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THE RELATIONSHIP BETWEEN IRON NUTRITION AND THE FACTORS PRESENT IN VEGETABLE AND CEREAL FOODS THAT AFFECT IRON ABSORPTION

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A thesis submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, for the Degree of Doctor of Philosophy in Medicine.

Johannesburg, 1986

For Andrew, Alexandra and Jeremy.

This thesis is my own work. No part of it has been presented at any other University. The information used in this thesis was obtained while I was employed by the University of the Witwatersrand.



M.A. Gillooly

ABSTRACT

The iron nutrition of populations is largely determined by the quality of the diet consumed. As this is frequently cereal or vegetable based, the interplay of the different enhancers and inhibitors that influence iron absorption from the "common pool" of non-haem iron assumes a profound significance in iron nutrition. This thesis took advantage of the widespread occurrence of iron deficiency among Indian women in Natal to establish more clearly the factors in frequently consumed foods which affect iron absorption.

In all the studies performed, iron absorption was measured using the method of extrinsic labelling of non-haem iron. The absorption values were then calculated on the basis that 100% of the absorbed radioactivity was present in the haemoglobin of circulating erythrocytes. The iron status of the subjects was evaluated by measuring their haemoglobin, serum iron concentration and unsaturated iron binding capacity. Iron stores were assessed using the serum ferritin value. The individual absorption results were made comparable by correcting them to a 40% reference absorption from 3 mg iron given as $FeSO_4$ with 30 mg ascorbic acid. This value is the average percentage absorption found in individuals who lack iron stores but who are not yet anaemic.

Initially non-haem iron absorption from a variety of vegetable meals was measured with the aim of correlating the

chemical composition of the foodstuff with the bioavailability of its intrinsic iron. Using a basic rice meal the addition of 1 g of certain organic acids (ascorbic acid, citric acid, L-malic acid and tartaric acid) led to a marked improvement in iron absorption. The same applied to vegetables containing these acids:- all those vegetables associated with a moderate or good iron bioavailability contained appreciable amounts of one or more of these organic acids. In contrast, those vegetables associated with poor bioavailability of the iron tended to have a low content of the carboxylic acids and a high phytate content. Poor iron absorption values were also obtained from vegetables having high total polyphenol contents. The significant inverse correlation between iron absorption and total polyphenol content (r Ø,859; $p < Ø,ØØ1$), was however even greater for the non-hydrolysable polyphenol content of the vegetables (r Ø,9Ø1; $p < Ø,ØØ1$).

Having found that the phytate and polyphenol composition of vegetables had a marked effect on iron absorption, a series of studies evaluating cereals was carried out. Modifying the polyphenol and phytate contents of sorghum by pearling procedures substantially altered iron absorption. When the content of these two inhibitors was reduced, iron absorption increased significantly. Iron absorption from a polyphenol-free cultivar of sorghum was superior to that from a cultivar containing polyphenols and iron was poorly absorbed from the phytate rich pearlings of

the strain that was free of polyphenols. The effect of fibre, which is an important component of cereals, on iron absorption was found to vary according to the constituent examined. Both hemicellulose and lignin depressed iron absorption, whilst pectin, guar gum, gum tragacanth and microcrystalline cellulose did not alter the bioavailability of iron.

Finally, a series of studies was designed in order to examine the effect of ascorbic acid on improving iron bioavailability from a soy bean based infant milk formula. Soy is potentially a valuable source of high quality protein, as well as of iron but the iron contained in soy is not well absorbed. This is of some concern, since soy is used as a substitute for milk in some infant formulas. The marked inhibitory effect of soy protein on iron absorption was confirmed in the present study. While a concentration dependent improvement in iron bioavailability was demonstrated with increasing amounts of ascorbic acid, the increase was far less than was noted with a cow's milk based formula. It was concluded that soy infant formulas should contain at least 12 mg iron/100 g formula, and that ascorbic acid should be added in a molar ratio of 4:1 for optimal absorption of iron from them.

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PREFACE

Iron deficiency is the most common nutritional disorder present in the world and one to which enormous attention has been directed. The major aim has been to find effective ways of preventing it and thereby of freeing entire populations of the broad spectrum of problems arising from the condition. As iron balance is determined largely by absorption of the metal rather than excretion, the interplay between the various inhibitors and promoters of iron absorption present in foods must profoundly influence iron nutrition. The series of studies described in this thesis was undertaken in order to examine systematically what factors in commonly consumed vegetables and cereals either promote or depress iron absorption. It was felt that the identification of foods that promote iron absorption from the diet might make it possible to improve iron nutrition in vulnerable groups by increasing the intake of such foods. Conversely it might be possible to prevent iron overload in predisposed individuals by adding foods to the diet which inhibit iron absorption.

In Chapters I and II of this thesis the current knowledge pertaining to internal and external iron exchange has been reviewed. The disorders of iron nutrition with particular reference to iron deficiency are discussed in Chapter III. In this section the methods for defining iron status are described. In addition the problems associated

with iron deficiency have been reviewed. Chapter IV contains details of the various laboratory methods used in the second half of the thesis. This second section comprises Chapters V, VI and VII, which contains the results of studies in which the absorption of iron from numerous foodstuffs was measured. Chapter V evaluates the effect of different organic acids, phytates and polyphenols on iron absorption from vegetables, whilst the factors leading to poor iron absorption from cereals are discussed in Chapter VI. The important promoting effect of ascorbic acid on iron absorption is emphasized in Chapter VII, in which iron absorption from infant milk formulas has been evaluated. In this chapter data are also presented which show the inhibitory effect of soy protein on iron absorption. Finally there is an appendix, comprising the individual results obtained in the iron absorption studies reported in the second half of the thesis.

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Chapter One

INTERNAL IRON EXCHANGE

1 INTERNAL IRON EXCHANGE

1.1 Plasma Iron

Iron plays a central role in human physiology and fulfils most of its functions in combination with protein. Some of the major iron containing proteins are haem proteins, in which various porphyrins complex with iron and form structures the configuration of which allows them to slot neatly into a fold or crevice in a protein chain (Wrigglesworth & Baum, 1980). Of the total complement of about 60 mg/kg of body iron (i.e. about 4 g), approximately 1 g exists in a reversible complex with proteins, the function of which is iron storage (Brittenham et al, 1983). The other major metalloproteins are haemoglobin and myoglobin, which contain about 60% and 6% of the total body iron respectively. Both of these compounds are capable of reversible oxygen binding, either to transport bound oxygen in the erythrocyte as oxyhaemoglobin, or to function as an oxygen store in muscle. The largest group of haemproteins, however, are enzymes involved in oxidizing reactions, even though they only contain about 5% of the total body iron. In this respect, the cytochromes, which by the reversible valency charges of their haem iron are able to catalyse electron transfer reactions, are crucial for normal cellular respiration (Nicholls & Elliot, 1974). The metabolic pathways followed by iron comprise an internal loop, in which the iron released from catabolized cells is constantly being reutilized, and an external loop characterized by the

meticulous control of food iron absorption to balance basal iron losses of about 1 mg per day.

Ferrokinetic analyses have shown that the largest fraction of plasma iron (24 mg/day) is destined for the synthesis of haemoglobin in developing erythrocytes, whilst about 5 mg/day is exchanged between the iron transport protein, transferrin, and parenchymal tissues (Bothwell et al, 1979). Finally a small amount of plasma iron (3 mg/day) passes into the extravascular tissues (Finch et al, 1970). In striking contrast to this is the small amount of daily external iron exchange.

1.1.1 Distribution of plasma iron by transferrin

Iron is distributed within the body by the plasma and over 95% of the plasma iron is bound to a specific transport protein apotransferrin (Bothwell et al, 1979). It is synthesized in the liver at a rate which varies inversely with the hepatocyte ferritin concentration (Morton & Tavill, 1977; Morton & Tavill, 1978). This protein, which has a molecular weight of 76,000 (Mann et al, 1970) has evolved by the contiguous duplication of the structural gene for an ancestral protein that had a single iron-binding site and contained approximately 340 amino acid residues (Mac Gillivray et al, 1982). Transferrin, an alpha-1 globulin, is a single chain polypeptide that has two iron binding sites, an acid labile N terminal and an acid stable C terminal (Aasa et al, 1963; Aisen et al, 1966; Huebers & Finch, 1982). Thus the transferrin pool is not homogenous

but is composed of apoferric, monoferric and diferric transferrin (Huebers et al, 1981; Finch & Huebers, 1982; Huebers et al, 1983a). The two sites differ in the tightness with which they bind iron and in their spectroscopic properties (Aisen, 1982) and though they function equivalently as iron donors (Huebers et al, 1981b), it is possible that they behave differently in accepting iron from storage and absorption sites (Zapolski & Princiotto, 1980; Marx et al, 1982). In contrast to previous work (Williams and Moreton, 1980), Huebers and co-workers (1984b) have found that the distribution of iron between the two binding sites of transferrin following iron-loading is random. This applied whether the iron loading was achieved by increasing intestinal absorption or by loading from the reticuloendothelial system.

The protein fulfils the crucial role of distributing absorbed iron in proportion to need. In this regard, the iron requirements of the erythroid marrow greatly exceed those of other tissues. In addition, transferrin minimises iron losses by depositing surplus iron in tissues designed for iron storage (Bothwell et al, 1979). Iron is bound to transferrin in the ferric form but should it attach in the ferrous state the latter is rapidly oxidised (Bothwell et al, 1979). The bond, though extremely tight, still permits the effective delivery of iron to tissues that require it. The unusual character of this strong bond is critically dependent on the concomitant binding of an anion, usually

bicarbonate (Aisen, 1982), which possibly locks iron into its binding site by coordinating both to the metal and the protein (Schlabach & Bates, 1975). Without this anion the iron-binding function of transferrin is lost (Aisen, 1980). Analysis of transferrin reveals that the binding sites for the ferric and carbonate ions are probably located near the junction of two peptide fragments joined by disulfide bridges 3 in the N-domain of the molecule, with a similar arrangement existing in the C-domain of the protein (Chasteen, 1983).

1.1.2 Interaction between transferrin and the erythroid cells

Transferrin bound iron appears to be the only acceptable form of iron for erythroid cells (Eldor et al, 1970), with three main steps to the interaction between the transferrin-iron-complex and erythroid cells. Absorption of the complex onto the red cell membrane occurs first. This is a rapid process, which is dependent on the transferrin concentration and which is probably due mainly to electrostatic forces (Kornfeld 1969; Morgan 1974). During a fixation phase the transferrin-iron-complex binds to specific receptors on the red cell membrane (Bothwell et al, 1979; Finch and Huebers 1982; Morgan 1983; Harding and Stahl 1983; Egyed 1984). The neutral pH at which this occurs precludes the binding of apotransferrin (Dautry-Varsat et al, 1983). Finally the complex is internalized by a process of receptor mediated endocytosis (Hemmaplardh and Morgan,

1977; van Renswoude et al, 1982; Enns et al, 1983; Hopkins and Trowbridge, 1983; Iacopetta and Morgan, 1983a; Takahashi and Tavassoli, 1983; Yamashiro et al, 1984). Transferrin endocytosis is a temperature dependent mechanism requiring metabolic energy (Light and Morgan, 1982; Blight & Morgan, 1983).

The exact intracellular pathway followed by the transferrin complex after endocytosis has not been fully established. However, it appears that transferrin and the receptor are located in small vesicles and tubules in the periphery of the cell (Harding et al, 1983; Hopkins, 1983; Hopkins and Trowbridge, 1983; Yamashiro et al, 1984). These structures then possibly fuse together or with larger vesicles to form tubulovesicular structures variously termed endocytic vesicles (Tycko et al, 1983), receptosomes (Pastan and Willingham, 1981) or CURL (Compartment of Uncoupling Receptor and Ligand) (Geuze et al, 1983). The low pH (5.55) within these endocytic vesicles, which is generated by an ATP-dependent acidification mechanism (Yamashiro et al, 1983), provides the necessary environment for iron to be released from transferrin (Dautry-Varsat et al, 1983; Harding and Stahl, 1983; Morgan, 1983; Edit. Nutr. Rev. 1984; Paterson et al, 1984; Veldman et al, 1984). It also provides the conditions for apotransferrin to remain bound to its receptor (Dautry-Varsat et al, 1983). Having been released from transferrin, iron can then be conveyed across the vesicular membrane and be incorporated into haem in the

mitochondria of the cell (Light and Morgan, 1982). Pyrophosphate may function as a ligand in delivering iron to the mitochondria (Konopka et al, 1980; Konopka et al, 1981; Nilsen and Romslo, 1984).

The tubular portions of the endocytic vesicle which separate from the vesicular area, permit the receptor to escape lysomal degradation (Geuze et al, 1983). Apotransferrin and its receptor can then undergo a recycling process. This exocytic pathway involves small vesicles and tubular structures that accumulate near the Golgi complex (Hopkins 1983; Willingham et al, 1984; Yamashiro, et al, 1984). These para-Golgi structures have an average pH of 6.4 (Yamashiro et al, 1984). Ultimately apotransferrin and its receptor are returned to the cell surface, where after exocytosis, apotransferrin dissociates from its receptor at a neutral pH (Dautry-Varsat et al, 1983; Morgan, 1983; Harding and Stahl 1983; Harding et al, 1983). Apotransferrin is then free once again to bind iron and the free receptor on the cell surface is available for another cycle of receptor mediated endocytosis (Baker et al, 1982; Sibille et al, 1982).

The number of specific membrane receptors appears to determine the relative amount of iron delivered to any particular tissue (Finch & Huebers, 1982). The gene for expression of these cell surface glycoproteins has been assigned to human chromosome 3 (Enns et al, 1982; Miller et al, 1983; van de Rijn et al, 1983; Huerre et al, 1984), and

it has been shown that the receptor binds two molecules of transferrin (Enns & Sussman, 1981a). The question of whether the transferrin receptor is the same for all human tissue is being studied. Those found on human reticulocytes and human placental trophoblast cells appear to be extremely similar (Seligman et al, 1979; Enns & Sussman, 1981b). The human transferrin receptor is a transmembrane glycoprotein comprising two disulfide bonded subunits, each having a molecular weight of 90,000. It has an unusual configuration with a small N-terminal cytoplasmic domain and an extracellular C-terminal domain of 672 amino acids (Schneider et al, 1984). One transferrin molecule binds to each subunit of the receptor (Huebers and Finch, 1984). As the cells of the erythroid series mature, so does the number of transferrin receptors decline, with the maximum number, and hence iron uptake, being measured in intermediate normoblasts (Frazier et al, 1982; Iacopetta et al, 1982; Iacopetta and Morgan, 1983a; Parmley et al, 1983). Mature erythrocytes no longer bind transferrin and lack detectable levels of membrane receptor (Jandl & Katz, 1963; Enns & Sussman, 1981a; Frazier et al, 1982; Parmley et al, 1983). The main molecules involved in the regulation of the number of transferrin receptors in haemopoietic cells appear to be haem, iron and protoporphyrin IX (Louache et al, 1984). Whilst haem and iron decrease the synthesis of receptors, the presence of protoporphyrin IX increases their production. The number of transferrin receptors may also be

influenced by the presence of chelatable iron (Bridges and Cudkowicz, 1984) as well as by the intracellular haem content (Ward et al, 1984).

Although the major factor determining the delivery of iron to a cell is the number of transferrin receptors on the cell membrane, the degree of saturation of the transferrin molecule also plays a part. Transferrin molecules are preferentially selected over apotransferrin molecules to bind to the cell receptor (Baker et al, 1982) and the monoferric molecules are far less effective than the diferric species in donating iron (Huebers & Finch, 1982). Diferric iron exchange occurs at a more rapid rate than does monoferric iron exchange (Huebers et al, 1981) and the uptake of iron from diferric transferrin by reticulocytes is twice that from the monoferric form at the same iron concentration, both in vitro and in vivo (Christensen et al, 1978; Iacopetta and Morgan, 1983b). This is probably due to a competitive interaction in iron delivery at a common membrane receptor binding site for transferrin (Huebers et al, 1983a; Huebers et al, 1984a; Cazzola et al, 1985). One implication of this iron unloading behaviour is an increase in plasma iron delivery to tissues as a function of increasing transferrin saturation, irrespective of any change in receptor number (Huebers and Finch, 1984). No functional difference has been found to exist in vitro between the two iron-binding sites of transferrin with respect to iron delivery (Van der Heul et al, 1981; Delaney

et al, 1982; Heaphy and Williams, 1982; Young, 1982; van der Heul et al, 1984).

1.1.3 Plasma transferrin concentration

The plasma transferrin concentration may be determined by measuring the maximum iron-binding capacity of the plasma, since transferrin is the only high affinity iron binder present (Bothwell et al, 1979). In normal adults the average total iron binding capacity of plasma is about 330 /ug/dl (SD about 25 /ug/dl) (Laurell, 1947). The inverse relationship existing between body iron stores and the total iron-binding capacity makes iron deficiency the commonest cause of hypertransferrinaemia, when the value may rise to between 370 and 580 /ug/dl (Laurell, 1952; Lipschitz et al, 1971; Ballas, 1979). During pregnancy a rise in the plasma total iron-binding capacity is associated with the mobilization of storage iron to supply the needs of the maternal bone marrow and foetus (Bothwell et al, 1979). Hypotransferrinaemia may occur as the result of poor protein nutrition as in Kwashiorkor (McFarlane et al, 1970), in protein losing states such as protein losing enteropathies and the nephrotic syndrome (Gitlin et al, 1956; Hancock et al, 1976; Warshaw et al, 1984) and in acute or chronic inflammatory disorders such as rheumatoid arthritis and hepatic disease (Laurell, 1952; Brendstrup, 1953).

1.1.4 Plasma iron concentration

Plasma iron concentrations are similar in the two sexes despite great differences in iron stores. The mean and standard deviation values are 115 (±31) /ug/dl in normal adult males and 115 (±40) /ug/dl in normal females (Bothwell et al, 1979). Due to a circadian fluctuation in the reticuloendothelial cell release of iron, (Lipschitz et al, 1971; Uchida et al, 1983) the plasma iron concentration peaks in the morning and reaches a nadir in the evening, with the pattern being reversed in individuals who work all night and rest during the day (Fillet et al, 1974). The plasma iron pool of about 3 mg is small when compared with a plasma iron turnover of about 35 mg daily (Bothwell et al, 1979). Plasma iron levels do vary with age, being high in the cord blood of newborns (148 - 193 /ug/dl) (Weipple et al, 1973) and then declining to about 50 /ug/dl at three to six months (Sturgeon, 1954). The two year old child has a plasma iron level of about 100 /ug/dl (Sturgeon, 1954). In aged individuals the plasma iron concentration tends to be lower than in adulthood.

Hypoferraemia exists when the plasma iron level falls below 40 /ug/dl, or 50 /ug/dl on repeated determinations (Bothwell et al, 1979), and most commonly arises from the depletion of body stores (Brittenham et al, 1981). The defective reticuloendothelial release of iron that accompanies acute and chronic inflammatory conditions such as infective disorders, rheumatoid arthritis, malignancies,

trauma and surgery is associated with a depression in the plasma iron concentration (Brendstrup, 1953; Laurell, 1947; Konijn & Hershko, 1977). A comparable situation exists in ascorbic acid deficiency, and whilst this is corrected by administering the vitamin, the defective release in inflammatory problems can only be improved by the use of glucocorticoids (Lipschitz et al, 1971; Bothwell et al, 1979). The reduced transferrin concentrations that occur in protein deficient states also lead to a fall in the plasma iron level but the percentage transferrin saturation remains normal under such circumstances (Lahey et al, 1958).

Elevation of the plasma iron level to greater than 150 /ug/dl on repeated determinations is defined as hyperferraemia (Bothwell et al, 1979). Hepatic disease and impaired erythropoiesis are the two major causes. In liver disease, ferritin with an unusually high iron content is released, while the increase in plasma iron parallels the degree of hepatic necrosis (Powell & Halliday, 1982). Since the transferrin concentration usually falls in liver disease, the percentage saturation is frequently raised due to the associated hyperferraemia (Bothwell et al, 1979). When the rate of erythropoiesis falls to below one third of normal the plasma iron level rises, probably because the release of iron to the plasma cannot be further reduced and because the erythropoietic marrow is the major tissue that assimilates and uses iron (Bothwell et al, 1979). An elevated serum iron level is therefore a feature of aplastic

anaemias (Finch et al, 1970) and marrow damage secondary to chemotherapeutic drugs (Lane et al, 1964). Ineffective erythropoiesis also leads to hyperferraemia, possibly due to a combination of altered iron exchange across cell membranes, increased reticuloendothelial cell iron release (Bothwell et al, 1979) and increased gastro-intestinal iron absorption (Erlandson et al, 1962).

The normal range of transferrin saturation is 20 - 55% and as this value depends upon the plasma iron concentration and the transferrin level, it also demonstrates a circadian fluctuation (Laurell, 1947). Its clinical importance lies in the fact that a saturation below 16% is inadequate to meet basal erythropoietic requirements (Bainton & Finch, 1964; Hillman & Henderson, 1969). As the plasma iron level declines, so does the amount of diferric iron available for the erythron (Huebers & Finch, 1982).

1.1.5 Internal iron kinetics

Plasma transferrin iron is derived mainly from the reticuloendothelial system. The major contribution is derived from the breakdown of haemoglobin in senescent red cells (15 mg per day), while a smaller amount arises from ineffective erythropoiesis (7 mg per day) (Cook et al, 1970). The other important pathway from which transferrin iron is obtained is the hepatocyte (7 mg/day). Hepatocyte storage of iron appears to be passive, with the net direction of flow depending on the plasma iron concentation. With iron depletion and a fall in plasma iron, the

hepatocyte releases iron to transferrin, while the converse applies to iron overload (Cook et al, 1970). However, hepatocyte iron uptake and release probably occur via separate mechanisms (Young & Aisen, 1980). Isolated rat hepatocytes appear to take up transferrin bound iron in a fashion similar to reticulocytes, with the process being dependent on temperature and involving a cell surface receptor (Young and Aisen, 1980). Baker and co-workers (1985) have however postulated that in addition to the transferrin-bound pool of iron, both a fixed pool in the form of ferritin as well as a chelatable pool that can be mobilized by chelators exist in hepatocyte cultures.Within the hepatocyte, transferrin is located specifically in the endoplasmic reticulum and the Golgi apparatus (Vassy et al, 1984). The third important pathway followed by plasma iron is the small exchange, (about 3 mg per day), between the intravascular compartment and the extravascular tissues (Finch et al, 1970). As the supply of iron to transferrin is closely matched to demand, there is very little variation in the plasma iron concentration unless marrow activity is markedly suppressed, red cell destruction is excessively high, or storage iron has been severely depleted.

The extreme economy shown by the body in its handling of iron is evident from the unidirectional flow of iron from the macrophage to transferrin which transports it to the erythropoietic marrow where it is incorporated into the developing red cells. Almost all the iron taken up by

immature red cells is converted to haemoglobin with about 10% being found in the non-haem form (Noyes et al, 1960). Muscle, which contains myoglobin and ferritin, and which makes up about one third of the body iron store, receives less than 6-7% of transferrin bound radioiron (Cheney et al, 1967). Iron uptake by the erythropoietic marrow and the hepatocyte is inversely related but uptake by both is increased when the transferrin iron saturation rises (Cook et al, 1973a; Page et al, 1984).

The exquisite control that matches iron absorption to meet tissue iron needs remains an enigma. Transferrin however plays a pivotal role in the efficient regulation of iron supply. Increasing the receptors for transferrin by exchange transfusion with reticulocyte rich blood in experimental animals is associated with a rise in plasma iron turnover and an increase in the amounts of iron mobilized from the reticuloendothelial cells and the intestinal mucosa (Finch et al, 1982). Noteworthy in this study was the fact that the plasma iron level did not alter significantly, which suggests that the very act of donating iron by transferrin in some way "activates" the transferrin procurement system (Finch et al, 1982; Finch & Huebers, 1982; Huebers & Finch, 1984). Iron uptake by individual tissues is determined by their transferrin receptor number, by the relative amounts of monoferric and diferric transferrin present in the circulation and by the concentration of iron in donor tissues (Huebers & Finch,

1984). Since the erythroid marrow is the only tissue that can modify its receptor number by cellular proliferation, erythropoiesis determines plasma iron turnover (Bauer et al, 1981; Huebers & Finch, 1982). A link appears to exist between the receptor number and the mucosal regulation of iron absorption, so that when erythropoiesis is increased, so too is intestinal absorption. In this way, iron supply is automatically adjusted to tissue needs (Bothwell et al, 1979; Huebers & Finch, 1982). An increase in the marrow blood flow, also appears to be an important mechanism for meeting the increased iron requirements of hyperplastic marrow (Celada et al, 1984).

Less than 2% (4.5 mg/dl) of the plasma iron is present as haemoglobin. This haemoglobin, which is derived from intravascular haemolysis, is complexed to a specific protein carrier, haptoglobin. The haemoglobin/haptoglobin complex is catabolised by hepatocytes (Goldfischer et al, 1970). The haem iron is liberated from the porphyrin ring by haem oxygenase (Bothwell et al, 1979) and is then either stored in the liver or returned to the plasma for reutilization (Keene & Jandl, 1965). Should the haem binding capacity of haptoglobin be exceeded, haemopexin, a beta-globulin, takes up the surplus haemoglobin and delivers it to the liver where it is catabolised (Hershko et al, 1972). Such a situation may arise in severe haemolytic conditions or following haemorrhage into tissue, for example in ectopic pregnancy (Bunn & Jandl, 1968).

Lactoferrin and ferritin are the remaining two forms in which iron is found in the plasma. Although the amount of ferritin in the plasma is small, it has great clinical significance. All the evidence suggests that it is mostly apoferritin that is in circulation (Brown et al, 1979).

1.1.6 Serum ferritin

Though the physiological significance of this small extracellular iron compartment is unclear, serum ferritin measurements are an invaluable means of evaluating iron status. Numerous authors have found that the serum ferritin level correlates closely with body iron stores (Addison et al, 1972; Cook et al, 1973b; Jacob et al, 1980; Jacobs & Dommisse, 1982; Milman et al, 1983) though unusual circumstances in which this does not occur have been reported (Rajantie et al, 1981; Rajantie & Siimes, 1983). The protein appears to be derived from reticuloendothelial cells (Siimes & Dallman, 1974), with the RNA for ferritin subunit synthesis apparently being represented both on polyribosomes bound to the endoplasmic reticulum, as well as on the free polyribosomes (Bomford & Munro, 1980; Cook & Skikne, 1982). Serum ferritin is composed mainly of the basic L subunit, and therefore on gel electrofocussing and immunologically, closely resembles natural liver apoferritin (Worwood, 1980). In contrast to tissue ferritin, the iron content of serum ferritin is low (Worwood et al, 1976; Zuyderhoudt et al, 1978; Cragg et al, 1981). Biochemically up to 70% of normal serum ferritin binds to concanavalin A

(Cragg et al, 1980). This fraction of serum ferritin has been found to contain a high molecular weight 'G' subunit which is glycosylated (Cragg et al, 1981). Some concanavalin A binding ferritin is released into the plasma following red blood cell phagocytosis by monocytes, which suggests that these cells may be a source of the glycosylated fraction (Worwood et al, 1984).

Glycosylation is a major factor in determing the clearance rate of plasma ferritin. The fraction binding to concanavalian A is cleared with a half life of about 50 hours, whilst the fraction that is not glycosylated is cleared with a half life of about 5 hours (Worwood et al, 1982). Tissue ferritin is cleared more rapidly and studies using ^{131}I labelled human spleen ferritin have shown that less than 1% of the labelled protein is present in the plasma after 5 hours (Cragg et al, 1983). Human plasma contains binders that are specific for tissue ferritins but not for human serum ferritin (Covell et al, 1983). Human heart ferritin reacts strongly with serum, whilst human spleen ferritin does so less strongly and human serum ferritin least of all (Covell et al, 1984). This interaction of 'H' subunit-containing ferritins with serum may also be partly responsible for the rapid clearance of tissue ferritins from the circulation (Covell et al, 1984). Serum ferritin appears to be cleared exclusively by the hepatic parenchymal cells which may utilize a specific receptor (Hershko et al, 1973; Halliday et al, 1979; Mack et

al, 1981; Mack et al, 1983) and it is possible that a mechanism resides within the liver that maintains the circulating ferritin concentration at a level appropriate to body iron stores (Mack et al, 1981).

The most important clinical and nutritional application of serum ferritin measurements is the fact that a low serum ferritin level in a patient with a low haemoglobin is diagnostic of iron deficiency anaemia. The lower cut off level is taken as 12 /ug/l (Cook & Skikne, 1982). Stainable iron cannot be demonstrated in the bone marrow at a serum ferritin concentration of about 20-25 /ug/l (Harju et al, 1984). The mean serum ferritin level in developed countries is 90 - 95 /ug/l in males and 25 - 30 /ug/l in females, reflecting mean storage iron values of 900 and 300 mg respectively (Cook et al, 1974; Worwood 1980). The way in which serum ferritin reflects movement of iron between the red cell and the storage compartment is clearly demonstrated during the first few months of life. Ferritin concentrations initially increase as foetal haemoglobin is broken down and then decrease as the stored iron is used for the synthesis of haemoglobin A (Worwood, 1980).

Inflammatory disease (Blake et al, 1981; Kitschke et al, 1982; Harju & Larmi, 1984) and hepatic disease (Prieto et al, 1975; Nakano et al, 1984) lead to a disproportionate increase in serum ferritin levels in relation to tissue stores (Hengeveld et al, 1982) and to possible alterations in its immunological properties (Arosio et al, 1981). In

inflammatory conditions, whether due to infection (Birgegard et al, 1979) or neoplasia (Ito et al, 1980; Patel et al, 1980; Jakobsen et al, 1982; Luger et al, 1983; Milman & Graudal, 1984), there is thought to be increased reticuloendothelial synthesis of ferritin (Konijn & Hershko, 1977) possibly on bound polysomes (Konijn et al, 1981). The high concentrations of serum ferritin in malignancy may however be due to a number of other causes. Tissue necrosis can lead to a direct release of cytosolic ferritin, while there may also be abnormalities in the synthesis of ferritin in malignant cells (Hann et al, 1980; Worwood, 1982). In liver disease the raised level of plasma ferritin seem to be due in part to the inflammatory component of the condition and in part to the release of ferritin into the plasma by damaged hepatocytes (Bothwell et al, 1979; Zuyderhoudt et al, 1980; Worwood, 1982). Elevated serum ferritin levels in alcoholics are related to the degree of hepatic damage (Lundin et al, 1981; Meyer et al, 1984). The ferritinaemia in patients with transfusional iron overload appears to be the combined result of increased ferritin synthesis and the release of intracellular ferritin from damaged cells (Worwood, 1980; Cazzola et al, 1982; Cazzola et al, 1983a). The presence of raised serum ferritin levels in haemodialysis patients may relate to increased iron stores as a result of blood transfusions or indicate a propensity to persistent hepatitis B infection (Lustbader et al, 1983).

1.2 Storage Iron

1.2.1 Function and distribution of storage iron

Any iron that is surplus to immediate functional need is stored in two related forms, a mobile soluble fraction known as ferritin, and as insoluble aggregates of haemosiderin (Worwood, 1982; Brittenham et al, 1983). Ferritin normally occurs in all mammalian cells, with the exception of specialized anuclear cells, such as those comprising the lens of the eye (Mason & Taylor, 1978; Fairbanks & Klee, 1981). It is possible that even in cells such as neurons, small amounts of ferritin serve to provide a readily available source of iron to supply the metabolic needs of the cell (Fairbanks & Klee, 1981). The ferritin found in immature red cells appears to function both as a possible intermediate in haem synthesis, (Fibach et al, 1983) and as an intracellular depot for iron in excess of that required for haemoglobin synthesis (Cazzola et al, 1983b; Grasso et al, 1984).

Iron stored as ferritin in the parenchymal cells of the liver accounts for about one third of the total amount of storage iron (Powell & Halliday, 1982; Wilms & Batey, 1983). The remainder of the body's reserve iron is stored as haemosiderin within the cells of the reticuloendothelial system in the liver, spleen, bone marrow and skeletal muscle (Bothwell et al, 1979; Powell & Halliday, 1982). The muscle storage iron pool is relatively non-miscible and even when required for haemoglobin formation may not be rapidly

mobilized (Torrance et al, 1968). In contrast, stores elsewhere provide a readily available source of iron for maintaining haemoglobin levels relatively constant, despite fluctuations in the supply of iron (Bothwell et al, 1979; Worwood, 1982).

It is biologically essential to store spare iron, for the interaction between ferrous iron and dioxygen leads to the production of superoxide, hydroxyl and other reactive radicals that can destroy nucleic acids, lipids and proteins found within the cell (Zamenhoff et al, 1954; Harrison, 1977; Crichton, 1979; Clegg et al, 1980; Whiting et al, 1981; Shull & Theil, 1983). In man, storage capacity is always maintained and the ferritin in a tissue is never fully saturated with iron, so that free iron may be incorporated immediately (Bomford & Munro, 1980). Should the amount of iron to be stored increase, apoferritin synthesis on the free polyribosomes is immediately increased (Konijn et al, 1973; Zähringer et al, 1977; Bomford & Munro, 1980). In the myocardium, where it is vital to maintain function in the face of iron deficiency, ferritin stores persist so that myoglobin and the cytochromes are maintained at a time when they may be much reduced in skeletal muscle (Dallman & Schwartz, 1965).

1.2.2 Nature of ferritin and haemosiderin

Ferric iron has a tendency to form polynuclear aggregates and while this may be an effective way of packing a large number of iron atoms into a relatively small space,

such aggregates would tend to coalesce and precipitate unless covered by a hydrophilic shell (Harrison, 1977). Thus ferritin and haemosiderin are distinguished by their solubility in aqueous tissue extracts, the soluble fraction being ferritin and the insoluble aggregates, haemosiderin (Brittenham et al, 1983). The ferritin molecule comprises a protein shell surrounding a "ferrihydrite" core of stored iron, which is associated with inorganic phosphate (Towe, 1981; Ford et al, 1984). Haemosiderin appears to be iron in a similar form to that of ferritin, for both give similar X-ray diffraction patterns and Mössbauer spectra (Fischbach et al, 1971). However, haemosiderin lacks the solubilising protein coat of ferritin (Fischbach et al, 1971) and its insoluble granules are probably derived from the breakdown of ferritin in secondary lysosomes (Harrison et al, 1980; Wixom et al, 1980; Hoy & Jacobs, 1981; Bell et al, 1984; Weir et al, 1984a). Haemosiderin thus appears to represent the end point of the intracellular storage iron pathway (Cook & Skikne, 1982) and unlike ferritin iron, it is not as readily mobilizable. In fact, haemosiderin may persist even when haemoglobin synthesis is limited by the availability of iron (Jacobs, 1980). This slow mobilization of haemosiderin iron has been found to occur in subjects being treated for hereditary haemochromatosis (Wagstaff et al, 1982).

1.2.3 Structure of the ferritin molecule

Ferritin molecules comprise a globular protein shell called apoferritin, with a central cavity within which iron

accumulates. Apoferritin has a molecular weight of approximately 444,000 and is about 130 A^{o} in diameter with a central cavity of about 75 A^{o} (Crichton & Bryce, 1970; Hoare et al, 1975; Harrison, 1977). The apoferritin shell has been extensively studied using horse spleen ferritin. It is composed of twenty four polypeptide chains or subunits (Crichton & Bryce, 1970). Each subunit has a molecular weight of 18,5000 and is best described as a bundle of four parallel alpha helices, with two shorter helices situated perpendicular to them and interconnected by non helical regions (Fairbanks & Klee, 1981; Heuterspreute & Crichton, 1981). The subunits are arranged in a symmetrical fashion to form a cubic structure with four monomers lying in the plane of each face of the cube (Harrison, 1976). The monomers are arranged spatially so that six channels with diameters of about 10 A^{o} lie between them (Banyard et al, 1978). These pores are a means of access for iron stores and other small molecules, such as sucrose (Harrison, 1977).

Apoferritin is a colourless molecule, whilst ferritin molecules with a full content of iron have a deep red-brown colour (Bothwell et al, 1979; Worwood, 1980). The ferritin molecule, though capable of holding 4,500 iron atoms as an inorganic complex (Clegg et al, 1980; Rice et al, 1983), usually does not contain more than about 2,000 molecules of ferricoxyhydroxide (Harrison et al, 1974). The core appears roughly spherical or cubic when it is filled with iron (Bothwell et al, 1979). The stored iron is present in the

form of microcrystals (Massover, 1973; Towe et al, 1981; Fairbanks & Klee, 1981; Ford et al, 1984; Treffry & Harrison, 1984b; Massover, 1985) and the size of these ferrihydrite crystals is limited to about 8 nm (8Ø A^{O}) by the protein shell (Treffry & Harrison, 1984b). Electron microscopy studies have shown that the electron dense cores of ferritin never touch and that adjacent cores are separated by the thickness of two protein shells (Massover, 1985).

1.2.4 Ferritin heterogeneity

Ferritin derived fom different organs (Arosio et al, 1978; Russell & Harrison, 1978; Wagstaff et al, 1978; Gowan et al 1983) and from serum (Cragg et al, 1981) isofocuses over a pH range of 4,8 to 5,8, giving several bands of isoferritins (Drysdale et al, 1977; Harrison, 1977; Massover, 1978; Arosio et al, 1978; Russell & Harrison, 1978; Wagstaff et al, 1978). On sodium sulphate or polyacrylamide gel electrophoresis most human tissue ferritins give two major subunit bands, with approximate molecular weights of 21,ØØØ and 19,ØØØ. These are designated H and L respectively (Arosio et al, 1978; Lavoie et al, 1978; Bomford et al, 1981). Two distinct forms of the H subunit have been found to occur in rat liver and HeLa cells (Watanabe & Drysdale, 1983). Variations in the proportion of H and L subunits give rise to the isoferritins, with the most acidic isoferritins of heart (pi 5,Ø) containing a high proportion of H subunits whilst that of liver (pi 5,9) is

composed almost entirely of L subunits. A spectrum of isoferritins exists between these two extremes, with those of kidney and spleen occupying an intermediate position (Bomford et al, 1977; Russell & Harrison, 1978; Bomford & Munro, 1980; Bomford et al, 1981). Although the two subunits share extensive sequence homologies, they probably arise from distinct mRNA species (Watanabe & Drysdale, 1981a) and the light subunit appears to be encoded for by a family of genes (Brown et al, 1983).

The acidic isoferritins contain a high proportion of iron rich molecules in contrast to the basic isoferritins in which there is a preponderance of iron poor molecules (Bomford et al, 1977; Treffry et al, 1984b). Iron loading of tissues produces an increase in the more basic (L-rich) isoferritins relative to the acidic (H-rich) species (Bomford et al, 1981; Treffry et al, 1984a,c). This is not due to differential rates of degradation, but rather to an alteration in the isoferritins being synthesized (Treffry et al, 1984a,c). The production of L-rich isoferritins in response to iron does not seem to confer any advantage in the storage of iron and it is possible that newly synthesized apoferritin molecules are less efficient in competing for iron than older molecules (Treffry et al, 1984b). The second effect of iron loading in rat liver is to increase the transfer of molecules from the acidic to the basic isoferritins after the two different species have been synthesized. These rat liver ferritin molecules undergo

post-assembly processing (Treffey & Harrison, 1980; Treffry et al, 1984c). Thus the main reasons for ferritin's heterogeneity relate to its iron content, its subunit composition (Jacobs, 1983a) and the proportion of polymers present in the molecule (Hoy & Jacobs, 1981).

The physiological significance of the isoferritins has still to be established. However, the acidic (H) reacting isoferritins derived from monocytes have been identified as having leukaemia associated inhibitory activity (Broxmeyer et al, 1981). These isoferritins are found in most haemopoietic cells, including lymphocytes, monocytes, neutrophil polymorphs, erythrocytes and erythroblasts (Ali et al, 1983; Dorner et al, 1980; Jacobs, 1983b; Jones et al, 1983; Peters et al, 1983). The latter provide one of the richest sources of acidic isoferritins within the bone marrow (Ali et al, 1983). Monocyte-macrophage derived acidic isoferritins exhibit a similar regulatory activity over granulocytic progenitor cells in vitro (Broxmeyer et al, 1982) and they have therefore been implicated in possibly playing a role in the pathogenesis of leukaemia (Broxmeyer et al, 1982; Broxmeyer et al, 1983; Jacobs, 1983a).

Ferritin is selectively distributed on lymphocyte and macrophage surface membranes. The ferritin found on the B cell surface may well be a recognition molecule, determining its site of migration (Cragg et al, 1984).

1.2.5 Red cell ferritin

Erythrocytes contain ferritin that is about ten times more reactive with antibody to heart than to spleen ferriin; (Jacobs et al, 1981; Peters et al, 1983). While the acidic isoferritin content is less closely related to iron status, the changes occuring in tissues in both iron deficiency and iron overload are reflected in the red cell basic isoferritin level (Cazzola et al, 1983 a & c; Peters et al, 1983). The major factor determining the red cell ferritin content is the level of transferrin saturation, which is in keeping with the competitive advantage of diferric transferrin in delivering iron to erythroid cells (Huebers et al, 1981b; Cazzola et al, 1983c). The two ferritin types within erythroid cells may have different metabolic functions, with the basic liver type acting as an intracellular depot of iron which is in excess of that required for haem synthesis. Heart-type ferritin, which is less influenced by iron status, may behave as an intermediate in the transfer of iron from the plasma membrane to the mitochondria for incorporation into haem (Cazzola et al, 1983c). The erythrocyte ferritin content has been shown to be valuable as a marker for excess tissue iron in the diagnosis of hereditary haemochromatosis and in monitoring treatment of the disease (van der Weyden et al, 1983).

1.2.6 Synthesis of ferritin

As apoferritin has a finite lifespan, it must be

constantly renewed (Bothwell et al, 1979). Rat liver apoferritin which has a half life of 50-75 hours, is synthesized mainly on the free polyribosomes (Drysdale & Munro, 1966; Konijn et al, 1973), with a smaller amount being synthesized on membrane bound polysomes (Puro & Richter, 1971; Konijn et al, 1973; Zähringer et al, 1977; Parmley et al, 1981b).

When iron enters a tissue, the ferritin content increases both as a result of incorporation of iron into pre-existing apoferritin and as the result of a dose dependent stimulation of apoferritin synthesis (Fineberg & Greenberg, 1955; Zähringer et al, 1975; Doolittle & Richter, 1981). This ability to synthesize apoferritin de novo when extracellular iron levels are high is a means of protecting cells from the toxic effects of iron. Likewise its mechanism of control appears to protect intranuclear DNA from exposure to iron, which in the presence of oxygen, would otherwise lead to the production of radicals that would destroy the nucleic acid (Shull & Theil, 1983).

Normally the induction of protein synthesis depends upon an increase in the availability of specific regions of DNA for transcription. This is thought to be brought about by the inducing agent (Shull & Theil, 1983). However, iron induced apoferritin biosynthesis is not controlled in this way (Drysdale & Shafritz, 1975; Zähringer et al, 1976b). The process is not depressed by Actinomycin D, which inhibits RNA synthesis (Zähringer et al, 1976b) and may even

be more pronounced in the presence of this drug (Rittling & Woodworth, 1984). Iron appears rather to influence the translation of mRNA, for ferritin synthesis is more sensitive to inhibitors of translation, than of total protein synthesis (Schaefer & Theil, 1981). Ferritin mRNA is an abundant species in polysomes (Watanabe & Drysdale, 1981b) and is efficiently translated in response to iron (Shull & Theil, 1982). Thus ferritin mRNA is stored in the cell in a form which becomes available for translation when iron is present in excess (Shull & Theil, 1983).

The ability of iron to improve the cell's storage facilities for the metal, has significance in that an increase in the concentration of iron can be accommodated on a regional basis (Bothwell et al, 1979). In developing erythroid cells iron is taken up and retained within ferritin after a phase of maximal apoferritin synthesis. With continuing cellular maturation both ferritin iron and that obtained directly from transferrin are used for haemoglobin synthesis (Konijn et al, 1979).

1.2.7 The incorporation and release of storage iron

Iron can become incorporated into apoferritin after the synthesis and assembly of the protein subunits (Lee & Richter, 1977). Iron is taken up as ferrous iron and binds in an intersubunit region where it can be oxidized to the ferric state. The apoferritin shell catalyzes this reaction (Bomford & Munro, 1980; Clegg et al, 1980; Crichton, et al, 1980a; Chasteen & Theil, 1982). Molecular oxygen functions

as the electron acceptor in the oxidation-reaction and following hydrolysis, ferric oxyhydroxide is generated and deposited in the interior of the protein shell (Crichton et al, 198Øa). A mixed valence dimer of ferrous and ferric iron may be an essential precursor to the core formation of ferric iron within the ferritin protein shell (Chasteen et al, 1985). Ferric oxyhydroxide forms a crystal nucleation centre, which allows for the growth of microcrystals (Bothwell et al, 1979). Iron may then be deposited progressively on the iron core particles until saturation is approached (Treffry & Harrison 1984b). Similarly, iron is stripped off the iron cores, layer by layer, with the atoms on the outermost layer being the most available for release (Harrison et al, 1974). Radioiron studies have confirmed this finding. Radioiron incorporated last is the first to be released (Hoy et al, 1974). The uptake of iron by apoferritin occurs within minutes (Fairbanks & Klee, 1981) and is a function of the ratio of iron atoms to ferritin molecules (Wauters et al, 1978; Crichton & Paques, 1977). Little is known about the number and types of iron binding sites on the apoferritin shell. There seems not only to be more than one type of metal binding site, but also more than one type of ferric iron binding site (Treffry & Harrison, 1984a).

Iron contained in ferritin and haemosiderin is readily available for use in functional iron compounds when required (Bothwell et al, 1979), with their rate of mobilisation and

release into the plasma being dependent upon the rate of erythropoiesis (Hillman & Henderson, 1969). In acute situations normal adult males can usually mobilize up to 60 mg storage iron a day to treble the rate of red cell production (Bothwell et al, 1979). Ferritin iron is released by a reduction of ferric iron to the ferrous form by NADH, in a reaction in which reduced flavin mononucleotide ($FMNH_2$) serves as a critical intermediate (Jones et al, 1978; Crichton et al, 1980b; Sirivech et al, 1974; Osaki & Sirivech, 1971). It is thought that the reduced flavins, with their small diameters of 1.3 nm, are able to traverse the channels of the apoferritin shell and to react directly with the iron contained within the crystalline lattice (Sirivech et al, 1974; Hoy et al, 1974). The mechanism for reducing the flavin mononucleotide involves the C-side of the inner membrane of the mitochondria. The rate at which iron can be mobilized from ferritin therefore depends upon the capability of the flavins to penetrate the outer membranes of the mitochondria and the inter-subunit channels of the ferritin protein shell (Ulvik & Romslo, 1979; Ulvik, 1983). The rate of mobilization also increases with the iron content of the ferritin molecule. It levels to a maximum in ferritins containing about 1200 iron atoms per molecule (Ulvik et al, 1981). Ferritin appears to function as an iron donor to the mitochondria for haem synthesis (Ulvik, 1981) which suggests the presence of a flavin dependent mitochondrial

ferrireductase (Ulvik, 1983). In inflammation, Biemond and co-workers (1984) have demonstrated that the superoxide anion O_2 produced by stimulated polymorphonuclear leukocytes is capable of reducing ferric iron within the ferritin core and thereby mobilizing iron from ferritin. It is apparent from animal studies, that the mechanisms for storage iron release from hepatocytes and reticuloendothelial cells are not identical (Bothwell et al, 1979).

Little is known concerning the conversion of the released ferrous iron to ferric iron for transport to the erythroid marrow by transferrin (Bothwell et al, 1979). Caeruloplasmin, which has ferroxidase activity, may be involved (Osaki et al, 1966; Friedman, 1983). However, the release of storage iron is not impaired in patients with Wilson's disease or copper deficiency (Dunlap et al, 1974). A non caeruloplasmin, copper-containing ferroxidase (ferroxidase 11) in serum has also been found to be capable of the in vivo mobilization of iron from tissue stores (Topham et al, 1980). Interestingly, probable linkage between the genetic loci for transferrin and caeruloplasmin seems to exist (Weitkamp, 1983). Lod score analyses have revealed a recombination frequency of approximately 10-15%.

Chapter Two

EXTERNAL IRON EXCHANGE

2 EXTERNAL IRON EXCHANGE

2.1 Iron Losses

Iron must be absorbed from the diet to replace obligatory losses, to provide for physiological requirements and to establish a reserve store in order to avoid the occurrence of iron deficiency.

2.1.1 Gastrointestinal losses

In classic experiments done by McCance and Widdowson it was clearly demonstrated that as the body's capacity to excrete iron is limited, the control of iron balance must reside more in the absorption of the metal by the gastrointestinal tract (McCance & Widdowson, 1937). In males, prepubertal girls and post-menopausal women about two thirds of the total daily iron loss is from the gastrointestinal tract (Green et al, 1968; Bothwell et al, 1979). This amounts to about Ø,6 mg of iron per day, of which the major part is derived from the haemoglobin of the erythrocytes found in the minimal daily blood loss of all healthy individuals. Only about Ø,14 mg originates from the desquamation of mucosal cells at the tips of the intestinal villi (Green et al, 1968). It is apparent that any increase in the amount of blood lost from the gut is likely to have a profound effect on iron balance.

2.1.2 Skin

Iron is lost from the external surface of the body by desquamation of the epidermis and its appendages, as well as in sweat (Bothwell et al, 1979). This, however, does not

represent an important source of iron loss. Green and co-workers (1968) have demonstrated that the theory of excessive sweating being a factor contributing to iron deficiency is unfounded. In their study, steam laundry workers in subtropical Durban were found to have a similar mean total daily iron loss of 1,Ø2 mg when compared with the Ø,95 mg loss in sedentary workers living in Seattle, Washington. The same group of investigators found that skin iron uptake, which must equal skin iron loss was Ø,2 - Ø,3 mg daily.

2.1.3 Urine

Less than Ø,1 mg iron is lost in the urine daily (Green et al, 1968; Man & Wadsworth, 1969). It is found within voided erythrocytes, leucocytes and epithelial cells that have disintegrated during or after exfoliation (Bothwell et al, 1979).

Thus obligatory basal iron losses in the male are approximately 1 mg daily, and those in women from the gut, skin and urinary tract about Ø,8 mg per day, owing to their smaller size (INACG report, 1981; Bothwell & Charlton, 1982).

It is the extra demands on iron balance imposed by menstruation and pregnancy that make women of childbearing age a group particularly vulnerable to iron deficiency.

2.1.4 Menstruation

Although the volume of menstrual blood lost is fairly constant in the individual woman from one period to another,

the range in normal women is wide with the distribution curve being skewed to the right (Hallberg et al, 1966a; Hallberg et al, 1966b). In two large groups of women studied, the one Swedish (Hallberg 1966b) and the other British (Cole et al, 1971), more than 10% had a monthly blood loss exceeding 80 ml, whilst the median blood loss in the two surveys was similar, being 30 ml for the Swedish group and 26,5 ml in the English one. This is equivalent to 12,15 mg of iron or 0,4 - 0,5 mg daily when averaged over the whole month. In women losing more than 80 ml monthly the loss is equivalent to more than 1 mg daily (INACG report, 1981; Hallberg 1982a). As this latter amount is greater than the amount of iron lost from all other sources, menstruation obviously plays a critical role in determining iron balance.

Various factors determine menstrual losses of which heredity is probably the most important (Rybo & Hallberg, 1966). Positive correlations with parity and body size have also been found (Cole et al, 1971). Contraceptive practices can also modify menstrual blood losses, with the amount being reduced in women using the combined varieties of oral contraceptive and increased to an average greater than 50 ml in those using intra-uterine devices (Cole et al, 1971; Heikkinen et al, 1983). It is unfortunate that the "pill" is used more widely in developed countries, where iron balance is far less precarious than in the many developing nations where intra-uterine devices are more commonly

employed (INACG report, 1981).

2.1.5 Pregnancy

Although pregnancy is associated with a temporary cessation of menstruation, the overall cost to the mother with regard to iron far exceeds that of the non-pregnant state. Requirements during the first trimester decline to about 1 mg daily. This represents the obligatory basal iron losses of Ø,8 mg daily with minimal foetal and red cell needs (about 3Ø - 4Ø mg) (INACG report, 1981). A major expansion in the maternal red cell mass of about 35% occurs at the start of the second trimester and continues well into the third (Lowenstein et al, 1962; Rovinsky & Jaffin, 1965; Lund & Donovan, 1967). Iron requirements during the second trimester therefore rise to about 5 mg daily to cover basal losses of Ø,8 mg daily and the needs of the red cell mass (3ØØ mg) and conceptus (115 mg). The degree to which the red cell mass expands is a direct function of the supply of iron. For example, the mean increase in haemoglobin iron in women receiving supplemental iron has been found to be 57Ø mg as compared with 262 mg in unsupplemented women (De Leeuw et al, 1966). Despite the massive increase in the red cell mass during the second trimester, the haemoglobin level in iron replete women falls by about 1 g/dl. This is due to the fact that the plasma volume increases to a greater degree than does the red cell mass (De Leeuw et al, 1966). The fact that the red cell mass increases at a time when the oxygen requirements of the foetus are still modest, supports

the theory that part of the increase is due to raised maternal oxygen consumption (Flanagan et al, 1966). The iron requirements of the conceptus, that is the foetus, the umbilical cord and the placenta are greatest during the third trimester, so that the amount of iron needed daily is still about 5 - 6 mg (INACG report, 1981). This iron is required to replace the basal losses of Ø,8 mg daily as well as to supply the needs of the red cells (15Ø mg) and the conceptus (223 mg) (Pritchard, 1965; Bothwell et al, 1979; INACG report, 1981).

The iron nutrition of the infant during pregnancy is not jeopardised by poor maternal iron status unless the mother is severly iron deficient and placental insufficiency occurs. In this sense, therefore, the baby's needs enjoy precedence over the mother's and the placenta continues to transfer iron to the foetus at the expense of maternal erythropoiesis (Singla et al, 1978;MacPhail et al, 198Ø; Bratlid et al, 198Ø; Gebre-Medhin & Birgegard, 1981). Finally, the losses of iron occurring with the haemorrhage that accompanies parturition and in the lochia during the puerperium, must be included in the iron cost of pregnancy. The amount lost by these routes is usually about 15Ø mg but should any operative procedure such as an episiotomy be performed, the amount is increased. The mean blood loss in an uncomplicated Caesarian section has been found to be 1ØØØ ml (Wilcox et al, 1959; Pritchard et al, 1962). Thus the total iron requirements for pregnancy of a 55 kg woman

amount to more than 1000 mg (WHO Tech. Rep. Ser. 1970), but the net cost above basal requirements is about 500 mg, since the iron required for expansion of the maternal red cell mass is largely returned to the stores after delivery (INACG report, 1981). In developing countries the body mass is about 20% less than the 55 kg value customarily used for Western women, and the iron requirements are proportionately less. However, food intake is also likely to be reduced in such populations so that supplemental iron is a crucial requirement during pregnancy (INACG report, 1981; Taylor et al, 1982b; Toe & Than, 1982; Srispandit et al, 1983; Wallenburg & van Eijk, 1984).

2.1.6 Lactation

Although the iron content of breast milk is low, being about 0,5 mg/l during the first month after birth and declining to about 0,3 mg/l between four and six months postnatally (Siimes et al, 1979), it is highly bioavailable to the infant (Saarinen et al, 1977; Garry et al, 1981). A low molecular weight ligand to which iron has been found bound in large amounts, possibly accounts for this high bioavailability (Weber et al, 1982). The average daily maternal iron losses from breast feeding are low, being about 0,1 mg at one month, 0,28 mg at two months and 0,27 mg at three months (Vuouri 1979). Lactation, therefore, does not usually represent a significant drain of iron, but it may do so in developing countries, where breast feeding is continued long after the recommencement of menstruation.

2.2 Changing Iron Requirements Occurring with Growth

While the foetus is in utero, iron is derived solely from the mother through the active transport function of the placenta. It is therefore in a privileged position insofar as iron nutrition is concerned (MacPhail et al, 1980). As was mentioned earlier, it is generally accepted that iron deficiency in the mother does not affect the infant's iron stores at birth (Dallman et al, 1980; Zitloun et al, 1983). Even in mature infants the most important influence on body iron stores is birth weight. The foetus acquires most of its iron during the last trimester of pregnancy, since this is the period of maximal weight gain (Burman 1982).

After birth, the infant faces extrauterine life with an endowment of iron from his mother of about 80 mg/kg (Widdowson & Spray, 1951; Josephs, 1959). Of this amount, 50 mg/kg is present in the iron of haemoglobin, 25 mg/kg exists as storage iron divided equally between the liver and other tissues, and 5 mg/kg is found in the erythroid cells of the marrow, in myoglobin and in various intracellular enzymes (Widdowson & Spray, 1951). Though the body iron content at birth is directly proportional to the birth weight of the infant (Widdowson & Spray, 1951), it is also affected by the initial haemoglobin mass, which in turn is critically influenced by the time of ligation of the umbilical cord (Burman 1971; Lanzkowsky 1976). The proportion of blood distributed between the infant and the placenta immediately after delivery is 67% and 33%

respectively. Uterine contractions rapidly propel blood along the umbilical veins from the placenta to increase the infants endowment to 87% over the next 3 minutes (Yao et al, 1969). Clamping the cord immediately after delivery may therefore reduce the infant's blood volume from 93 ml/kg to 70 ml/kg, which this represents the loss of a significant contribution of iron (Yao et al, 1969; Burman 1971; Lanzkowsky, 1976).

In the first six to eight weeks of life, erythropoiesis virtually ceases, and the haemoglobin concentration declines from about 17 g/dl to 11 g/dl (O'Brien & Pearson, 1971). Since this is associated with a rise in the serum ferritin concentration, it would appear that most of the iron released from haemoglobin is transferred to the storage compartment (Rios et al, 1975; MacPhail et al, 1980). Thereafter the red cell mass gradually expands at the expense of the infant's iron stores, which are usually depleted by six months of age. An adequate intake of iron is therefore essential. Between this time and the end of the first year of life when the body weight has reached about 10 kg (Bothwell et al, 1979). During the second year of life the growth rate slows and the body mass only increases by about 2,5 kg. Eighty mg of iron is necessary to support this growth and when added to the daily losses of about 0,2 mg per day, represents 0,4 mg per day (Bothwell et al, 1979). Should the child be receiving a well balanced diet, the iron required for growth can be easily obtained.

During childhood the body weight increases from the second year to the twelfth year by 2,5 - 2,75 kg per year. This is accompanied by a steady rise in iron losses from Ø,2 mg per day in infancy to about Ø,5 mg per day at the end of the childhood years. Since the haemoglobin level also increases by about 1 g/dl, iron requirements rise from Ø,5 mg to Ø,8 mg per day during this period (Bothwell et al, 1979).

Adolescence is accompanied by a growth spurt that increases the body mass of males more than that of females (Bothwell et al, 1979; Lanzkowsky 1982). This increase in lean body mass, together with a rise in the haemoglobin level of about 2 g/dl in males (Garn et al, 1981) and 1 g/dl in females, results in a total daily need of 1,6 mg per day. Once the growth spurt has ended in males, iron stores rise to the adult level of 1ØØØ mg and obligatory basal iron losses to 1 mg per day, necessitating an early adulthood requirement of about 1,2 mg per day. The onset of the menarche in females, supplants the need for the iron required for growth and represents a median extra requirement of about Ø,5 mg per day (Marshall & Tanner, 1969). When added to the basal iron losses of Ø,8 mg per day, the average daily iron needs of young menstruating women are about 1,4 mg per day. However, it must be remembered that there are many in whom monthly blood loss exceeds 3Ø ml (Baldwin et al, 1961; Cole et al, 1971; Hallberg et al, 1966b) so that daily requirements may be as much as 2,Ø mg per day in a proportion of women (INACG report,

1981). Faddish food selection may also influence iron nutrition during adolescence (Lanzkowsky 1982; Bothwell & Charlton, 1982) and contribute to the 10% prevalence of anaemia among American teenage girls and non-pregnant women (White 1968; White 1970). A similar situation exists in the United Kingdom (Kilpatrick & Hardisty, 1961; Jacobs et al, 1965; Barber et al, 1985). From serum ferritin measurements, adolescent girls are at a far greater risk for developing iron deficiency than are boys and menstrual blood loss is the single most significant factor in determining their iron status (Bailey et al, 1982; Milman & Ibsen, 1984).

2.3 Absorption of Iron from the Diet

The demonstration by McCance and Widdowson (1937) that the ability of the body to excrete iron is limited, pinpointed the absorptive process as the major factor in determining body iron content. The three major factors influencing the absorption of iron from the diet are the amount of iron ingested, its bioavailability, and the behaviour of the intestinal mucosa (Cook & Monsen, 1976).

2.3.1 Dietary iron content

The iron content of the diet is determined by its constituent foods. Typical Western meals contain 6 mg iron per 1000 kcal, with surprisingly little variation between different diets (Hallberg & Björn-Rasmussen, 1981). Some previous dietary surveys have overestimated the iron content of foods, since contaminant iron has been included in the

estimations (Bothwell et al, 1979). This extrinsic iron may have been derived from soil residues on vegetables and cereals or have been dissolved from the surface of cooking utensils and containers (Hallberg & Björn-Rasmussen, 1981; INACG report, 1982). It is generally of low bioavailability (Hallberg et al, 1983; Björn-Rasmussen, 1983), but may contribute to iron nutrition when the food or beverage being prepared has a low pH (Derman et al, 1980a).

2.3.2 Bioavailability of dietary iron

With regard to absorption there are two types of iron present in the diet; haem iron derived from haemoglobin and myoglobin and non-haem iron derived mainly from vegetables, cereals and fruits (Hallberg 1981a). Haem iron is not degraded within the lumen of the gut (Sanford, 1960) and is absorbed as haem, with the iron locked within the porphyrin ring (Weintraub et al, 1968). Its absorption is therefore not affected by the numerous factors present within the diet that are able to alter the absorption of non-haem iron (Turnbull et al, 1962; Turnbull, 1974). Though the contribution of haem iron to the total iron intake is small in relation to the amount of non-haem iron (10-15% in Western diets (Hallberg, 1981b)), its high bioavailability endows it with great nutritional significance (Layrisse & Martinez-Torres, 1972; Milman et al, 1984).

Radioisotopic studies in which the haem and non-haem iron present in foods have been separately labelled, have demonstrated that each forms a separate pool of iron

(Martinez-Torres & Layrisse, 1971; Cook et al, 1972; Björn-Rasmussen et al, 1973; Layrisse & Martınez-Torres, 1972). Hence all the non-haem iron in the diet forms a 'common pool' in that it is all absorbed to the same degree. In addition, a comparable, common intraluminal pool exists for haem iron absorption. There are exceptions to the intraluminal pool of non-haem iron. For example, the iron storage compounds, ferritin and haemosiderin are less well absorbed than the cereal iron in a meal (Layrisse et al, 1975; Martinez-Torres et al, 1976). Their absorption is however affected by promoting and inhibiting factors that may be present and the greater the concentration of enhancing ligands such as ascorbic acid, the more closely does the absorption of iron from these compounds approximate that of iron in the non-haem pool (Martinez-Torres et al, 1976; Derman et al, 1982).

A major factor determining iron absorption is the solubility of iron (Hungerford & Linder, 1983). This is related to particle size and in general the smaller the particle size, the more easily will it solubilize at the low pH of the stomach before it passes into the duodenum (Clydesdale, 1983). The insoluble iron compounds such as ferric pyrophosphate, ferric orthophosphate and ferric oxyhydroxide are all poorly absorbed (Cook et al, 1973c; Derman et al, 1977; Clydesdale, 1983), while ferric oxide is not absorbed at all (Derman et al, 1977). Iron absorption from Fe(lll)EDTA has been found to be somewhat better than

that of the intrinsic non-haem food iron. Presumably iron in this chelated form is partially protected from inhibitory ligands in the diet (MacPhail et al, 1981b; Candela et al, 1984). EDTA itself is capable of solubilizing 95% of iron bound to neutral detergent fibre, lignin and cellulose at pH 5 and 6,45 (Reinhold et al, 1981). Non-haem iron added to unmilled, unpolished rice is poorly exchanged with the intrinsic non-haem iron, probably due to impaired diffusion of iron across the dense outer aleurone layer (Björn-Rasmussen, 1973).

Non-haem iron must be in an ionized form before it can be absorbed (Bothwell et al, 1979). *In vitro* studies investigating the variables that are involved in the release of iron from compounds in foods, have been performed by mimicking the processes of digestion (Lock & Bender, 1980; Kojima et al, 1981; Miller et al, 1981; Schricker et al, 1981). Values for the amounts of ionizable iron obtained by these methods are usually greater than the actual amount of iron absorbed *in vivo* from the same foods (Martinez-Torres & Layrisse, 1973). This indicates that though *in vitro* studies provide an index of the maximum amount of iron available for absorption in a particular foodstuff, the released iron is vulnerable to a number of different exogenous and endogenous ligands. The profound modifying influence on iron absorption of these various promoting and inhibitory ligands is obvious from the different bioavailable nutrient density for iron in Western and Asian

meals. This is the amount of iron absorbed from a meal per 1000 kcal by subjects who are borderline iron deficient (Hallberg et al, 1983).

2.3.3 Exogenous iron ligands

2.3.3.1 Inhibitors of iron absorption: The interaction of inhibitors and promoters of iron absorption in the diet is central to the process of iron absorption, since the interaction of various ligands profoundly influences the bioavailability of the iron found in food (Bothwell et al, 1979; Lechner & Raja, 1984). When both promoting agents and inhibiting agents are present in a meal, their effects are antagonistic (Bothwell et al, 1979). On the basis of *in vitro* studies, the inhibitors of iron absorption are thought to form large insoluble complexes with iron, that are poorly absorbed (INACG report, 1982). The most frequently listed inhibitory substances are carbonates, oxalates, phosphates and phytates (Bothwell et al, 1979). The degree to which these compounds depress iron absorption *in vivo* is, however, uncertain. For example, inorganic phosphate, when administered alone, does not affect iron absorption but does decrease it when administered as the calcium salt (Monsen & Cook, 1976; Miller, 1983a). Calcium appears to depress the absorption of both ferrous and ferric iron in a dose related fashion (Ellis et al, 1982; Barton et al, 1983). Hallberg (1981a) has not found oxalate to depress non-haem iron absorption, when added in a dose of 100 mg to a mixed meal.

The effect of phytate on iron absorption is controversial and this may be due to the fact that a whole range of inositol polyphosphates occur in nature (Johnson & Tate, 1969). Monoferric phytate which is the major fraction of iron in wheat bran (Morris & Ellis, 1976; Morris & Ellis, 1982), is highly bioavailable (Lipschitz et al, 1979; Ellis & Morris, 1979; Clydesdale, 1983). In contrast, di- and tetraferric phytate are poorly available (Morris & Ellis, 1982). Studies performed in mice have not shown phytic acid to have any substantial effect on the absorption of ferric iron (Graf & Eaton, 1984). While sodium phytate has been shown to decrease iron absorption in man (Turnbull et al, 1962), in vitro studies have demonstrated that it behaves as a solubilizing agent of iron in the presence of neutral detergent fibre, lignin and cellulose (Reinhold et al, 1981; Platt & Clydesdale, 1984). Sandberg and co-workers (1982) failed to find any inhibition of iron absorption by wheat bran in ileostomy patients who were on a low fibre diet.

Because dephytinized wheat bran has been found to be capable of inhibiting iron absorption (Morris et al, 1980; Simpson et al, 1981) its inhibitory effect has been attributed to a water soluble, phosphate rich fraction (Simpson et al, 1981). Washing bran with water does not reduce its depressant effect, but boiling it with dilute hydrochloric acid does remove all the phytates and reverses the inhibition of iron absorption (Camire & Clydesdale, 1982; INACG report, 1982). Replacing the phytate in

hydrochloric acid washed bran with sodium phytate, restores the depressing effect of bran on non-haem iron absorption (INACG report, 1982). Though these studies seem to point to sodium phytate as the factor responsible, the relative concentration of phytate binding minerals such as Ca^{++}, Mg^{++} and Zn^{++}, may play an important role in limiting food iron bioavailability. They probably do this by forming insoluble complexes with the iron phytate complex (Subba Rao & Narasinga Rao, 1983).

It has also been postulated that the progressive depression in iron absorption which parallels the bran content of bread, may be due to its fibre composition (Björn-Rasmussen, 1974). In vitro studies have demonstrated that the fibre of wheat and maize binds iron (Ismail-Beigi et al, 1977; Reinhold et al, 1981). However work carried out by Anderson and co-workers (1983) indicates that wheat bran and the cell wall polysaccharides of bran are unlikely to exert a significant effect on iron absorption independently of the effect of the phytate present in the bran. Thus the data on the effect of fibre on iron absorption are conflicting. The matter is discussed further in Chapter 6 of this thesis, which describes the results of a careful investigation of the effects on iron absorption of the various components of 'fibre'.

One of the most important inhibitors of non-haem iron absorption is Indian tea (Disler et al, 1975a; de Alarcon et al, 1979; Roy & Mukerhjee, 1979; Hallberg & Rossander,

1982b; Acosta et al, 1984; Yau-tian, 1983). When tea is consumed with a variety of meals, insoluble iron tannates are formed (Disler et al, 1975b; Weber & Schwedt, 1984). Tannates are also present in coffee and when non-haem iron absorption from a hamburger meal was measured, tea reduced it by 61% and coffee by 33% (Hallberg & Rossander, 1982b). Morck and co-workers (1983) have also noted a concentration dependent inhibition of iron absorption by coffee. It is possible that it may exert this effect in a different fashion from tea by oxidizing iron to the less bioavailable ferric form (Kojima et al, 1981; Morck et al, 1983). Polyphenols, of which tannates are an example, are widely distributed in other foodstuffs and the poor absorption of the iron found in many of the legume and cereal staples consumed in India may be attributed to their polyphenol content (Narasinga Rao & Prabhavathi, 1982). The complex interplay between phytates, polyphenols and fibre insofar as iron absorption is concerned is examined in Chapters 5 and 6 of this thesis.

Soybean products are also associated with a depression of non-haem iron absorption (Cook et al, 1981; Morck et al, 1982) though they may improve the bioavailability of haem iron (Lynch et al, 1985). The iron-binding proteins in soy comprise two major fractions, a large protein or protein aggregate that elutes at > 600,000 daltons and a low molecular weight species that elutes in the range of 10,000 - 25,000 daltons (Smith, 1983). <u>In vitro</u> work has revealed

that the iron contained in the larger protein does not undergo complete exchange with extrinsically added iron (Smith, 1983). In iron deficient rats, soy protein isolate has been shown to affect iron absorption adversely in two ways (Thompson & Erdman, 1984). When it is present in a meal less iron is retained in comparison to a casein test meal. Furthermore, when the diet before and after the casein test meal is based on soy protein isolate then iron retention from the test meal is lower than when the diet is casein based. The mean value for iron absorption from whole boiled soybeans of 1,Ø6% (Morck et al, 1982) is very similar to the poor figures obtained from other legumes such as blackbeans - 1,5% (Cook et al, 1972) and 2,6% (Layrisse et al, 1969). Iron absorption from soy sauce, a condiment extracted from the fermentation of soy beans and flour, is greater, having a mean value of 6,95% (Yau-tian, 1983). This is of potential importance since, the high iron content of soybeans, as well as its high content of protein and fat (4Ø% and 2Ø% respectively), make the legume a valuable source of good quality protein (INACG report, 1982). Because of their nutritional properties, soy food products are used extensively by Asian populations (Chou, 1983; Yau-tian, 1983) and are also incorporated into Western diets where they are widely used as meat extenders. They may even be the sole source of protein and calories for infants with suspected or proven lactose intolerance, who are fed with soy based formulas (INACG report, 1982). As this latter

group is one critically vulnerable to iron deficiency, the problem was investigated in the present thesis. A means of improving iron absorption from soy based infant formulas is described in Chapter 7.

Eggs have been found to depress non-haem iron absorption, though the actual amount of iron absorbed increases slightly because of the high iron content of breakfasts that contain eggs (Rossander et al, 1979). Egg yolk contains a phosphoprotein which binds iron strongly (Halkett et al, 1958) and when heated egg albumin is fed with iron the low absorption of the latter is significantly increased, probably due to the inactivation of conalbumin. Milk has not been found to decrease iron absorption (Hallberg, 1981a; Hallberg & Rossander, 1982b), and has been used successfully as a vehicle for supplementing the diet of Mexican school-children with iron (Rivero et al, 1982). However, since dairy products add very little iron to a meal and since they do not improve absorption, meals in which they are the major protein source, are far less satisfactory from an iron nutritional standpoint than are meals containing equivalent portions of meat, fish or poultry (Cook & Monsen, 1976).

2.3.3.2 Enhancers of iron absorption: For a compound to be an enhancer it must promote solubility, it must maintain its stability during the process of digestion and it must release it to an acceptor in the intestinal mucosa (Clydesdale, 1983). The major promoters of iron absorption

are ascorbic acid and meat.

Ascorbic acid is a powerful reducing agent and also binds iron in equimolar concentrations to form readily absorbable complexes with ferric ions, thereby preserving iron solubility in the alkaline milieu of the duodenum (Conrad & Schade, 1968; Lynch & Cook, 1980; Nojeim & Clydesdale, 1981; Gorman & Clydesdale, 1984). The promoting effect of ascorbic acid on iron absorption appears to be dose related (Sayers et al, 1973; Björn-Rasmussen & Hallberg, 1974; Cook & Monsen, 1977; Derman et al, 1980b). In <u>in vitro</u> studies, Kojima and co-workers (1981) increased the percentage of soluble iron in a bean suspension from 10% to 50%, by raising the molar concentration of ascorbic acid from 0 to 10 mM. The International Nutritional Anaemia Consultative Group (1982) has indicated that the mean percentage of iron absorbed from soy protein containing foods increases in direct proportion to the molar ratio of ascorbic acid to iron. The beneficial effects of ascorbic acid on iron absorption has been demonstrated by comparing values from two meals of similar iron content (Hallberg, 1981a). By including cauliflower containing about 70 mg ascorbic acid into a vegetarian meal, non-haem iron absorption increased from 0,32 to 0,98 mg. A similar 3 fold increment was achieved by adding boiled cauliflower containing about 65 mg ascorbic acid to a simple Latin American meal (Hallberg & Rossander, 1984). Serving a meal with orange juice which contained about 70 mg ascorbic acid

also significantly increased iron absorption (Rossander et al, 1979; Hallberg & Rossander, 1982b).

Ascorbic acid has recently been shown to be effective in reversing to some degree the inhibitory effect of soy products on non-haem iron absorption (Morck et al, 1982; Rizk & Clydesdale, 1983; Weaver et al, 1984). This is predominantly a function of its reducing capabilities (Rizk & Clydesdale, 1984). Camire and Clydesdale (1982) have found that iron binding to wheat bran is completely inhibited by 60 mg ascorbate. With products having a high protein content, such as soy and beans, a low pH has been found necessary in vitro to permit effective solubilization of iron by ascorbate (Kojima et al, 1981; Rizk & Clydesdale, 1983). Clydesdale (1983) has postulated that since the protein in soy and beans is close to its isoelectric point at an alkaline pH of 6,0, this may prevent solubilization of iron by ascorbate. At a low pH (± 2,0), any fibre mineral complexes will have been solubilized already by pH alone and the increased solubilization then results from removal of iron from the protein by ascorbate. Crystalline ascorbic acid and the native ascorbic acid present in foods have about the same promoting effect (Hallberg, 1981a). Cooking and baking procedures can destroy the ascorbic acid and its effect on iron absorption (Sayers et al, 1974 a & b; Miller, 1983 a & b). Prolonged warming of meals reduces their ascorbic acid content significantly, with 75% of the ascorbic acid loss occurring within the first hour of

warming (Hallberg et al, 1982). This is associated with a reduction in iron absorption from such meals. Miller and co-workers (1981) have also found in in vitro experiments that the high levels of ascorbic acid added to processed meats do not affect the amount of dialyzable iron. This implies that such processing methods destroy the effectiveness of ascorbic acid as a reducing agent and chelator of iron. When another promoter such as meat is also present in a meal, the enhancing effect of ascorbic acid is diminished (Hallberg, 1981a), while the presence of an inhibitor such as tea counteracts its effect (Disler et al, 1975a). From the previous discussion it is therefore apparent that ascorbic acid plays a crucial role in diets containing little or no meat, such as are consumed by most of the world's population (INACG report, 1982; Cook et al, 1984).

In contrast to ascorbic acid, citric acid most effectively solubilizes iron from neutral detergent fibre, lignin and cellulose. It has a similar effect on beans at a high pH (5,Ø - 6,45) but loses its effectiveness with beans at a pH of 2,Ø (Kojima et al, 1981; Reinhold et al, 1981; Fernandez & Phillips, 1982a). These observations indicate its powerful binding strength (Clydesdale, 1983). There are, however, many other organic acids present in vegetables and fruits. Their role with respect to iron nutrition has been carefully dissected in Chapter 5, which is concerned with iron absorption from vegetables.

The significance of meat for iron nutrition is two fold. Not only does the absorption of haem iron appear to be a linear function of the amount of haem iron in the meal (Bezwoda et al, 1983), but there is also evidence that it potentiates the absorption of non-haem iron from other foodstuffs (Martinez-Torres & Layrisse, 1971; Layrisse & Martinez-Torres, 1972; Cook & Monsen, 1975; Martinez-Torres et al, 1975; Cook & Monsen, 1976; Hallberg & Rossander, 1982c; Hallberg & Rossander, 1984). The availability of haem iron ranges from 15% for iron replete individuals to 35% in those lacking iron stores (Monsen & Balintfy, 1982). Serving beef with an isolated soy protein meal has been found to improve marginally non-haem iron absorption from the legume (Morck et al, 1982).

The mechanism responsible for this effect is not known. Martinez-Torres and co-workers (1981) found increments of the same order in the absorption of non-haem iron when black beans were served with 100 mg of fish or with the relevant amino acids present in the same quantity of fish. Neither the basic amino acids nor the aliphatic and aromatic groups have been found to improve non-haem iron absorption and among the sulphur amino acid group only cysteine enhances vegetable iron absorption (Martinez-Torres & Layrisse, 1970; Martinez-Torres et al, 1981). Soluble ferric cysteine is stable over a pH range of 2,0 to 7,4 (Flynn et al, 1984). However, its effect is only significant if added to the meal after cooking, since it is oxidized to cystine by heating

(Martinez-Torres et al, 1981). The divalent amino acids asparagine and glycine have also been shown to increase iron absorption in rats (Christensen et al, 1984). If cysteine stability is preserved by feeding it in the peptide form as reduced glutathione, it improves both haem and non haem iron absorption (Layrisse et al, 1969). Cysteine is very effective in the solubilization of iron from neutral detergent fibre at a pH of 6,45 (100%) (Reinhold et al, 1981) but less so from beans at pH 2 (30%) (Kojima et al, 1981). It has no effect on lignin at pH 5,0 (Fernandez & Phillips, 1982). The release of amino acids and polypeptides in the upper small bowel from animal proteins may be a reason for their enhancing influence on iron absorption (Layrisse et al, 1969). The globin degradation products from haemoglobin may also enhance iron absorption by preventing the polymerization of haem (Martinez-Torres et al, 1981). Simulated digestion studies have shown that when meat or haemoglobin is digested, the end products are low molecular weight non haem compounds that are easily absorbed (Hazell et al, 1981). Haemoglobin is absorbed intact when digested alone but as a non haem iron complex when digested with meat (Hazell, 1982).

Studies have also been conducted to assess the effects of processing and cooking methods on iron absorption from meat. Nitrite cured meats, which account for about 7% of the total food supply in the U.S.A., probably have no detrimental effect on iron absorption (Lee & Greger, 1983).

Schricker and Miller (1983) have found that the haem iron concentration of ground beef samples falls by less than 10% when prepared conventionally, but drops by about 40% when cooked by baking or by microwave heat. Thus preparation of the meal by these methods may decrease its haem value, probably by oxidative cleavage of the porphyrin ring.

Alcohol is another promoter of iron absorption (Hallberg & Rossander, 1982b; Chapman et al, 1983). It is not a ligand and probably acts by stimulating gastric acid secretion, since the increased absorption of ferric chloride which it causes is not seen in achlorhydric patients (Charlton et al, 1964). Celada and co-workers (1978) have however found whisky to depress iron absorption.

When all the current information is taken together it shows that the bioavailability of dietary iron is largely determined by the complex interplay between the various promoting and inhibitory ligands present in commonly used foods and that variations in meal composition have a major impact on iron bioavailability (Schricker & Miller, 1982). It is therefore important to attempt to establish the roles that compounds present in food staples such as vegetables, cereals and legumes play in affecting iron absorption. This topic is explored further in chapters 5, 6 and 7 of this thesis.

2.3.4 Endogenous ligands

Ionic iron appears to bind with large molecular weight compounds in gastric juice, which prevent its precipitation

in the alkaline milieu of the upper small bowel. However, this process is not altered in any of the conditions known to affect iron absorption (Bothwell et al, 1979). Similarly, whilst the various biliary, pancreatic and intestinal secretions have obvious relevance to iron absorption in that they promote digestion with release of iron from food, there is no evidence that they contain any component that acts as a specific carrier of iron (Bezwoda et al, 1978). The proteolytic enzymes secreted by the pancreas may have an enhancing effect on iron absorption, since they release polypeptides and amino acids, that can act as promoting ligands for iron absorption (INACG report, 1981). Their role cannot however, be a major one, since iron absorption is not reduced in patients with chronic pancreatitis (Balcerzak et al, 1967; Kavin et al, 1967).

2.3.5 The role of the stomach in iron absorption

Gastric acid is one of the crucial factors necessary for optimal non-haem iron absorption. During peptic digestion of food in the stomach, haem is released from its bonds with globin (Conrad et al, 1966) and a proportion of the non-haem iron in food is rendered ionizable (Jacobs & Greenman, 1969). The importance of gastric acid seems to relate to the fact that polymeric iron complexes are less likely to form at a low pH (INACG report, 1981; Smith, 1983). This is supported by the finding that achlorhydric patients absorb ferric iron poorly, even when they are iron deficient (Cook et al, 1964; Jacobs et al, 1964). The defect

may be corrected by administering hydrochloric acid (Jacobs et al, 1964). Ferrous salts and haemoglobin iron are however adequately absorbed by achlorhydric patients (Jacobs et al, 1964). A further example of the importance of gastric acid in food iron absorption is the demonstration by Skikne and co-workers (1981) that small doses of cimetidine, which is an H_2 receptor antagonist, administered one hour before a meal produce a modest but significant decrease in non-haem iron absorption.

The reservoir function of the stomach is also important for haem and non-haem iron absorption (Stevens et al, 1959; Turnbull, 1965) and the bioavailability of iron is affected by the rate of gastric emptying. It has been found that if a neutral iron solution is acidified at pH 2,Ø it may take up to one hour to convert the iron from its polymerized form to a soluble one (Smith, 1983). Thus a meal comparing food items that affect residence time in the stomach differentially may influence the resolubilization of iron. It is therefore not surprising that iron deficiency is a complication of gastrectomy procedures that lead to an accelerated passage of the food bolus through the upper gastrointestinal tract (Bothwell et al, 1979). In the Polya gastrectomy, food is also deviated away from the normal site for iron absorption in the upper duodenum (Magnusson, 1976).

2.3.6 Gastrointestinal secretions and the absorption of haem compounds

Once haem containing compounds have been split in the

intestinal lumen into haem and globin, haem is taken up by the mucosal cells as the intact metalloporphyrin (Conrad et al, 1966). Some workers have identified a high affinity haem receptor on the brush border membrane of porcine duodenum which is remarkably similar to haemopexin (Gräsbeck et al, 1982). Having entered the mucosal cell, probably by endocytosis (Parmley et al, 1981a), an enzymatic process catalysed by microsomal haem oxygenase (Tenhunen et al, 1969; Raffin et al, 1974) splits iron from the porphyrin ring. This allows the iron to be transported rapidly into the circulation (Wheby & Spyker, 1981). Raffin and co-workers (1974) have demonstrated that the enzyme activity involved in haem iron absorption is increased in the iron deficient state. It is evident therefore that the iron in haem compounds is absorbed more efficiently than non-haem food iron and the factors known to influence the absorption of non-haem iron do not affect haem iron absorption (Turnbull et al, 1962). Meat has a second action in that it potentiates the absorption of both haem and non-haem iron (Hallberg et al, 1979a; Martinez-Torres & Layrisse, 1971). The bioavailability of haem iron in foods prepared from blood is much lower than if meat is included in the meal (Hallberg, 1981a), with figures of about 10% and 25% respectively (Hallberg et al, 1979a). About half the iron content of meat products is non-haem iron (Hallberg, 1981b).

2.3.7 The influence of mucosal factors

The most active sites of iron absorption are the duodenum and upper jejunum (Chirasiri & Izak, 1966; Richter & Lee, 1982; Parmley et al, 1984) and absorption then decreases progressively towards the terminal ileum (Van Campen & Mitchell, 1965). This is a property of the intestinal mucosa itself and not of the intraluminal pH; it applies both to haem and non-haem iron absorption (Howard & Jacobs, 1972). Though little is known about the molecular mechanisms of iron absorption, the process is usually divided into phases of mucosal uptake and mucosal transfer (Marx, 1979). Iron uptake refers to the entry of iron from the intestinal lumen into mucosal cells (Bothwell et al, 1979) and is a process that is dependent in part on the concentration of iron within the lumen (Höglund & Reizenstein, 1969). This relationship has been found to be linear over a Ø,1 to 5,Ø mM range in rats (Thomson & Valberg, 1971). The previous iron intake of experimental animals also has an effect upon the mucosal uptake of iron (Fairweather Tait & Wright, 1984). In contrast, the previous type of dietary protein intake has little influence on iron absorption (Johnson & Weaver, 1983).

In the proximal small intestine iron binds to surface glycoproteins located on the brush border of the mucosal cells (Kimber et al, 1973) and variations in their distribution and number may be part of the mechanism whereby absorption of food iron is altered to meet changing body

requirements (Bothwell et al, 1979; Muir & Hopper, 1985). Isolated brush border preparations, from the distal intestine of iron deficient animals bind more iron than those from animals with normal iron stores, while proximal iron binding is inhibited in iron loaded animals (Kimber et al, 1973; Barton et al, 1981). Cox and Peters (1980b) have found iron uptake by duodenal mucosal biopsy samples obtained from iron deficient patients to be 2 to 3 times greater than normal. Oral iron repletion restored uptake to normal values in these subjects even though mucosal cell iron levels remained low. Cox and Peters (1980b) postulated that iron deficiency reversibly induces brush border iron carriers, making the entry of iron into the intestinal cell a major regulatory step. The uptake of iron is a process known to be temperature dependent (Marx & Aisen, 1981; Simpson & Peters, 1984). In addition, metabolic energy is required for movement across the cell membrane (Kimber et al, 1973; Cox & O'Donnell, 1981).

Transfer refers to the movement of iron across the mucosal cell and into the body (Bothwell et al, 1979). While a proportion of the iron is transferred to the portal circulation within minutes, the overall process is a slow one which takes 12 - 24 hours. Some of the iron in mucosal cells is stored as ferritin and is discarded as cells exfoliate (Charlton et al, 1963; Conrad & Crosby, 1963; Wheby & Crosby, 1963). The amount of iron transferred depends upon the iron status of the individuals; - in those

who are iron deficient almost all the iron entering the mucosa is transferred, whilst in iron replete persons an increased amount of iron is sequestered within the mucosa as ferritin (Boender & Verloop, 1969).

There are two distinct iron-binding compartments in the intestinal mucosa, a storage compartment containing ferritin and a transport compartment involving a protein similar to transferrin (Huebers et al, 1976; Savin & Cook, 1980; Johnson et al, 1983). The source of mucosal transferrin has not yet been identified (Huebers et al, 1983b). It has a molecular weight of 78,000 and comprises two equal subunits so that each molecule can bind two atoms of iron (Pollack & Lasky, 1976). It is a system that is dependent upon metabolic energy for normal function (Manis & Schachter, 1962), and one that is capable of altering its capacity in accordance with body iron requirements (Forth & Rummel, 1973). Huebers and co-workers (1983b) have postulated a "shuttle" role for mucosal transferrin. According to this theory, apotransferrin secreted by mucosal cells reacts with food iron in the lumen to form transferrin. The iron-transferrin enters mucosal cells as an intact complex and can then be transfered to the interior or be retained within the cell as mucosal ferritin. When iron-transferrin has delivered its iron to the plasma the iron free transferrin is free to return to the intestinal brush border. Xanthine oxidase may be the enzyme located in the intestinal mucosa that promotes the incorporation of iron into transferrin

(Edit. Nutr. Rev. 1981; Topham et al, 1981). However, iron absorption is not impaired in rats depleted of xanthine oxidase (Kelley & Amy, 1984).

Haem iron uptake and transfer are maximal in the duodenum (Wheby, 197Ø), and once the iron has been freed from the porphyrin ring it appears to enter the same pool within the mucosal cell as non-haem iron. This means that not all the absorbed haem iron is necessarily transferred (Bothwell et al, 1979; Parmley et al, 1981a).

2.3.8 Regulation of iron absorption

The amount of iron that is absorbed each day from the diet is a function of the amount of available iron in the diet, the body's iron stores and the rate of erythropoiesis. The absorptive mechanism responds rapidly to fluctuations in iron requirements (Finch et al, 1982). If the body iron content is reduced, a high percentage of available iron is absorbed. In contrast, absorption falls as the body iron content rises (Kuhn et al, 1968; Bezwoda et al, 1979). Absorption of iron is therefore inversely related to the plasma ferritin concentration (Charlton et al, 1977; Bezwoda et al, 1979).

Ultimate control of the regulation of the rate of iron absorption is probably closely related to the iron content of individual tissues (Cavill et al, 1975) though a local role has been suggested for the intestinal mucosa (Kimber et al, 1973; Cox & Peters, 198Øb; Savin & Cook, 198Ø; Björn-Rasmussen, 1983). From studies in rats it has been found

that increasing receptors for transferrin bound iron by exchange transfusion with reticulocyte rich blood leads to an immediate increase in the donation of iron from tissues, including the mucosal cells of the gut (Finch et al, 1982). This occurs without there being a significant alteration in the plasma iron level (Rosenmund et al, 198Ø; Finch et al, 1982). Plasma iron turnover appears to be determined primarily by tissue iron requirements as expressed by the number of membrane receptors for the transferrin iron complex (Finch et al, 1982). Thus any increase in plasma iron turnover, whether due to enhanced erythropoietic activity or to a decrease in tissue iron content, will lead to increased iron absorption (Bothwell et al, 1983). The gut mucosa must therefore be seen as one of the tissues containing a labile pool of iron which is available to transferrin. The amount of iron donated by each tissue is proportional to the iron stores present in that tissue (Cavill et al, 1975). As iron uptake from transferrin is largely determined by the requirements of the erythroid precursors and as each tissue supplies iron to transferrin in proportion to its own pool (Hershko, 1977), the rate of erythropoiesis is the ultimate factor responsible for controlling iron absorption.

Chapter Three

DISORDERS OF IRON NUTRITION

3.1 The Problem of Iron Deficiency

Iron has been selected by evolution to form the "energy backbone" in vertebrate physiology and its essential role has become highlighted by the problem of nutritional iron deficiency, which currently affects more than 500 million people throughout the world (Finch & Huebers, 1982; Huebers & Finch, 1982). Whilst nutritional iron deficiency of a mild degree is widespread in the United States and Western Europe, it reaches its greatest prevalence and severity in the developing nations where cereal and vegetable staples form a large proportion of the daily caloric intake (Monsen et al, 1978; Bothwell & Charlton, 1982; Linpisarn et al, 1984; Mayet et al, 1985). The virtual disappearance of meat from the diet of these nations has given rise to a situation where the amount of iron that can be absorbed from the diet is inadequate to meet physiological requirements. A frequent problem compounding the drain imposed by basal iron losses is that caused by parasitic infestation. In this regard, hookworm infestation is especially important; it leads to intestinal blood losses that parallel the parasitic load (Layrisse et al, 1964). Since hookworm infestation is so common in developing nations, it can be regarded as a factor contributing to nutritional iron deficiency (Miller, 1979; Bothwell & Charlton, 1982).

3.1.1 The assessment of iron status

Because of the wide variation in haemoglobin levels between different normal subjects, the evaluation of the

iron status of an individual involves the measurement of additional parameters. These include the level of storage iron as reflected by the serum ferritin, the amount of iron bound to the transport protein, transferrin, and the concentration of free red cell protoporphyrin. Where only one of the measurements of the iron status is abnormal, iron deficiency is unlikely to be present, whilst its prevalence in a population will be far greater when two or more parameters are abnormal (Cook et al, 1976). If anaemia in an individual is defined as a haemoglobin level two standard deviations below the mean value in a population, there will be many subjects who have depressed formation of haemoglobin in new erythrocytes, even though their haemoglobin values lie within the normal range for the population (Hallberg, 1982b). Because of these overlaps it is helpful to divide the development of iron deficiency anaemia into three stages (Bothwell et al, 1979; Siimes et al, 1980; Cook, 1982).

The earliest stage is that of <u>storage iron depletion</u> in which the serum ferritin falls below 20 /ug/l. Though the iron reserves are virtually absent there is not yet a decrease in the supply of iron to the developing red cell. When there is no longer an adequate supply of iron entering the plasma, the serum iron concentration starts falling progressively. When it is less than 40 /ug/dl and the transferrin saturation has fallen to below 16%, the delivery of iron to the erythroid marrow becomes severely compromised (Bainton & Finch, 1964; Hillman & Henderson, 1969). At this

stage of iron deficient erythropoiesis not all the porphyrin being synthesized can be incorporated into haem so that red cell protoporphyrin concentrations rise above normal (Langer et al, 1972). If the figure exceeds 70 /ug/dl red cells, the iron supply to the marrow is considered to be suboptimal (INACG report, 1981). The third and final stage is overt iron deficiency anaemia. This occurs when the level of circulating haemoglobin has fallen below the "cut off" value for anaemia (Cook, 1982). The cut off value varies as a function of age and sex. The haemoglobin levels below which anaemia is likely to be present are 11 g/dl between 6 months and 6 years, 12 g/dl between 6 years and 14 years, 13 g/dl in adult males, 12 g/dl in non-pregnant women and 11 g/dl in pregnant females (WHO Tech Rep Ser 1972). Should this be accompanied by a plasma ferritin below 12 /ug/dl, a transferrin saturation less than 16% and a red cell protoprophyrin concentration greater than 100 /ug/dl (Bothwell et al, 1979), iron deficiency anaemia is extremely likely to be the cause. In the final analysis an increase in the haemoglobin level by 2 g/dl in response to a therapeutic trial of iron, is the ultimate confirmation that an anaemia is due to iron deficiency (Cook, 1982).

3.1.2 Prevalence of iron deficiency

The most vulnerable periods of life for the development of nutritional iron deficiency are during infancy, adolescence and the childbearing years in women. Growth is maximal during infancy and adolescence (Burman, 1982) and

the low birth weight infant, with its correspondingly smaller iron stores, will inevitably develop iron deficiency unless the diet is fortified with iron (Gorten, 1965; Lundström et al, 1977). The staple diet of the infant is milk, the iron content of which is low. Unfortified cow's milk contains 50 /ug per 100 ml, which is less than the 76 /ug per 100 ml present in breast milk (DHSS, 1980). About 50-80 % of the iron in breast milk is absorbed during the first three months of life (Saarinen et al, 1979; Garry et al, 1981); the percentage then declines to about 10% between three and six months, whether or not solid foods are introduced to the infant's diet (Garry et al, 1981). (This is in contrast to previous findings (Oski & Landau, 1980)). Iron absorption from cow's milk is about 10% and that from infant formulas between 4 and 7% (Burman, 1982), the latter being dependent on the molar ratio of ascorbic acid to iron in the preparation (Derman et al, 1980b). Gut losses of iron may be increased during infancy by diarrhoea (Elian et al, 1966), as well as by the use of cow's milk during the first 140 days of life (Fomon et al, 1981). The mechanism whereby this blood loss takes place is not known. In order to avoid iron deficiency in infancy it has been recommended that mature infants should receive iron supplementation of 1 mg per kg from at least four months of age (American Academy of Paediatrics, 1976). There is a high prevalence of iron deficiency in young children especially in developing countries and underprivileged communities (Bothwell et al,

1979; Gurney, 1983). Derman and co-workers (1978) found the prevalence of anaemia to be 70% in South African children of Zulu or Cape Coloured origin. The highest prevalence of anaemia in this extensive survey was found in the 12 to 14 month age group (Derman et al, 1978). Other workers have confirmed a high incidence of iron deficiency anaemia and storage iron depletion in Cape Coloured infants between 6 and 12 months of life (Kirsten et al, 1984). Dawson and Whimster (1980) have found iron deficiency to be a common problem among New Zealand children aged between 6 months and 6 years.

Hookworm infestation may also exacerbate the effects of poor iron nutrition since gastrointestinal blood loss parallels the parasitic load which is often considerable (Layrisse & Roche, 1964; INACG report, 1981). Nutritional iron deficiency becomes less prevalent towards the age of ten years, when a progressive increase in caloric intake coincides with a decline in the rate of growth (Bothwell et al, 1979).

The female during her reproductive years represents the second major target for iron deficiency. Her daily requirements exceed those of the male yet her caloric intake and hence her iron intake is usually lower. The situation is made even worse in poorer countries where the mother of a family is more likely to deprive herself of food in favour of her children and the wage earner. The unacceptably high prevalence of iron deficiency in this group has been made

evident in studies of South African women of Indian descent by Mayet and co-workers (1972; 1985) and by MacPhail and co-workers (1981a), who found a 14,4% prevalence of iron deficiency anaemia and a further 26% to have depleted iron stores as indicated by serum ferritin concentrations below 20 /ug/l. A similar survey conducted by Rougereau and co-workers (1982) revealed that 76% of Senegalese women had serum ferritin concentrations below 20 /ug/l and 58% had haemoglobin values below 12 g/dl. The exceptionally strenuous challenge of pregnancy with regard to iron nutrition is highlighted by the even greater prevalence of iron deficiency during this time. The prevalence in pregnant Indian women of haemoglobin concentrations below 11 g/dl has been found to vary from 56 to 80% (Sood et al, 1968; WHO Tech Rep Ser 1968). In South African women of Indian descent this value is about 40% (Mayet 1966). Even among women of privileged means iron deficiency occurs commonly (Finch et al, 1977).

The adult male is rarely affected by nutritional iron deficiency. Cook and co-workers (1971) found a prevalence of only 3,3% of iron deficiency and of 1,2% of iron deficiency anaemia in a study of 240 adult males. Where iron deficiency does occur a pathological cause for excessive blood loss is more probable, unless extremely unfavourable dietary circumstances have existed for a long time (Bothwell et al, 1979). In this latter connection, dietary iron deficiency appears to be a common cause of anaemia in adult

males in India (Mehta, 1982).

Finally, the elderly do not appear to represent a target for nutritional iron deficiency. Though extensive studies using all the parameters available to assess iron status have not been carried out in this group (Lynch et al, 1982), estimates based on serum ferritin concentrations suggest that the prevalence is below 5% (Morgan et al, 1973; Bothwell et al, 1979). Iron stores rise in post-menopausal women (Rubin et al, 1984) and this is reflected by an increase in the mean haemoglobin level and transferrin saturation and by a concomitant fall in iron absorption (MacPhail et al, 1981a). Marrow iron stores appear to be at least normal or even increased in the elderly (Marx, 1979). Where they are depleted due to iron deficiency this is invariably accompanied by a low serum ferritin concentration (Sharma & Roy, 1984). The haemoglobin concentration, though constant until the age of 70 years in males, shows a significant fall after this time (Lynch et al, 1982). Iron deficiency when found in the elderly should be regarded as pathological and not due to an age related impairment in food iron absorption (Hallberg, 1983; Ventura et al, 1983). In fact absorption is often increased, possibly as a result of ineffective erythropoiesis (Marx, 1979; Edit. Nutr. Rev. 1979).

3.1.3 The disabilities of nutritional iron deficiency

The most apparent physical consequences of iron deficiency are those attributable to anaemia. In pregnant

women in Malaya the maternal mortality rate was 15,5 per thousand among women having a haemoglobin level below 6,5 g/dl, as compared with 3,5 per thousand in the non-anaemic group (Llewellyn-Jones, 1965). The rate of premature deliveries as well as the prenatal mortality were also significantly increased in the anaemic patients. Unfortunately, as folate deficiency coexisted with iron deficiency in a number of the anaemic subjects, it is difficult to dissect out the role played by the existence of iron deficiency. While there is no question that severe nutritional iron deficiency is detrimental to health, evidence is now being provided which suggests that even mild degrees of iron deficiency may have metabolic consequences.

3.1.3.1 Exercise tolerance: It has been demonstrated that iron deficiency impairs exercise tolerance, particularly for brief and intense forms of work. Though this seems to be a function of the degree of anaemia (Dallman, 1982), the exact haemoglobin level at which the reduction in maximum work capacity becomes important has not yet been established (Bothwell et al, 1979). In studies on venesected male physical education students Ekblom and coworkers (1972) showed that the reduction in maximal oxygen uptake corresponded very closely to the percentage of total body haemoglobin removed and that the loss of a relatively small percentage of total body haemoglobin was associated with impaired performance. This has important economic implications for individuals who are manual labourers and

whose reimbursement is usually based on productivity. Basta and co-workers (1979) studied a group of male latex tappers, among whom anaemia and hookworm infestation were common, and found a close correlation between anaemia and low income. That manual labourers suffering from iron deficiency anaemia are less efficient workers than their normal counterparts has also been shown in female workers on tea plantations in Sri Lanka (Gardner et al, 1977; Edgerton et al, 1979). In both groups the performance of the iron deficient subjects improved when iron therapy was given, with the greatest improvement occurring in those who had the lowest initial haemoglobin concentrations. Improved exercise performance as measured by the Harvard Step Test (HST) has also been demonstrated in anaemic Guatamalan agricultural labourers. When they were treated with iron they showed a parallel increase in their HST scores and haemoglobin concentrations (Viteri & Torun, 1974). Though such measurable improvements in work capacity have all been attributed to the repletion of haemoglobin, it is possible that the treatment is correcting some other iron dependent metabolic process.

The importance of a small haemoglobin deficit in terms of oxygen transport is unlikely to be critical because of the compensatory mechanisms that safeguard the delivery of oxygen to tissues (Bothwell et al, 1979). These include adjustments in the affinity of haemoglobin for oxygen and alterations in the distribution of the cardiac output (Torrance et al, 1970; Finch & Lenfant, 1972). Increased

methaemoglobin reductase activity has been found to occur in rats at haemoglobin concentrations less than 10 g/dl (Hagler et al, 1981). Davies and co-workers (1973) detected a decrease in aerobic work capacity only if the haemoglobin level was below 12 g/dl. At 8 to 10 g/dl it was reduced by one quarter and below 8 g/dl by a third.

An attempt to explain the mechanisms responsible for the impaired work capacity in iron deficiency has been made from studies using rats. Important functional haem-containing compounds that become depleted include myoglobin, which stores oxygen for use during muscle contraction and cytochrome c, which is a component of the mitochondrial electron chain involved in oxidative metabolism (Dallman, 1982). Non-haem compounds, including the metalloprotein enzymes such as NADH, alpha-glycerophosphate-dehydrogenase and other iron dependent enzymes, are also decreased (Dallman, 1974; Bothwell et al, 1979). When depletion of these compounds occurs, it does not do so uniformly in all tissues. For example, the intestinal mucosa becomes depleted before skeletal muscle, while skeletal muscle is decreased before the myocardium (Dallman & Schwartz, 1965; Finch et al, 1976; McKay et al, 1983). The rate at which these compounds are restored in the different tissues after the administration of iron is also very variable. In tissues with a rapid turnover, such as the intestinal mucosa, the restoration of the cytochromes occurs rapidly, while the process is very slow in skeletal muscle (Dallman &

Schwartz, 1965). Siimes and co-workers (1980) found that below a threshold level of 25 mg dietary iron per kg in rats, haemoglobin, myoglobin and cytochrome c levels declined in a parallel fashion. Even the mildest degree of nutritional anaemia was accompanied by a decrease in muscle and intestinal cytochrome c. The fact that myoglobin levels only become significantly depressed at a dietary iron intake below 10 mg per kg, suggests that the reduced level of voluntary activity in iron deficient rats could be responsible (Edgerton et al, 1972; Beutler & Fairbanks, 1980). Finch and co-workers (1976) have found skeletal muscle from iron deficient rats to have reduced quantities of cytochrome c + c_1, cytochrome b_t and b_k and cytochrome a and a_3, although the reduction in the latter was less than that of the other cytochromes. Somewhat different findings have been reported by Hagler and co-workers (1981). They reported no decrease in cytochrome oxidase activity, although the components of the enzyme, cytochrome a and a_3, were not measured.

Impaired carbohydrate and fatty acid oxidation by the mitochondria of iron deficient rats, has also been found by a number of authors (Hagler et al, 1981; Baquer et al, 1982; Sochor et al, 1982; Sochor et al, 1983). This indicates that iron deficiency has deleterious effects at some point on the common oxidative pathway of pyruvate-malate and palmityl-carnitine substrates. The sites most likely to be involved are either the non-haem iron containing

dehydrogenases or the cytochromes. Heart cytochrome c also becomes depleted in iron deficiency. Myoglobin may be unaffected in the heart but it is significantly decreased in slow twitch red (soleus), fast twitch red (red fibre part of gastrocnemius) and mixed fibre muscles (gastrocnemius) (Hagler et al, 1981).

Oxidative phosphorylation in skeletal muscle, with alpha glycerophosphate as a substrate, becomes impaired in iron deficiency (Finch et al, 1979). This leads to the accumulation of lactic acid and to a deterioration in function. In iron deficiency anaemia the impairment may be compounded by a reduced ability of the red cells to clear carbon dioxide adequately from exercising muscles (Ohira et al, 1983). Comparable studies carried out in human subjects have demonstrated that blood lactate levels rise considerably higher in iron deficient subjects than in normal individuals, despite the fact that the two groups of iron deficient subjects studied were unable to cope with the same work load (Gardner et al, 1977). Further support for the crucial role that iron appears to play in oxidative metabolism has been provided by Schoene and co-workers (1983), who found that a two week course of oral iron therapy significantly decreased blood lactate levels in anaemic female athletes.

Heart muscle does not seem to be affected biochemically in the same way as skeletal muscle and this is compatible with its almost complete reliance on aerobic metabolism for

its production of energy (Finch et al, 1976; Ohira et al, 1982a; Strause et al, 1983). It is more able to retain essential iron and thereby its mitochondrial functions than either liver or skeletal muscle (McKay et al, 1983). However, patients with iron deficiency anaemia do show significant ST segment depression during exercise when compared with normal controls. The changes are reversed by total dose iron dextran therapy even before the haemoglobin level has risen significantly (Mehta et al, 1983).

Although a cause and effect relationship between alpha glycerophosphate oxidase activity and work ability appears to be consistent (Finch et al, 1976; Finch et al, 1979) subsequent studies have demonstrated the involvement of a broader range of oxidative enzymes in iron deficiency (Maguire et al, 1982). Muscle succinate dehydrogenase activity is drastically affected by iron deficiency. Since this enzyme, together with glycerol-3-phosphate dehydrogenase and NADH dehydrogenase, is vital for electron transport in the mitochondria and subsequent ATP production, its deficiency is probably the critical factor causing the early fatigue of iron deficient muscle (McKay et al, 1983). The same authors have not found glycerol-3-phosphate dehydrogenase activity to be significantly affected by iron deficiency. While NADH dehydrogenase levels are profoundly decreased in iron deficient muscle their restoration by iron supplementation does not appear to be directly linked to muscle work performance (Ohira et al, 1982b).

It is the capacity to use oxygen for oxidative phosphorylation that seems to make it possible to exercise for long periods. In contrast, the capacity to deliver oxygen (i.e. the haemoglobin concentration), is more closely associated with aerobic work capacity and with the VO_2 max (Dallman, 1982; Davies et al, 1981; Davies et al, 1982; Koziol et al, 1982). The point has been clearly demonstrated by Davies and co-workers (1982) who subjected iron deficient rats to exchange transfusion and thereby largely corrected their VO_2 max and aerobic work capacity. The procedure did not, however, significantly improve the endurance capacity of the iron deficient animals. Thus defects in VO_2 max during iron deficiency result mainly from decreased O_2 delivery, whilst decreased endurance capacity reflects impaired muscle mitochondrial function (Davies et al, 1984).

3.1.3.2 Cellular changes: Iron deficiency is associated with mucosal epithelial changes in the gastrointestinal tract that lead to gastric mucosal atrophy and diminished hydrochloric acid secretion. The achlorhydria in its turn further compromises the iron status of the individual by impairing the absorption of non-haem food iron (Bothwell et al, 1979). In addition, there is some evidence that lowered mucosal levels of intestinal disaccharidase may lead to impaired lactose absorption in young children with iron deficiency anaemia (Lanzkowsky et al, 1981; Edit. Nutr. Rev. 1982).

A number of morphologic abnormalities of epithelial structures have been described in iron deficiency. These include koilonychia, angular stomatitis and webbing of the oesophagus at the level of the cricoid plane (Bothwell et al, 1979). The thickness of the buccal mucosa is significantly reduced in patients with iron deficiency anaemia (Rennie et al, 1982; Ranasinghe et al, 1983). This is due to a decrease in the depth of the maturation compartment of the epithelium (Rennie et al, 1982) and to a decrease in the size of epithelial cells (Rennie et al, 1983). Structural abnormalities of the liver and myocardium have been described in rats (Dallman, 1974) and of the erythroblasts and lymphocytes in man (Dallman, 1974). These occur in association with a deficiency of tissue iron compounds.

Impaired proliferation of red cell precursors and lymphocytes has been ascribed to a defect in DNA synthesis, probably as a result of inhibition of ribonucleotide reductase (Hershko et al, 197Ø; Hoffbrand et al, 1976). This is in keeping with the observation that total amount of nucleotides is decreased in iron deficient red cells (Crowther & Cauchi, 1983). The reduced ^{51}Cr red cell survival time that occurs in iron deficiency anaemia (Loria et al, 1967) may arise from their increased vulnerability to peroxidative membrane damage. This is due to the diminished levels of glutathione peroxidase, catalase and vitamin E in red cells. Without them it is difficult to detoxify

intracellular peroxide (MacDougall, 1972; Rodvien et al, 1974; Lee et al, 1981; Levander et al, 1981). The elevated levels of protoporphyrin and copper found in iron deficient red cells may also play a role in initiating membrane lipid peroxidation (Jain et al, 1983). Evidence of peroxidative damage in the erythrocytes of iron deficient rats has been found from the high levels of malonyldialdehyde, an end product of lipid peroxidation (Jain et al, 1983; Yip et al, 1983). It is postulated that the substance can then react with the aminiphospholipids and proteins of the red cell membrane, to produce cross linking of its constituents. In this way its deformability is decreased and it is rendered more vulnerable to haemolysis (Jain et al, 1983). The characteristic microcytosis of iron deficiency anaemia also appears to be due to an abnormality of the red cell membrane rather than to a decrease of the cytosol content (Yoshimoto & Yawata, 1983). These workers have found the red cells of patients with iron deficiency anaemia to have a reduced total lipid content as well as diminished activities of sodium transport and ATP-ase.

3.1.3.3 Behaviour: There is growing evidence that verbal performance is impaired by iron deficiency and that at least some abnormalities can be corrected by the administration of iron (Dallman, 1982). Probably the best documented behavioural disturbance associated with iron deficiency is the compulsion to eat non-food material. This is called pica. That iron deficiency is the cause of this abberation

has been suggested by the prompt relief of the symptom when iron is administered (Coltman, 1969; Brown & Dyment, 1972; Johnson & Stevens, 1982). Dallman and co-workers (1975) have shown that iron deficiency in young rats causes depletion of cerebral iron which persists long after the repletion of iron in the tissues. The highest concentration of iron in the rat brain is located in the dopamine rich areas, and iron may be crucial for the synthesis or coupling of the D_2 dopamine binding sites (Youdim et al, 1981; Ashkenazi et al, 1982). It also plays a role in the storage of 5-hydroxy tryptamine (Kaladhar et al, 1982). If such a link between behavioural disturbance and biochemical abnormalities exists, its impact would be profoundly modified by the developmental state of the brain at the time of the insult (INACG report, 1981).

Studies in anaemic infants up to the age of two years have shown that iron therapy improves the results they achieve in the Bayley Test, which measures sensory and motor skills as well as language development (Oski & Honig, 1978; Walter et al, 1983). These improvements may take some time to occur (Lozoff et al, 1982 a,b,c). Deinard and co-workers' (1981) failure to observe any significant differences in cognitive development between iron deficient and iron replete non-anaemic infants has not been confirmed by other authors, who have found that the mental development index of non-anaemic, iron deficient infants improves significantly after iron therapy (Oski et al, 1983; Walter

et al, 1983). In a study of anaemic Indian children ranging in age from five to eight years, iron and folic acid supplementation improved both haemoglobin levels and intelligence test scores significantly (Seshadri et al, 1982). Careful neurological investigations in phlebotomized adult subjects have shown only EEG power asymmetries in the occipital electrodes to be related to the decline in iron status (Tucker et al, 1982).

The fact that iron deficiency is associated with other nutritional deficiencies, and that both are often the product of deprived socioeconomic backgrounds, makes the identification of the role of iron deficiency in impaired mental and behavioural function difficult (Brittenham, 1983).

3.1.3.4 Thermogenesis: Iron has recently been found to play a role in the maintenance of body temperature. When iron deficient rats where exposed to a temperature of 4 degrees centigrade they were unable to maintain their body temperature, despite a great elevation of nor-epinephrine (Dillman et al, 1979; Beard et al, 1982). The lesion responsible for the inability to maintain body temperature appears to be an impairment in the conversion of thyroxine to triiodothyronine (Dillman et al, 1980), possibly by an enzyme that is iron dependent. Though Beard and co-workers (1982) were able to restore a normal response to cold in iron deficient anaemic rats by transfusion, Martinez-Torres and co-workers (1984) found plasma norepinephine levels and

O_2 consumption to rise significantly in both anaemic and non-anaemic iron deficient subjects. This suggests that the metabolic changes observed in iron deficiency in man are due to diminished tissue iron dependent enzyme activity rather than to the presence of anaemia.

3.1.3.5 Infection: The role that iron deficiency plays in affecting an individual's susceptibility to and ability to cope with infection is not clear. A good deal of experimental evidence exists to suggest that there is a decreased resistance to infection in iron deficiency, which involves both lymphocyte and granulocyte function. Iron deficient children have been found to have a lower percentage of T-lymphocytes (MacDougall et al, 1975; Krantman et al, 1982), impaired lymphocyte transformation (Joynson et al, 1972; MacDougall et al, 1975) and a lower incidence of positive skin reactions to common antigens (Joynson et al, 1972; Chandra, 1973; MacDougall et al, 1975; Krantman et al, 1982). On the basis of studies in mice, Kuvibidila and co-workers (1981) have suggested that iron deficiency may act at the level of monocyte proliferation and lymphokine production. The production of lymphokines by helper T cells has been found to be decreased in iron deficiency. Since this is the means whereby helper T cells regulate the generation of humoral immunity, iron deficiency probably affects the link between these cell and B cells (Kuvibidila et al, 1982). The blastogenic response of splenic lymphocytes from iron deficient mice to T and B cell

mitogens has also been found to be impaired but may be restored by repletion with iron (Kuvibidila et al, 1983 a, b & c).

Granulocyte abnormalities that involve a diminished capacity to kill Escherichia coli and Staphylococcus aureus (Chandra, 1973; Srikantia et al, 1976) have been ascribed to a deficiency of the iron containing enzyme myeloperoxidase (Baggs & Miller, 1974; Prasad, 1979; Sinha & Swarup-Mitra, 1981), or to decreased activation of the hexose monophosphate shunt (Yetgin et al, 1979). Neutrophils derived from iron deficient rats exhibit markedly decreased myeloperoxidase activity (Mackler et al, 1984). The changes are rapidly reversed by the administration of iron (Chandra, 1973). Neutrophil lactoferrin, which is found in the specific granules of neutrophils, may also be an important component in the microbicidal activity of granulocytes. This iron containing protein provides iron for the generation of hydroxyl radicals during the process of phagocytosis (Ambruso et al, 1981). The toxic effects of the superoxide anion (O_2) and hydrogen peroxide (H_2O) produced by stimulated polymorphonuclear leukocytes and macrophages are increased when traces of iron are present because iron catalyzes the formation of the hydroxyl radical (Biemond et al, 1984). A reduction in lactoferrin in iron deficiency would therefore be expected to lead to reduced granulocyte activity.

Real evidence that iron deficiency is of clinical

importance in increasing one's vulnerability to infection is still lacking. One relevant finding has been the presence of iron deficiency in 23 out of 31 patients with chronic mucocutaneous candidiasis (Higgs & Wells, 1972). Administration of iron produced definite clinical improvement in three out of four patients. In a laboratory setting, Rennie and co-workers (1983) could find no difference in the incidence of experimentally induced oral fungal infection between anaemic and normal rats. However, once infected the former group recovered more slowly and seemed less able to rid themselves of the infection. The epithelial changes that occur in iron deficiency could however predispose to fungal infections. In Papau New Guinea, where iron lack is the main cause of anaemia, hospital surveys have shown significant anaemia to be present in infants admitted for treatment of pneumonia and meningitis (Oppenheimer, 1980).

There are paradoxical reports that increased levels of serum iron may have adverse effects on susceptibility to infection. Masawe and co-workers (1974) noted that treatment of iron deficiency increased the number of malarial attacks in East Africans, while Murray and co-workers (1980) found the administration of iron to milk drinking Africans increased their vulnerability to amoebiasis. The presence of siderosis in South African Blacks is thought to predispose to generalized Yersinia enterocolitica infection (Robins-Browne et al, 1979). In

neonates there is probably insufficient evidence to link iron therapy to an increased risk of infection and whilst parenteral iron therapy is best avoided (Oppenheimer & Hendrickse, 1983), oral iron supplementation in infants whose stores are marginal is likely to be of benefit (Stockman, 1981).

It seems probable that it is the restriction in available ionic iron in body fluids as a result of the presence of the iron binding proteins transferrin and lactoferrin that is important in protection against bacterial infection (Bullen et al, 1979; Marcelis, 1980; Bezkorovainy, 1981; Ballantyne, 1984; Letendre & Holbein, 1984). Serum albumin may act synergistically with transferrin to limit iron supply to invading micro-organisms (Konopka & Neilands, 1984) and interleukin-1 may also play a role in decreasing the plasma iron concentration (Klasing, 1984). Unsaturated transferrin has been shown to have anti-viral, (Martin et al, 1963) fungistatic (King et al, 1975) and bactericidal activities (Bullen & Rogers, 1969), while unsaturated lactoferrin appears to be partly responsible for the bactericidal effect of polymorphonuclear leukocytes on Pseudomonas aeruginosa (Bullen, 1981). Indeed, patients with leukaemia have a high percentage of saturation of their serum transferrin with iron, and this is associated with a diminished ability of their serum to inhibit the growth of Pseudomonas aeruginosa in vitro (Hunter et al, 1984).

In order to acquire iron, bacteria have to produce

chelating agents capable of removing the metal from the iron-binding proteins. Some of these have been identified. Enterobactin, which is synthesized by the bacteria of the Escherichia, Salmonella and Klebsiella groups (Rosenberg & Young, 1974), has been found to be extremely efficient at removing iron (Tidmarsh et al, 1983). Pyochelin is produced by Pseudomonas aeruginosa (Cox & Graham, 1979). Corynebacterium diphtheriae too possesses a siderophore-dependent iron transport system (Russel et al, 1984). Neisseria meningitidis however possesses a siderophore free, high affinity iron acquiring system with a preference for phosphate ester or carboxylic acid bound iron (Archibald & de Voe, 1980). Even the intraerythrocytic parasite, Plasmodium falciparum is capable of obtaining iron from transferrin (Pollack & Fleming, 1984). In a clinical setting, the liberation of haem compounds, e.g. haemorrhage into the peritoneal cavity associated with abdominal trauma, seems to enhance bacterial infection (Bullen, 1981; Helms et al, 1984).

3.2 The Problem of Iron Overload

In contrast to iron deficiency, iron overload is a rare condition (Bothwell et al, 1979) that exists when total body iron stores exceed about 4g (Finch & Huebers, 1982). It may be a primary phenomenon, as in the inherited inborn error of metabolism, hereditary haemochromatosis, or be secondary to a number of haematological diseases or to a high intake of

bioavailable iron (McLaren et al, 1983). Whilst a positive iron balance in the normal individual leads to a parallel enlargement in both parenchymal and reticuloendothelial iron stores, in the disease states leading to iron overload, selective storage may occur.

3.2.1 Disorders characterized by parenchymal iron overload

Most parenchymal iron is held by the hepatocyte and the prototype of parenchymal iron loading is hereditary haemochromatosis. That this is an inherited inborn error of metabolism is evident from the abnormalities in iron metabolism that can be consistently demonstrated among relatives of affected individuals (Beaumont et al, 1979; Simon et al, 1980; Bassett et al, 1981). Excessive amounts of iron are absorbed throughout life and deposited in the parenchymal cells of the liver, heart and endocrine glands. Deposits in the reticuloendothelial system are not prominent, which suggests that there may be a defect in the ability of these cells to store iron (Brink et al, 1977; Bothwell et al, 1979; Halliday & Powell, 1982). The disease is transmitted as an autosomal recessive trait (Cartwright et al, 1979; Simon et al, 1980), with the iron loading gene being located about 1 centimorgan from the HLA-A locus on the short arm of chromosome 6 (Edwards et al, 1980). Though HLA-A_3 is frequently found in patients with hereditary haemochromatosis (Simon et al, 1976; Doran et al, 1981; Zacchi et al, 1982), the relationship between the disease

and the HLA locus is one of linkage rather than a specific association with a particular HLA antigen (Kidd, 1979). Full phenotypic expression of this HLA-linked iron loading gene depends not only on the gene frequency in the population (Bothwell & Charlton, 1982) but also on exogenous factors, the most important of which is the amount and bioavailability of iron in the diet. Concomitant excessive alcohol ingestion which increases both the total iron load and the degree of tissue damage, may also play an important part in the progression and severity of the disease (Powell & Halliday, 1980).

Clinical expression of hereditary haemochromatosis is about ten times more frequent in males. This is due to the fact that the lower iron intakes and greater losses (e.g. menstruation, pregnancy and lactation) retard the accumulation of iron (Bothwell et al, 1979). Evidence concerning the nature of the metabolic disorder in the disease appears to focus on the handling of iron by the intestinal mucosa and by the reticuloendothelial cells. An increased iron uptake by the duodenal mucosal cells has been demonstrated by Cox and Peters (1980a) and a larger proportion than normal of iron taken up by the mucosal cells is transferred into the body (Boender & Verloop, 1969; Marx, 1979). Further evidence that disordered mucosal iron transport may be a primary abnormality in hereditary haemochromatosis has been furnished by Björn-Rasmussen (1980, 1983), who found very low post absorption excretion

values of orally administered radioiron in affected patients. This was in sharp contrast to the high values observed in patients with secondary iron overload. The apparent failure of both the intestinal and reticuloendothelial cells to store iron normally (Brink et al, 1977; Bothwell et al, 1979; Uchida, 1982) leads to saturation of the circulating transferrin, with deposition of iron in the parenchymal cells of the liver and other organs occurring as a passive consequence (Cook et al, 1973a). The build up of iron stores is reflected by a rise in the serum ferritin concentration and once hepatic fibrosis or cirrhosis has occurred, it usually exceeds 700 /ug/l (Bassett et al, 1984). Because the transferrin saturation is high and most of the plasma iron is bound to diferric transferrin, erythroblasts of patients with iron overload take up more iron than that required for haem synthesis (Finch & Huebers, 1982; Cazzola et al, 1983b).

A predominantly parenchymal distribution of iron deposition is also found in patients suffering from anaemias characterized by ineffective erythropoiesis such as homozygous thalassaemia and sideroblastic anaemia (Modell, 1979). There are a number of reasons for this. In beta - thalassaemia major the transferrin is fully saturated and offloads its iron on parenchymal tissues. In addition, there are also small quantities of free iron in circulation which are also deposited (Hershko et al, 1978; Graham et al, 1979; Bothwell, et al, 1979). The increased plasma iron

turnover, the elevated saturation of transferrin (Cook et al, 1973a), the presence of excessive dietary iron absorption secondary to increased erythropoiesis (Heinrich et al, 1973; Solomon et al, 1981; Halliday & Powell, 1982; Vatanavicharn et al, 1983) and the iron derived from blood transfusions all contribute to the parenchymal overload (Bothwell et al, 1983).

3.2.2 Disorders characterized by reticuloendothelial iron overload

In striking contrast to this situation is the reticuloendothelial pattern of deposition of iron derived from transfusions in patients with aplastic anaemia and other causes of a hypoplastic marrow (Finch & Huebers, 1982; Halliday & Powell, 1982). Each unit of blood contains about 225 mg of iron, and as the ability of the body to excrete iron is limited, the iron liberated from the haemoglobin of the transfused cells is deposited in the reticuloendothelial system (Bothwell et al, 1979). Whilst deposits of haemosiderin in the reticuloendothelial system appear to be relatively innocuous, it is the presence of large deposits of iron in the parenchymal cells of the heart, liver and endocrine glands that are responsible for the clinical manifestations of iron overload (Bothwell et al, 1979).

3.2.3 Alcoholic cirrhosis and iron overload

Patients with alcoholic cirrhosis may also have varying degrees of iron overload, (Finch & Huebers, 1982), which may be due to enhanced intestinal iron absorption (McLaren et

al, 1983). No relationship between the amount and duration of ethanol consumption and the liver iron concentration has however been demonstrated in alcoholics and patients with idiopathic haemochromatosis (Chapman et al, 1982). The mechanism of the increased iron absorption in alcohol abuse is not understood, although it is possible that the ineffective erythropoiesis and elevated plasma iron-turnover resulting from disordered folate metabolism could be responsible (Celada et al, 1979; Conrad & Barton, 1980; Bothwell et al, 1983). However, Chapman and co-workers (1983) postulate that the increased liver iron found in about 30% of alcoholics arises from a cause other than excessive absorption, since they found no difference in iron absorption values between non-anaemic alcoholics and normal controls. Iron absorption was, however, significantly increased in alcoholics with anaemia when they were compared with iron deficient control subjects. Prolonged alcohol abuse leads to the appearance of sialic acid deficient transferrin which has been shown to selectively deposit iron in the hepatocyte (Regoeczi et al, 1984). This may be a significant factor in the development of hepatic siderosis in alcohlism. Some patients with cirrhosis who have undergone portocaval shunting procedures may develop a syndrome indistinguishable from hereditary haemochromatosis, (Baker & Cohen, 1972) and though the levels of storage iron are not as great as those found in the inherited disease, the time required for the development of cardiac dysfunction

and diabetes may be as short as 3 years (McLaren et al, 1983).

3.2.4 Porphyria cutanea tarda

Porphyria cutanea tarda, in which haem synthesis is defective due to a deficiency of the hepatic enzyme uroporphyrinogen decarboxylase (Kushner et al, 1976; Felsher et al, 1982), also leads to some increase in parenchymal iron (Disler et al, 1984), but the amount rarely exceeds 4 g (Lundvall et al, 197Ø). The enzyme defect has also been demonstrated in the erythrocytes of most North American patients (Kushner, 1982; Felsher et al, 1982). The presence of this inherited enzyme deficiency appears to act in concert with exogenous factors, such as excessive alcohol ingestion or the use of oestrogen containing oral contraceptives, to produce the phenotypic stigmata of the disease (Grossman et al, 1979).

3.2.5 Iron overload in Blacks

Mention must finally be made of the unique situation of iron overload found in South African Blacks, who consume home brewed beer that contains high concentrations of iron in a highly bioavailable form (Bothwell et al, 1964; Derman et al, 198Øa). Iron overload becomes manifest in the reticuloendothelial system as well as the hepatic parenchyma. Once cirrhosis has developed, the capacity of the cirrhotic liver to store iron is overwhelmed and haemosiderin is deposited in the parenchymal cells of other organs (Isaacson et al, 1961; Bothwell et al, 1979). The

presence of excessive ferric iron deposits in the tissues alters the metabolism of ascorbic acid, accelerating its catabolism to carbon dioxide and oxalic acid (Lynch et al, 1967; Hankes et al, 1974). There is *in vitro* evidence that ferric iron catalyses the first step in the oxidative sequence, and biochemical evidence of ascorbic acid deficiency is present in virtually all adults with iron overload. In addition, frank scurvy is not unusual in late winter or early spring (Seftel et al, 1966). The presence of chronic ascorbic acid deficiency is one of the aetiological factors leading to the development of osteoporosis in siderotic Black patients (Seftel et al, 1966). With increasing urbanization, the prevalence and severity of siderosis among South African Blacks is declining. This is due to the substitution of aluminium for iron containers and to a considerable change in the drinking habits, with a preference now for conventional Western liquors (MacPhail et al, 1979).

3.2.6 Effects of iron overload

The clinical manifestations of iron overload depend largely upon the rate of accumulation of the iron and on the amount found in the parenchymal cells (Modell, 1979; Milder et al, 1980; Finch & Huebers, 1982). Acute iron poisoning rapidly leads to a necrotizing gastritis and enteritis (Aldrich, 1958), haemorrhagic necrosis of the hepatic parenchymal cells (Luango & Bjornson, 1954) and parenchymal cell damage in the heart, brain, kidneys and lungs (Aldrich,

1958). The rapid accumulation of iron in thalassaemia major leads to early clinical evidence of parenchymal iron overload, with hepatic fibrosis developing in early childhood. Gonadal failure secondary to pituitary involvement appears in adolescence and death ultimately results from a fatal cardiomyopathy (Modell, 1979).

In HLA-related iron overload, clinical evidence of the disease may not appear until the age of 5Ø to 6Ø years (Milder et al, 198Ø). The spectrum of pathology is variable and includes hepatic cirrhosis (Finch & Finch, 1955), diabetes due to failure of pancreatic islet cell function (Bothwell et al, 1979), cardiomyopathy with cardiac failure and disturbances of rhythm (Finch & Finch, 1955) and hypogonadism due to reduced pituitary secretion of luteinizing hormone (Stocks & Powell, 1972; Bezwoda et al, 1977; Altman et al, 198Ø; Kelly et al, 1984). Arthropathy (Schumacher, 1964; Schumacher, 1982; Bjelle et al, 1982; Adamson et al, 1983) and pigmentation of the skin (Finch & Finch, 1955) are also commonly present. Although alteration in the hydroxylation of Vitamin D to 25-hydroxy vitamin D by the liver has been postulated as being an aetiological factor in the generation of bone disease. Chow and co-workers (1985) have not found levels of 1,25-dihydroxy vitamin D_3 to be reduced.

The mechanisms of iron toxicity in iron overload states have not been clearly elucidated. Enzymic analyses have shown increased activities of acid hydrolases and enhanced

lysosomal fragility, while histologically the hepatic lysosomes in both hereditary haemochromatosis and in transfusional iron overload contain large amounts of iron (Peters & Seymour, 1976). Non-transferrin bound iron present in the serum of patients with hereditary haemochromatosis is readily taken up by the hepatocyte and deposited in lysosomes (Batey et al, 1981). Bacon and co-workers (1983) have demonstrated the ability of excess iron to induce hepatic lipid peroxidation in both the mitochondrial and microsomal fractions of hepatocytes. It is possible that the accumulation of iron beyond the capacity of the cell to store it in a non-toxic form initiates cell damage by promoting lipid peroxidation. As a consequence, there is disruption of the lysosomes with release of their destructive enzymes (McLaren et al, 1983). The fibrosis which affects organs containing excessive amounts of storage iron is probably due to direct stimulation of collagen synthesis by iron (Iancu & Neustein, 1977).

Chapter Four

METHODS

4. METHODS

The present chapter includes details of all the methods that were used in the various studies that make up the second part of this thesis.

4.1 Haemoglobin Estimations

These were carried out using the cyanmethaemoglobin technique.

4.2 Serum Iron Concentrations

Serum iron concentrations were measured using the International Committee for Standardization in Haematology (ICSH 1978a) method. Initially the plasma is acidified with a mixture of I M hydrocholoric acid and 10% trichloracetic acid in order to dissociate the iron-transferrin complex and to precipitate proteins. The simultaneous addition of a 3% thioglycollic acid solution ensures complete reduction of the dissociated transferrin iron to the ferrous form (Fielding, 1980). Deproteinization is a crucial step in measuring serum iron for it removes substances such as haemoglobin, bilirubin and plasma lipids which would otherwise increase the optical density of the plasma (Bothwell et al, 1979). The optically clear supernatant is then treated with buffered bathophenanthroline sulfonate and the absorption of the ferrous complex measured at 535 nm. Two millilitres of a standard iron solution containing 40 /umol/l iron in 5 mmol/l hydrochloric acid, and of distilled

water for blank are treated in the same way as the serum samples. The serum iron concentration may then be calculated by multiplying by 40 the ratio of the differences of the absorption of the test sample and the blank to the standard sample and the blank. Because of the marked diurnal variation in the plasma iron concentration, blood samples were all taken during mid-morning.

4.3 Unsaturated Iron-Binding Capacities (UIBC)

These were measured using the ICSH method (1978b). This is an adsorbant method in which iron in excess of the binding capacity of transferrin is added. The compound used to saturate transferrin is radio-labelled ferric chloride in a dilute hydrochloric acid solution. Magnesium carbonate which strongly adsorbs the unbound ionic iron is then added, and the samples centrifuged to remove the iron. The unsaturated iron-binding capacity is then measured directly by counting the activity present in the supernatant. The ratio of the counts in the test sample to those of 2 ml of the saturating iron solution will give the UIBC value, after background activity has been subtracted.

4.4 Total Iron-Binding Capacities (TIBC)

The TIBC was calculated by summing the values obtained for serum iron concentration and the UIBC. The TIBC reflects the level of transferrin and gives the total number of iron binding sites on the transport protein, whilst the

serum iron concentrations reflects the number of atoms actually bound (Kimber et al, 1983).

4.5 Percentage Saturation of Transferrin

The percentage saturation was calculated by multiplying the ratio of serum iron to the total iron binding capacity (TIBC) by 100.

$$\text{Transferrin saturation (\%)} = \frac{\text{Serum Iron}}{\text{TIBC}} \times 100$$

4.6 Serum Ferritin Concentrations

The Enzyme-linked Immunosorbant Assay (ELISA) of Conradie and Mbhele (1980) was the method used for measuring serum ferritin concentrations in the subjects. In this method, which is comparable in its accuracy to the radio-immunoassay techniques, antiserum to immunologically pure human spleen ferritin is raised in rabbits and collected by plasmaphoresis. The rabbit antiferrin IgG is adsorbed onto polyvinylchloride microtitre plates and this then comprises the solid phase. A conjugate of highly purified horseradish peroxidase (HRPO) and rabbit IgG anti-ferritin is used in conjunction with a chromogen substrate in order to detect the ferritin present in the standards and samples which has bound to the solid phase. The enzymic reaction is stopped by adding 1,5 N HCl to the wells at timed intervals. The resulting colour intensity, which is proportionate to the

amount of ferritin present, is read colorimetrically at 492 nm.

4.7 Measurement of Iron Absorption

Iron absorption from the test meals was measured using the method of extrinsic labelling of non-haem iron. Immediately before the meal was eaten 1 ml of a solution containing 3 mg Fe as $FeSO_4.7H_2O$ or as $FeCl_3.6H_2O$ and 3 μCi radioiron was thoroughly mixed with each portion. One meal was labelled with 3 μCi ^{59}Fe and the other with 3 μCi ^{55}Fe (Radiochemical Centre, Amersham, Berks, England).

Two weeks later blood for the determination of ^{59}Fe, ^{55}Fe, haemoglobin, serum iron, unsaturated iron-binding capacity and serum ferritin was obtained from all the subjects after they had fasted overnight. Each person then drank a standard 3 mg dose of iron as a solution of $FeSO_4.7H_2O$ labelled with 3 μCi ^{59}Fe and containing 30 mg ascorbic acid. The ascorbic acid prevents the oxidation of ferrous iron in the alkaline milieu of the intestine which leads to the formation of non absorbable ferric iron complexes (Marx & Stiekema, 1982). Only water was permitted during the following 3 hours. Blood samples were obtained 14 days later and iron absorption from the standard reference dose was determined from the increment in ^{59}Fe in the blood. This gave a measure of each person's absorption capacity and by expressing the absorption of iron from the meals in relation to the reference dose it was possible to

compare results in individuals of differing iron nutritional status (Cook et al, 1969; Rossander et al, 1979; Hallberg, 1981a).

Duplicate 10 ml blood samples and duplicate portions of standard iron solutions were prepared for differential radioactive counting using the method of Eakins and Brown (1966). By this technique, whole blood is digested in concentrated sulphuric and nitric acids and afterwards the iron is precipitated twice with ammonium hydroxide. The iron precipitate is then dissolved with concentrated phosphoric acid. The activities of ^{55}Fe and ^{59}Fe in the processed samples were determined in Insta-Gel scintillant (Packard Instrument Co., Downers Grove, Illinois), using a liquid-scintillation spectrometer (Packard-Tri-Carb AAA Spectrometer Model No. 3375) which automatically corrected for quenching. The counting efficiency at optimal gain and window settings was 24% for ^{55}Fe and 42% for ^{59}Fe. The ^{59}Fe activity in 4 ml blood samples collected immediately before the reference iron salt was administered and two weeks later was assessed against suitable standards using a Packard Auto-Gamma scintillation spectrometer No. 5320 with a counting efficiency of 60%. The absorption values were calculated on the basis that 100% of the absorbed radioactivity was present in the haemoglobin of the circulating erythrocytes and that the blood volume for each subject was 65 ml/kg body-weight.

$$\text{Absorption (\%)} = \frac{a_B \times BV}{A \times k}$$

a_B = activity per ml of blood

BV = blood volume (ml)

A = total radioactivity administered

k = fraction of absorbed radioactivity incorporated into circulating red cells

The calculated food iron absorptions were then corrected to an absorption of 40% for the reference salt as follows:-

$$\text{Corrected food iron absorption} = \frac{\text{Calculated food iron absorption}}{\text{Calculated reference iron salt absorption}} \times 40$$

A value of 40% was selected as the reference point, since it represents the amount absorbed from the reference dose by subjects who lack iron stores, (i.e. whose ferritin is less than 30 /ug/l)) but who are not yet anaemic (Hallberg, 1981a). The corrected food iron absorptions would therefore be expected to provide a sensitive indicator of the relative bioavailablity of iron in different meals. Insofar as the ferritin results were concerned, the iron absorption values were expressed as the geometric means and standard deviation ranges, since there was considerable variation with positive skewing in the individual experiments.

4.8 Polyphenyl Content of Vegetables

The total and extractable polyphenol contents of the vegetables were measured by a modification of the method of Singleton and Rossi (1965). Ten g of each vegetable were boiled in water and then homogenized. Thereafter Ø,1 ml of the food homogenate was made up to 1Ø ml with Ø,5 ml Folin-Ciocalteu reagent, 1,5 ml sodium carbonate solution and 7,9 ml water. After incubating the solutions at 75 degees C for two hours the samples were cooled and centrifuged. The supernatants were read at 765 nm against the blank.

4.9 Polyphenol Content of Cereals

Polyphenols in the cereals were measured by the modified DMF-FAC procedure of Daiber (1975). This method forms part of the investigation of the ability of oligomeric condensed tannins, (i.e. polyphenols) to inhibit the diastatic enzymes in sorghum malts. Finely ground sorghum grain is extracted with 75% dimethylformanide (DMF) and then centrifuged. The polyphenols present in the nuclear layer of the grain are then quantitatively determined by reacting an aliquot of the centrifuged sample with 3,5% ferric ammonium citrate (FAC) followed by an 8% NH_3 solution. For the blank the FAC reagent is replaced by water. The absorbance is determined at 525 nm and compared to identically treated tannic acid standards.

4.10 Phytate Content of Cereals

The phytate content of the cereals was measured by the method of Wheeler and Ferrel (1971), in which an acid extract of the grain is made and the phytate is precipitated as its ferric salt. The phytate phosphorus is then determined as inorganic phosphate after digestion of the washed precipitate.

4.11 Statistical Methods

The significance of differences between the corrected absorptions of the two isotopes used in each study was calculated using Student's T test for paired observations.

4.12 Ethical Considerations

Approval for the studies to be performed was obtained from the Committee for Research on Human Subjects of the Faculty of Medicine, University of the Witwatersrand, Johannesburg. Written consent was obtained from all subjects after the nature of the investigation had been fully explained to them by an Indian social worker. Each subject took part in one experiment only. It was calculated that if each test dose was completely retained, the total radiation dosage would be 143 mrems (Bothwell et al, 1979) which is 28 % of the annual maximum permissible dose for members of the public (International Commission for Radiation Protection, 1960; South African Bureau of Standards, 1972). In practice the percentage absorbed is

much less, which makes the radiation exposure proportionately smaller.

Chapter Five

THE EFFECT OF ORGANIC ACIDS, PHYTATES AND POLYPHENOLS ON THE ABSORPTION OF IRON FROM VEGETABLES

5. THE EFFECT OF ORGANIC ACIDS, PHYTATES AND POLYPHENOLS ON THE ABSORPTION OF IRON FROM VEGETABLES

5.1 Introduction

The high prevalence of iron deficiency in developing nations probably correlates far more closely, with the quality of the diet consumed than with the total amount of iron contained in the diet (Cook, 1983). Thus the absorption of iron from the non-haem pool is critically important in areas where reliance is placed upon vegetables as the major source of calories. As has been previously mentioned, the major enhancers of iron absorption are ascorbic acid (Sayers et al, 1973, 1974 a & b; Disler et al, 1975c; Derman et al, 1977; Hallberg & Rossander, 1982a; Monsen, 1982) and meat (Layrisse et al, 1973; Cook & Monsen, 1976; Hallberg & Rossander, 1982a). The diets of poorer nations contain little or no meat and this confers on ascorbic acid a crucial role in determining the iron nutritive value of a meal. It is however only one of the several organic acids found in fruit and vegetables and there is little information concerning the possible role of others.

Inhibitors of iron absorption present in vegetable foods may also exert a profound influence on iron nutrition. Sodium phytate has been reported to have such an effect (Turnbull et al, 1962; Hallberg, 1981a), and it occurs in a number of plants as a phosphorus reserve storage compound (van Soest, 1978) Another established inhibitor is the

tannin occurring in Indian tea (Disler et al, 1975 a & b; Hallberg & Rossander, 1982b; Acosta et al, 1984; Yau-tian, 1983). Other tannins or polyphenols are present in many vegetable foods, but there is little information concerning any influence they might exert. A detailed study of iron absorption from a number of vegetables was therefore undertaken in order to obtain some insight into the relative contributions of these various factors to the bioavailability of dietary non-haem iron.

5.2 Materials and Methods

5.2.1 Subjects

A group of 180 parous Indian housewives took part in these studies. None of the subjects was pregnant or lactating and all were unpaid volunteers. Their ages ranged between 21 and 76 years (mean 39 years) and all belonged to a low socio-economic group living in municipal housing schemes in Chatsworth and Merebank, near Durban. It has previously been established that iron deficiency is a common problem among the women of this community (Mayet et al, 1972; MacPhail et al, 1981a).

5.2.2 Preparation and administration of the meals

The absorption of radio-labelled-iron that had been mixed with the vegetables listed in Tables 5.2 and 5.3 (see pp. 117 and 119), or with rice, (Table 5.1, pp. 114) was measured. In each absorption study two meals were consumed on consecutive mornings after an overnight fast. Only water

Table 5.1 The effect of different organic acids on the absorption of iron (3 mg) as $FeSO_4.7H_2O$ from a basic rice meal (215 g/subject) in fasting female subjects

(Mean values and standard deviations)

Organic acid	No. of subjects	Haemoglobin (g/dl)		Transferrin saturation (%)		Serum ferritin (µg/l)		Iron absorption*					
								Reference Salt‡		Rice Meal		Rice meal with organic acid	
		Mean	SD	Mean	SD	Geometric mean**	± 1SD	Geometric mean**	± 1SD	Geometric mean**	± 1SD	Geometric mean**	± 1SD
	25	12,6	1,8	26,2	14,9	14,7	(4,3-50,3)	32,0	(19,5-52,5)	3,1	(1,6-5,9)	-	-
Ascorbic acid 15mg	9	14,6	0,8	35,3	11,6	42,6	(19,9-91,0)	24,2	(14,2-41,3)	-	-	8,1	(3,3-19,6)
Citric acid 36mg	7	11,3	1,9	17,7	17,6	6,7	(1,8-25,5)	31,8	(19,7-51,5)	4,9	(2,3-10.1)	9,9	(5,2-18,8)
Citric acid 1g	9	12,6	1,5	25,6	12,1	18,2	(4,5-73,0)	27,2	(16,9-43,6)	2,8	(1,6-5,1)	8,5	(4,7-15,4)
Tartaric acid 1g	11	13,2	1,2	22,0	10,4	19,1	(4,7-78,0)	36,7	(17,7-76,1)	4,1	(2,5-13,3)	9,6	(3,7-24,8)
Malic acid 1g	8	12,9	0,8	24,8	9,7	11,5	(5,4-24,6)	32,1	(22,1-46,6)	4,8	(2,6-9,1)	9,5	(5,3-16,9)

** Geometric means and SD ranges used because values were positively skewed.
‡ 3 mg iron as ferrous ascorbate given in the fasting state.
* Individual results adjusted to 40% reference absorption.

was permitted during the meal and for a period of 3 h afterwards.

5.2.2.3 Vegetable meals: Each subject received a meal that was equivalent to 50 g of the uncooked vegetable. Preparation was as follows: 750 g portions of the vegetables were weighed (after peeling in the case of pumpkin, carrots, potato, beetroot and turnip) and cooked in 1500 ml boiling water with 5 g table salt (sodium chloride) for 20 min. Lentils were cooked in 2500 ml water for 40 min. The cooked vegetable was homogenized in a Waring blender to the consistency of a thick soup, and each subject consumed 100 g of it after the radio-labelled-iron had been mixed in.

5.2.2.4 Rice meals: Polished parboiled rice (1 kg) was boiled for 20 min in 2000 ml water containing 20 g table salt. After boiling, 75 g yellow margarine and 150 g sucrose were added. Each person received 215 g of the 'pudding' with the radio-labelled-iron solution thoroughly stirred in, on one of the two occasions together with the organic acid under investigation. However, tannic acid was given as a drink with sugar and water, since a dark colour developed when added to the meal that made the meal appear unpalatable.

5.3 Results

5.3.1 The effect of various organic acids on iron absorption from a rice meal

As expected, iron absorption from the unsupplemented

rice was low, ranging between 1,6% and 5,9% (See Appendix 5.5). In the first study the amount of ascorbic acid present in an equivalent weight of potatoes (i.e. 15 mg) was found to increase iron absorption to 8,1%. An equimolar amount of citric acid (BDH Lab. Chemicals Div., Poole, Dorset) produced a mean absorption of 9,9%, but this was not significantly higher than from the unsupplemented rice (t 1,51, p > Ø,1). However, a highly-significant improvement occurred when the amount of citric acid was increased to 1 g, the absorption trebling from 2,8% to 8,5% (t 4,47, p < Ø,ØØØ5). When 1 g tartaric acid (Analytical Grade; Merck, Darmstadt) was substituted, the mean absorption increased from 4,1% to 9,6% (t 2,5, p < Ø,Ø25), and with 1 g L-malic acid (Sigma Chemical Co., St Louis) it rose from 4,8% to 9,5% (t 2,8, p < Ø,Ø25).

In a final experiment, 1 g calcium oxalate (BDH Chemicals, Poole, Dorset) was added to cabbage and the effect on the absorption of iron was examined. There was a moderate depression from a geometric mean of 32,Ø% (SD 19,2% - 53,2%) to 19,5% (SD 1Ø,3% - 37,Ø%) (t 3,1, P < Ø,Ø1).

5.3.2 Effect of sodium phytate and of tannic acid on iron absorption

The effect of two possible inhibitors of iron absorption was tested by adding them to broccoli (Table 5.2). In the first experiment, 2 g sodium phytate reduced the geometric mean iron absorption from 15,2% to 3,5% (t 4,8%, p < Ø,ØØØ5), while in the second experiment 5ØØ mg of

Table 5.2 The effect of sodium phytate and of tannic acid on the absorption of iron (3 mg) from 100 g broccoli puree in fasting female subjects

(Mean values and standard deviations)

	Haemoglobin (g/dl)		Transferrin saturation (%)		Serum ferritin (µg/l)			Iron absorption*	
No. of subjects	Mean	SD	Mean	SD	Geometric Mean‡	± 1SD	Vegetable	Geometric Mean‡	± 1SD
19	12,9	1,9	24,6	13,1	23,3	(7,0-77,1)	Broccoli	15,2	(6,8-34,3)
							Broccoli + 2 g sodium phytate	3,5	(1,9-12,2)
8	12,7	1,3	27,4	7,4	23,0	(11,4-46,5)	Broccoli	25,7	(13,0-51,3)
							Broccoli + 500 mg tannic acid	1,5	(0,3-6,7)

* No reference absorption studies were done and the absorption results are, therefore, uncorrected.
‡ Geometric means and SD ranges used because values were positively skewed.

the polyphenolic compound tannic acid diminished it from 25,7% to 1,5% (t 6,Ø, p < Ø,ØØØ1).

5.3.3 Factors affecting the absorption of iron from a number of vegetables

There was a wide range in iron absorption from the seventeen vegetables studied (Table 5.3), the lowest being wheat-germ (Ø,7%) and the highest turnip (32,7%) and sauerkraut (32,7%). Since it seemed likely that these variations might, in part at least, be due to differences in the concentrations of inhibitors and enhancers of iron absorption in individual vegetables, an attempt was made to relate the absorption findings to the concentrations of phytate and organic acids contained within them according to published tables (Diem & Lentner, 197Ø; Paul & Southgate, 1976) (Table 5.4).

The vegetables could be divided into two distinct groups with regard to iron absorption. In the first, which included wheat germ (Ø,7%), aubergine (Ø,7%), butter beans (1,2%), spinach (1,4%), brown lentils (2,4%), beetroot greens (2,4%) and green lentils (3,2%), the bioavailability of the iron was poor. In the second group the bioavailability was moderate or good, namely carrot (9,8%), potato (11,5%), beetroot (18,5%), pumpkin (2Ø,6%), cauliflower (21,3%), tomato (22,4%), broccoli (26,Ø%), cabbage (32,Ø%), turnip (32,7%) and sauerkraut (32,7%). All except one of the second group of vegetables contain reasonable quantities of one or more of the organic acids,

Table 5.3 The absorption in fasting female subjects of iron (3 mg) as $FeSO_4.7H_2O$ from 100 g puree made up from different vegetables

(Mean values and standard deviations)

	Haemoglobin (g/dl)		Transferrin saturation (%)		Serum ferritin (µg/l)		Iron absorption*				
							Reference Salt‡				
No. of subjects	Mean	SD	Mean	SD	Geometric mean‡*	± 1SD	Geometric mean‡*	± 1SD	Vegetable	Geometric mean‡*	± 1SD
6	11,0	4,8	20,3	7,5	17,7	(4,1-76,3)	41,8	(22,8-76,7)	Wheat germ	0,7	(0,3-1,4)
7	12,9	0,6	20,6	8,6	9,4	(4,1-21,9)	43,9	(24,8-77,9)	Aubergine	0,7	(0,2-2,6)
6	11,0	4,8	20,3	7,5	17,7	(4,1-76,3)	41,8	(22,8-76,7)	Butter beans	1,2	(0,7-2,0)
7	13,5	0,7	29,7	6,7	49,2	(27,3-88,7)	27,1	(18,1-40,6)	Spinach	1,4	(0,6-3,2)
11	12,1	1,7	22,0	11,9	7,7	(1,7-34,1)	34,8	(14,7-82,5)	Brown lentils	2,4	(0,5-10,5)
4	13,9	0,6	22,3	7,7	21,2	(11,4-39,6)	22,9	(10,4-50,3)	Beetroot greens	2,4	(0,3-17,5)
11	12,1	1,7	22,0	11,9	7,7	(1,7-34,1)	34,8	(14,7-82,5)	Green lentils	3,2	(0,9-10,9)
9	12,9	2,5	25,4	8,2	24,7	(14,9-40,7)	33,9	(19,3-59,7)	Carrot	9,8	(5,2-18,7)
9	14,6	0,8	35,3	11,6	42,6	(19,9-91,0)	24,2	(14,2-41,3)	Potato	11,5	(5,7-23,3)
25	13,7	1,6	22,6	8,8	18,1	(7,2-45,4)	31,3	(15,5-63,2)	Beetroot	18,1	(7,2-45,4)
7	12,9	0,6	20,6	8,6	9,4	(4,1-21,9)	43,9	(24,8-77,9)	Pumpkin	20,6	(15,7-27,0)
20	13,6	1,3	27,1	11,5	19,4	(7,1-52,9)	24,3	(13,1-45,2)	Cauliflower	21,3	(9,4-48,5)
8	13,4	1,1	32,0	13,1	17,3	(7,0-42,6)	29,5	(16,5-52,6)	Tomato	22,4	(11,0-45,6)
8	13,9	0,9	25,9	7,1	31,5	(10,8-91,4)	24,6	(16,4-36,9)	Broccoli	26,0	(13,9-48,6)
6	13,0	0,9	24,0	10,4	13,0	(6,4-26,4)	58,9	(35,4-98,1)	Cabbage	32,0	(19,2-53,2)
10	14,6	1,8	23,9	9,5	18,6	(6,2-56,0)	48,9	(35,3-67,9)	Turnip	32,7	(19,2-55,6)
6	13,9	0,3	27,2	6,3	29,3	(12,3-70,0)	30,6	(17,2-54,3)	Sauerkraut	32,7	(20,8-51,2)

‡ Geometric means and SD ranges used because values were positively skewed.
‡ 3 mg iron as ferrous ascorbate given in the fasting state.
* Individual results adjusted to 40% reference absorption.

Table 5.4 Phosphorus, polyphenol and organic acid contents of vegetables

	Phosphorus (mg/kg)*	Polyphenols (mg/g)		Organic acids (mg/kg edible portion)*			
		Total	Extractable	Malic	Citric	Oxalic	Ascorbic
Wheat germ	11180	8,4	2,0	0	3400	0	0
Aubergine	150	3,4	0,4	170	0	70	50
Butter beans	3200	3,5	0,6	-	-	-	0
Spinach	510	6,9	1,1	90	800	4600	500
Brown lentils	3800	6,8	1,8	0	0	0	0
Beetroot greens	40	6,6	2,3	0	0	9200	300
Green lentils	3800	6,3	2,9	0	0	0	0
Carrot	360	0,9	0,2	24	900	330	100
Potato	530	0,9	0,7	0	5100	60	200
Beetroot	330	2,4	0,9	0	1100	3400	100
Pumpkin	440	0,7	0,6	150	0	0	90
Cauliflower	560	1,6	0,9	390	2100	0	780
Tomato	270	0,6	0,3	150	3900	80	230
Broccoli	780	3,2	2,8	120	2100	0	1130
Cabbage	270	0,7	0,6	0	0	0	460
Turnip	300	0,9	0,6	230	0	0	360
Sauerkraut	180	0,6	0,4	0#	0#	0#	140

* Values from Diem & Lentner (1970) and Paul & Southgate (1976).
\# Lactic acid 1600 mg/kg.

malic, citric and ascorbic acids. The exception was sauerkraut, which contains a large amount of lactic acid.

The role of organic acids was further defined in another experiment in which broccoli was fed with and without the cooking water in which it was boiled, since a significant proportion of the acids in the broccoli would be dissolved in the water (See Appendix, Table 5.24). Haematological information on the eight housewives who took part in this study were as follows: mean haemoglobin concentration 13,9 g/dl (SD Ø,9), mean transferrin saturation 25,9% (SD 7,1), geometric mean serum ferritin concentration 31,5 /ug/l (SD 1Ø,8 - 91,4), and geometric mean reference absorption 24,6% (SD 16,4% - 36,9%). When the broccoli was given with the cooking water the corrected geometric mean iron absorption was 26,Ø% (SD 13,9 - 48,6) compared with 9,8% (SD 5,6 - 17,1) when the water was discarded (t 7,41, $p < Ø,ØØ5$).

No relationship between oxalate content and iron absorption emerged when the three vegetables that contained large amounts of oxalate were examined: Iron absorption was poor from spinach and beetroot greens and good from beetroot. On the other hand the phosphorus and, therefore, presumably the phytate contents, were high in wheat germ, butter beans and lentils, in all of which the iron bioavailability was low.

The results of a further experiment raised the possibility that the polyphenols present in certain

vegetables might also exert a profound effect on iron absorption. A 10 g ferric chloride/l solution was added to individual vegetables, since it has previously been shown that when ferric salts are added to a solution containing polyphenols, a bluish-black discoloration develops due to the formation of coloured iron complexes (Seikel, 1964; Disler et al, 1975a). Marked darkening of aubergine, spinach, green and brown lentils and beetroot greens was noted. This was of interest, since the bioavailability of the iron in each had been found to be low. In contrast, a vegetable such as pumpkin, from which iron was well absorbed, did not darken noticeably when iron was added. The total polyphenol content of all the vegetables was therefore measured (Table 5.4) and a significant inverse correlation with iron absorption was noted ($r = 0{,}859$; $p < 0{,}001$). It seemed that the effect was due to condensed rather than to hydrolysable polyphenols, since the inverse correlation was even closer ($r = 0{,}901$; $p < 0{,}001$) when the amounts of extractable polyphenols had been subtracted (Fig. 5.1). In these analyses square-root conversion of the values was used as this normalized the values which were otherwise positively skewed.

5.4 Discussion

Iron deficiency has been shown to be a common problem in the Indian population in which this series of studies was undertaken, with a 26% prevalence in the females (Mayet et

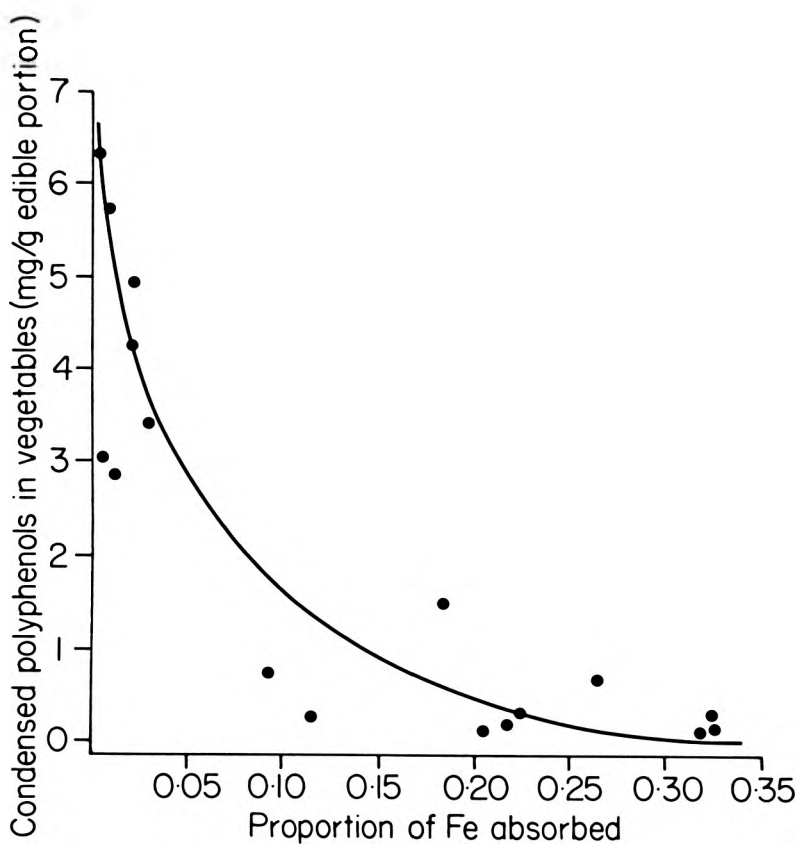


Figure 5.1 The inverse correlation between the condensed polyphenol content of vegetables (total-extractable polyphenols) and iron absorption from them. The line is derived from the least squares regression after square-root transformation (r - Ø.9Ø).

al, 1972; MacPhail et al, 1981a). These findings were confirmed by our present results, which showed that the geometric mean serum ferritin level in most of the groups of women was a good deal less than the mean normal value of 35 /ug/l.

In each experiment, 3 mg inorganic iron labelled with radioactive-iron was added to the meal and the absorption of the isotope measured. The results reflected the absorption of not only the added iron but also the intrinsic vegetable iron, since it has previously been well established that there is free isotopic exchange within the lumen of the gastrointestinal tract under such circumstances (Bothwell et al, 1979; Hallberg, 1981a).

In the first seris of studies, different organic acids were added to a rice meal to examine their individual effects. Citric, tartaric and malic acids in 1 g quantities were each found to increase iron absorption significantly. These findings may well have relevance to iron nutrition, since citrus fruits, such as oranges, lemons and grapefruit, contain a minimum of 10 g citric acid/kg, while malic acid is found in the deciduous fruits, such as plums, peaches and apples, in concentrations ranging from 3 g/kg to greater than 10 g/kg (Diem & Lentner, 1970; Paul & Southgate, 1976). Tartaric acid is not present in vegetables, but is found in concentrations ranging from 1630 - 2340 mg/kg in white wines (Diem & Lentner, 1970), and may play a part in the high bioavailability of the iron they contain.

Although the concentrations of individual organic acids in vegetables are not notably high, they usually contain more than one of them and much of the considerable variation in the bioavailability of the iron given with different vegetables in the present study could be ascribed to differences in the content of malic, citric and ascorbic acids (Table 5.4). Carrots, potato, beetroot, pumpkin, cauliflower, broccoli, tomato, cabbage and turnip were associated with good iron bioavailability and contained one or more of the organic acids in reasonable concentrations. This has relevance for potato especially since it provides more iron in the diet of the United Kingdom than any other single vegetable (Fairweather-Tait, 1983 a & b). The method of preparation of the vegetables also has relevance to iron nutrition, for frying has been shown to decrease significantly the iron content in both the cortical and pith areas of potatoes (Mandy & Ponnampalam, 1983).

Ascorbic acid appeared to be the major promoting agent in cabbage, and malic acid in pumpkin, but in other vegetables it was not possible to dissect out the individual effects of the different acids. The good absorption of iron from sauerkraut may have been due to its high lactic acid content, since this acid has previously been shown to be an effective promoter (Derman et al, 1980a). The high bioavailability of iron in some of these vegetables was underlined by the fact that the absorption from cabbage, turnip and sauerkraut was as much as 80% of that of a 3 mg

reference dose of ferrous ascorbate. Lactic acid, malic acid and ascorbic acid when studied in vitro have been found to form stable bonds with ferric iron (Gorman & Clydesdale, 1984). This would ensure that these enhancing ligands maintain iron in a soluble form both in food, as well as in the gastrointestinal tract. The thermodynamic stability constants of malic acid and ascorbic acid are extremely similar (1,41 x 10^3 and 1,90 x 10^3 to 2,61 x 10^4 respectively), while that for lactate is lower (2,40 x 10^2). Measurement by the same workers of the rate of iron exchange from the carboxylic acid to apotransferrin, yielded slopes in the following descending order: ascorbic, lactate, malate and citrate.

Meat is an important dietary constituent for adequate iron nutrition, since it not only potentiates the absorption of the non-haem iron in the meal, but it also contains haem-iron which is well absorbed since it is not vulnerable to any inhibitory ligands that may be present (Layrisse et al, 1969; Cook & Monsen, 1976; Monsen et al, 1978). The low meat intake of many populations has, therefore been regarded as one of the factors responsible for the widespread occurrence of dietary iron deficiency and the critical importance for iron nutrition of the ascorbic acid content of such diets has been stressed. In the present series of experiments, however, it was possible to show that ascorbic acid is just one of several organic acids which potentiate non-haem iron absorption, although it is the most potent of them.

The one organic acid that was found to have a moderate depressing effect on iron absorption was oxalic acid. However, its role, if any, in modifying the bioavailability of iron in vegetables was not clearly elucidated. While the addition of calcium oxalate, which is one of the two major salts of oxalic acid in vegetables (Diem & Lentner, 1970; Paul & Southgate, 1976), to broccoli caused some depression in iron absorption, there was a wide range of absorption from vegetables containing large amounts of oxalate. The bioavailability of the iron in these vegetables therefore appeared to depend more on the presence of other inhibitors and enhancers. Two factors possibly responsible for the poor bioavailability of iron in some of the vegetables were identified. Sodium phytate and the polyphenol, tannic acid, were shown in separate experiments to inhibit the absorption of vegetable iron, which is in line with previous findings (Hallberg & Solvell, 1967; Disler et al, 1975 a & b). It therefore seemed probable that the presence of certain phytates and polyphenols in food might have a similar effect and, indeed, iron absorption was found to be uniformly poor in those vegetables such as wheat germ, butter beans and lentils that contain large amount of phosphorus, presumably mostly in the form of phytates (van Soest, 1978). Lynch and co-workers (1984) have also found that many legumes are a poor source of dietary iron. Iron absorption ranged between 0,84 and 1,91% when measured from soy beans, black beans, lentils, mung beans and split peas.

As far as polyphenols are concerned, we have previously shown that tea, which contains tannates, is a potent inhibitor of iron absorption (Disler et al, 1975 a & b; Hallberg & Rossander, 1982b; Yau-tian, 1983; Acosta et al, 1984). Suggestive evidence that polyphenols, of which tannates are an example, might be important inhibitors of vegetable iron absorption, was obtained when iron was added to two vegetables, namely aubergine and spinach. The bluish-black colour that developed suggested that they might contain significant amounts of phenolic compounds (Seikel, 1964), and that iron was poorly absorbed despite the presence of adequate organic acids and little phytate. It was then observed that the other vegetables, including brown and green lentils and beetroot greens, that showed marked colour changes on the addition of iron, were also associated with poor iron bioavailability. The total and extractable phenol contents of all the vegetables on which iron absorption measurements had been done were therefore determined and a strong inverse correlation between the total polyphenol content and iron absorption was found ($r = 0{,}859$, $p < 0{,}001$). The fact that the inverse correlation was even greater when the extractable polyphenol content had been subtracted from the total value ($r = 0{,}901$; $p < 0{,}001$) suggested that it was the non-hydrolysable condensed polyphenols that were responsible for the inhibitory effect on iron absorption.

The present findings have a relevance wider than that

of the bioavailability of the iron contained within the individual vegetables tested. The iron content of vegetables varies considerably, and in some is low. However, the effect of the inhibitors and enhancers of iron absorption which they contain is not confined to their endogenous iron, but extends to the whole pool of non-haem-iron in the particular meal of which they form a part. In this context vegetables such as broccoli, cauliflower, cabbage and turnip exert a positive effect on iron nutrition, while the opposite is the case with wheat germ, aubergine, butter beans, lentils and spinach.

5.5 Summary

1. Non-haem iron absorption from a variety of vegetable meals was studied in parous Indian women, using the erythrocyte utilization of radioactive iron method.
2. The studies were undertaken to establish whether iron absorption could be correlated with the chemical composition of the foodstuff.
3. Addition of the following organic acids commonly found in vegetables, improved the geometric mean iron absorption from a basic rice meal as follows: from 2,8% to 8,5% with 1 g citric acid, from 3,1% to 8,1% with 15 mg ascorbic acid, from 4,8% to 9,5% with 1 g L-malic acid, from 4,1% to 9,6% with 1 g tartaric acid. The only exception was oxalic acid; the addition of 1 g calcium oxalate to cabbage (Brassica oleraceae) was associated with some depression in Fe

absorption from 32,Ø% to 19,5%.

4. There was a marked inhibition of the geometric mean absorption when 5ØØ mg tannic acid was added to a broccoli (Brassica oleraceae) meal (1,5 % v 25,7 %). Sodium phytate (2 g) caused a similar, though less profound inhibition (3,5% v 15,2%)

5. When 3 mg ferrous sulphate was added to different vegetables the geometric mean absorption varied widely. Vegetables of low iron bioavailability were wheat germ (Triticum aestivum) Ø,7, aubergine (Solanum melongena) Ø,7%, butter beans (Phaseolus lunatus) 1,2%, spinach (Spinacea oleraceae) 1,4%, brown lentils (Lens culinaris) 2,4%, beetroot greens (Beta vulgaris) 2,4% and green lentils (Lens culinaris) 3,2%. In contrast, bioavailability was moderate or good with carrot (Daucus carota) 9,8%, potato (Solanum tuberosum) 11,5%, beetroot (Beta vulgaris) 18,1%, pumpkin (Cucurbita mixta) 2Ø,6%, cauliflower (Brassica oleraceae) 21.3%, tomato (Lycopersicon esculentum) 22.4%,broccoli (Brassica oleracea) 26,Ø%, cabbage 32,Ø%, turnip (Brassica rapa) 32,7% and sauerkraut 32,7%.

6. All the vegetables associated with moderate or good iron bioavailability contained appreciable amounts of one or more of the organic acids, malic , citric and ascorbic acids.

7. Poor iron bioavailability was noted in vegetables with high phytate contents (e.g. wheat germ Ø,7%, butter beans 1,2%, brown lentils 2,4% and green lentils 3,2%).

8. The fact that a number of vegetables associated with low

iron absorption turned bluish black when iron was added to them suggested that the total polyphenol content in them was high. The vegetables included aubergine, spinach, brown lentils, green lentils and beetroot greens. When the total polyphenol content in all the vegetables tested was formally measured, there was a significant inverse correlation (r Ø,859, p < Ø,ØØ1) between it and iron absorption. The inverse correlation between the non-hydrolysable polyphenol content and iron absorption was r Ø,9Ø1, p < Ø,ØØ1).

9. The major relevance of these findings is the fact that the total absorption of non-haem iron from a mixed diet may be profoundly influenced by the presence of single vegetables with either marked enhancing or inhibiting effects on iron bioavailability.

Chapter Six

FACTORS AFFECTING THE ABSORPTION OF IRON FROM CEREALS

6. FACTORS AFFECTING THE ABSORPTION OF IRON FROM CEREALS

6.1 Introduction

The diets of poorer populations in many parts of the world are predominantly cereal based, with rice, maize, and wheat forming the major staples. Although these diets appear to contain sufficient iron (Apte & Iyengar, 1970; Hallberg, 1974), only 1, 3, and 5% of the iron in rice, maize and wheat respectively have been found to be absorbed (Martinez-Torres & Layrisse, 1973). While it has been shown that some of these absorption values may be too low because of contamination with dirt containing insoluble iron (Hallberg, 1981a), there can be no question that cereal iron is poorly bioavailable, and may be insufficient to meet physiological requirements (Acosta et al, 1984). This is because cereals contain substances that inhibit iron absorption (Bothwell et al, 1979). Like the better-known cereal staples, sorghum (Sorghum vulgare) contains inhibitory substances, and iron absorption from sorghum porridge is very poor (Derman et al, 1980a). Iron deficiency has been shown to be rampant among Cameroon children below 5 years of age who are reared on a sorghum based diet (Delpeuch et al, 1980). Because all the non-haem iron in a meal forms a common pool within the intestinal lumen, these inhibitory substances affect the absorption of not only the cereal iron but also the other non-haem food iron, and in addition any added non-haem iron (Hallberg, 1974, 1981a). Their influence on iron nutrition is therefore

critical.

Three groups of substances found in cereals, namely phytates, polyphenols, and fibre, have been proposed as inhibitors of iron absorption. Many cereals contain large amounts of phytate (inositol hexaphosphate), which as previously indicated serves as the phosphate reserve for the plant seedling (Van Soest, 1978; Graf and Eaton, 1984). There is evidence that phytate may interfere with iron absorption (Radhakrishnan & Sivaprasad, 1980; Ellis et al, 1982), presumably by forming an insoluble iron-phytate complex. A variety of plant polyphenols, particularly those occurring in the form of non-hydrolysable tannins, are potent inhibitors of iron absorption (Disler et al, 1975a & b). Recent interest in dietary fibre has revealed that certain fibre components influence the absorption of minerals (Cummings, 1978; Sandstead et al, 1978; Wiemer et al, 1981). Many of these fibres are indigestible polysaccharides, and it has been claimed that even digestible polysaccharides, such as rice starch, may inhibit iron absorption to some extent (Hallberg et al, 1978). In the present study an attempt was made to evaluate the relative roles of phytate, polyphenol and fibre on non-haem iron absorption.

6.2 Materials and Methods

6.2.1 Subjects

158 parous Indian housewives who took part in the

present study all belonged to a low socio-economic group living in municipal housing schemes in either Chatsworth or Merebank, near Durban. As before, none of the subjects was pregnant or lactating and all were unpaid volunteers.

6.2.2 Grain and commercial cereal products

Two types of sorghum grain were used. The first was a bird proof sorghum, cultivar SSK-52 (Stoffberg, Transvaal). It was dark red-brown and had a high polyphenol content, whilst the second, albino sorghum, a white grain sorghum cultivar 766W (CIBA-Geigy), had a very low polyphenol content. Both sorghum varieties had a high phytate content. The polyphenols are located mainly in the outer layers of the sorghum grain and the phytate in the layers just below this. It is thus possible to remove most of the polyphenols and increasing amounts of phytate by removing the outer layers of the grain. This process, known as pearling, was carried out using a Miag laboratory rice pearler (Buhler-Miag, Muhlenbau and Industrie G.m.b.H., Braunschweig,West Germany). The pearled bird-proof sorghum used in the study was treated for 30 min. This removed 55% of the grain, leaving only 4% of the 16290 mg polyphenol/kg and 8% of the 5390 mg phytate/kg that had been present in the whole grain. Pearling the albino sorghum for 20 min removed 40% of the grain and left a grain containing 14% of the original phytate (1520 mg/kg) whilst the pearlings (the removed outer layers) had 9040 mg phytate/kg. The whole grain and the pearled grain were milled to a flour using a coffee mill.

One other form of sorghum used in these studies was a commercially available breakfast food, Maltabella (Hinds Bros, Durban), which is prepared by milling malted sorghum and adding sodium chloride.

6.2.3 Preparation and administration of the meals

In each iron absorption study, two meals were consumed on consecutive mornings after an overnight fast. No additional food or drink except water was permitted during the meal and for a period of 3 h afterwards.

6.2.3.1 Sorghum porridge: Sorghum (450g; 30 g/subject) was made into a paste with 750 ml water. This was cooked in 2250 ml boiling water containing 10 g table salt for 20 min to make a porridge, of which each subject was given 200 g. Where tea was served with malted sorghum, 75 g tea leaves (Pot O'Gold: O K Bazaars Ltd., Johannesburg) were infused with 3000 ml boiling water to give 200 ml/subject.

6.2.3.2 Oat porridge: Oat meal (Jungle Oats, Cape Town; 450 g; 30 g uncooked meal per subject) was made into a paste with 500 ml water and added to 2250 ml boiling water containing 10 g table salt and cooked for 20 min to make a porridge. Each person was given 200 g cooked porridge together with 10 g sucrose.

6.2.3.4 Wheat flour and bran meal: Snow Flake cake flour (South African Milling Co, Johannesburg; 450 g; 30 g uncooked flour per subject) was made into a paste with 750 ml water and then cooked for 20 min in 2250 ml boiling salted water. Each subject received 200 g porridge. On the

second day, 90 g wheat bran (6 g/subject) was added to 360 g Snow Flake cake flour (24 g/subject) and made into a porridge. Both meals were eaten with 10 g sucrose.

6.2.3.4 Broccoli meal: Each subject received a meal made up from 50 g uncooked broccoli. It was cooked in boiling water for 20 min and then homogenized to the consistency of a thick soup. Radioactive iron was added just before serving; it was added alone on one morning and together with 2 g sodium phytate (sodium salt of inositol hexaphosphoric acid BDH Chemicals Ltd, Poole, Dorset) on the other.

6.2.3.5 Fibre meals: Several meals were prepared using constituents of the dietary fibre complex.

Individual 5 g portions of microcrystalline cellulose (Sigma cell type 50; Sigma Chemical Company, St Louis, Missouri) were weighed into glasses and 200 ml water containing 15 g sucrose and 3 mg iron as $FeSO_4.7H_2O$ labelled with 3 μCi radio iron was added to each and mixed well immediately before it was consumed.

The effect of 5 g apple pectin (250 grade; BDH Chemicals) was assessed in two studies. In the first, the jelly was prepared by dissolving 70 g pectin in 400 ml distilled water containing 140 ml ethanol, 210 g sucrose and 42 mg iron as $FeSO_4.7H_2O$ labelled with 42 μCi $^{59}FeCl_3.6H_2O$. After removing the ethanol by boiling, the jelly was refrigerated overnight. Each subject received 70 ml of the jelly. In the second study the apple pectin jelly was prepared by adding 5 g apple pectin very slowly to 150 ml

cold water and then adding 100 ml boiling water for each subject. The mixture was sweetened with 15 g sucrose and a drop of apple green food colouring was added to each bowl.

A separate guar gum meal was prepared for each subject. Guar gum (Jaguar, Stein Hall S A (Pty) Ltd) 5 g was sprinkled over 250 ml cold water and allowed to settle overnight at room temperature. White sugar (15 g) and a drop of an apple green food colouring were used to sweeten and colour the mixture in each bowl.

A meal of gum tragacanth was prepared by slowly dissolving 70 g finely powdered gum tragacanth (Merck, Darmstadt, West Germany) in 2,000 mls water. The dissolved material was refrigerated and before dividing it into 100 g meals the mixture was sweetened and flavoured by adding 200 g sugar and 5 ml caramel essence.

The effect of a highly purified hemicellulose preparation from psyllium seeds (Plantago ovata) was tested. Each meal was prepared separately by adding 200 ml water to 5 g Mucilose (Winthrop Laboratories, New York).

In a final experiment the effect of cocoa (Cadbury, Bournville, Birmingham) was assessed by measuring the change in iron absorption which occured when 10 g cocoa was added to 250 ml full cream milk containing 3 mg iron and three teaspoons of sugar. Although cocoa is not usually considered to be a high fibre food, it contains (g/kg) 40 cellulose, 110 non-cellulosic polysaccharides and 280 lignin, giving it a total fibre content of 430 g/kg

(Southgate et al, 1976).

The total iron content of all the test meals was between 3 and 4,2 mg.

6.3 Statistical Considerations

The significance of differences between the absorption of the two isotopes used in each study was calculated using Student's t test for paired observations, except in the case of malted sorghum where the unpaired t test was used because the results were not based on direct comparison in the same individuals.

6.4 Results

6.4.1 Effects of polyphenols and phytates on iron absorption from sorghum

In the first study, iron absorption from whole bird-proof sorghum was compared to that from 55% pearled bird-proof sorghum in sixteen subjects (Table 6.1). Although absorption was poor in each case, removing most of the polyphenol and phytate by pearling did significantly improve iron absorption from a geometric mean of 1,7% to 3,5% (t 3,9, $p < 0,005$). Since sorghum appeared to contain insignificant quantities of promotors of iron absorption, such as ascorbic acid, it was felt that more clear-cut evidence of the effects of the removal of inhibitors might be obtained if a small dose of a known enhancer of iron absorption was present in the meal. An amount of ascorbic

Table 6.1 The effect of the phytate and polyphenol contents of sorghum (Sorghum vulgare) on the assumption of iron (3 mg) as ferric chloride

(Mean values and standard deviations)

No. of subjects	Haemoglobin (g/dl) Mean	SD	Transferrin saturation (%) Mean	SD	Serum ferritin (µg/l) Geometric mean‡	± 1SD	Iron absorption* Reference Salt† Geometric mean‡	± 1SD	Test substance	Geometric mean‡	± 1SD
16	12,7	1,5	26,9	16,3	31,7	(13,3-75,3)	34,4	(20,0-59,0)	Whole bird-proof sorghum + Pearled bird-proof sorghum	1,7 3,5	(0,7-4,1) (1,5-8,1)
12	14,0	1,2	25,3	9,1	26,1	(10,7-75,3)	41,1	(25,7-65,7)	Whole bird-proof sorghum #+ Pearled bird-proof sorghum	2,4 6,3	(0,7-7,3) (2,5-14,4)
13	12,2	1,7	22,9	17,1	11,9	(2,9-48,5)	29,3	(17,3-49,5)	Whole bird-proof sorghum #+ Whole albino sorghum #	1,9 4,3	(0,6-5,9) (1,8-10,3)
10	12,9	1,5	25,2	10,6	18,1	(4,5-72,6)	34,0	(21,1-54,8)	Albino sorghum pearlings #+ Pearled albino sorghum #	1,5 3,5	(0,9-2,4) (2,3-5,1)
16	13,2	1,7	24,9	13,1	25,0	(8,7-71,3)	34,9	(17,5-69,8)	Broccoli o+ Broccoli + 2 g sodium phytate	18,5 3,7	(8,9-38,2) (0,9-16,0)
13	13,5	1,2	25,9	6,5	16,1	(3,6-72,5)	45,0	(35,9-56,4)	Malted sorghum #	2,4	(1,1-5,1)
18	13,9	2,5	30,8	19,3	-	-	30,5	(13,5-68,7)	Malted sorghum + 50 mg ascorbic acid ⁂	9,4	(4,6-19,5)
7	12,9	1,6	27,6	13,2	9,1	(2,0-40,2)	36,6	(18,5-72,4)	Malted sorghum + 50 mg ascorbic acid + tea ⁂	4,0	(2,0-8,2)

\+ The difference was statistically significant ($p < 0,05$).
‡ Geometric means and SD ranges used because values were positively skewed.
† 3 mg iron as ferrous ascorbate given in the fasting state.
# Meal served with an equimolar amount of ascorbic acid (9,5 mg) to iron.
* Individual results adjusted to 0,40 reference absorption.
o Broccoli (Brassica oleracea) included because of its high iron bioavailability.
⁂ 3 mg iron as $FeSO_4.7H_2O$ used.

acid equimolar with the added iron (9,5 mg) was therefore included with each meal in subsequent studies. When the first experiment was repeated in twelve subjects using this approach, the geometric mean iron absorption for whole grain was 2,4% versus 6,3% for pearled grain (t 3,4, p < Ø,Ø1). In a third experiment using thirteen subjects, the geometric mean iron absorption from polyphenol-poor albino whole grain sorghum was found to be 4,3% versus 1,9% from the polyphenol-rich bird-proof sorghum (t 2,78, p < Ø,ØØØ5). The geometric mean iron absorption, from pearled albino sorghum in a further ten subjects was 3,5% versus 1,5% from a porridge prepared from the pearlings (t 8,4, p < Ø,ØØØ5). Since the pearlings were rich in phytate, an experiment was carried out on sixteen subjects to assess the effects of sodium phytate on the absorption of iron from a broccoli meal of high iron-bioavailability. The addition of sodium phytate was associated with a significant decrease (t 6,13, p < Ø,ØØØ5) in the geometric mean iron absorption (18,5% versus 3,7%).

The effect of ascorbic acid on iron absorption from malted sorghum porridge was studied in further experiments. Increasing the dose of ascorbic acid from 9,5 to 5Ø mg was associated with a significant rise in the geometric mean iron absorption from 2,4% (thirteen subjects) to 9,4% (eighteen subjects) (t 3,33, p < Ø,ØØ5). The enhancing effect of the larger dose of ascorbic acid was effectively eliminated in seven subjects by serving the porridge with a

cup of tea; the geometric mean iron absorptions were 9,4% and 4,Ø% respectively (t 38,1, p < Ø,ØØØ5).

The ability of ascorbic acid to overcome the effects of the inhibitors of iron absorption present in cereals was shown also to extend to oats (See Appendix Table 6.11). In seven subjects the geometric mean absorption from oat porridge was Ø,9% v 5,7% after adding an equimolar amount of ascorbic acid (t 12,25, p < Ø,ØØØ5).

6.4.2 Effect of various types of fibre on the absorption of iron

The presence of wheat bran in a porridge prepared from white cake flour reduced the geometric mean iron absorption from 11,6% to 4,3% (t 5,2, p < Ø,ØØ5) Table 6.2. A comparison was then made in twelve subjects between the absorption of iron in apple pectin jelly and in microcrystalline cellulose slurry. The corrected geometric mean iron absorptions were high on both occasions, being 24,1% and 35,6% respectively. Although the difference was statistically significant (t 5,41, p < Ø,ØØØ5), this may have been due to the method of preparation, since the geometric mean iron absorption from apple pectin jelly was higher when ethanol was not used in preparing it. In the next study the alcohol-free preparation was compared with guar gum in ten subjects, and the geometric mean iron absorption values were 34,2% and 39,2% respectively (t 1,Ø3, p > Ø,1). In eight subjects there was no significant difference between the geometric mean iron absorption from

Table 6.2 The effect of various types of 'fibre' on the absorption of iron (3 mg) as ferrous sulphate

(Mean values and standard deviations)

No. of subjects	Haemoglobin (g/dl) Mean	SD	Transferrin saturation (%) Mean	SD	Serum ferritin (µg/l) Geometric mean‡	± 1SD	Iron absorption* Reference Salt Geometric mean‡	± 1SD	Test substance	Geometric mean‡	± 1SD
8	12,7	Ø,6	18,9	8,9	16,7	(5,4-51,9)	34,1	(14,8-78,5)	White flour +	11,6	(8,8-14,9)
									White flour + 6 g bran	4,3	(3,Ø-6,1)
12	12,9	1,8	27,9	10,3	10,4	(2,8-38,5)	37,7	(23,7-60,Ø)	Cellulose (5 g)	35,6	(26,3-48,2)
									Apple pectin (5 g)	24,1	(15,2-38,3)
10	12,6	1,5	34,3	16,1	16,8	(4,6-61,5)	21,8	(12,7-37,6)	Guar gum (5 g)	39,2	(18,6-82,8)
									Apple pectin (5 g)	34,2	(18,4-63,5)
8	14,7	1,Ø	29,8	7,8	20,6	(10,5-40,8)	25,6	(17,Ø-38,7)	Gum tragacanth (2,5 g)	25,1	(10,8-58,2)
									Cellulose (5 g)	28,2	(14,6-54,7)
6	12,2	1,6	25,3	9,3	12,8	(1,6-100,1)	18,9	(12,4-29,Ø)	Hemicellulose #+	5,3	(1,9-14,3)
									Cellulose (5 g)	20,5	(9,Ø-46,7)
8	13,5	1,8	26,3	7,4	22,5	(9,4-54,2)	45,9	(25,6-82,8)	Milk +	6,3	(2,5-15,6)
									Milk + 10 g cocoa (279 g lignin/kg)	3,5	(1,7-7,4)

\+ The difference was statistically significant ($p < Ø,Ø5$).
‡ Geometric means and SD ranges used because values were positively skewed.
‡ 3 mg Fe as ferrous ascorbate given in the fasting state.
\# Mucilose (Winthrop Laboratories, New York).
* Individual results adjusted to 40% reference absorption.

gum tragacanth (25,1%) and from cellulose (28,2%); (t Ø,44, p > Ø,1). However, when Mucilose (hemicellulose) was substituted for the gum tragacanth in another six subjects, the mean geometric iron absorption was significantly lower (5,3%) compared with the cellulose (2Ø,5%) (t 2,95, p < Ø,ØØØ5). In a final experiment in nine subjects, the addition of cocoa to milk reduced the geometric mean iron absorption significantly from 6,3% to 3,5% (t 2,Ø3, p < Ø,ØØØ5)

6.5 Discussion

This study was carried out on a female population in which iron deficiency, usually of mild degree, is common (Mayet et al, 1972; MacPhail et al, 1981a). The diets consumed by these people of Indian origin are in many respects similar to those in India, where cereals and legumes are important staples (Narasinga Rao & Prabhavathi, 1982). The bioavailability of the iron in these staples is low and this has stimulated a good deal of research into the reasons and ways of overcoming it.

6.5.1 The effect of phytate and polyphenols on iron absorption

One potent inhibitor of iron absorption is bran (Bjorn-Rasmussen, 1974), and this was confirmed in the present study. It has been suggested that its action may be due to some factor or factors present in vegetable fibre. Reinhold and co-workers (1981) have shown that the fibre present in

wheat and maize binds iron. The effect cannot be ascribed entirely to its phytate content, since the capacity of bran to bind iron (Reinhold et al, 1981) and to inhibit iron absorption persists even after the phytate has been removed (Morris & Ellis, 1980; Simpson et al, 1981). Conflicting evidence regarding the role of phytate has been provided by Fairweather-Tait (1982) who found that increasing the amount of dietary fibre in bread without altering its phytate content, failed to reduce iron absorption in rats. In other experiments the particle size of bran has been reduced in order to increase the surface area of the phytate-containing aleurone layer. This comprises the bran of the wheat kernel (Schaller, 1978) and by exposing it to the intestinal mucosa it might be expected to increase hydrolysis of the phytate. However, the manouvre was not found to improve iron absorption in rats (Caprez & Fairweather-Tait, 1982). There is nevertheless reason to believe that phytates can inhibit iron absorption, since both diferric and tetraferric phytate are poorly bioavailable (Ellis & Morris, 1979), as is the iron in various vegetables with high phytate contents (e.g. wheat germ, butter beans and lentils.

Certain polyphenols also exert an inhibitory effect on iron absorption. Important examples are the tannin in tea (Disler et al, 1975 a & b; Rossander et al, 1979; Hallberg & Rossander, 1982b; Yau-tian, 1983; Acosta et al, 1984) and coffee (Morck et al, 1983), but as has been discussed in Chapter 5, many other vegetable foodstuffs contain similar

polyphenols in quantities which correlate inversely with the bioavailability of the iron they contain. Narasinga Rao & Prabhavathi (1982) have postulated that decortication of legumes should enhance iron absorption, since most of the tannins reside in the seed coat.

The present study systematically examined the effect on iron absorption of the polyphenol and phytate in sorghum. When amounts of both compounds were reduced to low levels by pearling, there was a significant increase in iron absorption from 1,7% to 3,5%. This difference was still present when absorption was enhanced by adding ascorbic acid, a known enhancer of iron absorption (Sayers et al, 1973, 1974 a & b; Derman et al, 1977). When only an equimolar amount (9,5 mg) was added, absorption increased from 2,4% to 6,3%. A much greater enhancement of iron absorption from 2,4% to 9,4% was found when 50 mg of ascorbic acid was added to a meal of malted sorghum, thus confirming that the effect was dose related (Derman et al, 1980a). This enhancement was compromised by taking tea with the meal, with absorption dropping to 4,0%. These results illustrate the interplay of inhibitory and promoting ligands on the bioavailability of iron.

An albino sorghum cultivar containing phytate but no polyphenol was used to study the relative inhibitory effects of these two compounds on iron absorption. The significantly higher geometric mean absorption (4,3%) from this grain in comparison with that from the bird-proof cultivar (1,9%),

suggested that the polyphenol in the latter cultivar was a more potent inhibitor. Evidence that phytate was also an inhibitor was obtained by pearling the albino sorghum to give fractions of high and negligible phytate content and showing that iron was absorbed better from the latter (3,5% versus 1,5%). More direct evidence was obtained by adding 2 g sodium phytate to a broccoli meal; it decreased iron absorption from 18,5% to 3,7%. This effect may explain the low iron absorption of Ø,9% from oatmeal, a cereal having a particularly high phytate content (approximately 1Ø mg/kg). Addition of ascorbic acid, equimolar to added iron, increased absorption to 5,7% indicating that ascorbate can overcome the inhibitory effect.

6.5.2 The effect of the components of fibre on iron absorption.

Since pearling also causes a reduction in the fibre content of cereal grains, an attempt was made to identify components of fibre that inhibit iron absorption. Previous evidence that fibre may be of importance rests on the previous observation of an inverse exponential correlation between the bran content of bread and the absorption of iron (Björn-Rasmussen, 1974). Certain components of fibre have the property of cation exchange (Eastwood & Mitchell, 1976) which may be responsible for the occurrence of a negative calcium balance observed by Cummings (1978) in students fed an increased amount of whole wheat and bran. The major fibre of many vegetable foods is cellulose, which does not bind

cations (Van Soest, 1978) and which in the present study did not appear to inhibit iron absorption. In three experiments the absorption of iron given with microcrystalline cellulose was 35,6%, 28,2% and 20,5%. The weak bond between iron and cellulose has been confirmed by the in vitro work of Fernandez and Phillips (1982) and Platt and Clydesdale (1984) who have shown that phytate, citrate and EDTA completely solubilize the iron from cellulose. It has been postulated that the iron binding of the neutral detergent fibre of wheat (0,38 mg Fe/g) is related to the unsubstituted uronic groups of hemicellulose (Reinhold et al, 1981). The neutral detergent fibre contains only the insoluble plant cell walls and not the more soluble wall constituents, such as guars and pectins (Van Soest, 1978), which would not be expected to affect iron absorption. This prediction was substantiated in the present study. Neither guar gum, which is a plant mucilage contained in the endosperm of the fodder legume, Cyamopsis tetragonolobus, nor gum tragacanth, another mucilaginous type of fibre, inhibited iron absorption. The geometric mean absorptions were 392% and 25,1% respectively compared with a figure of 28,2% from cellulose. Although Monnier and co-workers (1980) claimed that apple pectin inhibited iron absorption no such evidence was obtained in the present study. The mean iron absorptions in two groups of women were 24,1% and 34,2% respectively. In this connection, Baig and co-workers (1983) have not found that citrus pectin affects iron

absorption or turnover. Cook and co-workers (1983) found 1,89% of iron to be absorbed from pectin muffins, and 2,26% from cellulose muffins.

Of some interest were the findings with Mucilose, which is a highly refined form of hemicellulose consisting of pentosans,hexosans and galactans obtained from psillium (Plantago ovata) (Fernandez & Phillips, 1982). Absorption from mucilose was significantly less than from cellulose (5,3% versus 20,5%). This finding suggests that hemicellulose may act as an inhibitor of iron absorption. Of possible relevance in this regard may be the findings of Holloway and co-workers (1980). Whilst pectin is only degraded in the large bowel (Holloway et al, 1983) many of the hemicelluloses of fruit and vegetables are degraded in the small intestine (Holloway et al, 1980). Hemicellulose may therefore exert its metal sequestering ability by altering active ionic membrane transport sites in the villi of the small intestine in a fashion analagous to that found by Schwartz and co-workers (1982) in rats.

Because of the extremely high lignin content of cocoa its effect on iron absorption was examined. Lignin is thought to have two functional types of binding sites, A and B (Platt & Clydesdale, 1984), or high affinity and low affinity (Fernandez & Phillips, 1982). The high affinity or Type A site maintains iron in an insoluble form, whilst the Type B or low affinity site has a lower binding constant with iron than phytate phosphate groups and its iron can be

solubilized by phytate. Addition of 10 g cocoa to 250 ml milk decreased iron absorption from 6,3% to 3,5%. It is possible that this decrease in iron absorption was due to the lignins, some of which have a polyphenolic structure, including groups of the gallic acid type. In studies using a malted cocoa drink (20 g powder) fortified with ferric orthophosphate (0,5 mg Fe/g powder) and ^{58}Fe as the extrinsic label, Fairweather-Tait and co-workers (1983) found 25% of the iron to be absorbed when the drink was served with ascorbic acid (1 mg/g powder); 24% when only $FePO_4$ was present, and 23% with $FeSO_4$. The interpretation of these data is not clear.

6.6 Summary

1. Non-haem-iron absorption from a variety of cereal and fibre meals was measured in parous Indian women, using the erythrocyte utilization of radioactive iron method.

2. The studies were undertaken to establish whether alteration of the phytate and polyphenol content of sorghum (Sorghum vulgare) affected iron absorption from sorghum meals, and to assess the influence of fibre on iron absorption.

3. Removing the outer layers of sorghum grain by pearling reduced the polyphenol and phytate content by 96 and 92% respectively. This treatment significantly increased the geometric mean iron absorption from 1,7% to 3,5% (t 3,9, $p < 0,005$).

4. The geometric mean iron absorption from a sorghum cultivar

that lacked polyphenols (albino sorghum) was 4,3%, which was significantly greater than the 1,9% absorbed from bird-proof sorghum, a cultivar with a high polyphenol content (t 2,78, p < 0,0005).

5. Iron was less well absorbed from the phytate-rich pearlings of the albino sorghum than from the pearled albino sorghum (1,5% versus 3,5%) (t 8,4, p < 0,0005)). Addition of sodium phytate to a highly iron-bioavailable broccoli (Brassica oleracea) meal reduced iron absorption from 18,5% to 3,7%.

6. The geometric mean iron absorption from malted sorghum porridge was 2,4% when 9,5 mg ascorbic acid was added and 9,4% when the ascorbic acid was increased to 50 mg (t 3,33, P < 0,005). This enhancing effect of 50 mg ascorbic acid was significantly depressed to 4,0% by tea (t 38,1, p < 0,0005).

7. Wheat bran significantly decreased the geometric mean iron absorption from white flour from 11,6% to 4.3% (t 5,2, p < 0,005).

8. Some of the constituents of the dietary fibre complex, such as apple pectin, guar gum, gum tragacanth and microcrystalline cellulose, did not inhibit iron absorption. On the other hand, hemicellulose and lignin decreased absorption. The geometric mean absorption of iron fed with hemicellulose was 5,3% versus 20,5% with microcrystalline cellulose (t 2,95, p < 0,0005). Addition of cocoa, which contains approximately 280 g lignin/kg, reduced the

geometric mean iron absorption from milk from 6,3% to 3,5% (t 2,Ø3, p < Ø,ØØØ5).

9. The results obtained in these studies suggest that the low bioavailability of iron in the presence of sorghum is due to several factors, including the presence of the inhibitory substances phytate and polyphenols, and insufficient quantities of promoting substances such as ascorbic acid. Some components of dietary fibre may also contribute to the poor bioavailability of iron in sorghum meals. Of the various constituents of the fibre complex that were tested only lignin and hemicellulose were shown to have an inhibitory effect on iron absorption. While these latter results are in agreement with in vitro observations (Fernandez & Phillips, 1982), their relevance to iron nutrition remains to be defined.

Chapter Seven

THE RELATIVE EFFECT OF ASCORBIC ACID ON IRON ABSORPTION FROM SOY AND MILK BASED INFANT FORMULAS

7.1 Introduction

Iron deficiency is the most commonly recognised nutritional deficiency in both developing countries and affluent societies. It is particularly prevalent among infants and young children because rapid growth imposes large requirements for iron and many infant diets contain only a marginal supply of iron. In this context, the use of infant formulas based on soy as a source of protein is of some concern, since there is evidence that iron absorption in the presence of soy products is poor (Morck et al, 1981). There are two ways in which the low bioavailability of iron in soy products might be improved. Firstly, such products could be fortified with greater amounts of iron, and secondly, a promoter of iron absorption, such as ascorbic acid, could be added (Morck et al, 1981). The present study compared the absorption of iron from a soy based infant milk formula with that from a similar product based on cow's milk, and examined the effect of varying concentrations of ascorbic acid.

7.2 Materials and Methods

7.2.1 Subjects

The subjects who participated in the studies were 64 parous Indian house-wives living in municipal housing schemes in Chatsworth and Merebank, near Durban. This community was selected, since there is a high prevalence of iron deficiency amongst the women (Mayet et al, 1972;

MacPhail et al, 1981a).

7.2.2 Composition of milk formulas

The soy-based formula used in this study was a soy protein isolate in powder form, designed specifically for infants and children intolerant to lactose or cow's milk. The formula, which contained 6 mg iron/100 g as ferrous sulphate, was prepared for the present studies without ascorbic acid by Nestlè Infant and Dietetic Services, Johannesburg, South Africa. The preparation contained soy protein hydrolysate, vegetable oils, and maltodextrin and was supplemented with minerals and vitamins. The major components were fat (25%), protein (14%), and carbohydrate (55%). It contained 3% minerals, 3% moisture and had an energy value of 500 kcal/100 g. The normal dilution was 670 kcal/l and it was prepared by dissolving 134 g powder in 900 ml of previously boiled water.

The milk-based formula ("Lactogen" Nestlè, Randburg, South Africa) was a commercially available infant formula prepared from cow's milk modified by partial replacement of the butter fat by corn oil and by the addition of carbohydrates, vitamins and mineral salts. The overall composition was similar to that of the soy formula (24% fat, 16% protein, 52% carbohydrate, 4% minerals, 3% moisture, 490 kcal/100 g). However, it contained no iodine and there were minor differences in the zinc, sodium, potassium and chloride concentrations. The basic preparation used in these studies contained 6 mg iron and 40 mg ascorbic acid per 100 g.

7.2.3 Administration of formulas

Absorption of iron from two different formulas was measured in each group of subjects. The milk drinks were consumed on consecutive mornings after an overnight fast, and no food or drink other than water was permitted for four hours thereafter. Each portion, prepared by dissolving 50 g of the "milk" powder in 150 ml water, was labeled with 3 /uCi of either ^{55}Fe or ^{59}Fe (Radiochemical Centre, Amersham, Berks, England). One formula was tested using one isotope and on the following day the second formula was given labeled with the other isotope. The radioiron, which was prepared in 1N HCl, was stirred into the drink immediately before consumption.

7.3 Results

In the first three studies (Table 7.1, studies 1,2 & 3) the effect of various levels of ascorbic acid on the absorption of iron from soy and milk-based formulas was compared. In study 1 the absorption of iron from the standard milk formula containing 20 mg ascorbic acid (40 mg/100 g) was 6,9% (SD range 1,9-25,3%), which was more than three times greater than that from the soy formula containing no ascorbic acid 1,5% (SD range 0,6 - 3,7%) ($t = 5,75$ $p < 0,0005$). In the next study (2) both formulas had the same ascorbic acid content (20 mg) (40 mg/100 g) but iron absorption from the milk formula was 5,3% (SD range 1,9 - 14,6%), which was more than twice that from the soy

Table 7.1 Iron absorption from soy and milk formulas containing 3 mg iron, 50 g formula and varying amounts of ascorbic acid

(Mean values and standard deviations)

Study number	No. of subjects	Haemoglobin (g/dl) Mean	Haemoglobin (g/dl) SD	Transferrin saturation (%) Mean	Transferrin saturation (%) SD	Serum ferritin (µg/l) Geometric Mean*	Serum ferritin (µg/l) ± 1SD	Ascorbic Acid Content (mg)	Iron absorption* Test substance	Iron absorption* Geometric Mean‡	Iron absorption* ± 1SD
1	13	13,4	(1,1)	27	(11)	20,4	(5,6-74,8)	0	Soy	1,5	(0,6-3,7)
								20	Lactogen	6,9	(1,9-25,3)
								30	Reference	23,0	(14,7-36,1)
2	12	12,0	(1,3)	21	(8)	12,1	(4,0-36,2)	20	Soy	2,4	(0,5-11,0)
								20	Lactogen	5,3	(1,9-14,6)
								30	Reference	34,0	(18,2-63,5)
3	7	12,9	(1,3)	31	(11)	20,5	(7,9-53,3)	40	Soy	7,2	(3,8-13,6)
								40	Lactogen	19,5	(7,9-47,9)
								30	Reference	32,2	(16,4-63,2)
4	11	11,8	(1,5)	21	(8)	20,6	(7,6-54,6)	0	Soy	2,6	(1,1-5,7)
								20	Soy	3,4	(1,3-8,8)
								30	Reference	31,0	(22,2-43,5)
5	9	12,7	(0,7)	31	(11)	34,8	(18,3-66,3)	0	Soy	0,6	(0,2-1,7)
								40	Soy	4,5	(1,9-10,9)
								30	Reference	35,0	(18,5-67,0)
6	12	12,1	(1,6)	24	(9)	16,4	(4,5-59,9)	40	Soy	4,9	(1,6-15,8)
								80	Soy	5,2	(1,5-17,9)
								30	Reference	35,0	(18,0-70,0)

* Individual results adjusted to a 40% reference absorption.

‡ Geometric mean and SD ranges used because values were positively skewed.

formula 2,4% (SD range Ø,5-11,Ø%)) (t = 2,93; p < Ø,Ø25). Increasing the ascorbic acid content to 4Ø mg (8Ø mg/1ØØ g) in both formulas (Study 3) only served to emphasise the enhanced iron absorption from the milk-based preparation. Figures were 19,5% (SD range 7,9 - 47,9%) for the milk based formula and 7,2% (SD range 3,8 - 13,6%)) (t = 2,8, p < Ø,Ø25) for the soy.

The remaining studies (Table 7.1, studies 4,5 & 6) were designed to show the effect of increasing ascorbic acid concentrations on iron absorption from the soy-based formula. Study 4 demonstrated that the addition of 2Ø mg ascorbic acid (4Ø mg/1ØØ g) to the soy formula did not significantly improve the absorption of iron (3,4% (SD range 1,3 - 8,8%)) compared to that from the same preparation containing no ascorbic acid (2,6% (SD range 1,1 - 5,7%)) (t = 1,6, p > Ø,1). However, increasing the level of fortification to 4Ø mg ascorbic acid (8Ø mg/1ØØ g) in Study 5 significantly improved absorption from Ø,6% (SD range Ø,2 - 1,7%) to 4,5% (SD range 1,9 - 1Ø,9%) (t = 8,24, p < Ø,ØØØ5). An increase in the ascorbic acid content from 4Ø mg to 8Ø mg (16Ø mg/1ØØ g) failed to enhance iron absorption further. The figures were 4,9% (SD range 1,6 - 15,8)% and 5,2% (SD range 1,5 - 17,9%) respectively (t = Ø,4, p > Ø,1) (Study 6).

In a final analysis, an overall comparison was made between the effects of varying doses of ascorbic acid on the absorption of iron from the soy and milk formulas. To make the comparison possible, all the individual results obtained

in the 6 studies were standardized to a reference absorption of 40%. Since reference absorptions were not obtained in 11 subjects, the analysis was based on the results obtained in 53 patients. The geometric mean iron absorption from the soy-based formula was very low (1,8% (SD range 0,6 - 5,2%)) when no ascorbic acid was present and rose only modestly when ascorbic acid was added in concentrations varying between 40 mg/100 g and 160 mg/100 g (Fig. 1). Statistical comparison of these groups showed that the addition of ascorbic acid in a concentration of 40 mg/100 g did not significantly increase iron absorption (3,3%, SD range 1,2 - 9,6%) ($t = 1,8$, $p > 0,07$), but raising the concentration to 80 mg/100 g did so (6,9% (SD range 2,9 - 16,3%)) ($t = 2,4$, $p < 0,02$). Increasing the concentration to 160 mg/100 g did not improve absorption further (7,7% (SD range 2,4 - 24,8%)) ($t = 0,4$, $p > 0,7$). In contrast, iron absorption from the milk formula was markedly affected by the concentrations of ascorbic acid present. An increase in the ascorbic acid concentration from 40 mg/100 g to 80 mg/100 g was associated with a more than two fold increase in iron absorption from 10,5% to 24,2%. An analysis of variance showed that the overall results fell into three groups. In the first group, absorption from the milk-based formula containing 40 mg ascorbic acid/100 g did not differ significantly from absorption from the soy-based formulas containing 80 mg and 160 mg ascorbic acid/100 g ($F = 1,55$, $p > 0,2$). Absorption from this group was significantly better than that from a

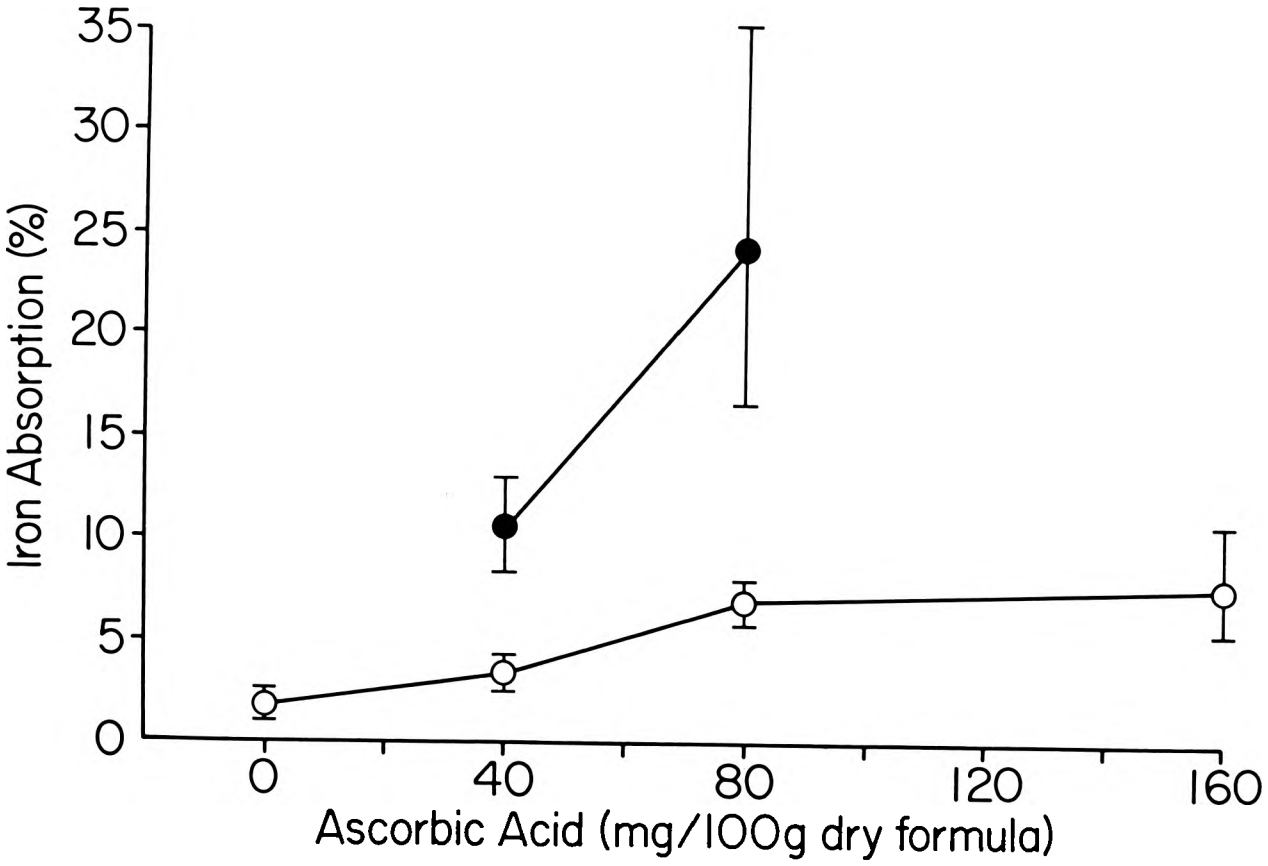


Figure 7.1 The effect of various concentrations of ascorbic acid on the geometric mean absorption of iron (± SE) from a soy based infant milk formula containing 3 mg iron as ferrous sulphate and 50 g dry powder (o) and from a similar product based on milk ("Lactogen") (o). To make comparisons possible all absorption values were corrected to a reference absorption of 40%. (The geometric mean reference absorptions in the various groups studied varied between 23,0 and 35,0%).

second group comprising the soy-based formulas containing either no ascorbic acid or 40 mg/100 g ($F = 25,85$, $p < 0,002$). Finally, iron absorption from the milk-based formula with 80 mg ascorbic acid/100 g was significantly better than that from all other combinations ($F = 15,96$, $p < 0,002$).

7.4 Discussion

The full-term infant receives a generous and relatively fixed iron supply of 75 mg/kg from the mother (Widdowson & Spray, 1951). During the first two months of life the infant relies largely on stored iron for its requirements but the demands of erythropoiesis and growth rapidly deplete these stores and between the second to six month there is an increasing dependence on dietary iron which is largely derived from milk, milk products and soy-based products (Andelman & Sered, 1966). It is currently estimated that soy-based preparations account for 10 to 20% of infant formula sales in the United States. They are used extensively to avoid suspected or proven allergies to milk, while soy is also a major constituent of infant protein supplements (INACG report, 1982). However, there is currently some concern as to whether such products may have a deleterious effect on iron nutrition, since there is evidence that soy inhibits iron absorption (Morck et al, 1981; Morck & Cook, 1981; Hallberg & Rossander, 1982c). The iron-binding protein in soy comprises two fractions, one

that elutes in the range of 10,000-25,000 daltons and the other having a molecular weight of >600,000 (Smith, 1983). The protein binds iron through one or more carboxyl groups (Fujita et al, 1982).

Earlier studies on the absorption of iron in the presence of soy products gave conflicting results, with some showing good bioavailability (Layrisse et al, 1969; Sayers et al, 1973) and others relatively poor bioavailability. The confusion was resolved in a series of studies by Cook and co-workers (Morck et al, 1981; Morck & Cook, 1981; Cook et al, 1981; Morck et al, 1982). They demonstrated that the bioavailability of iron in corn-soy-milk was low (Morck et al, 1981) and that iron absorption from meals containing isolated soy protein was significantly less than from meals containing egg albumen or casein (Cook et al, 1981). In further studies, they showed that the inhibitor of iron absorption was not removed by purification but could be partially overcome by ascorbic acid (Morck et al, 1982). This latter observation was of interest because it may explain why Rios and co-workers (1975) in an earlier study were not able to show a difference in iron absorption when they compared a milk and soy-based formula. Ascorbic acid was present in both formulas.

The enhancing effect of ascorbic acid on the absorption of iron from vegetable meals has been amply demonstrated in a number of studies (Sayers et al, 1973; Sayers et al, 1974 a & b; Derman et al, 1977). The fact that the enhancing

effect of ascorbic acid on iron absorption is dose related (Derman et al, 1980b) prompted the present investigation, since it was felt that it might be possible to overcome the inhibitory effect of soy on iron absorption from soy-based formulas by having an appropriate amount of ascorbic acid present in the formula. Ascorbic acid supplementation of soy protein isolates has been demonstrated in in vitro studies to produce substantial increases in soluble iron in a concentration dependent fashion (Rizk and Clydesdale, 1983). In a study evaluating the effect of soy protein concentrate on long term protein nutrition in 6 adult men, iron absorption calculated by the difference between total body iron losses and the decline in storage iron, was found to be about 16% from soy concentrate. Each meal was supplemented with 75 mg ascorbic acid and there was a wide variation obtained between and within subjects involved (Istfan et al, 1983b). Where no supplemental ascorbic acid was added, iron absorption from the soy concentrate and that from an equivalent milk based diet were the same (Istfan et al, 1983a). In the present study a comparison was made between a soy-based formula and a similar formula in which milk was present instead of soy. Several points of interest emerged. Iron absorption from the soy based formula was very low when no ascorbic acid was present. At the concentration of ascorbic acid which is used for commercial fortification in the products tested (40 mg/100 mg), iron absorption was significantly better from the milk formula

and the difference was even greater when the level of ascorbic acid concentration was increased to 80 mg/100 g. The inhibitory effect of soy on iron absorption was still apparent when the concentration of ascorbic acid was increased to 160 mg/100 g formula.

In an attempt to express the present results in figures relevant to iron deficient subjects, all values were corrected to a reference absorption of 40% (Fig. 7.1). This figure was chosen since it is the amount of iron absorbed by subjects with early iron deficiency (Hallberg, 1981a). If the WHO/UNICEF recommended amount of supplement (100 g) were consumed daily by a year old infant then the following amounts of iron would be absorbed from the soy-based formula with increasing amounts of ascorbic acid:- no ascorbic acid -0,11 mg, 40 mg/100 g -0,20 mg, 80 mg/100 g -0,41 mg and 160 mg/100 g -0,46 mg. In contrast, figures of 0,63 mg and 1,45 mg were obtained with the milk formula when the levels of ascorbic acid fortification were 40 and 80 mg/100 g respectively. When these figures are considered against the background of the 1 year old infant's daily iron requirements of 1,5 mg, it can be seen how inadequate the absorption figures for the soy formula actually were. For such products, which represent an important potential source of dietary protein, to be adequate from the point of view of iron nutrition, certain adjustments seem appropriate. For example, the soy-based formula tested in the present study was found to be inadequate both with regard to the amounts

of iron and ascorbic acid that it contained. More satisfactory absorption figures might be anticipated if the level of iron fortification were to be doubled from 6 mg/100 g to 12 mg/100 g, and the weight ratio of ascorbic acid to iron of approximately 6 to 1, were to be increased to 12 to 1.

7.5. Summary

1. The effect of varying concentrations of ascorbic acid on the absorption of iron from a soy-based infant milk formula containing 6 mg iron/100 g was examined in 64 adult Indian females.

2. The corrected geometric mean absorption from the basic soy formula was only 1,8%. Addition of ascorbic acid in a concentration of 40 mg/100 g, did not significantly increase absorption (3,3%; $t = 1,8$, $p > 0,07$) but raising the concentration to 80 mg/100 g, did so (6,9%; $t = 2,4$, $p < 0,02$).

3. No further significant increase was noted when the concentration of ascorbic acid was increased to 160 mg/100 g (7,7%; $t = 0,4$, $p > 0,7$).

4. The inhibitory effect of soy on iron absorption was further demonstrated by a direct comparison between the soy based formula and a similar product based on cow's milk. The comparison was made at two concentrations of ascorbic acid, 40 mg/100 g and 80 mg/100 g. Geometric mean iron absorption figures for the soy formula was 2,4% and 7,2% ($t = 2,8$, $p < 0,02$) as compared with corresponding values of

5,3% and 19,5% for the milk formula ($t = 3,4$, $p < 0,02$)

5. The present results confirm the marked inhibitory effect of soy protein on iron absorption and suggest that such formulas should contain at least 12 mg iron /100 g together with ascorbic acid in a weight ratio of 12:1, if they are to be adequate in terms of iron nutrition.

APPENDIX

The following appendix includes the individual haematological and absorption results obtained in the different iron absorption studies.

Chapter 5. Table 5.5 Absorption of iron (3mg) as $FeSO_4.7H_2O$ from a basic rice meal (215g/subject) in fasting female subjects

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*	
					Rice meal	Reference salt+
1	12,8	64	18	22	3,5	37,9
2	13,Ø	162	54	26	5,4	17,9
3	9,6	25	6	3	21,8	62,6
4	13,Ø	79	25	3Ø	4,5	15,9
5	9,6	29	6	3	3,2	36,Ø
6	12,3	46	1Ø	4	5,Ø	32,2
7	8,5	25	5	1	2,1	42,3
8	12,3	1Ø7	28	26	9,5	18,7
9	12,7	82	2Ø	7	2,Ø	33,7
1Ø	12,5	1Ø8	33	16	5,4	23,6
11	9,1	3Ø	7	2	3,7	65,9
12	13,1	72	18	21	3,7	27,1
13	14,7	2Ø1	51	35Ø	2,6	11,6
14	13,9	1Ø9	26	9	2,6	24,6
15	12,2	59	21	19	2,6	31,Ø
16	12,8	1Ø1	26	3Ø	5,1	34,4
17	13,Ø	164	4Ø	69	8,Ø	3Ø,5
18	13,7	147	32	16	1,5	47,8
19	14,8	169	44	29	2,Ø	34,1
2Ø	14,Ø	127	38	21	3,1	54,2
21	11,Ø	75	6	7	2,8	75,1
22	14,7	115	36	11	2,5	49,8
23	15,3	1Ø4	36	31	2,1	33,9
24	14,8	221	48	42	3,9	11,7
25	13,4	77	21	18	8,6	4Ø,5
Mean(±SD)	12,6(1,8)	99,9(54,3)	26.2(14.9)			
# Geometric mean (± 1SD)				14,7(4,3-5Ø,3)	3,1(1,6-5,9)	32,Ø(19,5-52,5)

* Individual results adjusted to a 4Ø% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.6

The absorption of iron (3 mg) as $FeSO_4.7H_2O$ from a rice meal (215 g/subject) with 15 mg ascorbic acid in fasting female subjects

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*	
					Rice meal	Reference salt+
1	13,5	205	58	49	17,1	9,1
2	15,1	90	25	72	1,7	13,0
3	14,2	99	25	13	26,4	25,2
4	13,5	121	43	16	10,8	31,6
5	14,7	68	24	76	2,7	22,6
6	14,3	93	31	145	6,2	31,5
7	15,3	96	30	30	8,7	24,5
8	15,8	118	36	45	12,0	30,6
9	15,3	124	46	42	3,4	57,3
Mean(SD)	14,6(0,8)	112,7(38,9)	35,3(11,6)			
#Geometric Mean (± 1SD)				42,6(19,9-91,0)	8,1(3,3-19,6)	24,2(14.2-41,3)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.7

Absorption of iron (3mg) as $FeSO_4.7H_2O$ from a rice meal (215g/subject) with and without 36 mg citric acid, in fasting female subjects

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Rice meal	Rice + 36mg citric acid	Reference salt+
1	12,8	64	18	22	3,5	4,4	37,9
2	13,Ø	162	54	6	5,4	18,Ø	17,9
3	9,6	25	6	3	21,8	11,8	62,6
4	13,Ø	79	25	3Ø	4,5	11,4	15,9
5	9,6	29	6	3	3,2	4,3	36,Ø
6	12,3	46	1Ø	4	5,Ø	22,8	32,2
7	8,5	25	5	1	2,1	8,8	42,3
Mean(SD)	11,3(1,9)	61,4(48,9)	17,7(17,6)				
# Geometric Mean (± 1SD)					6,7(1,8-25,5)	4,9(2,3-1Ø,1)	9,9(5,2-18,8)	31,8(19,7-51,5)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg Fe as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.8

Absorption of iron (3mg) as $FeSO_4.7H_2O$ from a rice meal (215g/subject) with and without 1 g citric acid, in fasting female subjects

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Rice meal	Rice + 1 g citric acid	Reference salt+
1	12,3	107	28	26	0.9	6,9	18,7
2	12,7	82	20	7	2,5	14,1	33,7
3	12,5	108	33	16	5,4	11,3	23,6
4	9,1	30	7	12	3,7	3,4	65,9
5	13,1	72	18	21	3,7	22,0	27,1
6	14,7	201	51	350	2,6	3,5	11,6
7	13,9	109	26	9	2,6	7,0	24,6
8	12,2	59	21	19	2,6	10,6	31,0
9	12,8	101	26	30	5,1	10,8	34,4
Mean(SD)	12,6(1,5)	96,6(47,4)	25,6(12,1)				
# Geometric mean (± 1SD)				18,2(4,5-73,0)	2,8(1,6-5,1)	8,5(4,7-15,4)	27,2(16,9-43,6)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.9

Absorption of iron (3mg) as $FeSO_4.7H_2O$ from a rice meal (215g/subject) with and without 1g tartaric acid, in fasting female subjects

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Rice meal	Rice + 1g tartaric acid	Reference salt+
1	14,2	73	16	19	46,6	51,Ø	3Ø,2
2	14,5	9Ø	21	8	4,Ø	6,7	75,3
3	13,1	45	9	4	2,6	4,5	85,2
4	12,1	57	13	5	17,5	8,5	39,3
5	14,2	75	2Ø	23	11,8	14,5	24,5
6	14,6	1Ø7	38	46	3,6	17,1	3Ø,2
7	11,9	1Ø5	25	9	2,1	6,6	46,5
8	11,7	88	4Ø	352	Ø,9	2,4	5,8
9	14,6	91	23	22	1,7	3,4	59,8
1Ø	12,4	91	28	111	1,2	8,8	52,6
11	12,2	51	9	5	3,9	42,6	34,Ø
Mean(SD)	13,2(1,2)	79,4(21,Ø)	22,Ø(1Ø,4)				
# Geometric mean (± 1SD)				19,1(4,7-78)	4,1(2,5-13,3)	9,6(3,7-24,8)	36,7(17,7-76,1)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.10

Absorption of iron (3mg) as $FeSO_4.7H_2O$ from a rice meal (215g/subject) with and without 1mg malic acid, in fasting female subjects

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Rice meal	Rice + 1 g malic acid	Reference salt+
1	13,4	87	25	21	2,9	3,6	29,1
2	11,7	34	8	3	4,0	7,7	38,7
3	13,3	153	42	15	4,6	21,6	34,0
4	13,9	102	29	13	2,6	15,8	30,9
5	13,5	74	19	11	21,3	16,5	16,8
6	13,1	127	28	6	5,9	9,4	43,6
7	13,2	98	26	10	4,8	7,4	55,9
8	11,5	77	21	37	3,6	6,1	23,4
Mean(SD)	12,9(0,8)	94,0(34,8)	24,8(9,7)				
# Geometric mean (± 1SD)				11,5(5,4-24,6)	4,8(2,6-9,1)	9,5(5,3-16,9)	32,1(22,1-46,6)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.11

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100g cabbage puree with and without 1g calcium oxalate.

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Cabbage + 1g calcium oxalate	Cabbage	Reference salt+
1	12,4	46	10	4	29,0	50,5	88,6
2	13,9	120	25	17	08,0	20,5	55,3
3	13,2	147	38	25	23,8	59,9	26,9
4	11,7	61	14	9	32,5	23,2	107,5
5	13,1	134	26	12	35,3	44,3	70,6
6	13,9	117	31	26	08,7	16,8	41,8
Mean(SD)	13,0(0,9)	104,2(41,0)	24,0(10,4)				
# Geometric mean (± 1SD)					13,0(6,4-26,4)	19,5(10,3-37,0)	32,0(19,2-53,2) 58,9(35,4-98,1)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.12

The effect of 2g sodium phytate on the absorption of iron (3mg) from 100g broccoli puree in fasting female subjects

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*	
					Broccoli	Broccoli + 2 g sodium phytate
1	10,2	32	9	2	28,6	7,9
2	13,6	176	39	16	25,7	2,1
3	13,9	113	28	21	12,8	2,4
4	8,6	26	5	2	13,7	17,7
5	14,1	84	20	13	7,5	0,3
6	12,2	51	17	31	23,5	4,6
7	13,6	83	27	70	10,2	2,2
8	15,9	112	28	71	17,3	4,0
9	11,7	95	23	179	3,7	0,3
10	14,3	202	59	57	18,4	12,6
11	12,3	194	37	16	36,4	10,3
12	14,1	56	16	61	24,2	3,7
13	12,6	52	11	7	44,2	1,9
14	12,9	82	26	132	2,6	0,6
15	9,8	53	13	9	35,9	2,2
16	11,6	55	14	2	29,9	15,3
17	15,8	115	37	43	17,4	11,8
18	13,1	130	37	39	14,2	12,6
19	14,1	93	21	20	3,9	2,8
Mean(SD)	12,9(1,9)	94,9(51,6)	24,6(13,1)			
# Geometric mean(± 1SD)				23,3(7,0-77,1)	15,2(6,8-34,3)	3,5(0,9-12,2)

* No reference absorption studies were done and the absorption results are therefore uncorrected
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.13

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100g broccoli puree with and without 500mg tannic acid

Subject number	Haemoglobin (g/dl)	Serum iron (/ug/dl)	Transferrin saturation (%)	Serum ferritin (/ug/l)	Percentage absorption* Broccoli with 500mg tannic acid	Broccoli
1	13,2	96	25	23	6,0	42,9
2	10,3	87	33	29	0,1	18,2
3	13,2	90	23	36	1,9	3,9
4	13,1	127	40	25	1,1	20,3
5	11,4	78	18	5	0,1	36,7
6	14,2	101	22	26	6,3	37,1
7	12,9	128	34	56	2,0	37,3
8	13,6	87	24	18	2,3	27,9
Mean(SD)	12,7(1,3)	99,3(18,7)	27,4(7,4)			
+ Geometric mean (± 1SD)				23,0(11,4-46,5)	1,5(0,3-6,7)	25,7(13,0-51,3)

* No reference absorption studies were done and the absorption results are therefore uncorrected
\+ Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.14

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from butterbeans and wheatgerm

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Wheatgerm	Butterbeans	Reference salt+
1	16,3	114	27	104	0,2	0,6	28,0
2	10,1	45	9	4	1,1	0,8	58,4
3	12,3	110	25	12	8,0	1,1	37,7
4	8,3	65	14	5	1,1	1,7	75,6
5	15,7	87	20	11	1,4	2,6	71,6
6	13,4	85	27	111	0,4	1,5	15,9
Mean(SD)	11,0(4,8)	84,3(26,3)	20,3(7,5)				
# Geometric mean (± 1SD)					17,7(4,1-76,3)	0,7(0,3-1,4)	1,2(0,7-2,0)	41,8(22,8-76,7)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.15

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100g puree made from pumpkin and aubergine

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Aubergine	Pumpkin	Reference salt+
1	11,9	93	28	43	1,3	18,7	20,4
2	13,5	81	19	6	2,2	34,2	78,0
3	12,8	48	9	3	1,4	24,3	80,1
4	12,7	50	11	9	0,1	22,6	40,5
5	13,5	92	26	8	2,5	13,8	75,8
6	12,8	115	32	17	1,3	25,1	26,4
7	13,2	75	19	7	0,2	17,0	30,7
Mean(SD)	12,9(0,6)	79,1(24,1)	20,6(8,6)				
# Geometric Mean (± 1SD)				9,4(4,1-21,9)	0,7(0,2-2,6)	20.6(15,7-27,0)	43,9(24,8-77,9)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.16 The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from spinach

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Spinach	Reference salt+
1	12,6	84	23	94	1,2	36,1
2	14,2	87	32	56	1,7	14,2
3	13,5	136	39	16	4,0	41,7
4	14,4	100	29	68	3,0	23,9
5	12,5	111	29	74	1,4	42,1
6	13,7	58	20	47	0,3	20,9
7	13,5	127	36	35	0,8	23,8
Mean(SD)	13,5(0,7)	100,4(26,9)	29,7(6,7)			
# Geometric mean(± 1SD)				49,2(27,3-88,7)	1,4(0,6-3,2)	27,1(18,1-40,6)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.17

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100g puree made from green and brown lentils

Subject number	Haemoglobin (g/dl)	Serum iron (/ug/dl)	Transferrin saturation (%)	Serum ferritin (/ug/l)	Percentage absorption* Green lentils	Brown lentils	Reference salt+
1	10,2	36	10	2	4,5	4,2	25,6
2	11,4	68	26	42	0,2	0,1	56,6
3	14,9	113	28	40	1,5	2,1	79,7
4	12,5	78	16	3	16,5	13,5	21,4
5	12,9	119	33	43	2,5	2,4	04,4
6	12,8	74	16	2	9,6	10,4	43,2
7	13,8	79	32	23	4,7	1,5	24,9
8	13,9	126	46	36	4,6	2,4	100,8
9	9,3	32	7	2	6,4	6,3	64,6
10	10,5	51	12	2	1,3	0,8	49,0
11	11,3	58	16	2	4,7	4,4	24,5
Mean(SD)	12,1(1,7)	75,8(32,1)	22,0(11,9)				
# Geometric Mean (± 1SD)				7,7(1,7-34,1)	3,2(0,9-10,9)	2,4(0,5-10,5)	34,8(14,7-82,5)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg Fe as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5 Table 5.18 The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from beetroot greens

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Beetroot greens	Reference salt+
1	14,4	63	19	49	13,5	16,9
2	14,3	56	15	11	14,4	38,4
3	14,0	79	22	21	0,8	48,5
4	13,0	111	33	18	0,3	8,7
Mean(SD)	13,9(0,6)	77,3(24,5)	22,3(7,7)			
# Geometric mean (± 1SD)				21,2(11,4-39,6)	2,4(0,3-17,5)	22,9(10,4-50,3)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.19

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from carrot

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Carrot	Reference salt+
1	15,0	53	16	21	14,2	23,5
2	16,2	131	39	36	12,1	31,9
3	8,5	152	30	14	02,0	42,0
4	14,2	113	27	62	19,0	12,2
5	12,6	101	34	42	06,0	46,6
6	12,1	65	18	24	05,6	65,8
7	11,0	127	28	17	08,2	18,5
8	11,4	68	16	17	10,6	60,0
9	15,2	80	21	18	21,3	45,7
Mean(SD)	12,9(2,5)	98,9(34,3)	25,4(8,2)	24,7(14,9-40,7)		
# Geometric mean (± 1SD)					9,8(5,2-18,7)	33,9(19,3-59,7)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg Fe as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.20

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from potato

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Potato	Reference salt+
1	13,5	205	58	49	16,3	9,1
2	15,1	90	25	72	05,0	12,9
3	14,2	99	25	13	35,6	25,1
4	13,5	121	43	16	14,2	31,6
5	14,7	68	24	76	04,1	22,8
6	14,3	93	31	145	08,6	31,4
7	15,3	96	30	30	28,6	24,4
8	15,8	118	36	45	13,7	30,5
9	15,3	124	46	42	67,5	57,2
Mean(SD)	14,6(0,8)	112,6(38,9)	35,3(11,6)			
# Geometric mean(± 1SD)					42,6(19,9-91,0)	11,5(5,9-23,3) 24,2(14,2-41,3)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.21

Absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from beetroot

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Beetroot	Reference salt+
1	15,7	121	32	10	61,0	29,1
2	15,8	128	34	56	11,5	66,5
3	15,2	89	26	22	13,8	43,5
4	16,4	154	37	96	28,9	49,1
5	13,4	101	29	35	59,3	29,7
6	10,1	28	6	2	31,4	53,2
7	14,1	85	17	8	21,6	66,6
8	15,5	90	21	18	17,5	75,5
9	14,6	69	18	12	26,1	56,9
10	14,8	69	19	34	4,3	42,0
11	14,4	63	19	49	14,8	16,9
12	14,3	56	15	11	30,7	38,4
13	14,0	79	22	21	2,6	48,5
14	13,0	111	33	18	28,4	8,7
15	12,3	49	10	5	9,6	32,6
16	13,2	69	23	32	17,2	9,6
17	12,1	53	12	5	70,4	18,6
18	12,4	51	12	5	4,4	53,9
19	10,2	68	20	62	16,8	5,3
20	14,3	118	30	12	0,9	44,6
21	14,0	71	21	16	3,6	24,7
22	13,5	144	41	22	0,9	45,2
23	13,6	81	23	30	24,8	48,4
24	11,7	54	16	41	40,0	24,2
25	12,8	98	28	22	76,0	11,8
Mean(SD)	13,7(1,6)	84,0(31,6)	22,6(8,8)			
# Geometric mean(± 1SD)					18,1(7,2-45,4)	14,7(4,3-50,5) 31,3(15,5-63,2)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg Fe as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5 Table 5.22 The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from cauliflower

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Cauliflower	Reference salt+
1	13,7	340	56	8	28,0	10,0
2	14,4	81	22	2	36,4	44,1
3	16,0	155	42	30	26,1	9,5
4	13,8	95	25	29	18,8	8,9
5	12,3	49	10	5	25,3	32,6
6	13,2	69	23	32	38,8	9,6
7	12,1	53	12	5	77,2	18,6
8	12,4	51	12	5	19,1	53,9
9	14,3	118	30	12	35,9	44,6
10	14,0	71	21	16	2,9	24,7
11	13,5	144	41	22	13,3	45,2
12	13,6	81	23	30	29,8	48,4
13	11,7	54	16	41	18,5	24,2
14	12,8	98	28	22	92,5	11,8
15	13,2	152	35	110	8,4	22,3
16	15,2	80	25	38	4,9	17,6
17	14,5	129	27	31	27,0	38,8
18	11,3	120	36	34	31,5	28,9
19	15,2	86	21	27	12,3	46,2
20	15,5	156	36	76	17,1	32,6
Mean(SD)	13,6(1,3)	109,1(65,2)	27,1(11,5)			
# Geometric mean(± 1SD)				19,4(7,1-52,9)	21,3(9,4-48,5)	24,3(13,1-45,2)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.23 The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from tomato

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Tomato	Reference salt+
1	14,4	113	47	49	6,2	34,9
2	12,1	47	13	4	35,9	76,0
3	14,3	57	16	19	45,1	37,6
4	15,0	142	46	42	16,8	32,1
5	13,5	147	43	35	12,7	30,1
6	11,9	82	28	7	14,5	32,2
7	13,1	139	35	10	44,9	10,4
8	13,2	103	28	21	43,0	17,7
Mean(SD)	13,4(1,1)	103,8(38,8)	32,0(13,1)			
# Geometric mean (± 1SD)					17,3(7,0-42,6)	22,4(11,0-45,6) 29,5(16,5-52,6)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.24

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100g puree made from broccoli, served with and without its cooking water

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Broccoli without cooking water	Broccoli with cooking water	Reference salt+
1	13,7	104	32	17	7,5	16,9	40,6
2	12,2	56	11	6	9,7	24,0	18,6
3	13,6	118	33	8	22,9	28,9	35,7
4	13,8	84	24	53	5,4	18,7	14,9
5	15,4	98	25	83	5,7	10,5	24,2
6	14,6	85	26	59	14,3	41,0	20,5
7	13,7	87	24	50	15,8	47,7	16,7
8	14,4	114	32	91	8,8	18,4	40,3
Mean(SD)	13,9(0,9)	93,3(19,9)	25,9(7,1)				
# Geometric mean(± 1SD)				31,5(10,8-91,4)	9,8(5,6-17,1)	26,0(13,9-48,6)	24,6(16,4-36,9)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.25 The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 1ØØ g puree made from turnip

Subject number	Haemoglobin (g/dl)	Serum iron (/ug/dl)	Transferrin saturation (%)	Serum ferritin (/ug/l)	Percentage absorption* Turnip	Reference salt+
1	15,7	121	32	1Ø	56,4	29,1
2	15,8	128	34	56	28,Ø	66,5
3	15,2	89	26	22	26,8	43,5
4	16,4	154	37	96	33,9	49,1
5	13,4	1Ø1	29	35	75,1	29,7
6	1Ø,1	28	6	2	47,Ø	53,2
7	14,1	85	17	8	28,7	66,6
8	15,5	9Ø	21	18	24,9	75,5
9	14,6	69	18	12	38,8	56,9
1Ø	14,8	69	19	34	1Ø,1	42,Ø
Mean(SD)	14,6(1,8)	93,4(35,4)	23,9(9,5)			
# Geometric mean(± 1SD)					18,6(6,2-56,Ø)	32,7(19,2-55,6) 48,9(35,3-67,9)

* Individual results adjusted to a 4Ø% reference absorption

\+ 3 mg Fe as ferrous ascorbate given in the fasting state

\# Geometric mean and SD ranges used because values were positively skewed

Chapter 5
Table 5.26

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g puree made from sauerkraut

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*	
					Sauerkraut	Reference salt+
1	14,1	105	27	96	27,3	42,2
2	14,2	145	34	13	18,8	58,4
3	13,9	69	17	15	52,7	27,1
4	13,5	190	31	13	53,9	39,6
5	13,7	108	31	51	20,1	11,0
6	14,0	91	23	51	41,4	28,1
Mean(SD)	13,9(0,3)	118,0(43,2)	27,2(6,3)			
# Geometric mean(± 1SD)				29,3(12,3-70,0)	32,7(20,8-51,2)	30,6(17,2-54,3)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.3

The absorption in fasting female subjects of iron (3mg) as $FeCl_3.6H_2O$ from 200 g porridge made from whole grain sorghum and 54.9% pearled sorghum

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Whole sorghum	Pearled sorghum	Reference salt+
1	13,8	111	28	37	1,2	4,5	13,9
2	12,7	157	54	27	6,0	2,7	39,1
3	12,4	53	12	12	1,4	1,0	37,7
4	12,4	74	23	61	0,7	1,2	29,3
5	14,3	100	28	66	0,5	1,8	25,0
6	10,2	43	10	14	0,6	2,6	87,9
7	11,2	30	7	7	0,5	3,5	66,8
8	14,0	227	68	29	0,4	2,5	27,0
9	14,2	119	31	150	3,7	8,1	19,3
10	13,7	87	22	54	1,2	1,6	50,1
11	13,0	111	30	58	2,5	4,2	31,2
12	13,3	95	27	33	1,1	1,4	30,9
13	13,3	99	21	17	2,6	13,9	28,4
14	9,1	45	9	8	8,4	14,2	65,0
15	12,6	147	40	78	2,3	2,6	15,2
16	13,8	99	20	46	2,6	5,6	63,0
Mean(SD)	12,7(1,5)	99,8(49,3)	26,9(16,3)				
# Geometric mean(± 1SD)				31,7(13,3-75,3)	1,7(0,7-4,1)	3,5(1,5-8,1)	34,4(20,0-59,0)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.4

The absorption in fasting female subjects of iron (3mg) as $FeCl_3.6H_2O$ from 200g porridge made from whole grain and 54.9% pearled sorghum, with 9,5 mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Whole grain	Pearled sorghum	Reference salt+
1	13,1	132	31	8	1,3	2,8	79,6
2	13,6	65	16	10	2,2	11,5	38,7
3	13,9	64	18	35	3,6	6,4	33,1
4	15,6	124	33	27	1,4	1,8	86,5
5	15,2	74	19	35	10,9	19,5	34,0
6	16,0	131	36	71	3,2	8,9	32,2
7	12,9	59	15	32	0,2	9,1	40,9
8	12,5	106	33	120	2,1	4,5	18,3
9	13,8	71	18	12	1,3	1,9	50,9
10	13,5	96	30	30	1,5	4,8	53,6
11	15,3	104	39	54	5,9	10,0	21,4
12	13,6	75	16	7	15,4	17,7	55,2
Mean(SD)	14,0(1,2)	91,8(27,2)	25,3(9,1)				
# Geometric mean(± 1SD)					26,1(10,7-75,3)	2,4(0,7-7,3)	6,3(2,9-14,4)	41,1(25,7-65,7)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.5

The absorption in fasting female subjects of iron (3mg) as $FeCl_3.6H_2O$ from 200g porridge made from whole 'bird-proof' sorghum and whole albino sorghum with 9,5mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Bird-proof sorghum	Albino sorghum	Reference salt+
1	14,2	228	67	17	5,8	8,1	45,1
2	13,6	83	25	96	2,5	2,3	22,0
3	12,9	94	32	105	1,3	0,9	23,7
4	11,3	46	10	7	2,2	20,8	22,6
5	12,3	59	14	6	6,3	9,9	75,6
6	11,1	57	14	8	0,1	2,5	19,1
7	12,7	63	15	9	1,2	2,5	63,4
8	7,7	23	5	1	0,7	3,3	23,7
9	13,2	152	35	10	10,4	12,5	11,3
10	11,0	52	11	6	1,1	4,4	30,7
11	13,6	142	40	54	1,8	6,6	30,0
12	12,7	81	21	34	3,9	3,6	21,5
13	11,9	41	9	2	1,9	1,6	45,3
Mean(SD)	12,2(1,7)	86,2(56,7)	22,9(17,1)				
# Geometric mean(± 1SD)				12,1(2,9-48,5)	1,9(0,6-5,9)	4,3(1,8-10,3)	29,3(17,3-49,5)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.6

The absorption in fasting female subjects of iron (3mg) as $FeCl_3.6H_2O$ from 200g porridge made from pearled albino sorghum and its pearlings, with 9,5mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Albino pearlings	Pearled albino sorghum	Reference salt+
1	9,6	34	7	2	1,1	3,1	73,9
2	13,2	115	26	30	1,1	2,9	36,1
3	13,3	96	32	50	1,6	2,8	20,1
4	12,9	144	32	15	1,1	3,9	55,6
5	13,0	100	32	210	1,0	1,6	27,9
6	13,0	60	15	22	1,5	2,9	60,4
7	12,5	121	37	26	1,1	3,9	36,6
8	13,5	105	23	14	2,5	5,3	25,8
9	15,9	133	36	25	4,7	7,2	21,6
10	12,8	54	12	2	1,2	3,5	20,3
Mean(SD)	12,9(1,5)	96,2(36,0)	25,2(10,6)				
# Geometric mean(± 1SD)				18,1(4,5-72,6)	1,5(0,9-2,4)	3,5(2,3-5,1)	34,0(21,1-54,8)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.7

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 100 g broccoli puree with and without 2 g sodium phytate

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Broccoli	Broccoli with 2 g sodium phytate	Reference salt+
1	10,2	32	9	5	11,4	0,4	100,0
2	13,6	176	39	16	35,0	2,9	29,4
3	13,9	113	28	21	8,7	1,6	58,7
4	14,1	84	20	13	3,2	0,4	95,1
5	12,2	51	17	31	32,6	1,4	28,8
6	13,6	83	27	70	10,9	0,5	88,9
7	15,9	112	28	71	16,9	1,7	23,9
8	11,7	95	23	179	23,7	5,5	29,2
9	14,3	202	59	57	37,3	0,8	47,3
10	14,1	56	16	9	6,5	0,6	22,7
11	12,6	52	11	5	51,3	2,8	14,3
12	9,8	53	13	43	30,4	1,8	47,3
13	11,6	55	14	39	27,0	13,9	44,2
14	15,8	115	37	20	26,1	17,6	26,8
15	13,1	130	37	61	17,8	15,8	31,9
16	14,1	93	21	7	19,8	14,3	7,8
Mean(SD)	13,2(1,7)	93,8(47,0)	24,9(13,1)				
# Geometric mean(± 1SD)					25,0(8,7-71,3)	18,5(8,9-38,2)	3,7(0,9-16,0) 34,9(17,5-69,8)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.8

The absorption in fasting female subjects of iron (3mg) as $FeCl_3.6H_2O$ from 200 g porridge made from malted sorghum with 9,5mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Malted sorghum	Reference salt+
1	13,8	110	27	14	2,1	49,5
2	13,5	135	22	2	2,6	54,3
3	13,1	91	19	2	1,6	45,5
4	14,4	142	29	8	4,5	60,2
5	13,8	121	31	36	9,9	40,9
6	13,1	124	35	29	0,9	29,2
7	11,7	129	31	24	4,1	53,8
8	15,7	87	26	270	2,0	35,9
9	14,2	118	30	39	0,7	38,7
10	11,5	90	17	-	6,9	45,7
11	14,3	124	33	26	5,0	60,9
12	12,2	83	14	2	2,7	49,9
13	14,2	77	23	49	1,0	34,3
Mean(SD)	13,5(1,2)	110,1(21,8)	25,9(6,5)			
# Geometric mean(± 1SD)				16,1(3,6-72,5)	2,4(1,1-5,1)	45,0(35,9-56,4)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.9

The absorption in fasting female subjects of iron (5mg) as $FeSO_4.7H_2O$ from malted sorghum with 50mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*	
					Malted sorghum with 50 mg ascorbic acid	Reference salt+
1	15,5	94	16	-	11,2	71,9
2	15,4	80	21	-	5,2	8,9
3	15,4	102	35	-	13,5	33,3
4	15,9	184	91	-	15,0	44,3
5	14,4	162	47	-	6,3	44,2
6	15,1	140	36	-	20,3	60,9
7	13,7	92	27	-	25,2	34,4
8	10,2	112	22	-	11,7	72,2
9	20,4	157	42	-	11,3	12,2
10	10,2	30	7	-	15,6	8,5
11	13,4	84	18	-	5,0	35,5
12	13,6	134	22	-	2,2	10,4
13	14,9	113	39	-	8,9	23,1
14	10,3	65	12	-	16,7	15,0
15	14,1	183	47	-	8,7	64,5
16	13,5	84	22	-	6,2	13,7
17	11,0	74	15	-	23,6	83,5
18	13,1	111	36	-	2,1	80,6
Mean(SD)	13,9(2,5)	111,5(41,9)	30,8(19,3)			
# Geometric mean(± 1SD)					9,4(4,6-19,5)	30,5(13,5-68,7)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.10

Absorption in fasting female subjects of iron (5mg) as $FeSO_4.7H_2O$ from malted sorghum served with tea

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*	
					Malted sorghum with 50 mg ascorbic acid and tea	Reference salt+
1	13,6	134	22	1	6,6	71,9
2	14,9	113	39	2	12,1	8,9
3	10,3	65	12	74	5,6	33,3
4	14,1	183	47	22	2,9	44,3
5	13,5	84	22	16	1,8	44,2
6	11,0	74	15	2	4,3	60,9
7	13,1	111	36	2	1,7	34,4
Mean(SD)	12,9(1,6)	109,1(40,6)	27,6(13,2)			
# Geometric mean(± 1SD)				9,1(2,0-40,2)	4,0(2,0-8,2)	36,6(18,5-72,4)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg Fe as ferrous ascorbate given in the fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.11

The absorption of iron (3mg) as $FeSO_4.7H_2O$ from 200g oat porridge with and without 9,5 mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*	
					Oats	Oats and 9,5 mg ascorbic acid
1	12,7	105	26	20	6,1	42,1
2	13,4	91	25	27	0,1	0,6
3	11,4	46	12	11	1,3	15,7
4	14,8	115	36	38	0,5	2,6
5	15,0	69	23	42	1,5	5,2
6	13,8	110	35	10	0,7	5,4
7	15,0	84	25	36	0,7	4,7
Mean(SD)	13,7(1,4)	88,6(24,7)	26,0(8,0)			
+ Geometric mean(± 1SD)				23,0(12,7-41,6)	0,9(0,3-3,0)	5,7(1,5-21,3)

* No reference absorption studies were done and the absorption results are therefore uncorrected
\+ Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.12

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 200g white flour porridge with and without 6g bran

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* White flour	White flour + 6g bran	Reference salt+
1	12,8	45	14	40	14,5	5,0	36,5
2	12,6	86	31	72	9,0	5,8	18,0
3	11,9	33	9	12	13,2	2,8	133,0
4	12,9	56	14	16	8,7	6,1	10,0
5	11,7	59	19	20	10,3	2,4	29,5
6	13,4	84	22	7	11,7	4,1	73,5
7	13,5	108	32	39	18,6	4,2	16,8
8	13,0	40	10	2	8,9	6,0	65,9
Mean(SD)	12,7(0,6)	63,9(26,2)	18,9(8,9)				
# Geometric mean(± 1SD)					16,7(5,4-51,9)	11,6(8,8-14,9)	4,3(3,0-6,1)	34,1(14,8-78,5)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.13

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 5g cellulose and pectin

Subject number	Haemoglobin (g/dl)	Serum iron (/ug/dl)	Transferrin saturation (%)	Serum ferritin (/ug/l)	Percentage absorption* Pectin	Cellulose	Reference salt+
1	13,3	150	31	11	25,7	33,8	43,3
2	12,5	129	27	2	40,8	63,4	25,9
3	12,9	80	26	10	30,3	40,6	31,8
4	14,8	163	46	21	30,4	50,0	13,2
5	9,3	95	24	2	24,3	29,3	82,6
6	14,4	92	23	110	13,1	21,9	32,7
7	11,9	68	18	9	30,3	34,9	27,8
8	15,3	171	42	9	22,5	31,8	48,3
9	13,6	114	34	58	7,8	36,4	41,7
10	13,5	115	30	4	39,9	22,6	48,8
11	10,0	30	7	2	39,9	44,3	59,3
12	13,4	78	27	19	25,4	37,1	40,3
Mean(SD)	12,9(1,8)	107,1(41,6)	27,9(10,3)				
# Geometric mean(± 1SD)					10,4(2,8-38,5)	24,1(15,2-38,3)	35,6(26,3-48,2) 37,7(23,7-60,0)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.14

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 5g Guar gum and Pectin

Subject number	Haemoglobin (g/dl)	Serum iron (/ug/dl)	Transferrin saturation (%)	Serum ferritin (/ug/l)	Percentage absorption* Guar Gum	Pectin	Reference salt+
1	14,3	121	37	24	13,9	23,9	25,1
2	9,9	43	9	2	1Ø,2	98,3	34,5
3	12,9	148	4Ø	19	97,6	79,1	16,5
4	1Ø,5	49	12	2	21,9	34,6	48,7
5	13,1	18Ø	48	29	63,1	38,3	18,Ø
6	14,3	13Ø	42	1Ø7	3Ø,7	14,9	18,6
7	12,8	16Ø	54	37	51,1	32,2	34,Ø
8	14,Ø	65	18	14	71,6	46,8	29,4
9	12,3	93	33	11	32,3	29,1	14,4
1Ø	12,4	145	5Ø	55	12,2	13,8	7,4
Mean(SD)	12,6(1,5)	113,4(48,3)	34,3(16,1)				
# Geometric mean(± 1SD)					16,8(4,6-61,5)	39,2(18,6-82,8)	34,2(18,4-63,5) 21,8(12,7-37,6)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.15

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 2,5 g Gum tragacanth and 5 g Cellulose

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Gum tragacanth	Cellulose	Reference salt+
1	14,7	68	19	38	47,9	66,2	13,5
2	14,6	97	23	15	5,3	22,7	32,4
3	14,9	105	26	27	25,6	11,8	21,5
4	13,6	105	29	42	15,1	17,6	44,0
5	15,2	126	41	30	24,3	41,1	32,1
6	12,9	130	33	5	38,2	15,3	23,0
7	16,1	107	27	20	19,2	29,5	37,1
8	15,4	164	40	17	89,4	69,0	16,4
Mean(SD)	14,7(10,1)	112,8(28,0)	29,8(7,8)				
# Geometric mean(± 1SD)					20,6(10,5-40,8)	25,1(10,8-58,2)	28,2(14,6-54,7) 25,6(17,0-38,7)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg Fe as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.16

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 5g Hemicellulose and Cellulose

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* Hemicellulose**	Cellulose	Reference salt+
1	14,5	110	23	86	1,5	13,1	15,1
2	12,7	103	29	11	22,9	63,0	13,3
3	12,5	92	24	13	10,0	12,4	40,6
4	12,9	125	36	15	3,2	38,1	13,2
5	9,8	45	9	01	2,6	27,9	21,9
6	11,0	100	31	80	7,5	6,9	19,6
Mean(SD)	12,2(1,6)	95,8(27,3)	25,3(9,3)				
# Geometric mean(± 1SD)				12,8(1,6-100,1)	5,3(1,9-14,3)	20,5(9,0-46,7)	18,9(12,4-29,0)

**Mucilose (Winthrop Laboratories, New York)
* Individual results adjusted to a 40% reference absorption
+ 3 mg iron as ferrous ascorbate given in fasting state
# Geometric mean and SD ranges used because values were positively skewed

Chapter 6
Table 6.17

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 250 mls milk with and without 10 g cocoa

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption*		
					Milk	Milk + 10 g cocoa	Reference salt+
1	13,4	130	36	51	6,5	2,1	39,1
2	13,8	105	24	18	13,6	5,4	53,0
3	16,3	125	33	16	13,0	4,3	26,5
4	11,4	64	21	15	7,7	4,5	42,8
5	14,2	119	30	53	1,4	6,1	31,9
6	15,1	117	31	7	10,7	4,1	89,4
7	13,0	66	19	10	12,3	3,9	23,3
8	10,6	70	21	81	1,4	0,7	127,3
Mean(SD)	13,5(1,8)	99,5(28,2)	26,3(7,4)				
# Geometric mean(± 1SD)				22,5(9,4-54,2)	6,3(2,5-15,6)	3,5(1,7-7,4)	45,9(25,6-82,8)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 7
Table 7.2

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 50g SOJ DIAT 7B without ascorbic acid and from 50g Lactogen containing 20mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* SOJ DIAT 7B	Lactogen	Reference salt+
1	13,3	109	31	141	0,2	1,4	-
2	13,2	123	27	84	0,9	3,2	10,6
3	14,6	125	29	26	3,0	23,1	22,1
4	13,9	109	33	19	1,0	0,9	33,6
5	13,3	88	20	9	2,0	5,7	14,2
6	15,7	167	49	135	0,5	0,7	-
7	13,7	115	28	36	1,0	7,8	-
8	12,4	149	40	13	4,7	9,6	38,5
9	14,2	128	30	55	5,0	8,2	22,0
10	12,7	107	25	3	1,4	8,7	40,1
11	11,6	64	13	6	2,7	24,3	25,7
12	12,9	71	15	4	2,1	21,3	-
13	12,2	63	11	8	1,9	15,8	18,6
Mean(SD)	13,4(1,1)	109,1(31,4)	27(11)				
# Geometric mean(± 1SD)					2,4(5,6-74,8)	1,5(0,6-3,7)	6,9(1,9-25,3)	23,0(14,7-36,1)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 7
Table 7.3

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 50g of SOJ DIAT 7B and Lactogen, both with 20 mg of ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* SOJ DIAT 7B	Lactogen	Reference salt+
1	11,4	109	31	9	0,2	2,4	33,6
2	13,0	79	24	45	0,4	3,0	14,3
3	14,1	102	28	16	0,5	1,7	-
4	12,0	101	26	9	14,3	13,2	84,4
5	12,0	89	24	10	7,0	5,5	50,5
6	14,0	104	26	23	2,3	4,5	23,7
7	11,4	76	24	32	1,4	1,2	-
8	10,0	30	6	1	11,2	16,5	54,2
9	11,8	87	27	39	1,0	3,6	-
10	10,3	33	7	4	13,3	13,1	60,5
11	12,3	64	18	25	2,5	3,0	15,9
12	11,3	59	15	6	9,2	24,1	24,1
Mean(SD)	12,0(1,3)	77,8(26,7)	21,3(8,1)				
# Geometric mean(± 1SD)					12,1(4,0-36,2)	2,4(0,5-11,0)	5,3(1,9-14,6)	34,0(18,2-63,5)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 7
Table 7.4

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 50 g SOJ DIAT A and Lactogen, both with 40 mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* SOJ DIAT A + 40 mg ascorbic acid	Lactogen + 40 mg ascorbic acid	Reference salt+
1	13,4	108	39	29	4,9	28,7	8
2	10,8	41	9	3	26,8	50,0	58
3	11,6	123	31	11	6,5	4,7	27
4	13,1	158	42	37	3,6	7,1	29
5	13,2	135	39	40	7,9	21,8	42
6	15,3	128	32	31	5,7	21,8	44
7	12,7	100	24	35	7,2	46,6	53
Mean(SD)	12,9(1,3)	113,3(36,9)	30,9(11,4)				
# Geometric mean(± 1SD)					20,5(7,9-53,3)	7,2(3,8-13,6)	19,5(7,9-47,9)	32,2(16,4-63,2)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 7
Table 7.5

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 50g SOJ DIAT 7B with and without 20mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* SOJ DIAT 7B	SOJ DIAT 7B + 20mg ascorbid acid	Reference salt+
1	12,3	59	20	52	5,4	6,5	-
2	12,7	72	22	40	4,7	9,1	-
3	13,1	69	20	14	2,8	1,0	16,0
4	13,5	98	30	46	0,8	1,5	26,0
5	11,2	51	13	8	3,9	5,6	42,0
6	10,1	62	14	4	1,2	0,9	30,0
7	13,1	103	33	52	0,8	1,5	36,0
8	12,0	89	24	62	1,1	1,8	38,0
9	11,2	78	18	14	9,9	8,0	39,0
10	8,6	35	8	6	3,4	12,7	-
11	12,3	127	31	25	2,9	5,2	-
Mean(SD)	11,8(1,5)	76,6(26,2)	21,2(7,9)				
# Geometric mean(± 1SD)				20,6(7,8-54,6)	2,6(1,1-5,7)	3,4(1,3-8,8)	31,0(22,2-43,5)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg Fe as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 7
Table 7.6

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 50g SOJ DIAT A with and without 40mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* SOJ DIAT A	SOJ DIAT A with 40mg ascorbic acid	Reference salt+
1	11,6	48	14	55	0,7	3,8	42,0
2	12,9	95	21	14	3,5	23,5	86,0
3	12,2	109	35	41	0,5	2,9	22,0
4	13,1	90	29	36	0,3	3,7	20,0
5	13,8	117	26	22	1,3	6,3	57,0
6	12,0	122	48	61	0,7	2,1	14,0
7	13,2	111	30	103	0,5	1,7	33,0
8	12,5	152	47	28	0,1	2,6	24,0
9	13,2	122	29	17	0,9	13,7	82,0
Mean(SD)	12,7(0,7)	107,3(28,5)	31(11,0)				
# Geometric mean(± 1SD)					34,8(18,0-66,0)	0,62(0,2-1,7)	4,5(1,9-10,9)	35,0(18,5-67,0)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

Chapter 7
Table 7.7

The absorption in fasting female subjects of iron (3mg) as $FeSO_4.7H_2O$ from 50g SOJ DIAT A with 40 mg and 80 mg ascorbic acid

Subject number	Haemoglobin (g/dl)	Serum iron (µg/dl)	Transferrin saturation (%)	Serum ferritin (µg/l)	Percentage absorption* SOJ DIAT A + 40 mg ascorbic acid	SOJ DIAT A + 80 mg ascorbic acid	Reference salt+
1	11,9	80	21	17	1,4	1,8	29
2	13,0	135	37	24	1,7	1,4	13
3	7,8	30	6	1	23,6	37,9	102
4	13,9	88	24	76	8,2	5,6	7
5	12,9	118	36	26	2,9	1,9	31
6	13,1	106	30	35	5,6	6,8	30
7	10,2	47	9	2	19,8	22,2	36
8	11,7	91	27	37	8,5	13,4	14
9	12,5	80	24	35	2,2	2,8	7
10	12,1	117	27	6	20,8	26,1	102
11	13,1	101	25	34	0,6	1,3	37
12	12,9	95	23	25	5,8	2,1	46
Mean(SD)	12,1(1,6)	90,7(29,5)	24,0(9,0)				
# Geometric mean(± 1SD)				16,4(4,5-6,0)	4,9(1,6-15,8)	5,2(1,5-17,9)	35,0(18,0-70,0)

* Individual results adjusted to a 40% reference absorption
\+ 3 mg iron as ferrous ascorbate given in fasting state
\# Geometric mean and SD ranges used because values were positively skewed

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