hepcidin Increased expression of transferrin receptors Mobilisation of depots – reduced ferritin levels Increased intestinal uptake and transfer of iron (possibly induced at serum ferritin levels <60 µg/L) Reduced saturation of serum transferrin Functional defects in iron dependent activities Defective haemoglobin synthesis (increased zinc protoporphyrin) Reduced haemoglobin (anaemia) Impaired muscle metabolism Secondary functional defects in the metabolism of other nutrients Cellular and tissue architectural and functional damage

Establishment of reference values for markers used to assess iron status

4.4 Iron status is a qualitative concept as there are no good dose-response data to determine thresholds for adverse events associated with iron deficiency or excess.

4.5 The selection of reference markers has been limited by their accessibility and by the reliability of their measurement. Each of the markers has limitations and is subject to other influences apart from those directly involved in the supply, acquisition, distribution, deposition and use of iron. There is no good single marker of iron deficiency, adequacy or excess. Any assessment of iron status depends on an integrated interpretation of a battery of markers which represent functional, storage, distribution, regulatory or adaptive mediators.

4.6 The reference ranges are not diagnostic; they essentially describe sufficiency or adequacy. Values outside the reference ranges do not necessarily indicate deficiency or excess; however, the further a value lies from the reference limits, the more likely it is to represent a systemic deficiency (and a need for more exogenous iron) or an excess of iron.

4.7 Two approaches have been used to establish thresholds and reference values for these markers. The first is to determine values in a sample of healthy subjects who are considered to be representative of the population from which they are taken and who are unlikely to be iron deficient or have a high iron body burden; cut-offs are then based on 90 or 95% confidence intervals (normative population

method). This method requires, or assumes, the exclusion or absence of individuals with iron deficiency or iron overload from the selected population sample and an assumption that the rest of the sample population is otherwise "healthy" and unaffected by any conditions which may affect the markers being measured.

4.8 The second approach is to compare concentrations of any iron marker from individuals identified with iron deficiency anaemia (based on the absence of stainable iron in the bone marrow and/or a positive response to iron supplementation) with those in non-iron deficient individuals (non-responders to iron supplementation). Cut-offs can then be established from the distribution of values for the two groups. Lower levels determined in this way are more likely to reflect values which are representative of functional outcomes of iron deficiency. However, with the exception of serum ferritin, this approach has not been widely used to assess markers of iron deficiency.

4.9 The cut-offs selected for markers of iron status can vary between laboratories and are not based on the appearance of functional deficits. The limits simply indicate values which signal the possibility of iron depletion, deficiency or excess.

4.10 A low value for a single marker has a low diagnostic efficiency for iron deficiency. Approaches based on combinations of markers have been explored to improve the specificity and sensitivity of diagnostic approaches (see paragraphs 4.34–4.36). Markers need to be evaluated according to their functional relevance, potential confounders and relevance of the indicative risk.

Markers of iron status

4.11 Markers that have been used to assess iron status are categorised according to whether they represent: a functional use of iron in the synthesis of haemoglobin; the distribution or transport and supply of iron to tissues; and iron deposits in tissues. Their representative reference ranges, use and limitations are summarised in Table 4.

Table 4: Markers used for assessment of body iron status (adapted from British Nutrition Foundation, 1995)

Measurement	Representative reference range (adults)	Confounding factors	Diagnostic use
Functional iron			
Haemoglobin concentration		Other causes for anaemia besides iron deficiency; a reciprocal relationship with iron stores should be expected in all anaemias, except in IDA.	Assess severity of IDA; response to a therapeutic trial of iron confirms IDA. Not applicable to assessment of iron overload.
Males Females	130–180 g/L 120–160 g/L		
Red cell indices			
MCV* MCH	84–99 fl 27–32 pg	May be reduced in other disorders of haemoglobin synthesis (e.g., thalassaemia, sideroblastic anaemias) in addition to ID.	
Tissue iron supply			
Serum iron Saturation of transferrin	10–30 µmol/L 16–50%	Normal short term fluctuations mean that a single value may not reflect iron supply over a longer period. Both measures reduced in chronic disease.	Raised saturation of transferrin used to assess risk of tissue iron loading (e.g. in haemochromatosis or iron-loading anaemias).
Serum transferrin receptor	2.8–8.5 mg/L*	Directly related to extent of erythroid activity as well as being inversely related to iron supply to cells.	Decreased saturation of transferrin, reduced red cell ferritin, increased zinc protoporphyrin, and increased serum transferrin receptors indicate impaired iron supply to the erythroid marrow. Serum transferrin receptors may have particular value in identifying early iron deficiency and, in conjunction with serum ferritin, distinguishing this from anaemia of chronic disorders.
Red cell zinc protoporphyrin*	<70 µmol/mol Hb (<800 µg/L red cells)	Stable measures: reduced iron supply at time of red cell formation leads to increases in free protoporphyrin and hypochromic red cells, and reduced red cell ferritin. However, values may not reflect current iron supply.	
Red cell ferritin (basic)	3–40 ag/cell		
% hypochromic red cells	<6%	May be increased by other causes of impaired iron incorporation into haem (e.g., lead poisoning, aluminium toxicity in chronic renal failure, sideroblastic anaemias).	

Measurement	Representative reference range (adults)	Confounding factors	Diagnostic use
Iron in tissues			
Serum ferritin			
Males Females	15–300 µg/L 15–200 µg/L	Increased: as an acute phase protein and by release of tissue ferritins after organ damage.	All measures are positively correlated with iron stores except TIBC which is negatively correlated. Serum ferritin is of value throughout the range of iron stores. Quantitative phlebotomy, liver iron concentration, chelatable iron and MRI are of value only in iron overload. Bone marrow iron may be graded as absent, normal or increased and is most commonly used to differentiate ACD from IDA.
Tissue biopsy iron – liver (chemical assay)	3–33 µmol/g dry wt	Potential for sampling error on needle biopsy, especially when this is <0.5 mg, or liver is nodular; but remains the "gold standard" in iron overload.	
Bone marrow (Perls' stain)			
Quantitative phlebotomy	<2g iron		
Serum TIBC (may be measured directly or calculated from transferrin concentration)	50–70 µmol/L**		In IDA, a raised TIBC is characteristic.
Urine chelatable iron (after 0.5 g IM desferrioxamine)	< 2 mg/24h		
Non-invasive imaging MRI	–	Not yet sufficiently sensitive and reproducible for quantitation of normal levels of storage iron. Useful for detecting iron overload.	
SQUID (magnetic susceptibility)		Sensitive, accurate and reproducible but only a few machines in the world.	

ACD, anaemia of chronic disease; Hb, haemoglobin; IDA, iron deficiency anaemia; IM, intramuscular; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MRI, magnetic resonance imaging; TIBC, total iron-binding capacity.

* There is a major problem with the different units and reference ranges for the various assays in use (Akesson *et al*, 1999; Worwood, 2002a).

** No internationally accepted cut-off values for MCV, TIBC, or zinc protoporphyrin have been developed because of analytical differences between laboratories and because these indicators can be influenced by variations in the conditions under which the blood samples were collected (e.g., fasting/non-fasting, time of day) and by the methods used for transportation, storage and processing.

Characterising and detecting iron depletion and deficiency

4.12 The definitive diagnostic measure of iron deficiency in individuals and populations is to monitor changes in haemoglobin concentration in response to oral iron supplementation (WHO, 2001): an increase of at least 10 g/L in haemoglobin following 1–2 months of supplementation is indicative of iron deficiency.

4.13 A direct way to assess iron stores is to measure iron concentration in bone marrow using the Prussian blue stain, which detects the presence of iron which has not been incorporated into haemoglobin: absence of stainable iron indicates depletion of iron stores. This is widely considered as the gold standard for evaluating iron depletion and deficiency; however, its use in population surveys is limited by its impracticality and cost. The most commonly used markers for population surveys are considered below.

Functional iron

Haemoglobin

4.14 Haemoglobin and haematocrit have been widely used to assess anaemia, which is a functional consequence of iron deficiency. However, these measurements lack sensitivity[29] because anaemia associated with nutritional iron deficiency is usually mild resulting in extensive overlap between healthy and iron deficient populations. The specificity[30] of these measurements are also poor since there are many other causes of anaemia (DeMaeyer and Adiels-Tegman, 1985). The shape and size of the red cells and other indicators of iron deprivation are necessary to determine whether low haemoglobin concentrations are due to iron deficiency in a population. Even then, other causes of anaemia, including other nutritional deficiencies, may co-exist.

4.15 Internationally accepted values have been developed for diagnostic and screening purposes which correspond to the concentration of haemoglobin below which the functional consequences of anaemia are encountered but the levels themselves are not necessarily those at which specific functional defects arise. The WHO criteria for anaemia are provided in Table 5.

Table 5: Haemoglobin thresholds used to define anaemia at sea level (WHO, 2001)

Group/Age	Haemoglobin (g/L)
Children 0.5–4.99 years	110
Children 5–11.99 years	115
Children 12–14.99 years	120
Non-pregnant women (>15 years)	120
Pregnant women	110
Men (>15 years)	130

4.16 The WHO criteria of anaemia are widely used although there is evidence of significant racial differences in "normal" haemoglobin values. In the USA, data from national surveys have generally shown that individuals of African extraction with adequate iron stores have haemoglobin concentrations which are 4–10 g/L lower

29 The sensitivity represents the proportion of a population with a certain characteristic (e.g., disease, health status) that are correctly identified by the measurement. It refers to the proportion of true positives that are correctly identified by the measurement.

30 The specificity of a measurement represents the proportion of a population correctly identified without a certain characteristic. Specificity refers to the proportion of true negatives that are correctly identified by the measurement.

than those of Caucasian origin (Perry *et al*, 1992). Haemoglobin concentrations are also influenced by smoking and altitude (WHO, 2001), and individual haemoglobin concentrations show strong tracking, implying genetic influences.

4.17 A gradation of anaemia into mild, moderate and severe has been used by the WHO according to whether haemoglobin concentrations are respectively above 80%, between 80 and 60%, or below 60% of the appropriate population threshold. These cut-offs have been used in characterising the global burden of anaemia but they are arbitrary and have no functional correlates (DeMaeyer, 1989). However, such derived values have been used to inform the management of anaemia in pregnancy.[31]

Tissue iron supply

Zinc protoporphyrin

4.18 Measurement of zinc protoporphyrin (ZPP) has largely replaced the free erythrocyte protoporphyrin assay which was previously used in population surveys; for example, in the National Health and Nutritional Examination Survey (NHANES) II and NHANES III in the USA. Protoporphyrin IX is a precursor of haemoglobin. In the absence of iron, zinc is inserted into the protoporphyrin molecule instead. Therefore the amount of ZPP in red cells increases with increasingly marked iron deficiency. The cut-offs for increased values recommended by the WHO (2001) for ZPP are >61 µmol/mol haem (under 5 years) and >70 µmol/mol haem (over 5 years). Although ZPP is a sensitive indicator of iron deficient erythropoiesis, concentrations are also raised in the iron deficiency and anaemia due to infection and inflammation.

Serum iron

4.19 Serum iron has very limited value in assessing iron deficiency or chronic excess in population studies because concentrations within an individual are affected by other influences such as diurnal and post-prandial variations, and concentrations are rapidly reduced following infection or inflammation as part of the acute phase reaction.

Transferrin saturation

4.20 Measurement of transferrin saturation (serum iron/total iron binding capacity x 100%) is a more sensitive and specific indicator of iron deficiency than serum iron alone (Bainton and Finch, 1964). However, the limitations of using transferrin saturation are similar to those of serum iron.

4.21 Although total iron binding capacity (or transferrin concentration) specifically increases in iron deficiency, measurements of total iron binding capacity are rarely used alone as reliable reference ranges are not available because of analytical differences between laboratories.

4.22 Transferrin saturation below 16% indicates the point at which iron deficient erythropoiesis develops (Bainton and Finch, 1964) and allows assessment of iron deprivation based on a reduction of the transport pool. Koerper and Dallman (1977) defined

31 Severe anaemia in pregnancy is defined as haemoglobin <70 g/L and requires medical treatment. Very severe anaemia in pregnant women (defined as haemoglobin <40 g/L) is a medical emergency.

the lower limit of the 95% range for normal infants and children (aged 0.5–18 years) as 7%. Cut-off values have also been derived from NHANES II and NHANES III data (1–2 years <10%; 3–5 years, 12%; 6–15 years, 14%; over 16 years, 15%).

Serum transferrin receptor

4.23 Cells increase their production of transferrin receptors when they need iron. Circulating receptors are derived from cellular transferrin receptors which are mostly released from developing red cells. Iron deficiency causes increased synthesis and the assay for serum transferrin receptors provides an indicator of the adaptations of cells, particularly red cell precursors, to acquire iron. The use of this assay is limited because there is a variety of assay reagents, units of measurement and reference ranges and no agreed methodological approach for measuring serum transferrin receptors or establishing reference ranges (Worwood *et al*, 2000). However, a recombinant soluble transferrin receptor preparation has now been established as the first WHO reference reagent for serum transferrin receptor assays (Thorpe, 2010). The availability of a reference reagent should facilitate the use of the assay in population studies of iron status (see paragraph 4.32).

4.24 Concentrations of serum transferrin receptors are higher in infants than adults (Choi *et al*, 1999; Olivares *et al,* 2000; Suominen *et al,* 2001) and decrease steadily throughout childhood to adult values. Concentrations are also higher at high altitudes (Allen *et al*, 1998).

4.25 The serum transferrin receptor assay provides a sensitive indicator of iron deficient erythropoiesis and is relatively unaffected by inflammatory phase reactions. Serum transferrin receptors, however, represent tissue requirements for iron rather than deficiency *per se*. They are therefore raised in the absence of iron deficiency when iron needs are increased for other reasons such as growth, erythropoiesis to compensate red cell breakdown in other conditions, and some malignancies. Consequently, in clinical practice, measurement of serum transferrin receptors has not enhanced the sensitivity and specificity for detecting iron deficiency in patients with the anaemia of chronic disease (Worwood, 2005).

Total body iron depots

Serum ferritin

4.26 Serum ferritin concentration reflects systemic ferritin iron depots. Phlebotomy studies have estimated that, in normal individuals without infection, 1 μg/L of serum ferritin represents about 8 mg of stored iron (Walters *et al*, 1973). This approximation does not take account of body weight. In healthy individuals, total body iron stores may be calculated from measurements of serum ferritin and serum transferrin receptors (see paragraph 4.32) (Cook *et al,* 2003).

4.27 Cut-offs for serum ferritin concentration have been derived by examining the highest values found in patients with iron deficiency anaemia, defined as microcytic anaemia with either an absence of storage iron in the bone or a subsequent response to therapeutic iron (Worwood, 1979). Hallberg *et al* (1993a) measured serum ferritin concentrations in healthy women with no stainable iron in the bone marrow. Both these approaches suggested a limit of 15 μg/L and this cut-off has been widely

used in population surveys in Scandinavia and other parts of Europe to identify iron depletion (Hallberg, 1995). In the USA, a slightly lower limit of 12 µg/L has been used (Looker *et al*, 1997). These different thresholds reflect methodological variations and their application (e.g., for clinical purposes or for population surveys). Lower values have generally been recommended for children. The WHO criteria used to define depleted storage iron are 12 µg/L for children under 5 years and 15 µg/L for males and females over 5 years (WHO, 2001).

4.28 It is important to recognise that low serum ferritin concentrations represent low iron depots in tissues. They do not represent a functional deficiency of iron. For example, Cook *et al* (1976) showed there was little difference in the prevalence of iron deficiency anaemia[32] in women with a single low result for serum ferritin, erythrocyte protoporphyrin or transferrin saturation, compared with subjects with normal results for all three tests.

4.29 Since ferritin behaves as an acute phase reactant, serum concentrations are increased in response to acute and chronic inflammation and even a mild infection can raise concentrations (Hulthen *et al*, 1998). This means that serum ferritin concentrations can be in the normal range even when iron depots are low. This limits the sensitivity of serum ferritin as an indicator of iron deficiency or excess, particularly in populations of regions where infectious diseases are common.

4.30 Markers that can be used concomitantly for detection of infection and/or inflammation include C-reactive protein (CRP) which provides an indication of acute disease, and α-1-acid glycoprotein (AGP) which provides a marker of chronic infection (although this assay is not widely used in the UK). The acute phase protein most commonly used for this purpose is CRP. Although CRP concentration rises quickly in response to inflammation, it also falls quickly. It has been suggested that AGP concentration, which is slower to respond to inflammation, may be a better indicator than CRP because it remains at a higher concentration for a longer time (WHO/CDC, 2004). Although high sensitivity CRP assays may improve the value of the CRP assay, such measurements will not detect minor infections that may increase ferritin levels (Hulthen *et al*, 1998) and, ideally, a health questionnaire needs to be completed for each subject to identify possible infection.

4.31 It has also been proposed that a higher threshold of <30 µg/L should be recommended in the presence of infection, but this applies only for children aged under 5 years (WHO, 2001). There has been no consensus regarding a threshold for adults with infections. This approach and these values have not been validated. Weiss *et al* (2005) have described a means of differentiating iron deficiency anaemia

32 Defined as transferrin saturation <15%, red cell protoporphyrin >1 mg/L packed red blood cells, serum ferritin <12 µg/L.

and the anaemia of inflammation by using the ratio of serum ferritin and transferrin receptor values, serum ferritin cut-off values of <30 µg/L, and an acute phase protein as a marker of inflammation.

Serum transferrin receptor/serum ferritin ratio

4.32 The logarithm of concentrations (µg/L) of serum transferrin receptors/serum ferritin ratio may provide a quantitative measure of iron stores in normal subjects (Cook *et al*, 2003). These values may be positive (stores present) or negative (reflecting a loss of haemoglobin iron) and are related to body weight. The usefulness of this ratio has been demonstrated in studies of iron supplementation and fortification (Cook *et al,* 2003). A study assessing iron deficiency in the US population (NHANES) (Cogswell *et al,* 2009) reported that the serum transferrin receptors/serum ferritin ratio appeared to be less affected by inflammation than the "ferritin model" (see paragraph 4.35). However, the serum transferrin receptors/serum ferritin ratio applies to healthy subjects and its validity has not yet been established in populations with a high degree of infection. It is unlikely that direct confirmation of the validity of the relationship will be possible for neonates, children and pregnant women. Secondly, until the assay is standardised, it will be difficult to compare results between studies and to use this approach extensively in population studies.

Hepcidin

4.33 Hepcidin is being explored as a marker of changes in iron metabolism which may indicate systemic need for the element. Early studies on hepcidin were difficult to interpret because the assays used measure prohepcidin rather than hepcidin. Specific assays for hepcidin have shown significant correlations between circulating concentrations of immunoreactive plasma hepcidin and serum ferritin in some (Roe *et al*, 2009; Zimmermann *et al,* 2009) but not all (Young *et al*, 2009) studies and poor correlations with the other customary markers (haemoglobin; transferrin receptors) used to assess iron status (Dallalio *et al*, 2003; Young *et al*, 2009). Additionally, serum hepcidin concentrations respond inversely to iron absorption in human adults including those who are iron replete (Zimmermann *et al,* 2009; Young *et al,* 2009). Hepcidin is therefore a potential marker of homeostatic adaptation which may enhance the characterisation of the lower adaptive thresholds of the homeostatic range of adequate iron supply and systemic burden. However, hepcidin concentrations are also increased by inflammation and there is still a need to standardise the assay methods for hepcidin and to validate these within the context of measuring iron status in all population groups, and at the upper end of the homeostatic range.

Multiple indices: using a combination of parameters to assess iron depletion and deficiency

4.34 All the individual indices used to measure biochemical iron status have limitations in terms of their sensitivity and specificity and no single indicator can assess the entire range of deficiency. A combination of haemoglobin and other indicators have been used to better characterise iron depletion and deficiency, and to distinguish between anaemia associated with iron deficiency from that associated with other causes.

4.35 A multiple analyte approach was developed for the analysis and interpretation of NHANES II data (Expert Scientific Working Group, 1985). Two models, the ferritin model and the mean red cell volume (MCV) model, were used to estimate the prevalence of abnormal values. The ferritin model used serum ferritin, transferrin saturation and erythrocyte protoporphyrin as indicators while the MCV model used MCV, transferrin saturation and erythrocyte protoporphyrin. Participants with two or more abnormal values were considered to be iron deficient. Relatively large differences between the two models in prevalence estimates for potential iron deficiency were observed in males aged 11–14 years and females aged 15–19 years. This would be expected because low serum ferritin concentrations detect early stages of reduced iron stores and by implication iron depletion, whereas mean red cell volume is reduced when haemoglobin synthesis is impaired in iron deficiency.

4.36 A WHO/CDC consultation, which reviewed the various indicators used to assess the iron status of populations, recommended that measurement of haemoglobin concentration could provide useful information about the severity of iron deficiency when used with other measurements of iron status (WHO/CDC, 2004). Serum ferritin concentration was considered the best single indicator of iron status except in populations with widespread infection; in such populations, measurement of transferrin receptor concentration was also recommended. Transferrin receptor concentration is not increased with inflammation which makes it possible to distinguish between iron deficiency and inflammation when both transferrin receptor and serum ferritin concentrations are measured. Serum ferritin concentration, together with haemoglobin concentration, was considered to be the most useful indicator to assess the response to an intervention to treat iron deficiency.

Assessment of iron status in infants and young children

4.37 For children aged 6 months to 5 years, current WHO guidelines (2001) recommend a cut-off value for anaemia as haemoglobin concentration of 110 g/L, while depleted iron stores are defined as a ferritin value below 12 µg/L.

4.38 The ESPGHAN[33] Committee on Nutrition has criticised these values, suggesting that they do not represent current knowledge of developmental aspects of erythropoiesis and iron metabolism. Consequently, they are too high for the first two years of life and overestimate the prevalence of anaemia and iron deficiency (Aggett *et al*, 2002). The Committee identified the need for evidence to demonstrate the concentrations of haemoglobin and ferritin associated with functional defects that are responsive to iron therapy and which can therefore be regarded as indicators of deficiency.

33 European Society for Paediatric Gastroenterology, Hepatology and Nutrition.

4.39 Many studies have suggested lower haemoglobin reference values based on the distribution of the parameters in groups of infants that are likely to be iron replete. These cut-offs are, however, not based on functional criteria such as stainable iron in bone marrow aspirates (Aggett *et al*, 2002) or on any systematic study of the response to iron supplementation.

4.40 A study in Sweden and Honduras characterised iron replete infants using three markers of iron adequacy, and lower cut-off levels (-2 standard deviations) were identified by the normative population method (Domellof *et al*, 2002b). For haemoglobin, the values identified were 105 g/L at 4 and 6 months and 100 g/L at 9 months. For ferritin, the suggested values were 20 µg/L at 4 months, 9 µg/L at 6 months, and 5 µg/L at 9 months (Table 6). In this study, haemoglobin response to iron supplementation was also used to identify infants with iron deficiency, but gave a higher haemoglobin cut-off (113 g/L) than the normative population method. In the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC), a large randomly selected representative population sample in the UK (n=1175), the 5th percentile for haemoglobin was 97 g/L at 8 months and 100 g/L at 12 and 18 months (Emond *et al*, 1996; Sherriff *et al*, 1999); ferritin concentrations were 16 µg/L at 8 and 12 months and 12 µg/L at 18 months.

Table 6: Suggested cut-off values for iron deficiency at 4, 6 and 9 months based on iron replete, breast fed infants (Domellof *et al*, 2002b)

Iron marker	4 months	6 months	9 months
Haemoglobin g/L	<105	<105	<100
Mean cell volume fL	<73	<71	<71
Zinc protoporphyrin µmol/mol haem	>75	>75	>90
Ferritin µg/L	<20	<9	<5
Transferrin receptor mg/L*	>11	>11	>11

* Ramco assay (cannot be compared directly with other assays but should relate to the assay of Flowers *et al*, 1989). For infants (aged 8–15 months), the upper reference value (95% CI) for serum transferrin receptor is 13.5 mg/L (Olivares *et al*, 2000).

Assessment of iron overload

4.41 In genetic haemochromatosis (type 1), measurement of transferrin saturation provides an early indication of the risk of iron accumulation and measurement of serum ferritin concentration provides an indication of developing iron overload. Subjects may have slightly higher blood haemoglobin levels and mean cell volume or mean cell haemoglobin (Barton *et al*, 2000) but these are not diagnostically useful. Serum transferrin receptors also do not have diagnostic value; although these are lower in subjects with iron overload, there is considerable overlap with concentrations in subjects with normal iron depots (Baynes *et al*, 1994; Ledue and Craig, 1995; Looker *et al*, 1999).

4.42 Increased hepatic iron deposition and concentration is not fully reflected by serum ferritin concentrations during the early stages of excessive iron accumulation, for example in patients with haemochromatosis (homozygous for C282Y) and for

heterozygotes (Edwards *et al*, 2000). This is because serum ferritin is usually derived from phagocytic cells of the reticuloendothelial system, whereas excess iron is first deposited in the ferritin pool of hepatic parenchymal cells (Worwood, 1990).

4.43 Suggested upper reference thresholds for serum ferritin concentrations are uncertain and have also varied widely. In the UK, values of >300 µg/L for men and postmenopausal women (200 µg/L for premenopausal women) have been suggested (Dooley and Worwood, 2000); the same thresholds were used in the Haemochromatosis and Iron Overload Screening (HEIRS) study of 100,000 adults in the USA (Adams *et al*, 2005). For men, cut-off values ranging from 200 µg/L (Mainous *et al*, 2002) to 400 µg/L (Looker and Johnson, 1998) have been applied. For premenopausal women, a concentration of 200 µg/L has commonly been selected, although 100 µg/L has also been used (Asberg *et al,* 2001).

4.44 In the USA approximately 6% of adults in NHANES III (1988–94) had a transferrin saturation of >45% (Looker and Johnson, 1998); however this cut-off is associated with a low specificity for haemochromatosis. A threshold as high as 62% has been applied (Edwards, 1988) and intermediate thresholds of >55% (men) and 50% (women) have also been used (Dooley and Worwood, 2000). In the HEIRS study (Adams *et al*, 2005) and in a systematic review for the US Preventative Services Task Force (Whitlock *et al*, 2006), a transferrin saturation threshold of 50% was selected for men and 45% for women. Unsaturated iron-binding capacity is as sensitive and specific as transferrin saturation but reference ranges vary widely for different assay systems (Adams *et al*, 2000; Jackson *et al*, 2001; Murtagh *et al*, 2002).

4.45 Results from studies which have examined the association of HFE genotype and iron status have generally found slightly but significantly higher values for serum iron and transferrin saturation in heterozygotes for either C282Y or H63D compared with those lacking these variants but no significant difference between heterozygotes and "wild type" individuals for serum ferritin concentration (Burt *et al,* 1998; Datz *et al*, 1998; Beutler *et al,* 2000; Jackson *et al*, 2001; van Aken *et al*, 2002; Chambers *et al*, 2003; Raddatz *et al*, 2003; Sanchez *et al*, 2003).

Summary and conclusions

4.46 Iron status represents a risk assessment of adequacy, deficiency and excess. There are a number of haematological and biochemical indicators which are used to assess iron status. These include serum transferrin (transport iron capacity), serum ferritin (systemic iron depots), haemoglobin (functional iron), zinc protoporphyrin (haemoglobin synthesis) and serum transferrin receptors (tissue needs for iron). Most assessments combine ferritin and haemoglobin concentrations as markers of the deposition of iron in tissues and iron utilisation.

4.47 Iron status is essentially a qualitative concept. It cannot be precisely quantified because of difficulties in determining accurate thresholds for adaptive responses and for adverse events associated with iron deficiency or excess. The thresholds selected for use are not based on functional outcomes. Reference ranges indicate iron sufficiency and values outside the ranges do not necessarily define iron deficiency or excess. Assessment of status for iron, as for other micronutrients,

requires an approach based on integrated use of the markers according to their functional relevance and potential confounders. However, because they each measure different aspects of iron metabolism, the iron related markers do not always correlate well. The full potential and role of hepcidin concentrations in assessing iron status remains to be characterised fully.

4.48 Reference ranges for the indices are affected by analytical differences between laboratories and other conditions such as diurnal and post-prandial variations. Changes in analytical methodology and quality control limit the value and reliability of using historical data to analyse trends and comparisons with more recent studies.

4.49 No single marker of iron metabolism is considered ideal for the assessment of iron deficiency, adequacy or excess, as all the individual indices have limitations in terms of their sensitivity and specificity. However, in accordance with pragmatic decisions made by others such as the WHO/CDC (2004) and COMA (DH, 1994), haemoglobin (functional iron) and ferritin (iron depots), in combination, are considered to be the most useful indicators of iron status. Although these markers are useful for field work, they need to be used critically in developing and monitoring interventions in practice, and in developing policy. A relationship between iron markers and outcomes can only be expected to exist in specific circumstances such as iron deficiency or increased iron need.

4.50 The WHO criteria for identification of anaemia, irrespective of cause, are haemoglobin concentrations of: children under 5 years, 110 g/L; children 5–11.99 years, 115 g/L; children 12–14.99 years and non-pregnant females over 15 years, 120 g/L; males over 15 years, 130 g/L. The WHO criteria used to define depleted storage iron are serum ferritin concentrations of: children under 5 years, <12 µg/L; males and females over 5 years, <15 µg/L.

4.51 The best, and possibly only, definitive diagnostic measure of iron deficiency is to systematically monitor the response of haemoglobin concentration to iron supplementation.

5 Iron in the diet

Dietary iron

5.1 Dietary iron exists in two main forms: haem iron and non-haem iron. Haem iron is found almost entirely in food of animal origin. Non-haem iron is found in animal and plant tissues as Fe^{2+} bound to insoluble proteins, phytates, oxalates, phosphates and carbonates, and as ferritin. The richest sources of non-haem iron include cereals, vegetables, nuts, eggs, fish and meat. The contribution of these foods to dietary iron intakes in the UK is described in section 9.

5.2 Iron is also added to a number of foods during its manufacture (iron fortificants) and is available in supplemental form (capsules, tablets or other over-the-counter preparations containing iron either alone or in combination with other micronutrients).

5.3 In some developing countries, contamination iron from mechanical or chemical reactions occurring during food production and preparation may be an important source of iron (Adish *et al*, 1999; Kroger-Ohlsen *et al*, 2002). The iron content of food is increased by being cooked in cast-iron cookware, particularly when acidic foods are cooked for extended periods of time (Brittin and Noassaman, 1986; Fairweather-Tait *et al,* 1995). Contamination iron is not considered to be a significant source of iron in the UK since the use of cast-iron cookware is not widespread in the UK.

5.4 There is no direct method for measuring the haem iron content of meats and foods. Values are derived by measuring the inorganic iron and total iron content of a food and assuming the difference represents the amount of haem iron present. The haem iron content of meat is usually estimated to be 40% of total iron (Monsen *et al,* 1976); however, the percentage of total iron present as haem in different non-milk animal food products is very variable. Later estimates of mean haem iron content of beef have ranged from 64 (Valenzuela *et al*, 2009) to 78% (Lombardi-Boccia *et al*, 2002) of total iron and from 52 to 83% of total iron in other red meats (Lombardi-Boccia *et al*, 2002).

5.5 Processes that are most likely to increase the amount of iron available for absorption by degrading inhibitors of iron uptake (e.g., phytates and phosphates) include milling, soaking, cooking, germination, fermentation and heat.

Bioavailability of dietary iron

5.6 The terms bioavailability and absorption are often used interchangeably in the literature. Absorption refers to the passage of a nutrient, or other dietary constituent, from the intestinal lumen into the body. It usually comprises the uptake of a nutrient into the enterocytes of the gut mucosa and its subsequent transfer across the cell and into the body. Bioavailability describes the systemic

utilisation of a dietary nutrient and refers to the proportion of a nutrient that is taken up and transferred by the intestinal mucosa and subsequently used systemically.

5.7 Haem and non-haem iron are transported into the intestinal mucosal cells by independent mechanisms (see section 2). The intestinal uptake and transfer of iron is dependent on the body's need for iron. The availability of dietary iron for uptake by the gut mucosa is affected by the chemical form of iron (haem iron or inorganic non-haem iron) and the character of the complexes of inorganic iron with other dietary constituents.

5.8 Dietary components that affect iron absorption have been identified and partly characterised from single meal studies using isotope labels. Single meal studies have shown that haem iron is more efficiently absorbed from the diet (20–30%) than non-haem iron (5–15%) (Martinez-Torres and Layrisse, 1971; FAO, 1988). Non-haem iron absorption in single meal studies is very variable and is influenced by the balance of dietary factors enhancing (e.g., meat, ascorbic acid) and inhibiting (e.g., phytic acid, soy protein, polyphenols, calcium) absorption (see paragraphs 5.20–5.38). With the exception of calcium, which has an inhibitory effect on both haem and non-haem iron (Hallberg *et al*, 1991), absorption of haem iron is not affected by other components of the diet. Interactions between iron and other micronutrients (zinc and copper) are considered in section 6 (paragraphs 7.9–7.15).

5.9 Homeostatic control of non-haem iron absorption is more pronounced than that of haem iron. Lynch *et al* (1989) reported that absorption of non-haem iron from a standard meal containing 4.8 mg/L of iron was nearly 10-fold higher with decreasing serum ferritin concentrations (from 100 to 10 µg/L) compared to a 2–3-fold increase for absorption of haem iron. Hallberg *et al* (1997) reported that fractional absorption of haem and non-haem iron was similar (about 40%) at serum ferritin concentrations of 10 µg/L. At higher serum ferritin concentrations, absorption of both haem and non-haem iron was decreased but the reduction was greater for non-haem. At serum ferritin concentrations of 15, 20 and 30 µg/L, haem iron absorption was 40%, 80% and 140% higher than that of non-haem iron. Non-haem and haem iron form a common pool after uptake into the enterocyte, so the difference in their absorption is probably due to differences in the processes involved in the uptake of the two forms of iron by the enterocytes.

5.10 Since the systemic need for iron is the major determinant of iron uptake and transfer, bioavailability is not an absolute characteristic of a food or diet *per se*. However, as systemic needs for iron increase, the type of diet and its influence on the bioavailability of iron becomes increasingly relevant. For example, it could be of greater importance for growing children or in populations with iron deficiency secondary to intestinal parasites and infection, more particularly when the iron content of the diet is low. The presence of iron deficiency in a population where there is no evidence of any diseases that affect iron metabolism suggests that dietary iron supply may be inadequate in terms of absolute amounts of absorbable iron relative to iron requirements.

5.11 In general, iron compounds that are water-soluble have the highest bioavailability, followed by those that are soluble in dilute acid (equivalent to gastric conditions) (Hurrell, 1997). Compounds that are insoluble in water or dilute acid have a lower bioavailability because the iron precipitates and cannot be taken up by the enterocyte.

Measuring bioavailability and absorption of dietary iron

5.12 Dietary iron "absorption" has been measured in a number of ways, including chemical balance studies, radio or stable isotope studies, whole-body counting after ingestion of a radio label, plasma appearance curves of whole iron with or without stable or radio labels, and the incorporation of an iron tracer into a functional pool of iron, usually haemoglobin. Each of these methods provides different information and the results have different implications and limitations.

5.13 The influence of dietary factors on iron bioavailability and absorption has been determined from single meals containing isotope labels which are consumed by subjects following an overnight fast.

5.14 The true effect of diet on iron bioavailability can only be assessed when the technique used is not limited by the intestinal setting for iron absorption, i.e., in individuals with a high setting for iron uptake and transfer (for example, iron deficient individuals). If iron bioavailability is assessed in individuals in whom intestinal uptake and transfer of iron is down regulated, i.e., normal healthy individuals whose only requirements for iron are to replace systemic losses, the values will probably underestimate the potential bioavailability of iron from food.

5.15 Single meal absorption studies are therefore useful for comparing and ranking iron bioavailability from various foods but such studies do not provide absolute values for bioavailability which can be reliably translated to dietetic and nutritional practice. An additional uncertainty about many reported bioavailability values that have been determined from single meal studies is that they do not allow for intestinal adaptation of iron uptake, transfer and systemic use, in response to the different quantitative and qualitative exposures to iron.

5.16 The only effective method of determining iron bioavailability, i.e., the amount available for systemic utilisation, is to measure the fraction of dietary iron that is absorbed and incorporated into haemoglobin as a functional measure of iron utilisation. Foods or meals are intrinsically or, more commonly, extrinsically tagged with radioisotopes of iron (^{59}Fe and ^{55}Fe) and the percentage of the isotope incorporated into haemoglobin is measured 14 days later (Cook *et al*, 1969). This is based on the assumption that most (80–100%) of the absorbed iron is incorporated into haemoglobin in newly formed erythrocytes (Cook *et al*, 1991). Enriched amounts of naturally occurring low-abundance stable isotopic tracers (^{54}Fe, ^{57}Fe, ^{59}Fe) can also be used; because these markers avoid the use of ionising radiation, they are preferred for vulnerable groups such as pregnant women and children.

5.17 A once frequently used technique to measure iron absorption is whole-body counting in which foods or meals labelled with a radioisotope of iron are consumed and the fraction of radioactivity remaining in the body after about 14 days is measured using a whole-body counter. This approach actually measures iron retention but, because there is usually no means of iron excretion, iron retention can be assumed to represent iron absorption. Iron absorption has also been calculated as the difference between oral iron intake and faecal excretion of an isotope label. This method requires a study period of sufficient duration to ensure the complete faecal loss of iron trapped in the gut mucosa, otherwise it only measures the luminal disappearance of iron.

5.18 The influence of systemic iron needs on iron absorption means that absorption values of individuals in studies can only really be compared if allowance is made for inter-individual variability. Three methods have been developed to enable a standardised approach:

- Use of a reference dose of inorganic iron which is compared to the source of iron under investigation (Layrisse *et al*, 1969); results for each individual are presented as ratios.
- Measurement of absorption from a reference dose of inorganic iron and correction of food absorption data to a mean reference value of 40% by multiplying by 40/R, where R is the reference dose absorption. The reference value of 40% represents the amount of iron which corresponds to absorption by subjects with borderline iron stores (Magnusson *et al*, 1981).
- Correction of dietary absorption data to a value corresponding to low levels of iron stores, i.e., serum ferritin concentrations of 40 µg/L (Cook *et al*, 1991) or 30 µg/L (Reddy *et al*, 2000).

Dietary factors influencing iron absorption and bioavailability

Studies of iron absorption based on single meals

5.19 Although single meal absorption studies can control for all confounding variables and provide a robust method for ranking the relative absorption and evaluating dose-response, they cannot be used with confidence to predict the efficiency of iron absorption from foods because they do not take account of adaptive, absorptive and homeostatic responses that might occur if the foods or diets were consumed over a more extensive time period.

Enhancers of iron absorption and bioavailability

5.20 The most widely researched enhancers of non-haem iron absorption and bioavailability are meat and ascorbic acid (vitamin C).

Meat

5.21 Meat and fish contain haem iron, which is relatively well absorbed (20–30%); they also enhance non-haem iron absorption from foods consumed at the same time (Cook and Monsen, 1976; Hallberg and Rossander, 1984; Layrisse *et al*, 1968; Martinez-Torrez

et al, 1971) in a dose-dependent manner (Baech *et al*, 2003; Cook and Monsen, 1975; Layrisse *et al*, 1984). Hallberg and Rossander (1984) found non-haem iron absorption increased 2.5-fold ($p<0.01$) after addition of meat (75 g) to a meal of maize, rice and black beans.

5.22 It has been suggested that meat enhances absorption of non-haem iron only when ingested with notably inhibitory foods (Bjorn-Rasmussen and Hallberg, 1979). Hurrell *et al* (1988) reported that addition of beef (92 g) to maize porridge significantly increased non-haem iron absorption 3-fold but had no effect on a bread meal made from wheat flour. Maize has a greater inhibitory effect on non-haem iron absorption than wheat and it has been estimated that the percentage of non-haem iron absorbed from wheat meals is six times that from maize meals (International Nutritional Anaemia Consultative Group, 1982).

5.23 The mechanism for the enhancing effect of meat on non-haem iron absorption or the component(s) of meat responsible for the enhancing effect is unclear. Attention has focused mainly on protein digestion products of meat including cysteine-containing peptides (Taylor *et al*, 1986). Armah *et al (*2008) reported that L-α-glycerophosphocholine (GPC), a hydrolytic product of a phospholipid found in muscle tissue, significantly enhanced non-haem iron uptake *in vitro* and enhanced iron absorption from vegetarian lasagne (added in a molar ratio of GPC to iron of 2:1) in women (n=13) with serum ferritin concentrations <30 µg/L. However, Troesch *et al* (2009) reported that the addition of GPC (in a molar ratio of 4:1) did not significantly increase iron absorption from maize porridge. The maize porridge used in the study by Troesch *et al* (2009) contained higher levels of inhibitors of iron absorption than the vegetarian lasagne used in the study by Armah *et al* (2008), so it is possible that the inconsistent findings were due to differences in the food matrix.

Ascorbic acid

5.24 The primary mechanism for the enhancing effect of ascorbic acid on iron absorption is thought to be by formation of a more soluble complex than iron alone and by its ability to reduce Fe^{3+} to Fe^{2+}, the form in which iron is taken up into the mucosal cells (Plug *et al,* 1984).

5.25 Cook and Monsen (1977) reported that non-haem iron absorption from a semi-synthetic meal increased in a dose-dependent manner over a range of 25–1000 mg of added ascorbic acid. A review of 24 studies (Bendich and Cohen, 1990) reported a clear dose-response effect of ascorbic acid on non-haem iron absorption which appeared to level off at doses above 500 mg; however, the authors noted that the sample size at high doses of ascorbic acid was too small to reach firm conclusions.

5.26 The influence of ascorbic acid on increasing non-haem iron absorption is reported to be most effective with meals containing high levels of phytates and polyphenols, which are inhibitors of iron absorption (Hallberg *et al,* 1989; Siegenberg *et al*, 1991).

5.27 The effects of ascorbic acid and meat in facilitating non-haem iron absorption are not additive. Cook and Monsen (1977) reported that addition of ascorbic acid (100 g) to a meal containing beef increased absorption 1.67-fold compared to a 4-fold increase observed when the same amount was added to a meal without beef.

5.28 Erythorbic acid, a stereoisomer of ascorbic acid used as an antioxidant in processed foods, is a stronger enhancer of non-haem iron absorption than ascorbic acid (Fidler *et al,* 2004). Other organic acids occurring naturally in fruit and vegetables (including malic, citric and tartaric acid) have also been shown to increase non-haem iron absorption (Gillooly *et al,* 1983; Ballot *et al*, 1987). Lactic acid, which is produced during the brewing process, is one of the factors responsible for the high absorption of iron from maize and sorghum beers in southern Africa (Derman *et al*, 1980).

Inhibitors of iron absorption and bioavailability

Phytic acid

5.29 Phytic acid (myoinositol hexaphosphate and some lesser phosphorylated forms of myoinositol), found in whole-grain cereals, legumes, nuts and seeds, has been shown to be a dose-dependent inhibitor of non-haem iron absorption (Hallberg *et al*, 1989). Addition of 2, 25 and 250 mg doses of phytate phosphorus (phytate P) to phytate-free bread rolls significantly reduced iron absorption by 18, 64 and 82% respectively (Hallberg *et al*, 1989). Many Western-style meals contain 10–100 mg of phytate P, and vegetarian meals can contain 250 mg; an 80 g wheat roll made with 70% extraction flour contains 30 mg phytate P, and a roll made with 80% extraction flour contains 60 mg.

5.30 The inhibitory effects of phytates on iron absorption can be ameliorated by ascorbic acid (Hallberg *et al*, 1989; Siegenberg *et al*, 1991). Hallberg *et al* (1989) estimated that approximately 80 mg of ascorbic acid (more than is usually contained in a 100 g portion of fruit) would be needed to counteract the inhibitory effect of 25 mg of phytate P, and that very large amounts (several hundred mg) would be required to overcome the inhibitory effects of high phytate diets (250 mg phytate P). Siegenberg *et al* (1991) reported that 30 mg of ascorbic acid was required to overcome the inhibitory effects of 58 mg of phytate P contained in a bread roll made of maize bran.

5.31 Meat has also been shown to ameliorate the inhibitory effect of phytate (Hallberg *et al*, 1989; Baech *et al,* 2003). Addition of 75 g of beef to a meal containing inhibitors of iron absorption increased absorption of non-haem iron 2.5-fold (Hallberg *et al*, 1989). Baech *et al* (2003) reported no effect when 25 g of pork was added to a meal containing 62 mg of phytate P; however, addition of 50 g and 75 g of pork significantly increased absorption 1.4- and 1.6-fold respectively.

Soy protein

5.32 Soy protein products have been reported to inhibit non-haem iron absorption (Cook *et al*, 1981). Although they contain high levels of phytate, which is an important inhibitor of iron absorption (see paragraphs 5.29–5.31), phytate-free soy proteins have also been shown to inhibit iron absorption. Hurrell *et al* (1992) examined the effect of soy protein isolates on non-haem iron absorption from a liquid meal to which either egg white (control) or soy protein isolate was added. Reduction of the phytate content of the soy isolate from its native amount (4.9–8.4 mg/g isolate) to 0.2 mg/g resulted in a significant 2.3-fold increase in iron absorption. After complete removal of the phytate from the soy protein isolate, iron absorption was approximately 55% of the absorption from the egg white control meal, suggesting that soy protein itself is inhibitory.

5.33 Lynch *et al* (1994) demonstrated that a protein-related moiety in soy protein isolate had an inhibitory effect on non-haem iron absorption independently of phytate. After dephytinisation of the two main protein fractions in soy beans, conglycinin and glycinin, the glycinin fraction was only inhibitory in the presence of phytate; however, the phytate-free conglycinin fraction had an inhibitory effect similar to that of phytate, suggesting that the conglycinin fraction in soy protein inhibits iron absorption.

Polyphenols

5.34 Polyphenols, found particularly in tea (Disler *et al,* 1975) and coffee (Morck *et al*, 1983), have a dose-dependent effect on non-haem iron absorption, with coffee having approximately half the inhibitory effect of tea. Other beverages such as red wine (Bezwoda *et al*, 1985), cocoa (Gillooly *et al*, 1984) and herb teas (Hurrell *et al*, 1999) have also been shown to inhibit non-haem iron absorption. Black tea polyphenols are more powerful inhibitors than those from herb teas, cocoa or wine, possibly due to higher levels of galloyl esters (Hurrell *et al*, 1999). Hurrell *et al* (1999) reported that 20–50 mg total polyphenols reduced non-haem iron absorption from a bread meal by 50–70%, while 100–400 mg total polyphenols (equivalent to one cup of tea/ instant coffee) reduced non-haem iron absorption from a bread meal by 60–90%.

5.35 Although polyphenols bind strongly to protein, the addition of milk to black tea or coffee does not reduce their inhibitory effect (Hurrell *et al,* 1999). Ascorbic acid has been shown to counteract the inhibitory effects of the polyphenol tannin on non-haem iron absorption: 50 mg of ascorbic acid was required to overcome the effects of >100 mg tannic acid (Siegenberg *et al,* 1991).

Calcium

5.36 Both supplemental and dietary calcium has been reported to reduce absorption of non-haem iron (Monsen and Cook, 1976; Deehr *et al*, 1990; Hallberg *et al,* 1991; Gleerup *et al,* 1995; Minihane and Fairweather-Tait, 1998) and haem iron (Hallberg *et al,* 1993b). The mechanism for the inhibitory effect of calcium on iron absorption is unknown. It has been suggested that calcium and iron may compete for binding with one or more substances that are important in the absorption pathway (Hallberg *et al*, 1991).

5.37 Hallberg *et al* (1991) reported that addition of calcium (40–600 mg) to wheat rolls significantly reduced iron absorption; the extent of inhibition was clearly dose-related up to levels of 300 mg. The inhibiting effect was greater when calcium was added to the dough, as it reduced phytate degradation during fermentation and baking. Addition of 40 mg and 300–600 mg of calcium to dough reduced iron absorption by approximately 40 and 75–80% respectively. Addition of 40 mg of calcium after baking had no effect on iron absorption; however, addition of increasing amounts of calcium after baking, up to 300 mg, successively inhibited iron absorption by approximately 60%. The decrease in iron absorption between 300 and 600 mg of added calcium was not statistically significant. Consumption of milk or cheese (equivalent to 165 mg of calcium) with the rolls reduced iron absorption by 57 and 46% respectively.

5.38 The inhibiting effect of calcium appears to be more pronounced in simple meals (such as bread rolls) compared to more complex meals. Galan *et al* (1991) found no difference in iron absorption (haem or non-haem) from a typical French meal (comprising meat, vegetables, potatoes, cheese, bread and fruit) when it was consumed with or without 150 ml of milk or yoghurt. Since the calcium content of the basal meal was 320 mg, maximal inhibition of iron absorption may already have occurred prior to the addition of the milk or yoghurt. However, iron absorption from a meal low in calcium (hamburger, string beans and potatoes) was not reduced when it was consumed with milk (250 ml) (Hallberg and Rossander, 1982).

Studies of iron absorption and bioavailability based on the whole diet

5.39 A number of studies have suggested that single meal studies overestimate or underestimate the magnitude of the effect of dietary modulators on non-haem iron absorption. This could be because absorptive efficiency is maximised after an overnight fast, effects of key modulators may be diluted substantially when they are consumed with other foods as part of the whole diet and because the intestinal setting for uptake and transfer of iron has time to adapt to the change of diet over longer periods.

5.40 Tidehag *et al* (1996) compared non-haem iron absorption from a morning meal with that from all three meals of the day for two consecutive days (n=10; 34–65 years). Meals were extrinsically labelled and iron absorption was determined from whole-body counting. On a low-fibre diet, absorption was approximately 78% greater from the morning meals than from all meals during the two-day period. With a high-fibre diet, absorption from the morning meals was 48% more than the average from all meals, but this difference was not significant.

5.41 Cook *et al* (1991) compared the bioavailability of non-haem iron over a two-week period with that from single meals. Extrinsically tagged bread rolls were consumed with each meal and bioavailability was measured by erythrocyte incorporation of radioactivity. Participants (n=45; 21–40 years) modified their diets to either maximally enhance[34] or inhibit[35] absorption of non-haem iron. Mean iron bioavailability from a diet modified to maximise iron absorption was 2.5-fold higher than from a diet of very low bioavailability. When the same diets were compared using single meals, iron absorption was five times higher from the meal modified to maximise non-haem iron absorption compared to the meal high in inhibitors of non-haem iron absorption.

5.42 Hunt and Roughead (2000) compared short term measurements of iron absorption with longer term measurements in men (n=31; 32–56 years) fed diets containing either high amounts of enhancers or inhibitors of iron absorption[36] for 12 weeks. All foods consumed during this period were isotopically labelled. Haem and non-haem iron

34 All meals were required to contain ≥90 g meat, poultry, or fish and enough vegetables/fruit, or citrus juice, to provide ≥100 mg ascorbic acid. Tea/coffee with meals, eggs, foods with high bran content were not permitted.

35 No meat products (very limited amount of fish or chicken allowed); no fresh vegetables/fruits or citrus juices. Consumption of dairy products, eggs, legumes, cereals and foods with high bran content were encouraged.

36 Meals contained different amounts of meat, ascorbic acid, whole grains, legumes and tea.

absorption was measured at 0 and 10 weeks by whole-body counting. With time, there was a significant decrease in non-haem iron absorption on the diet high in enhancers of iron absorption and a significant increase in non-haem iron absorption on the diet high in inhibitors of iron absorption. After 10 weeks, a 5-fold difference in non-haem iron absorption between the two diets at the beginning of the study was significantly reduced to a 2-fold difference. There was no significant difference in haem iron absorption from the two diets or any adaptation in absorption with time.

5.43 Cook and Reddy (2001) evaluated the effect of ascorbic acid on non-haem iron bioavailability from a complete diet over 3 five-day periods. Participants (n=12; 20–38 years) consumed extrinsically tagged bread rolls with each meal during the dietary period and non-haem iron bioavailability was determined from erythrocyte incorporation of the radioisotope. Diet was self-selected for the first five-day period and then altered to maximally increase or decrease ascorbic acid for the second and third dietary periods. Mean ascorbic acid intake was 90 mg/day with the self-selected diet, 247 mg/day with the maximal ascorbic acid diet, and 51 mg/day with the decreased ascorbic acid diet. No significant difference was found in iron bioavailability between the three dietary periods.

5.44 Reddy *et al* (2006) examined the effect of meat intake on non-haem iron bioavailability over 3 five-day dietary periods. Participants (n=14) initially consumed a self-selected diet followed by diets to eliminate or maximally increase intakes of meat and seafood. A radiolabelled wheat roll was consumed with each of three meals during the different diets and iron bioavailability was measured by erythrocyte incorporation. Although meat intake was significantly different between the three diets (0 g, no meat; 136 g, self-selected; 222 g, high-meat), there were no differences in non-haem iron bioavailability.

5.45 Variations in calcium intake (280, 684 and 1,281 mg/day), achieved by modifying food selection, did not affect total dietary non-haem iron bioavailability over 3 five-day periods (n=14; 19–37 years) (Reddy and Cook, 1997). The diet was labelled during each five-day period by consumption of a radioisotopically tagged bread roll with each meal and iron absorption was measured by erythrocyte incorporation of radioactivity.

5.46 Grinder-Pedersen (2004) examined the effects of consuming three different sources of calcium with three main meals over 4 days, on non-haem iron absorption in women (n=14; 21–34 years) with relatively low iron stores (serum ferritin ≤40 µg/L). The basic diet, which was low in calcium (224 mg/day), was supplemented with either one glass of milk (826 mg/day calcium), calcium lactate (802 mg/day calcium), or a milk-mineral isolate (801 mg/day calcium). Meals were extrinsically labelled with a radioisotope of iron and absorption was determined from whole-body retention of radioactivity. There were no significant differences in non-haem iron absorption between the different diets.

Models to predict iron bioavailability

5.47 Several models have been developed to estimate iron bioavailability from different meals and diets based on the serum ferritin concentration of the individual, the type of iron (haem or non-haem), and that take various account of the presence of enhancers or inhibitors of iron absorption (Monsen *et al*, 1978; Hallberg and Hulthen, 2000; Reddy *et al*, 2000).

5.48 Prediction equations to estimate iron bioavailability from meals have limitations. They are based on iron absorption from single meals which may overestimate the effects of enhancers and inhibitors and do not take account of dietary complexity and variability or long term adaptation to iron absorption. For example, Beard *et al* (2007) compared a number of prediction equations with the change in serum ferritin concentrations of women (n=317) taking part in a nine-month feeding trial in the Philippines to assess the efficacy of iron fortified rice. Analysis of six equations showed highly significant differences in the predicted efficiency of iron absorption, and none agreed with dietary iron utilisation based on improvement in serum ferritin concentration.

The influence of enhancers and inhibitors of iron absorption on iron status

Epidemiological studies

5.49 Evidence on the relationship between dietary modulators of iron absorption and iron status is based mainly on observational data which have a number of limitations. Although epidemiological studies take account of adaptive responses and the complexity of the whole diet, unless good quality dietary information is collected in conjunction with appropriate and sensitive measures of the systemic need for iron, correlations between dietary constituents and iron status can be misleading.

5.50 Multiple days of dietary records are required to obtain accurate estimates of habitual iron intake; 12 days is probably the shortest study period needed to provide a good representation of individual iron intake (Bingham, 1987). A questionnaire based on food frequency has been developed which measures iron intake on a meal-by-meal basis and also measures concomitant intake of inhibitors and enhancers (Heath *et al*, 2005), but this method requires further validation. Most studies have used dietary records of less than 12 days or questionnaires not specifically validated for iron intake. Additionally, there are only limited food composition data for some modifiers of iron absorption such as phytate and polyphenols.

5.51 A measurable effect of dietary enhancers and inhibitors may only be observed in individuals with a systemic need for iron and, as a consequence, an elevated absorptive capacity rather than in populations where the majority of the population is iron replete (Garry *et al,* 2000). The relationship between iron intake and iron status is also complicated by a number of confounding factors which influence iron absorption, such as age, homeostatic metabolic responses, menstrual losses (in women) and genetic influences (e.g., HFE and other polymorphisms).

5.52 Indices of iron status, such as serum ferritin and haemoglobin concentration, are also affected by other factors. Serum ferritin concentrations are raised when haemoglobin synthesis is inhibited, by the acute phase reaction in response to infection and inflammation, and in liver damage. Concentrations of haemoglobin can be a reflection of low vitamin B_{12} or folic acid intakes, haemoglobinopathies, and a variety of other diseases. Additionally, most studies have collected only one blood sample, which does not take account of day-to-day variability in iron status measurements.

Cross-sectional studies

5.53 Numerous cross-sectional studies have examined the association between total dietary iron intake and dietary modulators of iron absorption on biochemical markers of iron status (mainly serum ferritin and haemoglobin concentration). These studies suffer from a number of limitations including narrow range of exposures, small sample sizes, inadequate dietary assessment methods, and variability in the allowance made for other factors that affect iron status (e.g., most studies did not measure markers of inflammation/infection which are also associated with increased serum ferritin concentration).

5.54 Most cross-sectional studies have reported better iron status with increased meat intake (Worthington-Roberts *et al*, 1988; Leggett *et al*, 1990; Fleming *et al*, 1998; Galan *et al*, 1998; Gibson *et al*, 1999; Heath *et al*, 2001; Milman *et al*, 2004) and haem iron intake (Davis *et al*, 1992; Preziosi *et al*, 1994; Fleming *et al*, 1998, Galan *et al*, 1998) although some studies found no association (Fogelholm *et al*, 1993; Garry *et al*, 2000; Cowin *et al*, 2001).

5.55 Findings from cross-sectional studies assessing the effects of total iron intake, ascorbic acid, calcium and polyphenols, have been inconsistent. Most cross-sectional studies assessing the effects of phytate consumption on markers of iron status did not find an association.

Prospective studies

5.56 A limited number of prospective studies have investigated the association between dietary factors and iron status. For details of the prospective studies considered in this report, including iron status of participants, dietary assessment methods, and allowance made for other factors affecting iron status, see Annex 5 (Table A4).

5.57 A prospective study in Costa Rica (Munoz *et al*, 1988) investigated the association between coffee consumption and iron deficiency anaemia (haemoglobin <110 g/L, serum ferritin <10 µg/L) among low income women (n=48; 17–30 years) during late pregnancy/early lactation. All women were receiving iron supplements (60 mg/day elemental Fe) by six months of pregnancy. There were no differences between coffee drinkers (>450 ml/day; ≥ 10 g/day ground coffee) and non-drinkers (0 ml/day) in consumption of energy, protein, ascorbic acid, iron, red meat, and dark green vegetables. In multivariate analysis, haemoglobin concentrations were significantly lower in coffee drinkers at eight months gestation ($p<0.001$) and at one month postpartum (p not provided); breast milk iron concentration was also significantly lower in the coffee drinkers group at one month postpartum. Infants whose mothers

consumed coffee had significantly lower birth weight (p<0.001) and haemoglobin concentration (p<0.05) at 1 month of age; this association was independent of maternal iron status and birth weight. There were no differences in maternal and infant serum ferritin concentrations between the two groups.

5.58 A 10-year longitudinal study in the USA (n=125; 60–93 years) investigated the influence of haem and non-haem iron intake on body iron stores (ferritin bound iron) (Garry *et al*, 2000) which were estimated from serum ferritin concentrations[37] (Cook *et al,* 1986). No significant association was found between dietary iron intake (haem or non-haem) and estimated iron stores. However, supplemental iron intake was significantly associated (p=0.04) with increased iron stores (estimated body ferritin was 64 mg greater for individuals consuming more than 18 mg/day of supplemental iron compared to those not consuming iron supplements).

5.59 Backstrand *et al* (2002) investigated the association of usual diet with haemoglobin and serum ferritin concentrations in a cohort of women (n=125; 16–44 years) in Mexico. Higher plasma ferritin concentrations were significantly associated with increasing intakes of non-haem iron (p=0.003) and ascorbic acid (p=0.0395). Risk of low plasma ferritin concentration (<15 µg/L) was not associated with intake of non-haem iron (odds ratio [OR], 0.92; 95% CI, 0.83–1.01; p=0.09), haem iron (OR, 1.26; 95% CI, 0.77–2.05; p=0.36) or ascorbic acid (OR, 0.98; 95% CI, 0.94–1.00; p=0.13). No association was found between risk of low haemoglobin concentration (<130 g/L) and intakes of non-haem iron (OR, 0.95; 95% CI, 0.86–1.05; p=0.3) and haem iron (OR, 1.21; 95% CI, 0.74–1.98; p=0.45); however, each additional mg of ascorbic acid was associated with a 7% reduction in risk of low haemoglobin concentration (OR, 0.93; 95% CI, 0.89–0.97; p=0.0009).

5.60 Liu *et al* (2003) examined dietary variables affecting serum ferritin concentrations of postmenopausal women in the USA followed for 10 years (n=620; 44–69 years). Higher serum ferritin concentrations were significantly associated with increasing intakes of haem iron (mainly from red meat) (p trend=0.01) and iron supplements (p trend=0.02). Dietary intakes of non-haem iron, calcium, vitamin C and coffee were not associated with serum ferritin concentrations.

5.61 A study in Sweden (Öhlund *et al*, 2008) evaluated factors affecting haemoglobin and serum ferritin concentrations in children (n=127) aged 6–12 months who were followed up to age 4 years. Haemoglobin concentration at 4 years was significantly related to previous haemoglobin concentration (at 6, 12, 18 months) and mothers' haemoglobin concentration (measured when child was 4 years). No associations were found between haemoglobin concentration and mean daily intake of iron, meat, ascorbic acid, calcium or dairy products. A significant association was found between serum ferritin concentration and meat intake in boys only (p=0.015).

37 For participants with serum ferritin concentrations >12µg/L, the following equation is used: iron stores (mg) = 400 x (lnSF – ln12), where 400 is the proportionality constant, ln is the natural logarithm and SF is serum ferritin. Serum ferritin concentrations of all participants in the study were >12µg/L. This formula is based on quantitative phlebotomy studies indicating that 1 µg/L of serum ferritin corresponds to 8 mg of storage iron (Walters *et al*, 1973).

Intervention studies

5.62 Further details of the studies considered, including baseline serum ferritin and haemoglobin concentrations, are provided in Annex 5 (Table A5).

Meat

5.63 Lyle *et al* (1992) compared the effects of supplemental iron intake with increased meat consumption on serum ferritin and haemoglobin concentrations of previously sedentary women in the USA (n=60) participating in a 12-week programme of moderate aerobic exercise (30 minutes, 3 days/week). Participants were assigned to one of five groups: 50 mg/day iron supplement and a diet containing low food-iron; 10 mg iron supplement and low food-iron diet primarily from non-haem sources; placebo; high food-iron diet (mainly from meat) and muscle-food supplements; and a control group with a free-choice diet and no exercise. Mean dietary intakes of total iron for the groups were 57.8, 17.5, 8.8, 11.8 and 8.0 mg/day respectively. There were group differences in serum ferritin concentration at baseline.

5.64 After 12 weeks, the haemoglobin concentration of the high food-iron and muscle-food supplement group was significantly higher than for all other groups ($p<0.009$). The serum ferritin concentrations of the high food-iron/meat supplement group and the 50 mg/day iron supplement group were significantly higher ($p<0.02$) than that of the control group; serum ferritin concentrations of the high food-iron/meat supplement group were also significantly higher ($p=0.04$) than those of the placebo group.

5.65 Engelmann *et al* (1998) examined the effect of increased meat intake on haemoglobin and serum ferritin concentrations of partially breast fed infants in Denmark (n=41; age, 8 months). Infants were randomised to either a low-meat (10 g/day) or a high-meat (27 g/day) group for 2 months. There were no significant differences in total iron intake between the two groups as infants in the low-meat group consumed more commercial gruel which is fortified with iron. At the end of the intervention period there was a significant difference ($p=0.008$) in the change in haemoglobin concentration which decreased by 4.9 g/L (range, -12.9 to 5.6 g/L) in the low-meat group and by 0.6 g/L (range, -12.1 to 7.3 g/L) in the high-meat group. There was no significant difference in change in serum ferritin concentration between the two groups.

5.66 In the USA, Hunt and Roughead (1999) compared the effect of consuming a lacto-ovovegetarian (0 g/day meat) or non-vegetarian (184 g/day meat) diet for 8 weeks on serum ferritin concentrations of women (n=21; 20–42 years) in a random crossover design. Type of diet was found to have no effect on serum ferritin concentrations.

5.67 Wells *et al* (2003) compared the effect of vegetarian and beef-containing diets on serum ferritin and haemoglobin concentrations of men (n=21; 59–78 years) undergoing resistance training (3 days/week) in the USA. All participants consumed a vegetarian diet supplemented with textured vegetable protein (0.6 g protein/kg body weight/day) for 2 weeks before being assigned to a beef-containing or vegetarian diet for 12 weeks. In addition to the self-selected vegetarian diet, the

beef group received 0.6 g protein/kg body weight/day of beef products and the vegetarian group received the equivalent amount as texturised vegetable protein. After 12 weeks, serum ferritin concentrations significantly decreased in both groups ($p<0.01$). There was a significant increase ($p<0.01$) in haemoglobin concentrations of the beef group compared to the vegetarian group, which remained stable.

5.68 Another study in the USA (Snetselaar *et al*, 2004) compared serum ferritin concentrations of adolescents (n=86; age not specified) randomised to an eating pattern emphasising either beef or poultry and fish for 3 months. Participants were instructed to consume the study meat five times per week and the comparison meat no more than twice a week. After 3 months, the beef group increased their mean consumption of beef by 21 g/day (to 66 g/day) and decreased their mean poultry/fish intake by 13 g/day (to 18 g/day). The poultry/fish group increased their mean consumption of poultry/fish by 8 g/day (to 50 g/day) and decreased their mean beef intake by 4 g/day (to 36 g/day). There were significant differences between groups in the amount of beef and poultry/fish consumed. After 3 months there was a significant difference between groups in serum ferritin concentration which was unchanged for the beef group and decreased for the poultry/fish group.

5.69 In Denmark, Tetens *et al* (2007) compared the impact of a meat- or vegetable-based diet for 20 weeks on the serum ferritin and haemoglobin concentrations of women (n=57; 19–39 years) with low iron status (serum ferritin ≤30 µg/L; haemoglobin ≥120 g/L). Participants allocated to the meat-based diet consumed their usual diet as well as 150 g/day of meat; participants in the vegetable-based diet group consumed vegetable products with energy and iron content similar to that of the meat products and were instructed to consume no more than 250 g of meat/week. At the end of the intervention period, serum ferritin and haemoglobin concentrations were unchanged in the meat-based diet group; there was a significant decline in serum ferritin concentrations ($p<0.001$) and haemoglobin concentrations ($p=0.003$) in the vegetable-based diet group.

5.70 A 7-week controlled feeding study in the USA (Hunt *et al*, 1995) which compared the effect of a high-meat (289 g/day) and low-meat (38.5 g/day) diet on haemoglobin and serum ferritin concentrations of postmenopausal women (n=14; 51–70 years) reported a significant decrease in serum ferritin concentrations with the high-meat diet; haemoglobin concentrations were unaffected by either diet.

Ascorbic acid

5.71 Cook *et al* (1984) reported no change in serum ferritin concentrations of adults (n=17; age, 20–30 years) in the USA, when meals were supplemented with 2 g/day of ascorbic acid (1 g with each of two meals) for 16 weeks. The lack of effect was not due to adaptation to the ascorbic acid as enhancement of iron absorption by ascorbic acid measured from single test meals was observed at the beginning and end of the 16-week period. There was also no significant effect on serum ferritin concentrations of four iron deficient subjects (mean serum ferritin concentration <6 µg/L) who continued to receive ascorbic acid for a further 20 months.

5.72 In a double-blind placebo-controlled trial in Ireland (Malone *et al*, 1986), healthy women (n=58; age, 17–21 years) were supplemented with either 300 mg/day of ascorbic acid (100 mg with each of three meals) or placebo for 8 weeks. At the end of the supplementation period, serum ferritin concentrations were unchanged in both groups.

5.73 In a study by Hunt *et al* (1990) in the USA, premenopausal women (n=11; age, 22–36 years) with low to moderate serum ferritin concentrations (8.5–55 µg/L) were depleted of iron stores by consuming a low-iron diet and undergoing phlebotomy until serum ferritin concentrations were less than 8.5 µg/L. Participants then consumed a low iron bioavailability diet (minimal meat and ascorbic acid, containing 13.7 mg of iron/2000 kcal) which was supplemented with 1500 mg/day ascorbic acid (500 mg with each of three meals) or placebo, for 5.5 weeks in a controlled metabolic environment. At the end of the supplementation period, there was a significant improvement ($p<0.05$) in haemoglobin concentrations of the ascorbic acid supplemented group but serum ferritin concentrations were unchanged.

5.74 In another study by Hunt *et al* (1994a), premenopausal women (n=25; age, 20–45 years) with low serum ferritin concentrations (3.5–17.7 µg/L) consumed either a diet with low iron availability or a typical Western diet for 10 weeks, which was supplemented with 1500 mg/day ascorbic acid (500 mg with each of three meals) or placebo for 5 out of 10 weeks using a double-blind crossover design. Participants were provided with all meals during the study. Ascorbic acid supplementation had no effect on serum ferritin concentration in either dietary group; however, serum ferritin concentrations were slightly higher with ascorbic acid ($p<0.06$) when data from both diets were combined.

5.75 In a placebo-controlled intervention trial in Mexico (Garcia *et al,* 2003), women (n=36; mean age, 28 years) with serum ferritin concentrations <12 µg/L consumed either 50 mg ascorbic acid (as limeade containing 25 mg with each of two meals a day) or placebo, 6 days/week, for 8 months. Stable isotope studies over 14 days had previously shown that this treatment more than doubled iron absorption of women with serum ferritin concentrations <12 µg/L (Diaz *et al*, 2003). After 8 months, no difference was found between the two groups in haemoglobin or serum ferritin concentrations.

Calcium

5.76 Sokoll and Dawson-Hughes (1992) investigated the effect of calcium supplementation (1000 mg/day) on serum ferritin and haemoglobin concentrations of premenopausal women (n=109; age, 18–52 years) in the USA. Calcium (500 mg) was consumed daily with each of two meals for 12 weeks; women in the control group were not given placebo tablets. At the end of the supplementation period, there were no significant differences in serum ferritin or haemoglobin concentrations for the treatment or control groups.

5.77 A randomised double-blind intervention trial in the USA (Ilich Ernst *et al*, 1998) examined the effect of calcium supplementation (1000 mg/day) over 4 years on serum ferritin concentrations of girls (n=354) who were premenarcheal (age 8–13

years) at baseline. Calcium or placebo tablets were taken after breakfast and before bedtime. No significant difference was found in serum ferritin concentration between the placebo and intervention group at 0, 1, 2, 3 and 4 years.

5.78 Kalkwarf and Harrast (1998) examined the effect of 6 months of calcium supplementation on serum ferritin concentrations in lactating and non-lactating women (n=187; mean age, 31 years) in the 6–12 month postpartum period. Half the women in each feeding group were randomly assigned to either 1000 mg/day calcium carbonate or a placebo, in a double-blind design. There were no significant differences between the four groups in baseline haemoglobin concentrations; however, baseline serum ferritin concentrations were significantly higher (p=0.044) in the lactating women[38] (44.0 µg/L) compared to non-lactating women (34.5 µg/L). At the end of the intervention, there was no significant effect of calcium supplementation on serum ferritin or haemoglobin concentrations in either group.

5.79 A study in the UK investigated the effects of calcium supplementation (1200 mg/day) for 6 months on serum ferritin and haemoglobin concentrations of adults (n=24; mean age, 43 years) with serum ferritin concentrations >12 µg/L (Minihane and Fairweather-Tait, 1998). Participants consumed 400 mg of calcium with each of three meals daily. The control group did not receive any dietary intervention. After 6 months, there was no significant effect of calcium supplementation on serum ferritin or haemoglobin concentrations.

5.80 A review examining the effect of calcium supplementation on the iron status of women (Bendich, 2001) concluded that long term consumption of calcium supplements has no effect on various indicators of iron status, including serum ferritin concentration.

Phytate

5.81 Lind *et al* (2003) examined the effect of reducing the phytate content of infant cereals on serum ferritin and haemoglobin concentrations of infants (n=267; age, 6 months). In a double-blind design, infants were randomly allocated to three cereal groups containing different amounts[39] of phytate for 6 months: a commercial milk-based cereal drink (MCD) and porridge (CC group), phytate-reduced MCD and phytate-reduced porridge (PR group); or milk-based infant formula and porridge (IF group). After 6 months, serum ferritin concentrations were significantly lower (p<0.05) in all the groups compared to baseline but there were no differences in serum ferritin concentrations between the three groups. Haemoglobin concentrations were significantly lower (p<0.05) in the CC and PR groups compared to baseline and significantly lower (p=0.015) in the IF than the PR group.

5.82 Bach Kristensen *et al* (2005) examined the effects of long term (4 months) consumption of fibre-rich wheat bread (300 g), prepared with and without phytase (an enzyme that breaks down phytate), on serum ferritin and haemoglobin

38 Post-partum iron stores of lactating women are usually higher than those of non-lactating women because of the delayed return of menstruation.

39 Mean daily intake of phytate for the different diets at 6–8 months was:, 124 µmol/day on the CC diet; 48 µmol/day on the PR diet; and 26 µmol/day on the IF diet. At 9–11 months, mean daily phytate intake was: 189 µmol/day on the CC diet; 36 µmol/day on the PR diet; and 62 µmol/day on the IF diet.

concentrations of women (n=41; age, 19–37 years). The molar ratio of phytic acid to iron was 8.5:1 in the wheat bread and 6.7:1 in the wheat plus phytase bread. At the end of the intervention period, serum ferritin concentrations were significantly decreased ($p<0.001$) in both groups but there was no difference between groups. Haemoglobin concentrations were unchanged in both groups.

Limitations of intervention studies

5.83 Overall, short or long term intervention studies of modulators of dietary iron absorption have not shown corresponding changes in haemoglobin or serum ferritin concentrations. However, this might be expected if the studies were not carried out in populations with an increased need for iron since the systemic need for iron is the most important determinant of iron absorption from the diet. Most studies were carried out in iron replete Western populations who are unlikely to have a physiological response to additional iron in the diet. Participants may already have been consuming diets containing promoters of non-haem iron absorption which would minimise any further effects.

5.84 Although beneficial effects of enhancers and inhibitors of iron absorption on markers of iron status might be expected in studies with iron deficient individuals, the studies which included menstruating women, who tend to have low iron stores due to blood losses, and the study in Mexico of women with low serum ferritin concentration (mean = 6 µg/L) also showed no beneficial effects of modulators of non-haem iron absorption on markers of iron status.

5.85 Most of the intervention studies had small sample sizes (n=11–354) and may not have had sufficient power to detect small or moderate associations.

Fortification iron

5.86 Fortification of foods with iron (addition of iron to foods) has been the main approach used to improve the supply of iron in the diet. Iron has also been added to foods to replace iron lost during processing (restoration) and to ensure nutritional equivalence of products replacing common foods in the diet (e.g., meat substitutes).

5.87 The most common food vehicles used for iron fortification are staples such as wheat flour because they are widely consumed. In developing countries, condiments such as salt, sugar, fish sauce, soy sauce and curry powder have also been used. For infants and children, breast milk substitutes (infant formulas) and cereal-based complementary foods can be fortified with iron.

Food fortification policies

5.88 Iron is lost during the processing of wheat flour. In the UK, mandatory addition of iron to white and brown flour was introduced in 1953 to restore it to the level found in 80% extraction flour. The Bread and Flour Regulations (1998)[40] require all

40 Statutory Instrument 1998 No. 141. United Kingdom.

flour other than wholemeal flour to contain not less than 1.65 mg iron/100 g flour, regardless of intended use. The forms of iron used for fortification of flour include ferric ammonium citrate, ferrous sulphate and elemental iron powder.

5.89 The composition of infant formula and follow-on formula is controlled by European legislation (Directive 2006/141/EC)[41] which stipulates that the iron content of cows' milk-based infant formulas should be between 0.07 and 0.3 mg/100 KJ (0.3–1.3 mg/100 kcal).[42] Regulations also recommend the amount of iron in follow-on formulas and other breast milk substitutes.

5.90 In the UK, many breakfast cereals are fortified on a voluntary basis with levels usually in the range of 70–120 mg/kg (EVM, 2003). Other foods fortified with iron on a voluntary basis include cereal bars, beverages and some weaning foods. Information on the contribution of fortificant iron to iron intakes in the UK is provided in section 9.

Bioavailability of iron fortification compounds

5.91 Intestinal uptake, systemic transfer and utilisation of iron fortificants are subject to the same regulatory factors that affect iron found naturally in foods, i.e., systemic need for iron, composition of the diet, and the physico-chemical characteristics of the fortificant (Hurrell, 1997).

5.92 The iron compounds used for fortification can be divided into four groups depending on their solubility in water and acid: freely water-soluble (ferrous sulphate); poorly water-soluble but soluble in dilute acids such as gastric juice (ferrous fumarate); water-insoluble and poorly soluble in dilute acid (ferric pyrophosphate, elemental iron powders); and protected iron compounds (NaFeEDTA[43]) (Hurrell, 1997).

5.93 Iron fortificants differ in their relative bioavailability and their potential to cause unfavourable sensory changes such as discoloration of the food vehicle and rancidity. The water-soluble iron compounds are the most bioavailable. Compounds which are water-insoluble but soluble in acid are also well absorbed as they dissolve in gastric juice. Elemental iron powders and iron phosphate compounds, which are water-insoluble and poorly absorbed in dilute acid, have the lowest and most variable bioavailability (Hurrell, 2002).

5.94 The bioavailability of iron fortification compounds is usually compared with that of ferrous sulphate (Hurrell, 2002). Widespread use of ferrous sulphate is limited because it can cause unfavourable colour changes and rancidity in the food vehicle if it is stored for long periods. It is usually used to fortify foods stored for short periods, such as breast milk substitutes.

41 Implemented by the Infant Formula and Follow-on Formula Regulations (England) 2007 and equivalent regulations in the devolved administrations.

42 For a typical infant breast milk substitute with an energy content of 680 kcal/L, this is equivalent to 2.04–8.84 mg/L of iron.

43 Sodium iron ethylenediaminetetraacetate.

5.95 Elemental iron powders (electrolytic, hydrogen/carbon monoxide reduced, carbonyl) are most widely used for fortification of cereal products since they do not cause organoleptic changes, which increases their consumer acceptance and shelf-life. The different processes used to produce elemental iron powders affect their shape, surface area and porosity. These differences influence their solubility in gastric acid and, consequently, their bioavailability (Hurrell, 1997). An expert panel[44] which considered the nutritional benefit of elemental iron for cereal flour fortification concluded that only electrolytic iron powder had been demonstrated to be a useful fortificant (Hurrell *et al,* 2002). This was based on limited evidence: an efficacy study of infants (Walter *et al,* 1993); a human bioavailability study which found that absorption of electrolytic iron powder was 75% of that of ferrous sulphate (Forbes *et al,* 1989); and rat haemoglobin repletion studies (Hurrell *et al,* 2002).

5.96 Wheat flour is one of the most difficult vehicles to fortify with iron because it contains high levels of phytates. Although iron fortification of wheat flour is practised in several countries, the beneficial effect of this health measure on iron status is not clear (see paragraphs 5.106–5.110). Hydrogen reduced elemental iron is used to fortify flour in the UK and is also added to breakfast cereals and other fortified food products. Lack of evidence about the effectiveness of iron fortification of flour on iron status in the UK led to a recommendation by COMA in 1981 that fortification of flour with iron should no longer be mandatory (DH, 1981). Studies which have investigated the effect of iron fortification on markers of iron status are considered in paragraphs 5.98–5.102.

5.97 The WHO/FAO (2006) recommends that the order of priority for iron compounds used for fortification should be ferrous sulphate, ferrous fumarate, encapsulated ferrous sulphate/fumarate, electrolytic iron at twice the dose of ferrous sulphate/fumarate, ferric pyrophosphate at twice the dose of ferrous sulphate/fumarate, and NaFeEDTA for high-phytate cereal flours.

Effect of iron fortification on iron status

Efficacy trials of iron fortification

5.98 The majority of trials examining the effect of iron fortified foods on markers of iron status were conducted in developing countries. Results from these trials suggest that water-soluble iron compounds such as ferrous sulphate added to salt (Zimmermann *et al*, 2003) or wheat flour (Zimmermann *et al*, 2005; Sun *et al*, 2007), and iron chelators such as NaFeEDTA added to soy sauce (Chen *et al*, 2005) or wheat flour (Andang'o *et al*, 2007; Sun *et al*, 2007), are associated with an improvement in markers of iron status.

5.99 A number of trials have examined the efficacy of elemental iron powders, which are most widely used to fortify flour in national fortification programmes. Details of these trials, including sample size, duration, and baseline iron status of participants,

44 SUSTAIN (Sharing United States Technology to Aid in the Improvement of Nutrition) is a non-profit organisation whose mission is to improve nutrition in developing countries through applications of food science and technology.

are provided in Annex 5 (Table A6). Seven out of eight trials reported efficacy of electrolytic iron and three reported efficacy of hydrogen-reduced iron. All reported on haemoglobin concentration and six on serum ferritin concentration. The amount of additional iron provided by the foods (mainly wheat flour) fortified with elemental iron ranged from 3.7 to 20 mg/day.

5.100 Results from these trials are inconsistent. Five out of eight trials found no significant effect on haemoglobin concentration (Nestel *et al*, 2004; van Stuijvenberg *et al*, 2006; Andang'o *et al*, 2007; van Stuijvenberg *et al*, 2008; Biebinger *et al*, 2009), and five out of six trials found no significant effect on serum ferritin concentration (van Stuijvenberg *et al*, 2006; Andang'o *et al*, 2007; Sun *et al,* 2007; van Stuijvenberg *et al*, 2008; Biebinger *et al,* 2009). Two trials reported a significant improvement in haemoglobin concentration with electrolytic iron (Walter *et al*, 1993; Sun *et al*, 2007) and one trial reported a significant increase in serum ferritin concentration with both electrolytic and reduced iron (Zimmermann *et al,* 2005).

5.101 Baseline status of trial participants did not influence the amount of iron absorbed from foods fortified with elemental iron. Findings from a short term absorption study (Moretti *et al,* 2006) suggest that adaptive up regulation of iron absorption in response to low iron status is less effective for poorly water-soluble iron compounds than for readily soluble compounds such as ferrous sulphate. Iron deficient individuals may be less able to increase iron absorption of elemental iron because of the poor dissolution of the iron powders in the gastric contents.

5.102 The additional doses of iron provided by fortification ranged from 10 to 20 mg/day in the studies that found a significant improvement in haemoglobin or serum ferritin concentration, and 3.7 to 14 mg/day in studies that reported no effect.

Trials comparing fortified versus unfortified breast milk substitutes for prevention of iron deficiency in infants

5.103 Iron fortified breast milk substitutes and complementary foods are frequently recommended to combat iron deficiency during infant development. In the EU, all infant breast milk substitutes are fortified with iron to a typical level of 6–7 mg/L (see Annex 4 for recommendations for the iron content of infant formulas and follow-on formulas). Findings from studies investigating the effectiveness of iron fortified breast milk substitutes have proved equivocal.

5.104 Stevens and Nelson (1995) reported no difference in mean haemoglobin or median serum ferritin concentrations of infants aged 6 months (n=92) after being fed either iron fortified with ferrous sulphate (12 mg/L iron) or unfortified (1 mg/L iron) follow-on milk for 12 months. There was also no difference between groups in the proportions with anaemia (haemoglobin <110 g/L) or low serum ferritin concentrations (<10 µg/L).

5.105 Gill *et al* (1997) investigated the efficacy of iron fortified follow-on formula in preventing iron deficiency in infants (n=406) aged 6 months who were receiving either infant formula or unmodified whole cows' milk. Those receiving formula were randomly assigned to either iron fortified (12.3 mg/L iron) or non-fortified (1.4

mg/L iron) formula for 9 months. Infants already receiving cows' milk continued with this feed. Significant differences between the groups were observed at 15 months for haemoglobin (<110 g/L in 33% of infants fed cows' milk, 13% in those fed non-iron fortified formula, 11% in those fed iron fortified formula) and serum ferritin concentrations (<10 µg/L in 43% of infants fed cows' milk, 22% and 6% in those fed non-iron fortified formula and iron fortified formula respectively).

Effectiveness of countrywide iron fortification strategies

5.106 Although iron fortification of cereal flour (wheat and maize) and other food products is practised in several countries as a strategy to combat iron deficiency, there is limited evidence of a beneficial effect on iron status at a population level. No large-scale fortification programmes have formally evaluated their impact on iron status.

5.107 In Denmark, flour was fortified with carbonyl iron (30 mg/kg flour) from 1954 until the discontinuation of mandatory fortification in 1987. A study which compared serum ferritin concentrations of a cohort of men and women (n=238; age, 35–65 years) in 1987/88 and six years later (1993/94), reported a significant increase in postmenopausal women and an increasing trend over time for men (p=0.07) (Osler *et al*, 1999). A population survey of Danish men reported no difference in the prevalence of depleted iron stores (serum ferritin <16 µg/L) and iron deficiency anaemia (serum ferritin <13 µg/L; haemoglobin <129 g/L) in 1994 (n=1332; age, 40–70 years) compared with 1984 (n=1044; age, 30–60 years) (Milman, 1999). The prevalence of depleted iron stores (serum ferritin <16 µg/L) and iron deficiency anaemia (serum ferritin <13 µg/L; haemoglobin <121 g/L) in women in 1994 (n=1319; age, 40–70 years) and 1984 (n=880; age, 30–60 years) was also unchanged (Milman *et al*, 2000).

5.108 In Venezuela, mandatory fortification of corn flour with ferrous fumarate (50 mg/kg flour) and voluntary fortification of wheat flour (20 mg ferrous fumarate/kg flour) was introduced in 1993. Surveys of schoolchildren (aged 7, 11 and 15 years) before fortification in 1992 (n=282) and one year after fortification in 1994 (n=317) showed a significant reduction in the prevalence of iron deficiency (serum ferritin <12 µg/L) from 37 to 16%, and in the prevalence of anaemia (defined as haemoglobin: 115 g/L, children 7 years; 120 g/L, females 11 and 15 years; 125 g/L, males 11 years; 130 g/L, males 15 years) from 19 to 9%. There was no significant difference in the prevalence of iron deficiency anaemia (haemoglobin as above and serum ferritin <12 µg/L) between 1992 and 1994 (Layrisse *et al*, 1996). Later surveys, however, in 1997, 1998 and 1999 reported that the prevalence of anaemia had increased to pre-fortification levels while serum ferritin concentrations were unchanged (Layrisse *et al*, 2002).

5.109 In Brazil, fortification of wheat flour with iron (42 mg/kg flour; type of iron not specified) became mandatory in 2004. A study which assessed the impact of iron fortification on haemoglobin concentrations of children under 6 years of age reported no effect at 12 and 24 months post-fortification (Assunção *et al*, 2007).

5.110 The limited impact of iron fortification programmes on markers of iron status may be due to a number of factors including widespread use of elemental iron powders which are poorly absorbed, insufficient intakes of the fortified food, or inadequate level of fortification. The impact of iron fortification will also depend on the proportion of anaemia in the population that is due to iron deficiency.

Supplements

5.111 Many non-haem iron supplements are available over the counter from chemists, supermarkets and health food shops. The most common forms are ferrous sulphate, ferrous fumarate, ferrous gluconate, ferrous glycine sulphate and iron polysaccharide (Fairweather-Tait and Teucher, 2002). The bioavailability differs, but all are generally better absorbed than slow-release capsules or multivitamin/multimineral supplements (Dawson *et al*, 1998).

5.112 Iron supplements are usually used as a short term measure to provide extra iron when iron levels are low. Commercially available prophylactic doses used to prevent deficiency usually range between 7 and 50 mg/day. Supplemental intakes above the Guidance Level[45] of 17 mg/day are not advised in the UK (EVM,[46] 2003). During pregnancy, iron supplements are recommended for women with haemoglobin concentrations outside the normal UK range for pregnancy (i.e., 110 g/L during the first trimester and 105 g/L at 28 weeks) (NICE, 2008).

5.113 Information on the contribution made by supplements to iron intake in the UK can be found in section 9.

The effect of vegetarian diets on iron status

5.114 Vegetarian diets contain, almost entirely, non-haem iron and generally higher quantities of inhibitors of iron absorption, such as phytate (associated with legumes, including soy, and whole-grain cereals).

5.115 Several, mostly small, cross-sectional studies, have compared the iron intake and/or iron status markers of vegetarians with, otherwise broadly similar, non-vegetarians (see Annex 5, Table A7). Results from these studies show that dietary iron intakes of vegetarians are on average similar, or sometimes higher, than those of non-vegetarians. In the Oxford cohort of the European Prospective Investigation of Cancer and Nutrition (EPIC), estimated iron intakes among women (n=43,582) were 12.6, 12.8, 12.6 and 14.1 mg/day for meat eaters, fish eaters, lacto-ovovegetarians and vegans respectively (Davey *et al*, 2003).

45 The Guidance Level is the amount that would not be expected to cause any adverse effects in the majority of people (see paragraph 7.1).

46 Expert Group on Vitamins and Minerals.

5.116 A comparison of the haemoglobin concentrations of vegetarians and non-vegetarians shows that they are similar in the two diet groups; however, in several studies, mean values are slightly lower in vegetarians. Although serum ferritin concentrations are consistently significantly lower in vegetarians compared to non-vegetarians, they are usually within the reference ranges.

5.117 An intervention study (Hunt and Roughead, 1999) comparing the effect of consuming a lacto-ovovegetarian or non-vegetarian diet for 8 weeks on serum ferritin concentrations of women (n=21; age, 20–42 years) in a random crossover design (see paragraph 5.66) reported that the type of diet had no effect on serum ferritin concentrations.

Summary and conclusions

5.118 Iron is present in foods as haem or non-haem iron compounds. Haem iron is found almost entirely in foods of animal origin as haemoglobin and myoglobin. Non-haem iron is found in animal and plant tissues.

5.119 The efficiency of intestinal absorption of iron from food is principally influenced by systemic iron needs. More iron is absorbed from the diet in a state of iron deficiency and less is absorbed when iron stores are replete.

5.120 The bioavailability of dietary iron, i.e., the amount available for systemic utilisation, is affected by the chemical form of iron. The absorption of haem iron from the diet is more efficient than the absorption of non-haem iron.

5.121 Evidence from single meal studies suggests that a number of dietary components increase or reduce non-haem iron bioavailability. The main enhancers of non-haem iron absorption are meat and ascorbic acid (found in fruit and vegetables). The main inhibitors of non-haem iron absorption are calcium, phytates (found in cereals and legumes) and phenolic compounds (found in tea, coffee and other beverages).

5.122 The individual effects of dietary factors which influence iron absorption may be reduced when they are consumed as part of a whole diet. This might reflect interactions between the various ligands for iron and their combined influence on its presentation for mucosal uptake. Additionally, evidence from whole diet studies over a number of days or weeks, suggests that the overall effect of enhancers and inhibitors on iron absorption is considerably less than predicted from single meal studies; this is probably because of mucosal adaptation.

5.123 The effects of dietary modulators of non-haem iron absorption on markers of iron status (usually haemoglobin and serum ferritin concentration) are difficult to ascertain in epidemiological studies. An important consideration is the difficulty of obtaining accurate exposure data because of the quality of dietary assessments, limited food composition data for some modifiers of iron absorption, and interactions between enhancers and inhibitors of non-haem iron absorption. Observational studies are also affected by a number of confounding factors such as disease which can raise serum ferritin concentrations. Such studies usually assume a direct relationship between markers of iron status and risk of iron deficiency or excess; however,

markers of iron status are influenced by homeostasis and the relationship between exposure to dietary iron and body burden across the spectrum of iron status is not linear.

5.124 Evidence from a limited number of prospective studies suggests that dietary inhibitors and enhancers of iron absorption do not substantially influence iron status.

5.125 Long term intervention studies of enhancers and inhibitors of iron absorption have, overall, not shown a corresponding change in iron status parameters. A measurable effect of dietary modulators may only be observed in individuals with increased systemic iron needs and, as a consequence, higher absorptive capacity. Most intervention studies were carried out in iron replete Western populations who are less likely to have a physiological response to additional dietary iron. It is also possible that the lack of effect on serum ferritin concentration is because of relative insensitivity of serum ferritin concentration to changes in iron depots.

5.126 The lack of sustained effect of enhancers and inhibitors on markers of iron metabolism and use raises uncertainties regarding the relevance of iron bioavailability for meeting iron requirements in the UK and of specific dietary advice regarding enhancers and inhibitors of iron absorption. With regard to calcium, which has been shown to inhibit iron absorption in single meal studies, dietary advice to reduce consumption on this basis would have to be balanced against its dietary essentiality for skeletal development and maintenance.

5.127 The main determinant of the amount of dietary iron absorbed by the body is the systemic need for iron. Absorption will also be influenced by the absolute amount of iron in the diet and the overall composition of the meal rather than a single food item that enhances or inhibits iron absorption. UK and Western diets include a broad range of foods containing iron. They also include various modulators of iron absorption which may lead to complex interactions between enhancers and inhibitors of iron absorption. Consequently, the bioavailability of dietary iron may have little influence on iron status of the UK population.

5.128 Although the systemic need for iron is the main determinant of the amount of iron absorbed from the diet, iron bioavailability could become a limiting factor in certain circumstances (e.g., for individuals with an increased need for iron, particularly when the iron content of the diet is low). The effects of enhancers and inhibitors of iron absorption may also be more important in developing countries where populations are at greater risk of iron intakes insufficient to meet requirements since diets are plant-based, more limited and monotonous; they also contain higher levels of inhibitors, lower levels of enhancers, and less haem iron. Under these circumstances, the imbalance between requirements and absorption may lead to iron deficiency.

5.129 In the UK, addition of iron to white and brown wheat flour is mandatory. A number of foods, including breakfast cereals, are fortified with iron on a voluntary basis. The iron compounds used for fortification vary in their relative bioavailability and their potential to cause unfavourable sensory changes to the food vehicle. Elemental iron powders, which are less soluble than other iron fortificants and have the

lowest bioavailability, are widely used to fortify foods and for flour fortification programmes because they do not cause organoleptic problems during storage. Evidence from efficacy trials and experience of countrywide fortification strategies suggest that foods fortified with this form of iron may be of little practical use in improving iron status.

5.130 Breast milk substitutes in the UK, which are also fortified with iron on a mandatory basis, are often recommended to prevent iron deficiency during infant development; however, their usefulness in improving iron status of infants is uncertain.

5.131 Studies comparing the iron intake and status of vegetarians with non-vegetarians have generally shown no significant differences in dietary iron intake or haemoglobin concentrations. Although serum ferritin concentrations are consistently statistically significantly lower in vegetarians, they are usually within the reference ranges.

5.132 The inter-relationship between the different factors that affect iron absorption such as individual iron requirements, inter-individual variability in absorption, iron intake and dietary type (i.e., haem or non-haem) will influence iron status. Overall, despite extensive study of the factors that influence the bioavailability of iron, it is not possible to quantify predictively the bioavailability of iron from a diet or from particular foods. However, the available data enable a qualitative ranking of the effects of various foods on the efficiency of iron absorption from foods and diets that can inform dietary strategies.

6 Health consequences of iron deficiency

Physiological consequences of iron deficiency

Metabolic response to iron deficiency

6.1 Iron deficiency results in functional defects which arise from reduced synthesis of haemoglobin and myoglobin with impaired peripheral distribution of oxygen and reduction in the activities of the iron dependent enzymes (see Annex 2). Many metabolic and biochemical processes and pathways, especially oxidative metabolism, are affected which may lead to suboptimal physiological function and ill health. Of particular concern are effects on physical work capacity, reproductive efficiency, cognitive and psychomotor development (which are considered in this section) and immune function and infection (which are considered separately in section 8).

Development of iron deficiency

6.2 The development of iron deficiency can be considered in three stages (Charlton and Bothwell, 1982) corresponding to the sequential effects on the systemic iron stores, the supply of iron to the tissues, and the subsequent impairment of iron dependent functions. As iron deficiency becomes more severe relative to the systemic needs, the tissue ferritin depots become depleted and there is a concomitant and an increased risk of defective functional outcomes.

6.3 Depletion of cellular iron depots leads to mobilisation of iron depots from macrophages or hepatocytes, up regulation of iron uptake and transfer by the intestine, decreased serum ferritin concentration, and reduced stainable iron in bone marrow smears.

6.4 Impaired supply of iron to tissues results in a decrease in serum iron concentrations, a progressive fall in serum transferrin saturation, and an increase in serum transferrin concentration, in numbers of transferrin receptors on tissue surfaces, and in concentrations of serum transferrin receptor. Decreases in transferrin saturation below 16% lead to defective erythropoiesis (Bainton and Finch, 1964).

6.5 Progressive iron deficiency leads to a decrease in haemoglobin concentrations and in circulating numbers of precursor red cells (reticulocytes), and iron dependent functions are affected (Hercberg and Galan, 1989).

Causes of iron deficiency and anaemia

6.6 Globally, anaemia is one of the most prevalent nutritional deficiency diseases. Iron deficiency is one possible cause of anaemia. However, in the literature, anaemia is often equated with iron deficiency without full characterisation of the anaemia and of the assumed nutritional iron deficiency.

6.7 Iron deficiency is not necessarily caused by an inadequate dietary intake of iron or reduced dietary availability of iron. Other causes of iron deficiency and anaemia include impaired absorption and increased blood losses due to menstruation or gastrointestinal blood loss caused by gastrointestinal disease (Rockey and Cello, 1993). Gastrointestinal blood loss, associated with use of non-steroidal anti-inflammatory drugs such as aspirin, may be an important cause of iron deficiency in older people (Fleming *et al,* 2001). In many parts of the world, haemolysis caused by malaria (Fleming, 1981; WHO, 2000) and intestinal blood loss caused by helminthiasis (Roche and Layrisse, 1966; Crompton and Nesheim, 2002) are major causes of anaemia but are of less relevance in the UK.

6.8 Although iron deficiency anaemia may respond to iron supplements, these do not necessarily treat the underlying primary cause and may not treat the entire nutritional deficit. However, this is the nutritional state in which the adverse effects attributed to iron deficiency are studied.

Iron and physical work capacity

6.9 Since iron is required for oxidative energy production, it is proposed that anaemia (irrespective of its cause) and iron deficiency affect work capacity by two separate mechanisms: anaemia reduces the oxygen transport capacity of the circulation, which impairs aerobic capacity, while iron deficiency reduces tissue oxidative capacity, which impairs endurance capacity and energetic efficiency of muscles (Davies *et al*, 1984). These impairments could lead to reductions in work productivity and voluntary activity, with economic and social implications.

6.10 A number of studies in animals and humans have investigated the relationship between iron deficiency and work capacity. In many of these studies, iron deficiency is poorly characterised and anaemia is often assumed to be caused by iron deficiency without corroborative data (measurement of markers of iron deficiency or sufficiency, such as serum ferritin concentration). There are also difficulties in grading iron deficiency and thresholds of iron marker values associated with adverse outcomes because the available data are presented discontinuously in bands or categories of values. This problem is evident in all the health consequences of iron deficiency considered in this section. In addition, most studies were carried out in developing countries where there are multiple nutritional and socioeconomic deprivations which could also affect physical work capacity.

6.11 The evidence for a causal relationship between iron deficiency and physical work capacity has been systematically reviewed by Haas and Brownlie (2001) who evaluated both animal (nine studies) and human studies (five cross-sectional studies; 15 intervention trials). Five aspects of work capacity were examined: aerobic capacity, endurance capacity, energetic efficiency, voluntary activity and work productivity. The human studies were divided into laboratory studies and field studies of workers in Africa, China, Indonesia, Venezuela and Sri Lanka. Findings from this review are summarised below.

Aerobic capacity

6.12 Aerobic capacity is assessed using the maximum oxygen consumption (VO_2 max) test which measures oxygen uptake at maximum physical exertion, usually on a treadmill or cycle ergometer. The Harvard step test is commonly used in the field and involves measuring heart rate responses to a fixed workload.

6.13 Studies in animal models have demonstrated an association between haemoglobin concentration and aerobic capacity, with severity of anaemia directly proportional to the degree of impairment in aerobic capacity. Perkkio *et al* (1985a) demonstrated a nonlinear relationship between haemoglobin and aerobic capacity: a decrease in haemoglobin concentration from 140 to 80 g/L was accompanied by a linear decline (of about 16%) in aerobic capacity; below 70 g/L, however, the decline was much steeper, suggesting a threshold below which aerobic capacity cannot be sustained. Iron supplementation of iron deficient rats was found to return aerobic capacity and haemoglobin concentration to control values after 3 days, whilst endurance and muscle oxidative enzymes returned to control values after 5 days (Davies *et al*, 1982). In another study (Davies *et al*, 1984), inducing iron deficiency anaemia (haemoglobin <80 g/L) reduced aerobic capacity by 50%, which was restored to control values after iron supplementation. Findings from these studies suggest that haemoglobin is the primary determinant of aerobic capacity.

6.14 Phlebotomy studies in humans suggest that decreases in haemoglobin concentrations are associated with impaired aerobic capacity, which is proportional to the severity of haemoglobin reduction. Woodson *et al* (1978) reported that an acute reduction in mean haemoglobin concentration from 153 g/L to 104 g/L reduced aerobic capacity by 16% and that the decrease was proportional to the haemoglobin reduction. In another study (Celsing *et al*, 1986), reduction in mean haemoglobin concentration from 146 g/L to 110 g/L was associated with an 18% reduction in aerobic capacity. Iron deficiency without anaemia was not associated with impaired aerobic capacity (Klingshirn *et al*, 1992; Lukaski *et al*, 1991; Newhouse *et al*, 1989; Zhu and Haas, 1998).

6.15 Findings from two randomised double-blind placebo-controlled trials (Gardner *et al*, 1975; Ohira *et al*, 1979) reported improvements in various measures of aerobic activity after iron treatment (intramuscular or intravenous injection of iron dextran) of individuals with low haemoglobin (60–80 g/L). The studies did not assess iron deficiency without anaemia.

Endurance capacity

6.16 Endurance capacity is the maximum length of time an individual can sustain a given workload and is dependent on both oxygen delivery and oxygen use capacities of the working muscle. It is assessed by progressively increasing exercise intensity during fixed intervals of long duration and measuring time to exhaustion or by using shorter tests with higher workloads. Another test measures submaximal work on a cycle ergometer in which resistance and number of revolutions are fixed but pedal speed is at the subject's discretion (Zhu and Haas, 1998; Hinton *et al*, 2000).

6.17 All of the reported animal studies observed a significant association between haemoglobin concentration (<120 g/L) and endurance capacity. Edgerton *et al* (1972, 1977) and Ohira *et al* (1981) reported a significant correlation between run time to exhaustion and haemoglobin concentration. Perkkio *et al* (1985b) reported that endurance capacity was correlated with cytochrome c concentration[47] and that the relationship became stronger with decreasing concentration, supporting the suggestion that reduced oxidative capacity mediates the impairments in endurance.

6.18 Endurance capacity is difficult to measure accurately in human studies because performance is highly dependent on subject motivation.

6.19 Four trials considered the effect of iron deficiency without anaemia (haemoglobin >120g/L) on endurance capacity (Celsing *et al*, 1986; Rowland *et al*, 1988; Klingshirn *et al*, 1992; Zhu and Haas, 1998). Only one of these (Rowland *et al*, 1988) reported an association between iron deficiency without anaemia and endurance capacity: an increase in mean serum ferritin concentration (from 8.7 to 26.6 µg/L) was associated with improvements in endurance capacity ($p<0.01$).

6.20 Hinton *et al* (2000) observed that iron supplementation (20 mg/day for 6 weeks) of non-anaemic iron deficient women (haemoglobin >120 g/L, serum ferritin <16 µg/L; n=42) for 4 weeks increased endurance capacity (time to complete a 15 km cycle ergometer test) compared to the placebo group when controlling for pretreatment levels. Iron supplementation significantly increased ($p<0.05$) serum ferritin in the supplemented group (14.5 µg/L) compared to the placebo group (8.11 µg/L); however, all participants had also undertaken an aerobic training regimen during the trial and endurance capacity was significantly improved in both supplemented and placebo groups compared to baseline.

Energetic efficiency

6.21 Energetic efficiency is the amount of energy required to perform a given amount of external work. Energy expenditure is usually measured by calorimetry and external work is assessed at the same time by physical work on a cycle ergometer or a treadmill. In field studies, energy expenditure is measured by monitoring heart rate, and work output is measured by practical items of output (e.g., amount of tea picked).

6.22 A double-blind randomised trial (Zhu and Haas, 1998) of iron deficient women (haemoglobin >120 g/L, serum ferritin <16 µg/L; n=37) reported that iron supplementation (27 mg/day) for 8 weeks significantly reduced total energy expended during a fixed-distance cycle ergometer test and that energetic efficiency was significantly related to serum ferritin concentration.

6.23 In a 12-week trial of Chinese female cotton mill workers (n=80) with iron deficiency (haemoglobin ≥120 g/L; serum ferritin <12 µg/L) and iron deficiency anaemia (haemoglobin <120 g/L; serum ferritin <12 µg/L), earnings per unit of energy expended over 8 hours of work were significantly improved in the group

47 Cytochrome c is a marker of the oxidative capacity of muscle mitochondria.

supplemented with iron (60 mg/day if haemoglobin ≥100 g/L, serum ferritin <12 µg/L; 120 mg/day if haemoglobin <100g/L, serum ferritin <12 µg/L) compared to the placebo group, resulting in an increase in production efficiency of 17% (Li *et al*, 1994). The iron supplemented group also reported an increase in time engaged in leisure activities and an increase in energy expended during those activities.

Voluntary activity

6.24 Iron deficiency may affect voluntary activity by causing early fatigue. Voluntary activity is assessed by activity wheels in animal studies and by time allocation questionnaires and heart rate monitoring in human studies.

6.25 Two studies in rats assessed the relationship between haemoglobin concentration and voluntary activity (Edgerton *et al*, 1972; Hunt *et al*, 1994b). Both reported significant reductions in voluntary activity after inducing iron deficiency and iron deficiency anaemia (by feeding iron deficient diets) which was directly related to haemoglobin concentration. Edgerton *et al* (1972) reported that voluntary activity began diverging from control values (130 g/L) at haemoglobin concentrations of 70–80 g/L; Hunt *et al* (1994b) reported that voluntary activity was reduced in rats with iron deficiency (haemoglobin, 152 g/L, liver iron 1.36 µg/g dry weight) compared to control animals (haemoglobin, 159 g/L, liver iron 4.58 µg/g dry weight), which was reduced further by iron deficiency anaemia (haemoglobin, 52 g/L, liver iron 0.79 µg/g dry weight).

6.26 Edgerton *et al* (1979) observed that voluntary activity of Sri Lankan female tea plantation workers (haemoglobin, 110 g/L; n=18) was increased after 2–3 weeks of iron supplementation (40 mg/day). Li *et al* (1994) also reported an increase in voluntary activity of iron supplemented female cotton mill workers (see paragraph 6.23).

Work productivity

6.27 Productivity has been measured in jobs that involve producing a commodity that can be easily quantified over a specified time. Studies which evaluated the effects of iron deficiency on economic productivity were all conducted in developing countries and include studies of rubber tree tappers, tea pickers, cotton mill workers and cigarette rollers.

6.28 An important problem with these studies is that productivity is influenced by a number of factors. For example, it can be affected by motivation which is strongly influenced by production incentives which could, in turn, have an effect on the effort expended. Weaker people could achieve the same productivity as stronger people by expending more energy at work. Type of labour can also affect the mechanism by which iron affects productivity: physically demanding work requiring high aerobic capacity could be impaired by anaemia, while less strenuous work could require greater endurance and might be affected by iron deficiency. Impaired productivity during shorter, more physically demanding work may be easier to assess than longer, less strenuous tasks which may be affected by motivation.

6.29 Cross-sectional studies have reported significantly higher work productivity in female cigarette rollers (n=230) with haemoglobin concentrations above 120 g/L compared to those with haemoglobin less than 120 g/L (Untoro *et al*, 1998) and in female jute factory workers (n=92) with haemoglobin concentration >110 g/L compared to those with haemoglobin <110g/L (Scholz *et al*, 1997).

6.30 A trial in Indonesia (Basta *et al,* 1979) reported a 17% increase ($p<0.05$) in work output of anaemic rubber tree tappers (haemoglobin <130 g/L; n=302) receiving iron (100 mg/day for 60 days) compared to those receiving a placebo. In another study of anaemic female tea pickers (haemoglobin 102–114 g/L; n=199) in Sri Lanka (Edgerton *et al*, 1979), significantly more tea was picked by workers supplemented with iron (40 mg/day for 30 days) compared to those who had received placebo. Li *et al* (1994) reported a 17% significant increase ($p<0.01$) in the production efficiency (productivity/energy expended) of Chinese female cotton mill workers (n=80) after 12 weeks of iron supplementation (60 mg/day if haemoglobin ≥100 g/L, serum ferritin <12 µg/L; 120 mg/day if haemoglobin <100g/L, serum ferritin <12 µg/L) compared to the change in the placebo group; however, there were no differences in productivity. Although the women were paid by the quantity and quality of yarn produced, productivity was limited by the speed of the machines used in the mill.

Other studies

6.31 Studies since the review by Haas and Brownlie (2001) have mainly examined the effect of iron deficiency on endurance capacity during aerobic exercise training. In a double-blind randomised controlled trial (Brutsaert *et al,* 2003), iron supplementation (20 mg/day for 6 weeks) of iron depleted women (haemoglobin >110 g/L; serum ferritin <20 µg/L; n=20) was associated with a significant improvement ($p=0.01$) in muscle fatigue resistance in the iron supplemented group but not in the placebo group; however, there was no improvement in measures of iron status. In another randomised controlled trial (Brownlie *et al*, 2004), iron depleted women (haemoglobin >120 g/L; serum ferritin <16 µg/L; n=41) were supplemented with either iron (16 mg/day) or placebo for 6 weeks; significant treatment effects of iron supplementation were observed in participants with baseline transferrin receptor concentration >8.0 mg/L. Hinton and Sinclair (2007) examined the effect of iron supplementation (30 mg/day for 6 weeks) compared to placebo on iron deficient men (haemoglobin >130 g/L; serum ferritin <16 µg/L; n=3) and women (haemoglobin >120 g/L; serum ferritin <16 µg/L; n=17) with previous aerobic exercise training. Iron supplementation significantly increased serum ferritin concentration ($p=0.01$) and endurance capacity ($p=0.01$) compared to placebo treatment.

6.32 Gera *et al* (2007) systematically reviewed randomised placebo-controlled trials investigating the effect of iron supplementation on physical performance (assessed by heart rate, treadmill endurance time, blood lactate levels or oxygen consumption) in children and adolescents. The pooled analysis showed beneficial effects of iron supplementation on blood lactate levels and treadmill endurance time. The findings were not considered conclusive due to the limited data: only three trials (n=106) were included in the analysis; details were not available about other factors affecting

physical performance, e.g., energy adequacy or illness; and participants in one of the trials were adolescent athletes who had received one month of physical training, which is also likely to improve physical performance.

Summary and conclusions

6.33 Evidence from animal models and human studies suggests that decreases in haemoglobin concentration are associated with impairments in various aspects of physical work capacity, including aerobic capacity, endurance capacity, energetic efficiency, voluntary activity and work productivity. Clear thresholds cannot be determined because of interspecies differences, uncertainties in the analyses of ferritin and haemoglobin, variabilities in the study participants, and also difficulties in measuring the outcomes, and in combining data from various studies because of the way in which the data are presented. A grey area within which functional defects have been observed are haemoglobin concentrations at or below 110–120 g/L and ferritin concentrations at or below 16–20 µg/L. These values accord with cut-offs below which beneficial effects of iron supplementation on work capacity have been reported. However, the absence of dose-response data makes it difficult to derive diagnostic thresholds.

6.34 Studies in humans suggest that a reduction in haemoglobin concentration below about 110 g/L is associated with reduced aerobic capacity, although there is no clear threshold for this effect. There is no clear evidence that iron deficiency in the absence of anaemia has adverse effects on physical work capacity.

6.35 Although there is a limited amount of evidence that iron deficiency in the absence of anaemia (haemoglobin >120 g/L; serum ferritin <16 µg/L) might impair endurance capacity, this needs further substantiation.

6.36 Overall there are insufficient data to assess the effects of iron deficiency or iron deficiency anaemia on energetic efficiency or voluntary activity.

6.37 Very few studies have examined the effects of anaemia (all-cause and iron deficiency anaemia) on work productivity and all have been conducted in developing countries in subjects with haemoglobin concentrations below those usually observed in the UK. Overall, there are insufficient data to assess the effects of iron deficiency on work productivity.

6.38 There are a number of uncertainties and difficulties in interpreting the data examining the relationship between iron status and physical work capacity. In addition to physiological factors, physical work capacity can be influenced by social, economic and motivational factors. Most field studies have been carried out in developing countries where populations are associated with multiple deprivations (nutritional, social and economic) which can all affect work capacity. Studies have also used different criteria to classify iron deficiency/iron deficiency anaemia, treatment duration and dose have varied considerably, a number of different test protocols have been used, and sample sizes were very small in most studies.

Maternal iron status and pregnancy outcome

6.39 During pregnancy, iron is needed to support fetal growth and development, the placenta, expansion of maternal red cell mass and to cover the iron lost in blood during delivery (see paragraphs 3.35–3.38). In normal pregnancy, plasma volume increases steadily until 32–34 weeks causing a fall in maternal haemoglobin concentration although red cell mass actually expands during the second and third trimesters. Separate cut-off points have been established to diagnose anaemia during pregnancy because of the changes in the relative balance between plasma volume and red cell mass (see paragraphs 3.39–3.40).

Epidemiological studies

6.40 Epidemiological studies have suggested a relationship between maternal haemoglobin concentration during pregnancy and birth outcome. Haemoglobin concentrations at either the low or high end of the distribution have been associated with increased risk of low birth weight (i.e., small for gestational age, <2.5 kg; or preterm, <37 weeks gestation) and perinatal mortality (Rasmussen, 2001).

6.41 Due to the range of physiological changes associated with pregnancy (plasma volume expansion and the corresponding haemodilution), markers of iron metabolism are difficult to interpret during this time. Birth outcome is also affected by a wide array of health behaviours, as well as by demographic, socioeconomic and nutritional factors. These confounders are rarely fully accounted for or considered in epidemiological studies of the effect of maternal iron deficiency on fetal and early infant development (US Preventive Services Task Force, 1993).

6.42 Increased haemoglobin concentrations during pregnancy are not caused by high intakes of dietary or supplemental iron but are more likely to be associated with pathophysiological processes which in turn cause a smaller plasma volume expansion and poor reproductive outcomes (Koller *et al*, 1979; Lu *et al*, 1991). Inadequate plasma volume expansion is associated with restricted fetal growth and low birth weight (Dunlop *et al*, 1978) or preterm birth (Forest *et al*, 1996). Failure of plasma volume expansion has also been associated with a greater than 3-fold increase in the incidence of pre-eclampsia in pregnancy (Murphy, 1986). In developed countries it is likely that high haemoglobin concentrations are themselves not the cause of low birth weight and preterm labour but are the result of reduced plasma volume secondary to other causes of failed pregnancies (Steer, 2000).

6.43 Garn *et al* (1981) analysed pregnancy outcome data in the USA (n=50,000) and reported that unfavourable pregnancy outcomes (low birth weight, preterm birth, fetal death) were minimal at maternal haemoglobin concentrations of 110–120 g/L for Caucasians and 90–100 g/L for African Americans but were increased at or below haemoglobin concentrations of 90 g/L and at or above 130 g/L. In the UK, Murphy *et al* (1986) analysed haemoglobin concentration at first antenatal attendance (n=54,382). The highest rates of low birth weight, preterm birth and perinatal mortality were associated with maternal haemoglobin concentrations below 104 g/L or above 132 g/L, irrespective of whether first antenatal attendance was in the first or second trimester. An analysis of 153,602 pregnancies in the UK

(Steer *et al*, 1995) reported highest birth weight at lowest maternal haemoglobin concentrations of 85–95 g/L. The incidence of low birth weight and preterm labour was minimal at lowest haemoglobin concentrations between 95 and 105 g/L; however, this observation is limited as haemoglobin concentrations were not measured at a consistent gestational period.

6.44 A prospective study of pregnant women (n=829) in China (Zhou *et al*, 1998) observed an association between haemoglobin concentration in early pregnancy (second gestational month) and rates of low birth weight and preterm birth. Compared to women with haemoglobin concentrations between 110 and 119 g/L, the risk of low birth weight was significantly increased in women with haemoglobin concentrations between 100 and 109 g/L (relative risk, 2.73; 95% CI, 1.01–7.39), and 90 and 99 g/L (relative risk, 3.27; 95% CI, 1.09–9.77). The risk of preterm birth was also significantly increased in women with haemoglobin concentrations between 90 and 99 g/L (relative risk, 2.63; 95% CI, 1.17–5.90) and <90 g/L (relative risk, 3.73; 95% CI, 1.39–10.23). The relative risks of preterm birth associated with haemoglobin concentrations <100 g/L in the first trimester were not significant in the second trimester (fifth gestational month) or third trimester (eighth gestational month), but the relation with low birth weight remained significant in the second and third trimester. The minimum risk of low birth weight was at haemoglobin concentrations of 110–119 g/L.

6.45 The epidemiological studies have a number of limitations which complicate their interpretation. In most populations, women who are anaemic also have other factors associated with a risk of adverse pregnancy outcomes which can be difficult to control for (see paragraph 6.41). Another difficulty is that the physiological changes that occur during pregnancy obscure the usual relationship between haemoglobin concentration and other markers of iron metabolism. Additionally, haemoglobin concentrations in early pregnancy will differ from those in the second trimester (when plasma volume expansion is at its peak and haemoglobin concentration is therefore reduced) and the third trimester (when plasma volume is constant and red cell mass increases). There is little consistency between studies in the time point at which haemoglobin concentration was assessed. This is particularly problematic when the lowest measurement of haemoglobin concentration is used because this occurs at different times during pregnancy. Another difficulty with interpreting data is that many older studies did not discriminate between infants who were small for gestational age and those that were preterm as causes of low birth weight. The clearest associations between pregnancy outcomes and haemoglobin values are probably best observed when the latter are measured during the first trimester of pregnancy (Scholl, 2005).

6.46 It is not possible to identify upper or lower thresholds for haemoglobin concentrations which have been associated with adverse birth outcomes in observational studies as these have varied across studies and have been measured at different stages of pregnancy.

Intervention trials of iron supplementation in pregnancy

6.47 A Cochrane review (Pena-Rosas and Viteri, 2009) of 49 trials (n=23,200) evaluated the effects of iron supplementation alone or in combination with folic acid during pregnancy on premature delivery (<36 weeks gestation), birth weight, low birth weight (<2500 g) and haematological parameters. The authors observed that the majority of trials focused on maternal changes in haemoglobin, that the data on pregnancy outcomes were limited, and that there was significant heterogeneity across most prespecified outcomes. Women receiving daily iron supplements (alone or in combination with folic acid) had higher haemoglobin concentration at term than women who did not receive supplements. Daily iron supplementation was also associated with a greater likelihood of haemoglobin concentration >130 g/L at term as well as during the second and third trimesters. Iron supplementation (alone or with folic acid) had no effect on risk of premature delivery or low birth weight; however, infants whose mothers received iron with folic acid during pregnancy were heavier than those who had not received iron with folic acid.

Summary and conclusions

6.48 Epidemiological data suggest that maternal haemoglobin concentrations (usually in the first or early second trimesters) at either the low or high end of the distribution during pregnancy are associated with increased risk of adverse birth outcomes, i.e., low birth weight, preterm birth and increased perinatal mortality. However, these are not necessarily related to a causal relationship with iron supply or nutrition. Physiological changes which occur during pregnancy make it difficult to interpret markers of iron metabolism at this time since haemoglobin concentrations fall during early pregnancy due to plasma volume expansion. High haemoglobin concentrations during pregnancy are usually caused by inadequate plasma volume expansion which is also associated with adverse birth outcomes.

6.49 Intervention studies of routine iron supplementation during pregnancy have not shown beneficial or adverse effects on pregnancy outcomes such as premature delivery or birth weight. Although iron supplementation (daily or intermittent) during pregnancy may be associated with higher maternal haemoglobin concentration at term, daily iron supplementation may also increase the risk of high haemoglobin concentration (above 130 g/L) during the second and third trimesters of pregnancy.

6.50 Evidence supports the current position that nutritional strategies should address women's nutritional needs throughout their reproductive years and not just when they are pregnant, and of having no specific DRVs for iron for pregnant and lactating women (DH, 1991). Evidence also supports the recommendation by NICE (2008) that iron supplementation should not be offered routinely to all pregnant women but should be considered for women identified with haemoglobin concentrations below 110 g/L in the first trimester and 105 g/L at 28 weeks.

Cognitive, motor and behavioural development in children

6.51 There is an extensive body of research considering the relationship between iron deficiency anaemia and cognitive, motor and behavioural development in children. While most researchers conclude that iron deficiency anaemia causes poor cognition in school-aged children, the effect on younger children remains controversial. Some researchers consider iron deficiency anaemia causes poor child development (e.g., Walker *et al*, 2007) while others have concluded that there is inadequate evidence of an effect (e.g., Sachdev *et al*, 2005). Many studies share common design flaws which limit their interpretation. Iron deficiency anaemia is associated with a large number of socioeconomic and biomedical disadvantages that can themselves affect children's development but are difficult to control for in observational studies or uncontrolled treatment trials. Double-blind randomised controlled trials of iron supplementation, treating or preventing iron deficiency anaemia, provide the most accurate way of determining whether iron deficiency is a cause of poor cognitive, motor and behavioural development.

6.52 Many factors are associated with both iron deficiency anaemia and cognitive function. These include low socioeconomic status (Owen *et al*, 1971); poverty (Czajka-Narins *et al,* 1978); poor quality of stimulation in the home (de Andraca *et al,* 1990); low levels of maternal education (de Andraca *et al,* 1990; Idjradinata and Pollitt, 1993) and IQ (Lozoff *et al,* 1991); maternal depression (de Andraca *et al*, 1990); low birth weight (<2.5 kg) and early weaning (Lozoff *et al,* 1991); parasitic infection (Ramdath *et al*, 1995); and undernutrition (Grantham-McGregor and Ani, 2001a).

6.53 Based on data from the National Diet and Nutrition Survey (NDNS) of children aged 1.5 to 4.5 years in Great Britain (n=1859) (Gregory *et al*, 1995), Thane *et al* (2000) reported that the parents of children with haemoglobin concentrations below 110 g/L were more likely to be receiving benefits, be unemployed, and have lower educational attainment levels and incomes than the parents of non-anaemic children. Iron deficient children were less likely to have been breast fed and more likely to be poor feeders. A greater proportion of children with low serum ferritin concentrations (<10 µg/L) were also from homes whose household heads had poor occupational status. Both groups consumed more cows' milk and less meat and fruit. Blood specimens were not available for young children in the Low Income Diet and Nutrition Survey (LIDNS) (Nelson *et al*, 2007b) due to a low response rate.

6.54 The relationship between iron and physical growth of children is considered in paragraphs 7.84–7.93.

Iron in the brain

6.55 The amount of iron in the brain increases throughout childhood and early adult life: about 10% of the brain iron content at 20 years of age is present at birth and 50% is present at 10 years of age. Although iron is distributed throughout the brain, the highest concentrations are in the basal ganglia (caudate-putamen, globus pallidus and the substantia nigra) which effect fine control and integration of movement. The iron content in these areas (13–21 mg/100 mg) is comparable to that of the liver (Hallgren and Sourander, 1958).

6.56 Mechanisms for the uptake and transfer of iron into the brain, and for the regulation of the processes involved, are still being identified (Moos *et al,* 2007). Receptor-mediated uptake of iron transferrin occurs at the capillary blood brain barriers. The mechanisms for the differential distribution of iron in the brain have not been characterised but the overall processes involved are similar to those of other organs (Patel *et al*, 2002). It has been suggested that because transferrin is quickly saturated in cerebrospinal fluid, neurones might acquire some iron in the form of low molecular weight citrate and ascorbate iron II complexes, thereby increasing the risk of free radical damage in some circumstances (Sipe *et al*, 2002).

6.57 In the brain, iron is found predominantly in the oligodendrocytes which are the cells responsible for producing myelin, the lipid sheath that insulates nerve cells. Defective myelination is thought to be one mechanism by which iron deprivation might impair neurological function. Additionally, iron is a catalytic element in the synthesis of neurotransmitters such as dopamine and serotonin (Thompson *et al*, 2001).

6.58 Neuronal metabolism is also impaired by iron deficiency. Iron deprivation in young rats, induced by feeding their mothers iron deficient diets during gestation and lactation, has been associated with reduced cytochrome oxidase activity in the hippocampus (which processes information to create memory) and other areas of the brain (de Ungria *et al*, 2000), and with an altered neurochemical profile indicative of altered energy metabolism and neurotransmission (Rao *et al*, 2003). The hippocampus is thought to be important for spatial navigation. Persisting difficulty with spatial navigation has been observed in rodent models of early-life iron deficiency, which is analogous to observations of impaired spatial memory in adolescents who were iron deficient as infants (Lozoff, 2000).

Mechanism

6.59 There are several biologically plausible ways in which iron deficiency could affect cognitive development; most directly, changes may occur to the structure and function of the central nervous system (CNS).

Brain development

6.60 Animal research has provided evidence of changes to the brain in iron deficiency. There is also some evidence of changes to the CNS in children. Event-related potentials measure transient changes in electrical activity of the brain in response to a stimulus. Burden *et al* (2007) found that the development of event-related potentials associated with attention and recognition memory tasks were delayed in infants (aged 9 and 12 months) with iron deficiency anaemia.[48]

6.61 Other studies have examined auditory brain stem responses (ABRs), which provide a measure of the activation of the auditory pathway from the distal part of the acoustic nerve to the brain. The central conduction time (CCT), which is considered to be an indicator of CNS development, is exponentially reduced from birth to 24

48 Haemoglobin ≤105 g/L and ≥ 2 other abnormal iron status measures: mean corpuscular volume <74 fL, red cell distribution width >14%, zinc protoporphyrin/haem ratio >69 µmol/mol of haem, ferritin <12 µg/L, and transferrin saturation <12%. The comparison group of infants with iron sufficiency had haemoglobin 115 g/L and 1 abnormal iron indicator.

months. Roncagliolo *et al* (1998) found that the CCT was prolonged in infants (aged 6 months) with iron deficiency anaemia[49] compared to infants without iron deficiency anaemia, and that the differences were more pronounced 6 and 12 months after iron treatment. The authors suggested that the prolonged CCT may have been caused by impaired myelination, which has been observed in iron deficient animals (Yu *et al*, 1986). Peirano *et al* (2001) reported that children who had iron deficiency anaemia[50] in infancy also had longer latencies in visual-evoked potentials (VEPs) at 3–5 years of age. One study of infants who had iron deficiency anaemia[51] in infancy found that both ABRs and VEPs remained abnormal at 4 years of age (Algarin *et al,* 2003). However, another study (Sarici *et al,* 2001) reported no differences in ABRs of infants with iron deficiency anaemia (not defined) compared to controls. There is also evidence of changes in the autonomic nervous system that cause sleep disturbances (Peirano *et al,* 2001, 2007; Kordas *et al,* 2008).

6.62 These observations provide a plausible mechanism for causality. They also raise the possibility that there may be periods when the developing brain might be particularly sensitive to iron deprivation and suggest why it might not be possible to compensate early deficits by subsequent iron replenishment.

6.63 During the immediate postnatal period in the rat, a rapid increase occurs in the brain content of transferrin and iron. In young animals, however, iron seems to be preferentially distributed to the erythron over other organs, including the brain. Thus in early life it is conceivable that iron deficiencies might exist in tissues, such as the brain, in the absence of iron deficiency anaemia (Allen, 1997; Lozoff, 2000).

Behaviour

6.64 It has been hypothesised that the link between iron deficiency anaemia and poor cognitive development can be explained by functional isolation (Levitsky and Strupp, 1995). Anaemic children explore and move around their environment less than non-anaemic children and, in response, carers are less stimulating. Both behaviours may delay their acquisition of new skills.

6.65 Compared with non-anaemic children (haemoglobin >110–120 g/L) in the first two years, iron deficient anaemic children (haemoglobin, 100–110g/L and two markers of potential iron deficiency) are more fearful (Lozoff *et al*, 1982a, 1996), withdrawn, unreactive to usual stimuli (Lozoff *et al*, 1982a), unhappier (Lozoff *et al,* 1996; Walter *et al*, 1983), make fewer attempts at tasks, are less playful and have poorer attention (Lozoff *et al*, 1998). These behaviours were found to persist after iron treatment.

6.66 It remains possible that these behaviours could be due to deprived environments rather than iron deficiency anaemia. A study of undernourished children with similar behaviour found that behaviour was changed with stimulation alone (Grantham-McGregor *et al*, 1989).

49 Haemoglobin ≤100g/L and ≥ 2 iron measures – mean cell volume <70 fl, erythrocyte protoporphyrin >1.77 µmol/L red blood cells, serum ferritin <12 µg/L.

50 Haemoglobin ≤100 g/L at 6 months and <110 g/L at 12 and 18 months; and 2 out of 3 measures in the iron deficient range: mean cell volume <70 fL, erythrocyte protoporphyrin >1.77 µmol/L red blood cells, serum ferritin <12 µg/L.

51 Iron deficiency anaemia definition same as footnote 50.

Review of evidence

6.67 There are several reviews on the relationship between iron and cognitive development (Grantham-McGregor and Ani, 2001b; Sachdev *et al*, 2005; McCann *et al,* 2007; Lozoff, 2007; Martins *et al*, 2009).

6.68 Evidence from correlational and case-control studies suggests an association between iron deficiency and iron deficiency anaemia and cognitive and/or motor and/or behavioural development. Longitudinal studies have indicated that these effects may continue into primary school age. Findings from a longitudinal study with 19 years of follow-up (Lozoff, 2006) suggest that children from poor backgrounds may be more vulnerable to the long term effects of iron deficiency anaemia (haemoglobin ≤100 g/L; serum ferritin ≤12 µg/L; and either erythrocyte protoporphyrin ≥1 mg/L red blood cells or transferrin saturation <10%) on cognitive deficits than those from more affluent backgrounds.

6.69 While observational studies consistently show a relationship between iron and cognitive development, they suffer from a number of limitations including lack of control for social determinants and other confounding variables which may also affect child development (see paragraphs 6.51–6.52). Definitions of anaemia and iron deficiency also vary in most studies. Observational data are not considered further in this section as there is a large evidence base of intervention studies which have examined the relationship between iron and cognitive development.

Measures of child development

6.70 Most studies examining the relationship between iron and cognitive development in young children used the Bayley Scales of Infant Development (BSID) to assess outcome. The BSID consists of two age-standardised sub-scales, the Mental Development Index (MDI) and the Psychomotor Development Index (PDI). These indices give little indication of specific deficits. The ability of the BSID to predict future development in the first year of life is extremely limited, but increases in the second year (Colombo, 1993). However, in nearly all the studies considered in the following sections, the BSID were sensitive to differences between anaemic and non-anaemic groups. Studies in older children have generally used tests of intelligence, specific cognitive functions, or school achievement.

Intervention studies

6.71 A Cochrane review (Martins *et al,* 2009) of randomised controlled trials examining the effects of iron supplementation on psychomotor development and cognitive function in children under 3 years of age with anaemia (haemoglobin <105 g/L in most studies) or iron deficiency anaemia (haemoglobin <105 g/L and at least two abnormal measures of iron status in most studies) included five trials (n=180) of 5–11 days and two trials (n=160) of more than 30 days. The findings of the review suggest that short term iron treatment has no effect on psychomotor development; however, the confidence intervals around treatment effects were wide because of small sample sizes in the studies. The effect of longer term treatment was unclear because of inconsistency between study results and small sample sizes.

6.72 The key intervention studies considered in this section are treatment trials in iron deficient children (divided into short and long term treatment), trials in populations with mixed iron status, and preventive treatment trials with iron replete children. The treatment trials are further divided by age of subjects (under and over 3 years[52]). The majority of trials were carried out in developing countries. Details of the studies considered, including country, sample size, allowance for confounding factors, iron status, and specific cut-offs used to define iron deficiency/iron deficiency anaemia, are provided in Annex 6 (Tables A8–A13). The tables are based on those included in the systematic review by Grantham-McGregor and Ani (2001b).

Treatment trials in children ≤3 years

Short term trials in children with iron deficiency or iron deficiency anaemia (Table A8, Annex 6)

6.73 Seven early trials assessed short term effects (less than 2 weeks) of iron supplementation in young children (aged 6–26 months) with iron deficiency anaemia (Oski and Honig, 1978; Lozoff *et al*, 1982b; Oski *et al,* 1983; Walter *et al*, 1983; Driva *et al*, 1985; Lozoff *et al*, 1987; Walter *et al*, 1989). The findings suggest that short term treatment does not have beneficial effects on mental or motor development of children with iron deficiency or iron deficiency anaemia. However, the hypothesis has not been well tested; sample sizes were small in all the studies and none reported their statistical power to show differences. It is also unlikely that the type of skills measured by the BSID would improve substantially in 2 weeks.

Longer term trials in children with iron deficiency or iron deficiency anaemia (Table A9, Annex 6)

6.74 Of the 10 longer term trials (2–12 months duration) of children (aged 12–30 months), 7 were not randomised. Five trials used non-anaemic iron replete groups as controls (Lozoff *et al*, 1987; Walter *et al*, 1989; Lozoff *et al*, 1996; Harahap *et al*, 2000; Akman *et al*, 2004), based on the idea that the anaemic group would have an initial deficit and would catch up with iron treatment. However, evidence suggests that children with iron deficiency anaemia in poor circumstances do not develop similarly to non-anaemic children but actually increase their deficits over time (Lozoff *et al*, 2006); so even maintaining the same degree of deficit could be a treatment effect.

6.75 The treated anaemic groups did not catch up with the non-anaemic groups in 3 of the 5 studies (Walter *et al*, 1989; Lozoff *et al*, 1987, 1996) and caught up in 2 (Harahap *et al,* 2000; Akman *et al,* 2004). In the study by Akman *et al* (2004), non-anaemic iron deficient (haemoglobin >110 g/L; serum ferritin <12 µg/L) children were randomised to iron treatment or no treatment: an iron deficient anaemic group (haemoglobin <110 g/L; serum ferritin <12 µg/L) received iron treatment, and an iron replete non-anaemic control group (haemoglobin>110 g/L; serum ferritin>12 µg/L) received no treatment. The groups with iron deficiency anaemia and non-anaemic iron deficiency began with poorer development scores than the iron replete non-anaemic group. After treatment, the iron replete non-anaemic,

52 Children under 3 years of age undergo a period of very rapid brain growth when nutrition is likely to be particularly important and have long term effects. The tests used are also very different, i.e., they measure motor development rather than IQ.

the non-anaemic iron deficient, and the iron deficiency anaemia groups were all similar. Although the non-anaemic iron deficient children were randomised, "intent to treat analysis" was not reported.

6.76 Hasanbegovic *et al* (2004) compared the effects of iron supplementation in children with haemoglobin concentration less than 95 g/L (A) or between 95 and 110 g/L (B). Initially group A had lower mental and motor development scores than group B. The deficit increased after treatment, with only group B showing some improvement. The authors suggested that children with haemoglobin concentrations below 95 g/L had irreversible deficits.

6.77 The differences in failure of anaemic children to catch up with the non-anaemic children in these trials are not readily explained by duration of treatment or severity of anaemia. The poorer backgrounds of the anaemic children could explain some of the failure to catch up. Few trials comprehensively controlled for environmental factors.

Longer term randomised trials in children with iron deficiency or iron deficiency anaemia (Table A10, Annex 6)

6.78 Only 3 of the 10 treatment trials were double-blind randomised controlled trials (Aukett *et al*, 1986; Idjradinata and Pollitt, 1993; Stoltzfus *et al,* 2001). Idjradinata and Pollitt (1993) reported a significant effect in both mental and motor development after 4 months of treatment. Stoltzfus *et al* (2001) reported that iron supplementation for 12 months significantly improved language milestones regardless of initial haemoglobin concentration and improved motor milestones in children with initial haemoglobin concentrations below 90 g/L; however, the predictive validity of these developmental milestones are not well-established. In contrast, Aukett *et al* (1986) did not find a significant treatment effect although there was a suggestion of an effect in *post hoc* analyses. However, the Denver test which was used in this study is not sensitive to small differences.

Longer term randomised trials with children of mixed iron status (Table A11, Annex 6)

6.79 In these trials (6–12 months duration), the iron status of children (aged 6–11 months) varied from non-anaemic iron sufficient to anaemic iron deficient. Since it is unlikely that any benefits of iron treatment would be observed in iron replete children, the mean effect size would be expected to be small and studies would require larger sample sizes. Three randomised controlled trials were identified (Black *et al*, 2004; Lind *et al*, 2004; Olney, 2006).

6.80 Lind *et al* (2004) reported a significant treatment effect on motor but not mental development. Olney *et al* (2006) examined only time to begin walking and reported that the treated group walked sooner than the untreated group; however, the treatment group also received folic acid with the iron.

6.81 Black *et al* (2004), examined the effect of 6 months treatment with different micronutrients including iron alone, zinc alone, iron plus zinc, and a micronutrient mix (containing 16 vitamins and minerals including iron and zinc) in a sample of infants aged 6 months; 68% of infants were anaemic (haemoglobin <110 g/L). The four

groups were compared with a group receiving riboflavin. PDI scores declined in all the groups; however, the decreases were significantly smaller in infants who received iron and zinc together or with other micronutrients. The group receiving only iron declined less than the riboflavin group but the difference was not significant. It is unlikely that this study had sufficient statistical power to detect small differences.

Preventive trials with non-anaemic children (Table A12, Annex 6)

6.82 These studies are based on the theory that if the placebo group develops iron deficiency anaemia and iron deficiency is prevented in the treated group, it should be possible to determine the effect of iron deficiency anaemia. However, the design is only valid if a sufficient proportion of the placebo group become iron deficient, otherwise the impact would be expected to be small or absent.

6.83 Eight trials were identified (Heywood *et al*, 1989; Walter *et al*, 1989; Moffatt *et al*, 1994; Morley *et al*, 1999; Williams *et al*, 1999; Lozoff *et al*, 2003, Friel *et al,* 2001, 2003). The age of the children ranged from 2 to 9 months and duration of treatment was 5–13 months. Four of these studies are not included in Table A12 (Annex 6) (Heywood *et al,* 1989; Walter *et al*, 1989; Morley *et al*, 1999; Friel *et al*, 2001). The trial by Friel *et al* (2001) lacked internal validity as no differences were found in haemoglobin concentration between babies who received formula fortified with 20.7 mg/L or 13.4 mg/L of iron. The presence of malaria confused the results in the study by Heywood *et al* (1989) and analysis was not reported according to the original trial design in the study by Walter *et al* (1989). Morley *et al* (1999) found no benefits of treatment but since data on initial and final haemoglobin concentrations were lacking, it is not possible to determine how many children, if any, were initially iron deficient or if there was any difference in iron status at the end of the study.

6.84 The 4 remaining studies were randomised trials (Moffatt *et al*, 1994; Friel *et al*, 2003; Williams *et al*, 1999; Lozoff *et al*, 2003) and some beneficial response to iron treatment was found in all of them. Williams *et al* (1999) reported beneficial effects of iron treatment; however, since one group was given fortified formula while the other group was given money to buy cows' milk, participants were not blind to treatment. It is possible that other constituents in the formula could have been responsible for the observed benefits, or cows' milk could have reduced the absorption of other nutrients.

6.85 Lozoff *et al* (2003) reported that although iron supplementation had no effect on PDI, MDI or a test of recognition memory, there were improvements in speed of information processing, behaviour and age of crawling. However, as supplementation procedures were changed half-way through the study, and cows' milk was given to some of the control group, it is not possible to infer with confidence that iron treatment caused these differences.

6.86 Moffatt *et al* (1994) reported that children given iron fortified formula from 2 months of age for 13 months had significantly higher PDI scores than the placebo group at 9 and 12 months. The benefit was no longer significant at 15 months; however, the difference between the groups in iron status measures was smallest at 15 months. There was no significant effect on MDI. Friel *et al* (2003) reported that iron replete infants supplemented at 1 month of age for 5 months had significantly

higher PDI but not MDI scores at 12 months of age. The power of this study was limited because there was little difference between groups in iron status measures and sample sizes of the groups were small.

6.87 A study of very low birth weight infants (<1301 g) randomised to early (mean 14 days) or late (mean 61 days) iron supplementation reported that the early-supplemented children had slightly better (but not significant) neurological, cognitive, achievement and disability outcomes at 5 years of age (Steinmacher *et al,* 2007).

Summary of findings from supplementation trials with children ≤3 years

6.88 There is no clear evidence that short term (less than 2 weeks) iron treatment benefits psychomotor and mental development of anaemic children aged 3 years or under; however, this has not been rigorously tested.

6.89 Longer term (3–6 months) iron treatment trials that were not randomised are difficult to interpret because of differences in the development of non-anaemic and anaemic children who are usually from poorer social backgrounds.

6.90 Of 3 randomised long term trials (2–12 months) with anaemic children, 2 showed benefits in motor and mental or language development (Idjradinata and Pollitt, 1993; Stoltzfus *et al*, 2001).

6.91 Of the 3 randomised trials (6–12 months) of children with mixed iron status, 2 studies reported significant benefits to motor development only (Lind *et al*, 2004; Olney *et al*, 2006). The third reported beneficial effects on motor development from iron and zinc combined, but only a non-significant benefit from iron alone (Black *et al*, 2004).

6.92 Of the 8 preventive trials (5–13 months), only 2 (Moffatt *et al*, 1994; Friel *et al*, 2003) could be interpreted with confidence. Both reported beneficial effects on motor development only, but sample sizes were small and differences in iron status were limited, reducing the chances of finding other benefits.

Randomised controlled trials in children >3 years of age (Table A13, Annex 6)

6.93 Fourteen iron treatment trials with anaemic or iron deficient children over 3 years of age were identified. Duration of treatment was 2–6 months; the age of the children ranged from 3 to 18 years, although 2 studies included children aged 3 years or younger (Deinard *et al*, 1986; Metallinos-Katsaras *et al*, 2004). Two trials were not randomised (Pollitt *et al*, 1983; Pollitt *et al*, 1986); in one the method of assignment was not clear (Lynn and Harland, 1998); one reported no statistical analysis (Soemantri, 1989); and one had no placebo group (Seshadri and Gopaldes, 1989; study 1).

6.94 Nine of the studies were double-blind randomised controlled trials (Soemantri *et al*, 1985; Deinard *et al,* 1986; Seshadri and Gopaldes, 1989 [studies 2–4]; Pollitt *et al,* 1989; Soewondo *et al*, 1989; Bruner *et al*, 1996; Metallinos-Katsaras *et al*, 2004).

6.95 Seven of the 9 double-blind randomised controlled trials used cognitive tests as outcome measures. Seshadri and Gopaldes (1989) (study 2) reported that the children in the iron treated group were significantly better than the control group in verbal and performance tasks; however, children were given folic acid with iron which may have had an independent benefit. In study 3 (Seshadri and Gopaldes, 1989), the treated group improved significantly in most of the cognitive tests, while the placebo group did not. In study 4 (Seshadri and Gopaldes, 1989), iron treated children improved more than the placebo group in two of four tasks. Only study 4 (Seshadri and Gopaldes, 1989) reported differences between the groups in change of scores, but analysis was restricted to anaemic children.

6.96 Two studies reported significant improvements with iron treatment of children with iron deficiency anaemia in speed of processing (Metallinos-Katsaras *et al*, 2004) and with non-anaemic iron deficient girls in memory (Bruner *et al*, 1996) but not in other tests. No significant treatment effects were reported in 2 other trials (Deinard *et al*, 1986; Soewondo *et al*, 1989).

6.97 Two randomised controlled trials examined school achievement (Soemantri *et al*, 1985; Pollitt *et al,* 1989). A significant improvement with iron treatment was reported in the study by Soemantri *et al* (1985) but not in the study by Pollitt *et al* (1989). The criteria for iron deficiency anaemia was higher in the study by Pollitt *et al* (1989) (haemoglobin <120 g/L compared to <110 g/L) and haemoglobin concentration of the placebo group improved, reducing the difference in haemoglobin concentration between the treatment and placebo groups. The quality of schooling may also play a role.

Summary of findings from supplementation trials with children >3 years

6.98 Out of 8 randomised controlled trials in which cognitive tests were outcome measures, 2 reported clear benefits, 4 reported findings suggestive of benefits but did not report an analysis of change in scores between treated and placebo groups, and 2 reported no effects of iron treatment.

6.99 One of 2 randomised controlled trials assessing school achievement found benefits with iron treatment.

Limitations of supplementation trials

6.100 Common problems with the intervention studies were that they were often not double-blind and some were not randomised. Other studies were confounded by the use of breast milk substitutes, cows' milk or additional micronutrients. Many studies lacked statistical power because of small sample sizes or little difference between the groups in iron status measures even after treatment. Early studies often failed to conduct analyses by intention to treat or to control for initial scores. When batteries of tests were used, several different scores from each test were often examined, increasing the possibility of observing spurious significant effects. Events in early childhood may show immediate or delayed effects that are transient or sustained; however, few trials followed the children after treatment ceased. Lastly, there is a paucity of data on high-risk children (e.g., those with low birth weight, undernourished) as they were excluded from most studies.

Cut-off levels at which haemoglobin concentration affects development

6.101 Few investigators have determined the threshold levels of haemoglobin concentration which may be associated with developmental decline. Deficits in motor development have been reported at haemoglobin concentrations below 105–110 g/L, and declines in mental development have been reported at concentrations below 100–110 g/L, but the actual thresholds of haemoglobin concentration at which these deficits occur have not been identified.

6.102 Response to iron treatment of children with different haemoglobin concentrations provides some help with assessing the haemoglobin concentrations below which children's development is affected. However, it is possible that deficits are irreversible. There are currently no consistent findings from such trials.

6.103 Improvements in motor development following iron treatment were reported at haemoglobin concentrations below 80 g/L and benefits in language development at all concentrations (Stolzfus *et al*, 2001); however, these children had a high prevalence of malaria which reduces haemoglobin concentrations independently of iron and other nutritional deficiencies. Another trial (Bruner *et al*, 1996) found benefits (but only in one of many measures) in non-anaemic girls with reduced iron depots (haemoglobin >120 g/L; serum ferritin <12 µg/L; age, 13–18 years). Three smaller trials, however, did not find benefits of iron supplementation in non-anaemic, potentially iron deficient children aged over 3 years (Pollitt *et al*, 1989; Soewondo *et al*, 1989; Pollitt *et al*, 1985) or under 3 years (Idjradinata and Pollitt, 1993). In one study (Hasanbegovic *et al*, 2004), children with haemoglobin concentrations below 95 g/L had lower mental and motor development scores than those with concentrations between 95 and 110 g/L, and both were lower than non-anaemic children (haemoglobin >110 g/L), suggesting a relation between severity of iron deficiency and development.

6.104 Based on current data, it is difficult to derive thresholds of iron deficiency measures at which cognitive, motor and behavioural development might be at risk. However, the evidence suggests that the risks of such deficits are less at haemoglobin concentrations above 100–110 g/L than at concentrations below this range although the deficits may not be solely attributable to iron deficiency.

Summary and conclusions

6.105 Evidence from observational studies shows that iron deficiency and iron deficiency anaemia are usually associated with many psychosocial, economic and biomedical disadvantages, which can independently affect development. Iron deficient anaemic young children usually have poorer development concurrently and in the future than non-anaemic children. It remains possible that measured and unmeasured environmental variables could explain these findings.

6.106 Evidence from randomised controlled trials of iron supplementation suggests that iron deficiency anaemia is a cause of poor motor development in children in the first three years of life. The long term implications of these findings are unknown.

6.107 There are insufficient rigorous randomised controlled trials to assess whether iron deficiency or iron deficiency anaemia affects cognitive or language development in children aged ≤3 years. The relatively short duration of follow-up in the trials may explain the lack of observed effect. Also, in the trials of children with mixed iron status (varying from non-anaemic iron sufficient to anaemic iron deficient) and the preventive trials, there was generally very little difference in iron status measures between groups at the end of treatment.

6.108 There is evidence for a beneficial effect of iron treatment on cognitive development in anaemic older children. However, none of the trials reported long term follow-up of children to determine whether any benefits were sustained. There is insufficient evidence to determine the effect of iron treatment on school achievement.

6.109 Based on current evidence, it is not possible to derive thresholds of anaemia or iron deficiency measures at which cognitive, motor and behavioural development might be at risk, but early adverse effects do not appear to be present at haemoglobin concentrations above 110 g/L. However, adverse effects have been observed below this level (irrespective of cause).

7 Health consequences of high iron intake and high iron burden

Recommended upper intake levels for iron

UK

7.1 In May 2003, the Expert Group on Vitamins and Minerals (EVM) reported on the safety of vitamin and mineral supplements and recommended maximum advisable levels of intake. Safe Upper Levels (SULs) were established when supported by sufficient data. The SUL represents an intake that can be consumed daily over a lifetime without significant risk to health. A Guidance Level (GL) on the safe level of intake was set when the evidence base was inadequate to establish an SUL. GLs represent an approximate indication of levels that would not be expected to cause adverse effects; they are less secure than SULs because they are derived from limited data.

7.2 The EVM concluded that there were insufficient data to establish an SUL for iron, but set a GL for supplemental iron intake (i.e., in addition to dietary intake) of 17 mg/day[53] for adults. Iron supplements at doses of 50–220 mg/day and above are associated with gastrointestinal effects, including constipation, nausea, diarrhoea and vomiting (EVM, 2003).

USA

7.3 In the USA, the Institute of Medicine (IOM) set a Tolerable Upper Intake Level (UL) for total iron intake (from all sources) of 45 mg/day[54] for adults ≥19 years (IOM, 2001). The UL represents the highest level of daily nutrient intake over a lifetime that is likely to pose no risk of adverse health effects for almost all individuals in the general population. The UL for iron was based on gastrointestinal side effects. The IOM concluded that there were insufficient data to determine the UL based on the other effects that were considered (impaired zinc absorption, increased risk of cardiovascular disease and cancer, systemic iron overload).

Europe

7.4 In Europe, the Scientific Panel on Dietetic Products, Nutrition and Allergies concluded that the available data were insufficient to establish a UL for iron (EFSA,[55] 2004). Adverse gastrointestinal effects reported after short term oral dosage of supplemental iron (50–60 mg) were not considered a suitable basis to establish a UL for iron from all sources. A UL was not established based on increased risk

53 The GL is not applicable to those with a susceptibility to iron overload associated with haemochromatosis (see paragraphs 2.42–2.45).

54 The UL does not apply to individuals with haemochromatosis.

55 European Food Safety Authority.

of chronic diseases (CVD, diabetes, cancer) due to lack of convincing evidence, or with regard to iron overload, due to a poor correlation between iron intake and biochemical indicators of iron status.

Acute iron toxicity

7.5 Cases of severe acute iron toxicity usually occur in children, often from accidental ingestion of iron supplements used by a close relative or carer (Mills and Curry, 1994). Intakes of approximately 20 mg elemental iron/kg body weight precipitate intestinal effects, which develop over hours. Systemic manifestations occur at thresholds around 40–60 mg/kg body weight; exposures of about 100 mg/kg body weight are usually lethal. Initial effects include nausea, vomiting and loose stools caused by corrosive damage and haemorrhagic necrosis of the gastrointestinal mucosa. After 4–6 hours, evolving systemic effects following loss of the gastrointestinal barrier result in fluid loss and hypovolaemic shock, and a further increased systemic overload of iron with eventual multi-organ failure and death (Mills and Curry, 1994).

7.6 Although smaller acute exposures to iron do not cause such serious effects, the slow mucosal adaptation, either through systemic mediation or the local autonomous mechanism, to high iron intakes means that a surge of iron can enter the portal and systemic circulation. This may cause nausea, faintness and other relatively mild effects. Based on these observations, it has been proposed that intermittent use of iron supplements might increase systemic iron load which would increase the risk of systemic oxidative damage, particularly of the vascular endothelium and the liver. Such exposures and concepts have been based on the use of supplements rather than through the diet. These aspects are considered below.

Physiological consequences of high iron intakes and overload

7.7 Iron can exert a range of acute and chronic adverse effects by facilitating oxidative reactions or by competing with other transition metals of nutritional importance (e.g., copper, zinc) in a number of physiological processes.

7.8 Theoretically, iron may exert adverse effects under a number of conditions. It may have detrimental effects on the gut through direct interaction with the intestinal mucosa or on other tissues if regulation of iron absorption from the intestine is impaired or overloaded, if iron deposition in tissues is impaired, or if iron is inappropriately released from tissue depots (see section 2).

Interaction with other divalent metals

7.9 The intestinal uptake and transfer, systemic distribution, deposition and use of divalent cations such as zinc, iron and copper depend on a chain of binding and transport proteins which are able to distinguish between the metals on the basis of their relatively small physico-chemical differences. However, each particular step in the chain is not necessarily sufficiently selective to cope with increased amounts of other metals. Competitive interactions between similar metals (iron,

copper, manganese, cobalt, cadmium and lead) on these binding proteins have been demonstrated *in vitro,* and sometimes *in vivo* (Hill and Matrone, 1970). Biologically relevant interactions involve iron, zinc and copper (Kordas and Stoltzfus, 2004).

7.10 These interactions vary according to the oxidation state of the metals, their relative amounts (i.e., molar ratios), absolute amounts or chemical activities, the number and affinities of potential binding sites (e.g., uptake sites, physiological ligands and dietary ligands in the gut lumen and mucosa) and, in the nutritional context, the time over which the interaction is monitored. For example, interactions between elements may occur when they are presented in simple solutions but not when exposure is in the diet, which would reduce the chemical availability of the elements. The difficulty in unravelling such factors and interactions is illustrated by the uncertainty surrounding potential or hypothesised effects of increased iron intakes in early childhood on absorption and systemic use of zinc and copper (see paragraphs 7.12–7.15), and on growth (see paragraphs 7.84–7.93).

7.11 Antagonistic interactions between these minerals may have negative consequences on functional outcomes such as growth and development in populations with higher requirements such as infants, adolescents, and pregnant and lactating women (Sandström, 2001).

Interaction with zinc

7.12 Sandström *et al* (1985) examined the effect of iron on zinc absorption in humans (n=55). Zinc absorption was determined from measurement of whole-body retention 14 days after consumption of test solutions or meals labelled with a zinc radioisotope (^{65}Zn). Addition of iron to an aqueous solution of zinc (40 µmol/2.6 mg) in a 1:1 molar ratio reduced zinc absorption from 74 to 58%; increasing the iron:zinc molar ratio to 2.5:1 had no further effect; however, at a ratio of 25:1, zinc absorption was significantly reduced to 34%. Addition of iron to test meals at the same molar ratios of iron to zinc did not decrease zinc absorption (25, 23 and 22% respectively).

7.13 Crofton *et al* (1989) examined the converse interaction of the effect of zinc on iron absorption. Participants (n=16) ingested radiolabelled iron (^{59}Fe) with differing molar ratios of zinc and iron absorption was assessed by measuring plasma appearance of iron and ^{59}Fe content and whole-body retention of ^{59}Fe. Co-administration of 842 µmol of iron with 344 µmol of zinc had no effect on the plasma appearance of iron; however, at 421 µmol of iron, 421 µmol of zinc reduced the plasma appearance of iron ($p<0.002$), which was further reduced ($p<0.001$) by 1048 µmol of zinc (iron:zinc molar ratio 1:2.5). The amounts used were representative of doses used in nutritional supplementation and prophylaxis.

7.14 A review of randomised controlled trials assessing interactive effects of iron and zinc supplementation on markers of iron (serum ferritin and haemoglobin concentration) and zinc (serum zinc concentration) in children under 5 years and women of reproductive age (Fischer Walker *et al*, 2005) reported that, overall, trials of zinc supplementation alone (n=10) showed no effect on haemoglobin or serum ferritin concentration and trials of iron supplementation alone (n=10) showed no effect on serum zinc concentration. In the trials that assessed effects of addition of zinc to

iron supplementation (n=9), none reported negative effects on haemoglobin and/or serum ferritin concentrations in women. However, results were mixed in trials of children: in some trials improvement in serum ferritin and haemoglobin was less when zinc was added. Out of the trials which assessed the addition of iron to zinc supplementation (n=4), none found an effect on serum zinc concentration; however, these interactions are confounded by other factors, particularly the diet, and plasma zinc concentration may not be an adequate indicator of zinc body burden. There were insufficient data to assess the effects of joint iron and zinc supplementation on growth.

Interaction with copper

7.15 Studies using Caco-2 cells have shown that iron and copper directly compete for uptake across the apical membrane (Tandy *et al*, 2000; Arredondo *et al*, 2006) and studies in animal models have shown adverse effects of high levels of dietary iron on copper metabolism (Johnson and Hove, 1986; Storey and Greger, 1987). Only a small number of human studies have examined iron and copper interactions. Haschke *et al* (1986) reported that infants (n=7) fed formula containing low concentrations of iron (2.5 mg/L) absorbed significantly more copper ($p<0.01$) than when they were fed formula with high iron concentration (10.2 mg/L). Another study (Lönnerdal and Hernell, 1994) compared infants (n not specified) fed formula containing either 4 or 7 mg/L of iron with from 1.5 to 6 months of age; both formulas contained 0.04 mg/L of copper. Although serum copper concentrations were similar at the start of the study, infants fed the formula with the highest iron concentrations had significantly lower ($p<0.05$) serum copper levels at 6 months. Morais *et al* (1994) reported that serum copper concentrations in iron deficient (serum ferritin=3.9 µg/L; haemoglobin=102 g/L) children (n=31; median age 32 months) after two months of iron therapy (5 mg/kg/day) were significantly lower ($p=0.010$) than they were before iron therapy. In a study of low birth weight infants (n=55) randomly assigned to receive daily supplements of either 13.8 mg iron, 7 mg iron, or no elemental iron (iron edetate) from age 28 days to 20 weeks, activity of the copper-dependent enzyme, superoxidase dismutase, was significantly lower in infants given 13.8 mg iron compared to those in the group given 7 mg/day ($p<0.05$) or none ($p<0.01$) (Barclay *et al*, 1991).

The role of iron as a pro-oxidant

7.16 The ability of iron to gain or lose single electrons makes it an efficient catalyst for free-radical reactions. Fe^{2+} (ferrous form) and Fe^{3+} (ferric form) which have four and five unpaired electrons in each configuration respectively, and Fe^{4+} (ferryl species), can exist in biological systems. Ferryl species are generated when certain haem moieties react with hydrogen peroxide (H_2O_2).

7.17 Haemoglobin and myoglobin contain haem iron and undergo oxidation to form superoxide and Fe^{3+} protein; both can initiate damage when they react with peroxide. Haemoglobin and myoglobin will degrade to haem and iron ions, which can in turn stimulate lipid peroxidation, protein oxidation and DNA oxidation.

Haem iron can also catalyse the decomposition of pre-existing lipid peroxides to alkoxy and/or peroxyl radicals, causing cellular damage and leading to cell death (McCord *et al*, 1998).

7.18 Cells have evolved protective mechanisms to remove free radicals. These include superoxide dismutase which converts superoxide to H_20_2, and catalase, peroxidase and glutathione peroxidase that reduce peroxides and H_20_2. Under normal conditions these defence mechanisms are effective at scavenging free radicals generated by iron dependent and iron-independent mechanisms.

7.19 Free radical defence systems are impaired in certain diseases and it has been proposed that this is an important factor in pathogenic processes. Concentrations of antioxidants in animals fed an iron-rich diet have been shown to be lower than those in animals receiving less dietary iron (Kuratko, 1998). Evidence to support the role of antioxidant nutrients in humans is mainly associative. Intervention trials have shown either no beneficial effect or adverse effects of antioxidant nutrients on cancer risk (WCRF,[56] 2007).

Iron overload

7.20 Iron overload occurs when excess iron accumulates in the body. It can be caused by increased absorption of dietary iron or by parenteral iron loading (repeated blood transfusions) as there is no mechanism to excrete excess iron (see section 2).

Increased iron absorption

7.21 Iron absorption is increased with the haemochromatoses (see Table 1), rare genetic disorders of iron metabolism which include atransferrinaemia and aceruloplasminaemia, and with sub-Saharan dietary iron overload.

7.22 Chronic liver disease (e.g., alcoholic cirrhosis) and porphyria cutanea tarda (associated with homozygosity for HFE C282Y gene) may also be associated with iron loading. Ineffective erythropoiesis is associated with increased iron absorption in severe thalassaemia disorders (e.g., ß-thalassaemia major and intermedia) and in sideroblastic anaemias (either inherited or acquired).

Parenteral iron loading

7.23 The rate of iron loading through regular transfusions is considerably greater than the maximum possible through increased iron absorption. One unit of red blood cells delivers approximately 200 mg iron, which means that individuals with transfusion-dependent anaemias are at significant risk of iron overload. With congenital anaemias, such as ß-thalassaemia major, this can lead to the accumulation of up to 100 g iron by 20 years of age, by which time most patients would have died from toxic effects of the excess iron (Modell, 1979). Complications associated with iron overload include cardiac arrhythmias and heart failure, diabetes, delayed onset of puberty, and cirrhosis (Pippard, 1994).

56 World Cancer Research Fund.

Health consequences of high iron intakes

7.24 It has been hypothesised that high tissue iron concentrations carry an increased risk of: neoplasia (Nelson, 1992); atherosclerotic disorders (Sullivan, 1981; Kent and Weinberg, 1989); infection (Kent and Weinberg, 1989); neurodegenerative disorders (Thompson *et al*, 2001); and inflammatory conditions (Halliwell and Gutteridge, 1984).

7.25 This report focuses on the relationship of iron intakes and systemic iron with cancer and CVD risk as these were considered to be the two main issues of public health concern in the UK. Studies of colorectal cancer and meat intake are also considered because meat is almost exclusively the source of haem iron. Additionally, the effects of high exposures to iron on growth in children who are iron replete are also considered. Other conditions that have been associated with high iron intakes/systemic iron (such as Alzheimer's disease, Parkinson's disease, arthritis, diabetes mellitus) are only considered briefly.

Epidemiological studies of iron and chronic disease

7.26 The majority of data on the relationship between iron and chronic disease are based on epidemiological studies which have a number of important limitations. These include the difficulty of obtaining reliable estimates of iron exposure (intake) and accurate measures of iron burden, as well as confounding by other dietary and lifestyle factors that have been associated with chronic disease risk. Additionally, studies may not have sufficient power to detect associations of small or moderate magnitude as statistically significant.

7.27 Assessments of iron intakes in epidemiological studies are not very reliable because of errors in estimated intakes of foods and supplements, because values in food composition tables may not be accurate, and because of continually changing exposure to iron caused by constant introduction or removal of foods voluntarily fortified with iron. There is considerable variation in the haem iron content of meat (about 22–80% of total iron) (Lombardi-Boccia *et al,* 2002); however, most studies assume haem iron content to be 40% of total iron. There are also difficulties in classifying and assessing intakes of meat (see paragraphs 7.63–7.65), which is an important source of iron. Serum-based measures of iron depots, distribution and use are more objective, but there are problems with their interpretation (see section 4) and serum samples need to be collected some years before diagnosis of the disease.

7.28 Measures of iron intake and iron status are subject to confounding by both dietary and non-dietary variables. For example, high iron intakes in Western populations may be due to high intakes of meat and could therefore be associated with other nutritional factors related to meat, e.g., saturated fats. Associations between meat consumption and cancer risk might also be affected by other differences in diet, such as variations in fibre, fruit and vegetable consumption. Serum markers of iron metabolism are affected, for example, by blood loss (e.g., menstruation, gastrointestinal disease), diurnal variation, infection and inflammation.

7.29 Additional evidence for an effect of iron on disease risk comes from using an approach termed "mendelian randomisation"[57] (Davey-Smith *et al*, 2005) which is based on the principle that if genetic variants alter the level of a nutrient, or its biological effects, and the risk of disease, this suggests a role for the nutrient in disease aetiology. The main advantage of this approach is that it is not associated with many of the confounding lifestyle factors which complicate the interpretation of observational nutritional studies (Davey-Smith *et al*, 2005). However, whilst genetic studies may suggest a nutritional link with disease, this does not mean that changes in dietary intake would have similar effects. Studies reporting associations between chronic disease and genetic variants in iron absorption and metabolism (i.e., mutations of the HFE gene) were, therefore, not given as much weight in the overall evaluation of possible adverse effects of high intakes of dietary iron.

Iron and cancer

7.30 Although it is uncertain whether, or how, iron might be carcinogenic, it has been suggested that oxygen free radical formation catalysed by iron might play a role in this process (Toyokuni, 1996; Okada, 1996). Two main pathways have been suggested: increased DNA damage induced either directly or indirectly by impeding DNA repair, and modulation of nuclear redox sensitive transcriptional regulators through signal transduction mechanisms (Galaris and Evangelou, 2002). Haem iron, but not inorganic iron, also increases production of *N*-nitroso compounds in the lumen of the gastrointestinal tract (Cross *et al*, 2003); many *N*-nitroso compounds have been shown to be human and animal carcinogens (IARC[58], 1978). In addition, iron is a limiting nutrient for the growth and replication of cancer cells in the human body (Weinberg, 1984).

7.31 Higher concentrations of body iron might increase cancer risk by increasing oxidative stress to cells and by providing iron for growth and replication of cancer cells. Animal studies have shown that iron is essential for proliferation of neoplastic cells (Siegers *et al*, 1991). Iron supplementation was found to enhance the rate of tumour growth in mice with chemically induced colonic neoplasia (Siegers *et al*, 1992); the increase in tumour rate was dependent on the iron concentration in the diet. Seril *et al* (2002) found that a 2-fold iron enriched diet significantly increased the incidence of ulcerative colitis-associated colorectal tumours in mice. Hann *et al* (1988) observed that tumour growth in mice inoculated with colonic adenoma-carcinoma cells was decreased after they were fed an iron deficient diet.

7.32 Some epidemiological studies have suggested an association between blood donation (which reduces body iron) and lower cancer risk (Merk *et al*, 1990; Kato *et al*, 2007). Zacharski *et al* (2008) analysed cancer risk in patients with peripheral arterial disease (n=1277; mean age 67 years) participating in a randomised controlled trial on the effect of iron reduction by phlebotomy (at six-month intervals) on vascular outcomes (Zacharski *et al*, 2007). Mean serum ferritin concentrations between the iron reduction and control groups were similar at baseline but were significantly

57 Named after Mendel's second law of independent inheritance of characteristics.

58 International Agency for Research on Cancer.

lower in the iron reduction group after phlebotomy (79.7 µg/L versus 122.5 µg/L; p<0.001). After a mean follow-up of 4.5 years, cancer risk was reported to be lower in the iron reduction group (hazard ratio, 0.65; 95% CI, 0.43–0.97; p=0.036).

7.33 Attention has mainly focused on the relationship between iron and colorectal cancer, based on the hypothesis that high intakes of iron might increase colorectal cancer risk by intraluminal (Graf and Eaton, 1985) or systemic effects (Stevens and Kalkwarf, 1990). Since most dietary iron is not absorbed, luminal exposure to excessive dietary iron may result in direct oxidative damage to the colorectal lumen.

Epidemiological studies of iron and colorectal cancer

7.34 The relationship between dietary iron and cancer was considered by COMA in their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), which reviewed studies on iron and cancer published up to 1996. Based on the available evidence at that time, COMA concluded that high iron stores may be related to increased risk of colorectal cancer, but since iron stores may not be related directly to iron intake, further prospective studies were required to confirm whether iron intake has a role in colorectal cancer.

7.35 The World Cancer Research Fund (WCRF) has also reviewed the evidence for an association between iron and colorectal cancer (WCRF, 2007). Four cohort studies on iron intake and colorectal cancer (Wurzelmann *et al*, 1996; Glynn *et al*, 1996; Kato *et al*, 1999; Konings *et al*, 2002) and 1 cohort study on haem iron intake and colorectal cancer (Lee *et al*, 2004) were considered. Four studies suggested an increased risk of colorectal cancer among people with the highest iron intake compared to those with the lowest intake, which was statistically significant in 2 studies (Wurzelmann *et al*, 1996; Lee *et al*, 2004). The WCRF concluded that there was limited evidence that foods containing iron are a cause of colorectal cancer and commented that the evidence was "sparse, of poor quality, and inconsistent".

7.36 In this report, consideration of the relationship between iron and colorectal cancer is based on prospective studies published since 1996[59] which have examined the relationship between colorectal cancer and dietary iron (including supplements) and serum ferritin concentration (although serum ferritin concentrations do not necessarily represent the amount of iron to which the colorectal mucosa has been exposed). Studies of individuals heterozygous for hereditary haemochromatosis were also considered. Details of these studies, including sample size, duration of follow-up, and allowance made for confounding factors, can be found in Annex 7 (Tables A14–A17).

7.37 Five prospective studies (Annex 7, Table A14) have reported on dietary iron and colorectal cancer; most studies did not include iron intake from dietary supplements. Six out of the 7 relative risks reported were above 1; of these, one was significant. The median relative risk was 1.08.

59 COMA considered studies published up to 1996 (DH, 1998).

7.38 Four prospective studies have reported on dietary haem iron and colorectal cancer risk (Annex 7, Table A15). Two of these studies (Lee *et al*, 2004; Larsson *et al*, 2005) assumed haem iron content from all types of meat to be 40%, while in the other 2 studies (Balder *et al*, 2006; Kabat *et al*, 2007) haem iron content was estimated according to the type of meat. Kabat *et al* (2007) found similar results using the two different methods. Five of the 6 relative risks were above 1; none were significant. The median relative risk was 1.26.

7.39 Two small prospective studies (Kato *et al*, 1999; Cross *et al*, 2006) have reported on serum ferritin concentration and colorectal cancer risk (Annex 7, Table A16). Both observed a significant reduction in risk with increasing serum ferritin concentrations. In the study by Kato *et al* (1999), the authors suggested that low ferritin concentrations might have been caused by blood loss associated with preclinical colorectal cancer because the average time between blood donation and cancer diagnoses was only 4.7 years. Cross *et al* (2006) suggested that low serum ferritin concentrations observed in their study might indicate that less iron was absorbed and therefore more was present in the gut lumen where it could exert direct oxidative damage; that it could be due to increased iron requirement for tumour growth; or, although all cases were diagnosed at least five years after blood collection, that bleeding from a tumour could explain the lower serum ferritin concentrations because of the long induction period (5–10 years) associated with colorectal cancer.

7.40 Most studies assessing the relationship between iron intake or iron stores and colorectal cancer risk were relatively small and, therefore, probably had insufficient statistical power to detect small or moderate associations.

Hereditary haemochromatosis and cancer

7.41 Hepatocellular carcinoma, the main form of liver cancer, is very strongly associated with haemochromatosis. Bradbear *et al* (1985) first quantified the excess risk for hepatocellular carcinoma as 200-fold in patients with genetic haemochromatosis. Subsequent studies have confirmed this strong association (Hsing *et al,* 1995; Fracanzani *et al*, 2001) and indicated that the increased risk generally follows the development of cirrhosis.

7.42 The risk for cancers other than hepatocellular carcinoma in patients with hereditary haemochromatosis has also been investigated in several small studies. Bradbear (1985) reported no excess of non-hepatocellular cancers, based on eight cancers, in a cohort (n=208) of patients with hereditary haemochromatosis. Fracanzani *et al* (2001) reported that the risk for non-hepatic cancers among 230 patients with hereditary haemochromatosis compared to 230 patients with non iron–related chronic liver disease was 1.8 (95% CI, 0.8–4.0). Geier (2002) reported that, among 59 patients with hereditary haemochromatosis, there were 13 non-hepatocellular cancers and a standardised incidence ratio of 1.40 ($p<0.04$).

Heterozygosity for type 1 hereditary haemochromatosis and colorectal cancer

7.43 Seven studies have assessed the association between heterozygosity for genetic haemochromatosis and colorectal cancer (Annex 7, Table A17).

7.44 Six out of the 8 relative risks reported were greater than 1; one of these was statistically significant (Nelson *et al*, 1995). The median relative risk was 1.05. Most studies were relatively small and may not have had sufficient power to detect small or moderate associations.

7.45 In the study which reported a significantly increased risk of colorectal cancer associated with C282Y heterozygosity (Nelson *et al*, 1995), colorectal cancer risk was assessed from postal questionnaires sent to individuals homozygous for genetic haemochromatosis regarding the health histories of their parents (who were assumed to be heterozygotes). Their spouses were asked to complete questionnaires on the health histories of their parents (who were assumed not to be heterozygotes). Heterozygosity for hereditary haemochromatosis was not confirmed by DNA analysis and colorectal cancer was not confirmed by hospital records.

7.46 Robinson *et al* (2005) reported that although C282Y or H63D heterozygosity was not associated with colorectal cancer risk, individuals who were compound heterozygotes (C282Y/H63D) were at increased risk of colorectal cancer compared with those with a single mutation (odds ratio, 3.03; 95% CI, 1.06–8.61; p=0.038); however, this finding did not reach statistical significance after adjustment for multiple *post hoc* testing.

Meat and colorectal cancer

7.47 A number of plausible biological mechanisms have been suggested for an association between meat and colorectal cancer. Consumption of red and processed meat, but not white meat or fish, is associated with increased endogenous production of potentially carcinogenic *N*-nitroso compounds in the colon (Bingham *et al*, 2002). One mechanism proposed for the association is that the presence of haem iron, which is found in higher amounts in red and processed meat, enhances nitrosation. It has also been proposed that nitrites or nitrates which are added to some processed meat as preservatives could increase exogenous exposure to *N*-nitroso compounds or their precursors. Heterocyclic amines and polycyclic aromatic hydrocarbons, which are formed when foods are cooked at very high temperatures, have also been proposed to increase the risk of colon cancer (Sugimura, 2000); however, their production is not specific to the cooking of red and processed meat. Toxicological data examining whether differences in colorectal cancer risk between white, red and processed meat can be explained on the basis of cooked food mutagens, preservatives, *N*-nitroso compounds or haem iron can be found in Annex 8. There are currently no convincing data that provide an explanation for the observed differences in colorectal cancer risk between high consumers of white meat/fish and high consumers of red and processed meat.

7.48 It has also been suggested that the fat contained in red meat could affect colorectal cancer risk by increasing production of secondary bile acids which have been associated with promoting colon cancer (Narisawa *et al*, 1978). In addition, people eating diets high in red meat may eat fewer foods, such as fruit and vegetables, which might be protective against cancer risk (WCRF, 2007).

7.49 Several studies have examined the association between red and processed meat intake and colorectal cancer risk. There are a number of difficulties in interpreting the results from these studies including lack of consistency in definitions of red and processed meat, adequacy of dietary assessment methods, and variability in quantification of intakes. These limitations are discussed in further detail in paragraphs 7.62–7.70.

7.50 The relationship between meat intake and colorectal cancer was previously considered by COMA in their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), which reviewed epidemiological studies published up to 1996. The report concluded that evidence from cohort studies was: inconsistent for an association between total meat consumption and risk of colorectal cancer; moderately consistent for a positive association between consumption of red or processed meat and risk of colorectal cancer; and moderately consistent that poultry (white meat) and fish were not associated with colorectal cancer risk. COMA recommended that average consumption of red and processed meat (90 g/day cooked weight or 8–10 portions/week which was current at that time) should not increase and that higher consumers (above 140 g/day or 12–14 portions/week) should consider reducing their intakes.

7.51 A meta-analysis of 13 cohort studies published up to 1999 (Sandhu *et al*, 2001) reported that an increase in intake of 100 g/day of all meat significantly increased risk of colorectal cancer by 12–14%, 100 g/day red meat significantly increased risk by 13–17%, and 25 g/day increase in intake of processed meat was associated with an increased risk of 49%.

7.52 A subsequent meta-analysis included nine cohort studies of red meat and seven cohort studies of processed meat published from 1973 to 1999 (Norat *et al*, 2002). Compared to lowest level of intake, the highest level of red meat consumption was associated with a significant increase in colorectal cancer risk (relative risk, 1.27; 95% CI, 1.11-1.45). The relative risk for an increase of 120 g/day of red meat was 1.22 (95% CI, 1.05–1.41). Processed meat was also associated with a significant increase in risk: relative risks were 1.39 (95% CI, 1.09–1.76) for highest compared to the lowest level of intake and 1.54 (95% CI, 1.10–2.17) for an increase of 30 g/day of processed meat. No significant association was found between total meat consumption and colorectal cancer risk.

7.53 Another meta-analysis (Larsson and Wolk, 2006), which updated and expanded the previous analyses to include all prospective studies published up to March 2006 (15 studies of red meat; 14 studies of processed meat), included up to six times as many cases of colorectal cancer (7367 cases; n=1,042,824) compared to the two earlier meta-analyses. The relative risk of colorectal cancer for individuals in the highest category of red meat consumption compared with those in the lowest category

was 1.28 (95% CI, 1.15–1.42); an increase of 120 g/day of red meat was associated with a relative risk of 1.28 (95% CI, 1.18–1.39). The relative risk of colorectal cancer for individuals with the highest compared to the lowest processed meat intake was 1.20 (95% CI, 1.11–1.31) and an increase of 30 g/day of processed meat intake was associated with a relative risk of 1.09 (95% CI, 1.05–1.13).

7.54 The relationship between red and processed meat intake and colorectal cancer risk was also considered by the WCRF (2007). Sixteen cohort studies (published from 1975 to 2005) on red meat intake and colorectal cancer risk were identified. All showed an increased colorectal cancer risk for highest versus lowest intake, which was significant in 4 studies. Meta-analysis of 7 studies that measured red meat intake in times/week and 3 studies that measured red meat intake in g/day reported relative risks of 1.43 (95% CI, 1.05–1.94) per times/day and 1.29 (95% CI, 1.04–1.6) per 100 g/day. Fourteen cohort studies (published from 1990 to 2005) on processed meat and colorectal cancer risk were considered. Twelve studies showed an increased colorectal cancer risk for highest versus lowest intake which was statistically significant in 3 studies. A meta-analysis of 5 of these studies reported a relative risk of 1.21 (95% CI, 1.04–1.42) for 50 g/day of processed meat intake.

7.55 The WCRF concluded that red and processed meat "is a convincing cause of colorectal cancer" and recommended that the population average consumption of red meat[60] should be no more than 300 g (cooked weight) per week and that very little, if any, should be processed. On an individual basis, the report recommended that consumption of red meat should be less than 500 g (cooked weight) per week, with very little or any processed meat. The report also concluded that there was limited evidence to indicate that eating fish protects against colorectal cancer and that there was insufficient evidence to draw conclusions on the association between poultry (white meat) and colorectal cancer risk.

Prospective studies of red and processed meat intake and colorectal cancer risk

7.56 Prospective studies published after 1996[61] on the association between red meat and processed meat intake and colorectal cancer are considered in this report. Details of these studies are provided in Annex 7 (Tables A18–A19).

Red meat intake and colorectal cancer risk (Annex 7, Table A18)

7.57 Twenty-one prospective studies have been published since 1996 (including some updated analyses from previously published cohorts). Two of these studies (Balder *et al,* 2006; Sato *et al*, 2006) considered the risk of colorectal cancer with total meat consumption rather than only red meat. Twenty-one out of the 25 relative risks reported were greater than one, 3 significantly so (Chao *et al*, 2005; Larsson *et al*, 2005; Cross *et al*, 2007). The median relative risk for highest versus lowest red meat intake was 1.17. The increased relative risk was statistically significant in 1 out

60 Defined as beef, pork, lamb and goat, including that contained in processed foods.

61 COMA considered cohort studies published up to August 1996 (DH, 1998).

of the 4 largest studies (Wei *et al*, 2004; Chao *et al*, 2005; Norat *et al*, 2005; Cross *et al*, 2007); the trend was statistically significant in 2 of the 4 studies and close to significance in 1 study.

7.58 Significantly increased colorectal cancer risk was associated with red meat intakes of ≥ 114 g/day for men, ≥ 80 g/day for women (Chao *et al*, 2005); ≥ 94 g/day (Larsson *et al*, 2005); and 62.7 g per 1000 kcal (equivalent to 145 g/day for men and 102 g/day for women[62]) (Cross *et al*, 2007). In all the studies which reported a significantly increased risk of colorectal cancer, processed meat was included under the category of red meat.

Processed meat intake and colorectal cancer risk (Annex 7, Table A19)

7.59 Fourteen prospective studies have been published since 1996 (including some updated analyses from previously published cohorts). Thirteen out of the 18 relative risks for highest compared to lowest processed meat intake were greater than 1; of these, 5 were significant (English *et al*, 2004; Wei *et al*, 2004; Norat *et al*, 2005; Oba *et al*, 2006; Cross *et al,* 2007). The median relative risk was 1.16. The relative risk was statistically significant in 3 out of the 4 largest studies (Wei *et al*, 2004; Chao *et al*, 2005; Norat *et al*, 2005; Cross *et al*, 2007), and the trend was statistically significant in all the studies.

7.60 Increased colorectal cancer risk was significantly associated with processed meat intakes of ≥ 20.3 g/day (Oba *et al*, 2006); ≥ 29 g/day (English *et al*, 2004); ≥ 80 g/day (Norat *et al*, 2005); 22.6 g per 1000 kcal (equivalent to 52.3 g/day for men and 36.9 g/day for women[63]) (Cross *et al*, 2007); and 5 times per week or more (Wei *et al*, 2004).

Colorectal cancer risk in vegetarians

7.61 A pooled analysis (Key *et al*, 2009a) of individual participant data (n=61,566) from 2 studies in the UK which examined cancer incidence in vegetarians (Sanjoaquin *et al*, 2004; Key *et al*, 2009b) observed no significant difference in colorectal cancer incidence between meat eaters and vegetarians (relative risk in vegetarians compared to meat eaters = 1.12; 95% CI, 0.87–1.44) after 12 years of follow-up. However, the analysis did not fully characterise meat consumption patterns and the total meat intake of non-vegetarians was much lower than the average UK intakes of meat consumers reported in the National Diet and Nutrition Survey[64] (Henderson *et al*, 2002).

62 Based on average total energy intake of 2313 kcal/day for men and 1632 kcal/day for women reported in the 2000/01 NDNS survey (Henderson *et al*, 2003b).

63 See footnote 62.

64 In the study by Key *et al* (2009b), the median meat intake of non-vegetarians was 78.1 g/day for men and 69.7 g/day for women compared to 204 g/day for men and 135 g/day for women reported in the National Diet and Nutrition Survey. In the study by Sanjoaquin *et al* (2004), meat intakes of non-vegetarians were not quantified: meat consumption was divided into three categories of intake: not eaten; eaten less than daily; eaten daily.

Limitations in interpreting the results from prospective studies on red and processed meat intake and colorectal cancer risk

7.62 The majority of prospective studies published after 1996 suggest that high intakes of red and processed meat are associated with an increased risk of colorectal cancer. Although the association was not statistically significant in most studies, the sample sizes in the majority of studies may not have been large enough to ensure adequate power to detect a significant association.

7.63 There are a number of methodological inconsistencies between the different studies which make comparisons difficult. These include adequacy of the dietary assessment methods to obtain reliable estimates of red and processed meat intake; lack of consistency in the categorisation of red and processed meat; and variability in the reporting of quantities of red and processed meat intake.

7.64 In most studies, red meat and processed meat intake is based on a single dietary assessment at the start of the study. This does not take account of changes in dietary patterns over a number of years and could therefore be an unreliable estimate of intake over the specified follow-up period. The relevant period between dietary intake and development of cancer is also uncertain and dietary intakes estimated at baseline may not be the relevant period for dietary assessment in relation to cancer risk.

7.65 There are also considerable inconsistencies between studies in categorisation and definition of red and processed meat: some studies collected detailed information of the foods included under the red and processed meat categories, while others used very broad classifications (e.g., beef, pork, lamb). In addition, some separated red and processed meat categories and only included fresh or untreated red meat in the red meat category, while other studies included processed meat under the red meat category.

7.66 Another difficulty is the variable ways in which quantities of red and processed meat intake were reported. While most studies compared highest versus lowest intake in grams per day/week/month, some reported intake as grams per 1000 kcal, frequency of intake per week/month, or servings per day. There are also large differences in the quantiles of intake between studies so that the amounts in the lowest quantiles in some studies are higher than the top quantiles in others. For red meat, intakes in the highest quantiles ranged from more than 40 to 158 g/day and in the lowest quantile from 10 to 61 g/day; processed meat intakes ranged from 16.3 to 80 g/day or more in the top quantiles and from 0 to 12 g/day in the lowest quantiles.

7.67 Some studies also analysed the association between colorectal cancer risk and red/processed meat intake for men and women combined, although intake in the highest and lowest quantiles differed by sex (Brink *et al*, 2005; Chao *et al*, 2005). In the study by Chao *et al* (2005), a significant association was found for men and women combined in the highest compared to the lowest quintile of red meat intake; however, intakes in the highest quintile were above 114 g/day for men and above 80 g/day for women.

7.68 All these inconsistencies make it difficult to quantify a level of red or processed meat intake that may be associated with colorectal cancer risk and to make recommendations on levels of consumption. The potential effect of a recommendation to reduce the consumption of red meat and processed meat intake on iron and zinc intakes in the UK is considered in section 10.

7.69 Although results from prospective studies of dietary fibre and colorectal cancer are inconsistent, it has been suggested that higher intakes of foods containing fibre may protect against colorectal cancer risk (WCRF, 2007; Bingham *et al,* 2003). The fibre content of the diet might be a surrogate marker for another plant component such as phytate, which has been shown to reduce the incidence of experimentally induced large intestinal tumours in animal studies (Shamsuddin and Ullah, 1989); however, only eight of the 21 studies on red meat intake and colorectal cancer risk and five of the 14 studies on processed meat intake and colorectal cancer risk adjusted for fibre intake.

7.70 There are also a number of other factors that have been associated with colorectal cancer risk. These include genetic predisposition, high total fat intake, low fruit and vegetable intake, low physical activity, and meat preparation and cooking methods. Although most of the newer larger studies have controlled for confounding factors, other studies have varied in the adjustments made. Additionally the effects of residual confounding cannot be excluded.

Iron and cardiovascular disease

7.71 Premenopausal women have a lower incidence and mortality from coronary heart disease (CHD) compared to men and to postmenopausal women (Wingard *et al*, 1983; Lerner and Kannel, 1986). It has been proposed ("iron hypothesis") that this is due to higher levels of stored iron in men and postmenopausal women (Sullivan, 1981). The iron hypothesis suggests that menstrual iron loss protects against CHD and that depletion of body iron stores through blood donation would also protect against CHD risk (Sullivan, 1991).

7.72 Two prospective studies reported that, after adjustment for other CVD risk factors, blood donation was associated with a reduced risk of CVD (Meyers *et al*, 1997; Salonen *et al*, 1998a). Meyers *et al* (1997) reported that the occurrence of CVD events in a cohort of men and women (n=3855) followed for 5–8 years was significantly lower only in non-smoking male blood donors compared to non-donors (odds ratio, 0.67; 95% CI, 0.45–0.99). Salonen *et al* (1998a) reported a significantly reduced risk of acute myocardial infarction in blood donors compared to non-donors (relative hazard, 0.12, 95% CI, 0.02–0.86; p=0.035) in a cohort of men (n=2862) followed for 9 years. However, a much larger prospective study of men (n=38,244) followed for 4 years found no association between blood donation and risk of myocardial infarction or fatal CHD (Ascherio *et al,* 2001).

7.73 A meta-analysis of prospective studies (Danesh *et al*, 1999) assessed the association between CHD and markers of iron status (serum ferritin, transferrin saturation, total iron binding capacity, serum iron concentration). In 5 studies which assessed

CHD and serum ferritin concentration (570 cases; mean follow-up of 8 years), the combined risk ratio for individuals with serum ferritin ≥200 µg/L compared to those <200 µg/L at baseline was not significantly different from unity (risk ratio, 1.03; 95% CI, 0.83–1.29). Combined analysis of 3 studies comparing total dietary iron intake of individuals in the top third of intake with those in the bottom third (2,535 cases; mean follow-up of 10 years) did not find an increased CHD risk (risk ratio, 0.84; 95% CI, 0.66–1.06).

7.74 A randomised controlled trial (Zacharski *et al*, 2007) of patients with peripheral arterial disease (n=1277; mean age 67 years) reported that, compared to controls, a reduction of body iron stores[65] by phlebotomy (over an average of 3.5 years) did not decrease all-cause mortality or death plus non-fatal myocardial infarction and stroke. However, the study was underpowered overall and particularly underpowered to definitively assess outcomes in younger patients and smokers.

7.75 The mechanism by which iron could affect heart disease risk is not clear. Based on evidence from cell and animal studies, it has been suggested that iron might play a role in the development of atherosclerosis by promoting oxidation of low density lipoprotein cholesterol through catalysing the formation of free radicals or promoting free radical mediated myocardial damage following an ischaemic event (de Valk and Marx, 1999).

Prospective studies of iron and cardiovascular disease

7.76 Details of the studies considering the association between iron and CVD risk are provided in Annex 7 (Tables A20–A24). Most studies had relatively small sample sizes and probably had limited statistical power to detect small or moderate associations between iron and CVD risk. Other limitations associated with epidemiological studies of iron and chronic disease are considered in paragraphs 7.26–7.28.

7.77 Nine prospective studies have reported on total dietary iron and risk of CHD (Annex 7, Table A20). Three out of the 10 relative risks comparing highest versus lowest iron intake or incremental increases in intake were greater than 1, of which one was significant. The median relative risk was less than 1 (0.91).

7.78 Five prospective studies have reported on the association between haem iron intake and CVD risk (Annex 7, Table A21). Only 2 of the studies explained the method used to calculate the haem iron content of foods containing iron. Four out of the 5 relative risks for highest versus lowest haem iron intake were greater than 1 and all were significant; the median relative risk was 1.48. It is possible that the increased risk could be due to other components of meat (the main source of haem iron) associated with increased CVD risk, such as saturated fats or an unmeasured component. Although all the studies adjusted analyses for saturated fat intake, the possibility of residual confounding by saturated fats cannot be excluded because

65 Mean serum ferritin concentration was 58 µg/L in patients assigned to undergo iron reduction and 122 µg/L in controls.

of unreliable estimates of both haem iron and saturated fat intakes. The observed association could also be due to unidentified dietary or lifestyle factors associated with meat intake.

7.79 Fifteen prospective studies have reported on the association between serum ferritin concentration and CVD risk (Annex 7, Table A22). Of the 17 relative risks comparing individuals with serum ferritin measurements in the top quantiles with those in the bottom, with serum ferritin above 200 µg/L compared to those below 200 µg/L, or the effect of incremental increases in serum ferritin concentration, eight were above 1, and two were significantly so. The median relative risk was 1.00.

Heterozygosity for genetic haemochromatosis and cardiovascular disease

7.80 Studies examining the association between heterozygote carriers of genetic haemochromatosis and CVD risk are very diverse and have considered various CVD endpoints or early and late onset disease. Although there are some prospective studies, the majority are case-control studies.

7.81 A meta-analysis of epidemiological studies (1998–2005) examining the relationship between HFE mutations and CHD reported that C282Y or H63D heterozygotes were not at increased risk for CHD (van der A *et al*, 2006), but statistical power was limited for most studies. Seven prospective studies, 11 case-control studies and 1 cross-sectional study were included in the meta-analysis of the C282Y mutation; and 2 prospective studies, 8 case-control studies and 1 cross-sectional study were included in the meta-analysis of the H63D mutation. The pooled estimate was 1.02 (95% CI, 0.94–1.11) for C282Y heterozygotes and 1.03 (95% CI, 0.96–1.11) for H63D heterozygotes.

7.82 Eight prospective studies (1999–2007) have reported on C282Y heterozygosity and CVD risk (Annex 7, Table A23). Six of the 9 relative risks reported were greater than 1; two of these were significant. The median relative risk was 1.25.

7.83 Four prospective studies have reported (2002–2007) on H63D heterozygosity and CVD risk (Annex 7, Table A24). Two of the 5 relative risks reported were greater than 1; none were significant. The median relative risk was 0.98.

Other effects of high exposures to iron

Negative effects of iron supplements on growth

7.84 Some studies have reported an adverse effect of iron supplements on growth in iron replete infants (Idjradinata *et al*, 1994; Dewey *et al*, 2002; Majumdar *et al*, 2003, Lind *et al,* 2004). A systematic review of 25 randomised controlled trials (mostly from developing countries) evaluating the effect of iron supplementation on physical growth of children (weight-for-age, weight-for-height, height-for-age) reported no effect (Sachdev *et al*, 2006). However, there was significant heterogeneity between trials and sensitivity analysis of the trials assessing height-for-age showed that iron supplementation had a negative impact on linear growth of children from

developed countries (p=0.018) but had no significant effect in developing countries. Iron supplementation for more than 6 months was associated with slower linear growth (p=0.039).

7.85 A study from Indonesia reported that among iron replete infants (haemoglobin >120 g/L; serum ferritin >12 µg/L; age, 12–18 months; n=47), randomised to receive iron supplement (3 mg/kg/day) or placebo for 4 months, the rate of weight gain was significantly lower in those receiving iron supplements compared to those receiving placebo (Idjradinata *et al*, 1994). No difference was found in linear growth but this is difficult to measure at this age.

7.86 Results from a double-blind randomised controlled trial suggest a negative effect of iron supplements on linear growth and head circumference during infancy (Dewey *et al*, 2002). Full-term infants in Sweden (n=101) and Honduras (n=131) were randomly assigned at 4 months of age to three interventions groups: iron supplement (1 mg/kg/day) from 4 to 9 months; placebo 4 to 6 months and iron 6 to 9 months; or placebo from 4 to 9 months. All infants were exclusively or near exclusively breast fed (≤15 ml/day of foods/fluids other than breast milk and no iron fortified foods) until 6 months of age. Among the Swedish infants, gains in length and head circumference were significantly lower in those receiving iron supplements compared to those receiving placebo from the age of 4–9 months, particularly for the 6–9-month period. In Honduras, a negative effect of iron supplements on linear growth was only observed from 4 to 6 months among those with initial haemoglobin concentration of 110 g/L or above. There were no differences in head circumference among treatment groups.

7.87 A randomised controlled trial in India (Majumdar *et al*, 2003) of infants (age, 6–24 months; n=150) reported that, compared to the placebo group, weight gain and linear growth was significantly decreased in iron replete infants (haemoglobin>110 g/L; serum ferritin>12 µg/L; n=50) who were supplemented with iron (2 mg/kg/day). A significant improvement in weight gain and physical growth was seen with iron supplementation (6 mg/kg/day) of iron deficient children (haemoglobin=50–110 g/L; serum ferritin <12 µg/L) compared to placebo.

7.88 A secondary analysis of a large randomised trial on the effects of iron and zinc supplementation on growth and development of Indonesian infants (Lind *et al*, 2004) examined the effect of iron supplementation (10 mg/day) on the growth of iron replete infants (haemoglobin ≥113 g/L; serum ferritin ≥33 µg/L; n=154). Weight gain from age 6 to 12 months and mean weight at 12 months were significantly lower (p<0.001) in the iron replete infants supplemented with iron compared to non-supplemented iron replete infants. There was no difference in linear growth between the two groups. Serum zinc levels were lower in the supplemented iron replete children (9.7 µmol/L versus 10.5 µmol/L; p=0.04).

7.89 In a randomised controlled trial in Egypt (Abdelrazik *et al,* 2007), exclusively breast fed infants (n=248; age, 4–6 months) were supplemented with iron (1 mg/kg/day) or placebo for 12 months. Infants in the iron treatment group were stratified according to whether or not they were malnourished (based on anthropometric parameters) and further stratified into those with haemoglobin concentration above or below

100 g/L; all infants in the placebo group had haemoglobin values above 100 g/L. After 6 months of treatment, weight and length gain was significantly higher ($p<0.05$) in the iron treated group as a whole compared to the placebo group. Within the iron treated group, the increments in weight and length were significantly higher ($p<0.05$) in malnourished than nourished infants. Weight gain was greater ($p<0.01$) in malnourished infants with haemoglobin <100 g/L compared to those with haemoglobin >110 g/L; this difference was not observed in well-nourished infants. No effects were reported on head circumference.

7.90 A randomised trial in Brazil (Silva *et al*, 2008) examined the effects of different iron doses (1 mg/kg/day, 2 mg/kg/day, or 25 mg/week) on the growth of iron replete infants (haemoglobin ≥110 g/L; age, 5–6.9 months; n=114) supplemented for 16 weeks. The study did not include a control group. At the end of the intervention there were no statistical differences between groups in weight and length gain.

7.91 A randomised controlled trial in the US (Ziegler *et al,* 2009) which assessed the effect of iron supplementation at an early age on iron status also monitored effects on growth. Full-term exclusively breast fed infants aged 1 month (n=75) received iron (7 mg/day) as a multivitamin preparation (also containing vitamins A, C and D) or placebo (multivitamin preparation without iron) until 5.5 months of age. At the end of the intervention period, there were no significant differences in weight or length gain between the two groups. The authors noted that the study may not have been sufficiently powered to detect effects of iron supplementation on growth. There may also have been no difference in growth between the two groups because the infants probably did not need exogenous iron at this age (see paragraphs 3.9–3.13).

7.92 It is difficult to compare iron doses between studies or derive thresholds at which iron treatment might have adverse affects on the growth of iron replete children because the iron dosage in most studies varied according to body weight.

7.93 It is possible that iron supplementation of iron replete children inhibits absorption of other essential nutrients required for growth, such as zinc (see paragraphs 7.9–7.15). Although there were no differences in plasma zinc concentration between the iron treated and placebo treated groups in the study by Dewey *et al* (2002), plasma zinc concentration is not an adequate indicator of marginal zinc deficiency. The data on the effect of nutrient interactions on growth are limited as most studies considered the effects on biochemical indicators.

Neurodegenerative conditions

7.94 Iron acts as a catalyst in the biosynthesis of the neurotransmitters dopamine and serotonin and is also important in the formation of myelin by oligodendrocytes (Thompson *et al*, 2001). Abnormally high amounts of iron have been detected in brain material from individuals with neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Qian and Shen, 2001).

7.95 There appear to be no epidemiological data to support a role for dietary iron in neurodegenerative conditions. When regulation of systemic iron is under normal homeostatic control, it is unlikely that excess dietary iron will have any effect on

cognitive function or neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease. It is possible that local iron homeostasis is disrupted by disease processes associated with neurodegenerative disorders rather than iron *per se* being a primary cause of the pathogenesis.

7.96 There is also no convincing evidence for an association between HFE mutations and either Alzheimer's or Parkinson's disease (Guerreiro *et al,* 2006).

Arthritis

7.97 Arthropathy is a common and early clinical manifestation of haemochromatosis. There is, however, little available data regarding iron overload or the frequency of heterozygosity for haemochromatosis in arthritis patients (Worwood, 2002b).

7.98 Rheumatoid arthritis is a chronic inflammatory disease that predominantly affects the small joints of the body. It is associated with disturbed metabolism of iron as manifested by the anaemia of chronic disorders. Iron accumulates in the rheumatoid synovial membrane and is largely present within ferritin and haemosiderin; these are labile and the associated iron can have a catalytic oxidant capability leading to a cascade of oxidative damage to lipids, proteins and DNA (Halliwell and Gutteridge, 1985).

7.99 There are limited data on dietary risk factors associated with rheumatoid arthritis and the existing evidence is inconclusive (Choi, 2005). A prospective nested case-control study in the UK reported that patients who developed inflammatory polyarthritis (n=88) had a significantly higher median intake of red meat (p=0.04) and red meat combined with meat products (p=0.02) than controls (n=176). Individuals with the highest intakes of total red meat combined with meat products were at increased risk of inflammatory polyarthritis compared to those with the lowest intakes (OR, 2.3; 95% CI, 1.1–4.9). However, no association was found with iron intake (Pattison *et al*, 2004). A prospective study in Denmark (n=57,053) found no associations between risk of rheumatoid arthritis and intakes of iron or meat (Pedersen *et al*, 2005). Another prospective study in the USA (n=82,063) also found no significant associations between rheumatoid arthritis and intakes of total dietary iron, iron from foods, iron from supplements, haem iron, red meat, or total meat (Benito-Garcia *et al,* 2007).

Diabetes mellitus

7.100 Diabetes mellitus is a clinical manifestation of haemochromatosis which has led to the suggestion that high iron depots may increase the risk of developing diabetes; however, diabetes is a relatively late feature of haemochromatosis. A review (Saudek and Charache, 1992) concluded that the prevalence of haemochromatosis among diabetic patients was low (1%). Results from later studies have been inconsistent. An analysis of diabetic (types 1 and 2) patients (n=727) in the UK reported that the prevalence of haemochromatosis was 0.1% (Turnbull *et al,* 1997). Another study in Italy (Conte *et al,* 1998) compared the prevalence of haemochromatosis in diabetic (types 1 and 2) patients (n=894) with matched controls (n=467) and reported an overall prevalence of 1.34% in the diabetic patients compared to 0.2% in the

reference group; the odds ratio for haemochromatosis in association with diabetes was 6.3 (95% CI, 1.1–37.7). When only patients with type 2 diabetes were considered, the prevalence of haemochromatosis was 1.54% and the odds ratio of haemochromatosis with type 2 diabetes was 7.3 (95% CI, 1.3 to 41.9). The authors concluded that haemochromatosis was under diagnosed in patients with diabetes.

7.101 Studies have also examined whether iron depots in individuals without haemochromatosis are associated with diabetes. A prospective study in Finland (Salonen *et al*, 1998b), which followed 1038 men over a period of four years, reported that men with high iron depots (transferrin receptors to ferritin ratio <9.4 µg/µg) were 2.4 times more likely to develop diabetes than men with lower iron values (95% CI, 1.03–5.5; p=0.04). A larger prospective study of women (n=32,826) followed for 10 years (Jiang *et al*, 2004a) reported that, after adjustment for other risk factors for diabetes and for inflammation, those with plasma ferritin concentration in the highest quintile (≥107 µg/L) compared to the lowest quintile (<21 µg/L) were at significantly increased risk of developing diabetes (relative risk, 2.61; 95% CI, 1.68–4.07; p<0.001).

7.102 A subsequent prospective study of men and women (n=15,792) followed for 8 years (Jehn *et al*, 2007) reported that the risk of diabetes was significantly increased in those with plasma serum ferritin concentration in the top quintile (355 µg/L) compared to those in the bottom quintile (20 µg/L) after adjustment for age, ethnicity, smoking and alcohol intake (hazard ratio, 1.74; 95% CI, 1.14–2.65; p<0.001). However, after adjustment for factors associated with metabolic syndrome (HDL cholesterol, waist circumference, hypertension, glucose level, triglyceride level), no relation was found between plasma serum ferritin concentration and diabetes risk (hazard ratio, 0.81, 95% CI, 0.49–1.34; p trend=0.87).

7.103 Another prospective study examined whether dietary iron intake or blood donation was related to diabetes risk in a cohort of men (n=38,394) followed for 12 years (Jiang *et al*, 2004b). After adjustment for other risk factors for diabetes, no association was found between total iron intake and risk of diabetes; however, men in the highest quintile of haem intake (median, 1.9 mg/day) compared to those in the lowest quintile (median, 0.8 mg/day) were at increased risk of diabetes (relative risk, 1.28; 95% CI, 1.02–1.61; p trend=0.045). After further adjustment for red meat intake, no association was found between haem iron and diabetes risk (relative risk, 0.96; 95% CI, 0.74–1.23; p trend=0.58). Haem iron intake from sources other than red meat (such as chicken and fish) was not associated with diabetes risk. No association was found between blood donation and diabetes risk.

Summary and conclusions

7.104 In the UK, the GL[66] for supplemental iron intake (i.e., additional to iron intakes from foods) is 17 mg/day for adults (EVM, 2003). The GL is based on adverse gastrointestinal effects associated with intakes of supplemental iron (50–220 mg/day).

66 The GL is based on limited data and represents an approximate indication of intakes that would not be expected to produce adverse effects.

7.105 Evidence on the relationship between iron and chronic disease is based on epidemiological studies, which have a number of limitations. The most important considerations are reliable assessments of dietary iron intake and body iron burden and their confounding by other dietary and lifestyle factors. Another important consideration is that most studies were relatively small and, therefore, may not have had adequate statistical power to detect small or moderate associations.

7.106 A limited number of prospective studies have examined the association between colorectal cancer risk and dietary intakes of total/haem iron; serum ferritin concentration; and heterozygosity for hereditary haemochromatosis. Epidemiological prospective studies suggest that increased dietary intakes of total or haem iron might be associated with increased risk of colorectal cancer. The increased risk was not significant in most studies. Two small prospective studies indicate that high body burden of iron is not associated with increased colorectal cancer risk. Overall, there are insufficient data on the association between intakes of total dietary iron, haem iron, ferritin concentration and colorectal cancer risk to reach clear conclusions.

7.107 Overall, the evidence suggests that heterozygosity for hereditary haemochromatosis might be associated with increased risk of colorectal cancer although the association was not significant in most studies. There are insufficient data to reach clear conclusions.

7.108 Meat, particularly red meat, is the main source of haem iron. Several epidemiological studies have investigated the relationship between consumption of red and processed meat and colorectal cancer risk. Results from these studies have consistently suggested that high intakes of red and processed meat are associated with increased colorectal cancer risk. Although the increased risk was not statistically significant in most studies, this may be due to lack of statistical power. Overall, the available evidence suggests that red and processed meat intake is probably associated with increased colorectal cancer risk. The evidence for an increased risk is not unequivocal since it is based on observational studies which means that the effects of confounding by other lifestyle factors associated with increased colorectal cancer risk cannot be excluded. It is not possible to identify if there is a dose-response or a threshold level of red or processed meat associated with increased colorectal cancer risk because of a number of limitations in the available data, including lack of consistency in categorisation and quantification of red and processed meat intake.

7.109 Evidence from observational studies of total iron intake or body iron burden and CVD do not suggest an association. Evidence from a limited number of prospective studies suggests that high intakes of haem iron increase CVD risk. It is possible that the increased risk could be due to other components of meat (which is the main source of haem iron) associated with CVD risk, such as saturated fats, or dietary and lifestyle factors associated with meat intake. Further long term prospective studies, with more accurate and reliable measures of haem iron intake, are required to confirm this finding.

7.110 Studies of HFE heterozygosity and CVD risk suggest that C282Y heterozygotes, but not H63D heterozygotes, may be at increased CVD risk; however, there are insufficient data to reach clear conclusions.

7.111 Evidence from randomised controlled trials suggests, overall, that iron supplementation may have detrimental effects on the physical growth of iron replete infants and children (haemoglobin >110 g/L and serum ferritin >12 µg/L in most studies). Further studies are required to characterise this effect.

7.112 A number of common neurodegenerative conditions (e.g., Parkinson's disease and Alzheimer's disease) are associated with iron accumulation in the brain. There is no evidence to show dietary iron is associated with these conditions.

7.113 There are insufficient data to draw conclusions on an association between iron intake and rheumatoid arthritis.

7.114 There is insufficient evidence to draw conclusions on the association between body iron burden or iron intake and diabetes mellitus in the general population. There is also insufficient evidence to reach clear conclusions on whether being homozygous or heterozygous for either HFE C282Y or HFE H63D variants of hereditary haemochromatosis is associated with an increased risk of diabetes.

8 Effect of iron deficiency and excess on immunity and infection

8.1 A role of iron in immunity and infection is supported by a large body of literature from animal studies investigating the effects of iron deficiency and excess on immune cell functions and whole-body immune responses (Kuvibidila and Baliga, 2002).

The immune response

8.2 A wide range of cells are involved in the immune response. A key role of neutrophils and monocytes is to engulf bacteria by the process of phagocytosis. They are then destroyed primarily by reactive oxygen species produced in the respiratory (also called oxidative) burst. Once a pathogen is engulfed by an antigen-presenting cell (primarily dendritic cells, monocytes and macrophages), antigenic peptides derived from the digested pathogen are presented to antigen-specific T lymphocytes. This causes activation of cell-mediated immunity.

8.3 The precise phenotype of T lymphocytes that are stimulated by antigen-presenting cells depends upon the nature of the antigen (e.g., if it is bacterial or viral in origin). Bacterial antigens typically lead to stimulation of helper T lymphocytes, while viral antigens stimulate cytotoxic T lymphocytes. The process of T lymphocyte proliferation increases the number of antigen-specific T lymphocytes following infection.

8.4 Activation of antigen-presenting cells and T lymphocytes results in increased production of peptide mediators, termed cytokines, which act to regulate the activities of various cell types and to coordinate the overall response. The principal cytokine involved in regulating T lymphocyte proliferation is interleukin-2 (IL-2). However, the precise phenotype of T cells that develop is determined by the mixture of cytokines produced by the antigen-presenting cell and the T lymphocyte. Interferon-γ (IFN-γ) produced by type-1 helper T lymphocytes activates macrophages to induce bacterial killing and activates B lymphocytes to induce production of some classes of antigen-specific antibodies (immunoglobulins). Many other cytokines are involved in regulating the functions of immune cells and in coordinating the immune response.

Effects of iron deficiency on immune function

8.5 A number of studies have assessed the impact of iron deficiency anaemia (typically haemoglobin <100g/L plus one or more measure of iron deficiency including serum iron, total iron-binding capacity, transferrin saturation) on different aspects of immune function. Results from these studies have shown decreases in: the proportion of T lymphocytes in the blood (Swarup-Mitra and Sinha, 1984; Kemahli *et al*, 1988); the lymphocyte proliferative response to antigens (Swarup-Mitra and Sinha, 1984; Ahluwalia, 2004); and secretion of IL-2 (Galan *et al*, 1992; Thibault *et*

al, 1993). Neutrophil function (respiratory burst, bacterial killing) is also impaired (Yetgin *et al*, 1979; Walter *et al*, 1986). B cell functions (antibody responses) seem little affected by iron deficiency (MacDougall *et al*, 1975; Bagchi *et al*, 1980; Krantman *et al,* 1982; Prema *et al,* 1982). There are a limited number of studies on the impact of iron deficiency on the functions of monocyte/macrophages, none on antigen presentation, and few studies of cytokines other than IL-2.

8.6 Several studies have reported that immune impairments in iron deficient subjects were restored to control values after iron therapy (Bhaskaram and Reddy, 1975; MacDougall *et al*, 1975; Sawitsky *et al,* 1976; Yetgin *et al*, 1979; Swarup-Mitra and Sinha, 1984; Walter *et al,* 1986; Chwang *et al*, 1988), suggesting a pathogenic role of iron deficiency.

8.7 Most of these studies involved small numbers of participants and were mainly conducted in children, often from developing countries where confounding factors might include the presence of infections and the co-existence of other or multiple nutrient deficiencies. Findings from these studies may not be relevant to UK populations. Additionally, many of these studies were carried out when immunological knowledge and techniques were less well developed, which limits the conclusions that can be drawn from their findings.

Effects of iron overload on immune function

8.8 There are fewer studies on the effects of iron overload on immune function and most have been carried out in patients with hereditary haemochromatosis or with transfusional iron overload associated with conditions such as ß-thalassaemia.

8.9 Patients with iron overload due to multiple transfusions generally have reduced proportions of T lymphocytes (Gugliemo *et al*, 1984; Kaplan *et al*, 1984; Dwyer *et al*, 1987) and reduced lymphocyte proliferative responses (Hernandez *et al*, 1980; Munn *et al*, 1981; Dwyer *et al*, 1987; Escalona *et al*, 1987). The effect of hereditary haemochromatosis on T cell proliferative responses is not clear (Bryan *et al*, 1984, 1991).

8.10 Limited data indicate that circulating B cell numbers (Bryan *et al*, 1991) or immunoglobulin concentrations (Bryan *et al*, 1984) are not affected in haemochromatosis patients but are increased in patients with ß-thalassaemia and sickle cell disease (Glassman *et al*, 1980; Dwyer *et al*, 1987; Escalona *et al,* 1987). There are reports of impaired phagocytosis of bacteria by monocytes in haemochromatosis patients (van Asbeck *et al*, 1982, 1984); and in patients with ß-thalassaemia, phagocytosis was found to be normal but bacterial killing was impaired (Van Asbeck *et al*, 1984; Ballart *et al*, 1986). Phagocytosis and bactericidal capacity by neutrophils is also impaired in patients with ß-thalassaemia (Waterlot *et al*, 1985; Cantinieaux *et al*, 1987, 1990).

Effects of iron supplementation on infection

8.11 A consequence of impaired immunity may be an increased susceptibility to infectious pathogens. Iron is required for both the host immune response and by pathogens for growth and replication. The acute phase recompartmentation of

iron in response to inflammation and stress (see section 2) might be a host defence strategy to deprive invading pathogens of iron. It is therefore possible that iron supplements might exacerbate infections by providing iron to pathogens.

8.12 The immune response to infections is often pathogen-specific and the components of the host response will have different susceptibilities to iron deprivation. Additionally pathogens have different dependencies on endogenous iron-containing enzymes and/or on host iron dependent functions.

8.13 A systematic review of 28 randomised controlled trials on the effect of iron (supplements, fortified foods/beverages and parenteral administration) on the incidence of infectious illness in children (Gera and Sachdev, 2002) reported that iron supplementation did not increase the risk (incident rate ratio, IRR) of non-diarrhoeal (IRR, 0.97; 95% CI, 0.95–1.06, p=0.99), respiratory tract (IRR, 0.98; 95% CI, 0.90–1.06; p=0.54), lower respiratory tract (IRR, 0.97; 95% CI, 0.83–1.23; p=0.93) or other (IRR, 1.04; 95% CI, 0.98–1.11; p=0.20) infections in children. However, risk of developing diarrhoea was significantly increased (IRR, 1.11; 95% CI, 1.01–1.23; p=0.04).

8.14 A trial of breast fed infants in Sweden (n=101) and Honduras (n=131) found no difference in risk of diarrhoea in infants supplemented with iron (1 mg/kg body weight/day) from 4 to 6 or 4 to 9 months of age compared to infants not receiving supplements (Dewey *et al*, 2002). However, an interaction was found between iron supplementation and initial haemoglobin concentration: for infants with haemoglobin concentration less than 110 g/L at 4 months, diarrhoea was less common among those supplemented with iron compared to those who received placebo (odds ratio, 0.21; 95% CI, 0.04–0.95; p=0.04); for infants with initial haemoglobin concentration above 110 g/L at 4 months, diarrhoea risk was significantly greater among those supplemented with iron (odds ratio, 2.4; 95% CI, 1.0–5.8; p=0.046).

8.15 A study in Bangladesh of infants (age, 6 months; n=799) supplemented with iron (20 mg/week, alone and in combination with 20 mg/week of zinc) for 6 months reported no differences in risk of diarrhoea or acute lower respiratory infection between the iron supplemented and control groups (Baqui *et al*, 2003). However, among infants who were less well-nourished (on the basis of weight-for-age Z score below -1), the group supplemented with iron and zinc combined were at significantly decreased risk of severe diarrhoea (IRR, 0.70; 95% CI, 0.54–0.92) and severe acute lower respiratory infection (IRR, 0.60; 95% CI, 0.40–0.90).

8.16 Some studies have suggested that iron supplementation to manage iron deficiency in populations where malaria is a risk increases susceptibility to malaria and other infections (Oppenheimer *et al*, 1986a, b; Smith *et al*, 1989; van den Homberg *et al*, 1996). However, several studies have not reported an increased incidence of malaria (Menendez *et al*, 1997; Berger *et al*, 2000) or other infections (Harvey *et al*, 1989; Berger *et al*, 2000) with oral iron supplementation in countries where malaria is prevalent.

8.17 The systematic review by Gera and Sachdev (2002) (see paragraph 8.13), which included 5 trials assessing malaria incidence, reported that iron supplementation did not affect the incidence rate of malaria (IRR, 1.07; 95% CI, 0.94–1.24; p=0.35).

This finding was confirmed by 2 further trials. In Kenya, Verhoef *et al* (2002) reported that malaria risk was not increased in anaemic children (haemoglobin concentration<100g/L; age, 2–36 months; n=328) given iron supplements (6 mg/kg/week) for 12 weeks compared to those given placebo. A trial in Zanzibar (Mebrahtu *et al*, 2004) reported no effect of long term iron supplementation (10 mg/day for 12 months) on prevalence of malaria infection in children aged 4–71 months (n=614) compared to placebo (94% of the children had haemoglobin concentrations <110 g/L at baseline).

8.18 Two parallel randomised controlled trials assessed morbidity and mortality effects of iron (12.5 mg/day) and folic acid (50 µg/day) supplementation (with and without zinc) on infants (age, 1–35 months; n=24,076) in Zanzibar (Sazawal *et al*, 2006) where malaria transmission is high and occurs all year, and infants (age, 1–36 months; n=26,250) in Nepal (Tielsch *et al*, 2006) where malaria incidence is low. Average follow-up was for approximately 1 year. The trial in Zanzibar reported that, compared to the placebo group, infants supplemented with iron and folic acid were at significantly increased risk of adverse events (relative risk, 1.12; 95% CI, 1.02–1.23; p=0.02) and hospital admission (relative risk, 1.11; 95% CI, 1.01–1.23; p=0.03). Mortality risk was also increased but was not significant (relative risk, 1.15; 95% CI, 0.93–1.41; p=0.19). The trial in Nepal reported no differences in mortality or morbidity outcomes between the supplemented and placebo groups.

8.19 The WHO and UNICEF (2006) subsequently advised that, in areas with high prevalence of malaria and other infectious diseases, iron and folic acid supplementation should be targeted at children who are anaemic and at risk of iron deficiency and that they should receive concurrent protection from malaria and other infectious diseases. Optimal dose, duration, and mode of iron supplement delivery were identified as key research questions.

8.20 Another trial of children aged 0.5–15 years (n=855) in Peru (Richard *et al*, 2006) assessed the effect of daily supplementation (for 7 months) with iron (15 mg/day) alone or combined with zinc (20 mg/day) on morbidity outcomes. Compared with placebo, iron supplementation was associated with an increased incidence of malaria in children aged 5 years and above (IRR, 1.76; 95% CI, 1.14–2.70; p=0.010). Iron combined with zinc did not affect malaria incidence. Among children aged 9 years or over, iron (alone and in combination with zinc) significantly increased diarrhoea risk (iron: IRR, 1.72; 95% CI, 1.06–2.79; p=0.03/iron + zinc: IRR, 1.99; 95% CI, 1.18–3.34; p=0.009). Iron supplementation (alone and in combination with zinc) had no effect on incidence of acute lower respiratory infection.

Iron and human immunodeficiency virus (HIV) infection

8.21 Iron enhances transcription of HIV *in vitro*, probably by increasing oxidative stress leading to activation of the transcription factor, nuclear factor kappa B (Sappey *et al*, 1995; Boelaert *et al*, 1996a, b). Conversely, iron chelation with deferoxamine[67] (DFX) in cell culture inhibits HIV replication and decreases apoptosis of helper T lymphocytes.

8.22 A cross-sectional study in Zimbabwe (Friis *et al*, 2003) examined the association between serum ferritin concentration and HIV infection in pregnant women (n=526). After controlling for serum alpha-1-antichymotrypsin concentration (an acute phase reactant), mean viral load was lowest in women with serum ferritin concentrations below 6 µg/L and increased with increasing serum ferritin concentrations (p=0.08). Another cross-sectional study of HIV-infected women (n=483) in Malawi found no association between serum ferritin concentration and HIV progression or viral load (Semba *et al*, 2001). However, such data may be influenced by the anaemia of chronic disease associated with HIV infection.

8.23 A retrospective analysis of HIV-infected patients (n=348) in the USA found that the risk of death was greater in those with the highest bone marrow macrophage iron content[68] compared to those with normal or low iron contents (hazard ratio, 2.1; 95% CI, 1.3–3.5; p=0.003) (de Monye *et al*, 1999). Infections caused by *Candida* spp, *Pneumocystis carinii* and *Mycobacterium* spp were also more common in patients with the highest bone marrow macrophage iron stores than in those with low or normal stores ($p \leq 0.006$). However, the macrophage iron deposits might again reflect disease severity and the effect of HIV on the metabolism of iron rather than the effect of iron on HIV.

8.24 A study in France (Costagliola *et al*, 1994) reported that the rate of progression of HIV disease among patients (n=64) with thalassaemia major was inversely proportional to DFX dose ($p<0.002$).

8.25 A prospective study in the USA reported a significant protective relationship between increasing intakes of iron and development of AIDS in HIV-infected men (n=296) followed over 6 years (Abrams *et al*, 1993). After adjustment for age, smoking, energy intake and health status at baseline, men with iron intakes of 23–33 mg/day were significantly associated with an increased progression to AIDS compared to men with iron intakes ≥ 49 mg/day (hazard ratio, 2.0; 95% CI, 1.2–3.5; p trend=0.02).

8.26 A small trial of HIV-infected adults in Kenya (n=32) found no effect of 60 mg iron/twice weekly for 4 months on HIV-1 viral load (Olsen *et al*, 2004). Kupka *et al* (2007) followed a cohort of HIV-infected pregnant Tanzanian women (n=584) from 12 to 27 weeks gestation to 30 weeks postpartum. All the women received 120 mg/day of iron during their pregnancy. Serum ferritin concentration was not associated with HIV-related mortality or HIV progression.

67 Deferoxamine is a widely used chelating agent used to remove excess iron in thalassaemia patients with transfusional iron overload.

68 Bone marrow macrophage iron stores were graded on a scale of 1–5; patients with grades 4–5 iron stores (high) were compared to those with grade 0–2 iron stores (normal or low).

8.27 Haptoglobin (Hp), a plasma protein which removes free haemoglobin from the circulation (Langlois and Delanghe, 1996), exists as three major phenotypes (Hp 1-1, Hp 2-1, Hp 2-2). In men, the Hp 2-2 phenotype has been shown to be associated with increased serum ferritin concentrations and with increased macrophage iron accumulation (Langlois *et al*, 2000; Delanghe and Langlois, 2002). Delanghe *et al* (1998) reported that HIV-infected males with the Hp 2-2 phenotype in Belgium (n=493) and Luxembourg (n=160) had higher plasma HIV levels (p=0.03), a greater increase in plasma HIV level over one year (p=0.003), higher mortality (relative risk, 1.78; 95% CI, 1.25–2.54; p=0.0001) and shorter median survival time compared to those with the Hp 1-1 or 2-1 phenotypes. Friis *et al* (2003) reported that the viral load of HIV-infected pregnant Zimbabwean women with the Hp 2-2 phenotype was twice that of women with Hp 1-1 phenotype (95% CI, 1.4–4.0; p=0.002).

Iron and tuberculosis (TB)

8.28 *Mycobacterium tuberculosis* grows within macrophage phagosomes. The mycobacterium appears to evade host defence by preventing phagosome acidification and lysosome fusion and to acquire iron from host endosomal holotransferrin (Boelaert *et al*, 2007).

8.29 Iron has been shown to enhance the growth of *Mycobacterium tuberculosis* both *in vitro* (Cronje *et al*, 2005) and in animal studies (Lounis *et al*, 2001). *In vitro* studies have shown that excess iron impairs the anti-microbial cytotoxic effects of macrophages against pathogens (Moyo *et al*, 1997).

8.30 There are limited human data on the relationship between iron status and TB. Iron overload due to excessive dietary intakes has been associated with increased risk of progression and death from TB. Dietary iron overload is common in rural populations of southern Africa and is caused by consumption of traditional alcoholic beverages which are brewed in iron drums and cans (Bothwell *et al*, 1964) (see paragraph 2.47). In southern Africa, a high incidence of iron overload has been reported in patients dying from TB (Moyo *et al*, 1997; Lounis *et al*, 2001).

8.31 A prospective study in Zimbabwe of TB patients (n=98) and controls (n=98) examined whether previous dietary exposure to iron was a risk factor for active TB (Gangaidzo *et al*, 2001). Patients were followed from one week to nine months after start of treatment. After controlling for HIV status and liver function, increased dietary iron (defined as an estimated lifetime consumption of more than 1000 L of traditional beer) was significantly associated with a 3.5-fold increase in the odds of developing active TB (95% CI, 1.4–8.9; p=0.009). However, increased dietary iron in this population was from an alcoholic beverage and alcoholism has also been associated with increased TB risk (Feingold, 1976).

8.32 A study in Indonesia reported no difference in distribution of Hp phenotypes in TB patients (n=97) and healthy controls (n=126) (Grange *et al*, 1985). Kasvosve *et al* (2000) reported that the distribution of Hp phenotype of TB patients in Zimbabwe (n=98) was not significantly different from healthy controls (n=98); however, during 18

months of follow-up after start of treatment, the odds of dying from tuberculosis were 6-fold higher in patients with Hp 2-2 phenotype compared to those with Hp 1-1 phenotype (95% CI, 1.04–35.1; p=0.04).

Summary and conclusions

8.33 Iron deficiency anaemia (typically defined in most studies as haemoglobin <100 g/L plus one or more markers of iron deficiency including serum iron, total iron binding capacity, transferrin saturation) and iron overload (due to multiple blood transfusions in ß-thalaessemia patients) impair some aspects of immune function. The functional consequences of these impairments on morbidity are unclear.

8.34 It has been suggested that iron supplementation may favour infectious pathogens by providing them with a supply of iron which is required for their growth and replication. In human studies, the effect of iron supplementation on morbidity and mortality from infections is uncertain. Most studies have been carried out in children and in developing countries. The evidence suggests that iron supplementation does not increase the risk of non-diarrhoeal or respiratory tract infections in children but may increase risk of diarrhoea.

8.35 Studies which have examined whether iron supplementation increases malaria risk are inconsistent. It is also not clear whether iron supplementation increases risk of infectious diseases in areas where malaria incidence is high. The WHO/UNICEF advise that in regions of the world where the prevalence of malaria and infectious disease is high, iron supplementation should be targeted at young children with anaemia or those at risk of iron deficiency.

8.36 There is currently insufficient evidence to draw conclusions on the relationship between iron supplementation and HIV or TB.

8.37 Most studies on iron and infection have been conducted in developing countries where there are multiple nutrient deficiencies which may also affect resistance to infection. Many studies do not take these factors into account. However, the relevance of most of these studies to the UK may be limited.

8.38 On balance, there is little evidence to suggest that providing iron supplements to children in the UK would have any adverse effects on infectious disease incidence or morbidity. Some evidence suggests that iron supplementation might have adverse effects in some subgroups of the population, e.g., those with HIV and children at risk of diarrhoea.

9 Dietary iron intakes and iron status of the UK population

9.1 Nationally representative data on the iron intakes and iron status of the UK population were drawn from surveys of adults and children living in Great Britain and the UK. Data on the general population were obtained from the National Diet and Nutrition Surveys (NDNS), a series of stand-alone cross-sectional surveys, each covering a different age group in Great Britain: children aged 1½–4½ years in 1992/93 (Gregory *et al*, 1995); people aged 65 years and over in 1994/95 (Finch *et al*, 1998); young people aged 4–18 years in 1997 (Gregory *et al*, 2000); and adults aged 19–64 years in 2000/01 (Henderson *et al*, 2002; Henderson *et al*, 2003a; Rushton *et al*, 2004). An earlier survey of adults aged 16–64 years was conducted in 1986/87 (Gregory *et al*, 1990). Data on low income populations (aged 2 years and above) in the UK in 2003/05 were obtained from the Low Income Diet and Nutrition Survey (LIDNS) (Nelson *et al*, 2007a; Nelson *et al*, 2007b). Data from these surveys were used to assess the adequacy of iron intakes and iron status of the UK population. No nationally representative data are available for infants and children under 1½ years or for pregnant or lactating women.

9.2 The NDNS is now a continuous rolling cross-sectional survey of people aged 18 months and older in the UK. Data on food consumption and nutrient intakes from the first year of the NDNS rolling programme (2008/09) have been published[69] (Bates *et al*, 2010) and data on iron intakes are included for information in Annex 10. However, they were not used in this report to assess the dietary adequacy of iron intakes in the UK because the data are preliminary and sample sizes are currently too small for robust subgroup analysis.

Assessment of iron intakes

9.3 In the NDNS series, diet was assessed by weighed records of all foods consumed over 7 consecutive days for adults aged 19–64 years and young people aged 4–18 years; and 4 consecutive days for adults over 65 years and children aged 1½–4½ years. In the LIDNS, diet was assessed by 24-hour recall on 4 non-consecutive days including, where possible, a weekend day.

9.4 Iron intake data from these surveys were compared against the DRVs for iron intake (see Table 2). Difficulties associated with the use of dietary surveys to assess the adequacy of nutrient intakes against DRVs include reliability of food composition tables and misreporting of food consumption, which reduces confidence in the accuracy of population mean intakes and can affect the distribution of measured intakes. Participants may forget or purposely omit to record some items consumed so their intake is under-reported; over-reporting can also occur if, for example, food

69 Blood sampling data from the rolling programme have not been published because the sample size for blood specimens is currently too small for meaningful analysis.

left on the plate is not taken into account. Additionally, judgements of dietary adequacy of iron intakes against DRVs only take limited account of the amount of iron absorbed from the diet. Iron absorption of 15% was assumed in the derivation of the UK DRVs. This was based on consumption of a diverse diet and may not apply to subgroups of the population with different dietary patterns (DH, 1991). This assumption also does not take account of absorptive adaptation according to systemic iron needs. The limitations of the DRVs for iron are detailed in section 3.

Assessment of iron status

9.5 In this report, internationally accepted reference values for iron sufficiency published by the WHO (2001) (Table 7) were used to evaluate the haemoglobin and serum ferritin concentrations of the UK population. Haemoglobin concentrations below the WHO thresholds were used to define anaemia, and serum ferritin concentrations below the WHO thresholds were used to define iron deficiency or to identify a risk of iron deficiency because of low ferritin depots of iron. Haemoglobin and serum ferritin concentrations were measured for all age groups of the population in the NDNS and LIDNS; where age bands overlapped with more than one WHO age band, both thresholds were used to assess adequacy of iron status (Table 8).

9.6 Combinations of biochemical indicators have been proposed and used to define iron deficiency (see paragraphs 4.34–4.35), including: ferritin, transferrin saturation, erythrocyte protoporphyrin, and mean cell volume; and transferrin saturation and erythrocyte protoporphyrin. Although many of these indicators were measured in the NDNS and LIDNS, multiple indices were not used in this report because of the lack of internationally agreed cut-off levels.

Table 7: WHO (2001) thresholds for adequate haemoglobin and serum ferritin concentration

Group/Age	Haemoglobin (g/L)	Serum ferritin (µg/L)
Children 0.5–4.99 years	110	12
Children 5–11 years	115	15
Children 12–14 years	120	15
Non-pregnant women over 15 years	120	15
Men over 15 years	130	15

Table 8: Thresholds of haemoglobin and serum ferritin concentration used to assess adequacy of iron status of the UK population according to age bands in the NDNS and LIDNS

Group/Age	Haemoglobin (g/L)	Serum ferritin (µg/L)
Children 1½–4½ years	110	12
Children 4–6 years	110 and 115	12 and 15
Children 7–10 years	115	15
Children 11–14 years	115 and 120	15
Non-pregnant women over 15 years	120	15
Men over 15 years	130	15

Iron intakes of the UK population

9.7 The iron intakes of the UK population are tabulated in Annex 9 (Tables A28–A35).

Dietary sources of iron

9.8 In the NDNS (Annex 9, Table A28), iron fortified cereals and cereal products were the main contributors to iron intakes of adults aged 19–64 years (45% of total intake); major contributors within this group were breakfast cereals and white bread. Substantial contributions were also made by meat and meat products (19% in men; 15% in women) and vegetables, including potatoes (16% in men, 19% in women). The contribution made by cereals to iron intakes was greater in younger and older age groups (47–53%) while that from meat and meat products was lower in younger children (14%). Milk and milk products provided approximately 5–6% of iron intakes for children aged 1½–4½ years and adults aged 65 years and over, living in institutions, but less for other age groups.

9.9 In the LIDNS (Annex 9, Table A29), iron fortified cereals and cereal products were the main contributors to iron intakes in adults over 19 years (41% of total) and children aged 2–18 years (51% of total). Other sources were meat and meat products. The contribution made by meat and meat products to overall iron intakes was greater for adults (21%) than for children (17%) and for men (23%) compared to women (21%). Vegetables, including potatoes, were also major contributors to iron intakes of adults and children (19%).

Iron intake from all sources

9.10 In the NDNS (Annex 9, Table A30), mean intakes of iron from all sources (food and supplements) for adults 19 years and over were 10–15 mg/day for men and 8–13 mg/day for women. Average intakes tended to be at the higher end of the range for adults aged 25–64 years and at the lower end for adults aged under 25 years and over 65 years. Average iron intakes of children aged 1½–4½ years were 5–6 mg/day; intakes of young people aged 4–18 years were 8–13 mg/day for boys and 7–9 mg/day for girls. Differences in mean iron intake between the sexes and at different ages were less apparent after correction for energy intake, indicating that the iron density of the diet was similar in males and females at all ages (about 1.25 mg/MJ/day or 0.3 mg/1000 kcal/day).

9.11 Adults aged 65 years and over living in institutions had lower iron intakes than free-living adults (8.6 versus 10.0 mg/day) which were largely related to lower energy intakes.

9.12 In the LIDNS (Annex 9, Table A31) average intakes from all sources were between 10 and 12 mg/day for men (19 years and over) and about 9 mg/day for women (19 years and over). Average intakes were 8–9 mg/day for children aged 2–10 years and 9–11 mg/day for older children aged 11–18 years. In all age groups, males had higher mean intakes of iron than females.

Intake of haem iron

9.13 Haem iron, which is present in meat and fish, is, if needed, absorbed more efficiently than non-haem iron and is considered more bioavailable (see section 5). Haem iron contributed approximately 4–6% of total iron intake in the NDNS (Annex 9, Table A32) and 4–9% in the LIDNS (Annex 9, Table A33). This is lower than the amount usually cited (10%–12%) for a diet with substantial amounts of red meat (Hunt *et al,* 1999).

9.14 The distribution of haem iron intakes in the LIDNS was skewed, with median intakes 9–28% lower than mean intakes depending on age and sex.

Iron intake from supplements

9.15 In the NDNS, iron from supplements contributed a relatively small proportion of total daily iron intake in most age groups. As a consequence, average iron intakes from all sources were similar to intake from food sources. The exception was for adult women: supplements contributed 21% of total iron intake for women aged 35–49 years; 12% for women aged 19–24 years; and 11% for women aged 50–64 years.

9.16 Supplements made a greater contribution to iron intakes of men, but not women, in the 2000/01 adult survey than in the 1986/87 survey. For men, supplements contributed 6% of total iron intake in 2000/01 compared to 2% in 1986/87; for women, supplements contributed 15% of total intake in 2000/01 compared to 14% in 1986/87.

9.17 In the LIDNS, supplements contributed 4% and 7% of total iron intakes for men and women respectively.

Intake of fortificant iron

9.18 The main sources of fortificant iron in the UK diet are white and brown wheat flours, which are fortified with iron on a mandatory basis, and a number of other foods, particularly breakfast cereals, which are fortified with iron on a voluntary basis. The contribution of fortificant iron is included in the iron content of specific food items in food composition databases, and to the estimation of total iron and non-haem iron intakes. Values in food composition tables may not be accurate, however, because of the continual introduction and withdrawal of food products voluntarily fortified with iron. The NDNS and LIDNS do not provide information on the contribution of iron from fortified foods to total iron intakes; however, cereals and cereal products provide approximately 50% of average daily iron intakes for all population groups. Breast milk substitutes are a source of fortificant iron in the diet of infants in the UK (see paragraphs 9.50–9.51).

Iron intakes of subgroups of the population

9.19 The NDNS is not large enough to obtain representative data on subgroups of the population. However, in additional analyses of NDNS data from the surveys of children aged 1½–4½ years and young people aged 4–18 years, a vegetarian diet or a diet avoiding meat were not associated with lower than average total iron intakes

(Thane *et al*, 2003; Thane and Bates, 2000); and girls aged 11–18 years who smoked had lower total iron intakes than those who did not (Thane *et al*, 2003). There was no evidence of associations between ethnicity and total iron intakes among children aged 4–18 years, but children aged 1½–4½ years in Caucasian or Black households had lower iron intakes than those in Asian households.

9.20 In the LIDNS, sample sizes for ethnic groups were small and should be interpreted with caution. For adults aged 19 years and over, mean iron intakes were not significantly different by ethnic group. Mean intakes were 12.2 mg/day for Asian men (n=35) compared to 11.0 mg/day for Caucasian men (n=876). Mean iron intakes were 9.5 mg/day for Asian women (n=58), 7.9 mg/day for Black women (n=49), and 8.7 mg/day for Caucasian women (n=1713). For girls and boys aged 2–18 years and Black men, sample sizes were too small (n<30) to compare intakes by ethnic groups.

Comparison of iron intakes with Dietary Reference Values

9.21 Comparison of iron intakes against the DRVS for iron should be interpreted with caution because the DRVs for iron are derived from uncertain data (see paragraph 3.2).

Comparison of mean daily intakes as percentage of the Reference Nutrient Intake (RNI)

9.22 In a population in which nutrient requirements are normally distributed, the RNI represents the amount of a nutrient that is likely to meet the needs of 97.5% of the population (DH, 1991). This means that most individuals in a population will have a requirement that is below the RNI. However, the DRVs of females of reproductive age are not normally distributed because menstrual blood losses follow a right-skewed distribution (see paragraphs 3.29–3.30). Although this was taken into account in calculating the RNI for women of reproductive age (see paragraph 3.6), it is estimated that approximately 10% of women with the highest menstrual losses may have requirements above the RNI.

9.23 In the NDNS (Annex 9, Table A34), average daily intakes of iron were above the RNI, or above 90% of the RNI, for all age groups except children aged 1½–3½ years (73–81% of RNI), girls aged 11–18 years (about 60% of RNI), and women aged 19–49 years (66–87% of RNI).

9.24 In the LIDNS (Annex 9, Table A35), average daily iron intakes were above the RNI for males in all age groups. For females, only girls aged 2–10 years and women 50 years and over had daily intakes at or above the RNI; mean daily intakes were 63% of the RNI for girls aged 11–18 years and approximately 60% of the RNI for women aged 19–49 years.

Iron intakes below the Lower Reference Nutrient Intake (LRNI)

9.25 The LRNI represents the amount of a nutrient that is likely to meet the needs of only 2.5% of the population (DH, 1991). This means that in a normal population, 2.5% would be expected to have requirements below the LRNI. Intakes below this level

are almost certainly inadequate for most individuals. Low intakes of iron would be of public health concern only when the prevalence of intakes below the LRNI exceeds 5% of the population (WHO, 2001).

9.26 In the NDNS (Annex 9, Table A34), population groups with substantial proportions with iron intakes below the LRNI were children aged 1½–3½ years (12–24%), girls aged 11–18 years (44–48%), and women aged 19–49 years (25–40%).

9.27 In the LIDNS (Annex 9, Table A35), a higher proportion of females aged 11–49 years (39% of girls aged 11–18 years; 50% of women aged 19–49 years) had iron intakes below the LRNI compared to those aged 50 years and over (13%, 50–64 years; 5%, 65 years and over). A higher proportion of males aged 11–18 years (14%) had intakes below the LRNI compared to those aged 2–10 years (2%) and 19 years and over (3–5%).

9.28 Both the NDNS and LIDNS indicate that substantial proportions of some population groups in the UK have iron intakes that are below the LRNIs set for iron. The high proportion of toddlers and females with intakes of iron below the LRNI suggests that the iron DRVs for these population groups may be too high. The DRVs for iron are derived from limited data (see section 3); they also assume that only 15% of iron is absorbed from the diet which does not take account of absorptive adaptation to increased systemic iron needs.

Iron status of the UK population: evidence of anaemia, iron deficiency and iron deficiency anaemia

9.29 Haemoglobin and ferritin values in the UK are tabulated in Annex 9 (Tables A36–A41). Caution should be exercised in the interpretation of these data (see section 4). Neither marker, alone or in combination, can necessarily diagnose iron deficiency but they can be used as indicators of individuals at risk of deficiency. Data on haemoglobin and serum ferritin concentrations of children and young people aged under 19 years in the LIDNS are not included in this report because the response rate for blood specimens in this age group was low and, as a consequence, may not be representative.

Haemoglobin concentration

9.30 In the NDNS (Annex 9, Table A36), groups which had the highest proportions with haemoglobin concentrations below WHO cut-offs for anaemia were adults aged 65 years and over in institutions (39% of women; 52% of men), free-living adults aged 75 years and over (13–38%), and girls aged 4–6 years (15% based on 115 g/L cut-off; 9% based on 110 g/L cut-off). For adults aged 19–64 years, the prevalence of anaemia was higher in women (7–9%) compared to men (0–4%). In adults aged 75 years or over the prevalence was greater in men (16–38%) than women (13–16%). For adults aged 65–74 years, the proportion with haemoglobin concentrations below the WHO cut-offs for anaemia was similar for men (7%) and women (6%).

9.31 In the LIDNS (Annex 9, Table A37), the highest proportions of adults with haemoglobin concentrations below WHO cut-offs for anaemia were among men aged 65 years and above (20%); prevalences were much lower for men aged 19–64 years (0–5%). In contrast, prevalence of anaemia was higher in younger women aged 19–49 years (12–18%) than women aged 50–64 years (6%) and women 65 years and over (9%).

Serum ferritin concentration

9.32 In the NDNS (Annex 9, Table A38), the highest proportions with ferritin concentrations below the WHO cut-offs indicating iron deficiency were found in children aged 1½–4½ years (25–34%); girls aged 11–14 years (12%) and 15–18 years (24%); women aged 19–24 years (16%) and 35–49 years (13%); and free-living women 75 years and over (12–14%).

9.33 In the LIDNS (Annex 9, Table A39), the highest proportions with serum ferritin concentrations below WHO cut-offs were found in women aged 19–34 years (21%) and 35–49 years (14%); the proportion was lower in women aged 50 years and over (4–5%). For men aged 19 years and over, the proportion with serum ferritin concentrations below WHO cut-offs ranged between 0 and 5%.

Haemoglobin and serum ferritin combined (iron deficiency anaemia)

9.34 Based on the WHO thresholds of haemoglobin and serum ferritin concentrations used to define iron deficiency and anaemia, the prevalence of iron deficiency anaemia in the NDNS (Annex 9, Table A40) was 5% or above for children aged 1½–2½ years (6%), females aged 15–18 years (5%) and 35–49 years (5%), free-living adults aged over 85 years (6%), and men aged 65 years or over living in institutions (5%).

9.35 The prevalence of iron deficiency anaemia for these population groups is not clearly consistent with their mean iron intakes which were similar for all children aged 1½–4½ years (5–6 mg/day) and slightly higher for females aged 35–49 years (13 mg/day) than for females aged 19–34 years (10 mg/day) and 50–64 years (12 mg/day). The findings are consistent with the groups having high proportions with intakes below the LRNI for children aged 1½–2½ years (24%) and girls aged 15–18 years (48%). They are not consistent for women aged 35–49 years who had a lower proportion with intakes below the LRNI (25%) than women aged 25–34 years (40%); however, the proportion of women aged 25–34 years with iron deficiency anaemia was only 2%. They are also not consistent for free-living adults aged 85 years and over (4–10% below LRNI) or men aged 65 years and over living in institutions (4–5% below LRNI).

9.36 In the LIDNS (Annex 9, Table A41) the prevalence of iron deficiency anaemia was above 5% for women aged 19–49 years (9–11%) and 65 years and over (6%). The prevalence of iron deficiency anaemia in women aged 19–49 years and 65 years and over is also not clearly consistent with the mean intakes which were similar for all women aged 19–65 years and over (9 mg/day). The findings are consistent with the proportions with intakes below the LRNI for women aged 19–49 years which was highest for this group (50%). They are not consistent for women aged 65 years and over as the proportion in this age group with intakes below the LRNI was 5%.

9.37 Findings from the NDNS and LIDNS broadly show that men (under 85 years) in the UK are not at risk of iron deficiency anaemia and that some groups of women of reproductive age (15–50 years), particularly those from low income groups, may be at increased risk. This is consistent with increased blood losses due to menstruation in this age group making them more vulnerable to iron deficiency anaemia. Iron deficiency anaemia observed in some adult groups aged 65 years and over (free-living men and women aged 85 years and over; men aged 65 years and over living in institutions) is consistent with blood loss due to gastrointestinal disease or medication (e.g., aspirin) in older people.

Relationship between iron status markers and iron intakes

9.38 As would be expected in a population which has an adequate iron supply, few significant associations between haemoglobin and serum ferritin concentrations and intakes of total iron, non-haem iron or haem iron were observed in any of the surveys in the NDNS series.

9.39 The relationship between dietary iron intake and biochemical markers of iron status may also be complicated by a number of confounding factors which affect both iron absorption and biochemical markers of iron status (see paragraphs 5.51–5.52). There are also problems associated with obtaining reliable estimates of dietary iron intake in dietary surveys (see paragraph 9.4). Additionally, measurement of only one blood sample will not take account of diurnal and day-to-day variability in haematological markers of iron status.

9.40 Correlation coefficients were statistically significant in a few age and sex categories. Significant positive correlations were observed between haemoglobin concentration and dietary intakes of haem, non-haem and total iron in children aged 1½–4½ years ($p<0.05$) and in boys ($p<0.01$) and girls ($p<0.05$ for total and non-haem iron; $p<0.01$ for haem iron) aged 4–18 years. These relationships were apparent when all ages were combined but rarely reached significance when the age groups were considered separately. In adults aged 19–64 years, there was a significant positive association between haemoglobin concentration and haem iron intake for women ($p<0.05$) but not for men. Intakes of non-haem iron and total iron were negatively correlated with haemoglobin concentration in men aged 19–24 years ($p<0.05$) but positively correlated with haemoglobin concentration in men aged 25–34 years ($p<0.01$). In free-living adults aged 65 years and over, positive correlations were found between haemoglobin concentration and haem iron intake for men ($p<0.01$) and with total, haem and non-haem iron for women ($p<0.01$). In adults aged 65 years and over living in institutions, no significant relationship was observed between haemoglobin concentration and iron intake for men but positive associations were observed with total and non-haem iron for women ($p<0.05$).

9.41 There were no significant associations between serum ferritin concentrations and iron intakes in children aged 1½–4½ years. In young people aged 4–18 years, serum ferritin concentration was positively associated with intakes of total ($p<0.05$), haem ($p<0.01$) and non-haem ($p<0.05$) iron in boys, but only with haem iron intakes in girls ($p<0.01$). For adults aged 19–64 years, serum ferritin concentration was positively

correlated with haem iron intake in men aged 19–24 years ($p<0.05$) and 35–49 years ($p<0.01$) and in women aged 50–64 years ($p<0.01$), but no correlations were observed for total iron or non-haem iron. In free-living adults aged 65 years and over, ferritin concentration was positively associated ($p<0.05$) with haem iron intake in both men and women, and also with total and non-haem iron in women ($p<0.01$). No significant associations were observed between serum ferritin concentration and iron intakes of adults aged 65 years and over living in institutions.

9.42 Correlation coefficients for haemoglobin and serum ferritin concentrations with dietary intakes of iron are not reported in the LIDNS. Mean intakes of iron were lowest for boys aged 11–18 years and men aged 65 years and over. These findings are consistent with prevalence of haemoglobin concentrations below cut-off points, which were highest in these groups, but are not consistent with serum ferritin concentrations. In women, low reported iron intakes (as a percentage of the RNI) amongst those aged 19–34 years and 35–49 years were consistent with the high prevalence of serum ferritin concentrations below 15 µg/L and haemoglobin concentrations below 120 g/L in these age groups although not to the extent suggested by the dietary data.

Further analysis of specific age-groups in the NDNS series

9.43 Further analyses of NDNS data in relation to risk factors for poor iron status have been carried out for children aged 1½–4½ years (Thane *et al*, 2000; Thane and Bates, 2000), children aged 4–18 years (Thane *et al*, 2003) and older people aged 65 years and over (Doyle *et al*, 1999).

9.44 Thane *et al* (2000) reported that among children aged 1½–4½ years, significantly higher proportions with haemoglobin concentrations <110 g/L were found in those: whose head of household was unemployed; with low household incomes; whose mothers had low educational attainment; and those who had never been breast fed. The proportion of children with ferritin concentrations <10 µg/L was significantly greater in those whose head of household did manual work, had never worked, or was in the armed forces. The proportion of children with both haemoglobin concentrations <110 g/L and ferritin concentrations <10 µg/L was greater in households receiving benefits and in those whose mothers had low educational attainment.

9.45 The highest proportion of children with either haemoglobin concentrations <110 g/L or serum ferritin concentrations <10 µg/L were those in the lowest quintile of meat and fruit intake and those consuming large quantities of milk and milk products (Thane *et al*, 2000). Serum ferritin concentrations were lower in children who were vegetarian, and were significantly lower in children under 3 years (Thane and Bates, 2000). A greater proportion of girls aged 11–18 years who did not eat meat had haemoglobin concentrations below 115 g/L (11–12 years) or 120 g/L (13 years and over) and serum ferritin concentrations <15 µg/L (Thane *et al*, 2003).

9.46 Doyle *et al* (1999) reported that the haemoglobin concentration of adults aged 65 years was negatively associated with intakes of dairy foods, calcium and tea, and positively associated with intakes of meat, poultry, vegetables, fibre and alcohol, although these associations were not significant in all age and sex groups. A significant positive association was reported between serum ferritin concentrations and vitamin C and vegetable intake.

Iron status of minority ethnic groups

9.47 Representative data on biochemical markers of iron status in minority ethnic groups are not available in the NDNS and LIDNS. The Health Survey for England (HSE) 2004 provides information on haemoglobin and serum ferritin concentrations of minority ethnic groups (Black Caribbean, Black African, Indian, Pakistani, Bangladeshi, Chinese and Irish) aged 16 years and over. However, the HSE did not use combined haemoglobin and serum ferritin concentrations to assess the prevalence of iron deficiency anaemia. Anaemia was defined as haemoglobin concentration less than 120g/L for both men and women[70]. Iron deficiency was defined as serum ferritin concentrations below the upper cut-off for the bottom quintile of the general population range in 1998[71]. The distribution of the data was not reported in the HSE so it was not possible to interpret and compare the HSE data with the NDNS in which combined ferritin and haemoglobin concentrations enable iron deficiency anaemia to be identified using the WHO criteria (see Table 7).

9.48 The prevalence of haemoglobin concentrations below 120g/L (irrespective of cause) reported in the HSE ranged from 0 to 4.4% among men. In women, it was lowest in Irish (5.7%) and Chinese (7.3%) women and higher in Black Caribbean (16.4%), Pakistani (20.5%), Bangladeshi (22.5%), Black African (25.8%) and Indian (29%) women. This compares to 6-16%[72] for females aged 15 years and over with haemoglobin concentrations below 120 g/L reported in the NDNS.

9.49 The prevalence of low serum ferritin concentrations among men from minority ethnic groups in the HSE was lowest in Chinese (3%) and highest in Indian (33%) men and ranged between 15 and 19% in the other groups. Among women, the prevalence was lowest in Chinese and Irish women (14%); it was higher in Black Caribbean (19%), Black African (24%), Bangladeshi (29%) and Pakistani (38%) women and highest in Indian women (48%).

70 In the NDNS, haemoglobin concentrations below the WHO thresholds were used to define anaemia: men, 130g/L; women, 120g/L.

71 The actual value was not specified.

72 Does not include institutionalised women aged 65 years and over.

Iron intake and status in infants and young children up to 18 months

Iron intakes of infants in the UK

9.50 There are very few national and international data on the nutritional adequacy of the diet of infants up to 18 months of age. In the UK, the only nationally representative survey of food and nutrient intakes in infants aged 6–12 months was conducted in 1986 (Mills and Tyler, 1992). This survey reported that mean iron intake was 8.1 mg/day (above the RNI of 7.8 mg/day), median intake was 7 mg/day, and 15% of infants had intakes below the LRNI (4.2 mg/day). Average daily intakes of iron were significantly lower in infants aged 9–12 months (6.7 mg) than in infants aged 6–9 months (9.3 mg). For most infants, commercial baby foods, including infant formulas, represented the major source of iron; older infants obtained a large proportion of iron from "family foods". Most iron consumed was non-haem rather than haem iron.

9.51 Findings from the Infant Feeding Survey 2005 (Bolling *et al*, 2007) suggest considerable changes in infant feeding practices since the survey in 1986 (Mills and Tyler, 1992). For example, between 1995 and 2005, there was a significant increase in the incidence of breast feeding[73] in the UK: in 1995, 34% of mothers did not breast feed at all and gave infant formula as the sole source of nutrition from birth, compared to 24% in 2005; the proportion of mothers who gave their babies cows' milk in some form by 8–10 months of age was 61% in 1995 compared to 39% in 2005; and in 1995, 56% of mothers introduced solid foods by 3 months of age compared with 10% in 2005. The effects of these changes on nutrient intake and status of infants is unknown.

Prospective studies examining iron status of infants in the UK and Europe

9.52 A number of small prospective studies have assessed the iron status of infants. Iron intakes were usually not reported in these studies.

9.53 A study which followed infants (n=198) from before 4 to 24 months of age (Taylor *et al*, 2004) reported that mean daily iron intakes met the RNI at 4 and 8 months (except for non-meat eaters) but were below the RNI in all groups by 12 months. At 4, 12 and 24 months, haemoglobin concentrations were below the WHO threshold for anaemia (110 g/L) for 34, 23 and 13% of infants respectively. At 12 and 24 months, 4.2% and 2.8% of infants had low serum ferritin concentrations (<10 µg/L).

9.54 Another UK study (Sherriff *et al*, 1999), reported little variation in haemoglobin concentrations of infants (n=1175) from 8 to 18 months. However, mean serum ferritin concentrations changed rapidly over this period: 36.6 µg/L at 8 months, 32.8 µg/L at 12 months, and 27.5 µg/L at 18 months. The authors of the study suggested that ferritin concentration as an indicator of iron status should be used with caution.

73 Incidence of breast feeding is defined as the proportion of babies who were breast fed initially. This includes all babies who were ever put to the breast, even if this was on only one occasion.

9.55 A study in Ireland (n=76) which followed infants from birth to 36 months, reported that at 12 months, the quantity of cows' milk consumed was negatively associated with haemoglobin ($p<0.000$) and serum ferritin ($p<0.001$) concentration. A significant but less strong association was found with early introduction of cows' milk (Freeman *et al*, 1998). At 24 and 36 months, the most significant predictor of iron status was earlier iron status.

9.56 Male *et al* (2001) investigated the effects of various factors, including diet, on the iron status of European infants (n=488) at 12 months of age. The prevalence of iron deficiency anaemia (haemoglobin <110 g/L; serum ferritin <10 µg/L) in this study (2.3%) was lower than prevalence data reported for UK population-based studies (Emond *et al*, 1996; Sheriff *et al*, 1999; Gregory *et al*, 1995; Gregory *et al*, 2000). Low socioeconomic status and maternal education were associated with low iron status primarily as a result of feeding practices. Of the dietary factors investigated, early introduction of cows' milk was the strongest negative determinant of iron status while use of iron fortified formula was the main factor positively influencing iron status. Other dietary factors, including breast feeding, were not significant determinants of iron status at 12 months of age. Data from a study of Swedish infants (n=76) reported that 26% were iron-depleted (serum ferritin <12 µg/day), although 91% were receiving iron fortified cereal and follow-on formula (Persson *et al*, 1998). The authors suggested that high intakes of phytate-rich complementary foods (e.g., infant cereals) from 6 months of age may have reduced the bioavailability of iron from the diet.

Iron status of infants and children from minority ethnic groups

9.57 The first nationally representative survey of infant feeding practices in families of south Asian (Bangladeshi, Indian or Pakistani) origin living in England was conducted between 1994 and 1996 (DH, 1997). Haemoglobin and serum ferritin concentrations were analysed when the children (n=1057) were aged 2 years (Lawson *et al*, 1998). Mean haemoglobin concentrations were 116 g/L for Bangladeshi and Indian children and 114 g/L for Pakistani children compared to 120 g/L for children aged 1½–2½ years in the NDNS. Twenty-nine per cent of Pakistani children, 25% of Bangladeshi and 20% of Indian children had haemoglobin concentrations <110 g/L compared with 12% of children aged 1½–2½ years in the NDNS. The dietary factors found to have a significant negative impact on haemoglobin concentrations of Bangladeshi and Pakistani children included the introduction of cows' milk at 9 months; Indian children were more likely to have a lower haemoglobin concentration if mainly cows' milk was given at 15 months. The two most significant factors affecting serum ferritin concentrations of children in all groups were introduction of cows' milk at 15 months, and the amount of cows' milk consumed at 2 years of age (Bangladeshi and Pakistani children consuming more that 600 ml/day and Indian children consuming more than 700 ml/day were at greater risk of serum ferritin concentrations <10 µg/L).

9.58 A number of smaller-scale local studies conducted in England have reported a high prevalence of anaemia (haemoglobin <110 g/L) in young Asian children (Grindulis *et al*, 1986; Duggan *et al*, 1991) and a higher prevalence in Asian children compared with white children living in the same area (Ehrhardt, 1986; Warrington and Storey,

1988; Morton *et al,* 1988; Mills 1990; Marder *et al*, 1990). In contrast, a study (n=150) investigating the relationship between anaemia (haemoglobin <110 g/L) at 14 months and risk of anaemia at 2 years in children attending a deprived inner city practice found no significant difference in the prevalence of anaemia between ethnic groups (James *et al*, 1995).

Summary and conclusions

9.59 Iron fortified cereals, including bread, are the main contributors to iron intakes of the UK population. Meat/meat products and vegetables (including potatoes) also make substantial contributions to dietary iron intakes.

9.60 Assessing the adequacy of iron intakes of the population using DRVs is limited by a number of factors, including the accuracy of dietary measurements, reliability of food composition data, and assumptions about the amount of iron absorbed from the diet and intestinal adaptation in response to systemic needs.

9.61 In the general population, groups with substantial proportions below the LRNI for iron are children aged 1½–3½ years (12–24%), girls aged 11–18 years (44–48%) and women aged 19–49 years (25–40%). In low income populations, females aged 11–49 years have the highest proportions with intakes below the LRNI (39% of girls aged 11–18 years; 50% of women aged 19–49 years).

9.62 Groups with the highest prevalence of haemoglobin concentrations below WHO thresholds for anaemia in the general population were adults aged 65 years and above living in institutions (39–52%), free-living adults aged 75 years and over (13–38%), and girls aged 4–6 years (15% based on the higher threshold of 115 g/L; 9% based on the lower threshold of 110 g/L). In low income populations, men aged 65 years and above had the highest proportions (20%) with haemoglobin concentrations below the WHO cut-offs.

9.63 Measures of serum ferritin concentrations suggest that in the general population, children aged 1½–4½ years (25–34%), girls aged 11–18 years (12%), women aged 19–24 years (16%), women aged 35–49 years (13%), and free-living women aged 75 years and over (12–14%) may be at risk of iron deficiency. In low income groups, women aged 19–49 years (14–21%) are at greatest risk of iron deficiency.

9.64 The prevalence of iron deficiency anaemia (both haemoglobin and serum ferritin concentrations below WHO thresholds) in the general population ranged between 0 and 6% according to age and sex. Population groups with the highest prevalence of iron deficiency anaemia (5–6%) were children aged 1½–2½ years, girls aged 15–18 years, women aged 35–49 years, men aged 65 years and over living in institutions, and free-living adults aged 85 years and over. In low income groups, the prevalence of iron deficiency anaemia was highest for women aged 19–39 years (9–11%) and 65 years and over (6%).

9.65 The prevalence of iron deficiency anaemia in the different population groups is not clearly consistent with their dietary iron intakes. However, the NDNS and LIDNS both broadly show that adult men under 65 years in the UK are not at risk of iron

deficiency anaemia and that women aged 15–50 years are at increased risk. This is consistent with increased iron losses in this age group due to menstrual blood loss. Iron deficiency anaemia observed in some adults aged 65 years and over is consistent with blood loss due to gastrointestinal disease or medication in older age groups.

9.66 Although data from the NDNS and the LIDNS indicate that iron intake and iron status may be of public health concern for some population groups in the UK, this is dependent on the confidence placed on the DRVs for iron intake (which are based on limited data and do not take account of absorptive adaptation to increased iron needs) and on iron status criteria (which are not associated with functional outcomes). The large disparity between the high proportions of toddlers and females aged 11–50 years with intakes below the LRNI and the relatively low prevalence of females with iron deficiency anaemia in the general population suggest that the DRVs set for these groups may be too high.

9.67 In the NDNS, few consistent associations were observed between markers of iron status and intakes of total iron, non-haem iron or haem iron. This would be expected in a population with an adequate supply of dietary iron.

9.68 Data on iron intakes and status of infants up to 18 months of age are limited. Findings from the only nationally representative survey of infants up to 18 months suggest that 15% of infants aged 6–12 months have intakes below the LRNI. The effect of changes in infant feeding practices since the survey was carried out in 1986 is unknown.

9.69 There are few representative data on the iron intakes and biochemical markers of iron status of minority ethnic groups. Limited data from the NDNS and LIDNS suggests that iron intakes of minority ethnic groups are not less than those of the general population. Data from the HSE suggest that the prevalence of haemoglobin concentrations below 120g/L (irrespective of cause) was low in men (0-4%) from minority ethnic groups; for women, it was lowest in Irish and Chinese women (6-7%) and ranged from 16% in Black Caribbean women to 29% in Indian women. The prevalence of serum ferritin concentrations below the unspecified upper limit of the bottom fifth of values in the general population (in 1998) was lowest in Chinese (3%) and highest in Indian men (33%). Among women, the corresponding prevalence was higher in south Asian groups (29-48%) than Black Caribbean (19%), Black African (24%), Chinese (14%) or Irish (14%). The prevalence of iron deficiency anaemia was not reported.

9.70 Representative data on the iron status of infants and young children from minority ethnic groups are limited. A nationally representative survey in 1996 showed that the prevalence of haemoglobin concentrations below 110 g/L was 20–29% in Bangladeshi, Indian and Pakistani children aged 2 years compared with 12% of children aged 1½–2½ years in the NDNS.

10 The potential impact of reducing red and processed meat consumption on intakes of iron and zinc

Modelling exercise

10.1 Red meat is a source of iron[74] and zinc[75] in the UK diet. In 2000/01, meat and meat products[76] contributed about 17% of total iron intake and 34% of total zinc intake of adults aged 19–64 years (Henderson *et al*, 2003a).

10.2 Epidemiological data suggest that red and processed meat consumption is probably associated with an increased risk of colorectal cancer (see section 7). In their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), COMA concluded that reducing intakes of red and processed meat would probably reduce the risk of colorectal cancer. However, the Committee was concerned about possible adverse implications of a reduction in meat consumption on micronutrient status, particularly iron status, and recommended that the wider nutritional implications of any reduction in red and processed intake should be assessed.

10.3 Data from the NDNS series suggest that some subgroups of the UK population might be iron deficient or at risk of iron deficiency (see section 9). A recommendation to reduce consumption of red and processed meat in order to decrease colorectal cancer risk could therefore have a negative impact on iron and zinc intakes in the UK by increasing the proportion of the population with intakes below the LRNIs for these nutrients. Low intakes of nutrients are usually considered to be of public health concern when the prevalence of intakes below the LRNI exceeds 5%[77] (WHO, 2001). It is estimated that 1% of men and 25% of women in the UK (aged 19-64 years) have mean daily iron intakes below the LRNI for iron and about 4% of both men and women have mean daily zinc intakes below the LRNI for zinc (Henderson *et al,* 2003a).

10.4 A modelling exercise was therefore undertaken to explore the possible implications of a reduction in red and processed meat consumption on the:

- mean iron and zinc intakes of adults;
- proportion of adults with intakes below the LRNIs for iron and zinc.

74 Contains 0.5–3.0 mg iron per 100 g cooked red meat (FSA, 2002).

75 Contains 1.7–9.0 mg zinc per 100 g cooked red meat (FSA, 2002).

76 Includes white meat; composite dishes also contained non-meat components.

77 In a normal population, 2.5% would be expected to have requirements below the LRNI.

10.5 The modelling exercise also investigated the impact of a reduction in red and processed meat consumption on mean intakes of vitamin D because meat and meat products[78] contribute approximately 24 and 18% to dietary vitamin D intakes of men and women respectively (Henderson *et al*, 2003a). The effects on vitamin D intakes are described in Annex 11.

10.6 The modelling exercise only considered the impact of a reduction in red and processed meat consumption on intakes of iron, zinc and vitamin D. It did not attempt to assess the effects on iron, zinc, or vitamin D status.

10.7 The impact of reducing red and processed meat intakes of children was not assessed since children are not at increased risk of colorectal cancer and any recommendation for a reduction in red and processed meat consumption would apply only to adults.

Methods and assumptions

10.8 The potential effects of reducing red and processed meat consumption of adults were assessed for the following maximum daily intakes: 180, 120, 100, 90, 80, 70, 60, 50 and 0 g. The intakes of consumers exceeding each maximum level were reduced to the maximum level; intakes of those consuming below each maximum level were left unchanged.

Assessment of red and processed meat consumption in the UK

10.9 The impact of a dietary recommendation to reduce consumption of red and processed meat on the iron and zinc intakes of adults in the UK was investigated by modelling intake data from the 2000/01 NDNS of adults aged 19–64 years[79] (Henderson *et al*, 2002).

10.10 Although there are more current data on red and processed meat consumption from year 1 (2008/09) of the NDNS rolling programme (Bates *et al*, 2010), which are compared with intakes in 2000/01 (see Table 10), they were not used in the modelling exercise because of important insecurities in the data[80] (see Annex 12).

Categorisation of red and processed meat

10.11 Epidemiological data vary in their definition and categorisation of red and processed meat (see paragraph 7.65). Red meat usually refers to beef, goat, lamb and pork; processed meat refers to meat (usually red) that has been preserved by smoking, curing, salting or addition of preservatives (WCRF, 2007). In the modelling exercise, red meat was not separated on the basis of whether it was processed or unprocessed because toxicological data suggest no clear evidence to explain

78 Includes white meat.

79 The effects of reducing red meat consumption were not modelled for adults aged 65 years and over since the data were collected in 1994/95 and are likely to be out of date.

80 For example, the sample size is currently too small to disaggregate into men/women and consumers/non-consumers; diet was assessed by unweighed estimates of foods consumed over 4 consecutive days, including both weekend days, when people tend to eat more meat (especially on Sunday).

an association between colorectal cancer risk and presence of preservatives (see Annex 8). Therefore, in the modelling exercise, the term "red meat" also includes processed meat.

Estimates of total red meat consumption

10.12 The 2000/01 NDNS of adults (19–64 years) only provides estimates of total meat and meat dishes consumed (204 g/day for men; 135 g/day for women). However, composite meat dishes (e.g., lasagne, pies), which also contain non-meat components, are reported as total amount of meat consumed, resulting in an overestimation of meat consumption. To obtain more realistic estimates of total red meat consumption, the actual amount of red meat contained in composite meat dishes was quantified (see Annex 11 for further details). The same method was used to disaggregate the red meat content of composite dishes in the NDNS rolling programme.

Estimates of total iron and zinc intakes from red meat and products

10.13 Intakes of iron and zinc from total red meat consumed were assessed from estimates of typical iron and zinc content of each meat type together with consumption estimates of each meat type (see Annex 11 for further details of methods and assumptions).

Results of modelling exercise

Total red meat consumption and intakes of iron and zinc from total red meat (2000/01)

10.14 Estimates of the amount of total red meat consumed by adults in the UK in 2000/01 and intakes of iron and zinc from red meat is shown in Table 9.

10.15 It can be seen from Table 9 that for consumers of red meat, mean total red meat consumption in 2000/01 was approximately 88 g/day for men and 52 g/day for women (70 g/day for men and women combined).[81] This is lower than the previous estimate of 90 g/day reported by COMA (DH, 1998) because COMA's estimate included non-meat components of composite dishes, which would overestimate red meat consumption.

10.16 Mean intake of iron from total red meat consumption is estimated to be 1.5 mg/day for men and 0.9 mg/day for women; therefore the amount of iron obtained per gram of total red meat is approximately 0.017 mg.

81 Average consumption of total red meat for the UK population is 66 g/day. Average consumption of red meat is 85 g/day for males and 47 g/day for females (i.e., 97% of males and 91% of females are consumers of red meat).

Table 9: Total red meat consumption and intakes of iron and zinc from total red meat (2000/01)[82]

	Mean (95% CI)	2.5th percentile	25th percentile	Median	75th percentile	97.5th percentile
Total red meat intake (g/day)						
Men	88 (84.3-91.1)	9	51	81	115	208
Women	52 (49.7-54.4)	3	27	48	70	133
Men and Women	70 (67.6-72.1)	5	37	62	94	182
Iron intake from total red meat (mg/day)						
Men	1.5 (1.5-1.6)	0.1	0.8	1.3	2.0	4.2
Women	0.9 (0.8-1.0)	0.0	0.4	0.8	1.2	2.7
Men and Women	1.2 (1.2-1.3)	0.1	0.5	1.0	1.6	3.6
Zinc intake from total red meat (mg/day)						
Men	3.3 (3.2-3.4)	0.3	1.9	3.0	4.5	7.8
Women	2.0 (1.9-2.1)	0.1	0.9	1.8	2.8	5.8
Men and Women	2.6 (2.6-2.7)	0.1	1.2	2.3	3.6	7.2

10.17 Mean intake of zinc from total red meat consumption is estimated to be 3.3 mg/day for men and 2.0 mg/day for women; therefore the amount of zinc obtained per gram of total red meat is approximately 0.038 mg.

Comparison of total red meat intakes from the 2000/01 NDNS and the NDNS rolling programme year 1 (2008/09)

10.18 Due to differences in data collection methods in the 2000/01 NDNS and the NDNS rolling programme (see footnote 80), data on total red meat intakes from the two surveys were adjusted to facilitate comparison (see Annex 12 for further details). The adjusted estimates are shown in Table 10.

Table 10: Mean total red meat consumption (g/day) of adults in 2000/01 and 2008/09

	Mean (95% CI)	2.5th percentile	25th percentile	Median	75th percentile	97.5th percentile
NDNS 2000/01 (reanalysed to 4 days)						
Men	90.5 (90–91)	10.5	46.2	81.8	121.0	238.8
Women	54.6 (54–55)	3.7	25.0	47.8	75.6	146.5
Men and Women	69.6 (69.4-69.9)	5.8	31.9	58.8	95.6	197.4
NDNS rolling programme year 1 (2008/09) (reweighted by day of week)						
Men	101.4 (93–109)	14.7	57.3	98.6	135.2	238.4
Women	63.7 (58–70)	1.65	26.0	54.7	90.0	162.9
Men and Women	83.1 (78-79)	3.86	40.0	73.2	114.7	221.0

82 Consumers of red meat only: 94% of survey participants.

10.19 Findings from the first year (2008/09) of the NDNS rolling programme suggest that current consumption of red meat is about 10 g/day higher than it was in 2000/01, an increase of 12% for men and 17% for women. However, due to differences in methodologies between the surveys, it was not appropriate to perform a statistical test of significance. Therefore, these findings should be interpreted very cautiously.

Effects of reducing total red meat consumption

10.20 The effect of reducing total red meat intakes of consumers (in 2000/01) to different maximum levels on mean intakes of iron and zinc is shown in Table 11. The impact of these reductions on the proportion of the population with intakes below the LRNI for iron and zinc is shown in Table 12.

10.21 The information presented in Tables 11 and 12 is described in paragraphs 10.22–10.48.

Table 11: Effect of reducing total red meat consumption on mean iron and zinc intakes of adults

	Estimated mean iron intake from all foods (mg/day) (95% CI)		Estimated mean zinc intake from all foods (mg/day) (95% CI)	
	Men	Women	Men	Women
2000/01 NDNS	13 (12.9–13.5)	10 (9.8–10.3)	10 (10.0–10.4)	7.5 (7.2–7.5)
Maximum red meat intake (g/day)				
180	13 (12.8–13.5)	10 (9.8–10.3)	10 (9.9–10.4)	7.4 (7.2–7.5)
120	13 (12.7–13.4)	10 (9.8–10.3)	9.9 (9.7–10.1)	7.4 (7.2–7.5)
100	13 (12.6–13.3)	10 (9.8–10.2)	9.7 (9.5–9.9)	7.4 (7.2–7.4)
90	13 (12.6–13.2)	10 (9.7–10.2)	9.5 (9.4–9.7)	7.3 (7.1–7.4)
80	13 (12.5–13.1)	10 (9.7–10.2)	9.4 (9.2–9.6)	7.2 (7.1–7.3)
70	13 (12.4–13.0)	9.9 (9.7–10.2)	9.2 (9.0–9.4)	7.1 (7.0–7.3)
60	13 (12.3–12.9)	9.9 (9.6–10.1)	8.9 (8.7–9.1)	7.0 (6.9–7.2)
50	13 (12.2–12.8)	9.8 (9.6–10.1)	8.7 (8.5–8.9)	6.9 (6.8–7.0)
0	12 (11.4–12.1)	9.2 (9.0–9.5)	7.0 (6.8–7.2)	5.6 (5.5–5.7)

Table 12: Effect of reducing total red meat consumption on the proportion of adults with intakes of iron and zinc below the LRNI

	Estimated % of population exceeding threshold		Estimated % with iron intakes below LRNI (95% CI)		Estimated % with zinc intakes below LRNI (95% CI)	
	Men	Women	Men	Women	Men	Women
2000/01 NDNS	–	–	0.9 (0.2–1.5)	25 (22.0–22.7)	3.7 (2.4–5.0)	3.8 (2.5–5.1)
Maximum red meat intake (g/day)						
180	5	0.3	0.9 (0.2–1.5)	25 (22.0–22.7)	3.9 (2.6–5.2)	3.9 (2.6–5.1)
120	20	4.1	1.0 (0.3–1.7)	25 (22.4–28.1)	3.9 (2.6–5.2)	3.9 (2.6–5.1)
100	33	8.3	1.0 (0.3–1.7)	25 (22.6–28.3)	4.0 (2.7–5.3)	3.9 (2.6–5.17)
90	42	12	1.0 (0.3–1.7)	25 (22.6–28.3)	4.1 (2.8–5.5)	3.9 (2.6–5.1)
80	50	17	1.0 (0.3–1.7)	26 (22.7–28.4)	4.4 (3.0–5.8)	3.9 (2.6–5.1)
70	58	23	1.0 (0.3–1.7)	26 (23.3–29.1)	5.5 (3.9–7.0)	3.9 (2.6–5.1)
60	66	33	1.0 (0.3–1.7)	27 (23.7–29.5)	6.1 (4.5–7.8)	4.1 (2.8–5.4)
50	74	43	1.0 (0.3–1.7)	27 (24.3–30.1)	9.5 (7.3–11.2)	5.0 (3.6–6.4)
0	97	91	2.8 (1.7–3.9)	32 (29.3–35.4)	29 (26.3–32.5)	20 (17.3–22.5)

At levels of total red meat consumption in 2000/01

10.22 For consumers of red meat, mean iron intake from all foods (excluding supplements) in 2000/01 was approximately 13 mg/day for men and 10 mg/day women. Mean intake of iron from red meat was 1.5 mg/day for men and 0.9 mg/day for women (see Table 9). This means that red meat contributes approximately 12% and 9% to total iron intake in men and women respectively.

10.23 The mean zinc intake from all foods (excluding supplements) was 10 mg/day for men and 7.5 mg/day for women. The mean intake of zinc from red meat was 3.3 mg/day for men and 2 mg/day for women (see Table 9). This means that red meat contributes about 32% and 27% to total zinc intake in men and women respectively.

10.24 It is estimated that 0.9% of men and 25% of women have iron intakes below the LRNI for iron and 3.7% of men and 3.8% of women have zinc intakes below the LRNI for zinc.

Reduction of total red meat consumption to a maximum of 180 g/day

10.25 Approximately 5% of men and 0.3% of women consume more than 180 g/day of red meat.

10.26 Mean total iron intake would not change (13 mg/day for men; 10 mg/day for women). Mean total zinc intake would not change for men (10 mg/day) and would be reduced from 7.5 to 7.4 g/day for women.

10.27 The proportion of the population with intakes below the LRNI for iron (0.9% of men; 25% of women) would not change. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 3.9% for men and from 3.8 to 3.9% for women.

Reduction of total red meat consumption to a maximum of 120 g/day

10.28 Approximately 20% of men and 4% of women consume more than 120 g/day of red meat.

10.29 Mean total iron intake would not change (13 mg/day for men; 10 mg/day for women). There would be a reduction in mean total zinc intake from 10 to 9.9 mg/day for men and from 7.5 to 7.4 mg/day for women.

10.30 The proportion of the population with intakes below the LRNI for iron would increase from 0.9 to 1% for men and remain at 25% for women. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 3.9% for men and from 3.8 to 3.9% for women.

Reduction of total red meat consumption to a maximum of 100 g/day

10.31 Approximately 33% of men and 8% of women consume more than 100 g/day of red meat.

10.32 Mean total iron intake would not change (13 mg/day for men; 10 mg/day for women). Mean total zinc intake would be reduced from 10 to 9.7 mg/day for men and from 7.5 to 7.4 mg/day for women.

10.33 The proportion of the population with intakes below the LRNI for iron would increase from 0.9 to 1% for men and remain at 25% for women. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 4.0% for men and from 3.8 to 3.9% for women.

Reduction of total red meat consumption to a maximum of 90 g/day

10.34 Approximately 42% of men and 12% of women consume more than 90 g/day of red meat.

10.35 Mean total iron intake would not change (13 mg/day for men; 10 mg/day for women). Mean total zinc intake would be reduced from 10 to 9.5 mg/day for men and from 7.5 to 7.3 mg/day for women.

10.36 The proportion of the population with intakes below the LRNI for iron would increase from 0.9 to 1% for men and remain at 25% for women. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 4.1% for men and from 3.8 to 3.9% for women.

Reduction of total red meat consumption to a maximum of 80 g/day

10.37 Approximately 50% of men and 17% of women consume more than 80 g/day of red meat.

10.38 Mean total iron intake would not change (13 mg/day for men; 10 mg/day for women). Mean total zinc intake would be reduced from 10 to 9.4 mg/day for men and 7.5 to 7.2 mg/day for women.

10.39 The proportion of the population with intakes below the LRNI for iron would increase from 0.9 to 1% for men and from 25 to 26% for women. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 4.4% for men and from 3.8 to 3.9% for women.

Reduction of total red meat consumption to a maximum of 70 g/day

10.40 Approximately 58% of men and 23% of women consume more than 70 g/day of red meat.

10.41 Mean total iron intake would be unchanged for men (13 mg/day) and reduced from 10 to 9.9 mg/day for women. Mean total zinc intake would be reduced from 10 to 9.2 mg/day for men and from 7.5 to 7.1 mg/day for women.

10.42 The proportion of the population with intakes below the LRNI for iron would increase from 0.9 to 1% for men and from 25 to 26% for women. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 5.5% for men and from 3.8 to 3.9% for women.

Reduction of total red meat consumption to a maximum of 60 g/day

10.43 Approximately 66% of men and 33% of women consume more than 60 g/day of red meat.

10.44 Mean total iron intake would be unchanged for men (13 mg/day) and reduced from 10 to 9.9 mg/day for women. Mean total zinc intake would be reduced from 10 to 8.9 mg/day for men and from 7.5 to 7.0 mg/day for women.

10.45 The proportion of the population with intakes below the LRNI for iron would increase from 0.9 to 1% for men and from 25 to 27% for women. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 6.1% for men and from 3.8 to 4.1% for women.

Reduction of total red meat consumption to a maximum of 50 g/day

10.46 Approximately 74% of men and 43% of women consume more than 50 g/day of red meat.

10.47 Mean total iron intake would be unchanged for men (13 mg/day) and reduced from 10 to 9.8 mg/day for women. Mean total zinc intake would be reduced from 10 to 8.7 mg/day for men and from 7.5 to 6.9 mg/day for women.

10.48 The proportion of the population with intakes below the LRNI for iron would increase from 0.9 to 1% for men and from 25 to 27% for women. The proportion with intakes below the LRNI for zinc would increase from 3.7 to 9.5% for men and from 3.8 to 5% for women.

Interpretation of results from the modelling exercise

10.49 The modelling exercise shows that red meat makes a greater contribution to total zinc intake (32% for men; 27% for women) than to total iron intake (12% for men; 9% for women).

10.50 Reducing red meat consumption of consumers in the upper range of the distribution of intakes to an average of 90 g/day (COMA recommendation, 1998) or 80 g/day would have a minimal effect on total iron and zinc intakes or on the proportion of adults with iron or zinc intakes below the LRNI.

10.51 A reduction in red meat consumption to 70 g/day (WCRF recommendation, 2007) would have a minimal effect on the proportion of adults with iron intakes below the LRNI for iron; however, the proportion of men with zinc intakes below the LRNI would increase to over 5%.

10.52 The potential effects of a reduction in red meat consumption on overall intakes of iron and zinc, and on the proportion of adults with intakes below the LRNI for iron and zinc, are probably overestimates of real-life effects since the modelling exercise did not attempt to incorporate the effects of replacing the reduction in total red meat consumption with other foods (e.g., white meat/poultry) which also contain iron and zinc.

Limitations of the modelling exercise

10.53 The modelling exercise was not able to consider the impact of a reduction in red meat consumption on iron, zinc, or vitamin D status.

10.54 The modelling did not consider the possible effect of a recommendation to reduce red meat intakes on low red meat consumers because it was assumed that the advice would be taken up only by high red meat consumers. However, it is possible that any advice to reduce red meat consumption could result in further reductions, or complete removal, of red meat from the diets of low red meat consumers.

10.55 No allowance was made for the effects of dietary iron bioavailability: for example, the potential higher bioavailability of iron from red meat and the lower bioavailability of iron from iron fortified cereals and cereal products (which make the largest contribution to iron intakes). This is because dietary iron bioavailability will be affected to a variable extent by individuals' need for iron, mucosal adaptation, and other components of the diet; iron bioavailability of iron fortified cereals will depend on the solubility of the iron compound used for fortification. The iron intakes used in the modelling exercise therefore represent the actual amount contained in foods and not the amount available for systemic use.

10.56 The modelling exercise did not take any account of adaptive increases in dietary iron absorption in response to decreases in iron and zinc intakes.

10.57 The potential beneficial impact of reducing intakes of red meat on other aspects of public health was not considered. Some red and/or processed meat can contain high amounts of salt and saturated fats. Depending on the nutrient profile of foods replacing red meat in the diet, a reduction in red meat consumption could also lead to reductions in intakes of salt, total energy and saturated fat. These reductions could therefore have significant public health benefits on the UK population by contributing to a decrease in high blood pressure, obesity and CVD (DH, 1994; SACN, 2003).

Summary and conclusions

10.58 Data from the 2000/01 NDNS indicate that average total red and processed meat consumption of adult consumers of red and processed meat is approximately 70 g/day (88 g/day, men; 52 g/day, women). More recent data, from year 1 (2008/09) of the NDNS rolling programme, suggest that intakes of red and processed meat have increased by about 10 g/day (101 g/day, men; 64 g/day, women) since 2000/01. However, it is not known if the observed increase is statistically significant and important insecurities in the data from year 1 of the rolling programme limit confidence in these findings.

10.59 Based on data from the 2000/01 NDNS, red and processed meat makes a greater contribution to total zinc intake from all foods (32% for men; 27% for women) than to total iron intake (12% for men; 9% for women).

10.60 Reducing the red meat intakes of adults consuming red meat in the upper range of the distribution of intakes, down to 80 g/day, would have little impact on the proportion of adults who currently have iron or zinc intakes below the LRNIs for iron and zinc. Further reduction to 70 g/day would have a minimal effect on the proportion of adults with iron intakes below the LRNI, but the proportion of men with zinc intakes below the LRNI would increase to just over 5%.

11 Overall summary and conclusions

11.1 In their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), COMA concluded that lower consumption of red and processed meat would probably reduce the risk of colorectal cancer. The Committee recommended that intakes of red and processed meat should not rise, and that adults with intakes greater than the average (then estimated to be 90 g/day cooked weight), especially those in the upper reaches of the distribution of intakes, should consider a reduction. Since red meat is a source of iron as well as other micronutrients (such as zinc) in the UK diet, COMA recommended that the "implications of a reduction in meat consumption on other aspects of health, particularly iron status" should be reviewed.

11.2 Following the COMA recommendation, the key issues considered in this report were: iron in the diet; the health consequences of iron deficiency and iron excess; and adequacy of iron nutrition in the UK. Since red and processed meats are the main source of haem iron, the health consequences of high intakes of these foods and the potential effect of reducing intakes of red and processed meat on the iron and zinc status of the UK population were also considered.

11.3 Iron is an essential nutrient which is required: as haemoglobin in red blood cells for transporting oxygen from the lungs to the tissues; in the form of myoglobin for storage and use of oxygen in muscles; and as a component of a number of enzymes which are essential for many metabolic and synthetic functions.

11.4 Iron is also potentially toxic because free iron (i.e., not bound to proteins or other organic molecules) promotes free radical reactions. In humans, the risk of toxicity from iron is minimised by tight regulation of both the amount of iron that enters the body and by means of a series of proteins which bind iron, carry it in the circulation, and distribute it to functional sites or to deposits where it is maintained in a safe form.

11.5 The body has no means of excreting excess iron. Iron surplus to immediate requirements is deposited in tissues as ferritin. Serum concentrations of ferritin reflect the levels deposited in tissue and can be used as indicators of potential excess and deficiency of iron. However, since ferritin is an acute phase reactant, it can only be used in the absence of infection and inflammation.

11.6 The body's iron content is recycled between its various functional forms, particularly that in red blood cells and tissue deposits. Increased needs for iron are met initially by increased release of iron from ferritin depots and then by increased absorption. The absorption of iron from the diet (i.e., the uptake by gut mucosa and subsequent transfer into the body) is determined primarily by the body's need for iron to replace the small amounts lost from skin, hair, gut lining and menstruation, and to supply any additional amounts needed for growth and reproduction. In healthy individuals, there is no risk of iron overload from customary dietary intakes of iron because the amount of iron absorbed and the amount in the body are tightly regulated.

11.7 Polymorphisms in genes involved in regulating iron metabolism can affect iron absorption. The most frequent form of hereditary haemochromatosis, an autosomal recessive disease, is caused by mutation of the gene coding for HFE protein. This results in excessive absorption of dietary iron leading to the accumulation of high levels of iron in the body, which can lead to tissue and organ damage. Two common mutations of this gene, C282Y and H63D, have been identified. Although heterozygotes have altered iron metabolism, this does not predispose them to excessively accumulate iron.

11.8 Current Dietary Reference Values (DRVs) for iron are derived from limited data. They are based on estimates of the amount of iron required to replace basal and menstrual iron losses[83] and for growth. The estimates are based on an assumed absorption of 15% from the diet. This percentage is derived from short term studies carried out in iron replete individuals in whom iron absorption would be down regulated. Such studies do not allow for adaptive responses that occur over a longer time period than that needed for a single meal study, nor for the nature of this adaptation in response to systemic needs for iron. The DRVs for iron may be too high (particularly for girls and women of reproductive age) because the assumptions about the amount of iron absorbed and the degree of intestinal adaptation are cautious. There are currently insufficient new data to inform a reassessment of the DRVs for iron; however, improved understanding of iron metabolism could enable a reappraisal of existing data.

11.9 The necessity of DRVs for infants aged 0–6 months is questionable because healthy term infants are born with sufficient systemic iron to meet their functional needs in the first six months of life. Therefore they have little, if any, dependence on iron from breast milk or breast milk substitutes. Evidence from randomised controlled trials suggests that a delay in clamping the umbilical cord after birth until it has stopped pulsing (about 2–3 minutes) is associated with higher systemic iron depots in the first six months of life; however, it may also increase the risk of jaundice requiring phototherapy. It is not known if there are any long term beneficial effects.

11.10 Iron status describes whether an individual has too little, enough or too much iron in their body for their needs. A number of haematological and biochemical markers are used to assess iron deficiency, adequacy, or excess. The markers are categorised according to whether they represent a functional use of iron (haemoglobin), synthesis of haemoglobin (zinc protoporphyrin), supply of iron to tissues (iron bound to transferrin), or iron depots in tissues (serum ferritin). No single marker of iron metabolism is considered ideal for the assessment of iron deficiency or excess since all the individual indices have limitations in terms of their sensitivity and specificity. However, in this report, in agreement with international practice, a combination of haemoglobin (functional iron) and serum ferritin (iron depots) were considered to be the most useful indicators of iron deficiency, adequacy and excess.

83 The DRVs assume a normal distribution; however, the distribution of iron requirements for women of reproductive age is skewed because menstrual losses are highly skewed. The DRVs for women of reproductive age takes account of the skewed requirement.

11.11 The reference ranges for markers of iron metabolism define iron sufficiency. They do not define iron deficiency or iron excess as the thresholds selected for use are not based on functional defects. For example, low serum ferritin concentrations indicate low iron depots in tissues but they do not necessarily represent a functional deficiency of iron. It is not clear at which level above or below the reference range for serum ferritin there is an increased risk of an adverse outcome. Similarly, the thresholds used to define anaemia do not correspond to concentrations of haemoglobin below which functional consequences of anaemia occur. The reference limits only indicate the possibility of iron depletion, deficiency or excess. Individuals with values either above or below the reference ranges may still be healthy.

11.12 Iron is present in foods as haem or non-haem iron. Haem iron is found almost exclusively in foods of animal origin. Non-haem iron is found in animal and plant tissues, fortified foods and supplements. The most important determinant of dietary iron absorption is systemic iron need: more iron is absorbed from the diet in a state of iron deficiency and less is absorbed when iron depots are replete.

11.13 Iron bioavailability refers to the proportion of iron that is taken up and transferred into the body by the intestinal mucosa and is subsequently used systemically. Haem iron is absorbed more efficiently from the diet than non-haem iron. A number of dietary components have been shown to increase (e.g., ascorbic acid, meat) or reduce (e.g., calcium, phytates, phenolic compounds) non-haem iron absorption from single test meals. However, single meal absorption studies do not take account of adaptive absorptive responses to qualitative and quantitative changes in the diet. Studies over longer periods indicate that single meal studies overestimate the effects of enhancers and inhibitors of iron absorption.

11.14 Observational data have not shown a relationship between systemic markers of iron status and intakes of total iron or enhancers and inhibitors of iron absorption. Long term intervention studies have also, overall, not shown a corresponding change in markers of iron status. This raises uncertainties regarding the importance of dietary advice in the UK to maximise iron absorption: for example, eating cereal sources of iron with foods rich in vitamin C or avoiding drinking tea with meals.

11.15 There are no data to indicate that the bioavailability of dietary iron is a significant factor in the pathogenesis of anaemia and iron deficiency in the UK population. UK diets contain a broad range of foods containing iron and various enhancers and inhibitors of iron absorption.

11.16 The main approach used to increase the iron supply of the UK population has been the fortification of foods with iron. Iron fortification of white and brown wheat flour (to replace iron lost during processing) and breast milk substitutes is mandatory in the UK. A number of other foods, including breakfast cereals, are fortified on a voluntary basis. The iron compounds used for fortification of foods vary in their availability for intestinal uptake. Elemental iron powders, which have the lowest solubility, are widely used to fortify foods because they have a longer shelf-life than other more soluble iron fortification compounds.

11.17 Although iron fortified foods, especially cereals, make a substantial contribution to iron intakes in the UK, evidence from efficacy trials and from countries with national fortification policies suggests that foods fortified with elemental iron make little practical contribution to improving iron status, even in individuals with increased systemic iron needs. This is probably due to their low solubility and consequently low intestinal uptake. Iron fortified breast milk substitutes are frequently recommended to prevent iron deficiency during infant development; however, their usefulness in improving iron status of infants under and over 6 months is uncertain.

11.18 Results from several, mainly cross-sectional, studies suggest that dietary iron intakes of vegetarians are similar to those of non-vegetarians. Although evidence indicates that serum ferritin concentrations of vegetarians, which are usually within the reference ranges, are statistically significantly lower than in non-vegetarians, haemoglobin concentrations are similar in both diet groups.

11.19 Causes of iron deficiency include inadequate intakes of iron, impaired absorption and increased blood losses due to menstruation or gastrointestinal disease. Increased systemic need for iron leads to mobilisation of iron depots from macrophages or hepatocytes and up regulation of iron absorption. Progressive iron deficiency leads to anaemia and reduced numbers of circulating precursor red cells, and iron dependent functions are affected. Anaemia has been reported to have adverse effects on physical work capacity, pregnancy outcomes and cognitive, motor and behavioural development in children.

11.20 Evidence from animal and human studies suggests that decreases in haemoglobin concentration are associated with impairments in various aspects of physical work capacity. However, in many studies iron deficiency is poorly characterised and anaemia is often assumed to be caused by iron deficiency. Most studies were carried out in developing countries where there are multiple nutritional deficiencies and socioeconomic deprivations which could confound the relationship between iron and physical work capacity. Clear thresholds associated with adverse outcomes cannot be determined because the data are presented discontinuously.

11.21 The available data suggest functional defects associated with physical work capacity at haemoglobin concentrations at or below 110–120 g/L and ferritin concentrations at or below 16–20 µg/L. Human studies suggest that aerobic capacity is reduced at haemoglobin concentrations below about 110 g/L; however, there is no clear evidence that iron deficiency in the absence of anaemia has adverse effects on aerobic capacity. There is a limited amount of evidence suggesting that iron deficiency in the absence of anaemia (haemoglobin >120 g/L; serum ferritin <16 µg/L) might impair endurance capacity, but this needs further substantiation. Overall, there are insufficient data to assess the effects of iron deficiency or iron deficiency anaemia on energetic efficiency or voluntary activity, or work productivity. Studies examining effects of anaemia (all-cause and iron deficiency anaemia) on work productivity have been conducted in developing countries in subjects with haemoglobin concentrations below those usually observed in the UK.

11.22 Data from observational studies have suggested that maternal haemoglobin concentrations at either the low or high end of the distribution during pregnancy are associated with increased risk of adverse birth outcomes such as low birth weight, preterm birth and perinatal mortality. The physiological changes that occur during pregnancy, such as plasma volume expansion and haemodilution, make it difficult to interpret the markers of iron metabolism during this time. High haemoglobin concentrations during pregnancy are generally not caused by high intakes of dietary or supplemental iron but are the result of inadequate plasma volume expansion, which is also associated with adverse birth outcomes. Intervention studies of routine iron supplementation during pregnancy have reported no beneficial or adverse effects on pregnancy outcomes.

11.23 Evidence from randomised controlled trials of iron supplementation suggests that iron deficiency anaemia is a cause of poor motor development in children in the first three years of life but the long term effects are unknown. There is insufficient evidence from rigorous randomised controlled trials to determine whether iron deficiency or iron deficiency anaemia affects cognitive or language development in children aged 3 years or under. Evidence from randomised controlled trials suggests that iron treatment has beneficial effects on cognitive development in anaemic children aged over 3 years but it is not known whether these benefits are sustained in the long term. Based on current evidence, it is not possible to derive thresholds of iron status at which cognitive, motor and behavioural development might be at risk; however, risks appear to be less at haemoglobin concentrations above 110 g/L.

11.24 There are a number of difficulties in interpreting and comparing the data examining adverse effects of iron deficiency and iron deficiency anaemia. This is because most studies have been conducted in developing countries where populations are associated with multiple nutritional deficiencies combined with social and economic deprivations. All these factors, which usually accompany iron deficiency, are potential confounders because they may be independently associated with adverse effects on physical work capacity, pregnancy outcomes, and cognitive, motor and behavioural development in children. Another difficulty is that iron deficiency and iron deficiency anaemia are often poorly characterised in these studies: many only measure haemoglobin and assume this represents iron deficiency. Another assumption is that dietary iron deficiency is the cause of this anaemia rather than loss of iron secondary, for example, to blood loss. Additionally, sample sizes are small and different reference ranges and cut-off points have been used.

11.25 Acute high doses of iron can cause intestinal mucosal damage and systemic toxicity. Lower exposures may interfere with the intestinal uptake, transfer and systemic use of copper and zinc. High systemic iron burden is also associated with adverse effects arising from degradation of tissue ferritin and subsequent free radical damage of surrounding tissues.

11.26 In the UK, the evidence for adverse effects of iron was considered insufficient to establish a Safe Upper Level (SUL[84]). Instead, a Guidance Level (GL[85]) of 17 mg/day of supplemental iron (i.e., in addition to iron intake from foods) was recommended for adults, based on gastrointestinal effects. In the USA, a Tolerable Upper Intake Level (UL[86]) of 45 mg/day for total iron intake (i.e., from food and supplements) was set for adults, which was also based on gastrointestinal effects. A UL for iron has not been set in Europe as adverse gastrointestinal effects were not considered a suitable basis to establish a UL for iron from all sources and there were insufficient data regarding other risks.

11.27 It has been proposed that high iron intakes or high body iron burden may increase the risk of colorectal cancer, cardiovascular disease (CVD), infection, neurodegenerative disorders and inflammatory conditions. It has also been proposed that iron supplementation may have a negative effect on the physical growth of iron replete infants and children.

11.28 There is a limited amount of epidemiological evidence on the association between iron intakes and high iron depots and colorectal cancer risk. The available data suggest: increased dietary intakes of total or haem iron might be associated with increased colorectal cancer risk, however, confounding by other dietary and lifestyle factors is possible; high iron depots are not associated with increased colorectal cancer risk; and heterozygosity for hereditary haemochromatosis may increase colorectal cancer risk. Overall, there are insufficient data on the association between colorectal cancer risk and dietary intakes of total iron, haem iron, iron status, or heterozygosity for hereditary haemochromatosis to reach clear conclusions.

11.29 Meat, particularly red meat, is almost exclusively the source of haem iron. In their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), COMA concluded that there was moderately consistent evidence from cohort studies of a relationship between red and processed meat consumption and colorectal cancer. The substantial body of prospective epidemiological data that has accumulated since the COMA report in 1998 consistently indicates an increased colorectal cancer risk associated with high intakes of red and processed meat. Overall, the available epidemiological evidence suggests that red and processed meat intake is probably associated with increased colorectal cancer risk. The evidence for an increased colorectal cancer risk is not unequivocal since it is based on prospective observational studies; so effects of confounding by other dietary or lifestyle factors associated with meat consumption and colorectal cancer risk cannot be excluded. Although a number of plausible biological mechanisms have been proposed to explain the association between red meat and colorectal cancer risk, none is supported by convincing evidence.

84 The SUL represents the amount of a nutrient that can be consumed daily over a lifetime without significant risk to health and is based on adequate available evidence.

85 The GL is based on limited data and represents an approximate indication of intakes that would not be expected to cause adverse effects.

86 The UL represents the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population.

11.30 It is not possible to identify if there is a dose-response or a threshold level of red and processed meat which may be associated with increased colorectal cancer risk because of a number of limitations in the data.

11.31 The available epidemiological evidence on total iron intake or body iron and CVD does not suggest an association. The evidence examining the association between haem iron intake and CVD risk is limited to a few studies which suggest, overall, that high intakes of haem iron are associated with increased CVD risk. However, it is possible that this could be due to other components of meat (the main source of haem iron) associated with CVD risk, such as saturated fats or other dietary and lifestyle factors associated with meat intake. Studies of HFE heterozygosity and CVD risk suggest that C282Y heterozygotes (but not H63D heterozygotes) may be at increased risk of CVD, but there are insufficient data to reach clear conclusions.

11.32 Evidence suggests that iron supplementation may have a negative effect on the physical growth of iron replete infants and children (haemoglobin >110 g/L; serum ferritin >12 µg/L) but further studies are required to characterise this effect. There is insufficient evidence to suggest that high iron intakes or high iron depots increase the risk of diabetes mellitus in the general population or that homozygosity or heterozygosity for hereditary haemochromatosis increases diabetes risk. The limited amount of evidence for an association between iron intake and rheumatoid arthritis is inconclusive. There is no evidence to suggest that dietary iron is associated with Parkinson's disease or Alzheimer's disease.

11.33 Evidence from animal studies suggests that iron plays a role in immunity and infection. Human studies have shown that iron deficiency anaemia (typically defined as haemoglobin <100 g/L plus one or more measure of iron deficiency) and iron overload (due to multiple blood transfusions) impair some aspects of immune function. However, it is not known if these impairments increase susceptibility to infectious pathogens. Although it has been suggested that iron supplementation may decrease resistance to infection, evidence suggests that iron supplementation does not increase the risk of non-diarrhoeal or respiratory tract infections in children but may increase diarrhoea risk. It is not clear if iron supplementation increases risk of malaria or risk of infectious diseases in areas where malaria incidence is high. There is currently insufficient evidence to draw conclusions on the relationship between iron supplementation and HIV or tuberculosis. Most human studies on iron and infection have been conducted in developing countries where multiple nutrient deficiencies co-exist and which may also affect resistance to infection. There is no evidence to suggest that iron supplementation would have any effect on infectious disease incidence or morbidity in the UK. However, iron supplementation might have adverse effects in some subgroups of the UK population (e.g., those with HIV or children at risk of diarrhoea).

11.34 The National Diet and Nutrition Survey (NDNS) and the Low Income Diet and Nutrition Survey (LIDNS) provide nationally representative data on iron intakes and iron status in the UK for the general population and low income populations respectively. The NDNS formerly comprised a series of separate cross-sectional surveys covering different age groups in Great Britain: children aged 1½–4½ years

(1992/93); adults aged 65 years and over (1994/95); young people aged 4–18 years (1997); and adults aged 19–64 years (2000/01). The LIDNS covers low income people aged 2 years and above (2003/05) in the UK. Data from the NDNS series and the LIDNS were used to assess the adequacy of iron intakes and iron status in the UK.

11.35 The NDNS is now a continuous rolling programme of people aged 18 months and over living in the UK, and intake data from year 1 (2008/09) have been published. Although these data are more current, they were not used in this report to assess the adequacy of iron intakes of the UK general population because the data are preliminary and the sample size is currently too small for robust subgroup analysis.

11.36 Iron intakes in the UK were assessed against the DRVs for iron. Difficulties associated with using dietary surveys to assess the adequacy of nutrient intakes against DRVs include reliability of food composition tables and misreporting of food consumption. Additionally, assessment of adequacy of iron intakes against DRVs only takes limited account of the amount of iron absorbed from the diet.

11.37 Data from the NDNS and LIDNS show that iron fortified cereals, including bread, contribute about half of the iron intake of most of the population in the UK. The contribution that fortified foods make to the supply of iron available to the body for uptake, transfer and systemic utilisation, and their effect on iron status in the UK, is not known. Meat and meat products and vegetables also make substantial contributions to dietary iron intakes. Although haem iron is more bioavailable than non-haem iron, the NDNS did not find any significant associations between haem iron and markers of iron status (serum ferritin and haemoglobin).

11.38 Average iron intakes in the general population are near (>90%) or above the Reference Nutrient Intake (RNI)[87] for most population groups in the UK. Intakes below 90% of the RNI were reported for children aged 1½–3½ years (73–81%), girls aged 11–18 years (60%) and women aged 19–49 years (66–87%). Population groups with substantial proportions below the Lower Reference Nutrient Intake (LRNI)[88] were children aged 1½–3½ years (12–24%), girls aged 11–18 years (44–48%) and women aged 19–49 years (25–40%).

11.39 In low income groups, average daily intakes were above the RNI for all males. For females, average intakes of iron were at or above the RNI for girls aged 2–10 years and women aged 50 years and over; they were below the RNI for girls aged 11–18 years (63% of RNI) and women aged 19–49 years (about 60% of RNI). A high proportion of females aged 11–49 years (39% of girls aged 11–18 years; 50% of women aged 19–49 years) had intakes below the LRNI.

11.40 The WHO thresholds for iron deficiency (based on serum ferritin concentration) and anaemia (based on haemoglobin concentration) were used to identify the prevalence of iron deficiency and iron deficiency anaemia in the UK. However,

87 The RNI represents the amount of a nutrient that is likely to meet the needs of 97.5% of the population.

88 The LRNI represents the amount of a nutrient that is likely to meet the needs of 2.5% of the population.

these data should be interpreted with caution since neither marker, alone or in combination, necessarily diagnoses iron deficiency but simply indicates individuals at risk of deficiency.

11.41 In the general population, substantial proportions of children aged 1½–4½ years, girls aged 11–18 years, women aged 19–24 years and 35–49 years, and free-living adults aged 75 years and over, had serum ferritin concentrations below WHO thresholds indicating an increased risk of iron deficiency. In low income groups, women aged 19–49 years were at greatest risk of iron deficiency.

11.42 In the general population, risk of iron deficiency anaemia (haemoglobin and serum ferritin concentration below WHO thresholds) was highest (5–6%) for children aged 1½–2½ years, girls aged 15–18 years, women aged 35–49 years, men aged 65 years and over living in institutions, and free-living adults aged 85 years and over. In low income groups, a substantial proportion of women aged 19–39 years were at risk of iron deficiency anaemia (9–11%).

11.43 Although data from the NDNS and LIDNS suggest that considerable proportions of some population groups may have iron intakes below dietary recommendations for iron, this is not clearly consistent with the iron status data which suggests that for 95% of the general population, current intakes are adequate to maintain their iron status above internationally accepted criteria for iron deficiency anaemia. The high proportions of the population with intakes below the LRNI and the mismatch between the iron intake and iron status data suggest that the DRVs for iron may be too high. The DRVs are based on limited data and may not take full account of absorptive adaptation to increased iron needs.

11.44 The NDNS and LIDNS both broadly show that women aged 15–50 years are at increased risk of iron deficiency anaemia which is consistent with iron losses in this age group due to menstrual blood loss. Compared to those in the general population, more women of reproductive age from the low income population are at risk of iron deficiency anaemia. The reasons for this are not clear since mean iron intakes in both populations are similar. It is possible that intakes might be insufficient to compensate for higher pregnancy burden, and/or greater ill health in women from low income populations. Iron deficiency anaemia observed in some adults aged 65 years and over might be caused by decreased absorption of dietary iron due to gastric atrophy or increased blood loss due to gastrointestinal disease or medication. There are no data to clarify the aetiology of iron deficiency in the UK population.

11.45 Data from the NDNS and LIDNS suggest that iron intake and iron status in the UK may be of public health concern for toddlers, women of reproductive age, and some adult groups aged 65 years and over. However, this is dependent on the confidence placed on the DRVs for iron intake which are based on cautious assumptions, and on the iron status criteria for iron deficiency and iron deficiency anaemia which are not based on functional defects.

11.46 Limited data from the NDNS and LIDNS suggest that the iron intakes of minority ethnic groups are not lower than those of the general population. Representative data on biochemical markers of the iron status of minority ethnic groups are not

available in the NDNS and LIDNS. Data from the Health Survey for England show that the prevalence of haemoglobin concentrations below 120 g/L for men and women was low in men (0-4%) and Irish and Chinese women (6-7%) compared with Black Caribbean (16%), Pakistani (21%), Bangladeshi (23%) Black African (26%) and Indian women (29%). The prevalence of anaemia caused by iron deficiency was not reported. The prevalence of iron deficiency anaemia in infants and toddlers from minority ethnic groups is unclear because there are limited representative data. However a nationally representative survey of south Asian children aged 2 years found that 20-29% had haemoglobin concentrations below 110 g/L compared with 12% of children aged 1½–2½ years in the NDNS.

11.47 A modelling exercise (based on intake data from the 2000/01 NDNS of adults aged 19–64 years) to explore the potential effect of reducing red and processed meat consumption on intakes of iron and zinc suggests that red and processed meat makes a greater contribution to total zinc intake from all foods (32% for men; 27% for women) than to total iron intakes (12% for men; 9% for women). In 2000/01, the average consumption of total red and processed meat (consumers of red and processed meat only) in the UK was approximately 70 g/day cooked weight (88 g/day, men; 52 g/day, women). This is lower than the previous estimate of 90 g/day cited in the 1998 COMA report[89] because this figure included non-meat components of composite dishes such as meat products (e.g., sausage rolls, pies) and meals containing red meat (e.g., lasagne, stew) resulting in an overestimation of red meat consumption. In the 2000/01 NDNS, the average red and processed meat intake of consumers in the 75th percentile of the distribution of intakes was estimated to be 94 g/day cooked weight (115 g/day for men; 70 g/day for women).

11.48 The modelling exercise indicates that a reduction in the red and processed meat intakes of consumers in the upper ranges of the distribution, down to an average of 80 g/day, would have a minimal impact on the proportion of adults with average intakes below the LRNI for iron and zinc. Further reductions to an average of 70 g/day would have little effect on iron intakes, but the proportion of men with intakes of zinc below the LRNI may increase (from 3.7 to 5.5%).

11.49 Preliminary data from year 1 (2008/09) of the NDNS rolling programme suggest that mean intakes of red meat have increased by about 10 g/day (men, 101 g/day; women, 64 g/day) since 2000/01. However, these findings should be interpreted very cautiously because of important insecurities in the new data, including dietary assessment method and insufficient sample size to allow disaggregation.

11.50 In summary, there are a number of uncertainties which complicate a risk assessment of iron and health. The main sources of uncertainty are: difficulties in assessing dietary iron intakes; poor correlation between intakes and systemic iron load; difficulty in measuring adaptive and functional responses to variations in iron intake; uncertain and possibly conservatively high estimates of DRVs; lack of sensitive and specific markers to assess iron deficiency or excess; lack of consistent quality control and reference values in measurement of customary markers of iron

89 Department of Health. Nutritional Aspects of the Development of Cancer. Report on health and social subjects 48. London: TSO, 1998.

status; inadequate characterisation of the role 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; small sample sizes in most studies; and confounding by other dietary and lifestyle factors and by alterations in iron metabolism in response to infection. All these uncertainties make it difficult to determine dose-response relationships or confidently characterise the risks associated with iron deficiency or iron excess.

12 Recommendations

12.1 It is important to ensure that the UK population has a safe and adequate supply of iron to meet physiological requirements. It is recommended that a public health approach to achieving adequate iron status should emphasise the importance of a healthy balanced diet that includes a variety of foods containing iron. Such an approach is more important than focusing on particular inhibitors or enhancers of the bioavailability of iron from diets.

12.2 While substantial proportions of the UK population appear to have iron intakes below dietary recommendations for iron, this is not clearly consistent with the low prevalence of poor iron status (see paragraph 12.3). This might be because there are important uncertainties in the DRVs for iron intake which may be too high, particularly for girls and women of reproductive age. It is recommended that the DRVs for iron should be reviewed when more data become available (see research recommendations, paragraph 13.2).

12.3 Although there are many uncertainties in the data, about 95% of the UK population is iron replete.[90] However some population groups may be at risk of iron deficiency anaemia.[91] These include toddlers, girls and women of reproductive age (particularly those from low income groups) and some adult groups aged over 65 years.[92] It is recommended that health professionals be alert to the increased risk of iron deficiency anaemia in these groups. Those with signs and symptoms suggestive of iron deficiency anaemia should receive appropriate clinical assessment and advice, including dietary advice on how to increase their iron intakes and to consider use of iron supplements if required.

12.4 Current evidence does not support routine iron supplementation of pregnant women but this should be kept under review. The recommendation by NICE (2008) is therefore supported, that iron supplementation should not be offered routinely to all pregnant women but should be considered for women identified with haemoglobin concentrations below 110 g/L in the first trimester and 105 g/L at 28 weeks.

12.5 Red and processed meat is a source of iron in the diet of the UK population. COMA reported possible links between red and processed meat consumption and colorectal cancer risk in 1998 and the evidence that has accumulated since then generally supports this association. However, it is not possible to quantify

90 Haemoglobin and serum ferritin concentrations above the WHO thresholds used to define iron deficiency and anaemia (see footnote 91).

91 Haemoglobin and serum ferritin concentrations below the WHO thresholds used to define iron deficiency and anaemia. WHO criteria for iron deficiency are serum ferritin concentrations below the following thresholds: children aged under 5 years, 12 µg/L; children aged over 5 years and adults, 15 µg/L. WHO criteria for anaemia are haemoglobin concentrations below the following thresholds: children under 5 years, 110 g/L; children 5–11.99 years, 115 g/L; children 12–14.99 years and non-pregnant females over 15 years, 120 g/L; males over 15 years, 130 g/L.

92 Men aged 65 years and over living in institutions; free-living men and women aged 85 years and over.

the amount of red and processed meat that may be associated with increased colorectal cancer risk because of limitations and inconsistencies in the data. It may be advisable for adults with relatively high intakes of red and processed meat (e.g., it is estimated that those above the 75th percentile consume over 90 g/day) to consider reducing their intakes. Evidence from a theoretical modelling exercise indicates that a reduction in the red and processed meat intakes of high consumers, to the population average for adult consumers (about 70 g/day cooked weight in 2000/01), would have little impact on the proportion of the adult population with iron intakes below the LRNI. However, this estimate is based on data from 2000/01 and will need to be kept under review.

13 Research recommendations

13.1 A more coordinated approach to research on iron in the UK and elsewhere is required to characterise iron status, involving harmonisation of reference ranges and analytical quality control for markers of iron metabolism. Consistent study designs and protocols will enable better characterisation of functional thresholds in relation to iron sufficiency, deficiency or excess. This would improve the cost effectiveness of the research and enable research findings to be more relevant to public health needs.

13.2 Good quality dose-response data are required to enable a reassessment of the DRVs for iron. Knowledge of the systemic regulation and mediation of iron homeostasis should be applied to characterise better the responses to increased and reduced systemic needs for iron and the development, or better validation, of existing markers used to assess the adequacy of iron status in populations and individuals.

13.3 Future studies assessing the relationship between iron excess and chronic disease should employ a standardised approach to measure iron exposure and categorisation of red and processed meat and other sources of organic and inorganic iron. This, together with the maintenance and expansion of food composition databases, with particular reference to iron content, would improve the quality of dietary assessments of iron intake for studies relating to iron and chronic disease. Assessments of systemic iron depots in such studies should be based on measurement of serum ferritin concentration.

13.4 Iron intakes and iron status of vulnerable groups, particularly minority ethnic groups and infants aged up to 18 months, need to be better characterised.

13.5 An improved understanding is required of the factors underlying differences in the risk of iron deficiency anaemia between women of reproductive age from low income populations and those in the general population.

13.6 The extent to which foods fortified with iron contribute to the supply of absorbed iron and to achieving adequate iron status, particularly in vulnerable groups, should be assessed.

13.7 An improved understanding of the possible adverse effects of iron supplements on iron replete children is required.

13.8 Further randomised controlled trials with adequate power and sufficient duration are required to examine the effect of iron supplementation on mental development in children under 3 years old with iron deficiency anaemia.

13.9 Further studies are required on the benefits, risks and long term effects associated with a delay in clamping the umbilical cord after birth until it has stopped pulsing.

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SACN working procedures

Meetings

SACN Iron Working Group

1. The SACN Iron Working Group was established in December 2001. The working group met three times in 2002, 2003 and 2004, and twice in 2005. The work of the group was suspended in 2006 due to other SACN priorities. Work on the draft report was resumed in 2008 and the working group met once in that year. The working group also met once in 2009 to consider the responses received to the consultation on draft report (see below). The minutes of all the meetings are available on the SACN website (www.sacn.gov.uk).

SACN

2. The draft report was considered by the full committee in February 2005, February 2009 and February 2010.

Consultation

3. The draft report was posted on the SACN website on 17 June 2009. Interested parties were invited to submit comments relating to the science of the report by 23 September 2009.

4. Submissions were received from the following organisations and individuals.

    1. Agriculture and Horticulture Development Board – representing BPEX and EPBLEX (successors of Meat and Livestock Commission in England)
    2. British Nutrition Foundation (BNF)
    3. Committee on Carcinogenicity
    4. Committee on Toxicology
    5. Dean, Jennifer
    6. Food and Drink Federation (FDF)
    7. Health Food Manufacturers' Association (HFMA)
    8. McArdle, Professor Harry J, University of Aberdeen
    9. MRC Human Nutrition Research
    10. Northern Ireland Food Advisory Committee (NIFAC)
    11. Quality Meat Scotland (QMS)
    12. Rushton, Dr H, University of Portsmouth

13. Safe Food, Ireland

14. Scottish Food Advisory Committee (SFAC)

15. Vegetarian Society UK, Friends of the Earth, Sustain, and the Food for Life Partnership

5. The submissions can be viewed in full on the SACN website (www.sacn.gov.uk). The working group's response to all the submissions is also available on the SACN website.

Examples of functional iron-containing proteins in the body

Table A1: Examples of functional iron-containing proteins in the body (75 kg man)

Iron-containing protein	Function	Location	Iron content (mg)
Haem proteins			
Haemoglobin	Oxygen transport	Red blood cells	3000
Myoglobin	Oxygen storage	Muscle	400
		All tissues	c. 30
Haem enzymes			
Cytochromes a,b,c	Electron transfer Transfer of electrons to molecular oxygen at end of respiratory chain (also requires copper)		
Cytochrome C oxidase Cytochrome P450 + b5 Dcytb	Microsomal mixed function oxidases Phase I biotransformation of xenobiotics Ferrireductase (duodenal enterocytes)		
Catalase Peroxidases Myeloperoxidase	Hydrogen peroxide breakdown Peroxide breakdown Neutrophil bacteriocide		
Sulphite oxidase	Sulphites to sulphates		
Tryptophan 2,3-dioxygenase	Pyridine metabolism		
Iodase (iodoperoxidase)	Iodide to iodate		
Non-haem iron enzymes		All tissues	c. 30
Ribonucleotide reductase	Ribonucleotides → 2'-deoxyribnucleotides Synthetic phase of cell division		
(Iron-sulphur proteins) Aconitase Isocitrate dehydrogenase Succinate dehydrogenase NADH dehydrogenase	Citric acid cycle and initial steps of oxidative phosphorylation		
Aldehyde oxidase Xanthine oxidase	Aldehydes to carboxylic acids Hypoxanthine – uric acid		
Phenylalanine hydroxylase Tyrosine hydroxylase Tryptophan hydroxylase	Catecholamine, neurotransmitters, and melanin synthesis		
Prolyl hydroxylase Lysyl hydroxylase	Collagen synthesis, both depend on ascorbic acid		

International dietary reference values for iron

Table A2: International dietary reference values for iron (mg/day)

UK (Department of Health, 1991)		USA and Canada (Institute of Medicine, 2001)		FAO/WHO (2002)			EU (EC Scientific Committee on Food 1993)	
Age	Recommended Nutrient Intake (based on 15% absorption)	Age	Recommended Dietary Allowance (based on 18% absorption)	Age	Recommended Nutrient Intake (based on 15% absorption)	Recommended nutrient intake (based on 10% absorption)	Age	Population Reference Intake (based on 15% absorption)
0–3 months	1.7	–	–	–	–	–	–	–
4–6 months	4.3	0–6 months[1]	0.27	–	–	–	–	–
7–9 months	7.8	–	–	–	–	–	–	–
10–12 months	7.8	7–12 months[2]	11.0	6–12 months[5]	6.2	9.3	6–12 months[5]	6.2
1–3 years	6.9	1–3 years	7.0	1–3 years	3.9	5.8	1–3 years	3.9
4–6 years	6.1	4–8 years	10.0	4–6 years	4.2	6.3	4–6 years	4.2
7–10 years	8.7	–	–	7–10 years	5.9	8.9	7–10 years	5.9
				MALES				
11–14 years	11.3	9–13 years	8.0	11–14 years	9.7	14.6	11–14 years	9.7
15–18 years	11.3	14–18 years	11.0	15–17 years	12.5	18.8	15–17 years	12.5
19–50 years	8.7	19–50 years	8.0	18+ years	9.1	13.7	18+ years	9.1
50 + years	8.7	50+ years	8.0	–	–	–	–	–

UK (Department of Health, 1991)		USA and Canada (Institute of Medicine, 2001)		FAO/WHO (2002)			EU (EC Scientific Committee on Food 1993)	
Age	Recommended Nutrient Intake (based on 15% absorption)	Age	Recommended Dietary Allowance (based on 18% absorption)	Age	Recommended Nutrient Intake (based on 15% absorption)	Recommended nutrient intake (based on 10% absorption)	Age	Population Reference Intake (based on 15% absorption)
				FEMALES				
11–14 years	14.8	9–13 years[3]	8.0	11–14 years[6]	9.3	14.0	11–14 years[6]	9.3
15–18 years	14.8	14–18 years[3]	15.0	11 –14 years	21.8	32.7	11–14 years	21.8
19–50 years	14.8	19–50 years	18.0	15–17 years	20.7	31.0	15–17 years	20.7
50+ years	8.7	50+ years	8.0	18+ years	19.6	29.4	18+ years	19.6
–	–	Pregnancy[4]	27.0	Post-menopausal	7.5	11.3	Post-menopausal	7.5
–	–	Lactation (14–18 years)	10.0	Lactating	10.0	15.0	Lactating	10.0
–	–	Lactation (19–50 years)	9.0	–	–	–	–	–

1 No functional criteria of iron status have been demonstrated that reflect response to dietary intake in young infants. Recommended intakes are based on observed mean iron intake of infants principally fed human milk.

2 Based on 10% absorption.

3 Based on assumption that girls under 14 years do not menstruate and those above do. For girls under 14 years who are menstruating, the requirement is increased by approximately 2.5 mg/day of iron (assuming a median menstrual loss of 0.45 mg/day of iron).

4 The bioavailability in the first trimester is as estimated for non-pregnant females; in the second and third trimesters, it is increased to 25%.

5 Bioavailability during this period varies greatly.

6 Non-menstruating.

Existing public health advice to improve iron nutrition in the UK

1. Current public health measures to support/optimise iron nutrition in the UK include dietary advice aimed at infants, young children, pregnant women and the general population, as well as the mandatory addition of iron to white and brown flour and to breast milk substitutes.

Dietary advice for the general population

Meat consumption

2. The government currently advises that meat can be consumed as part of a healthy, balanced diet, and that it is a good source of iron, zinc, B vitamins and protein; however, due to its high saturated fat content, it should be eaten in moderation.

Maximising iron absorption

3. Single meal absorption studies have shown that certain foods and drinks can enhance or inhibit non-haem iron absorption from iron-containing foods (see section 5 of main report). Enhancers of non-haem iron absorption include meat and ascorbic acid (vitamin C); inhibitors of iron absorption include phytates (found in whole grains, legumes, nuts and seeds), polyphenols (found in tea and coffee), and calcium (found in milk and dairy products). Dietary advice for the general population regarding maximising iron absorption is to consume foods rich in vitamin C (such as a glass of fruit juice), and not to consume tea, coffee or dairy products at the same time as meals or foods containing iron.

Dietary advice for infants

4. UK health departments recommend that infants should be exclusively breast fed until the age of 6 months and that breast milk or breast milk substitutes should be the main drink until 12 months of age. This advice is based on evidence that breast feeding confers a number of health benefits to both the mother and infant, including the avoidance of iron deficiency anaemia during infancy, which is associated with the too early introduction of unmodified cows' milk and milk products (WHO, 2000). Women who are unable to breast feed are advised to use a commercial iron fortified breast milk substitute (DH, 1994). It is recommended that foods containing haem iron, such as meat and fish, should be introduced at approximately 6 months of age; that foods rich in vitamin C should be consumed with a meal to improve iron uptake; and that coffee/all types of tea (black, green and herbal) should be avoided until 24 months of age because of their inhibitory effect on iron absorption (see section 5 of main report).

5. The UK recommendations on infant feeding are similar to those made in the United States (AAP, 1999[93]), Canada (Canadian Paediatrics Society, 1991[94]), and by WHO (2001[95]).

Dietary advice for pregnant women

6. All pregnant women are advised to consume iron rich foods and to consume foods and drinks rich in vitamin C with meals, to improve iron absorption (see section 5 of main report). There is no universal recommendation regarding iron supplementation for pregnant women. The National Institute for Clinical Excellence (2008) recommends that iron supplementation should not be offered routinely to all pregnant women but should be considered for women identified with haemoglobin concentrations below 110 g/L in the first trimester, and 105 g/L at 28 weeks.

Food fortification policies

Flour

7. In the UK, fortification of white and brown flour is a mandatory requirement under food legislation (see Section 5 of main report). There is no legal requirement for fortification of other staple foods and ingredients, although compositional standards exist for a range of foods, including breakfast cereals.

Infant formulas, follow-on formulas and infant foods

8. Harmonised rules on the composition of infant formulas and follow-on formulas were adopted by the European Community in 1991 and 1996 (Directive 91/321 EEC). This directive has been amended several times to take account of scientific and technical developments. The composition of infant formula and follow-on formula is controlled by Directive 2006/141/EC, which is implemented by the Infant Formula and Follow-on Formula Regulations (England) 2007 and equivalent regulations in the devolved administrations. The revised directive stipulates that the iron content of cows' milk based infant formulas should be between 0.07 and 0.3 mg/100 KJ (0.3–1.3 mg/100 kcal). For a typical infant breast milk substitute with an energy content of 680 kcal/L, this is equivalent to 2.04–8.84 mg/L of iron. Regulations also recommend the amount of iron in follow-on formulas and other breast milk substitutes (see Table A3).

9. In the UK, all infant breast-milk substitutes are fortified with iron to a typical level of 6–7 mg/L. This is in accordance with fortification levels in most other European countries (range: 4–7 mg/L) but differs from the USA, where the majority of formulas are fortified at the upper level of current recommendations (10–12 mg/L). These formulas are not available in the UK.

10. Recommendations for the iron content of infant formulas and follow-on formulas, including those manufactured from soy proteins, are provided in Table A3.

93 American Academy of Pediatrics. Committee on Nutrition. Iron fortification of infant formulas. Pediatrics. 1999; 104:119–123.

94 Canadian Paediatric Society. Meeting the iron needs of infants and young children: an update. CMAJ. 1991; 144:1451–4.

95 World Health Organization. Iron Deficiency Anaemia. Assessment, Prevention and Control. A guide for programme managers. Geneva: WHO, 2001.

Table A3: Recommendations for the iron content of infant formulas and follow-on formulas

Type of formula	Iron content	
	mg/100 kcal	mg/100 kJ
Cows'-milk-based infant formula	0.3–1.3	0.07–0.3
Soy-protein-based infant formula*	0.45–2	0.12–0.5
Cows'-milk-based follow-on formula	0.6–2.0	0.14–0.50
Soy-protein-based follow-on formula*	0.9–2.5	0.22–0.650

Fortification of processed cereal-based foods and baby foods for infants and young children

11. According to the *Processed Cereal-based Foods*[96] and *Baby Foods*[97] for *Infants*[98] and *Young Children*[99] Regulations 2004 (as amended), the maximum limit for addition of iron in processed cereal-based foods and baby foods intended for infants and young children is 3 mg per 100 kcal.

96 Processed cereal-based foods are defined as "foods for particular nutritional use within the categories specified in Part 1 of Schedule 1 fulfilling the particular requirements of infants and young children in good health and intended for use by infants while they are being weaned, and by young children as a supplement to their diet or for their progressive adaptation to ordinary food."

97 Baby foods are defined as "foods for particular nutritional use fulfilling the particular requirements of infants and young children in good health and intended for use by infants while they are being weaned, and by young children as a supplement to their diet or for their progressive adaptation to ordinary food other than processed cereal-based foods."

98 "Infants" means children under the age of 12 months.

99 "Young children" means children aged between 1 and 3 years.

Studies considered in relation to iron in the diet

Table A4: Prospective studies of the association between dietary factors influencing iron status

Study/year/ country	Study population/ SF and Hb concentration/ Follow-up duration	Dietary assessment method	Markers of iron status	Total dietary iron	Non-haem iron	Dietary enhancers of iron absorption		Dietary inhibitors of iron absorption			Other factors considered	Comments
						Haem iron/ meat	Ascorbic acid	Calcium	Poly-phenols	Phytate		
Munoz *et al* (1988) COSTA RICA	Pregnant women (n=48) Age: 17–30 years Divided into coffee drinkers(≥450 ml/day) (n=22) and non-drinkers (0 ml/day) (n=26) SF (µg/L) (mean) Coffee drinkers: 16 Non-drinkers: 14 Hb Not provided Follow-up: 4 months	Three 24 hour recalls during last trimester and food frequency questionnaire. One 24 h recall postpartum All received prenatal iron supplements	SF Hb	N/A N/A	N/A N/A	N/A N/A	N/A N/A	N/A N/A	– –	N/A N/A	Intakes of energy, protein, ascorbic acid, iron, red meat, supplements. Age, parity, income, education, weight gain.	23% of coffee consumers had Hb below 110 g/L compared to 0% for non-drinkers.

Study/year/ country	Study population/ SF and Hb concentration/ Follow-up duration	Dietary assessment method	Markers of iron status	Total dietary iron	Non-haem iron	Dietary enhancers of iron absorption		Dietary inhibitors of iron absorption			Other factors considered	Comments
						Haem iron/ meat	Ascorbic acid	Calcium	Poly-phenols	Phytate		
Garry *et al* (2000) USA	Men and women (n=125) SF (µg/L) (mean) Women: 95.6 Men: 113 Follow up: 10 years	3-day food records (mean values for 3 separate years) (supplements included)	SF	–	–	–	N/A	N/A	N/A	N/A	Sex, age, BMI, energy intake, intakes of dietary and supplemental iron, Inflammation.	Significant positive association with supplemental iron intake.
Backstrand *et al* (2002) MEXICO	Women (n=125) Age:16–44 years SF (µg/L) (mean) Non-breast feeding: 16.4 Breast feeding: 21.9 Hb (g/L) (mean) Non-breast feeding: 33 Breast feeding: 130 Follow-up: 1 year	Diet history (>10 days dietary data collected over 1 year)	SF Hb Hct	– – –	↑ – –	– – –	↑ ↑ ↑	– – –	N/A N/A N/A	– – –	Age, breast-feeding status, BMI, time since birth of last child, season, haem and non-haem iron.	SF – significant positive association with breast feeding, age, more days since birth of last child.

Study/year/ country	Study population/ SF and Hb concentration/ Follow-up duration	Dietary assessment method	Markers of iron status	Total dietary iron	Non-haem iron	Dietary enhancers of iron absorption		Dietary inhibitors of iron absorption			Other factors considered	Comments
						Haem iron/ meat	Ascorbic acid	Calcium	Poly-phenols	Phytate		
Liu *et al* (2003) USA	Women (n=620) Age: 44–69 years (mean: 61.6 years) SF (median): 73.8 µg/L Follow-up: 10 years	Average of 3 food frequency questionnaires over 10 years (80 items initially; expanded to 116) (supplements included)	SF	–	–	↑	–	–	–	–	Energy intake, age, physical activity, BMI, aspirin use, PMH use, GI ulcer, haem iron, non-haem iron, iron supplements, alcohol, phytate, calcium, ascorbic acid, coffee	Significant positive association with age, BMI, supplemental iron intake and alcohol. Significant negative association with PMH use, aspirin use, and GI ulcer.
Öhlund *et al* (2008) SWEDEN	Infants (n=127) Age: 6–12 months SF (mean) (µg/L) Girls: 55.6 Boys: 35.04 Hb (mean) g/L Girls: 114.9 Boys: 115 Follow-up: 12 months	5-day food records	SF Hb MCV	– –	N/A N/A	↑ (boys) –	– –	– –	N/A N/A	N/A N/A	Growth, Hb concentration of parents, intakes of iron, meat products, ascorbic acid, calcium, milk, milk-based fortified cereals, milk-based cereal drinks, porridge	Iron status improved from 6 months to 4 years. Hb significantly associated with previous Hb at 6, 12 and 18 months. Mother's Hb correlated with child's Hb over time.

↑Significant positive association; ↓significant negative association; – no association; N/A not applicable as this factor not considered in study

Abbreviations: BMI, body mass index; EP, erythrocyte protoporphyrin; GI, gastrointestinal; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; PMH, postmenopausal hormone; SF, serum ferritin; TS, transferrin saturation.

Table A5: Intervention studies on the effect of enhancers and inhibitors of iron absorption on serum ferritin and haemoglobin concentrations

Study/year/country	Study population	Duration	Treatment groups	Serum ferritin (µg/L) initial/final	Haemoglobin (g/L) initial/final	Comments
			MEAT			
Lyle *et al* (1992) USA	Women (n=60) Mean age: 18.6 years	12 weeks	50 mg ferrous sulphate, low food iron diet and exercise	27/27.5 (NS)	126/124 (NS)	At baseline, group differences in SF. At 12 weeks, SF of 50 mg ferrous sulphate group and high food iron group significantly higher than the other group. At 12 weeks, Hb of high food iron group significantly higher than the other group.
			10 mg ferrous sulphate, low food iron diet (total 18 mg iron) and exercise	48.9/34.7 (NS)	129/125 (p<0.05)	
			Placebo, free choice diet and exercise	40/23.9 (p<0.05)	120/115 (p<0.05)	
			High food iron diet (18 mg) mainly from haem iron and exercise	23.7/29.2 (NS)	116/124 (p<0.05)	
			Free-choice diet and no exercise	22.2/20.4 (p<0.05)	121/121 (NS)	
Hunt *et al* (1995) USA	Postmenopausal women (n=14) Age: 51–70 years (mean: 62.9 years)	7 weeks	High meat (289 g/day)	–/74	N/A	Baseline measures of iron indices not provided. Significant decrease in SF with high meat diet (p=0.01).
			Low meat (38.5 g/day)	–/82		
			Low meat (38.5 g/day) + mineral supplements (748 mg/day potassium, 594 mg/day phosphorus, 3.3 mg/day iron, 55 mg/day magnesium, 5.5 mg/day zinc)	–/82		
Engelmann *et al* (1998)	Infants (n=41)	2 months	High meat diet (27 g/day)		Decreased by 0.6 g/L	Significant difference (p=0.008) in change in Hb.
DENMARK	Age: 8 months		Low meat diet (10 g/day)		Decreased by 4.9 g/L	No significant differences in SF.

Study/year/country	Study population	Duration	Treatment groups	Serum ferritin (µg/L) initial/final	Haemoglobin (g/L) initial/final	Comments
Wells *et al* (2003) USA	Men (n=21) 59–78 years Undergoing resistance training	12 weeks (plus 2 weeks baseline period when all consumed vegetarian diet	Beef (0.6 g/day protein/kg body wt) Texturised vegetable protein (0.6 g/day protein/kg body wt)	132/131 (p<0.01) 95/72 (p<0.01)	140/151 (p<0.01) 143/145 (p<0.01)	SF – significant decrease in both groups over time but not between groups. Hb – significant increase for both groups with time and between groups.
Snetselaar *et al* (2004) USA	Adolescents (n=86) (age not given)	3 months	Beef 5x/week and poultry/fish ≤2x/week Poultry/fish 5x/week and beef ≤2x/week	31.2/38.7 (NS) 32.5/26.7 (NS) Significant difference between groups in change in SF (p<0.01)	N/A	Median beef consumption of beef group increased beef consumption by 26 g. Median poultry/fish consumption increased by <10 g.
Tetens *et al* (2007) SWEDEN	Women (n=57) 19–39 years	30 weeks	Meat-based diet: usual diet + 150 g/day meat Vegetable-based diet: allowed to consume maximum of 250 g meat and 125 g fish per week	16.3/16.5 (NS) 17.3/11.2 (p<0.001)	126/125 (NS) 124/121 (p=0.003)	Significant difference between dietary groups in SF and Hb.
			ASCORBIC ACID			
Cook *et al* (1984) USA	Men and women (n=17) 20–30 years	16 weeks + 20 more months (n=9)	2000 mg/day ascorbic acid (2x500 mg with each of two meals)	46.3/43.9 (NS)	N/A	No significant effect on SF when ascorbic acid supplementation continued for additional 20 months with 5 iron-replete and 4 iron-deficient participants.
Malone *et al* (1986) IRELAND	Men and women (n=58) 17–21 years	8 weeks	300 mg/day ascorbic acid Placebo	27/31.3 (NS) 23/23 (NS)	N/A	Increase in the intervention group was significant at 10% level. Increase not significant when compared with change in serum ferritin in the control group over same period.

Study/year/country	Study population	Duration	Treatment groups	Serum ferritin (µg/L) initial/final	Haemoglobin (g/L) initial/final	Comments
Hunt *et al* (1990) USA	Women (n=11) 22–36 years	5.5 weeks	Diet containing 13.7 mg/2000 kcal + 1500 mg/day ascorbic acid Diet containing 13.7 mg/2000 kcal + placebo	Not provided	Not provided	Hb significantly higher in the group supplemented with ascorbic acid ($p<0.05$) Ascorbic acid had no effect on SF.
Hunt *et al* (1994) USA	Women (n=25) 20–45 years	10 weeks Crossover design	Diet of low iron bioavailability + 1500 mg/day ascorbic acid 5 weeks Placebo 5 weeks Typical Western diet + 1500 mg/day ascorbic acid 5 weeks Placebo 5 weeks	–/12.9 (NS) –/11.4 (NS) –/11.9 (NS) –/9.7 (NS)	–/132 (NS) –/131 (NS) –/132 (NS) –/130 (NS)	Combined data suggested slightly higher SF with ascorbic acid $p<0.06$.
Garcia *et al* (2003) MEXICO	Women (n=36) Mean age: 28 years	8 months	50 mg/day ascorbic acid as limeade Placebo group: lime-flavoured beverage	6.4/9.0 (NS) 6.2/8.7 (NS)	137/140 (NS) 139/137 (NS)	No significant differences in SF or Hb between groups after 8 months.
CALCIUM						
Sokoll and Dawson-Hughes (1992) USA	Women (n=109) 18–52 years	12 weeks	1000 mg/day calcium Control group did not receive placebo	34.9/–2.2% decrease (NS) 47.2/2.6% increase (NS)	135/1% increase (NS) 136/0.6% increase (NS)	No significant differences in SF and Hb between groups at end of intervention period.
Ilich-Ernst *et al* (1998) USA	Girls (n=354) Premenarcheal 8–13 years	4 years	1000 mg/day calcium Placebo	29.1/30.6 (NS) 29.3/29.5 (NS)	N/A	No significant differences between groups at end of intervention period.

Study/year/country	Study population	Duration	Treatment groups	Serum ferritin (µg/L) initial/final	Haemoglobin (g/L) initial/final	Comments
Kalkwarf and Harrast (1998) USA	Lactating (n=95) and non-lactating (n=92) women Mean age: 31 years	6 months	Lactating women 1000 mg/day calcium Placebo Non-lactating women 1000 mg/day calcium Placebo	Initial and final SF not provided by these groupings. After 6 months, no significant differences in SF between calcium supplemented and placebo groups.	135/133 (NS) 133/130 (NS) 132/129 (NS) 132/130 (NS)	At baseline SF significantly higher in lactating than non-lactating women.
Minihane and Fairweather-Tait (1998) UK	Men and women (n=24) Mean age 43 years	6 months	1200 mg/day Control group did not receive placebo	47/50 (NS) 40/38 (NS)	139/136 (NS) 143/139 (NS)	
			PHYTATE			
Lind *et al* (2003) SWEDEN	Infants (n=267) 6 months	6 months	Commercial milk-based cereal drink and porridge (containing 124 µmol/day at 6–8 months and 189 µmol/day at 9–11 months)	48.5/25.3 (p<0.05)	116/119 (p<0.05)	At 12 months of age, Hb significantly lower in infant formula group compared to phytate reduced group (p=0.015).
			Phytate reduced milk based cereal drink and phytate reduced porridge (containing 48 µmol/day at 6–8 m and 36 µmol/day at 9–11 months)	40.9/21.3 (p<0.05)	115/120 (p<0.05)	SF did not differ between groups.
			Milk based infant formula and porridge with usual phytate content (containing 26 µmol/day at 6–8 months and 62 µmol/day at 9–11 months)	44.1/25.2 (p<0.05)	115/117 (NS)	

Study/year/country	Study population	Duration	Treatment groups	Serum ferritin (µg/L) initial/final	Haemoglobin (g/L) initial/final	Comments
Bach Kristensen *et al* (2005) DENMARK	Women (n=41) 19–37 years	4 months	300 g/day fibre rich bread with reduced phytate (molar ratio of phytic acid:iron 8.5:1)	44/34 (p<0.001)	127/130 (NS)	No significant difference in SF between groups after 4 months.
			300 g/day fibre-rich bread (molar ratio of phytic acid:iron 6.7:1)	45/32 (p<0.001)	127/130 (NS)	

Abbreviations: Hb, haemoglobin; N/A, not applicable; NS, not significant; SF, serum ferritin; Zn, zinc.

Table A6: Efficacy and effectiveness trials of iron fortification

Study/year/country	Study population	Study length	Fortification vehicle/treatment groups	Iron dose (mg/day)	Findings	Comments
Walter *et al* (1993) CHILE	Exclusively breast-fed infants (n=136) Age: 4 months Mean Hb >95 g/L	11 months	Rice cereal: Unfortified Fortified with electrolytic iron (55 mg/100 g dry cereal)	 – 12	**Hb concentration** At 12 and 15 months of age, significantly higher ($p<0.05$) in infants fed fortified cereal. **SF concentration** Not reported **IDA**[1] Significantly higher ($p<0.01$) percentage of infants fed unfortified cereal developed IDA	
Nestel *et al* (2004) SRI LANKA	**Women** (n=1573) Mean age, 32±9 years Mean Hb (electrolytic iron gp): 128.5±17.5 g/L (*significantly higher than other 2 groups*) Mean Hb (reduced iron group) 125.2±16.2 g/L Mean Hb (control group) 122.8±17.5 g/L **Pre-school children** (n=745): Age: 9–71 months; mean, 46±16 months Mean Hb 120.6 ±12.2 g/L **Primary school children** (n=910): Age: 6–11 years; mean, 103±21 months Mean Hb 129.3 ±10.3 g/L	2 years	Wheat flour: Unfortified Fortified with either electrolytic iron(66 mg/kg) Fortified with reduced iron (66 mg/kg)	 – 9 9	**Hb concentration** *Pre-school children* No difference between flour groups ($p=0.284$) and those anaemic (Hb<110 g/L) at baseline ($p=0.952$) *Primary school children* No difference between flour groups ($p=0.183$)and those anaemic (Hb<120 g/L) at baseline ($p=0.712$) *Women:* No difference between flour groups ($p=0.775$) and those anaemic (Hb<120 g/L) at baseline ($p=0.650$) SF concentration Not reported	Dropouts: 62% preschool children 45% primary school children; 48% women

Study/year/country	Study population	Study length	Fortification vehicle/treatment groups	Iron dose (mg/day)	Findings	Comments
Zimmerman *et al* (2005) THAILAND	Women (n=330) Age: 18–50 years SF<25 µg/L (median 66 µg/L) Hb>90 g/L (mean 126±10 g/L)	35 weeks	Low extraction wheat flour biscuits and sweetened white bread: No fortification iron Fortified with ferrous sulphate Fortified with electrolytic iron Fortified with hydrogen-reduced iron	 – 10 10 10	**Hb concentration** No effect **SF concentration** Increased significantly (p<0.01) in all 3 fortification groups Relative efficacy compared to ferrous sulphate: Electrolytic iron – 77% Hydrogen-reduced iron – 49%	Dropouts: 34% Snacks made from low extraction wheat flour containing <0.1 g phytic acid/100 g
Van Stuijvenberg *et al* (2006) SOUTH AFRICA	Children (n=160) with low iron status (SF<20 µg/L) Age: 6–11 years	7.5 months	Brown bread made from wheat flour Unfortified Fortified with ferrous bisglycinate Fortified with electrolytic iron (35 mg/kg for 4.5 months; 70mg/kg for last 3 months)	 – 3.7[2] 3.7[3]	**Hb concentration:** Significant increase (p=0.001) only in ferrous bisglycinate group after adjustment for sex and baseline Hb concentration. Increase in Hb also significantly higher than that of electrolytic group (p=0.04) and control (p=0.01) **SF concentration:** No effect in any groups.	72% of children with SF <15 µg/L at baseline

Study/year/country	Study population	Study length	Fortification vehicle/treatment groups	Iron dose (mg/day)	Findings	Comments
Andang'o *et al* (2007) KENYA	Children (n=516) Age 3–8 years Hb>70 g/L	5 months	Maize flour porridge 5 times per week: Unfortified Fortified with NaFeEDTA (28 mg/kg) Fortified with NaFeEDTA (56 mg/kg) Electrolytic iron (56 mg/kg)	 – 3.5 7 7	**Hb concentration:** Compared to control significant increase in high NaFeEDTA group; no effect in low dose NaFeEDTA or electrolytic iron group **Plasma ferritin concentration:** Compared to control significant increase in high NaFeEDTA group; no effect in low dose NaFeEDTA or electrolytic iron group **Prevalence of IDA**[4] 89% lower in high dose NaFeEDTA group compared to controls; no change in low-dose NaFeEDTA or electrolytic iron groups **Prevalence of ID**[5] 91% lower in high-dose and 70% lower in low-dose NaFeEDTA; no change for electrolytic iron group.	Treatment effects of NaFeEDTA greatest for children with ID or IDA
Sun *et al* (2007) CHINA	Children (n=400) with IDA[6] Age: 11–18 years	6 months	Wheat flour No fortification iron Fortified with FeSO4 (30 mg/kg) Fortified with NaFeEDTA (20 mg/kg) Fortified with electrolytic iron (60 mg/kg)	 – 9 6 20	**Hb concentration:** Increased significantly ($p<0.05$) from month 2 in NaFeEDTA group, month 4 in ferrous sulphate group, month 6 in electrolytic iron group (increase significantly less than for other 2 iron groups). No change in control group. **SF concentration** Increased significantly ($p<0.01$) after 4 months in NaFeEDTA group and after 6 months in FeSO4 group. No change in control and electrolytic iron group.	

Study/year/country	Study population	Study length	Fortification vehicle/treatment groups	Iron dose (mg/day)	Findings	Comments
Van Stuijvenberg *et al* (2008)[7] SOUTH AFRICA	Children (n=361) Age: 6–11 years Hb≤125 g/L	34 weeks	Brown bread Unfortified Fortified with NaFeEDTA (10 mg/kg) Fortified with ferrous fumarate (20 mg/kg) Fortified with electrolytic iron (35 mg/kg)	 – 1.3 2.5 4.5	**Hb concentration** No significant intervention effect compared to control group. **SF concentration** No significant intervention effect compared to control group.	50% of children Hb <120 g/L; 47% SF<20 µg/L
Biebinger *et al* (2009) KUWAIT	Women (n=279) Age: 18–35 years SF<25 µg/L	22 weeks	Wheat flour based biscuits Unfortified Fortified with encapsulated ferrous sulphate and potassium iodate Fortified with hydrogen reduced iron	 – 7 14	**Hb concentration** No significant change in any group. **SF concentration** Significant increase ($p<0.001$) in encapsulated ferrous sulphate group; no increase in hydrogen-reduced group ID[8] prevalence No difference between groups	Dropouts: 35% Biscuits made from low extraction wheat flour containing <0.1 g phytic acid/100 g

Abbreviations: Hb, haemoglobin; NaFeEDTA, sodium iron ethylenediaminetetraacetate; SF, serum ferritin

1 Hb<110g/L + 2 out of 3 additional abnormal status measures (mean corpuscular volume<70 fl; transferrin saturation<10%; SF<10 µg/L).

2 2.5 mg/day for 4.5 months; 5 mg/day for last 3 months.

3 2.5 mg/day for 4.5 months; 5 mg/day for last 3 months.

4 For children under 5 years: plasma ferritin concentration <12 µg/L and haemoglobin concentration <110 g/L. For children over 5 years: plasma ferritin concentration <15 µg/L and haemoglobin concentration <115 g/L.

5 Plasma ferritin concentration <12 µg/L for children under 5 years and <15 µg/L for children 5 years or over.

6 Children ≤11 years: Hb, 115 g/L and SF, 15 µg/L; children >11 years: Hb, 120g/L; SF, 15 µg/L.

7 When NaFe EDTA (10 mg/kg) or ferrous fumarate (20 mg/kg) were added at levels that do not induce colour changes they were not efficacious in improving Hb or iron status in schoolchildren. Electrolytic iron fortified at same level as mandatory iron fortification in SA (35 mg/kg) showed it had no effect on iron and Hb status in schoolchildren who were iron-deficient.

8 Serum ferritin <15µg/L.

Table A7: Mean dietary iron intake, haemoglobin concentration and serum concentration ferritin in meat eaters and vegetarians

Study	Country	Participants (n)		Dietary iron (mg/day)		Haemoglobin (g/L)		Ferritin (µg/L)	
		Meat	Veg	Meat	Veg	Meat	Veg	Meat	Veg
WOMEN PREMENOPAUSAL									
Reddy and Sanders, 1990	UK	22 Caucasian	18 Caucasian	12.1	13.8	136	136	20	11*
			21 Indian		12.7		126*		8*
Alexander *et al*, 1994	New Zealand	36	36	13.5	15.5	–	–	34	14*
Donovan and Gibson, 1995	Canada	29	79	11.3	11.2	138	138	20	18
Ball and Bartlett, 1999	Australia	24	50	9.9	10.7	134	130	46	25*
Harvey *et al*, 2005	UK	30 (red meat)	30	10.9	14.5	134	135	7 (median)	11 (median)
		30 (poultry/ fish)		12.8		137		18† (median)	
WOMEN, ALL AGES									
Harman and Parnell, 1998	New Zealand	12	12	12.8	14.7	129	124	60	50
Haddad *et al*, 1999	US	10	15 vegans	15.3	17.6	133	132	22	27
MEN									
Alexander *et al*, 1994	New Zealand	14	14	17.4	20.2	–	–	105	37*
Harman and Parnell, 1998	New Zealand	11	12	16.2	15.5	142	151	148	80
Haddad *et al*, 1999	US	10	10	15.0	26.4	156	154	141	72*
Wilson and Ball, 1999	Australia	25	39	15.8	20.4	173	140*	121	64*
MEN AND WOMEN									
Gear *et al*, 1980	UK	264	91	–	–	143	139*	–	–
Helman and Darnton-Hill, 1987	Australia	37	93	–	–	–	–	70	45*
Hua *et al*, 2001	USA	30	30	–	–	–	–	72	35*
Li *et al*, 2000	Australia	60 (moderate meat)	43	16.8	20.5	149	142*	111	48*

* Significantly different from omnivores

† Significantly different from red-meat eaters

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Studies considered in relation to iron and cognitive function

Table A8: Short-term treatment trials in children aged ≤ 3 years with iron deficiency anaemia or iron deficiency

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Oski and Honig, 1978 USA	IDA (n=24) IDA treated IDA (n=12) IDA untreated (n=12) (IDA=Hb<105 g/L, MCV<74 serum Fe<15 µg/L, TS<13%)	9–26	DBRCT Treatment = IM Fe Placebo = IM saline Fe dose = enough to raise Hb to 120 g/L Duration: 5–8 days	Intercurrent illness or chronic disease	BSID IBR		**Baseline:** No significant differences in MDI or PDI between groups. **Treatment:** Change in MDI or PDI scores not significantly different between groups. Fe-treated group significantly increased in MDI. No significant change in PDI of either group. Treated group improved more than controls in reactivity ($p< 0.05$), gross and fine motor ratings ($p< 0.01$); attention not significantly different.	Small groups.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Lozoff *et al*, 1982a GUATEMALA	Total (n= 68) IDA treated (n=15) IDA placebo (n=13) NA treated (n=19) NA placebo (n=21) (IDA group = Hb≤105 g/L plus 2 of 3: SF≤12 µg/L, transferrin ≤10% , EP> 1 mg/L of packed cells) (Non-anaemic group = Hb≥120 g/L)	6–24	DBRCT Both IDA and non-IDA randomly assigned to Fe (5 mg/kg/day ferrous ascorbate) or placebo; Duration: 1 week	Hb ≤60 g/L, acute or chronic illness, birth complications, prematurity, congenital anomalies, retardation, malnutrition, birth weight <5 lb	IBR	7 non-IDA children	**Baseline:** significant differences between groups; IDA group more withdrawn, fearful, tense, unreactive to usual stimuli compared with non-IDA group. **Treatment:** No significant treatment effect. IDA group improved on all above measures with significant change in responsiveness and tension; non-IDA group were unchanged in 3 scales and deteriorated in 3 others. Only post-treatment difference was IDA group remained more fearful (p=0.06) .	Small groups.
Lozoff *et al*, 1982b GUATEMALA	As above	As above	As above	As above	BSID		**Baseline:** IDA significantly lower MDI and PDI than NA. **Treatment:** No significant treatment effect on MDI. All groups improved in MDI.	

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Oski *et al*, 1983 USA	NA (n=38) (Hb>110 g/L) divided into 4 groups: a) Fe-replete (n=10) b) Fe depleted (n=10) (SF<12 µg/L) c) Fe-deficient (n=10) (b+EP>0.3 mg/L) d) Fe-deficient (n=8) (c+MCV<70fL)	9–12	No randomisation All subjects received IM Fe Duration: 1 week	Prematurity, neonatal distress, congenital anomalies, chronic illness	BSID MDI; IBR		**Baseline:** MDI of group d less than group b (p=0.01) but a and b not different from c and d. Less involved (p<0.5), more solemn (p<0.054), attention, goal directed, responsivity, irritability not different. **Treatment:** Mean increase in MDI of groups a and b significantly less than groups c and d combined (p<0.01). Goal directness c+ d improved more than a + b (p< 0.05). All groups had normal SF levels.	Small groups; not randomised; no placebo group; some rating change differences not given.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Walter *et al*., 1983 CHILE (Subsample of Walter *et al*, 1989)	Total (n=37) Divided into 3 groups: IDA (n=10) NA ID (n=15) Fe-replete (n=12) (IDA = Hb<109 g/L and at least 2 abnormal Fe measures or a response in Hb or MCV to Fe treatment) (Fe-deficient = Hb ≥110 g/L but one or more abnormal Fe measures) (Fe-replete = Hb≥110 g/L, MCV≥0 70 fL, TS≥10%, SF≥10 µg/L)	15	Original cohort randomised at 3 months to Fe fortified or unfortified formula; At 15 months Fe status evaluated and all received 3–4 mg/kg/day ferrous sulphate. Duration: 11 days	BW < 2.5 kg, neonatal complications, chronic or congenital disorders, inadequate growth or development	BSID IBR		**Baseline:** IDA group had significantly lower MDI than Fe-replete group (p<0.0025); non-anaemic ID group not different. No significant group differences in PDI; IDA infants more unhappy than Fe-replete group (p< 0.05) on IBR. **Treatment:** Improvement in MDI larger in IDA group than Fe-replete group (p<0.05). Post test, Fe-replete group still had higher scores than IDA group (p< 0.05); NA ID group improved but not significantly different from Fe-replete group. No significant PDI differences. IDA improved in cooperativeness and attention on the IBR (p<0.05), but treatment effect not reported.	Small groups; not randomised; other behaviour ratings not reported.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Driva *et al*, 1985 GREECE	Total (n=48) NA (n=8) (Hb>110 g/L) IDA (n=40) (Hb<109 g/L): Group A, IDA Fe treated after first test (n=20) Group B, IDA Fe treated after 2nd test (n=20) No other Fe status cut-offs	3–25	RCT IDA tested 3 times, 10 days apart; randomly assigned to treatment after 1st or 2nd test NA treated after 1st test and tested twice. Treatment = IM Fe 50 mg; no placebo Duration: 10 days	None given	BSID	0	**Baseline:** no significant difference in MDI. **Treatment:** no significant treatment effect on PDI. Group A significantly improved between 1st and 2nd but not between 2nd and 3rd test; group B significantly improved between 2nd and 3rd test, not between 1st and 2nd. NA – no significant increase. Treatment effect not reported.	

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Lozoff *et al*, 1987 COSTA RICA	Total (n= 191) Five groups: a) IDA (n= 52) (Hb<105 g/L; 2 abnormal Fe status measures) b) Intermediate Hb and Fe-deficient (n=45) (Hb=106–119 g/L; 2 abnormal Fe status measures) c) Non-anaemic Fe-deficient (n=21) (Hb≥120 g/L; 2 abnormal iron status measures) d) Non-anaemic Fe-deficient (n=38) (Hb≥120 g/L; SF<12 µg/L) e) Fe-replete (n=35) (Hb≥120 g/L; normal measures of iron status	12–23	DBRCT Groups a and b randomised to IM Fe, oral Fe, or placebo; Groups c, d, e randomised to oral Fe or placebo; Treatment = 10 mg/ kg/day oral Fe or placebo; Dose of IM Fe = increase Hb level to 125 g/L Duration: 1 week	LBW, multiple pregnancy, perinatal complications, congenital anomalies, iron therapy after 6 months, IM Fe treatment at any age, acute or chronic ill health, retardation, abnormal Hb, or missing iron data	BSID	7%	**Baseline:** Hb<100 g/L significantly lower MDI than rest combined (p=0.0002). Hb<105 g/L significantly lower PDI than rest combined (p = 0.0001). **Treatment:** Significant increase in MDI for all groups (p<0.001); no significant treatment effect on PDI. Only Fe treated IDA group increased Hb by 10 g/L.	Study 1: short duration; Study 2: differences remained after controlling for covariates; not randomised.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Walter *et al*, 1989 CHILE	Total (n=196) IDA (n=39) Non-Fe-deficient (n=127) Fe-replete (n=30) (IDA = Hb<110 g/L + 2 or more abnormal Fe measures) (NA ID = Hb >110 g/L but not Fe-replete) (Fe-replete = Hb≥110 g/L, MCV ≥70 fL, TIBC<10%, SF≥10 µg/L+ <10 g/L increase in Hb after Fe treatment)	12	DBRCT All groups randomized to placebo or 15 mg of Fe^{2}+ Duration: 10 days	Same as above	BSID IBR	0	**Baseline:** IDA group had lower MDI than Fe-replete and NA ID group (p<0.01). IDA group scored lower on PDI than Fe-replete group (p< 0.01) and NA ID group (p<0.0001). PDI and MDI showed sigmoid curve relationship with Hb levels with intermediate point (Hb=105–109 g/L) significantly different (p< 0.05) from both extremes (i.e. < 105 or >110 g/L). **Treatment:** Study 1 – no significant treatment effect after 10 days.	

Also see Walter *et al* 1989 and Lozoff *et al* 1987 in Table A9.

Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomised controlled trial; EP, erythrocyte protoporphyrin, Fe, iron; Hb, haemoglobin; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron-deficient; IDA, iron deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin; TS, transferrin saturation; TIBC, total iron-binding capacity.

Table A9: Longer-term treatment trials in children aged ≤ 3 years with IDA or iron deficiency

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Walter *et al*, 1989 CHILE	Total (n=196) IDA (n=39) Non-Fe-deficient (n=127) Fe-replete (n=30) (IDA = Hb<110 g/L + 2 or more abnormal Fe measures) (NA ID = Hb >110 g/L but not Fe-replete) (Fe-replete = Hb≥110 g/L, MCV ≥70 fL, TIBC<10%, SF≥10 µg/L+ <10 g/L increase in Hb after Fe treatment)	12	**Study 1:** DBRCT All groups randomized to placebo or 15 mg Fe^{2+} until 15 months of age Duration: 3 months	Same as above	BSID IBR	0	**Baseline:** IDA group had lower MDI than Fe-replete and NA ID group (p<0.01). IDA group scored lower on PDI than Fe-replete group (p< 0.01) and NA ID group (p<0.0001). PDI and MDI showed sigmoid curve relationship with Hb levels with intermediate point (Hb=105–109 g/L) significantly different (p< 0.05) from both extremes (i.e. < 105 or >110 g/L). **Treatment:** No significant improvement in any group after 3 months.	Study 1, short duration; small groups. Study 2, not randomised.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Lozoff *et al*, 1987 COSTA RICA	Total (n= 191) Five groups: a) IDA (n= 52) (Hb<105 g/L and 2 other abnormal Fe status measures) b) Intermediate Hb and Fe-deficient (n=45) (Hb=106–119 g/L and 2 abnormal Fe status measures) c) Non-anaemic Fe-deficient (n=21) (Hb≥120 g/L and 2 abnormal iron status measures) d) Non-anaemic Fe-deficient (n=38) (Hb≥120 g/L and ferritin <12 µg/L) e) Fe-replete (n=35) (Hb≥120 g/L and normal measures of iron status	12–23	Groups a and b randomised to IM Fe, oral Fe, or placebo Groups c, d, e randomised to oral Fe or placebo Treatment = 10 mg/kg/ day oral Fe or placebo Dose of IM Fe = increase Hb level to 125 g/L After 1 week, IM treated groups (a and b) and Fe-replete group (e) given placebo; other groups (c and d) treated with oral Fe. No randomisation Treatment = 6 mg/kg/ day oral Fe or placebo Duration: 12 weeks	LBW, multiple pregnancy, perinatal complications, congenital anomalies, iron therapy after 6 months, IM Fe treatment at any age, acute or chronic ill health, retardation, abnormal Hb, or missing iron data	BSID	7%	**Baseline:** Hb<100 g/L significantly lower MDI than rest combined (p=0.0002); Hb<105 g/L significantly lower PDI than rest combined (p = 0.0001); **Treatment:** **Study 1** – Significant increase in MDI for all groups (p<0.001); no significant treatment effect on PDI. Only Fe treated IDA group increased Hb by 10 g/L. **Study 2** – No significant difference in change of scores between NA and IDA groups; IDA infants (<100 g/L) whose anaemia and Fe lack were corrected caught up to infants with initial Hb>100 g/L who became Fe-replete) but those remaining Fe-deficient continued to have lower MDI. IDA (<100 g/L) who became Fe-replete increased PDI significantly to catch up with NA Fe sufficient; those remaining Fe-deficient still had significantly lower PDIs. Increase in Hb of at least 10 g/L in 93% of Fe treated infants, 64% no longer anaemic but still Fe-deficient.	Study 1: short duration; Study 2: differences remained after controlling for covariates; not randomised.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Lozoff *et al*, 1998 COSTA RICA	As above	12–23	As study 2 above	As above	Free play; IBR; behaviour ratings and quality of maternal participation HOME	? 0	**Baseline:** IDA infants lower mental test scores but no differences in motor test scores. Also more wary and hesitant, maintained closer contact with caregiver, showed less pleasure and delight, tired easily, less playful and less attentive. **Treatment:** no significant treatment effect; formerly IDA continued to spend time near caregivers, attempted less tasks, more likely to be crying, irritable, asleep; less likely to play interactively. Post treatment, increase in Hb as above.	
Lozoff *et al*, 1996 COSTA RICA	IDA (n=34) NA (n=54) NA=Hb>125 g/L IDA=Hb ≤100g/L+ 2 of 3 measures – SF ≤ 12 µg/L, TS ≤ 10%, free erythrocyte protoporphyrin > 1 mg/L	12–24	IDA all treated Non-anaemic randomised to treatment or placebo Treatment = 6 mg/kg/ day Duration: 6 months	BW < 2500 g, birth complications, multiple pregnancy, acute or chronic health problems	BSID IBR		**Baseline:** IDA group significantly lower MDI than NA group; no group difference in PDI; IDA more fearful ($p<0.03$) and unhappy ($p<0.01$). **Treatment:** No significant treatment effect. IDA group disadvantaged in maternal education and home stimulation and less breast feeding. When controlled for all covariates, IDA not significantly different from NA group in MDI (limited power). No difference in IBR post treatment but treatment effect not reported.	No randomisation

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Harahap *et al*, 2000 INDONESIA	All children stunted or wasted. IDA from treatment groups 1 and 2 (n=18); NA from treatment group 3 (n=18). IDA = Hb<110 g/L, TS>16% or change in Hb>10 g/L	12-18	3 treatments: 1) Condensed milk+ micronutrient (12 mg Fe) 2) Skimmed milk and micronutrient (12 mg Fe) 3) Skimmed milk and placebo IDA groups 1 and 2 compared with NA group 3 matched for age and sex. Duration: 6 months	Chronic disease	BSID Response to novelty, object concept, motor milestones, activity at home, behaviour at home		**Baseline:** PDI and motor activity significantly different. Motor milestones, MDI, object concept, novelty recognition, all not significant. **Treatment:** IDA group improved significantly more than NA group in motor development. MDI, object concept, novelty recognition and milestones, all not significant.	Small groups, some children on ceiling of motor milestone scale. No randomised placebo anaemic group.
Hasanbegovic *et al* 2004 Sarajevu, Bolnicka	IDA = 90; A) 45 with Hb < 95 g/L B) 45 with Hb 95–110 g/L NA = 30 (Hb>110 g/L)	6–24	IDA all treated Duration: 3 months		BSID IDA given pre and post tests NA tested at baseline only		**Baseline:** IDA significantly lower in MDI and PDI than NA. Also A)lower than B) **Treatment:** MDI, PDI improved in B) but not in A).	No randomisation No post test in NA

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Akman *et al*, 2004 Turkey	1) 37 IDA= Hb<110 g/L 2) 40 NAID =Hb ≥ 110 g/L, SF ≤12 µg/L, MCV ≥ 70fl 3) 31 NA iron-replete (control) = Hb≥110 g/L	6–30	NAID only randomised to ferrous sulphate 6 mg/kg or no treatment All IDA treated All control no treatment Duration: 3 months	BW < 2500 g, no birth complications, multiple pregnancy, acute or chronic health problems	BSID Denver Screening test		**Baseline:** IDA and NAID significantly lower scores in MDI than controls, and IDA significantly lower PDI than controls. IDA and NAID had significantly lower scores in Denver than control. **Treatment:** initial 3 groups not different at end. Change significantly different across the 3 groups. No intent to treat analysis with randomised groups	Only NAID randomised No placebo

Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomized controlled trial; EP, erythrocyte protoporphyrin, Fe, iron; Hb, haemoglobin; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron deficient; IDA, iron deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; NAID, non-anaemic iron deficient; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin; TS, transferrin saturation; TIBC, total iron binding capacity.

Table A10: Longer-term randomised treatment trials in children aged ≤ 3 years with IDA or iron deficiency

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Aukett *et al*, 1986 UK	Total (n=110) Treated (n=54) Placebo (n=56) All with Hb=80–110 g/L (no other Fe cut off)	17–19	DBRCT Treatment = 24 mg Fe+10 mg vitamin C/day Placebo = 10 mg vitamin C/day Duration: 2 months	Hb < 80 g/L, lead poisoning, chronic health problems	Denver screening test Anthropometry	13	No significant treatment effect on psychomotor skills. No difference between those with Hb increase > 20 g/L and those with less. Expected rate of development was achieved by 31% of iron treated and 12% of the placebo group (p< 0.05). Hb increased by mean of 22 g/L in group receiving Fe and 0.3 g/L in placebo group.	Denver not sensitive; rate of development was a post-hoc analysis.
Idjradinata and Pollitt, 1993 INDONESIA	Total (n=126) IDA Fe treated (n=25) IDA placebo (n=25) NA ID, Fe treated (n=14) NA ID, placebo (n=15) NA Fe-replete, Fe treated (n=24) NA Fe-replete placebo (n=23) IDA = Hb ≤105 g/L, TS≤10%, SF≤12 µg/L Fe depleted=Hb≥120 g/L, TS≤10% , SF≤12 µg/L Fe-replete=Hb≥120 g/L, TS>10%, SF>12 µg/L.	12–18	DBCRT Stratified by Fe status group then randomised to treatment or placebo Treatment = Fe sulphate, 3 mg/kg/day Duration: 4 months	BW<2.5 kg, multiple pregnancy, congenital anomalies, perinatal complications, haemoglobinopathy, weight and height<2SD of reference standards, acute/chronic illness, Hb 10.5–120 g/L.	BSID	? 7 lost	**Baseline:** IDA groups scored significantly less in MDI than non-anaemic ID and Fe-replete groups and significantly less in PDI than ID and Fe-replete groups; latter two groups not significantly different. **Treatment:** significant treatment effect in IDA groups in MDI and PDI. No longer any differences between treated IDA and non-anaemic ID and Fe-replete groups. Fe-replete and ID groups had no significant treatment effect. Significant treatment effects on Hb concentration in IDA and ID group.	Previous delay reversed. Non-anaemic ID group small.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Stoltzfus *et al*, 2001 ZANZIBAR	685 randomised, 538 completed study 417 aged 12–48 months had language assessment 293 aged 12–36 months had motor assessment 97% , Hb<110 g/L 85% , malarial parasitaemia	12–48	DBCRT Received Fe supplement (10 mg/ day) or placebo and anthelmintic treatment (500 mg/day mebendazole) or placebo. (Children with Hb<70 g/L treated with 60 mg/day of Fe for 30 days in addition to randomly allocated iron). Duration: 12 months		Assessed by parental interview. Language milestones assessed in children 12–48 months and motor milestones assessed in those aged 12–36 months.	5% loss from language test 9% loss from motor test	**Baseline:** After adjustment for age, scores on motor and language scales significantly associated with Hb concentration. **Treatment:** significant Fe treatment effect on motor scores only in children with baseline Hb<90 g/L. Significant Fe treatment effect on language scores across Hb range. No significant anthelmintic treatment effect on motor and language milestones. No treatment effect on Hb concentration but significant effect on ferritin.	Motor improvement related to initial Hb concentration but language improvement was not.

Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomised controlled trial; EP, erythrocyte protoporphyrin; Fe, iron; Hb, haemoglobin; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron deficient; IDA, iron deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; NA ID, non-anaemic iron deficient; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin; TS, transferrin saturation; TIBC, total iron binding capacity.

Table A11: Longer-term randomised trials with children aged ≤ 3 years with mixed iron status

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Black *et al*, 2004 BANGLADESH	Total (n=221) subsample from larger study 5 groups: 1) 20 mg Fe + 1 mg riboflavin (n=49) 2) 20 mg Zn + 1 mg riboflavin (n=49) 3) 20 mg Fe + 20 mg Zn + 1 mg riboflavin (n=43) 4) Micronutrient mix (MM) (with 16 vitamins and minerals incl. 20 mg Fe, 20 mg Zn, 1 mg riboflavin) (n=35) 5) 1 mg riboflavin (n=45) All with Hb ≥90 g/L; approx 68% , Hb<110 g/L	6	DBRCT Treatments given weekly. All received 30 mg vitamin A at beginning of study. Duration: 6 months	Severely malnourished, neurologic disorders, physical disability, chronic illness Hb <90 g/L	BSID HOME scale Behaviour ratings: 3 factors Orientation-engagement Emotional regulation Motor quality	Initially 43 refused 28 absent + 125 dropped out (36%)	**Baseline:** Hb concentration not associated with any developmental or behavioural measures. **Treatment:** Significant group x time interaction for PDI and Orientation. PDI scores decreased significantly less in the Fe+Zn and the MM groups compared to riboflavin group. Fe group and Zn group ns MDI scores not affected by any treatment. Orientation decreased significantly less in the Fe and Fe +Zn groups than in the riboflavin group. No treatment effect on Hb concentration.	Small groups; controlled for number of covariates. Only iron effect was in orientation.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Lind *et al*, 2004 INDONESIA	Total (n=680) Each group (n=170) 4 treatment groups: 1) Fe (10 mg/day) 2 Zn (10 mg/day) 3) Fe (10 mg/day)+ Zn (10 mg/day) 4) placebo All with Hb>90 g/L 41% anaemic	6	DBRCT Each dose of all treatments also contained 30 mg ascorbic acid. Duration: 6 months	Chronic illness, twins, metabolic or neurologic disorder	BSID Behaviour ratings	25 (4%)	**Baseline:** No significant differences between groups. **Treatment:** Significant interaction between Fe and Zn treatment for PDI. Significant iron effect on PDI (p=0.042). no other group significant. No effect of Fe+Zn combined on PDI. No treatment effect on MDI or behaviour.	Controlled for number of covariates.
Olney *et al*, 2006 Zanzibar	n=404 103 = Fe 12.5 mg + folate (FeFA) 87= Zinc 10 mg 101= FeFA + zinc 114= placebo 65% anaemic	5–11	DBRCT Duration: until walked or up to 12 months		Age of walking By interview every 2 weeks	12%	**Treatment:** Fe (+/– zinc) had significant effect on age of walking. Improvement greatest in children with initial IDA zinc not significant .	

Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomised controlled trial; EP, erythrocyte protoporphyrin, Fe, iron; Hb, haemoglobin; Fe, iron; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron deficient; IDA, iron deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin; TS, transferrin saturation; TIBC, total iron binding capacity; Zn, zinc.

Table A12: Preventive trials with non-anaemic children aged ≤ 3 years

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Moffatt *et al*, 1994 CANADA	n=283 Blood not assayed on enrolment	< 2	DBRCT Randomised to: Fortified formula (12.8 mg/L Fe) or low iron formula (1.1 mg/L Fe) until 15 months of age. Duration: 13 months	Perinatal complications; congenital anomalies; BW <2.5 kg; prematurity	BSID; IBR	225, 204, 186 and 186 tested at 6, 9, 12 and 15 months respectively	PDI – significant treatment effect at 9 and 12 months but not at 15 months. MDI – no effect. Hb significantly higher in fortified group at each test. Percentage < 110 g/L in fortified and unfortified was 8.1 and 28.0 at 6 months, 8.1 and 18.6 at 9 months, 2.3 and 12.4 at 12 months and 2.6 and 10.4 at 15 months respectively.	Large loss. Difference between groups in iron status small.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Williams *et al*, 1999 UK	n=100 Infants who had started on unmodified cows' milk at 6 months 14.5% anaemic	5.7–8.6	RCT Randomized to: Fe fortified formula (1.2 mg Fe/100 ml) or usual cows' milk (0.05 mg Fe/100 ml) and given money to buy cows' milk Duration: until age 18 months Observed to age 24 months	Preterm, Hb <90 g/L, haemoglobinopathy, chronic ill health	Griffiths Scale	15%	Significant treatment effect on development. Mean developmental quotients fell in both groups but in fortified group they fell significantly less by 24 months ($p<0.05$). Difference not significant at end of treatment at 18 months. Drop in all subscale scores was less in fortified group but only significant in personal social subscale ($p<0.05$). At baseline no significant difference between groups in Hb concentration. Percentage with Hb < 110 g/L at 12 months was 2% in fortified and 33% in cow's milk group; at 18 months, fortified 0% and cow's milk 24%.	Subjects not blind to treatment; other constituents of formula may have caused the effect.
Morley *et al*, 1999 UK	n=493 from 3 centres Only one centre had Hb estimations	9	DBRCT Stratified by 3 areas and Asian/other, then randomised to: Cows' milk (0.05 mg Fe/L); Low Fe formula (0.9 mg Fe/L). High Fe formula (1.2 mg Fe/L) Duration: 9 months	Prematurity, BW < 2.5 kg; multiple pregnancy; previous iron supplement or blood transfusion; delayed development.	Pretest, Sherard's screen; post test. BSID	13%	No significant treatment effect on MDI or PDI. Similar ferritin levels in cows' milk and low Fe formula groups and both significantly lower than high Fe formula group ($p< 0.01$). Hb data missing from most at beginning and end of study, therefore % anaemic unknown.	Doubtful statistical power; large loss of Hb data.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Lozoff *et al*, 2003 CHILE	n=1657 Fe supplemented (n=1123) No added Fe (n=534)	6	6 groups varying in entrance criteria and supplementation procedures by time of enrolment 1991–94: Infants on ≥250 ml/day cow milk/formula assigned to high iron formula (12 mg/L) (n=430) or low iron formula (2.3 mg/L) (n=405) 1994–96: Infants on ≥250 ml/day cow milk/formula assigned to high iron formula (n=176) or unmodified cow milk and multivitamins with no iron (n=404) Infants on ≤250 ml/day cow milk/formula assigned to multivitamins with Fe (n=112) and multivitamins without Fe (n=130) Duration: 6 months	Premature; BW<3.0 kg low Hb, acute or chronic illness, congenital anomalies, IDA, iron therapy	BSID Fagan Test	?	No significant differences in mental or motor Bayley scores at 12 months. Fagan test – significant effect of Fe supplementation on looking time with non-supplemented infants looking longer. Significant effect of supplementation on age of crawling/creeping – non-supplemented infants crawled later. IDA in Fe supplemented group = 3.1% IDA in non-supplemented group 22.6%	Results should be interpreted with caution owing to possible confounding effects of cow's milk and breastfeeding with treatment.

Study/year/ country	Sample	Age (months)	Study design and treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Friel *et al*, 2003 CANADA	Total (n=77) Successfully breast feeding, healthy with Hb > 120 g/L SF>19 µg/L	1	DBCRT Received either Fe (7.5 mg/ day) or placebo until 6 months of age Developmental outcomes assessed between 12–18 months of age. (80% were 12 months) Duration: 5 months	Gestation<37 weeks; BW<2.5 kg; multiple pregnancy; major illness; major congenital anomaly	BSID Visual acuity	40%	**Baseline:** No significant difference between groups for Hb, MCV and plasma ferritin concentration. Developmental scores not assessed. **Treatment:** Fe treated group higher PDI ($p<0.05$) and visual acuity ($p=0.07$) than placebo group. No treatment effect on MDI. Fe treatment significantly improved MCV at 3.5 months and 6 months of age, and Hb at 6 months of age; no significant differences between groups in Hb, MCV, or SF at 12 months of age.	Small sample size; high dropout rate; By 6 m majority of children in both groups received formula containing Fe. Small difference in iron status.

Abbreviations: BSID, Bayley Scales of Infant Development; BW, birth weight; DBRCT, double-blind randomised controlled trial; Fe, iron; Hb, haemoglobin; IBR, Infant Behaviour Record; IDA, iron deficiency anaemia; MCV, mean corpuscular volume; MDI, mental development index; PDI, psychomotor development index; RCT, randomised controlled trial.

Table A13: Therapeutic treatment trials in children aged > 3 years

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Pollitt *et al*, 1983 USA	Fe depleted (n=15) NA (n=15) Fe depleted – change in TS-1 SD to+1.5 SD, mean Hb = 112 g/L NA = Hb>110 g/L, TS>20%	3–6 years	Both groups treated Treatment = 4–5 mg/ kg/day Fe Duration: 4–6 months	Physical handicap	Discrimination-learning tasks; Oddity learning; Short term memory; Stanford Binet IQ.	**Baseline:** IDA group took more trials to reach criterion in 1st part but not in reversal learning, IQ, short-term memory and oddity learning not significantly different. **Treatment:** No significant difference between groups in discrimination. Mean Hb increase in Fe depleted = 13 g/L	No randomisation; small groups.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Soemantri *et al*, 1985 CENTRAL JAVA	IDA (n=78) Fe-replete (n=41) IDA = Hb ≤110g/L, and TS ≤15% Fe-replete = Hb≥ 120 g/L TS ≥ 20%. Mean Hb: IDA = 97 g/L Fe-replete = 132 g/L	11 years	DBRCT Treatment: 10 mg/kg/ day ferrous sulphate Placebo = tapioca and saccharin Duration: 3 months	<80th percentile of weight and height; <85th percentile for MUAC, parasite egg after deworming, malaria, haematological diseases, severe illness, physical handicap, IQ<75	**Baseline:** Raven Progressive Matrices (IQ), Pre- and post-treatment: abbreviated standard achievement test, Bourden-Wisconsin test for concentration.	**Baseline:** IDA and NA not significantly different in IQ and concentration; NA group significantly higher school achievement than IDA group. **Treatment:** Fe treated IDA group improved significantly more in concentration and in school achievement than placebo IDA group. No significant difference between Fe treated and placebo NA groups. Post-treatment score of NA group still significantly better than Fe treated IDA group. Change in Hb Fe treated IDA = 26.7 g/L; Placebo IDA = –11.7 g/L; Fe treated NA = 7.6 g/L; Placebo NA = 6.7 g/L.	Clear treatment effect; ? control for school and grade level ; extremely low post test Hb in IDA placebo group.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Pollitt *et al*, 1985 EGYPT	IDA (n=28) Fe-replete (n=40) IDA = Hb≤115 g/L+ TS ≤25%, or SF ≤20 µg/L; Fe-replete = Hb>130 g/ L+ TS >25% or SF>12 µg/L	9.5 years	DBRCT Treatment: 50 mg ferrous sulphate/day Placebo: not stated Duration: 3–4 months	?	Matching familiar figure test.	**Baseline:** NA children had significantly greater efficiency than IDA children. **Treatment:** Efficiency of Fe treated IDA group significantly greater (p<0.05) than placebo treated IDA group. No significant effect on Fe-replete children.	Limited details (letter); clear treatment effect not reported; small groups.
Pollitt *et al*, 1986 GUATEMALA	IDA (n=25) NA (n=25) IDA: At baseline, Hb ≤100 g/L and EP>1 mg/L. Post treatment, Hb>110 g/L and EP≤175 or EP<100+Hb> 100 g/L or change inHb>20 g/L. NA: At baseline, Hb>110 g/L and EP≤1.5 mg/L. Post treatment, Hb>110 g/L and EP≤1.5 mg/L	3–6 years	Not randomised. All infants treated with Fe. Treatment: 3 mg/kg/ day ferrous sulphate Duration: 11–12 weeks	Birth weight < 2.5 kg, chronic illness, severe malnutrition, haematological disorder	Discriminant learning; short term memory; oddity learning tasks(measure attention, memory and conceptual learning).	**Baseline:** IDA group had more trials to criterion in the discrimination task compared with NA group (p<0.05); no significant difference between groups in memory and oddity tasks. **Treatment:** Discrimination test – IDA group improved significantly; no longer significant difference between groups. Memory test – no differences. Oddity test – NA group improved more than IDA (not significant) and had significantly better scores post treatment. Mean Hb increase in IDA = 29 g/L.	No randomisation.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Deinard *et al*, 1986 USA	IDA (n=25) Fe-deficient NA (n=45) NA Fe-replete matched to IDA group for sex, age mother's education, and race (n=?). IDA = Hb≤110 g/L, PCV ≤33%, EP≥35, MCV< 74fl, SF<20 µg/L; NA Fe-deficient = Hb≥ 110 g/L, PCV≥34%, EP = ≥35, MCV ≥ 75, SF<20 µg/L; NA Fe-replete = Hb≥110 g/L, PCV≥34%, EP<35, MCV ≥ 75, SF>20 µg/L.	18–60 months	Double blind intervention All IDA treated; NA Fe-deficient: alternately assigned to Fe (n=22) or placebo (n=23) NA Fe-replete: placebo Treatment = 6 mg/kg/ day elemental Fe Duration: 6 months	Gestational age ≥38 weeks, BW≥2.5 kg, head circumference, height and weight withinffl 2 SD of NCHS, chronic illness, developmental retardation	BSID MDI for infants 18–24 months; Stanford Binet IQ for children > 2 years; behaviour rating.	**Baseline:** No significant difference between IDA and Fe-replete group; Fe treated but not placebo Fe-deficient group significantly lower than Fe-replete group; IDA significantly less responsive to the environment and more unhappy. **Treatment:** Fe-replete group's score significantly improved at 3 and 6 months and significantly higher than IDA at 3 but not 6 months. IDA and Fe-deficient groups showed no significant improvement. NA group significantly more responsive to examiner than IDA (3 and 6 months), and more responsive to environment (baseline, 3 and 6 months) and emotional tone (baseline and 3 months). IDA Fe treated and both Fe-deficient groups showed complete haematological correction.	Wide age range. No analysis of group differences in change of scores.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Soewondo *et al*, 1989 INDONESIA	IDA (n=49) Fe depleted (n=57) Fe-replete (n=70) IDA = Hb< 10 g/L + two of SF<12 µg/L, TS<16%, EP>1.77 µmol/L; Fe depleted = Hb ≥110 g/L + two of above; Fe-replete = Hb≥110 g/L, + two of SF≥12 µg/L, TS ≥16%, EP≤ 1.77 µmol/L	IDA = 54 months NA = 58 months	DBRCT All groups received Fe or placebo. Treatment = 50 mg Fe/ day in syrup or placebo Duration: 8 weeks	?	Peabody Picture; vocabulary test (PPVT); two discrimination learning tasks; four oddity tasks.	**Baseline:** No difference between Fe-deficient and NA in any test. No differences between IDA and Fe-replete in PPVT or discrimination learning tasks; Fe-replete faster than IDA on 2 oddity tasks (p< 0.05). **Treatment:** PPVT no treatment effect; discrimination learning tasks remained too difficult; discrimination learning: treated IDA group earned significantly faster than treated Fe-replete group (p< 0.05); oddity task: no effect in trials 1 and 2; in trials 3 and 4 interaction between Fe treatment and group; Fe treated IDA group had significantly higher scores than Fe treated Fe-replete group and Fe-replete placebo group had higher scores than the IDA placebo group. Hb change in Fe treated IDA = 9 g/L; placebo IDA = 1 g/L.	Too many on floor of discrimination learning tasks, using subgroup who were on the test of doubtful validity.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Pollitt *et al*, 1989 THAILAND	IDA (n=101) Fe depleted (n=47) Fe-replete (n=1210) IDA = Hb<120 g/L+ two of: SF<10 µg/L, TS<16%, EP>700 µg/L; Fe depleted = Hb 120 g/L+ same as above; Fe-replete = Hb≥120 g/L+ two of: SF>9 µg/L, TS>15%, EP<701 µg/L	9–11 years	DBRCT All dewormed on enrolment and after 3 months Randomised to Fe or placebo before Fe status known, then divided into groups by iron status Treatment: 50 mg/day ferrous sulphate for 2 weeks, then 100 mg/day for 14 weeks Duration: 16 weeks	Thalassemia, cyanotic heart disease	Raven Progressive Matrices; Thai language and maths test; controlled for school and grade.	**Baseline:** IDA group scored significantly lower on IQ than NA control groups; IDA and Fe-deficient group scored lower on Thai language than NA controls; maths scores not significantly different. **Treatment:** no treatment effect; Fe status groups still significantly different in language and IQ after controlling for anthropometry and SES. Hb: IDA placebo and Fe treated groups increased by 14 and 20 g/L respectively; Fe depleted placebo and Fe treated groups increased by 1and 5 g/L respectively; both Fe-replete placebo and Fe treated decreased by 2 g/L.	Hb of IDA placebo group improved, therefore validity threatened ? due to deworming.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Seshadri and Gopaldas, 1989 (study 1) INDIA	n=94 IDA= Hb<110 g/L No other measure	5–8 years	Before Hb level known, children stratified by age, then every third child randomly assigned to control group and the next two to iron treatment group. Treatment = 20 mg Fe+ 0.1 mg/day folic acid Placebo not stated Duration: 60 days	Severe malnutrition (weight-for-age < 60% of NCHS standards).	Indian adaptation of WISC	**Baseline:** Anaemic children had significantly lower WISC scores than NA children only in 7-8-y-olds. **Treatment:** Fe treated group improved significantly in verbal, performance, and global IQ for all ages; no change in controls. In Fe treated group both IDA and NA children improved in IQ; improvement in global IQ of IDA children significantly higher than for NA children for the 7–8-y-olds only. Hb increased significantly in Fe treated group.	Analysis of treatment effect not reported by randomised groups; age groups too small to interpret separately; no placebo; folic acid may have independent benefits.
Seshadri and Gopaldas, 1989 (study 2) INDIA	n=28 (14 pairs of boys matched for IDA, height, weight, Hb, IQ, per capita income, and mother's educational level) IDA = Hb < 105 g/L	5–6 years	DBRCT Each pair randomized to iron or placebo Both groups dewormed. Treatment = 40 mg Fe+ 0.2 mg/day folic acid. Placebo = sugar. Duration: 60 days	Weight-for-age < 61% of NCHS standard; Draw-a-man IQ of <70 and >110.	Draw a man IQ; WISC	**Baseline:** No significant differences. **Treatment:** Both groups improved significantly in WISC. Fe treated group significantly better than controls in verbal and performance tasks. Mean change in Hb = +24 g/L in treated group and –8 g/L in controls.	Significance of group differences not reported; folic acid may have independent benefits; may have been over matched; small groups.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Seshadri and Gopaldas, 1989 (study 3) INDIA	n= 48 (16 groups of three, each matched for age, Hb level, and scores in cognitive function tests.) IDA = Hb<105 g/L; NA = Hb>115 g/L	8–15 years	DBRCT Each matched triplet randomised into 3 groups; Treatment (a) 30 mg/ day Fe; Treatment (b) 40 mg/ day Fe; Placebo = brown sugar; Duration: 60 days		Visual-recall; Digit-span; Maze (visual motor coordination); Clerical task.	**Baseline:** No differences because of matching. **Treatment:** Both Fe treatment groups significantly improved in all cognitive tests except for the maze test in the 30 mg group, no change in the placebo group (?significant difference). Compared with placebo, the 30 mg group had significantly higher scores in the clerical-task and visual-recall tests, and the 40 mg group had significantly higher scores in digit-span, mazes, clerical-task and visual-recall. IDA placebo boys showed no significant improvements; the 40 mg and 30 mg iron treated IDA group significantly improved in several tests.	No analysis reported of differences between groups in change of scores; may have over matched; small groups.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Seshadri and Gopaldas, 1989 (study 4) INDIA	n=130 65 pairs matched for age, and Hb.	8–15 years	DBRCT Matched pairs randomised to: Treatment = 60 mg/day Fe. Placebo = sugar tablets. Duration: 60 days	Family income > Rs500	Visual-recall; Digit-span; Maze; Clerical task.	**Baseline:** No differences in Hb or cognitive test scores in treated or placebo group or between IDA and NA groups. **Treatment:** Fe treated IDA children significantly better than placebo IDA children in overall scores and in clerical tasks, and mazes. Fe treated NA group improved significantly only in mazes.	Analysis not presented by randomised group; adding test scores of doubtful validity.
Bruner *et al*, 1996 USA	NA Fe-deficient girls (n=81); Treated (n=40) Placebo (n=41 IDA = Hb<120 g/L for white and 115 g/L for black girls Fe-deficient = normal Hb+ ferritin< 12 µg/L	13–18 years	DBRCT Treatment =260 mg/day Fe. Duration: 8 weeks.	Boys	Brief Test of Attention (BTA); Symbol Digits Modalities Test (SDMT); Visual Search and Attention (VSAT); Hopkins Verbal Learning Test (HVLT).	**Baseline:** No differences in haematologic and cognitive measures. **Treatment:** No significant effect on BTA, SDMT or VSAT. HVLT – iron treated group improved significantly more in score of 3 free recall items than the placebo group ($p< 0.02$). No significant difference in delayed recall or recognition parts of test. Hb and serum ferritin higher for Fe treated group.	Benefits limited to free recall; ferritin only other measure of iron status.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Lynn and Harland, 1998 ENGLAND	Treated (n=208) Placebo (n=205) 2.9% Hb < 120 g/L, 16.9% SF>12 µg/L.	12–16 years	Divided into 2 groups matched for age, sex and IQ; method of assignment not stated. Treatment = 17 mg Fe + 17 mg/day ascorbic acid. Duration: 16 weeks		Ravens Progressive Matrixes (IQ)	**Baseline:** Significant correlation between Hb and IQ ($p< 0.01$); ferritin and IQ not significant. **Treatment:** No significant difference between the groups. Sub group with ferritin <12 µg/L improved significantly more with treatment than placebo ($p= 0.02$); subgroups with ferritin 12–20 µg/L not different; treated subgroups with ferritin >20 µg/L improved significantly more than placebo ($p< 0.05$).	Moderate and high ferritin groups combined showed no treatment effects.

Study/year/ country	Sample	Age (years)	Study design and treatment	Exclusions	Outcome measures	Findings	Remarks
Metallinos-Katsaras *et al*, 2004 GREECE	IDA (n=21) Fe-replete (n=28) IDA=Hb<112 g/L and TS<16%, SF<12 µg/L or Hb increase>10 g/L after Fe supplementation Fe-replete = Hb>120 g/L and either TS>20% or SF>12 µg/L	3–4 years	DBCRT Treatment: 15 mg/day Fe and multivitamins Placebo (multivitamins only) Duration: 2 months	BW<2.5 kg; IQ≤1SD below age-adjusted mean; blood lead levels≥ 20 µg/day/L; head, weight and head circumference ≤10th percentile of US NCHS	Simple reaction time (SRT) test; continuous performance task (CPT); oddity learning (OL) tasks	**Baseline:** No significant differences between IDA and Fe-replete children in SRT and CPT scores, mean trials to criterion, or in proportion reaching criterion in any of the OL tasks. IDA group had significantly higher proportion correct on 1st OL task than Fe-replete group. **Treatment:** SRT – no treatment effect in IDA or Fe-replete children. CPT – Fe treated IDA children made significantly fewer errors of commission ($p<0.05$) and showed higher accuracy ($p<0.05$) and significantly more efficient ($p<0.05$) than Fe-replete children given placebo. OL – no treatment effect.	Small subgroups

Abbreviations: BSID, Bayley Scale of Infant Development; BW, Birth weight ; DBRCT, double-blind randomised controlled trial; EP, erythrocyte protoporphyrin; Fe, iron; Hb, haemoglobin; IDA, iron deficiency anaemia; IQ, intelligence quotient; MUAC, mid-upper arm circumference; NCHS, National Center for Health Statistics; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PCV, packed cell volume; SF, serum ferritin; SES, socioeconomic status; TS, transferrin; WISC, Wechsler Intelligence Scale for Children.

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Studies considered in relation to iron and risk of colorectal cancer and cardiovascular disease

Prospective studies of iron and colorectal cancer risk

Table A14: Total dietary iron and colorectal cancer risk

Study/year/country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Cancer site	Comparison (median intake or quantile range)	Adjustments	RR/OR/HR (95% CI)
Wurzelmann *et al*, 1996 USA	25–74	15	52 M+F	8,740 M+F	Proximal colon	Top fourth vs bottom fourth *(intakes in quartiles not specified)*	Age, sex.	1.44 (1.23–1.69) *p trend not given*
As above	As above	As above	57 M+F	As above	Distal colon	As above	As above.	1.03 (0.80–1.32) *p trend not given*
Kato *et al*, 1999 USA	34–65	4.7	105 F	523 F	Colorectum	Top fourth vs bottom fourth (intakes in quartiles not specified)	Age, beer intake, physical activity, family history CRC.	1.17 (0.6–2.3) *p trend=0.44*
Balder *et al*, 2006 NETHERLANDS	55–69	9.3	869 M	2,156 M	Colorectum	Top fifth (17 mg/day) vs bottom fifth (9.5 mg/day)	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol, vegetables.	1.34 (0.93–1.93) *p trend=0.12*
Balder *et al*, 2006 NETHERLANDS	As above	As above	666 F	2,215 F	As above	Top fifth (15 mg/day) vs bottom fifth (8.5 mg/day)	As above.	1.08 (0.72–1.62) p trend=0.90
Cross *et al*, 2006 FINLAND	50–69	14.2	130 M	260 M (smokers)	Colorectum	Top fourth (25 mg/day) vs bottom fourth (12.2 mg/day)	Age, education, BMI, smoking, physical activity, energy intake, alcohol, aspirin use.	0.4 (0.1–1.1) *p trend=0.06*
Kabat *et al*, 2007 CANADA	40–59	16.4	617 F	49,037 F	Colorectum	Top fifth (≥14.99 mg/day) vs bottom fifth (<11.90 mg/day)	Age, BMI, menopausal status, HRT, smoking, alcohol, education, physical activity; intakes of energy, fat, fibre, folic acid.	1.07 (0.8–1.43) *p trend=0.94*

Abbreviations: BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; HRT, hormone replacement therapy; OR, odds ratio; RR, relative risk

Table A15: Haem iron and colorectal cancer risk

Study/year/country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Cancer site	Comparison (median intake or quantile range)	Adjustments	RR/OR/HR (95% CI)
Lee *et al*, 2004* USA	55–69	15	438 F	33,967	Proximal colon	Top fifth (≥2.05 mg/day) vs bottom fifth (≤0.76 mg/day)	Age, energy, BMI, physical activity, smoking, alcohol, HRT, diabetes, intake of: saturated fat, calcium, vitamin E, folate, fibre, multivitamins.	1.41 (0.90–2.21) *p trend=0.24*
Lee *et al*, 2004** USA	As above	As above	303 F	As above	Distal colon	As above	As above.	0.65 (0.38–1.11) *p trend=0.09*
Larsson *et al*, 2005*** SWEDEN	40–75	14.8	547 F	60,886 F	Colon	Top fifth (≥2.06 mg/day) vs bottom fifth (<0.67 mg/day)	Age, BMI, education; intakes of energy, saturated fat, folate, calcium, fibre, zinc.	1.31 (0.98–1.75) *p trend=0.03*
Balder *et al*, 2006 NETHERLANDS	55–69	9.3	869 M	2,156 M	Colorectum	Top fifth (1.85 mg/day) vs bottom fifth (0.60 mg/day)	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol, vegetables.	1.32 (0.96–1.80) *p trend=0.08*
Balder *et al*, 2006 NETHERLANDS	As above	As above	666 F	2,215 F	As above	Top fifth (1.54 mg/day) vs bottom fifth (0.47 mg/day)	As above.	1.20 (0.86–1.69) *p trend=0.24*
Kabat *et al*, 2007 CANADA	40–59	16.4	617 F	48,049	Colorectum	Top fifth (>2.95 mg/day) vs bottom fifth (<1.58 mg/day)	Age, BMI, menopausal status, HRT, smoking, alcohol, education, physical activity; intakes of energy, fat, fibre, folic acid.	1.06 (0.8–1.42) *p trend=0.99*

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; HRT, hormone replacement therapy; OR, odds ratio; RR, relative risk

* Neither haem iron or zinc intake associated with risk of proximal colon cancer; however, when haem and zinc were mutually adjusted, both positive association of haem iron and inverse association of zinc intake were statistically significantly associated with proximal colon cancer (RR, 2.18 [1.24–3.86], p trend=0.01. Strength of associations of both haem iron intake and Zn intake became stronger with increasing levels of consumption.

** After mutual adjustment for haem iron and Zn, only inverse trend for Zn was statistically significant. Association not affected by alcohol consumption.

*** For women consuming 20 g or more per week alcohol, multivariate RR = –2.29 (1.25–4.21), p trend=0.007.

Table A16: Serum ferritin and colorectal cancer risk

Study/year/country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Cancer site	Comparison	Adjustments	OR (95% CI)
Kato *et al*, 1999 USA	34–65	4.7	105 F	523 F	Colorectum	Top fourth vs bottom fourth (not specified)	Age, beer intake, physical activity, family history CRC	0.40 (0.2–0.8) *p trend<0.01*
Cross *et al*, 2006 FINLAND	50–69	14.2	130	260	Colorectum	Top fourth (312 µg/L) v bottom fourth (59 µg/L)	Age, education, BMI, smoking, physical activity, energy intake, alcohol, aspirin use	0.4 (0.2–0.9) *p trend=0.09*

Abbreviations: BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; OR, odds ratio

Table A17: Prospective studies of C282Y heterozygosity and colorectal cancer risk

Study/year	Country	Cases	Non-cases	Odds ratio/Relative risk (95% Confidence interval)
Nelson *et al*, 1995	USA	47 M	26 M	1.28 (1.07–1.53)
As above	USA	45 F	36 F	1.08 (0.87–1.34)
Altes *et al*, 1999	Spain	116 M+F	108 M+F	0.86 (0.25–2.94)
Beckman *et al*, 1999	Sweden	173 M +F	294 M + F	1.02 (0.57–1.82)
Macdonald *et al*, 1999	Australia	229 M+F	228 M+F	0.90 (0.48–1.69)
Shaheen *et al*, 2003	USA	475 M+F	833 M+F	1.27 (0.83–1.95)
Van der A *et al*, 2003	Netherlands	240 F	635 F	1.20 (0.6–2.2)
Robinson *et al*, 2005	UK	327 M+F	322 M+F	1.01 (0.73–1.40)

Table A18: Prospective studies of red meat and colorectal cancer risk published after 1996

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR/HR/OR (95% CI)
Kato *et al*, 1997 USA	34–65	7.1	100 F	14,627 F	Colorectal cancer	Top fourth vs bottom fourth red meat *(intake in each quartile not provided)* *(classification of red meat not defined)*	Energy intake, age, place at enrolment, education.	1.23 (0.68–2.22) *p trend=0.55*
Chen *et al*, 1998 USA	40–84	13	212 M	221 M	Colorectal cancer	Top third (>1 serving/day) vs bottom third (≤0.5 serving/day) *(beef, pork, lamb, as a main dish, as a mixeddish, or as a sandwich, hot dogs)*	Age, smoking, BMI, physical activity, alcohol.	1.17 (0.68–2.02) *p trend=0.59*
Hsing *et al*, 1998 USA	≥35	20	145 M	17,488 M	Colorectal cancer	Top fifth (≥60 times/month) vs bottom fifth (<15 times/month) red meat *(beef, bacon, fresh pork, smoked ham)*	Age, smoking, alcohol, energy intake.	1.9 (0.9–4.3) *p trend=0.10*
Sellers *et al*, 1998 USA	55–69	10	241 F	34,975 F	Colon cancer	Top third (<7 servings/week) vs bottom third (≤3 servings/week) red meat *(includes liver, hamburger, beef stew, beef, venison)*	Age, energy intake, history of rectal colon polyps.	1.3 (0.8–2.0) *p trend=0.30*
Singh and Fraser, 1998 USA	≥25	6	157 M+F	31,894 M+F	Colon cancer	Top third (≥1 time/week) vs bottom third (never) *(beef, pork)*	Age, sex, BMI, physical activity, family history, smoking, alcohol, aspirin use.	1.41 (0.9–2.21) *p trend=0.46*
Pietinen *et al*, 1999 FINLAND	50–69	8	185 M (smokers)	26,926 M (smokers)	Colorectal cancer	Top fourth (99 g/day) vs bottom fourth (35 g/day) red meat *(beef, pork, lamb)*	Age, supplement group, smoking years, BMI, alcohol, education, physical activity, calcium intake.	0.8 (0.5–1.2) *p trend=0.74*

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR/HR/OR (95% CI)
Järvinen *et al*, 2001 FINLAND	Not specified	27–32	109 M+F	9850 M+F	Colorectal cancer	Top fourth (>206 g/day, M; 134 g/day F) vs bottom fourth (<94 g/day, M; <61 g/day, F) red meat *Classification of red meat not defined*	Age, sex, BMI, occupation, smoking, geographical area, energy intake, consumption of vegetables, fruit and cereals.	1.50 (0.77–2.94) *p trend not given*
Tiemersma *et al*, 2002 NETHERLANDS	20–59	9	102 M+F	537 M+F	Colorectal cancer	≥5 vs ≤3 times/week red meat *(fresh beef and pork)*	Age, sex, height, energy intake, alcohol.	1.6 (0.9–2.9) *p trend=0.10*
Flood *et al*, 2003 USA	61.9	8.5	487 F	45,009 F	Colorectal cancer	Top fifth (52.2 g/1000 kcal) vs bottom fifth (6.1 g/1000 kcal) red meat *(bacon, beef, hamburger, ham/other lunch meat, hot dogs, liver, pork, sausage, and meat components of beef stew, chilli, salad, spaghetti, vegetable soup)*	Energy intake, age.	1.10 (0.83–1.45) *p trend=0.39*
English *et al*, 2004[1] AUSTRALIA	27–75	9	451 M+F	36,661 M+F	Colorectal cancer	Top fourth (>126 g/day) vs bottom fourth (<57 g/day) red meat *(veal or beef schnitzel, roast beef or veal, beef steak, rissoles (meat balls), meatloaf; mixed dishes with beef, roast lamb or lamb chops, mixed dishes with lamb, roast pork or pork chops, and rabbit or other game)*	Age, sex, energy, fat, cereal products, BMI, physical activity.	1.4 (1.0–1.9) *p trend=0.20*
Wei *et al*, 2004 USA	40–75 M 30–55 F	14 M 20 F	1139 M+F	132,887 M+F	Colon cancer	Top fifth (≥5 times/week) vs bottom fifth (0 times/week) red meat *(beef, pork, lamb as main dish)*	Age, sex, BMI, physical activity, folate, calcium, alcohol, family history, height, smoking.	1.43 (1.00–2.05) *p trend=0.25*
As above USA	As above	As above	339 M+F	132,887 M+F	Rectal cancer	As above	As above.	0.90 (0.47–1.75) *p trend=0.55*

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR/HR/OR (95% CI)
Chan *et al*, 2005 USA	30–55	10	183 F	443 F	Colorectal cancer	>0.5 vs ≤0.5 servings/day red meat *(beef, pork, lamb as main dish)*	Age, BMI, family history, post-menopausal hormone use, previous endoscopy, multivitamin use, aspirin use, smoking.	1.21 (0.85–1.72) *p trend not given*
Chao *et al*, 2005 USA	50–74	8–9	1197 M+F	146,943 M+F	Colon cancer	Top fifth (M>800 g/week; F>560 g/week) vs bottom fifth (M≤180 g/week; F≤90 g/week) red meat *(bacon, sausage, hamburgers, cheeseburgers, meatloaf, or casserole with ground beef; beef [steaks, roasts etc., incl. sandwiches], beef stew or pot pie with veg; liver, pork [incl. chops, roast], hot dogs, ham, bologna, salami, lunch meat)*	Age, sex, BMI, energy, fruits, vegetables, high fibre grain foods, education, smoking, physical activity, multivitamin use, aspirin use, alcohol, HRT (women).	1.15 (0.90–1.46) *p trend=0.04*
As above USA	As above	As above	470 M+F	As above	Rectal cancer	As above	As above.	1.71 (1.15–2.52) p trend=0.007
Larsson 2005 SWEDEN	40–75	13.9	733 F	60,700 F	Colorectal cancer	Top fourth (≥94 g/day) vs bottom fourth (<50 g/day) red meat *(whole beef, chopped meat, minced meat, bacon, hot dogs, ham or other lunch meat, blood pudding, kidney, liver, liver pate)*	Age, BMI, energy, alcohol saturated fat, calcium, folate, fruits, vegetables, whole grain foods, education.	1.32 (1.03–1.68) *p trend=0.03*
Norat *et al*, 2005 EUROPE (10 COUNTRIES)	35–70	4.5	1329 M+F	476,711 M+F	Colorectal cancer	Top fifth (≥80 g/day) vs bottom fifth (<10 g/day) red meat *(all fresh, minced, and frozen beef, veal, pork, lamb)*	Age, sex, BMI, energy, fat, height, weight, physical activity, smoking, fibre, alcohol.	1.17 (0.92–1.49) *p trend=0.08*

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR/HR/OR (95% CI)
Balder *et al*, 2006 NETHERLANDS	55–69	9.3	869 M	2,156 M	Colorectal cancer	Top fifth (158 g/day) vs bottom fifth (56 g/day) total fresh meat *(meat that had not undergone preservation; includes beef, pork, minced meat, chicken, liver, and other meat, i.e. horse and lamb)*	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol, vegetables.	0.82 (0.62–1.08) p trend=0.15
As above	As above	As above	666 F	2,215 F	As above	Top fifth (146 g/day) vs bottom fifth (56 g/day) total fresh meat	As above	1.10 (0.80–1.51) *p trend=0.57*
Oba *et al*, 2006 JAPAN	≥35	7	111 M	13,783 M	Colon	Top third (56.6 g/day) vs bottom third (18.7 g/day) red meat *(beef, pork)*	Age, height, BMI, smoking, alcohol, physical activity.	1.03 (0.64–1.66) *p trend=0.86*
As above JAPAN	As above	As above	102 F	16,225 F	As above	Top third (42.3 g/day) vs bottom third (10.7 g/day) red meat *(meat classification as above)*	As above.	0.79 (0.49–1.28) *p trend=0.20*
Sato *et al*, 2006 JAPAN	40–64	11	474 M+F	41,361 M+F	Colorectal cancer	Top fourth (70.4 g/day) vs bottom fourth (40.3 g/day) total meat *(beef, pork, ham, sausage, chicken, liver)*	Sex, age, smoking, alcohol, BMI, education, family history, physical activity, intakes of fat, calcium and dietary fibre.	1.10 (0.80–1.51) *p trend=0.38*
Cross *et al*, 2007 USA	50–71	6.8	5107 M+F	440,640 M+F	Colorectal cancer	Top fifth (62.7 g/1000 kcal) vs bottom fifth (9.8 g/1000 kcal) red meat *(all types beef, pork, lamb; including bacon, ham, hamburger, hot dogs, liver, pork, sausage, steak. Also included meats added to complex food mixtures such as pizza, chilli, lasagne, and stew)*	Age, sex, education, family history, race, BMI, smoking, physical activity, total energy intake, alcohol, fruit and vegetable intake.	1.24 (1.12–1.36) *p trend<0.001*

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR/HR/OR (95% CI)
Kabat *et al*, 2007 CANADA	40–59	16.4	617 M+F	48,049 M+F	Colorectal cancer	Top fifth (>40.3 g/day) v bottom fifth (<14.25 g/day) red meat *(beef, pork, ham, bacon, pork-based luncheon meats, veal)*	Age, BMI, menopausal status, HRT, smoking, alcohol, education, physical activity; intakes of energy, fat, fibre, folic acid.	1.12 (0.86–1.46) p trend=0.66
Sørensen *et al*, 2008 DENMARK	50–64	6–10	379 M+F	769 M+F	Colorectal cancer	Per 25 g/day red meat *(beef, veal, pork, lamb, offal)*	Intake of poultry, fish, alcohol and fibre; BMI, HRT, smoking; mutually adjusted for fried and processed red meat.	1.03 (0.97–1.09) *p trend not given*

Abbreviations: BMI, body mass index; CI, confidence interval; HRT, hormone replacement therapy; HR, hazards ratio; OR, odds ratio; RR, relative risk or rate ratio

Table A19: Prospective studies of processed meat and colorectal cancer risk published after 1996

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)	Adjustments	RR/HR/OR (95% CI)
Kato *et al*, 1997 USA	34–65	7.1	100 F	14,627	Colorectal cancer	Top fourth vs bottom fourth *(intake in each quartile not provided)* *(ham, sausages)*	Energy intake, age, place at enrolment, education.	1.09 (0.59–2.02) *p trend=0.74*
Pietinen *et al*, 1999 FINLAND	50–69	8	185 M (smokers)	26,926 M (smokers)	Colorectal cancer	Top fourth (122 g/day) vs bottom fourth (26 g/day) (*classification of processed meat not defined*)	Age, supplement group, smoking years, BMI, alcohol, education, physical activity, calcium intake.	1.2 (0.7–1.8) *p trend=0.78*
Sellers *et al*, 1998 USA	55–69	10	241 F	34,975 F	Colon cancer	Top third (<1.5 servings/week) vs bottom third (≤0.5 servings/week) nitrate meat *(includes bacon, hot dogs, processed meats)*	Age, energy intake, history of rectal colon polyps.	1.00 (0.7–1.4) *p trend=0.90*

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)	Adjustments	RR/HR/OR (95% CI)
Knekt *et al*, 1999 FINLAND	Not specified	18–24	73 M+F	9912 M+F	Colorectal cancer	Top fourth vs bottom fourth cured meat and sausages *(intake in each quartile not defined)* *(classification of cured meat not defined)*	Age, sex, municipality, smoking.	1.84 (0.98–3.47) *p trend not given*
Flood *et al*, 2003 USA	61.9	8.5	487 F	45,009 F	Colorectal cancer	Top fifth (22.2 g/1000 kcal) vs bottom fifth (0.02 g/1000 kcal) processed meat *(bacon, ham/lunch meat, hot dogs, sausages)*	Energy, age.	1.00 (0.76–1.31) *p trend=0.22*
English *et al*, 2004 AUSTRALIA	27–75	9	451 M+F	36,661 M+F	Colorectal cancer	Top fourth (>29 g/day) vs bottom fourth (<9 g/day) processed meat *(salami, sausages, bacon, ham, corned beef, luncheon meats)*	Age, sex, energy, fat, cereal products, BMI, physical activity.	1.5 (1.1–2.0) *p trend=0.01*
Wei *et al*, 2004 USA	40–75 M 30–55 F	14 M 20 F	1139 M+F	132,887 M+F	Colon cancer	Top fifth (≥5 times/week) vs bottom fifth (0 times/week) processed meat *(classification of processed meat not defined)*	Age, sex, BMI, physical activity, folate, calcium, alcohol, family history, height, smoking.	1.33 (1.04–1.70) *p trend=0.008*
As above	As above	As above	339 M+F	132,887 M+F	Rectal cancer	As above	As above.	0.90 (0.52–1.57) *p trend=0.93*
Chao *et al*, 2005 USA	50–74	8–9	11,977 M+F	147,413 M+F	Colon cancer	Top fifth (M>240 g/week; F>120 g/week) vs bottom fifth (0 g/week) *(bologna, salami, lunch meat, bacon, sausage, hot dogs, ham)*	Age, sex, BMI, energy, fruits, vegetables, high fibre grain foods, education, smoking, physical activity, multivitamin use, aspirin use, alcohol, HRT (women).	1.13 (0.91–1.41) *p trend=0.02*
As above	As above	As above	470 M+F	146,943 M+F	Rectal cancer	As above	As above.	1.26 (0.86–1.83) *p trend=0.18*

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)	Adjustments	RR/HR/OR (95% CI)
Larsson 2005 SWEDEN	40–75	13.9	733 F	60,700 F	Colorectal cancer	Top fourth (≥32 g/day) vs bottom fourth (<12 g/day) processed meat *(bacon, hot dogs, ham, or other lunch meat, blood pudding)*	Age, BMI, energy, alcohol saturated fat, calcium, folate, fruits, vegetables, whole grain foods, education.	1.07 (0.85–1.33) *p trend=0.23*
Norat *et al*, 2005 EUROPE (10 COUNTRIES)	35–70	4.5	1329 M+F	476,711 M+F	Colorectal cancer	Top fifth (≥80 g/day) vs bottom fifth (<10 g/day) processed meat *(mostly pork and beef preserved by methods other than freezing, e.g., salting, smoking, marinating, air drying, heating: ham, bacon, sausages, blood sausages, meat cuts, liver pate, salami, bologna, tinned meat, luncheon meat, corned beef, and others)*	Age, sex, BMI, energy, fat, height, weight, physical activity, smoking, fibre, alcohol.	1.42 (1.09–1.86) *p trend=0.02*
Balder *et al*, 2006 NETHERLANDS	55–69	9.3	869 M	2,156 M	Colorectal cancer	Top fourth (≥20 g/day) vs bottom fourth (0 g/day) *(meat items that had undergone some form of preservation, i.e., smoking, fermentation, and/or treatment with nitrate and/or nitrite salt [curing])*	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol and vegetables.	1.18 (0.84–1.64) *p trend=0.25*
As above	As above	As above	666 F	2,215 F	As above	As above	As above	1.05 (0.74–1.48) *p trend=0.62*
Oba *et al*, 2006 JAPAN	≥35	7	111 M	13,783 M	Colon cancer	Top third (20.3 g/day) vs bottom third (3.9 g/day) processed meat *(ham, sausage, bacon, roasted pork)*	Age, height, BMI, smoking, alcohol, physical activity.	1.98 (1.24–3.16) *p trend<0.01*

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)	Adjustments	RR/HR/OR (95% CI)
As above	As above	As above	102 F	16,225 F	As above	Top third (16.3 g/day) vs bottom third (3.0 g/day) processed meat *(meat classification as above)*	As above.	0.85 (0.50–1.43) *p trend=0.62*
Cross *et al*, 2007 USA	50–71	6.8	5107 M+F	440,640 M+F	Colorectal cancer	Top fifth (22.6 g/1000 kcal) vs bottom fifth (1.6 g/1000 kcal) *(bacon, red meat sausage, cold cuts [red and white meat], ham, hot dogs. Also included meat added to complex food mixtures, e.g., pizza, chilli, lasagne, stew)*	Age, sex, education, family history, race, BMI, smoking, physical activity, total energy intake, alcohol, fruit and vegetable intake.	1.20 (1.09–1.32) *p trend<0.001*
Sørensen *et al*, 2008 DENMARK	50–64	6–10	379 M+F	769 M+F	Colorectal cancer	Per 25 g/day *(bacon, smoked ham, salami, frankfurter, Cumberland sausage, cold cuts, liver pate*	Intake of poultry, fish, alcohol and fibre; BMI, HRT, smoking; mutually adjusted for fried and processed red meat.	0.99 (0.84–1.16) *p trend not given*

Abbreviations: BMI, body mass index; CI, confidence interval; HRT, hormone replacement therapy; HR, hazards ratio; OR, odds ratio; RR, relative risk or rate ratio

Prospective studies of iron and cardiovascular disease (CVD) risk

Table A20: Total dietary iron and CVD risk

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR/HR (95% CI)
Salonen *et al*, 1992 FINLAND	42–60	3	51 M	1880 M	MI	1 mg/day increment	Age, BMI, smoking, HDL and LDL cholesterol, family history, blood pressure, diabetes, maximal oxygen uptake, diabetes, number of other risk factors (no other dietary factors).	Yes	1.05 (1.01–1.09)
Liao *et al*, 1994 USA	25–74	13	633 M	1,194 M	IHD	Top fourth vs bottom fourth (5 mg/day increment)	Age, blood pressure, serum cholesterol, education, smoking.	Yes	0.74 (0.55–0.99) *p<0.05* (0.97 (0.89–1.06))
As above	As above	As above	518 F	1,892 F	IHD	Top fourth vs bottom fourth (5 mg/day increment)	As above.	Yes	0.84 (0.62–1.15) *p<0.05* (0.91 (0.80–1.03))
Ascherio *et al*, 1994 USA	40–75	4	386 M	44,089 M	Coronary disease	Top fifth (37 mg/day) vs bottom fifth (11 mg/day)	Age, energy, BMI, smoking, alcohol intake, hypertension, diabetes, hypercholesterolemia, family history, profession; quintiles of intake of: vitamin E, total iron, haem iron, saturated fat and cholesterol.	Yes	0.73 (0.51–1.06) *p trend = 0.03*
Morrison *et al*, 1994 CANADA	35–79	15–17	? M+F	9920 M+F	MI	Not reported	Age, smoking, hypertension, serum cholesterol, diabetes.	Yes	No association (RR not reported)
Gartside and Glueck, 1995 USA	25–74	10	492 M+F	7,759 M+F	CHD	Top third (≥13.1 mg/day) vs bottom third (<8.4 mg/day)	Sex, physical activity, weight, alcohol, riboflavin intake, serum magnesium.	Yes	0.83 (0.66–1.03) p=0.097

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR/HR (95% CI)
Reunanen *et al*, 1995 FINLAND	45–64	13.8	984 M+F	11,204 M+F	CHD deaths	Top fifth vs bottom fifth	Age, serum cholesterol, hypertension, diabetes, obesity.	Yes	No association (RR not reported)
Klipstein-Grobusch *et al*, 1999a NETHERLANDS	≥55	3–7	124 M+F	4,678 M+F	MI	Top third (14.3 mg/day) vs bottom third (9.3 mg/day)	Age, sex, BMI, smoking, household income, education, alcohol; intakes of: ß-carotene, vitamins C and E, total fat, fat, saturated fat, cholesterol; antioxidant vitamin supplements.	Yes	1.11 (0.67–1.87) *p trend = 0.787*
van der A *et al*, 2005a NETHERLANDS	49–70	4.3	252 F	15,884 F	CHD	Top fourth (>11.43 mg/day) vs bottom fourth (<9.56 mg/day) per mg/day	Age, energy intake, BMI, smoking, physical activity, hypertension, hypercholesterolemia, saturated fat, carbohydrate, fibre, alcohol, ß-carotene, vitamins E and C.	Yes	0.98 (0.61–1.58) *p trend = 0.878* 0.92 (0.79–1.06)
Qi *et al*, 2007 USA	30–55	20	550 F (with type 2 diabetes)	5,611 F (with type 2 diabetes)	CHD	Top fifth vs bottom fifth (intake in quintiles not reported)	Age, BMI, smoking, smoking, alcohol, physical activity, diabetes; hypertension, hypercholesterolemia, HRT, CHD history, fibre, glycaemic load, polyunsaturated/saturated fat ratio, trans fat, multivitamins, vitamin C.	Yes	1.32 (0.95–1.84) *p trend = 0.04*

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; HDL, high density lipoprotein; HR, hazards ratio; HRT, hormone replacement therapy; LDL, low density lipoprotein; RR, relative risk or rate ratio

Table A21: Dietary haem iron and CVD risk

Study/Year/ Country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR/HR (95% CI)
Ascherio *et al*, 1994 USA	40–75	4	386 M	44,089 M	MI	Top fifth (2.1 mg/day) vs bottom fifth (0.7 mg/day)	Age, energy, BMI, smoking, alcohol intake, hypertension, diabetes, hypercholesterolemia, family history, profession; quintiles of intake of: vitamin E, total iron, haem iron, saturated fat and cholesterol.	Yes	1.48 (1.01–2.16) *p=0.03*
Klipstein-Grobusch *et al*, 1999a NETHERLANDS	≥55	3–7	124 M+F	4,678 M+F	MI	Top third (1.36 mg/day) vs bottom third (0.48 mg/day)	Age, sex, BMI, smoking, household income, education, alcohol; intakes of: ß-carotene, vitamins C and E, total fat, saturated fat, cholesterol; antioxidant vit amin supplements.	Yes	1.86 (1.14–3.09) *p trend = 0.01*
Lee *et al*, 2005 USA	55–69	15	1767 F	32,725 F	CVD deaths	Top fourth (2.43 mg*) vs bottom fourth (0.57 mg/day)	Age, energy intake, BMI, WHR, physical activity, smoking, alcohol, HRT, BP, sat fat, trans fat, polyunsaturated fat, folate, ß-carotene, vitamins E and C, non-haem iron, zinc.	Yes	0.94 (0.71–1.26)** *p trend = 0.82*
van der A *et al*, 2005a NETHERLANDS	49–70	4.3	252 F	15,884 F	CHD	Top fourth (>2.27 mg/day) vs bottom fourth (<1.28 mg/day) per mg/day	Age, energy intake, BMI, smoking, physical activity, hypertension, hypercholesterolemia, sat fat, carbohydrate, fibre, alcohol, ß-carotene, vitamins E and C.	Yes	1.65 (1.07–2.53) *p trend = 0.019* 1.15 (0.95–1.40)

Study/Year/ Country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR/HR (95% CI)
Qi *et al*, 2007 USA	30–55	20	550 F (with type 2 diabetes)	5,611 F (with type 2 diabetes)	CHD	Top fifth (2.83 mg/day) vs bottom fifth (1.70 mg/day)	Age, BMI, smoking, smoking, alcohol, physical activity, diabetes; hypertension, hypercholesterolemia, HRT, CHD history, fibre, glycaemic load, polyunsaturated/saturated fat ratio, trans fat, multivitamins, vitamin C.	Yes	1.43 (1.01–2.01) *p trend = 0.01*

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; HDL, high density lipoprotein; HR, hazards ratio; HRT, hormone replacement therapy; LDL, low density lipoprotein; RR, relative risk; WHR, waist to hip ratio

* *Upper half of top quartile*

** *Alcohol consumption 0–9 g/day; RR for alcohol consumption ≥10 g/day = 2.47 (1.10–5.55), p trend=0.04*

Table A22: Serum ferritin and CVD risk

Study/year/country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR/HR/OR (95% CI)
Frey and Krider, 1994 USA	42–60	5.2	32 M	266 M	MI	Difference in mean SF concentrations between cases and controls	Age	No	No association (RR not reported)
Salonen *et al*, 1994 FINLAND	42–60	5	83 M	1848 M	MI	≥ 200 µg/L vs < 200 µg/L	Age, markers of chronic inflammatory disease, CVD, pulmonary function; socioeconomic status, diabetes, family history of CVD, smoking, blood leucocyte count.	Yes	2.0 (1.2–3.1) *p=0.004*
Magnusson *et al*, 1994 ICELAND	25–74	8.5	81 M+F	1,955 M+F	MI	1 µg/L increment	Age, blood pressure, HDL cholesterol, total cholesterol, smoking.	No	0.999 (0.998–1.001) *p=0.23*
Manttari *et al*, 1994 FINLAND	40–55	5	134 M (with lipid abnormalities)	268 M (with lipid abnormalities)	CHD	≥85 µg/L vs ≤42 µg/L	Age, blood pressure, cholesterol, HDL-cholesterol, smoking.	No	0.78 (0.39–1.54) *p trend=0.5*
Aronow and Ahn, 1996 USA	62–100	3	235 M+F	342 M+F	CHD	M: >282 µg/L vs <282 µg/L F: >219 µg/L vs <219 µg/L	Age, sex, prior CHD.	No	1.0 (0.998–1.001) *p=0.61*
Kiechl *et al*, 1997 ITALY	40–79	5	401 M+F	425 M+F	Artheros-clerosis	1 SD increment (approx 166 µg/L)	Age, sex, baseline vascular status, alcohol.	No	1.50 (CI not reported) *p=0.0002*

Study/year/country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR/HR/OR (95% CI)
Marniemi *et al*, 1998 FINLAND	≥65	13	142 M+F	119 M+F	CVD deaths	Top third vs bottom third *(SF concentration in each tertile not reported)*	Age, sex, smoking, alcohol, BMI, CHD, hypertension, diabetes, serum cholesterol, HDL cholesterol, triglycerides.	No	0.65 (0.42–1.01) *p not reported*
Klipstein-Grobusch, 1999b NETHERLANDS	≥55	4	60 M+F	112 M+F	MI	>171 µg/L vs <77 µg/L	Age, sex, BMI, smoking, income, alcohol.	Yes	1.28 (0.98–1.67) *p trend=0.066*
Sempos *et al*, 2000 USA	45–74	12–16	254 M	404 M	CVD deaths	≥ 200 µg/L vs < 50 µg/L	Age, anaemia, blood pressure, hypertension, serum total cholesterol, smoking, diabetes, chronic conditions.	Yes	0.7 (0.4–1.3) *p trend=0.22*
As above	As above	As above	168 F	550 F	As above	≥ 200 µg/L vs < 50 µg/L	As above.	Yes	0.9 (0.4–2.1) *p trend = 0.92*
Fox *et al*, 2002 AUSTRALIA	20–79	3–4	235 M+F	1,796 M+F	CVD	> 300 µg/L vs ≤ 300 µg/L	Age, sex, BMI, blood pressure, diabetes, total cholesterol, HDL cholesterol, smoking, alcohol, Hb.	Yes	1.02 (0.69–1.50) *p not reported*
Knuiman *et al*, 2003 AUSTRALIA	40–89	17	217 M+F	450 M+F	CHD	Top third (M, >233 µg/L; F, >122 µg/L) vs bottom third (M, ≤126 µg/L; F, ≤49 µg/L)	Age, sex, BMI, BP, diabetes, total cholesterol, HDL cholesterol, smoking, Hb,	Yes	0.96 (0.60–1.5) *p not reported*

Study/year/country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR/HR/OR (95% CI)
van der A *et al*, 2005b NETHERLANDS	49–70	4.3	63 F	1134 F	Stroke	195 µg/L vs 51.8 µg/L <200 vs ≥200 µg/L	Age, BMI, alcohol, CRP, smoking, hypertension, hypercholesterolemia, diabetes, glucose, LDL and HDL cholesterol.	Yes	1.45 (0.87–2.42) *p trend=0.158* 1.77 (1.03–3.05)
van der A *et al*, 2006a NETHERLANDS	49–70	4.3	185 F	1134 f	CHD	137 µg/L VS <75.7 µg/L <200 vs ≥200 µg/L	Age, BMI, alcohol, CRP, smoking, hypertension, hypercholesterolemia, diabetes, glucose, LDL and HDL cholesterol.	Yes	0.55 (0.23–1.31) *p trend=0.142* 0.82 (0.35–1.95)
Galan *et al*, 2006 FRANCE	35–60	7.5	148 M	3,075 M	IHD	>160 µg/L vs <30 µg/L	Age, smoking, BMI, total cholesterol, serum triglycerides.	Yes	1.31 (0.52–3.27) *p not reported*
As above	As above	As above	39 F	6,655 F	As above	As above	As above + menopausal status.	Yes	2.18 (0.64–7.43) *p not reported*
Ekblom *et al*, 2007 SWEDEN	25–74	?	126 M+F	304 M+F	Ischemic stroke	Top fourth vs bottom fourth *(SF concentration in quartiles not reported)*	BMI, hypertension, smoking, diabetes, cholesterol, CRP, HFE C282Y and H63D.	Yes	0.80 (0.46–1.40) *p=0.250*
As above	As above	As above	27 M+F	As above	Hemorrhagic stroke	As above	As above.	As above	1.07 (0.17–6.94) *p=0.576*

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease, Hb, haemoglobin; HDL, high density lipoprotein; HR, hazard ratio; LDL, low density lipoprotein; OR, odds ratio; RR, relative risk; SF, serum ferritin.

Prospective studies of heterozygosity for hereditary haemochromatosis and CVD

Table A23: C282Y heterozygosity and CVD risk

Study/year/ country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Adjustments	Exclusion of chronic disease at baseline	RR/HR (95% CI)
Tuomainen *et al*, 1999 FINLAND	42–60		68 M	1082 M	MI	13 risk factors incl. age, WHR, HDL and VLDL cholesterol, socioeconomic status, blood pressure, smoking.	Yes	2.21 (1.05–4.67) *p=0.04*
Roest *et al*, 1999 NETHERLANDS	51–69	17	531 F	551 F	CVD deaths	Age, smoking, hypertension, obesity.	No	1.6 (1.1–2.4) *p=0.028*
Rasmussen *et al*, 2001 USA	45–64	2.9	243 F	535 F	CHD	No adjustments.	Yes	1.6 (0.88–2.91) *p value not reported*
Fox *et al*, 2002 AUSTRALIA	20–79	3–4	235 M+F	1796 M+F	CVD	Age, sex, BMI, blood pressure, diabetes, total cholesterol, HDL cholesterol, smoking, alcohol, Hb.	Yes	0.96 (0.65–1.42) *p value not reported*
Gunn *et al*, 2004 UK	45–64	4.9	482 M	1104 M	CHD	BMI, blood pressure, LDL and HDL cholesterol white cell count, fibrinogen, CRP.	No	0.87 (0.63–1.19) *p value not reported*
Ellervik *et al*, 2005 DENMARK	20–80	24	1035 M+F	8080 M+F	IHD	Sex, smoking, cholesterol, triglycerides, lipoprotein (a), diabetes, hypertension, BMI, fibrinogen, HRT, menopausal status.	Yes	1.1 (0.9–1.4) *p value not reported*
van der A *et al*, 2006b NETHERLANDS	49–70	4.3	211 F	1526 F	CHD	Age, BMI, smoking, hypertension, alcohol, diabetes, cholesterol, CRP.	Yes	1.25 (0.74–2.09) *p value not reported*
Ekblom *et al*, 2007 SWEDEN	25–74	?	231 M+F	550 M+F	Ischemic stroke	Age, sex.	Yes	0.74 (0.41–1.32) *p=0.332*
As above	As above		41 M+F	As above	Hemorrhagic stroke	As above.	Yes	1.34 (0.46–3.94) *p=0.352*

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; Hb, haemoglobin; HDL, high density lipoprotein; HR, hazard ratio; HRT, hormone replacement therapy; LDL, low density lipoprotein; RR, relative risk; VLDL, very low density lipoprotein WHR, waist to hip ratio

Table A24: H63D heterozygosity and CVD risk

Study/year/country	Age baseline (years)	Mean follow-up (years)	Cases	Non-cases	Outcome	Adjustments	Exclusion of chronic disease at baseline	HR/OR (95% CI)
Fox *et al*, 2002 AUSTRALIA	20–79	3–4	235 M+F	1796 M+F	CVD	Age, sex, BMI, blood pressure, diabetes, total cholesterol, HDL cholesterol, smoking, alcohol, Hb.	Yes	0.98 (0.72–1.34) *p value not reported*
Ellervik *et al*, 2005 DENMARK	20–80	24	1035 M+F	8080 M+F	IHD	Sex, smoking, cholesterol, triglycerides, lipoprotein (a), diabetes, hypertension, BMI, fibrinogen, HRT, menopausal status.	Yes	1.2 (1.0–1.4) *p values not reported*
van der A *et al*, 2006b NETHERLANDS	49–70	4.3	211 F	1526 F	CHD	Age, BMI, smoking, hypertension, alcohol, diabetes, cholesterol, CRP.	Yes	0.73 (0.43–1.24) *p value not reported*
Ekblom *et al*, 2007 SWEDEN	25–74	?	231 M+F	550 M+F	Ischemic stroke	Age, sex.	Yes	0.74 (0.49–1.12) *p=0.363*
As above	As above	?	41 M+F	As above	Hemorrhagic stroke	As above.	Yes	1.51 (0.69–3.27) *p=0.577*

BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HDL, high density lipoprotein; Hb, haemoglobin; HR, hazard ratio; HRT, hormone replacement therapy; OR, odds ratio

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Consideration of possible mechanisms to explain the association between colorectal cancer risk and red and processed meat intake

Background

1. Epidemiological data suggest that consumption of red and/or processed meat, but not white meat or fish, is associated with an increased risk of colorectal cancer (CRC). The definition of processed meat varies between studies, but is defined by the World Cancer Research Fund (WCRF) as meat that has been preserved by curing, smoking, salting or the addition of chemical preservatives.

2. This paper considers whether the differences in the risk of CRC attributed to red, processed and white meat can be explained by the presence of cooked food mutagens, haem iron or preservatives. It also considers whether, for modelling purposes (see Annex 11), processed and unprocessed red meat should be separated on the basis that differences in CRC risk are due to presence of preservatives in processed meat. More detailed background briefings considering the evidence for the association of cancer with food mutagens, haem iron and preservatives are attached as appendices (Appendices 1–4). Background data on nitrosamines and *N*-nitroso compounds are also considered since many of the proposed mechanisms involve their formation.

Can differences in CRC risk associated with red, white or processed meat consumption be explained by the presence of cooked food mutagens, haem iron or preservatives?

Cooked food mutagens

Heterocyclic amines

3. Heterocyclic amines (HAs) are formed from creatine and are therefore predominantly found in the muscle parts of meat and fish. The most commonly ingested HA is PhiP[100] which is more common in chicken than in other meats. Other heterocyclic amines can be formed in all meat types. Their formation is influenced by cooking temperature, cooking time and cooking method; some mutagens are more likely to

100 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

occur in certain types of meat or as a result of particular procedures, e.g., grilled steak or pan-fried burgers. Foods treated with preservatives such as ham or sausages tend to have lower levels of HAs, possibly due to the cooking methods used.

4. In the modelling exercise (see Annex 11), processed meat is defined as meat that has been treated with preservatives excluding salt and including some cured and smoked meat cuts. However, it is possible that mechanically processed (e.g. minced) meat could form more mutagens, due to the greater surface area being exposed directly to heat, but would be classed as unprocessed since no chemical preservatives have been used. This could affect interpretation and comparison of the epidemiological data.

5. Although HAs are genotoxins and known animal carcinogens, there are no convincing data suggesting a role in human CRC.

Polycyclic aromatic hydrocarbons

6. The other main class of cooked food mutagens is polycyclic aromatic hydrocarbons (PAHs) which are formed by incomplete pyrolysis of organic compounds. It is possible that people are more likely to char red meat, and therefore ingest more of these mutagens, compared to other meats (including processed meats in general or meats treated with preservative) but there are no data to support or refute this possibility. PAHs are widespread in foods and meat is not the major contributor to total dietary exposure. Regular consumers of barbecued foods may have higher intakes of PAHs but it is uncertain if this is due to the preferential consumption of a particular meat type. The role of PAH intake in the association of red meat consumption and cancer is unclear.

Haem iron

7. Haemoglobin content of red meat is much higher than that of white meat or fish. However, the majority of red meats treated with preservatives are made from pork which has lower levels of haem than other red meats.

Preservatives

8. There are no differences in the preservatives permitted for use in red and white meat. However, meat treated with preservatives is most likely to be red (bacon, ham, burgers etc.). Epidemiological data suggest that processed meat (as defined by WCRF) is associated with a higher risk of CRC than red meat. Although it is has been suggested that this is due to the use of nitrates and nitrites as preservatives, and the possible formation of nitrosamine, the data do not support this. While preservatives are permitted in processed meats, not all processed meats will contain them.

Conclusion

9. None of the above mechanisms appears to explain the epidemiological findings that red meat is associated with a higher risk of colorectal cancer than white meat or fish.

Are the different risks associated with red meats and meats treated with preservatives due to differences in the presence of cooked food mutagens?

10. There are no data which have directly compared red meats and meats treated with preservatives; however, it is possible that some foods considered to be processed (e.g., mixed products such as sausage) are less likely to form mutagens. It is possible that one of the meat categories might be preferentially cooked in ways that form particular mutagens, but no data have been identified that support this.

11. Processed meat is usually defined as meat that has been treated with preservatives. However, it is possible that mechanically processed (e.g., minced) meat, which would be would be classed as unprocessed, could form more mutagens due to the greater surface exposed to heat. This could affect interpretation and comparison of the epidemiological data.

Conclusion

12. Based on available data, the presence of cooked food mutagens does not explain the difference in risks associated with red and processed meats. However, the data are limited, and a direct comparison has not been done.

Are the different risks associated with unprocessed red meats and meats treated with preservatives due to the effect of haem iron?

13. It has been proposed that the presence of haem iron enhances nitrosation. There is evidence that both supports and contradicts this suggestion. However, intake of haem iron is likely to be higher from unprocessed meats than meats treated with preservatives since the latter tend to be pork rather than beef based and would contain lower levels of haem. Therefore the presence of haem iron does not explain the larger increase in CRC associated with processed meats (as defined by WCRF), unless there is some other interaction occurring, e.g., directly between nitrate preservatives, but this possibility has not been investigated.

Conclusion

14. The available data do not support different risks associated with red meat or meats treated with preservatives due to the effects of haem iron. However, the data are limited, and a direct comparison has not been carried out.

Are the different risks of colorectal cancer associated with unprocessed red meats and meats treated with preservatives due to the effect of preservative?

15. The main preservatives permitted for use in meat are ascorbic acid; benzoates and sorbates (which have limited permitted uses); and nitrates and nitrites, which are used in cured meats such as bacon and ham, though not all cured meats will contain

these preservatives. Some of the preservatives permitted for use in meats are also permitted for use in other food products. There are many sources of dietary nitrate including green leafy vegetables which are a particularly rich source. Nitrate and nitrite are also produced endogenously in the body as a result of intermediary metabolism.

16. Nitrosamines can be formed from nitrate via nitrite production. Following dietary exposure to nitrate, low levels of nitrosamines are formed and these vary between individuals, depending on nutritional and disease status. Nitrosamines have been measured directly in bacon and other cured, smoked or salted meats as well as malted beverages. Ascorbate is used in bacon to reduce nitrosamine formation. It has been suggested that the increased risk of stomach cancer associated with consumption of smoked preserved fish may be due to nitrosamine formation, but other factors such as PAHs and salt levels may also be involved. Evidence linking nitrosamines to CRC is much weaker. There is no clear evidence linking specific food types preserved with nitrates or nitrites, such as bacon or ham, with cancer. Nitrates and nitrites used in food as preservatives are not more likely to form nitrosamines than other sources of dietary nitrate, and more nitrate is actually ingested from vegetable sources than from processed meats. Similarly, nitrites are produced through endogenous conversion of nitrate at a much greater level than intakes from dietary nitrite.

Conclusion

17. The available data do not support the suggestion that the increased risk of colorectal cancer associated with processed meats is due to the presence of preservative.

Overall conclusion

18. There are no convincing data on cooked food mutagens, haem iron or preservative use which explain the differences in risk of CRC attributed to consumption or red, white or processed meat.

19. There are uncertainties in this assessment. These could be differences in potency of particular mutagens, or a combination of differing effects resulting in the findings seen in the epidemiology studies, but not in the individual mechanistic studies.

20. Current understanding of cooked food mutagens, preservatives or haem iron does not support the suggestion that their presence in meat provides a basis for separating red meat and meat treated with preservatives for modelling purposes.

Chemical Risk Assessment Unit
Food Standards Agency

Cooked food mutagens and cancer

1. Genotoxic carcinogens are formed during the heating of meat. All meats and meat products show some level of mutagenic activity following cooking. There are two main classes of mutagens in cooked meat: heterocyclic amines and polycyclic aromatic hydrocarbons.

Heterocyclic amines

2. Heterocyclic amines (HAs) are referred to as "thermic mutagens" which form at temperatures below 300°C, or "pyrolytic mutagens" which form at higher temperatures (Alajeos *et al*, 2008). More than 20 HAs have been isolated. The principal HAs are 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).

Formation of HAs

3. HAs are formed from natural constituents in foods via the Maillard reaction during heating (Skog, 1993). Creatine, sugars and amino acids (phenylalanine, threonine, alanine) are all precursors in their formation. The factors that influence the formation of mutagenic activity are cooking temperature, cooking time and cooking method (Skog, 1993). Mutagenic activity of cooked meats increases with increasing cooking temperature, which appears to be the most important factor, as well as with increased cooking time. Cooking methods influence mutagenic activity in terms of the temperature achieved. Cooking at temperatures of 200°C and above, such as contact frying, deep-fat frying, barbecuing and broiling (grilling), give much higher levels of mutagenic activity than other cooking methods, such as oven roasting, stewing, boiling and microwave cooking. Meats cooked at fast-food restaurants have been shown to contain lower levels of mutagens due to the short cooking times that do not allow HAs to form (Knize *et al*, 1995). The degree of "doneness" (closely related to cooking time and surface browning) is important to the formation of HAs. In addition, the greater the external charring of the meat (determined by cooking method), the higher the concentration of HAs.

4. The formation of HAs is also influenced by the amounts of the different precursors: fat, amino acid, glucose and creatine. Mutagenic activity is primarily found in the crust of cooked meat and fish, as well as in the pan residue, with negligible levels being reported in the inner parts. The formation of a crust is the result of the steady transportation of water and dissolved compounds to the surface by capillary flow, so that the precursors of HAs are present at or near the surface of the meat. Generally, levels of HAs are higher in cooked meats than in fish, and in pure meat than in mixed meat products such as sausage.

5. The definition of processed meat can vary. In principle, mincing of meat is a process, and if this results in a larger surface area for direct contact with heat during cooking, the HA content could be higher. However, there is no basis for assuming a difference in the HA content of meats containing preservatives and those that do not, if they have undergone the same mechanical treatment. For the purposes of the modelling in the SACN report (see Annex 11), processed meat is considered to be meat that has been treated with preservatives, excluding salt but including some cured meats. For example, meat that has been mechanically processed into burgers but has not been treated with preservatives would be classed as unprocessed.

6. The mutagens IQ, MeIQ, MeIQx, 4, 8-DiMeIQx and PhIP have all been found in cooked fish, chicken, pork and beef. PhIP is the most abundant mutagen in beef, chicken, pork, mutton and fish, followed by MeIQx. The mutagen PhIP seems to form more easily in chicken than in beef, pork or fish, whereas MeIQx formation is lower in cooked chicken than in cooked beef and pork. In a population-based study quantifying HAs in a range of meats (beef steak, beef hamburger, pork loin, pork sausages, chicken breast and lamb steak) cooked by a variety of methods (pan-frying, griddling, coating-frying and roasting), pan-fried chicken breast was the main source of HAs, and PhIP was estimated to be the most consumed HA (Busquets *et al*, 2009). Using data from cooking method questionnaires and food frequency questionnaires, Wu *et al.* (2006) examined the associations between exposure to HAs, meat intake and risk of distal colon adenoma. PhIP was by far the predominant HA, found primarily in broiled and grilled chicken and grilled steak. MeIQx and DiMeIQx were found at much lower levels than PhIP in all meats, with the highest levels found in pan-fried hamburger (MeIQx) and grilled chicken (DiMeIQx). These findings are in agreement with those of Martinez *et al* (2007), who found PhIP to predominate in grilled chicken and grilled steak; MeIQx was found predominantly in grilled and pan-fried hamburgers followed by grilled steak; and DiMeIQx was found predominantly in grilled hamburgers and grilled and pan-fried pork chops, followed by grilled chicken.

7. The levels of HAs in meat products such as sausages and ham made from pork and probably treated with preservatives (though this is not stated) are lower than those found in pan-fried beef or chicken. Levels in hot dogs or ham slices were low or undetectable; HA levels in sausage links or patties increased with doneness (Sinha *et al*, 1998).

8. The content of individual IQ compounds has been estimated to be less than 20 ng/day in fried beef and fish (summarised in Skog, 1993). PhIP is present in fried ground beef at 48.5 ng/day and 73 ng/day in barbecued salmon. PhIP levels in pork products are lower, being 4.8 ng/day and 30.3 ng/day in very well done pan-fried and oven-broiled bacon respectively. PhIP levels in cooked chicken have been reported to be approximately 20 ng/day, reaching 70 ng/day in pan-fried, skinless chicken breasts (Skog and Solyakov, 2002).

Dietary exposure to HAs

9. Assessing exposure to HAs is complex because of the high degree of variability from different cooking methods and preferences. Estimated potential intakes of total and individual HAs have been summarised by Alaejos *et al* (2008) (see Table A25). The data are limited and there is a high level of uncertainty.

Table A25: Estimated intakes of total and individual HAs

HA	Intake ng/day	Country
PhIp	5–300	Japan
	72	Sweden
	158.3	US
MeIQx	300–390	Japan
	72	Sweden
	52.1	US
DiMeIQx	16	Sweden
	3.5	US
Total HAs	8.53	Sweden
	330	Switzerland
	455	US

Adapted from Alajeos et al, 2008.

Metabolism and mutagenicity of HAs

10. HAs are genotoxic in bacterial systems and mammalian cells following metabolic activation (Alaejos *et al*, 2008). The major pathway for metabolic activation starts with the *N*-hydroxylation of the exocyclic amino group, mainly catalysed by cytochrome P450 1A2 (CYP1A2). These metabolites may directly react with DNA, but this step is generally followed by sulphationor acetylation by means of sulphotransferase1A1 or *N*-acetyltransferases.

11. The carcinogenic potency of HAs is dependent on the balance of their metabolic activation and detoxification by carbon oxidation, glucuronidation and sulphation at sites other than at the hydroxylamine (Gooderham *et al*, 2001). Variability in these enzymes between species and between humans will therefore influence the carcinogenic potency. Non-mutagenic HAs may enhance the mutagenicity of those that are mutagenic.

Carcinogenicity in animals

12. HAs have been demonstrated to be carcinogenic in rats, mice and monkeys (reviewed by Skog, 1993). Amino acid pyrolysates have been shown to induce primarily tumours of the liver and blood vessels in mice and tumours of the liver and intestines of rats. The thermic HAs (IQ, MeIQ or MeIQx) (see Table A26) also mainly produced liver tumours, but in addition tumours of the lung, forestomach, lymphoid tissues and haemopoietic system were observed in mice and tumours of the intestines, skin, Zymbal gland, mammary gland, clitoral gland and skin were reported in rats. PhIP has been reported to produce lymphomas in mice, and intestinal, colon and mammary gland tumours in rats. IQ has also been shown to produce liver tumours in monkeys.

Table A26: Tumours induced by HAs in animals

Compound	Species	Conc in diet (%)	Target organs
IQ	Mice	0.03	Liver, forestomach, lung
	Rats	0.03	Liver, small and large intestines, Zymbal gland, clitoral gland, skin
MeIQ	Mice	0.04, 0.01	Liver, forestomach
	Rats	0.04, 0.01	Large intestine, Zymbal gland, skin, oral cavity, mammary gland
MeIQx	Mice	0.06	Liver, lung, haemopoietic system
	Rats	0.04	Liver, Zymbal gland, clitoral gland, skin
PhiP	Mice	0.04	Lymphoid tissues
	Rats	0.01–0.04	Intestines
	Rats	0.04	Colon, mammary gland

(Adapted from Skog, 1993)

13. Thus, with the exception of liver tumours, a slightly different pattern of tumour occurrence is seen in different laboratory animals, suggesting that the tumours observed in humans could also differ. In general, it cannot be assumed that there is concordance between tumour sites in animal studies and in humans. The dietary concentrations of HAs used in feeding studies are in the range 0.06% to 0.1% (600 to 1000 mg/kg diet) which is significantly higher than estimated human intakes. Using standard default values, this is equivalent to doses of 30 to 50 mg/kg bw/day in older rats, compared to 7.5 ng/kg bw/day in humans (assuming an intake of 450 ng total HAs per day by an average 60 kg adult; a dose over 4 million times higher).

Studies in humans

14. HAs have to be activated by various enzymes, including cytochrome P450 (CYP) 1A2 and *N*-acetyltransferase-2 (NAT-2), before they can form DNA adducts. Enzyme activities vary between individuals suggesting that some individuals could be more susceptible to colorectal cancer due to enhanced enzyme activation.

15. A study of cancer patients given trace amounts of HAs prior to surgery reported that more PhIP than MeIQx was bound to DNA in the colon (Garner, 2004), suggesting that PhIP might be more important in the carcinogenic process than MeIQx. There was a 100-fold difference in binding between individuals, but there was no correlation between age, sex, site or severity of the cancer and the amount of HA binding to DNA. In a large study of colorectal cancer patients and controls, there was no difference in CYP1A2 and NAT-2 activity between cases and controls as assessed by measurement of urinary caffeine metabolism. CYP1B1 can also mediate the formation of *N*-hydroxy species and an association was found between a specific CYP1B1 genotype and higher levels of DNA adducts. However, since CYP1B1 expression was not associated with adduct formation, the significance of this observation with regard to increased individual risk was uncertain.

16. On the basis of small trials, it has been suggested that NAT2 "fast" acetylators show a greater association between HA intake and risk of CRC than slow acetylators. However, Barrett *et al* (2003) found no difference in the number of fast acetylators in CRC patients compared to matched controls, suggesting that "fast" acetylators

were not at increased risk of CRC even when exposed to high levels of HAs from well-cooked meat. In addition, high HA exposure from well-cooked meat (assessed by self-reported cooking preferences) was not associated with an increased CRC risk.

17. No difference was detected in the mutation frequency of the *hprt* gene in the DNA of white blood cells of 10 vegetarians compared to 14 meat eaters as assessed by comparison of cloning efficiency into selective and non-selective media (Gooderham and Boobis, 2004). However, it was noted that this finding could be due to small study size, young age of the volunteers, or use of the wrong marker for DNA damage. In this study, NAT-2 and CYP1A2 (enzymes involved in HA activation) were more active in healthy volunteers compared to CRC patients, again indicating that the role of HAs in the risk of CRC is uncertain.

Risk characterisation

18. HAs are known to be genotoxic *in vitro* and are able to produce tumours in laboratory animals. However, in humans the evidence that the increased risk associated with red meat consumption is due to HA intake is not convincing.

19. Comparison of the estimated human dietary exposure with the doses producing tumours in animal studies indicates that human exposure is at least 4 million times less than the doses used experimentally (30 mg/kg bw/day compared to 7.5 ng/kg bw/day) (see above).

Polycyclic aromatic hydrocarbons

Formation of polycyclic aromatic hydrocarbons

20. Polycyclic aromatic hydrocarbons (PAHs) are formed by incomplete combustion (pyrolysis) of organic compounds. They are formed whenever fossil fuels or vegetation are burned and can be present in a wide variety of cooked and processed foods, including meats, cereal products, oils and fats, fruits, vegetables, and sugars, as a result of environmental contamination or formation during drying or cooking. PAHs are ubiquitous in the environment and are found in water, soil and air. The two highest contributors to dietary PAH exposure are cereals and cereal products and seafood and seafood products. PAHs are found in smoked meat products and grilled or barbecued meats but these contribute only a small amount to total dietary exposure to PAHs. Benzo(*a*)pyrene (BaP) is the most widely studied and measured PAH.

21. Like HAs, PAH formation in food is dependent on the method of cooking, particularly the distance from the heat source. When meat is in direct contact with a flame, pyrolysis of the fats in the meat generates PAHs that become deposited on the meat. Even if the meat is not in direct contact with a flame, PAHs can be formed when fat/meat juices drip onto a hot fire/flame because flames containing PAHs are carried back to the meat and adhere to its surface. Increased fat content increases PAH formation. As with HAs, the content of PAHs could be higher in meat with a larger surface area in direct contact with the heat.

22. Cooking methods that result in the highest amounts of PAHs are barbecuing and grilling, especially when the meat is charred; charred meat of any type will contain PAHs. PAH levels increase with increasing cooking temperature. Using questionnaires to determine meat intake and preparation methods, Martinez *et al* (2007) estimated BaP intake from meat and meat sources to be 30.89 ng/day, with the largest contributions coming from grilled steak (20.64 ng/day or 67% of intake), followed by grilled chicken (7.47 ng/day or 24% of intake). Pan-fried bacon and sausage were estimated to provide 0.06 and 0.01 ng/day BaP respectively.

23. No direct comparison has been made but, as with HAs, there is no basis for assuming a difference in the PAH content of burgers or other meat products containing preservatives and those that do not since cooking procedures appear to be the most important determinant of PAH content.

24. In a study by White and colleagues (EFSA, 2008) only 3/77 retail samples contained detectable levels of BaP. In experiments to mimic home cooking practices, little evidence was found of BaP production. However, barbecuing beef burgers with charcoal plus woodchips gave the highest levels of PAH which increased with closeness to the heat source. In contrast, PAHs decreased with closeness to the heat source in sausages cooked over briquettes and with beef burgers, beef and salmon cooked over charcoal. Increased cooking time led to a moderate increase in PAHs in some foods but levels appeared to fall in beef burgers.

25. PAHs are also formed during the curing and processing of meats that use smoking as a preservation method: smoke containing PAHs is deposited on the surface of meats.

Dietary exposure to PAHs

26. Mean dietary exposure to BaP in EU countries for which data are available is 235 ng/day (3.9 ng/kg bw for a 60 kg adult) (EFSA, 2008). For a group of defined PAHs,[101] dietary exposure is 1729 ng/day (28.8 ng/kg bw for a 60 kg adult) and 3078 ng/day (51.3 ng/kg bw) for mean and high-level consumers respectively. For individual food groups, mean exposure is shown in Table A27.

27. It can be seen that the cereals, seafood, fish and vegetable groups make greater contributions to dietary PAH exposure than meat and meat products. However, regular consumers of home-barbecued food could be exposed to higher levels of PAHs.

101 Benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*] anthracene and indeno[*1,2,3-cd*]pyrene.

Table A27: Mean dietary exposure to PAHs from individual food groups

Category	Median consumption (g/day)	BaP (ng/day)	PAH (ng/day)
Cereals and cereal products	257	67	393
Sugar and sugar products (inc chocolate)	43	5	39
Fats (vegetable and animal)	38	26	239
Vegetables, nuts and pulses	194	50	378
Fruits	153	5	87
Coffee, tea, cocoa (expressed as liquid)	601	21	156
Alcoholic beverages	413	4	74
Meat and meat products	132	42	279
Seafood and seafood products	27	36	421
Fish and fishery products	41	21	210
Cheese	42	6	30

Carcinogenicity of PAHs in animals and humans

28. PAHs are carcinogenic by the oral, dermal and inhalation routes of exposure (SCF, 2002; EFSA 2008) and tumours are generally related to these routes. For example, gastric tumours occur after oral administration and skin tumours after dermal application. However, BaP and coal tar mixes have also been reported to produce tumours of the liver, lung, mammary gland, kidney and auditory canal. Studies with PAH-containing coal tar mixes suggest that different PAHs produce different tumour profiles. Few individual PAHs other than BaP have been tested by the oral route; however, dibenz[*a,h*]anthracene and benz[*a*]anthracene produced tumours of the gastrointestinal tract, lungs and liver in mice (SCF, 2002).

29. Based on evidence from occupational exposure and studies in laboratory animals, many PAHs including BaP are classified as known or probable human carcinogens (IARC, 2006). However, there are few human studies of associations between cancer and dietary exposure to PAHs; the majority of studies relate to occupational and environmental exposure (EFSA, 2008).

30. In a small case-control study, consumption of wine from bottles impregnated with tar increased the risk of stomach cancer, which was statistically significant only for men consuming more than 2L of wine per week (Lopez-Abente *et al*, 2001). However, the results could not be attributed to PAHs because of a number of limitations in the study. Another case-control study reported an increased risk of colorectal adenomas associated with benzo[*a*]pyrene intake from meat but more strongly with total intake from the diet (Sinha *et al,* 1998; Sinha *et al*, 2001; Sinha *et al*, 2005). In this US-based study, the BaP intake of 146 cases and 228 controls was assessed by means of a food frequency questionnaire, with additional questions on meat cooking preferences. Foodstuffs linked to the database were also analysed. In the controls, median BaP intake was 5 ng/day from meat and 73 ng/day from all food sources compared to 17 ng/day from meat and 76 ng/day from all food sources in the cases. Consumption of charcoal-grilled beef has been reported to increase the number of DNA adducts in the peripheral blood mononucleocytes of human volunteers (Fontana *et al,* 1999).

31. In a case-control study designed to investigate the proposed association between HAs and CRC (Sachse *et al*, 2002), the effect of polymorphisms in the genes for the enzymes CYP1A1*2B and GSTM1*2/*2 (glutathione-S-transferase M1, a phase 2 detoxifying enzyme, thought to be important in PAH metabolism) were examined. The study showed that inheritance of the CYP1A1*2B and the GSTM1*2/*2 "null" allele was associated with an increased risk of disease. This suggests that a genotype conferring increased activation and decreased detoxification respectively could increase susceptibility to the carcinogenic effects of PAHs; however, this would apply to PAHs from all sources, not just meat.

PAH metabolism

32. The majority of PAHs require metabolic activation for their toxic, mutagenic and carcinogenic action (reviewed by EFSA, 2008). In most cases, a CYP450-catalysed epoxide formation is the initial step followed by formation of highly reactive electrophilic metabolites capable of binding to cellular macromolecules, including nucleic acids. Three main metabolic pathways have been described. The most important of these is the bay region dihydrodiol epoxide pathway, responsible for the mutagenicity of many PAHs and which is catalysed by CYPs and epoxide hydrolase. A one-electron oxidation pathway leading to reactive PAH radicals and the formation of unstable DNA adducts and an ortho-quinone pathway also leading to reactive electrophilic PAH metabolites and stable and unstable DNA adducts have been described.

33. As noted above, PAHs such as BaP are activated by the bay region dihydrodiol epoxide route. The enzymes involved include CYP1A1, the most effective catalyst for most reactions, whilst CYP1A2 and CYP1B1 appear to be involved in BaP metabolism. The PAH-derived diols can then undergo phase 2 metabolism catalysed by sulphotransferases and uridine-diphosphate-glucuronyltransferases. Individual PAHs may undergo more than one pathway, and metabolic pathways other than the three noted above may be involved in metabolic activation. Many of the enzymes involved in the metabolism of PAHs have been shown to be polymorphic, suggesting that some individuals will have differing sensitivities to PAH exposure. Some genetic polymorphisms have been associated with an increased risk of cancer, but the role of these has not been completely elucidated because compensatory or alternative pathways may exist. It seems likely that polymorphisms may be more important at lower levels of PAH exposure.

Risk characterisation

34. It is not possible to link the increased risk associated with red meat consumption to PAH intake since there are no convincing quantitative data relating dietary exposure to PAHs in humans and risk of CRC. There are many dietary sources of PAHs; meat and meat products are lesser contributors to total PAH intake compared to other foods.

35. Comparison of the estimated human dietary exposure with animal studies indicates that there is a margin of exposure[102] (MOE) for BaP and defined groups of PAHs of 15,900–17,900 for average consumers but 10,000 or slightly less for high-level consumers, indicating possible concerns at high levels of intake (EFSA, 2008).

Conclusions

HAs

36. HAs are carcinogenic in laboratory animals producing liver and other tumours. It is not clear if they have a role in human cancer.

37. Meat is the only source of dietary HAs. The HA content tends to increase with cooking temperature, time and "doneness". The most abundant HA is PhIP which occurs at higher concentrations in chicken than in red meat. Meat products such as sausages and ham generally contain lower levels of HAs than other meats. There is no evidence that levels of HAs are different in meats containing preservatives compared with meats not containing preservatives.

38. Studies of CRC patients and controls indicate that HAs such as PhIP and MeIQx bind to DNA in the colon but there is no correlation between DNA binding and cancer. In addition, variants in the genes that could lead to greater activation of HAs did not differ significantly between CRC patients and controls. The studies reported that although meat consumption was associated with an increased risk of CRC, HAs were not major contributors to the risk.

39. There is no clear basis for separating meat which contains preservatives from meat which does not on the basis of its HA content.

PAHs

40. Some PAHs are known animal carcinogens producing a range of tumours, including tumours of the gastrointestinal tract, when administered orally. Some PAH mixtures are also proven human carcinogens via inhalation and dermal exposure. However, the role of dietary PAHs in humans has been less studied.

41. Meat and meat products represent only a small proportion of dietary PAH intake. Barbecuing can increase the PAH content of meat, but this is only a limited part of the total diet. Therefore any contribution of PAHs to cancer risk associated with red or processed meats is likely to be minor. There are no direct comparisons of PAH levels in preserved and unpreserved meat. It is unclear whether preserved meats or meat products are more likely to be barbecued or grilled, so there is no clear basis for separating these meats due to their PAH content. It is likely that meats which have been mechanically processed such as burgers may generate higher levels of PAHs through higher fat contents, but this would not be affected by preservative treatment.

102 The margin of exposure approach is a method by which the dose of a chemical known to cause tumours in a defined proportion of animals is compared to the level of human exposure. A margin of exposure of less than 10,000 is considered to indicate a potential concern for human health.

42. Humans are exposed to PAHs from a range of dietary and non-dietary sources and it is not possible to determine whether PAHs in cooked meats contribute to CRC risk.

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N-nitroso compounds and cancer

Occurrence of N-nitroso compounds

1. *N*-nitroso compounds (NOC), including nitrosamines, can be formed in the bodies of healthy individuals following the reaction of dietary nitrate and nitrite with primary, secondary and tertiary amines via a nitric oxide intermediary (Rostkowska *et al*, 1998). Nitrates and nitrites are found in a wide range of commonly consumed foods.

2. Studies in ileostomists have shown that nitrosamines are formed in the absence of colonic flora in the upper GI tract (Lunn *et al*, 2007) and are also produced endogenously in the stomach and colon of people who eat large amounts of red meat or take nitrate supplements. NOCs can also be produced in meat during the curing process or during smoking, drying and salting of foods such as fish and meat (WCRF/AICR, 2007).

3. Exposure to *N*-nitrosodimethylamine (NDMA), the most widely consumed and studied dietary NOC, has been reported to be <1 µg/day, whereas exposure to endogenously formed *N*-nitroso compounds (ENOC) was 93 µg/day (Jakszyn *et al*, 2006). Although NDMA is only one of many NOCs in foods, and represents a fraction of total NOC exposure, it has been used as an indicator of dietary exposure to total exogenous NOCs.

Evidence for the carcinogenicity of nitrosamines

Animal studies

4. Nitrosamines have been shown to produce a range of tumours in 40 animal species (Bartsch, 1991), including liver, stomach, forestomach and other tumours of the gastrointestinal tract, but there is little evidence of a link to CRC in humans or animal models (although it cannot be assumed that there is concordance between tumour sites in animals and humans). Individual exposure to endogenous nitrosamines is highly dependent on dietary modifiers as well as disease status. G-A transition mutations, in particular codons and DNA adducts characteristic of alkylating agents such as nitrosamines, are commonly found in CRC. Two nitrosamines (*N*-nitrosodiethylamine and NDMA) have been classified as probably carcinogenic to humans (group 2A) based on data from many animal species, with the main target organs being the liver, respiratory and upper digestive tracts and kidneys (IARC, 1998).

Human studies

5. In a cohort study in Finland (Knekt *et al*, 1999), 189 gastrointestinal cancers were identified during the 24 year follow-up period. Intakes of nitrate, nitrite and NDMA were estimated using a one-year dietary history interview. NDMA was assumed to be provided by smoked and salted fish (51.9%) and cured meats and sausages (48.1%). A significant positive association was observed between intake of NDMA and subsequent occurrence of CRC [RR between highest and lowest quartiles of intake

of 2.12; 95% CI, 1.04–4.33]. Of the various sources, intakes of smoked and salted fish were significantly associated with CRC [RR, 2.58; 95% CI, 1.21–5.51], whereas intakes of cured meat were non-significantly associated with CRC [RR, 1.84; 95% CI, 0.98-3.47]. No significant associations were observed with other cancers of the gastrointestinal tract or with nitrate and nitrite intake.

6. A nested case-control study of patients with gastric cancer (GC) assessed the exposure to dietary NDMA and ENOC through a dietary and lifestyle questionnaire (Jakszyn *et al*, 2006). Dietary intakes of nitrites and NDMA were estimated by matching food items on a country-specific questionnaire, with a food database of potential carcinogens. Dietary NDMA was not associated with an increased risk of GC [hazard ratio (HR), 1.00; 95% CI, 0.70–1.43 for an increment of 1.1 µg NDMA]. ENOC was positively, but not statistically significantly, associated with GC (HR, 1.18; 95% CI: 0.99–1.39 for an increment of 40 µg ENOC). When analysed by tumour site, ENOC was not associated with cardia[103] cancer risk but was significantly associated with non-cardia cancer risk (HR, 1.42; 95% CI, 1.14-1.78 for an increment of 40 µg ENOC). An association with non-cardia cancer was found amongst individuals with H. pylori infection (OR, 1.82; 95% CI, 1.32–2.51) in all models but not in non-infected individuals. Individuals with elevated serum vitamin C showed no association between ENOC and non-cardia GC, whereas those with low serum vitamin C showed a positive association (OR, 3.24; 95% CI, 1.77–5.93).

7. Studies have suggested that the conditions found at the gastro-oesophageal junction of the stomach (the cardia region) are optimal for the formation of nitrosamine compounds as the ascorbic acid to nitrite ratio is lowest at this point of the gastrointestinal tract, and the acidity and level of thiocyanate (a known catalyst for nitrosation of secondary amines by acidified nitrite) are sufficient to potentially promote this reaction (Suzuki *et al*, 2003).

8. Studies have shown that individuals consuming a high red-meat diet compared to those consuming a vegetarian diet have a higher percentage of faecal colonic exfoliated cells staining positive for O^6-carboxymethylguanine (O^6CMeG), a promutagenic DNA adduct which can be formed by many *N*-methyl-*N*-nitroso compounds. This adduct has been shown in *in vitro* assays to form G-A transitions and G-T transversions in adducted p53 cDNA and is not easily repaired by normal DNA repair mechanisms. The O^6CMeG adduct has been identified in many CRC cell lines (Nagasaka *et al*, 2008) and may be a possible mechanism for the relationship between high red-meat consumption and CRC (Lewin *et al*, 2006; Kuhnle *et al*, 2007).

9. Studies have also identified a link between consumption of cured and smoked meat and fish by pregnant mothers and young children with the development of childhood leukaemia and a protective effect of vegetables and bean-curd foods (Liu *et al*, 2009). There are a number of studies linking consumption of preserved meats by pregnant women (along with other sources of exposure to NOCs such as therapeutic drugs) with brain cancers in their children (McKean-Cowdin *et al*, 2003; Preston-Martin *et al*, 1982; Huncharek *et al*, 2003).

103 The area where the stomach joins the oesophagus.

International reviews

10. The World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) concluded in 2007 that there was limited evidence that processed[104] meats, but not red meats, were a cause of stomach cancer and that this was probably due to the formation of NOCs both in the meat and in the stomach (WCRF/AICR, 2007).

11. The WCRF/AICR also concluded that Cantonese-style salted fish, which is relatively high in nitrosamine compounds, is a probable cause of nasopharyngeal cancer. This was based on evidence that CYP2E1 is expressed in the nasopharynx, is involved in the metabolic activation of nitrosamines *in vivo,* and that individuals with a variant allele of CYP2E1 have been linked with an increased incidence of nasopharyngeal cancer.

12. For oesophageal cancer, the WCRF/AICR concluded that for both red meat and processed meat, there was limited evidence to suggest a causal link.

13. Based on data from cohort and case-control studies which showed a plausible dose-response relationship, the WCRF/AICR concluded that the evidence to suggest that processed and red meat are a cause of CRC was convincing but there are little data on the mechanism for this and whether this relates to the formation of nitrosamines.

Conclusion

14. There does not appear to be strong evidence to support a link between nitrosamine exposure (both exogenous and endogenous formation) and CRC. Although nitrosamine compounds may be more likely to be present in preserved meats (including smoked, cured and salted meats), evidence to link these foods with colorectal cancer is weak. There is a stronger association between preserved meats and stomach, oesophageal and other cancers compared to CRC. Exposure to *N*-nitroso compounds produced endogenously in the body appears to significantly exceed (~100 times for NDMA) that of *N*-nitroso compounds from foods.

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Meat, haem iron and cancer

1. Evidence suggests that diets high in red and/or preserved meat are associated with a higher risk of CRC compared with diets rich in chicken or fish. A major difference between red meat and white meat, such as chicken, is the levels of iron and haem which are approximately four and 10 times greater in red meat respectively (Cross *et al*, 2002, Santarelli *et al*, 2008; Sandhu *et al*, 2001; Norat *et al*, 2002, Larsson and Wolk, 2006, Norat *et al*, 2005; Lee *et al*, 2004; WCRF/AICR, 2007). Some studies have found no association between haem or iron intake and CRC (Kabat *et al*, 2007).

Foods containing iron

2. There is limited evidence suggesting that consumption of a diet high in total and haem iron may be associated with a higher risk of CRC. The WCRF concluded in 2007 that the evidence was "sparse, of poor quality and inconsistent" (WCRF/AICR, 2007). The definition of foods containing iron includes vegetables containing non-haem iron such as spinach.

Foods containing haem

3. The haem content of preserved and unpreserved meats from the same species appear to be comparable and therefore the haem content alone would not account for the different relative risks associated with non-preserved and preserved red meats. The majority of preserved meat products are of porcine origin and contain lower levels of haem iron than beef-based products.

Proposed mechanisms

4. A number of mechanisms have been proposed for a possible relationship between haem iron and CRC. These include the formation of *N*-nitroso-compounds (see Appendix 2 for more information on the possible carcinogenic activities of *N*-nitroso compounds), the catalysis of lipid peroxidation by iron/haem in the gut and subsequent DNA adducts caused by lipid radicals, and the nitrosylation of haem which may render it more cytotoxic than unnitrosylated haem. Some of the available studies are outlined below, grouped by proposed mechanism; these are prefaced by some general studies in which the most likely proposed mechanism was not specified by the authors.

General studies

5. Rats fed a low-calcium diet supplemented with haemin or haemoglobin and/or calcium, butylated hydroxyl anisole and rutin, and olive oil demonstrated that haemin and haemoglobin produced a statistically significant increase in numbers of total aberrant crypt foci (ACF) compared to controls. Calcium, antioxidants and olive oil reduced the total number of ACF found in the colons of these rats (Pierre *et al*, 2003).

A similar study by the same authors using meat instead of haemin/haemoglobin concluded that red meat in combination with a low-calcium diet promotes ACF formation in the colons of rats (Pierre *et al*, 2004).

6. Sesink *et al* (1999) compared rats fed a purified control diet or a diet containing protoporphyrin IX, ferric citrate or bilirubin with those consuming a purified diet containing haemin for 14 days. There were significant increases in proliferation of the colonic epithelium in rats on the haemin diet compared to control animals. Faecal water from the haemin-consuming groups was significantly more cytotoxic to erythrocytes compared to controls.

Nitrosation

7. In human studies, apparent total *N*-nitroso compounds (ATNC) were found to be significantly elevated following consumption of a haem-supplemented and high red-meat diet compared to a low meat diet (Cross *et al*, 2002; Cross *et al*, 2003; Kuhnle *et al*, 2007). Iron supplementation did not have an effect (Cross *et al*, 2002; Cross *et al*, 2003). The authors proposed that haem and not iron appears to enhance endogenous *N*-nitrosation to form mainly nitrosothiols under the acid conditions in the stomach which can then go on to form nitrosyl haem and other nitroso compounds in the alkaline and reductive conditions of the small and large intestines (Kuhnle *et al*, 2007; Kuhnle and Bingham, 2007). However, not all nitroso compounds are carcinogenic. An analytical study (Dennis and Clarke, 2006) was unable to characterise some of the nitroso compounds present in the ATNC.

8. A number of animal studies found an increase in faecal ATNC following consumption of grilled bacon or other preserved meats, but this was not accompanied by an increase in aberrant crypt foci (precancerous cells in the epithelium of the colon) (Santarelli *et al*, 2008).

Lipid peroxidation and oxidative stress

9. A human study and a rat study showed a significant increase in urinary markers of lipid peroxidation (4-hydroxynonenal metabolites or 8-*iso*-prostaglandin-F(2)α) following consumption of diets high in haem iron (Pierre *et al*, 2006).

10. Rats fed diets containing safflower oil (an oxidised refined PUFA) and haemoglobin at various levels for 36 weeks showed an increase in carcinoma of the colon compared to controls (however, this study did not follow the standard time frame for carcinogenicity studies). Peroxyl radicals were shown to be produced *in vitro* from oxidised PUFA in the presence of haemoglobin and oxidised PUFA was found to cause single strand breaks in DNA in the presence of haematin (Sawa *et al*, 1998).

11. An Ames test carried out using *S. typhimurium* strains sensitive to oxidative mutagens (TA102 and TA104) suggested that iron may cause redox cycling of bile acids in the presence of vitamin K1 and S9 mixture (Blakeborough *et al*, 1989).

12. *In vitro* studies using colon cancer cell lines incubated with haemoglobin and haemin showed that iron from both compounds was released and rapidly absorbed by the cells and resulted in increased proliferation and production of reactive oxygen species (Lee *et al*, 2006; Glei *et al*, 2006).

Nitrosylation of haem

13. In raw cured meat, myoglobin is nitrosylated and further cooking releases nitrosyl haem from the myoglobin. Nitrosylated haem has been shown to be weakly mutagenic in the Ames test, but has not been tested *in vivo* (Santarelli *et al*, 2008).

14. *N*-nitrosomorpholine (NMor) was formed *in vitro* in the presence but not absence of nitrosated haemoglobin at pH 6.8 (Lunn *et al*, 2007). In this study NMor was used as a marker for nitrosamine compounds in general.

Conclusion

15. There is some evidence that consumption of a diet rich in iron (haem and non-haem) may be associated with a higher risk of CRC although this evidence is considered "sparse, of poor quality and inconsistent" by the WCRF. A clear mechanism demonstrating either an effect of haem or non-haem iron or any differences in risks between preserved or unpreserved red meats has not been demonstrated by the studies above. It is possible that a combination of the above mechanisms is occurring or that different mechanisms may produce *N*-nitroso compounds which are more potent in their carcinogenic activity than those produced endogenously from sources such as vegetables. There is currently insufficient evidence to support these mechanisms.

16. These data do not provide a clear basis for separating preserved and unpreserved red meat for modelling purposes.

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Preservatives and cancer

1. Fresh meat is not permitted to contain additives (including preservatives). Additives can only be used in processed meats (including pre-packed fresh minced meat) and processed meat products.[105] Council Directive 95/2/EC on food additives other than colours and sweeteners details those additives approved for use in meat products. The additives classed as preservatives are E200 to E252. Other permitted additives include antioxidants and colours (EC, 1995).

2. In order for an additive to be approved for use, the applicant must submit sufficient safety data to demonstrate than no adverse effects would be expected under the conditions of use. The data may include acute and chronic studies and reproductive toxicity studies in animals as well as *in vitro* studies to identify genotoxic activity. The data are reviewed by independent expert committees such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA). If a substance is found to cause cancer in laboratory animals by a genotoxic mechanism, it would not be permitted for use as an additive in foods.

3. Concerns have been expressed regarding the potential carcinogenicity of nitrites/nitrates and benzoates and sorbates. These are considered below together with sulphates and sulphites.

Nitrate, nitrite and N-nitroso compounds

Regulatory aspects

4. Potassium nitrite (E249), sodium nitrite (E250), sodium nitrate (E251) and potassium nitrate (E252) are permitted in "a range of meat products, cured hams, etc." at levels of between 10 and 300 mg/kg (EC, 1995). They are also permitted in a small number of cheeses and preserved fish. These substances are added as preservatives in meat products, particularly cured meats such as ham and bacon, to reduce the growth of bacteria and therefore spoilage and health risks.

5. There is legislation in Europe limiting nitrate concentrations in vegetables (EC, 2005; EC, 2001).

105 Regulation (EC) 853/2004 laying down specific hygiene rules for food of animal origin provides the following definitions:
"fresh meat" means meat that has not undergone any preserving process other than chilling, freezing or quick freezing, including meat that is vacuum wrapped or wrapped in a controlled atmosphere;
"unprocessed products" means foodstuffs that have not undergone processing, and includes products than have been divided, parted, severed, sliced, boned, minced, skinned, ground, cut, cleaned, trimmed, husked, milled, chilled, frozen, deep-frozen or thawed;
"processed products" means foodstuffs resulting from the processing of unprocessed products. These products may contain ingredients that are necessary for their manufacture or to give them specific characteristics;
"processing" means any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of these processes.

International reviews and derivation of the ADI

6. The acceptable daily intake (ADI) is the amount of an additive that expert bodies consider can be consumed on a daily basis for a lifetime without any appreciable adverse effects. The ADI set for nitrate is 3.7 mg/kg bw/day (or 222 mg/day for an average 60 kg adult). The ADI for nitrate was set by the former Scientific Committee on Food (SCF) and reconfirmed by JECFA in 2002 (WHO, 2003). JECFA also set an ADI for nitrite of 0.07 mg/kg bw/day (or 4.2 mg/day for an average 60 kg adult) (WHO, 2003).

7. The ADI for nitrate was based on a two-year rat study and a sub-chronic (125 day) dog study both of which showed growth restriction at intakes of the nitrate ion above 370 mg/kg bw/day. The ADI for nitrite was derived from a two-year study in rats given sodium nitrite in their drinking water. Heart and lung toxicity were observed at doses of 100 mg/kg bw/day and above, and the no observed adverse effect level (NOAEL) was 10 mg/kg bw/day. No evidence of carcinogenic activity was observed in these studies (WHO, 2003). The EFSA Scientific Panel on Contaminants in the Food Chain concluded that there were no new data that would necessitate a revision in the ADI in the course of a recent evaluation of risks of nitrate in vegetables (EFSA, 2008).

Natural occurrence and metabolism of nitrate and nitrite

8. Nitrate can be produced endogenously through the L-arginine-NO-synthase pathway and is found naturally in vegetables, especially leafy vegetables such as cabbage and spinach. Nitrate can be converted to nitrite, nitric oxide and *N*-nitroso compounds in the body and these metabolites have the potential to cause adverse effects such as methaemoglobinaemia and carcinogenicity.

9. Nitrite may play a defensive role in the body through its antimicrobiological properties and nitric oxide may play a role in vasoregulation (EFSA, 2008), but these effects are not considered in the safety assessment of nitrate and nitrite in food.

10. In most individuals, 5–7% of dietary nitrate is converted to nitrite through the action of bacteria at the back of the tongue; for some individuals, with a high rate of conversion, this may be up to 20%. Reduction of nitrate to nitrite in the mouth accounts for 70–80% of the total nitrite exposure in humans, the remainder is from cured meats and other routes of metabolism in the body. The nitrite formed in the oral cavity is swallowed along with saliva, and food and enters the stomach where it can be converted to nitrogen oxides such as nitric oxide and subsequently may be metabolised to nitrosamines (EFSA, 2008).

Predicted exposures to nitrates and nitrites

11. An exposure assessment carried out by EFSA (2008) estimated that an individual consuming 400 g/day of mixed vegetables containing a typical median nitrate level and taking into account other sources of nitrate including drinking water and animal products would not exceed the ADI for nitrate. Estimated intakes of nitrate were approximately 157 mg/person/day (35–44 mg/person/day from non-vegetable

sources[106] and 113 mg/person/day from vegetables). Findings from the 1997 total dietary survey (TDS) showed that green vegetables, potatoes and other vegetables made the greatest contributions to nitrate exposure, contributing 21%, 33% and 15% respectively. The greatest contribution to dietary nitrites was from beverages (36%) followed by meat products (15%), miscellaneous cereals (14%) and milk (8%) (MAFF, 1998). Intakes of nitrate and nitrite from consumption of vegetables are likely to considerably exceed those from preserved meats. Concomitant consumption of vitamin C with foods containing nitrates appears to reduce levels of nitrosamine production by up to 50% (EFSA, 2008).

Studies of carcinogenicity and genotoxicity of nitrate and nitrite

12. Sodium nitrate was found not to be mutagenic in *in vitro* studies. Similar studies carried out on sodium nitrite showed mutagenic potential in one out of the two strains of *S. typhimurium* tested but overall, in their assessment of genotoxicity, JECFA considered that neither nitrate nor nitrite should be regarded as genotoxic carcinogens (WHO, 2003). JECFA and EFSA also looked at a number of long-term carcinogenicity studies on nitrate and nitrite. Both committees concluded that nitrate was not carcinogenic. The evidence for the carcinogenicity of nitrite was equivocal and based on a trend in the incidence of squamous cell papilloma and carcinoma of the forestomach, an endpoint that may not be relevant for humans.

13. Human epidemiology studies have shown no link between the incidence of cancer (multiple tissue sites) and nitrate intake from food and drinking water (EFSA, 2008; WHO, 2003).

Conclusion

14. Although epidemiological data suggest that high consumption of preserved meats are associated with greater CRC risk than red meat, the available evidence does not support the hypothesis that this is due to the presence of nitrate and nitrite preservatives. This is because green vegetables and potatoes are a much greater source of nitrate than preserved meats and endogenous conversion of nitrate to nitrite is a significantly greater source of nitrite than preserved meats.

Benzoates and sorbates

Regulatory aspects and international reviews

15. Sorbic acid (E200), potassium sorbate (E202), calcium sorbate (E203), benzoic acid (E210), sodium benzoate (E211), potassium benzoate (E212) and calcium benzoate (E213) are all approved additives which are permitted at levels *"quantum satis"*[107] for the surface treatment of dried meat products. These preservatives are used to a greater extent in carbonated soft drinks, and meat products are unlikely to be a significant source of these preservatives (EC, 1995).

106 Approximately 20 mg/person/day is contributed by water and the remainder from animal products (such as preserved meats, fish, cheese).

107 The amount that is needed.

16. In 1996, the JECFA set a group ADI for benzoate salts and benzoic acid of 5 mg/kg bw/day which was reconfirmed in 2001. This was based on a multigeneration long-term study in rats where no adverse effects were observed at intakes at or below 500 mg/kg bw/day. In 1973, the JECFA set a group ADI for sorbate salts and sorbic acid of 25 mg/kg bw/day based on a multigeneration long-term rat study where no treatment-related adverse effects were observed at intakes at or below 2500 mg/kg bw/day.

Committee on Mutagenicity assessment

17. In 2007, the Committee on Mutagenicity (COM) considered a study in modified yeast cells exposed to benzoate and sorbate preservatives that showed an increased level of DNA damage in exposed cells compared to controls. However, direct extrapolation of these results from the mutant yeast cells to mammalian cells *in vivo* was not possible because antioxidant and DNA repair mechanisms had been attenuated by the yeast cells. The committee concluded that, taking into account the large amount of toxicology data including rodent carcinogenicity studies, this study did not suggest a need for a full evaluation of the mutagenicity data on benzoate and sorbate preservatives (COM, 2007).

Conclusion

18. The above study together with the package of other data supplied prior to approval of these preservatives does not suggest a link between benzoate and sorbate preservatives and CRC.

Sulphates and sulphites

Regulatory aspects

19. Sodium sulphate and sodium hydrogen sulphate (E514), potassium sulphate and potassium hydrogen sulphate (E515), and calcium sulphate (E516) are permitted in all preserved meats but not fresh meats or poultry. Sodium sulphite (E221), sodium hydrogen sulphite (E222), sodium metabisulphite (E223), potassium metabisulphite (E224) and calcium sulphite (E226) are all permitted for use in burgers or breakfast sausages.

20. In 2000, JECFA assessed the safety of sodium sulphate and derived a temporary ADI "not specified" indicating no concerns over the use of this additive in foods when following good manufacturing practice (WHO, 2000). In 1999, JECFA reconfirmed the ADI set in 1986 of 0.7 mg/kg bw/day based on an eight-week animal study where gastric lesions were observed at doses above 70 mg/kg bw/day.

21. These substances, in common with all additives, have been rigorously assessed for safety by JECFA and other international experts. Sulphate compounds are endogenous in the body and long-term carcinogenicity studies using both sulphates and sulphites have shown no carcinogenic effect. Some *in vitro* studies of the mutagenicity of sulphites have produced positive results but these are not confirmed by *in vivo* data (WHO, 1986; WHO 1999).

Conclusion

22. No data have been identified that support a hypothesis that sulphate preservatives used in preserved meats could be linked to CRC.

Overall conclusion on preservatives

23. The data do not suggest that commonly used food additives used in preserved meats are the basis for the link between processed meat intake and CRC.

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Iron intakes and status of the UK population

1. Data on the general population from the National Diet and Nutrition Surveys (NDNS): children, 1½–4½ years (1992/93); adults, 65 years and over (1994/95); young people, 4–18 years (1997); adults, aged 19–64 years (2000/01).

2. Data on low-income populations (2 years and above) (2003–2005) from the Low Income Diet and Nutrition Survey (LIDNS).

Table A28: NDNS – Contribution (%) of food types to average daily intake of total iron

	Children aged 1½–4½ years		Young people aged 4–18 years		Adults aged 19–64 years		Adults aged 65+ years Free-living		Adults aged 65+ years Institutions	
	Males	Females	Male	Females	Males	Females	Males	Females	Males	Females
Cereals	49	48	55	51	44	45	48	47	50	50
White bread	8	8	11	11	10	8	10	9	10	10
Wholemeal bread	3	3	2	2	3	3	7	7	5	6
Soft-grain and other bread	–	–	–	–	3	3	–	–	–	–
Whole-grain and high-fibre breakfast cereals	11	10	12	9	12	13	10	12	8	9
Other breakfast cereals	10	10	17	14	6	7	5	5	9	8
Biscuits, buns, cakes, pastries	5	5	6	8	4	5	8	8	10	10
Milk and milk products	6	6	3	3	1	1	3	4	4	5
Eggs and egg dishes	2	3	2	2	3	3	3	3	4	4
Fat spreads	0	0	0	0	0	0	0	0	0	0
Meat and meat products	14	14	14	13	19	15	18	16	17	15
Fish and fish dishes	2	2	1	2	2	3	3	2	2	2
Vegetables (excluding potatoes)	7	7	7	8	9	11	8	10	8	8
Potatoes and savoury snacks	7	7	7	7	7	8	7	7	5	5
Fruit and nuts	3	3	1	2	2	3	3	3	3	3
Sugars, preserves, confectionery	4	3	4	4	2	2	1	1	1	1
Drinks	3	3	1	2	7	6	3	3	1	1
Miscellaneous	2	2	2	2	3	3	3	4	5	6
Total no. respondents (w)	–	–	–	–	*833*	*891*	*540*	*735*	*93*	*319*
Total no. respondents (unw)	*848*	*827*	*856*	*845*	*766*	*958*	*632*	*643*	*204*	*208*

* Includes soft drinks, alcoholic drinks, tea, coffee and water.

** Includes powdered beverages (except tea and coffee), soups, sauces, condiments and artificial sweeteners.

Table A29: LIDNS – Contribution (%) of food types to average daily intake of total iron

	Children (2–10 years)		Adults (19 years and over)	
	Boys	Girls	Men	Women
Cereals and cereal products	53	49	40	41
Milk and milk products	2	2	1	1
Eggs and egg dishes	2	2	4	3
Fat spreads	0	0	0	0
Meat and meat products	17	16	23	21
Fish and fish dishes	1	2	2	3
Vegetables excluding potatoes	7	8	10	10
Potatoes and savoury snacks	10	11	8	9
Fruit and nuts	1	2	2	2
Sugars, preserves and confectionery	3	3	2	2
Drinks*	1	2	5	4
Miscellaneous**	2	2	3	4
Total no. respondents (unw)	*439*	*493*	*946*	*1850*

* Includes soft drinks, alcoholic drinks, tea, coffee and water

** Includes powdered beverages (except tea and coffee), soups, sauces, condiments and artificial sweeteners

Table A30: NDNS – Total mean (median) iron intake from all sources and food sources

Age (years)	MALE				FEMALE			
	Mean (median) intake from all sources mg/day	Mean (median) intake from food sources, mg/day	Base *(w)*	Base *(unw)*	Mean (median) intake from all sources, mg/day	Mean (median) intake from food sources, mg/day	Base *(w)*	Base *(unw)*
Children 1.5–4.5	**5.7** (5.4)	**5.5** (5.4)	–	*848*	**5.4** (5.0)	**5.2** (5.0)	–	*827*
1.5–2.5*	5.0 (4.7)	4.9 (4.7)	–	*288***	5.0 (4.7)	4.9 (4.7)	–	*288***
2.5–3.5*	5.6 (5.4)	5.4 (5.3)	–	*303***	5.6 (5.4)	5.4 (5.3)	–	*303***
3.5–4.3	6.2 (5.9)	6.1 (5.9)	–	*250*	5.9 (5.5)	5.6(5.5)	–	*243*
Young people 4–18	**10.5** (9.9)	**10.4** (9.8)	3331	*856*	**8.5** (8.0)	**8.3** (7.9)	*3159*	*845*
4–6	8.3 (8.0)	8.2 (7.9)	1134	*184*	7.4 (7.1)	7.3 (7.1)	*656*	*171*
7–10	9.8 (9.3)	9.7 (9.3)	912	*256*	8.5 (8.2)	8.4 (8.2)	*866*	*226*
11–14	10.8 (10.4)	10.8 (10.4)	870	*237*	9.1 (8.6)	8.8 (8.4)	*821*	*238*
15–18	12.6 (11.7)	12.5 (11.6)	861	*179*	8.9 (8.2)	8.7 (8.0)	*816*	*210*
Adults 19–64	**14** (12.9)	**13.2** (12.6)	833	*766*	**11.6** (10.0)	**10.0** (9.6)	*891*	*958*
19–24	11.5 (11.3)	11.4 (11.2)	108	*61*	10.0 (9.3)	8.8 (9.1)	*104*	*78*
25–34	13.9 (12.8)	13.0 (12.5)	219	*160*	9.8 (9.0)	9.2 (9.0)	*210*	*211*
35–49	14.1 (13.2)	13.7 (13.1)	253	*303*	12.9 (10.5)	10.2 (10.1)	*318*	*379*
50–64	15.2 (13.6)	13.6 (13.3)	253	*242*	12.3 (11.0)	10.9 (10.6)	*259*	*290*
Adults 65 and over								
Free-living	**11.6** (10.6)	**11.0** (10.5)	540	*632*	**8.9** (8.4)	**8.6** (8.3)	*735*	*643*
65–74	11.9 (10.6)	11.1(10.5)	353	*271*	9.3 (8.7)	9.0 (8.6)	*409*	*256*
75–84	11.1 (10.7)	10.8 (10.5)	160	*265*	8.5 (8.1)	8.4 (8.1)	*249*	*217*
85 +	10.6 (9.7)	10.4 (9.7)	26	*96*	7.9 (7.6)	7.7 (7.5)	*77*	*170*
Institutionalised	**9.6** (9.3)	**9.6** (9.3)	93	*204*	**8.3** (7.9)	**8.2** (7.9)	*319*	*208*
65–84	9.6 (9.2)	9.6 (9.2)	57	*128*	8.7 (8.1)	8.6 (8.1)	*144*	*91*
85 +	9.7 (9.3)	9.6 (9.3)	36	*76*	8.0 (7.7)	7.8 (7.6)	*174*	*117*

* Data reported for boys and girls combined.

** Half of the base figure for the sum of boys and girls as data combined in report.

Table A31: LIDNS – Total mean (median) daily intake of iron (mg) from food sources only

Age (years)	Male	*Base (unw)*	Female	*Base (unw)*
Children 2–10	9.0 (8.6)	*239*	7.9 (7.7)	278
Young people 11–18	11.4 (10.6)	*200*	9.3 (9.0)	215
Adults 19–64				
19–34	11.6 (11.0)	*194*	8.5 (8.0)	483
35–49	10.8 (10.4)	*226*	8.7 (7.8)	494
50–64	11.5 (11.4)	*258*	8.8 (8.5)	336
Adults 65 and over	10.2 (9.8)	*268*	9.0 (8.6)	537

Table A32: NDNS – Average (median) daily intake of haem iron (mg)/contribution of average daily haem iron intake to total iron intake (%)

Age (years)	Males	Females
Children 1.5–4.5	0.2 (0.2)/3.5%	0.3 (0.2)/5.6%
Young people 4–18	0.4 (0.4)/3.8%	0.3 (0.3)/3.5%
Adults 19–64	0.8 (0.7)/5.7%	0.5 (0.5)/4.3%
Adults 65 and over		
Free-living	0.7 (0.6)/6%	0.5 (0.4)/5.6%
Institutionalised	0.6 (0.5)/6.3%	0.4 (0.4)/4.8%

Base numbers as in Table A30

Table A33: LIDNS – Average (median) daily intake of haem iron (mg)/contribution of average daily haem iron intake to total iron intake (%)

Age (years)	Males	Females
Children 2–10	0.4 (0.3)/4.4%	0.4 (0.3)/5.1%
Young people 11–18	0.6 (0.4)/5.3%	0.5 (0.4)/5.4%
Adults 19–64	0.9 (0.7)/7.8%	0.5 (0.5)/6.2%
19–34	0.8 (0.7)/6.9%	0.5 (0.5)/5.9%
35–49	0.8 (0.6)/7.4%	0.6 (0.5)/6.9%
50–64	1.0 (0.8)/8.7%	0.5 (0.4)/5.7%
Adults 65 and over	0.7 (0.6)/6.9%	0.6 (0.5)/6.6%

Base numbers as in Table A31

Table A34: NDNS – Average total daily iron intakes (from all sources) as a proportion of DRVs

Age (years)	Male		Female	
	%<LRNI	Mean intake as % of RNI	%<LRNI	Mean intake as % of RNI
Children 1.5–4.5				
1.5–2.5	24*	73	24*	73*
2.5–3.5	12*	81	12*	81*
3.5–4.5	4	95	4	92
Young people 4–18				
4–6	0	136	1	121
7–10	1	112	3	97
11–14	1	96	44	61
15–18	0	111	48	60
Adults 19–64				
19–24	3	133	40	68
25–34	0	160	40	66
35–49	1	163	25	87
50–64	1	174	4	137
Adults 65 and over				
Free-living				
65–74	0	133	4	102
75– 84	2	133	6	102
85+	4	133	10	102
Institutions				
65–84	4	111	3	95
85+	5	111	8	95

* Boys and girls combined.

Base numbers as in Table A30

Table A35: LIDNS – Average total daily iron intakes (from food sources) as a proportion of DRVs

Age (years)	Male		Female	
	%<LRNI	Mean intake as % of RNI	%<LRNI	Mean intake as % of RNI
Children 2–18				
2–10	2	120	2	107
11–18	14	101	39	63
Adults 19–65+				
19–34	5	134	49	58
35–49	5	124	52	59
50–64	4	133	13	99
65+	3	117	5	103

Base numbers as in Table A31

Table A36: NDNS – Proportion with haemoglobin (Hb) concentrations below WHO cut-offs*

Age (years)	Male			Female		
	Hb cut-off (g/L)	*Base*	% below cut-off	Hb cut-off (g/L)	*Base*	% below cut-off
1.5–4.5	110	*475 (unw)*	8.1	110	*476 (unw)*	9.1
4–6	110	*81 (unw)*	2.5	110	*76 (unw)*	9.1
	115		7.8	115		15.2
7–10	115	*176 (unw)*	1.4	115	*133 (unw)*	4.6
11–14	115	*166 (unw)*	3.1	115	*157 (unw)*	1.8
	120		8.1	120		4.3
15–18	130	*140 (unw)*	1.1	120	*156 (unw)*	9.1
19–24	130	*83 (w)*	0.0	120	*81 (w)*	6.9
25–34	130	*170 (w)*	2.1	120	*162 (w)*	9.1
35–49	130	*191 (w)*	3.7	120	*243 (w)*	9.0
50–64	130	*194 (w)*	3.4	120	*196 (w)*	6.7
65–74 free-living	130	*284 (w)*	7.0	120	*311 (w)*	5.9
75–84 free-living	130	*125 (w)*	16.0	120	*190 (w)*	13.1
85+ free-living	130	*20 (w)*	37.5	120	*53 (w)*	16.0
65+ Institutionalised	130	*147 (w)*	52.2	120	*135 (w)*	38.6

* *Except for children aged 1½–4½ years, weighting factors were used to adjust for over- or under-sampling of subgroups of the population, based on age, sex and socio-demographic factors.*

Table A37: LIDNS – Proportion with haemoglobin (Hb) concentrations below WHO cut-offs*

Age (years)	Male			Female		
	Hb cut-off (g/L)	*Base (unw)*	% below cut-off	Hb cut-off (g/L)	*Base (unw)*	% below cut-off
19–34	130	*67*	1	120	*205*	12
35–49	130	*99*	0	120	*250*	18
50–64	130	*135*	5	120	*181*	6
65+	130	*155*	20	120	*270*	9

* *Children aged 8–18 not included as sample sizes were too small.*

Table A38: NDNS – Proportion with serum ferritin (SF) concentrations below WHO cut-offs

Age (years)	SF cut-off (µg/L)	Male		Female	
		% below cut-off	*Base*	% below cut-off	*Base*
1.5–4.5	12	33.5	*467 (unw)*	25.1	*463 (unw)*
4–6	12	4.1	*65 (unw)*	6.3	*57 (unw)*
	15	9.8		9.2	
7–10	15	6.7	*141 (unw)*	2.9	*93 (unw)*
11–14	15	5.0	*137 (unw)*	11.5	*121 (unw)*
15–18	15	2.7	*110 (unw)*	23.8	*127 (unw)*
19–24	15	0.0	*85 (w)*	16.0	*80 (w)*
25–34	15	0.0	*169 (w)*	8.2	*156 (w)*
35–49	15	2.5	*186 (w)*	12.5	*238 (w)*
50–64	15	2.3	*194 (w)*	8.6	*195 (w)*
65–74 *(free-living)*	15	5.2	*272 (w)*	6.2	*306 (w)*
75–84 (*free-living)*	15	5.8	*121 (w)*	12.3	*177 (w)*
85+ *(free-living)*	15	7.4	*19 (w)*	14.3	*49 (w)*
65+ *(Institutions)*	15	8.0	*63 (w)*	10.2	*122 (w)*

Table A39: LIDNS – Proportion with serum ferritin (SF) concentrations below WHO cut-offs

Age (years)	SF cut-off (µg/L)	Male	Female
		% below cut-off	% below cut-off
19–34	15	0	21
35–49	15	0	14
50–64	15	4.9	5
65+	15	2.3	4

Base numbers as for table A37

Table A40: NDNS: Proportion with haemoglobin (Hb) and serum ferritin (SF) concentrations below WHO cut-offs

Age (years)	Male			Female		
	Hb cut-off (g/L)	SF cut-off (µg/L)	% below Hb and SF cut-offs	Hb cut-off (g/L)	SF cut-off (µg/L)	% below Hb and SF cut-offs
1.5–2.4	110	12	5.8	110	12	6.0
2.5–3.4	110	12	3.5	110	12	3.6
3.5–4.5	110	12	2.2	110	12	2.8
4–6	110	12	0.0	110	12	1.7
	115	15	0.0	115	15	1.7
7–10	115	15	0.6	115	15	0.0
11–14	115	15	1.2	115	15	1.9
15–18	130	15	0.0	120	15	5.0
19–24	130	15	0.0	120	15	3.8
25–34	130	15	0.0	120	15	2.0
35–49	130	15	0.9	120	15	4.8
50–64	130	15	0.5	120	15	2.9
65–74 (free-living)	130	15	1.4	120	15	1.6
75–84 (free-living)	130	15	0.5	120	15	3.3
85+ (free-living)	130	15	5.7	120	15	5.6
65+ (institutions)	130	15	5.0	120	15	3.3

Table A4 1: LIDNS: Proportion with haemoglobin (Hb) and serum ferritin (SF) concentrations below WHO cut-offs

Age (years)	Male			Female		
	Hb cut-off (g/L)	SF cut-off (µg/L)	% below Hb and SF cut-offs	Hb cut-off (g/L)	SF cut-off (µg/L)	% below Hb and SF cut-offs
19–34	130	15	0.0	120	15	9.1
35–49	130	15	0.0	120	15	10.5
50–64	130	15	0.0	120	15	2.9
65+	130	15	2.3	120	15	6.3

Preliminary iron intake data from year 1 of the NDNS rolling programme (2008/09)

Table A42: Contribution (%) of food types to average daily intake of total iron

Food type	1½–3 years	4–10 years		11–18 years		19–64 years	
		Males	Females	Male	Females	Males	Females
Cereal and cereal products (includes bread, pasta, breakfast cereals, biscuits, cakes)	50	56	52	48	48	38	36
Milk and milk products	6	2	3	1	2	1	1
Eggs and egg dishes	4	2	3	3	3	3	3
Fat spreads	0	0	0	0	0	0	0
Meat and meat products	12	13	14	20	19	23	19
Fish and fish dishes	2	2	2	1	2	3	3
Vegetables (including potatoes)	13	13	14	13	15	16	18
Fruits and nuts	5	2	3	1	2	2	3
Sugars, preserves, confectionery	2	2	2	2	2	2	2
Drinks (includes soft drinks, fruit juice, beverages, alcohol)	2	2	2	3	2	7	9
Total number respondents (unw)	*121*	*119*	*119*	*114*	*110*	*181*	*253*

Table A43: Average daily intake of iron (mg/day) from food sources only (median)

Age (years)	Males	*n*	Females	*n*
1.5–3 *(boys and girls combined)*	6.2 (6.0)		–	*121*
4–10	9.0 (8.8)	*119*	8.1 (8.0)	*119*
11–18	11.1 (11.1)	*114*	8.5 (8.3)	*110*
19–64	12.3 (11.8)	*181*	10.0 (9.9)	*253*

Table A44: Average total daily iron intakes (from food sources only) as a proportion of DRVs

Age (years)	Male		Female	
	%<LRNI	Mean intake as % of RNI	%<LRNI	Mean intake as % of RNI
1.5–3 *(boys and girls combined)*	6	89		
4–10	1	120	2	109
11–18	7	98	46	58
19–64	1	141	20	82

Modelling the impact of reductions in red and processed meat consumption on intakes of iron, zinc and vitamin D

1. The aim of this analysis was to estimate current consumption of red and processed meat and to use this information to model the impact of reducing red and processed meat consumption on iron and zinc intakes. An assessment was also made of the effect of reducing red and processed meat consumption on vitamin D intakes.

Background

2. Epidemiological studies suggest a link between red and processed meat consumption and risk of colorectal cancer (CRC). The available data suggest that processed meat consumption is associated with CRC risk independently of red meat. As these meats are a source of iron and zinc in the diets of the UK population, any recommendation to reduce consumption could increase the proportion of the population with intakes below the LRNI[108] for these nutrients. Red meat is also a dietary source of vitamin D, so reduced consumption could also reduce dietary vitamin D intake.

Methods

Defining categories for analysis

3. Processed meat typically refers to meat (usually red meat) that has been preserved by smoking, curing, salting, or by the addition of preservatives (WCRF, 2007); however, there is considerable inconsistency in the definition and categorisation of "processed meat" in epidemiological studies.

4. Following a review of the evidence by Food Standards Agency (FSA) toxicologists, it was advised that the available data do not support an association between increased CRC risk and the presence of preservatives (see Annex 8). The modelling exercise therefore considered the impact of a reduction in total red meat consumption (as defined in Appendix 1), as there was no clear basis for separating red and processed meat.

Estimates of total red meat consumption

5. Currently there are no accurate estimates for total red meat consumption in the UK. The National Diet and Nutrition Surveys (NDNS) provide estimates of total meat and meat dishes consumed. Red meat reported as consumed on its own, e.g. roast beef, can be isolated, but red meat products and meals containing red meat

108 The amount of a nutrient that is likely to meet the needs of only 2.5% of the population.

are composite dishes that also contain non-meat components (e.g., sausage rolls, lasagne and pies). These composite dishes are reported as total amount consumed, resulting in an overestimation of red meat consumption.

6. In order to obtain more realistic estimates of total red meat consumption, composite dishes were disaggregated, i.e., the actual amount of red meat within these products was identified. For recipe data collected in the survey, the meat component within the recipe was identified. For manufactured meat products and dishes reported within the NDNS, composition data and ingredient declaration were used to establish the percentage red meat content of brand leaders (Appendix 2).

7. The analysis focused on adults aged 19–64 years (Henderson *et al*, 2002) (data collected 2000/01). Data for the NDNS adults aged over 65 years were collected in 1994/95 and were not included within the analysis as they were considered to be of limited validity.

Assigning values for iron and zinc content of types of red meat and products

8. Typical iron and zinc values, obtained from *McCance and Widdowson's The Composition of Foods* meat supplement publications (Chan *et al*, 1995; Chan *et al*, 1996), were assigned to each meat type (Appendix 1, Table A46). These values were used, together with estimates of consumption of each meat type within all red meat containing foods, to estimate iron and zinc intakes from total red meat.

Estimation of total iron and zinc intakes

9. In order to assess total iron and zinc intakes from all foods, data from trade associations,[109] as well as retail label data, were checked for details of fortified foods currently available on the market to reflect current fortification practices, since these may have changed since the survey was carried out and, as a consequence, iron and zinc values for specific products may have changed. However, the amendments made to nutrient databank values made no difference to overall population iron and zinc intakes.

Modelling the effect of reduced total red meat consumption on iron and zinc intakes

10. The aim of the modelling exercise was to consider the effects of reducing total red meat consumption on mean intakes of iron and zinc and on the proportion of adults with iron and zinc intakes below the Lower Reference Nutrient Intakes (LRNI) for iron and zinc.

109 Personal communication with The Food and Drink Federation (FDF) October 2008 in relation to foods currently fortified with iron. The British Retail Consortium (BRC) was also contacted requesting information on foods fortified with iron.

11. The potential effect of reducing total red meat consumption was assessed for the following maximum levels per day: 180g, 160g, 140g, 120g, 100g, 90g,[110] 80g, 70g,[111] 60g, 50g and 0g. Intakes of consumers exceeding the maximum consumption of the different levels of red meat were reduced to the maximum, while intakes of consumers below the maximum were left unchanged.

Results

12. Estimated intakes of total red meat, iron and zinc and the effect of reducing total red meat consumption on mean intakes of iron and zinc and on the proportion of the population with intakes below the LRNI are presented in section 10 of the main report (Tables 11 and 12).

Modelling the effect of reduced total red meat consumption on mean intakes of vitamin D

13. The modelling exercise also considered the effect of reducing total red meat consumption on mean vitamin D intake. Vitamin D is found in a few foods, such as oily fish, fortified margarines and breakfast cereals; smaller amounts are found in red meat and egg yolk (Food Standards Agency, 2002). There are currently no dietary recommendations for vitamin D intake for the general population in the UK, as the main source of vitamin D is from the action of sunlight on the skin (DH, 1991)[112].

14. Low vitamin D status is apparent in some groups of the UK population[113] (Ruston *et al*, 2004). As meat is a dietary source of vitamin D (Henderson *et al*, 2003), the effect of reducing consumption of total red meat down to a maximum of 50g/day on mean intakes of vitamin D was assessed. Typical vitamin D values obtained from *McCance and Widdowson's The Composition of Foods* meat supplement publications (Chan *et al*, 1995; Chan *et al*, 1996) were assigned to each meat type (Appendix 1, Table A46).

15. The results of the analysis (for consumers of red meat only) were as follows:

- Estimated mean intake of vitamin D from the diet (excluding supplements) is 3.7 µg/day for men and 2.9 µg/day for women.
- The estimated mean intake of vitamin D from red meat is 0.68 µg/day for men and 0.39 µg/day for women, which accounts for about one-sixth of dietary vitamin D.
- Reducing total red meat consumption to a maximum of 100 g/day or 90 g/day would cause a reduction in mean vitamin D intakes: from a current intake of 3.7

110 Daily recommendations for total red meat consumption (maximum 90 g per day) – Committee on Medical Aspects of Food Policy (COMA) (DH, 1998). The COMA figure is based on almost no disaggregation.

111 Based on weekly recommendations for red meat consumption (maximum 500 g per week) by the World Cancer Research Fund (WCRF) (WCRF, 2007). The WCRF committee did not address the issue of disaggregation; however, the most recent studies included in their meta-analysis (i.e. EPIC studies) were based on some disaggregation.

112 Specific population groups at risk from low vitamin D status, i.e., those confined to the indoors, Asian women and children living in UK, pregnant and lactating women, and the elderly, are recommended to consume 10 µg/day vitamin D in the form of a vitamin supplement (DH, 1991).

113 Data from the NDNS suggest low vitamin status in 3% of children aged 4–6 years, 11% in 11–14 years, 13% in 15–18 years, 15% of adults aged 19–64 years, and 8% of adults over 65 years.

µg/day for men to 3.6 µg/day; and from 2.9 µg/day to 2.8 µg/day for women.

- Reducing total red meat consumption to a maximum of 80 g/day or 70 g/day would cause a reduction in mean vitamin D intakes: from a current intake of 3.7 µg/day for men to 3.5 µg/day; and from 2.9 µg/day to 2.8 µg/day for women.
- Reducing total red meat consumption to a maximum of 50 g/day would cause a reduction in mean vitamin D intakes: from a current intake of 3.7 µg/day for men to 3.4 µg/day; and from 2.9 µg/day to 2.7 µg/day for women.

Limitations of the modelling

16. See paragraphs 10.53–10.57 of the main report.

Summary of results

17. The estimated mean total red meat consumption (consumers of red meat only) in 2000/01 was 88 g/day for men and 52 g/day for women.

18. Red meat makes a greater contribution to total zinc intake from all foods than to total iron intake: the contribution of iron from total red meat to total iron intake is 12% for men and 9% for women; the contribution of zinc from total red meat to total zinc intake is 32% for men and 27% for women.

19. Reducing red meat to a maximum of 100, 90 or 80 g/day would have a minimal effect on the proportion of people with intakes below the LRNI for iron or zinc.

20. Reducing total red meat to a maximum of 70 g/day would have a minimal effect on the proportion of people with intakes below the LRNI for iron, but would increase the proportion of men with intakes below the LRNI for zinc from 3.7% to over 5%.

21. Red meat contributes approximately one-sixth of dietary vitamin D intake. Reducing total red meat to a maximum of 100, 90, 80 or 70 g/day would have little effect on dietary vitamin D intakes of men or women.

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Estimation of current red meat consumption and modelling the effect of reduced consumption on iron, zinc and vitamin D intakes

Table A45: Assumptions made in order to estimate current total red meat consumption in relation to colorectal cancer risk and the effect of reducing red meat consumption on iron, zinc and vitamin D intakes in UK adults

Subject	Assumption	Comments
	Estimation of red meat consumption	
Categorisation of total red meat	Risk factors for red and processed meat CRC risk • The epidemiological evidence indicates that increased consumption of red and processed meat increases the risk of colorectal cancer (CRC). • By contrast, an increased risk of CRC is not observed with increased total meat and therefore increased chicken or fish intakes are not considered to pose a risk. • The data suggests that the CRC risk is associated with "processed meat" independently of red meat consumption (see main report for details). • From a toxicological perspective, there are three possible mechanisms that may explain a link between red and processed meat and CRC: the first is related to haemoglobin levels; the second to compounds created on cooking; and the third is in relation to preservatives present in processed meats (see main report for details). • However, there seems to be no clear evidence to identify the most likely mechanism. • This modelling work therefore focused on total red meat consumption. • It was considered prudent, however, not to rule out any effect the presence of preservatives (excluding sodium chloride) may have on CRC risk. An exercise was therefore carried out to identify red meat and red meat products that do and do not contain preservatives. The details of this categorisation are not included within this annex. However, it should be noted that for composite meat products identified as containing preservatives (e.g., sausages) the weight of the whole product consumed was estimated rather than the weight of the meat within the product, to account for the role that the preservative-containing part of the product has on CRC risk. This is explained further below.	
	Total red meat The following types of meat were included within the estimation of total red meat consumption:	In order to decide whether certain meat products should be included within the analysis as the weight of the meat components of the products, or the total weight of the meat products themselves, it was necessary to identify whether the products contain preservatives.

	Carcass meats: Beef, lamb, pork, veal, mutton, venison, hare, goat, including kebab meat (doner, shish, kofte), sliced meats (including ham and similar products), grill steaks, rib steaks. **Offal**: Offal and offal component of meat products (including haggis, black pudding, brawn and faggots) from corresponding red meat animals. **Red meat component of meat products**: meat balls, beef paste, Cornish pasties, pork pies, sausage rolls, Scotch eggs. some beef burgers (purchased for home cooking from retail and from McDonalds and Burger King). **Red meat products:** The total weight of the following red meat products was included in the analysis as they were identified as containing preservatives: sausages, salami, frankfurters, corned beef, spam, pâté, meat loaf and beef burgers eaten out of the home (except McDonalds and Burger King).	**Beef burgers** The World Cancer Research Fund (WCRF) report on cancer (2007)[1] states that minced meat and hamburgers are sometimes considered processed if they are preserved chemically. Recent retail and manufacturer research indicates: • The majority of retail chilled burgers contain preservatives.[2] Retail frozen burgers generally do not contain preservatives.[2] • Market share data[3] indicates that the majority of burgers sold in retail are frozen. • Burgers sold in large fast food chains (McDonalds, Burger King) do not contain preservatives.[2] • Burgers sold through catering suppliers such as 3663 and Brakes are mostly frozen and some do contain preservatives[4] – estimated at about 50% of product types, although there are plans towards removing all preservatives from frozen burgers. The National Diet and Nutrition Survey (NDNS) nutrient databank does not classify burgers according to their preservative content, so it is necessary to make assumptions about the likelihood of burgers containing and not containing preservatives being classified in particular food codes. It is possible to identify food codes that are more likely to be used for retail burgers or those more likely to be used for burgers purchased for consumption out of the home – for example, the code for *Beef Burgers Economy Frozen Raw* is likely to be purchased in retail and consumed at home, whereas the code for *Cheeseburger Takeaway*, will have been consumed out of the home. In addition Burger King and McDonalds products are coded separately. For the purposes of the modelling work, it was assumed that: • All burgers purchased for home cooking from retail outlets do not contain preservatives. • All those consumed out of the home (excluding McDonalds and Burger King) do. • It is appreciated this was an over-estimation as many burgers consumed out of the home do not contain preservatives. • However, assuming that all burgers purchased for home cooking from retail do not, this was an under-estimation so these should have balanced each other out during the modelling. • These assumptions relate to beef burgers only, all other burgers (e.g., lamb) were assumed not to contain preservatives and the weight of the meat content alone was estimated.

Subject	Assumption	Comments
		Meatballs Meatballs were assumed not to contain preservatives as the majority available in retail do not. Therefore consumption of the meat within meatballs was estimated.[2] **Sausages** The WCRF report classifies *sausages and frankfurters, to which nitrates, nitrites or other preservatives are added*, as processed.[1] Most sausages available in the UK contain preservatives except for some frozen sausages.[2] Market share data[3] suggest more chilled sausages are purchased compared to frozen. As with burgers, the nutrient databank does not classify sausages according to preservative content; however, unlike burgers, there does not seem to be a difference between sausages eaten in the home or out of the home. As only a small proportion of frozen sausages are preservative free, it was assumed that all sausages contain preservatives.[2] It is appreciated that this may have been a slight over-estimation. "Sausages" refers to all meat sausages excluding frankfurters and salami. **Cornish pasties, pork pies, sausage rolls, Scotch eggs** A few brands of sausage rolls do contain preservatives; however, the majority do not.[2] Therefore these products were all considered not to contain preservatives and the weight of meat within these products was estimated. Although pork and egg pies tend to contain preservatives[2], the weight of the pork within the pie was used in the calculation rather than the preservative-containing part, as this was difficult to estimate from data provided within the ingredients list.
		Offal • Offal from red meat animals was included within the red meat category, including offal within products: haggis, black pudding, faggots and brawn. These products generally do not contain preservatives.[5] Some black pudding (Tesco) contains bacon,[2] and therefore contains preservatives; however, the majority do not contain bacon and therefore all black pudding was assumed not to contain preservatives and the consumption of the blood within the black pudding was estimated rather than consumption of the black pudding itself.

	Other meat Products **not** included in the estimation of total red meat: chicken, turkey, goose, duck, other wild birds e.g. pheasant, guinea fowl, partridge, pigeon, and rabbit, chicken and turkey burgers/ sausages, poultry offal. Also not included are: bacon flavour crisps, pork scratchings, lard, beef dripping, Bovril, gravy, gelatine, meat wastage (bones, skin etc.)	**Poultry burgers/sausages** • Although these products may be considered as "processed meat", poultry and other white meat are not considered a risk factor for CRC. • These products were not included within the analysis of total red meat.
		Poultry offal • Offal from poultry was not included within the estimation of total red meat. • Although likely to be very similar in composition to offal from red meat, it was considered more appropriate to be classified with poultry. • As it was likely to represent a small proportion of total offal consumed, this was not likely to have greatly affected the results.
	All products and dishes containing red meat were included in the analysis, although items such as meat stocks and beef extract were excluded.	• Within the nutrient databank there are a number of composite-meat-containing products, which do not have recipes assigned to them (generally retail or takeaway products or dishes). • Recipes have therefore been assigned to these products. This was done as follows: • Where known, the brand leader[3] was chosen for each product type and the stated % meat content used; e.g., for a lasagne ready meal, the meat content of the best-selling lasagne was used.[2,3] • Products for which market share data were not available, the % meat content from an equivalent known top-selling brand or retailer was used.[2] • Meat content was established from the label data obtained from retailer and manufacturer websites[2] or from supermarket searches where necessary. • Products that were no longer available on the market were compared with similar equivalent products. • As mentioned above for meat products containing preservatives, rather than estimating the meat content only, the content of the preservative-containing part of the meat product was estimated, thus including non-meat components. For example, consumption of *sausages* was estimated, not the consumption of *pork* within the sausages. • Similarly for burgers, those assumed to contain preservatives were considered 100% *beef burger,* whereas for those burgers assumed not to contain preservatives, % beef within the beef burger was estimated. This was also done for other meat products containing preservatives, including frankfurters, salami, corned beef, meat loaf, Spam and pâté. All were assumed to be 100% preservative-containing meat.

Subject	Assumption	Comments
		• The values obtained for meat content were broadly compared with TNS purchasing data 2008 provided by the Agriculture and Horticulture Development Board (AHDB).[6] Any major discrepancies were discussed with AHDB and the most appropriate figure was used.
	The % meat content was assumed to be % cooked weight except for updated recipes which use retail label data.	• The % meat content used was either established from recorded recipes within NDNS diaries and was therefore the equivalent % meat in the cooked dish (water loss on cooking has been taken into account). Alternatively, as described above, it was taken from retail label information. No attempt was made to adjust the meat content of these products for water losses on cooking.
		Iron content of foods
Natural iron content of foods	The analytical values within the nutrient databank for iron naturally present within foods reflect current values.	• The natural iron content of foods, including meat, was assumed not to have changed since analysis (early–mid 1990s) and inclusion within the NDNS nutrient databank. • There may have been recent changes within the meat industry towards leaner cuts of meat, or towards increased sales of organic less-lean meats, which would reduce/increase the fat content and therefore increase/reduce the iron content per 100 g, respectively. • However, changes such as these were not accounted for in the modelling due to lack of data on which to base any changes and the impact on overall iron intake would be small.
Iron content of types of red meat	The iron content of meat cuts/products were assumed to be standard average cooked values.	• The iron content of meat cuts used within the NDNS vary. • For the purposes of the modelling, standard average values for the iron content of *cooked* meat types were estimated using ranges of values from *McCance and Widdowson's Composition of Foods* meat supplements.[7,8] • Estimated standard iron values for each meat type using the modelling are listed in Table A46.
Foods fortified with iron – white and brown flour	Iron values for products containing flour within the nutrient databank were assumed to reflect current levels.	• Mandatory fortification of wheat flour with iron in the UK results in flour-containing products contributing to total iron intake. • The NDNS nutrient databank is updated as and when new analytical data become available. • Some food groups were last updated over 6 years ago and the proportion of flour within food products may have changed since analysis. • Data for flour within the databank is based on analysis carried out in 2003, but data for some flour-containing products, e.g., cakes and biscuits, are older.
	Potential for "overage"[9] of iron within flour- containing products was not taken into account.	As iron values for flour within the nutrient databank are analytical, the potential for overage would already have been captured within the analysis.

Foods fortified with iron – breakfast cereals	The number and types of breakfast cereals available on the market were assumed not to have changed since the NDNS.	• Current fortification practices were researched through retail and manufacturer websites[10] as well as information provided from trade organisations.[11] • Iron values were compared to those within the NDNS nutrient databank. Iron values for products within the databank for which the iron values have changed were updated to reflect current fortification practices. • It was not possible to account for new products available on the market since the NDNS survey was carried out (see above).
	For breakfast cereals a typical overage of 20% was applied.	• An overage is applied to breakfast cereals by manufacturers to account for raw material and process variability in order to meet the typical content as labelled. • The labelled value for iron is the total iron content of the product – natural and added.[12] As this overage is not added to account for degradation over time (as iron is stable and not prone to degradation), the full overage (20%) was assumed at the point of consumption.
Foods fortified with iron – other	The number and types of foods fortified with iron were assumed not to have changed since the NDNS. No adjustments were made for fortified foods introduced into the market since the NDNS, such as cereal bars, fruit juices etc.	Other fortified foods within the nutrient databank were compared with recent commercial data.[10] Any products already within the nutrient databank, i.e., consumed within the NDNS, which have recently become fortified or the level of fortification has changed, were updated. Although any changes made had a minimal effect on population iron intakes. **New products and changes in consumption patterns** • Each NDNS survey is accompanied by a nutrient databank which contains nutrient information for each food consumed within each survey. • The nutrient information in each databank is contemporaneous with the date of the survey so it is necessary to update the databank to take account of changes in fortification practices. • Some fortified foods such as cereal bars are more abundant than they were during data collection for the NDNS. • It is also not known in what quantity or frequency they are consumed, by which individuals or what foods they have taken the place of in the diet. • It is appreciated that excluding foods such as these may have resulted in an under-estimation in consumption of voluntarily fortified products. • Conversely, consumption of organic, simple foods (which are less likely to be fortified) is also likely to have increased, which may balance out any under-estimation.

Subject	Assumption	Comments
	Apart from for breakfast cereals, the potential for overage was not accounted for.	• Analytical values for iron were used where available in the nutrient databank. • For some fortified foods, only label data were available, which may have been a slight under-estimation of iron content as an overage may be applied by manufacturers in order to ensure that the label value for iron content is reached at time of consumption.
Zinc content of foods		
Zinc	Although the main focus of this modelling was to support the SACN report on iron and health, looking at the impact of reduced red meat consumption on iron intakes, red meat is also a source of zinc. Therefore an analysis of the impact of a reduction in red meat consumption was also assessed for zinc intake.	
Natural zinc content of foods	The analytical values within the nutrient databank for iron naturally present within foods reflect current values.	• As with iron, the natural zinc content of foods, including meat, was assumed not to have changed since analysis (early–mid 1990s) and inclusion within the National Diet and Nutrition Survey (NDNS) nutrient databank.
Zinc content of types of red meat	The zinc content of meat cuts/products was assumed to be standard average cooked values.	• The zinc content of meat cuts used within the NDNS vary. • For the purposes of the modelling, standard average values for the zinc content of *cooked* meat types were estimated using ranges of values obtained from *McCance and Widdowson's Composition of Foods* meat supplements.[7,8] • Estimated standard zinc values for each meat type used in the modelling are listed in Table A46.
Foods fortified with zinc	The number and types of foods fortified with zinc on the market were assumed not to have changed since the NDNS.	• The NDNS nutrient databank was checked for foods fortified with zinc. • Databank values were compared to label values on retailer and manufacturer websites[12] and the nutrient databank values were updated where appropriate. • Any fortified foods not contained within the databank were not accounted for. • Overage was not taken into account for foods fortified with zinc.

Vitamin D content of foods		
Vitamin D	As meat is also a dietary source of vitamin D, the effect of reducing red meat consumption was also assessed for vitamin D intakes.	
Natural vitamin D content of foods	The analytical values within the nutrient databank for vitamin D naturally present within foods reflect current values.	• As with iron and zinc the natural vitamin D content of foods, including meat, was assumed not to have changed since analysis (early–mid 1990s) and inclusion within the NDNS nutrient databank.
Vitamin D content of types of red meat	The vitamin D content of meat cuts/ products was assumed to be standard average cooked values.	• The vitamin D content of meat cuts used within the NDNS vary. • For the purposes of the modelling, standard average values for the vitamin D content of *cooked* meat types were estimated using ranges of values obtained from *McCance and Widdowson's Composition of Foods* meat supplements[7,8] and the NDNS nutrient databank. • Estimated standard vitamin D values for each meat type used in the modelling are listed in Table A46.
Other assumptions		
Other nutrients	The effect of reducing red meat consumption on the intake of other nutrients was not considered.	• Red meat is also considered a good source of protein and B vitamins (in particular B12)[13]; however, intakes of these nutrients are well above the Dietary Reference Values (DRVs).[14,15,16] • Red meat is also a source of selenium[15]; however, selenium was not included in the nutrient databank for NDNS adults 2000/01 as there was insufficient selenium composition data available at that time, so no estimate of selenium intake is available for that survey.
Processing losses	The potential for losses/gains in iron content as a result of food processing was not accounted for.	• Although iron can be both removed (through leaching) and added during processing (from equipment/instruments) (see main report for more details) this was not taken into account. • Where recipes contain ingredients fortified with iron such as flour and fortified breakfast cereals, additions/removal through cooking/processing were not taken into account. • The reason for this is that there is no standard factor that could be applied across products to take these losses or gains into account.

Subject	Assumption	Comments
Bio availability	Changes in the bioavailability of iron as a result of food processing have not been accounted.	• Cooking may increase iron bioavailability; heat processing can affect iron solubility and can denature haem iron. • Cooking and baking can also destroy ascorbic acid and reduce iron bioavailability (see main report for details). • These factors were not accounted for in the modelling.
	Iron intake was not split into haem and non-haem iron, but measured as total iron intake.	• In general haem iron is absorbed more efficiently than non-haem iron (see main report for details), • However, bioavailability was taken into account in setting the DRVs.[16] • Therefore for the purposes of this modelling, total iron intake was estimated, rather than splitting into haem and non-haem iron.
	The potential for oxidation/reduction of iron species and thus a change in bioavailability was not taken into account within the modelling.	
Under-reporting	Dietary intake data from NDNS series were assumed to represent usual intake. Potential for under-reporting was not considered.	• Dietary surveys such as the NDNS are prone to bias in reporting. • No attempt was made to adjust the energy and nutrient intakes presented in the NDNS report to take account of under-reporting.[14,15]
Population sample	The analysis used consumption data from the NDNS of adults aged 19–64 years[14,15] (data collected 2000/01) only. Data for the NDNS adults aged over 65 years[17] was collected in 1994/95 and considered of limited validity.	

Table A46: Standard iron, zinc and vitamin D values used for types of cooked meat [9, 10]

Meat	Iron (mg/100 g) cooked value	Zinc (mg/100 g) cooked value	Vitamin D (µg/100 g) Cooked value
Beef	2.3	5.8	0.6
Lamb (1)	1.8	4.1	0.5
Pork	1.0	2.9	0.8
Veal	1.0	3.5	1.5
Venison	5.1	3.9	0.5*
Ham (2)	0.9	2.1	0.8
Bacon	0.7	2.2	0.7
Sausages	1.1	1.3	1.1
Frankfurter (3)	1.1	1.4	0.5*
Salami (4)	1.3	3	0.5*
Haslet	1.9	1.5	0.2*
Polony	1.3	1.2	0.5*
Beef burger	2.7	6.2	1.9
Meat loaf	1.5	2.4	0.6*
Corned beef	2.4	5.5	1.3
Liver	12	8.6	0.8
Kidney	9	4	0.6*
Blood (5)	33	1.9	1.6
Pâté	0.5	2.7	1.2
Other offal (6)	3.4	3.6	Trace

(1) Assume values for goat are the same as for lamb as no nutrient data were available.

(2) Includes gammon, canned, Parma ham and pork shoulder.

(3) Frankfurter only (not including bun, ketchup or mustard).

(4) "Salami" includes pepperoni and chorizo.

(5) Based on the figures for cooked black pudding, assuming black pudding is 37% blood and the non-blood components of black pudding do not contribute to iron, zinc and vitamin D content.

(6) Offal encompasses brain, heart, tripe, lung, head, oxtail, tongue, sweetbread, trotters and tails.

* Vitamin D values for these products are denoted by "N" within McCance and Widdowson's Composition of Foods 9, 10 as the nutrient is present in significant quantities, but there is no reliable information on the amount. For the purposes of this modelling therefore, values have been taken from the NDNS nutrient databank, where estimates for vitamin D content of these products have been assigned for purposes of dietary analysis.

References

1. World Cancer Research Fund. Food, Nutrition, Physical Activity and the Prevention of Cancer: a global perspective. 2007 [available online] www.dietandcancerreport.org/

2. Retail and Manufacturer websites: www.asda.com; www.ocado.com; www.sainsburys.com; www.tesco.com; www.burgerking.com; www.mcdonalds.com; www.birdseye.co.uk; www.findus.co.uk; www.ginsters.com; www.turkeyfortoday.com; (accessed August 2008)

3. Taylor Nelson Sofres (TNS) market share data (2006).

4. Personal communication with 3663 and Brakes Food Service (August 2008).

5. Personal communication with The Bury Black Pudding Company (October 2008) and retailer and manufacturer websites: www.asda.com; www.ocado.com; www.sainsburys.com; www.tesco.com; www.farmingfriends.com; (accessed October 2008).

6. The Agriculture and Horticulture Development Board (AHDB) provided FSA with Taylor Nelson Sofres (TNS) estimates of % meat content based on purchasing figures for GB 2008.

7. Chan, W., Brown, J., Lee, S, and Buss, D.H. Meat, Poultry and Game. *Fifth supplement to 5th edition of McCance and Widdowson's The Composition of Foods.* Royal Society of Chemistry, Cambridge, 1995.

8. Chan, W., Brown, J., Church, S.M. and Buss, D.H. Meat Products and Dishes. *Sixth supplement to 5th edition of McCance and Widdowson's The Composition of Foods.* Royal Society of Chemistry, Cambridge, 1996.

9. Manufacturers often add an "overage" during fortification to ensure the label value is achieved at point of consumption. This is done as some vitamins and minerals are prone to degradation over time or losses during processing.

10. Retail and Manufacturer websites: www.asda.com; www.ocado.com; www.sainsburys.com; www.tesco.com; www.horlicks.co.uk; www.ovaltine.co.uk; www.slimfast.co.uk; (accessed August 2008).

11. Personal communication with the Food and Drink Federation (FDF). The British Retail Consortium (BRC) were also contacted requesting data (October 2008).

12. Personal communication with Kellogg's (August 2008).

13. Food Standards Agency advice [available online] http://www.eatwell.gov.uk (accessed March 2009).

14. Henderson L, Gregory J, Irving K, Swan G. National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 2: Energy, protein, carbohydrate, fat and alcohol intake. London: TSO, 2003.

15. Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G, Farron M. National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 3: Vitamin and mineral intake and urinary analytes. London: TSO, 2003).

16. Department of Health. Dietary Reference Values for Food, Energy and Nutrients in the United Kingdom. (Report on Health and Social Subjects, No. 41). London: HSMO, 1991.

17. Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G, Clarke PC. National Diet and Nutrition Survey: people aged 65 years or over. Volume 1: Report of the diet and nutrition survey. London: TSO, 1998.

Total red meat content of manufactured/retail foods within NDNS

1. Table A47 lists red meat containing food codes for retail/manufactured foods consumed within the NDNS and their corresponding estimated total red meat content. The methodology for estimating the meat content of these foods is explained in detail in Appendix 1. It should be noted that these are estimates based on data available from retailer and manufacturer websites, as well as food ingredient labels. Products that were no longer available or could not be found on the market were compared with similar equivalent products. These data were agreed in consultation with the Agriculture and Horticulture Development Board (AHDB).

Table A47: Estimated red meat content of manufactured/retail foods within the NDNS. The type of red meat within the food codes corresponds to the categories of red meat listed in Table A46 of Appendix 1

NDNS food code name	% red meat content
Bacon and cheese grills retail	48% bacon
Bacon and egg in a bun	33% bacon
Bacon and turkey in breadcumbs retail	55% bacon
Bacon crunchies coated breadcrumbs purchased	21% bacon, 8.6% pork
Baked beans in tomato sauce with pork sausages	17% sausage
Baked beans low fat sausage	22% sausage
Bbq sizzlers retail	58% pork
Beans baked with additions (burgers) not sausages	18% beef
Beef burger and onion fried not 100% meat	60% beef
Beef burger and onion grilled	77% beef
Beef burger economy fried	60% beef
Beef burger economy grilled	60% beef
Beef burgers economy frozen raw	60% beef
Beef burgers in gravy canned	40% beef
Beef burgers low-fat fried	80% beef
Beef burgers with onion frozen raw	77% beef
Beef casserole ready meal in gravy and veg	22% beef
Beef chicken and pork satay	22% pork, 22% beef
Beef cobbler with baked beans and carrots retail	12% beef
Beef curry as served with rice retail	6% beef
Beef curry frozen/chilled ready meal no rice	24% beef
Beef curry no rice retail	11% beef
Beef curry with rice ready meal retail	12% beef
Beef enchilladas ready meal retail	21% beef
Beef hot pot with pots ready meal retail	24% beef
Beef in red wine sauce with mashed potato retail	22% beef
Beef minced in gravy canned	75% beef
Beef minced pie filling canned	75% beef
Beef minced reformed pie filling + onion canned	75% beef
Beef oriental with rice retail	10% beef

NDNS food code name	% red meat content
Beef stew and dumplings frozen or chilled ready meal	10% beef
Beef stewed made w canned stewing steak + pulses	12% beef
Beef stewing reformed pie filling canned	75% beef
Beef wellington	31% beef
Black pudding fried	37% blood
Black pudding in batter takeaway	17% blood
Bolognese sauce canned	28% beef
Bridies scotch pies	14% veal
Burger king double supreme	63% beef
Burger king double whopper only	46% beef
Burger king double whopper with cheese only	43% beef
Burger king whopper only	30% beef
Burger king whopper with cheese only	27% beef
Cannelloni	15% beef
Cheeseburger takeaway not quarter pounder	39% beef burger
Chicago ribs pork and tvp in marinade retail	27% pork
Chicken and bacon lasagne purchased ready meal	3% bacon
Chicken and bacon pancakes retail	2% bacon
Chicken and bacon pasta gratin retail	3% bacon
Chicken in white sce ham mush and rice ready made	7% ham
Chicken with bacon, fromage frais, mash, reduced fat retail	2.7% bacon
Chicken, bacon, mushroom and cream pie	5% bacon
Chilli con carne canned	23% beef
Chilli con carne with rice ready meal	19% beef
Chilli con carne, no rice ready meal	35% beef
Chinese luncheon meat steamed	50% beef
Chinese meat buns	30% pork
Combination pizza deep pan base	2.8% salami
Combination pizza french bread base	3.4% salami
Combination pizza thin crispy base	3.4% salami
Corned beef and onion pie purchased	20% corned beef
Corned beef crispbake retail	38% corned beef
Corned beef pasty purchased	20% corned beef
Cornish pasty purchased	14% beef
Cornish pasty reduced fat retail	14% beef
Cumberland pie potato and cheese topping retail	19% beef
Doner kebab	50% lamb
Doner kebab with pitta ready purchased	27% lamb
Faggots in gravy ready meal	11% pork
Fortified pasta shapes with mini sausages	14% sausage
Fortified pasta shapes with mini sausages	14% bacon
Frankfurter in a bun with ketchup onions and mustard	18% frankfurter
Fresh egg pasta ravioli retail	6% pork, 2.4% beef
Gammon steaks in honey mustard and ginger retail	71% ham
Ham and pork chopped canned	50% pork, 50% ham
Ham cheese and leek pie retail	7% ham
Ham leek and potato pie retail	12% ham
Ham mushroom and cheese lattice retail	16% ham
Ham pate low fat purchased	100% pate
Hamburger big mac Mcdonalds	75% beef

NDNS food code name	% red meat content
Hamburger in bun takeaway	43% beef burger
Hamburger quarter pounder takeaway	67% beef burger
Hamburger quarter pounder with cheese takeaway	58% beef burger
Individual steak and kidney pie flaky	8.5% kidney, 21% beef
Individual steak pie flaky pur	30% beef
Irish stew canned	30% lamb
Kofte kebab	42% lamb
Kosher salami chicken and beef	40% beef, 20% salami
Lamb curry, takeaway, e.g., rojan josh, no rice	32% lamb
Lamb hot pot with pots ready meal	31% lamb
Lamb roast roll cooked	78% lamb
Lamb shepherd's pie retail	15% lamb
Lasagne frozen	16% beef
Lasagne frozen	3% bacon
Lasagne, reduced fat retail	14% beef
Liver and onion gravy retail	37% liver
Liver sausage	100% sausage
Low-fat liver pate	100% pate
Macaroni chicken and bacon ready meal retail	3% bacon
Meat balls and pasta/baked beans	7% beef
Meat balls in barbecue sauce	53% beef
Meat based pizza deep pan base	3.5% beef, 3% salami
Meat based pizza french bread based	2.6% beef, 2.6% ham
Meat based pizza thin crispy base	10% beef, 8% salami, 8% ham
Meat loaf delicatessen	100% meat loaf
Meat paste canned	36% beef
Meat paste not canned	37% beef
Meatballs in gravy with mashed potato ready meal	10% beef, 3.5% sausage
Mexican chilli slice retail	11% beef
Microwave sausages pork and beef	100% sausage
Minced beef and veg (pot peas carrots) ready meal	12% beef
Minced beef crispbakes oven baked purchased	12% beef
Minced beef pancakes grilled retail	12% beef
Minced beef pie pastry top and bot	18% beef
Minced beef pie purchased	18% beef
Minced beef pie purchased two crusts	18% beef
Minced beef pie top pastry	35% beef
Moussaka ready meal chill/frozen/long life	24% lamb
Oxtail soup canned	4% beef, 1% other offal
Pasta ravioli canned in tomato sauce	7% beef
Pasta spaghetti canned in bolognaise sauce	4% beef
Pizza ham deep pan	6% ham
Pizza ham mushroom chicken green pepper, deep pan	5% ham
Pizza ham thin base	9% ham
Pollack, chicken, prawn and salami paella retail	1.3% salami
Pork and egg pie	26% pork
Pork pie buffet	22% pork
Pork pie individual	26% pork
Pork pie sliced	30% pork
Pork roast dinner frozen ready meal	16% pork
Pork roast roll cooked retail	80% pork

NDNS food code name	% red meat content
Pork sausage snack bar meal	62% sausage
Pork steaks with honey and mustard retail	76% pork
Potato and corned beef pasty purchased	20% corned beef
Potato, bean and bacon melt retail	13% bacon
Quiche lorraine s/c pastry purchased	14% bacon
Quiche with beef, pork, ham and peppers	2% ham, 5% beef, 5% salami
Ravioli not canned	16% beef
Ready meal-steak in red wine + veg	18% beef
Roast beef dinner with yorkshire pud potatoes veg	8% beef
Roast beef in gravy purchased ready meal	54% beef
Roast pork in gravy frozen ready meal no pots/veg	54% pork
Samosa-meat filled	28% lamb
Sausage burger retail	44% sausage
Sausage hotpot with baked beans canned retail	16% sausage
Sausage in batter fry comm oil	54% sausage
Sausage roll flaky pastry purchased	28% pork
Sausage roll shortcrust pastry purchased	28% pork
Sausages in batter grilled retail	54% sausage
Scotch egg	26% pork
Scotch egg mini	28% pork
Shepherds pie frozen purchased ready meal	26% lamb
Shepherds pie frozen/chilled lamb ready meal	26% lamb
Shepherds pie with baked beans retail	12% lamb
Shish kebab	37% lamb
Sliced lamb roast dinner frozen retail	14% lamb
Spaghetti bolognaise low fat	11% beef
Spaghetti bolognaise ready meal	14% beef
Spare ribs in barbecue sauce no bones	27% pork
Spare ribs, barbecue style, e.g., takeaway, with bones	27% pork
Spinach and ham quiche retail	8% ham
Steak and kid pie 2 crusts s/c pastry not ind.	3% beef, 11% kidney
Steak and kidney pie 2 crust s/c	8.5% kidney, 21% beef
Steak and kidney pie canned	19% beef, 15% kidney
Steak and kidney pie filling can	60% beef, 15% kidney
Steak and kidney pudding canned	16% beef, 13% kidney
Steak pie canned steak lard golden churn in pastry	50% beef
Stewed meat (canned) and potato pie	14% beef
Stewing steak / meatballs in gravy canned	20% pork
Stewing steak canned with potatoes carrots and dump.	26% beef
Stewing steak in gravy pie filling canned	77% beef
Sweet and sour pork frozen ready meal no rice	36% pork
Sweet and sour pork with rice frozen ready meal	11% pork
Sweet and sour pork, battered with/without sauce	50% pork
Tagliatelle carbonara ready meal retail	7% bacon
Tagliatelle carbonara, reduced fat, ready meal, retail	12% ham
Turkey and bacon loaf retail	40% bacon,
Turkey and ham crispbakes retail	4% ham
Turkey and pork luncheon meat retail	29% pork
Veal jellied canned	85% veal
Xtra lean stewing steak in gravy canned	77% beef

Explanation of adjustment made to meat consumption estimates in the 2000/01 NDNS and the NDNS rolling programme year 1 (2008/09) to enable comparison between surveys

1. Estimates of red meat consumption for consumers only were derived for adults aged 19–64 years from the NDNS 2000/01 and the NDNS rolling programme year 1 (2008/09) using the same method to disaggregate the red meat content of composite dishes. However, differences between the two surveys in the data collection methods meant that the two estimates were not directly comparable:

   - While the NDNS of adults 2000/01 was based on a seven-day diary, the estimate from the NDNS rolling programme year 1 (2008/09) is based on a four-day diary. Estimates for mean consumption of foods excluding non-consumers will be higher when assessed over a four-day rather than a seven-day period, especially for foods that are not consumed every day.
   - Year 1 of the NDNS rolling programme over-sampled weekend days, so Saturdays and Sundays are over-represented in the dataset while weekdays are under-represented and Wednesdays are not sampled at all (as a consequence of always including weekend days in a four-day diary). NDNS 2000/01 has equal representation of days of the week, as a seven-day diary was used. Consumption of some foods and nutrients is known to differ between weekdays and weekend days.

2. To enable comparison between the two surveys, these methodological differences were addressed in the following ways:

   - Data from the NDNS 2000/01 were reanalysed on a four-day basis to make the estimates more comparable with the NDNS 2008/09 data. This was done by sampling four days from the seven-day record for each individual so that each day of the week appeared equally in the NDNS 2008/09 dataset. [For a more detailed explanation, see Appendix K of the year 1 (2008/09) findings from the NDNS rolling programme.[114]]

114 Bates B, Lennox A, Swan G. National Diet and Nutrition Survey. Headline results from Year 1 of the Rolling Programme (2008/209). A survey carried out on behalf of the Food Standards Agency and the Department of Health. Available online at http://www.food.gov.uk/science/dietarysurveys/ndnsdocuments/ndns0809year1

- The NDNS 2008/09 estimate was re-weighted by day of week to give a more even spread of days of the week. This was done by calculating mean red meat consumption for consumers by day of week and then calculating an average of the daily means. Wednesday was assumed to be similar to Tuesday and Thursday and was taken as an average of those two days.

3. Although this reanalysis addresses the main methodological differences between the two surveys and allows a direct comparison of the two estimates, another key methodological difference between the two surveys is that the 2000/01 NDNS was based on weighed records, whereas the NDNS 2008/09 is based on estimated records. It is not possible to adjust for this difference as the direction of the effect, if any, is not known.

Glossary

Absorption	Uptake of a nutrient into the intestinal mucosa and its subsequent transfer into the body.
Acute phase reactants	Series of proteins produced in the liver in response to infection and inflammation.
Aerobic	Living or occurring only in the presence of oxygen.
Amenorrhoea	Absence or abnormal stoppage of menstrual period.
Anaemia	Abnormally low level of haemoglobin in the blood. The WHO thresholds for anaemia are haemoglobin concentrations of: 110g/L, children under 5 years; 115 g/L, children 5–11.99 years; 120 g/L, children 12–14.99 years and non-pregnant females over 15 years; 130 g/L, males over 15 years.
Antibody	Immunoglobulin molecules that only interact with the antigen that induces their synthesis, or with closely related molecules.
Antigen	Substance (usually foreign) that prompts the generation of antibodies and induces an immune response.
Apical membrane	Layer of plasma membrane on the apical side (the side towards the lumen) of the epithelial cells in a body tube or cavity.
Apotransferrin	See transferrin.
Atrophy	Wasting away; diminution in the size of a cell, tissue, organ or part of one.
Autosomal dominant	A trait or disorder in which the phenotype is expressed in those who have inherited only one copy of a particular gene mutation.
Avogadro's number	Number of molecules in a mole (gram molecular weight) of a substance. It equals 6.02 x 1023 molecules.
Basal transporter	Translocates ferrous iron from enterocytes into the body; also known as ferroportin 1.
Bioavailability	Proportion of a nutrient that is taken up and transferred by the intestinal mucosa and subsequently used systemically in the body.
Carcinogen	Any substance which is directly involved or contributes to the development of cancer.

Catalyst	Substance that accelerates a chemical reaction, but is not consumed or changed in the process.
Ceruloplasmin	A ferroxidase found in plasma that may be involved in the oxidation of ferrous iron to ferric iron during binding to transferrin.
Chromosome	Physical structure found in the nucleus of cells consisting of a large DNA molecule organised into genes and supported by proteins.
Conceptus	Product of conception, i.e., embryo and membranes at all stages of intrauterine development.
Cytokine	Regulatory protein (e.g., interleukin, lymphokine) that is released by cells of the immune system and acts as an intercellular mediator in the generation of an immune response.
Deferoxamine (DFX)	Iron-chelating agent. Used in treatment of conditions associated with excessive iron storage and iron poisoning.
Dietary Reference Value (DRV)	Distribution of requirements in a group of individuals for a nutrient. It is assumed to be normally distributed. This gives a notional mean requirement or Estimated Average Requirement (EAR) with the Reference Nutrient Intake (RNI) defined as two notional standard deviations above the EAR; intakes above the RNI will almost certainly be adequate to meet the needs of 97.5% of the population. The Lower Reference Nutrient intake (LRNI), which is two notional standard deviations below the EAR, represents the lowest intakes which will meet the needs of approximately 2.5% of individuals in the group.
Dose response	Relationship in which a change in the amount, intensity or duration of exposure is associated with a change in risk of a specified outcome.
Endocytosis	Uptake of extracellular material by invagination of the cell membrane, which then breaks off to form a vesicle enclosing the material.
Endogenous iron	Iron originating from within the body.
Enterocyte	Epithelial cell found in the small intestine and colon that helps to break up food molecules and transports them into the body tissues.
Enzyme	Protein molecule produced by living organisms that catalyses chemical reactions of other substances without itself being destroyed or altered during the reactions.
Epithelial cell	Cell that lines the cavities and surfaces of structures throughout the body.

Erythroblast	Precursor cell that divides and matures to form an erythrocyte.
Erythrocyte	Mature red blood cell that contains haemoglobin to carry oxygen to the body tissues. Lifespan of approximately 120 days.
Erythropoiesis	Synthesis of new red blood cells in bone marrow.
Erythropoietin	Glycoprotein produced by the kidney that stimulates red blood cell formation.
Exogenous iron	Iron originating from outside of the body (e.g., from foods, supplements).
Extrinsic	Not contained in the body; of external origin.
Ferric iron	Oxidised form of iron (Fe^{3+}).
Ferritin	Main intracellular iron storage protein; soluble.
Ferrous iron	Reduced form of iron (Fe^{2+}).
Ferroxidase	Any enzyme that catalyses the oxidation of iron ions.
Ferryl species	Iron in Fe^{4+} form.
Fortification	Addition of nutrients to foods during the manufacturing process.
Free iron	Iron not bound to protein or other large molecular weight biomolecules.
Free radical	Atom or group of atoms that contains an unpaired electron. Very reactive, with short half-life.
Genome	The full DNA sequence of an organism.
Genotoxic	Ability of a substance to cause DNA damage, either directly or after metabolic activation.
Genotype	Genetic constitution of an individual as distinct from its expressed features.
Gestation	Length of a pregnancy from conception to birth.
Glycosylation	Enzymatic process that links saccharides to produce glycans, which are fundamental components of all cells.
Guidance Level (GL)	An approximate indication of levels of a nutrient that would not be expected to cause adverse effects; set when evidence is inadequate to establish a Safe Upper Level (SUL). GLs are less secure than SULs because they are derived from limited data.
Haem iron	Iron found in the haemoglobin and myoglobin of foods such as meat, poultry and fish.

Haem oxygenase	Enzyme that catalyses the oxidation of haem molecules to release ferric iron.
Haemochromatosis	Hereditary disease caused by mutation of the gene coding for the HFE protein. It is characterised by excessive absorption of dietary iron causing high levels of iron to accumulate in the body.
Haemoglobin	Protein composed of globin and haem that gives red blood cells their characteristic colour; functions primarily to transport oxygen from the lungs to the body tissues.
Haemosiderin	Intracellular iron storage protein formed when the potential to store iron as ferritin is exceeded; insoluble.
Haptoglobin	Plasma protein which removes free haemoglobin from the circulation.
Hepatocyte	A liver cell.
Hepcidin	Polypeptide; principal regulator of iron absorption.
Hephaestin	Protein involved in metabolism and homeostasis of iron and possibly copper. It is a transmembrane copper-dependent ferroxidase responsible for transporting dietary iron from intestinal enterocytes into the circulatory system.
Heterogeneous	Varied in content; composed of different parts.
Heterozygote	Individual with one normal and one altered form of a particular gene.
Holotransferrin	See transferrin.
Homeostasis	The process by which the internal systems of the body maintain a balance despite external conditions.
Homogeneous	Having parts which are all the same or which consist of only one substance.
Homozygote	Individual with two identical forms of a particular gene.
Hypoxia	An inadequate supply of oxygen to the tissues of the body.
Incidence	Measure of the risk of developing some new condition within a specified period of time.
Intestinal helminthiasis	Infection of the intestine by a parasitic worm.
Iron chelation	Chemical reaction in which there is a combination with iron to form a ring-shaped molecular complex within which the iron is firmly bound and isolated.
Iron deficiency	An absence of iron depots in the tissues.

Iron deficiency anaemia	Anaemia due to an inadequate supply of iron for blood cell production. This type of anaemia responds to iron therapy.
Iron overload	Accumulation of excess iron in body tissues. Usually occurs as a result of genetic disorders of iron metabolism.
Iron status	Describes whether an individual has too little, enough, or too much iron in their body for their needs.
Kupffer cells	Specialised macrophage cells located in the liver. They play a key role in normal physiology of the liver as well as participating in the immune response.
Lacto-ovovegetarian	Does not consume meat, poultry, game, fish, shellfish or crustacean; does consume dairy products and eggs.
Lipoprotein	Complex molecule that consists of a protein membrane surrounding a core of lipids. The principal means by which lipids are transported in the blood. The lipid proportion and density can vary, e.g., low-density lipoprotein.
Lymphocyte	Type of white blood cell that occurs in two forms: B-lymphocytes and T-lymphocytes. Collectively responsible for antibody production and direct cell-mediated killing of virus-infected cells and tumour cells.
Lysosome	Membrane-bound sac within cells that contains digestive enzymes. Liposomes digest material in food vacuoles, foreign particles entering the cell and, on the death of the cell, are involved in the breaking down of all cell structures.
Macrophage	Type of white blood cell derived from monocytes that engulf invading antigens and ultimately stimulate production of antibodies against the antigen.
Menarche	First menstrual period.
Menopause	End of a woman's reproductive phase. Cessation of menstrual cycle.
Menses	See menstruation.
Menstruation	Periodic loss of blood from the uterus of non-pregnant women of reproductive age. Average menstrual cycle is 28 days. Also called menses.
Mitochondria	Organelles found in most cells (but not red blood cells). Involved in several cellular metabolic activities including haem synthesis and the breakdown of glucose to produce energy.

Monocyte	Type of white blood cell that is part of the immune system. In response to inflammation signals they divide/differentiate into macrophages and dendritic cells to elicit an immune response.
Mucosal cells	Cells forming the mucosa or lining of the gastro-intestinal tract.
Mutagen	An agent, such as a chemical, ultraviolet light, or a radioactive element, that can induce or increase the frequency of mutation in an organism.
Myelin sheath	Insulating layer surrounding nerve fibre; facilitates transmission of nerve impulses.
Myelination	Acquisition, development or formation of a myelin sheath around a nerve fibre.
Myoglobin	Oxygen-transporting protein containing haem iron found in muscle cells.
Neoplasia	Abnormal new growth of tissues.
Neutrophil	Most common type of white blood cell in the body; engulfs (by phagocytosis) and kills microorganisms including viruses and bacteria. Life span of approximately 1–3 days.
Non-haem iron	Iron that is not bound to haem. Found in animal and plant tissues as Fe^{2+} bound to insoluble proteins, phytates, oxalates and carbonates and as ferritin.
Oxidation	Loss of electrons from the outer shell of an atom; often accompanied by the transfer of a proton and therefore involves the loss of a hydrogen ion. The loss of electrons or hydrogens in a chemical reaction.
Parenchymal cell	Cells forming the basic ground tissue of an organ.
Parenteral nutrition	Delivery of nutrients directly into the circulatory system by a dedicated venous catheter. Method of providing nutritional support to individuals when the gastrointestinal tract is not functioning or is inaccessible.
Pathogenesis	Origin and development of disease.
Phagocytic cell	White blood cells that can engulf (by phagocytosis) and destroy microorganisms including viruses and bacteria; cells in this category include neutrophils and monocytes.
Phagocytosis	Process by which a cell engulfs foreign matter (e.g. microorganisms) or debris (e.g., old red blood cells).

Phenotype	Observable physical and/or biochemical characteristics/symptoms of the expression of a gene under a particular set of environmental factors. Results from interaction between the genotype and the environment.
Phlebotomy	Withdrawal or removal of blood from the circulatory system through incision or puncture of a vein. Used in the treatment of haemochromatosis.
Phytate (phytic acid)	Principal storage form of phosphorus in many plant tissues, especially bran and seeds; an inhibitor of non-haem iron absorption.
Polymorphism	Natural variations in a gene, DNA sequence or chromosome.
Polyphenols	Group of chemical substances found in plants; characterised by the presence of more than one phenol unit or building block per molecule; inhibitors of iron absorption.
Postpartum	Time period shortly after childbirth.
Prevalence	Percentage of cases of a disease in a population at a given time.
Protoporphyrin	Chemical intermediate that combines with iron and protein to form haemoglobin, myoglobin and certain respiratory pigments.
Reticulo-endothelial (macrophage) system	Part of the immune system; comprises phagocytic cells located in different organs of the body.
Safe Upper Level (SUL)	Intake of a chemical or nutrient that can be consumed daily over a lifetime without significant risk to health; based on adequate available evidence.
Sequelae	Any abnormal condition that follows and is the result of a disease, treatment or injury.
Thalassaemia	Inherited blood disorder resulting in reduced synthesis of haemoglobin and subsequent chronic anaemia from blood cell destruction.
Total iron binding capacity	Measure of the binding capacity of transferrin for iron.
Transferrin	Protein synthesised in the liver that transports iron in the blood. It can bind two molecules of ferric iron. When it is not bound to iron it is known as apotransferrin; when it is bound to iron, it is known as holotransferrin.
Transgenic model	An organism for scientific study (e.g., mouse) that has had genes from another organism transferred into its genome.

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