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Iron deficiency and overload in relation to nutrition

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ABSTRACT

Nutritional iron intake in the Netherlands has been reviewed with respect to both iron deficiency and iron overload.
In general, iron intake and iron status in the Netherlands is adequate and therefore no change in nutrition policy is required.
The following aspects and developments, however, need to be emphasized.
Iron is an essential element of nutrition and is required for oxygen transport, cognitive development in children and several other processes and enzyme activities in the body.
The prevalence of iron deficiency is probably much lower than currently assumed. The general overestimation of iron deficiency is caused both by the incorrect comparison of the recommended daily intake with iron intake data and by the use of insufficiently specific diagnostic tests to determine real iron deficiency.
Iron overload is, on the contrary, more common than generally thought. About 10% of the general population is heterozygote for the C282Y mutation which can result in hemochromatosis, a hereditary iron overload disease. Iron overload is an established risk factor for cardiovascular disease. Recently, C282Y-heterozygotes have been suggested to be at higher risk, especially heterozygous men (> 20 years) and women (> 50 years).
In addition, unabsorbed intestinal iron has been suggested to increase the risk of colon cancer.
Iron supplementation or fortification of iron in functional foods should be avoided and discouraged until the risks of iron overload have been more clearly determined, since in the general population iron overload is associated with increased risk of several chronic diseases as well.

SAMENVATTING

De ijzer inneming via de voeding in Nederland is geëvalueerd waarbij zowel ijzertekort als ijzerstapeling in de beschouwing zijn betrokken. De gemiddelde dagelijkse ijzerinname en de ijzerstatus in Nederland lijken voldoende, zodat geen reden bestaat het voedingsbeleid t.a.v. ijzer te veranderen.
De volgende aspecten en ontwikkelingen verdienen echter de aandacht.
IJzer - een essentieel spoorelement - is nodig voor zuurstoftransport, cognitieve ontwikkeling van kinderen en verschillende enzymatische processen.
De prevalentie van ijzerdeficiëntie is waarschijnlijk veel lager dan momenteel wordt aangenomen. De oorzaak van de algemene overschatting van ijzerdeficiëntie is enerzijds de incorrecte vergelijking van de aanbevolen dagelijkse hoeveelheden met de gemiddelde dagelijkse inname en anderzijds het gebruik van minder geschikte laboratoriumtests voor het vaststellen van echte ijzerdeficiëntie.
De prevalentie van ijzerstapeling is groter dan momenteel wordt aangenomen. Ongeveer 10% van de bevolking is heterozygoot voor de C282Y mutatie die kan leiden tot hemochromatose, een erfelijke ijzerstapelingsziekte. IJzerstapeling is een bewezen risicofactor voor hart- en vaatziekten. Recent is aangetoond dat met name C282Y heterozygote mannen (vanaf 20 jaar) en vrouwen (vanaf 50 jaar) een sterk verhoogd risico hebben.
Een hoge concentratie niet-geabsorbeerd ijzer in de darm geeft mogelijk een verhoogde kans op darmkanker.
IJzersupplementatie aan voedingsmiddelen is hierdoor niet aan te raden totdat de risico's van ijzerstapeling zijn vastgesteld, aangezien ijzerstapeling ook in de algemene bevolking geassocieerd is met een verhoogd risico op verschillende chronische ziekten.

CONTENTS

1. INTRODUCTION

In a first report in this project "Health promoting compounds in nutrition", the risks of overdosing several active components in food supplements were considered (Siemelink et al. 2000). It was concluded that high iron intake may be toxic. As a result, the iron status in the general population was questioned because of the possible positive association between iron and several chronic diseases, the disease hereditary hemochromatosis and the contradictory point of view on widespread iron deficiency.
Therefore, the Directorate for Public Health of the Ministry of Health, Welfare and Sport initiated a detailed study of the nutritional iron status in the Netherlands in which aspects of both iron deficiencies and iron overload were taken into account.
The present report reviews the international literature with special emphasis on the situation in the Netherlands.

2. IRON ABSORPTION AND METABOLISM

Iron absorption is restricted to the villi of intestinal mucosa. Absorption rates of these cells depend merely on the iron homeostasis. A short-term increase in dietary iron is not absorbed as the mucosal cells possess already accumulated iron and will not increase additional uptake. As body iron stores decrease, the mucosa is signalled to moderately increase iron absorption. In response to a further decrease of iron the erythroid cells will signal the mucosa to increase iron absorption more significantly. By these mechanisms iron uptake is mainly regulated by the bodies' requirement (Andrews 1999, Bothwell 1995, Provan 1999, Uchida 1995).
Iron balance is primarily determined by iron absorption. No significant excretion mechanism of iron is present in humans. The uncontrolled excretion of iron occurs in the form of epithelial desquamation, perspiration or blood loss and is estimated at 1-1.5 mg per day (men and postmenopausal women: 0.5-1.5 mg per day; premenopausal women: 1-2.5 mg per day). Unabsorbed iron leaves the body through the feces (Crichton 1991, Anderson and Powell 1999, Griffiths et al. 1999, Nederlandse Voedingsnormen 1999, Crawford 1999, Edwards and Kushner 1993).

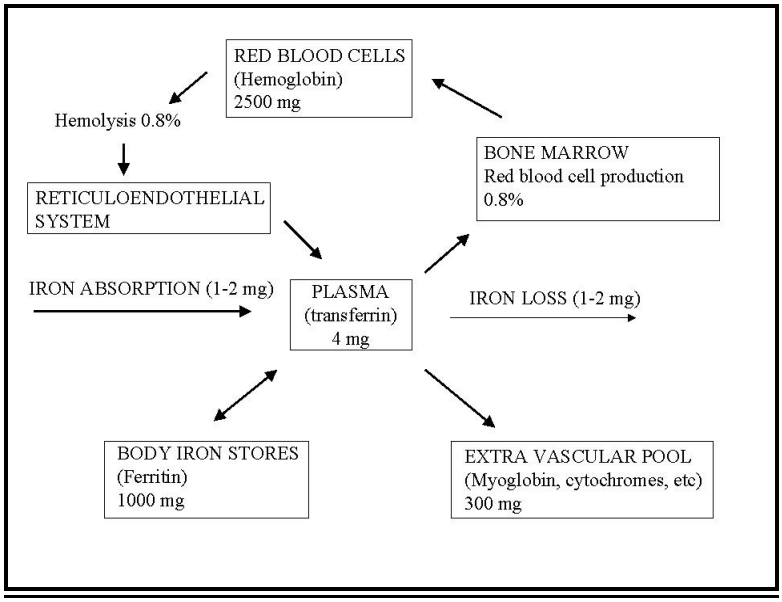

Figure 1: Daily turnover of iron in normal human adults

A well-balanced diet contains sufficient iron to meet body requirements. Based on iron turnover studies, the average required absorption has been estimated at 1-1.5 mg per day. As mean dietary bioavailability of iron was shown to be 10-15%, the recommended daily intake (RDA) has been established after extensive evaluation at a mean of 10 mg in 1989 by the Dutch Food Council (Nederlandse Voedingsnormen 1999). However, dietary iron absorption depends on body requirements, resulting in a low absorption rate since only a low amount of iron is required. If necessary, efficiency of iron absorption is increased to 20% or more during rapid growth periods in childhood and adolescence and during menstrual blood loss and pregnancy (Andrews 1999, Bothwell 1995, Provan 1999, Uchida 1995). The Dutch Health Council will reconsider the RDA for iron next year.
Important iron sources are bread, meat and meat products. Heme iron (red meat) is absorbed more easily than inorganic or non-heme iron (bread, cereals, rice and vegetables). Heme iron (predominantly Fe^{2+}) is 15-35% bioavailable whereas non-heme iron (mostly Fe^{3+}) is

absorbed for 2-20% (Sandstead 2000, Reddy et al. 2000, Nederlandse Voedingsnormen 1999).

Iron is predominantly associated with oxygen transport and oxidative conversions. The human body contains 40-50 mg iron/kg body weight. Most of the iron (about 30 mg/kg) is present in hemoglobin in circulating red blood cells. About 6 mg/kg is found in muscle cells as myoglobin and in various tissues and enzymes - including cytochromes, oxidases and peroxidases - as heme proteins. In addition, the biosynthesis of deoxyribonucleotides being precursors of DNA depends on iron containing enzymes such as ribonucleotide reductases (Fontecave 1999).
A small proportion of the body iron circulates in plasma bound to transferrin. In a normal situation transferrin is occupied by iron for 30%. If this percentage increases non-transferrin bound iron (NTBI) increases as well, resulting in increased oxidative stress levels (Cabantchik et al. 1999, de Valk et al. 1999). The remaining iron is stored in the liver, spleen, bone marrow and muscle in ferritin (Crichton 1991, Nederlandse Voedingsnormen 1999). In short, ferritin is an iron binding protein and is the main storage form of iron, transferrin is an iron transport protein in which iron circulates in the body.

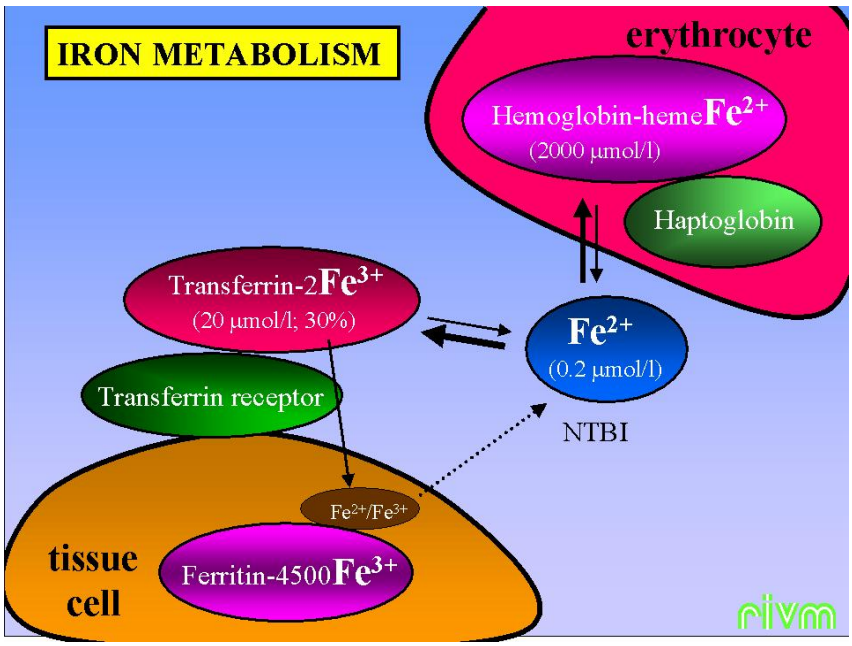

Figure 2: Iron metabolism in normal individuals

Iron uptake interferes with copper, zinc and manganese absorption and selenium bioavailability. A high iron content decreases the uptake of copper, zinc, manganese and selenium. High levels of copper and zinc, on the other hand, lower the iron absorption. Calcium, ascorbic acid, phytic acid and tea also influence iron absorption. Calcium, phytic acid and tea decrease iron uptake, whereas ascorbic acid increases iron absorption (De Sterke 1999, Meltzer and Alexander 2000, Reddy et al. 2000, Nederlandse Voedingsnormen 1999). However, recent clinical evidence shows that the combined effect of meal components has little impact on iron absorption (Reddy et al. 2000).

3. IRON DEFICIENCY

3.1 SYMPTOMS

Fatigue is the most common complaint of iron deficiency, along with paleness, physical uncomfort, decreased physical endurance and work capacity, sensitivity to cold, restless legs syndrome and decreased activity of the immune system.
The onset of iron deficiency is iron depletion in which iron stores are decreased without biochemical or clinical effects. Further decrease of iron stores results in iron deficiency, in which hemoglobin and ferritin levels are below normal and clinical signs are present. Finally, iron deficiency anemia, characterized by decreased iron contents in bone marrow, may be developed.

3.2 PREVALENCE

Prevalence of iron deficiency in developed countries has drastically declined in the past 15-20 years. Moreover, population based studies show that iron deficiency is now relatively uncommon (Niederau et al. 1998, Looker 1997, Borch-Iohsen and Trygg 1997), even among pregnant women (Roeser et al. 1999). In addition, as a result of public health programs in the past decades (nutritional advice, emphasis on breastfeeding and delayed introduction of cow's milk) and iron fortification of infant foods, prevalence of iron deficiency in children in Western countries nearly disappeared (Jonsson et al. 1991, Oski 1993, Nelson et al. 1993 and 1994, Cohen 1999). However, due to language or cultural barriers, prevalence of iron deficiency in children of ethnic groups may be slightly higher compared to children of the general population (Nelson et al. 1994, Cohen 1999).

It is difficult to estimate the prevalence of iron deficiency since the commonly used methods - both comparison of dietary intake data with the RDAs and laboratory diagnostic tests - are less suitable.
RDAs can only be indicative for the average required iron intake, since efficiency of dietary iron absorption varies and is adjusted to body requirements. It is therefore incorrect to assume that a mean daily intake below the RDA reflects iron deficiency, as is suggested in the study of Van Ooik et al. (1996) in which 63% of the study population (N=78) did not meet the RDA. If mean intake data from the Dutch Food Consumption Surveys (VCP 1998) are compared with RDAs, it is concluded that among certain subgroups (boys aged 1-3 and 13-18, women aged 1-3 and 10-49) iron intake is below the RDA. The authors conclude that the real significance of this finding can only be assessed after further biochemical investigation (Lowik et al. 1998).
Commonly used laboratory tests are not sufficiently specific to determine real iron deficiency and result in overestimation of iron deficiency prevalence. Prevalence in the Netherlands based on these methods have been reported from 0% (males, 22-55 y), 5% (females, >50 y), 11% (males, >50 y) to 16% (females, 22-50 y) (Brussaard et al. 1997). In other countries similar prevalences based on the same parameters were reported. When parameters of iron status are combined, the prevalence decreases substantially from 0.9% (men) and 4.1% (women) to 0.0% and 1.3%, respectively (Jonsson et al. 1991). The most conclusive laboratory test results in a much lower prevalence of iron deficiency as described by Roeser et al. 1999 for pregnant women. Based on ferritin data the prevalence was 16.4% whereas the

transferrin receptor test showed a prevalence of 0.9% iron deficiency. These observations have been summarized in Table 1.
Prevalence of iron deficiency in developing countries is still very high and may be attributed to poor dietary intake, parasite infections and malabsorptions (Iron Panel Australia, Crawford et al. 1999).

Table 1: Prevalence of iron deficiency in various countries based on routine laboratory methods

Study:	Sub group:	Prevalence of iron deficiency (%) based on:					Cut off values:
		Hemoglobin	Ferritin	Hemoglobin and ferritin	Hemoglobin, ferritin and TS	TfR	
Brussaard et al. 1997 (Netherlands, N=75)	F 20-49 F 50-69 M 20-49 M 50-79	5 8 1 5	16 5 0 11				Ferritin <10 ng/ml (F 20-49), <20 ng/ml (F 50-69, M 20-79) Hemoglobin <7.5 mmol/l (F 20-69); <8.1 mmol/l (M 20-79)
Roeser et al 1999 (Australia, N=175)	pregnant		16.4			0.9	Ferritin <10 µg/l; TfR >8 µg/ml
Looker et al 1997 (U.S., N=24894)	1-2 y 3-11 y M 12-69 y F 12-19 y F 20-49 y F 50-69 y				3 <1 <1 2-3 5 2		Hemoglobin <120 g/l; Ferritin <12 µg/l; TS <10-15%
Niederau et al 1994 (Germany, N=3012)	M F		0.5 6.0				Ferritin <12 µg/l
Nelson et al 1993 (U.K., N=399)	M 12-14 y F 12-14 y		0.7 3.8				Ferritin <12 µg/l
Jonsson et al 1991 (Iceland, N=1887)	M urban M rural F urban F rural	1.0 0.9 4.1 1.2			0.2 0.0 1.3 0.0		TS <16%; Ferritin <12 µg/l; Hemoglobin <130 g/l (M), <120 g/l (F)
Milman et al 1998 (Denmark, N=268)	F 18-30 y		9.7	2.2			Ferritin <13-16 µg/l; Hemoglobin <121 g/l
Milman et al 1999 (Denmark, N=1332)	M		0.45	0.15			Ferritin <13-16 µg/l; Hemoglobin <129 g/l

3.3 DIAGNOSIS AND TREATMENT

The major misunderstanding concerning iron deficiency is the assumption that low hemoglobin levels reflect low iron status (Iron Disorders Institute 1998, Crawford et al. 1999, Nelson et al. 1994, Roeser et al. 1999, De Sterke 1999). Low hemoglobin, low transferrin saturation (TS) and low serum iron and a high total iron binding capacity (TIBC) may indeed reflect a low iron status. However, the presence of chronic inflammation or even a simple infection results in the same values and is called anemia of chronic disease (ACD). In this immune regulated process, the body withholds iron from malignant cells, bacteria and viruses which need iron to grow or survive (Iron Disorders Institute 1998, Sempos et al. 1996, Weinberg 1999, Weiss 1999). As a result the iron is withdrawn from the circulation and stored into body tissues bound to ferritin.
A specific parameter to determine real iron deficiency is the soluble serum transferrin receptor (sTfR), which is elevated in iron deficient individuals (Roeser et al. 1999, Iron Disorders Institute 1998, Ferguson et al. 1992, North et al. 1997, Punnonen et al. 1997,

Provan 1999). The most conclusive test remains the determination iron contents in bone marrow which is, however, very invasive. The best current method for the diagnosis of real iron deficiency is the determination of red cell parameters, particularly the mean corpuscular volume (MCV) and serum ferritin combined with the determination of sTfR.
In practice, physicians may diagnose iron deficiency merely on hemoglobin values alone. As a result, prevalence of iron deficiency is generally overestimated. Hemoglobin determination remains a suitable tool (fast, cheap and easy) for a first screening, since an individual with an optimal hemoglobin value has no iron deficiency.

More misconceptions about iron deficiency occur. If iron deficiency is diagnosed - whether correctly or not - physicians often prescribe iron supplementation, sometimes without further investigation of the real cause of the iron deficiency (Iron Disorders Institute 1998, Crawford et al. 1999). Menstrual blood loss and poor dietary intake are often presumed to be the cause. Several studies show, however, that general infections, inflammation or gastrointestinal disease, including intestinal inflammation (e.g. celiac disease) and malignoma are very common (Niederau et al. 1998, Iron Panel Australia, Crawford et al. 1999, Kepczyk et al. 1999, Provan 1999, Uchida 1995). In older persons (>70 years) malignoma is a major cause of iron deficiency (Iron Panel Australia, Izaks et al. 1999). Supplementation of iron may be more harmful than beneficial under these circumstances. Firstly, body tissues which already contain excess iron will be loaded additionally with iron and secondly parasites or infections are supplied with enough iron to restore its pathological action.

Diseases associated with Anemia of Chronic Disease

Acute Infections	**Inflammatory Disorders**
Bacterial, fungal or viral	Rheumatoid Disease
	Systemic Lupus Erythematosus
Chronic Infections	Burns
Osteomyelitis	Severe Trauma
Infective Endocarditis	Acute and Chronic Hepatitis
Pelvic Inflammatory Disease	
Chronic Urinary Tract Infection	**Malignancy**
Tuberculosis	Carcinoma
Chronic Fungal Disease	Myeloma
	Lymphoma
Chronic Renal Failure	Leukemia

Therefore, further investigation of patients with iron deficiency may be of important benefit. Only for individuals correctly diagnosed with iron deficiency, iron supplementation can be probably recommended. The pharmaceutical formulations, however, have not been tested in a clinical setting as usual. Therefore the safety of high dosed iron supplementation (up to 600 mg/day) therapy is questionable in view of the risks of excess iron intake.
If iron deficiency is indeed caused by a diet low in iron, it is reasonable to assume that other micronutrients are deficient as well and a better diet should be recommended rather than supplementation (Crawford et al. 1999).

3.4 COMPLICATIONS

An optimal iron status is necessary for the development of cognitive and psychomotor development in children (Sandstead 2000, Scholl and Reilly 2000, Lozoff et al. 1991). Low iron uptake increases cadmium and lead absorption, possibly resulting in an increased heavy

metal load. Furthermore, iron is necessary for the production of DNA synthesizing enzymes and many other iron-containing or iron-dependent enzymes.
Low maternal hemoglobin or ferritin values early in pregnancy are associated with an increased risk of preterm delivery. Low iron status later in gestation is not associated with risk of adverse pregnancy outcome. In fact, a fall in hemoglobin and other iron parameters during the third trimester of pregnancy is due to normal physiological expansion of maternal plasma volume (Scholl and Reilly 2000).

4. IRON OVERLOAD

4.1 SYMPTOMS

Since no active excretion mechanism of iron exists, iron overload is caused by increased dietary iron absorption. Hereditary hemochromatosis (primary hemochromatosis) is characterized by excess iron uptake from a diet with normal iron contents. At present two major mutations have been characterized in the human gene for hemochromatosis (HFE), C282Y and H63D (Feder et al. 1996). The homozygote C282Y carriers have a higher prevalence to develop severe sympoms. An important cause of secondary hemochromatosis - in addition to multiple blood transfusions and thalassemia - is a long-term diet high in iron.
Symptoms of iron overload and hemochromatosis include chronic fatigue, arthritis, arthritic pain in finger joints, loss of libido or impotence, infertility, changes in skin color, heart arrhythmia, elevated blood sugar, diabetes and elevated levels of liver enzymes. Since iron builds up slowly, symptoms may be absent until the age of 40-50 years. But even without any symptoms, organ damage due to iron overload is already induced (Gezondheidsraad 1999, Swinkels and Marx 1999, Iron Disorders Institute 1998).

Symptoms of acute iron overload in children (an overdose of iron supplement pills) are nausea, vomiting, diarrhea, gastrointestinal bleeding and – more severe - shock, coma or death. Even children without immediate symptoms may develop gastrointestinal obstruction and liver damage. Iron poisoning has already resulted in 110,000 incidents since 1986, including 33 deaths, and is the leading cause of childhood poisoning (FDA 1997).

4.2 PREVALENCE

Recent epidemiological evidence indicates that iron overload is relatively common in Western populations (Niederau et al. 1998). A prevalence of 20% was found in a cohort of Danish men (N=1332) (Milman et al. 1999).

Hereditary hemochromatosis is the most common autosomal recessive genetic disease in populations of Northern European origin. Physicians usually fail to recognize hemochromatosis because the symptoms are rather non-specific and the disease was, until recently, believed to be rare. Therefore, patients are often diagnosed with diabetes, heart failure or liver disease (Gezondheidsraad 1999, Swinkels and Marx 1999, Iron Disorders Institute 1998).

Table 2: Prevalence of C282Y and H63D heterozygosity and homozygosity in various countries (YY and DD are homozygotes; CY and HD are heterozygotes; YCHD are compound heterozygotes)

Country	N	% YY	% CY	% DD	% HD	% YCHD	Reference
USA (white)	1450	0.4	8.9	3.5	23.9	2.4	McDonnell et al. 1999
USA (white)	193	0	14.0	3.6	23.3	1.0	Beutler et al. 1996
USA	142	0.7	14.7	2.8	23.2	3.5	Barton et al. 1997
USA (jewish)	381	0	2.6	1.6	16.3	0	Beutler et al. 1997
Canada	5211	0.3	7.1				Cassanelli et al. 1999
Australia	3011	0.5	14.1			2.2	Olynyk et al. 1999
Australia	502	1	12.4				Alanen et al. 1999
Australia	3011	0.5	12				Rossi et al. 1999
Denmark	219	0.7	13.7	0	12.3	0	Merryweather et al. 1999
S-Norway	505	0.4	14.9	1.4	20.2	2.2	Distante et al. 1999
Celtic	719	0.4	13.0				Lucotte et al. 1998
Nordic	379	1.3	10.3				Lucotte 1998
Anglo-sax	353	0.3	11.3				Lucotte 1998
N-Ireland	404	1.2	17.3		20.8		Ryan et al. 1998
S-Ireland	109	0	28.4		24.7		Borot et al. 1998
GB (S-Wales)	10556	0.7	15.2		30.6	2.4	Jackson et al. 2000
Jersey	411	1.0	11.4	2.9	20.9	3.2	Merryweather et al. 1998
France	254	0.8	13.8	2.4	23.6	3.5	Jezequel et al. 1998
France (Brittany)	1000	0.5	12				Jouanolle et al. 1998
France (Brittany)	1640	0.5	15				Jouanolle et al. 1999
N-Germany	157	1.3	9.6		23.5		Nielsen et al. 1998
Iceland	231	0.2	6.5	0.4	19.4	1.7	Merryweather et al. 1999
Faeroes	187	0.26	6.4	3.2	18.2	1.6	Merryweather et al. 1999
Greenland	200	0.05	4.5	0	9.0	0	Merryweather et al. 1999
Spain	485	0.2	4.1	4.1	33.8	1.4	Sanchez et al. 1998
Italy	2000	0	3.2		21.6		Cassanelli et al. 1999
Austria	271	0.4	7.4	3.0	19.9	0	Datz et al. 1997
S-Europe	534	0	5.1				Lucotte 1998
Russia	85	0	3.5				Lucotte 1998
Netherlands [exptd]		*0.5*	*10*	*3*	*22*	*2*	

The prevalence of hemochromatosis homozygosity is estimated at 0.5% whereas heterozygosity in the Netherlands is estimated at 10% of the general population (Swinkels and Marx 1999, De Sterke 1999, Slingerland et al. 1997) (see last row of Table 2).

4.3 DIAGNOSIS AND TREATMENT

An individual with iron overload has elevated serum ferritin and/or elevated transferrin saturation levels. Laboratory tests used for these determinations are commonly available and easy to perform. Fasting blood samples (12 hours or overnight) are recommended because serum iron parameters are influenced by diet (Niederau et al. 1998). Even more important is the determination of markers for infection or inflammation (Sempos et al. 1996, Beaton et al. 1989). When bacteria, viruses, harmful or malignant cells are present, the immune system decreases available body iron stores, resulting in elevated levels of ferritin, the iron storage source (Iron Disorders Institute 1998, Weinberg 1999). A patient may be incorrectly diagnosed with high iron levels if not corrected for these markers.

The only treatment of iron overload (and hemochromatosis) is removal of the excess iron by blood donation, called venesection or phlebotomy therapy (Gezondheidsraad 1999, Swinkels and Marx 1999, De Sterke 1999, Iron Disorders Institute 1998). Treatment is most effective when blood is removed in an early stage. This may well be before the onset of clinical symptoms.
Preferably, patients diagnosed with high iron levels and their first-degree relatives are genetically screened for the hemochromatosis mutation (Gezondheidsraad 1999, Swinkels and Marx 1999). The laboratory test is commonly available and easy to perform. In fact, screening of the whole population for the C282Y mutation is now being discussed, as an early detection of predisposition for iron overload has important public health benefits (Gezondheidsraad 1999, Cogswell et al. 1998).

4.4 COMPLICATIONS

4.4.1 NUTRIENT IMBALANCES

The amount of iron absorption influences the absorption ratio of other nutrients. Increased iron uptake results in a decreased absorption of zinc, copper and manganese. In addition, high iron absorption decreases selenium bioavailability (Meltzer and Alexander 2000).

4.4.2 ORGAN DAMAGE

In principle, organ damage due to excess iron intake is a problem of the general population but hemochromatosis homozygotes for the C282Y mutation are predominantly at risk of iron overload. About 20-50% of women and 50-70% of men who are homozygous will finally develop specific organ damage due to iron overload. Excess iron is stored in different organs including liver, heart and gallbladder, leading to liver cirrhosis and cancer, cardiovascular disease and possibly diabetes, respectively.
Individuals who are heterozygous for the C282Y mutation have significantly increased iron stores, as reflected in high serum ferritin and/or high transferrin saturation levels (Roest et al. 1999, Niederau et al. 1998, Olynyk et al. 1999, Distante et al. 1999, De Valk and Marx 1999, Bulaj et al. 1996, Rossi 2000, Merryweather-Clarke et al. 1998).
Increased iron status has been resulted in cardiovascular disease - including myocardial infarction and atherosclerosis - and to a lower extent in carcinogenesis. Hemochromatosis heterozygosity seems to be associated with diabetes (Salonen et al. 2000, Niederau 1999). The development of liver cirrhosis subsequently leading to liver cancer due to iron overload is evident (Gezondheidsraad 1999, Swinkels and Marx 1999, Iron Disorders Institute 1998).

- CARDIOVASCULAR DISEASE

In Appendix 1 the important epidemiological literature has been reviewed concerning iron status and risk of cardiovascular disease. The relative risks have been depicted in Figure 3. From these data it may be concluded that an increased serum ferritin level is an important independent risk factor for cardiovascular events. Particularly the most recent studies are well conducted and clearly show a positive association between iron status and cardiovascular disease (Roest et al. 1999, Klipstein et al. 1999, Tuomainen et al. 1999, Tuomainen et al. 1998, Kiechl et al. 1997, Meyers et al. 1997).

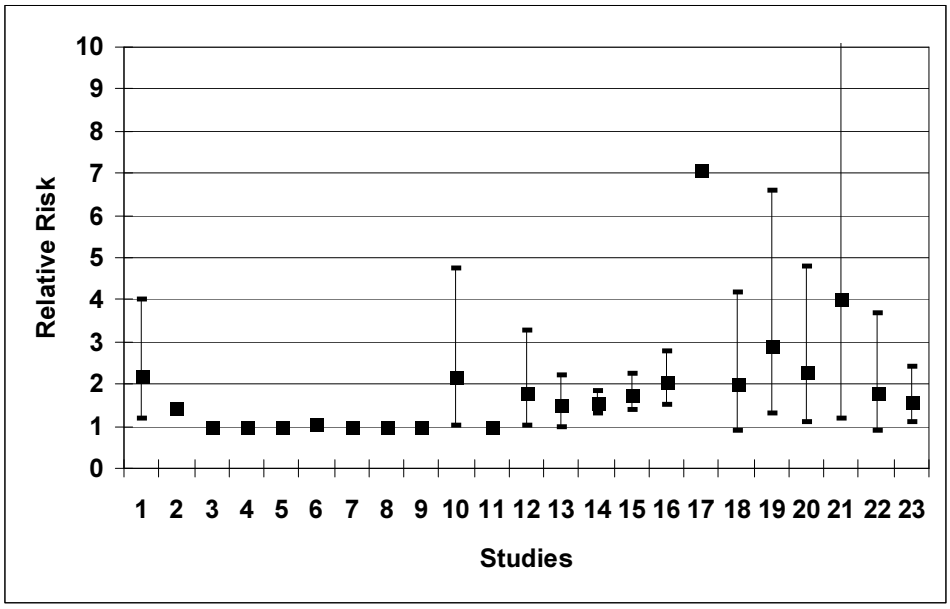

Figure 3: Relative risks (with 95% confidence limits) of cardiovascular diseases and iron intake or iron parameters (See Appendix for details)

Salonen et al (1992) published the first study that investigated the association between cardiovascular events and iron status. Men with serum ferritin levels of 200 μg/l or more were shown to have a 2.2 fold increased risk of myocardial infarction compared to men with serum ferritin levels below 200 μg/l. This increase was stronger in men with elevated serum LDL cholesterol. Roest et al (1999) showed that risk of cardiovascular death in women was increased significantly in heterozygotes compared to wild types (RR=1.6, adjusted for age, smoking, hypertension and obesity). This increased risk was particularly present (18.9 fold) in heterozygous individuals who were hypertensive and smoked compared to wild type non-hypertensive non-smokers. The study of Klipstein et al (1999) showed an increased risk (1.81) of myocardial infarction for serum ferritin levels of 200 μg/l or more compared to serum ferritin levels lower than 200 μg/l. Tuomainen et al (1999) investigated whether occurrence of the C282Y mutation was associated with an increased risk of first acute myocardial infarction in healthy men aged 42-60 years. A 2.3-fold risk of heterozygous men was shown. Tuomainen et al (1998) also found that the ratio between serum transferrin receptor (TfR) and ferritin was 28.6% lower among cases of acute myocardial infarction compared to controls. A low TfR/ferritin ratio is indicative for a high iron status. Low TfR/ferritin resulted in an increased risk (2.9 fold) of acute myocardial infarction compared to a high TfR/ferritin ratio, after adjustment for the known risk factors.
Moreover, regular blood donors had a substantially decreased risk (RR=0.14) of acute myocardial infarction compared to non-donors, indicating that periodic removal of iron may lower cardiovascular risk. Kiechl et al (1997) investigated the association between carotid atherosclerosis and serum ferritin levels in a prospective study. A strong association (OR=2.05) was present between ferritin and atherosclerosis progression through modification of the LDL cholesterol potential.
The mechanism by which iron overload probably functions is by inducing radical damage (oxidative stress). Iron catalyzes the oxidation of LDL, which is an important factor in the initiation and progression of atherosclerosis (De Valk and Marx 1999). In addition, iron

directly interacts with human platelet activity (Pratico et al. 1999), indicating that more mechanisms may exist.

- CANCER

Prolonged iron overload results in liver cirrhosis thereby increasing the risk of liver cancer substantially (Iron Disorders Institute 1998, Swinkels and Marx 1999, Gezondheidsraad 1999, De Sterke 1999). An association between nutritional iron intake and liver cancer has not been established.
Literature concerning iron overload and intestinal and colon cancer is less clear. Both animal and human studies indicate a positive correlation between excess iron intake and colorectal oncogenesis (Weinberg 1994). However, few epidemiological studies have been conducted (Harrison et al. 1997) and recent animal experiments gave inconsistent results (Rezazadeh et al. 1997, Lai et al. 1997). Furthermore, a high dietary iron intake increases the number of nuclear aberrations in the colon and decreases manganese superoxide dismutase (MnSOD) activity in rat colonic mucosa (Kuratko 1998). Since excess iron uptake reduces manganese absorption (the trace element required for MnSOD activity), this decrease in enzyme activity may be attributed to the lower availability of manganese (Kuratko 1998, Borrello et al. 1993). MnSOD is one of the important antioxidant enzymes that protects mitochondria from oxygen radical damage and a decreased MnSOD activity has been suggested to be involved in human carcinogenesis as well.
In a nested case-control study, serum ferritin was shown to be inversely correlated to the risk of colorectal cancer. When combined with a high fat diet, high iron intake increased colorectal cancer risk (Kato et al. 1999). Iron supplementation resulted in a drastically increased amount of fecal iron and nearly doubled the production of free radicals (Lund et al. 1999). A high iron intake in humans can therefore be a possible factor in the prevalence of colon cancer.

5. IRON INTAKE IN THE NETHERLANDS

According to the Dutch food consumption survey (VCP 1998), overall iron intake is 10.8 mg per day whereas the recommended daily allowance is 9 mg for males and 15 mg for females (see Table 4a). Iron intake is sufficient in all subgroups except for women aged 12-50 years. In this subgroup, mean intake ranges from 9 to 11 mg whereas 11 to 15 mg is recommended. This may implicate that premenopausal women have a suboptimal iron status (Van Ooik et al. 1996, Brussaard et al. 1997, Lowik et al. 1998). No evidence exists, however, to support this conclusion. In fact, when correct laboratory tests are used, it may be concluded that iron deficiency is relatively uncommon in developed countries. Particularly iron deficiency due to dietary factors seems to be rare. In conclusion, still more research is required to estimate the real prevalence of both iron deficiency and iron overload in order to determine the optimal daily intake in all subgroups.

At present, iron supplementation during pregnancy is being discussed. The association between iron deficiency in early gestation and adverse pregnancy outcome is well known. However, higher values of iron status during the third trimester (hemoglobin >105-130 g/l, ferritin >40 ng/l) are associated with increased risk of fetal death, preterm delivery and low birth weight as well. Although controlled trials of iron supplementation during pregnancy indeed showed increased maternal hemoglobin values at delivery, supplementation did not result in a decreased risk of preterm delivery or low birth weight (Scholl and Reilly 2000). Moreover, high iron intake may increase oxidative damage. Therefore, the advantages and risks of iron supplementation during pregnancy have to be investigated.

If not breastfed, infants aged 0-½ years need iron fortification which is common in formula. Lactating women possibly need iron supplementation due to increased requirements.

In the last 10 years, no trend is seen in iron intake in the Netherlands, although iron intake in males aged 13-22 years and females aged 13-16 years seem to decline in the past 10 years (Table 3). Additional statistical analysis is necessary to confirm this trend (VCP 1998).

Table 3: Iron intake in the past 10 years in the Netherlands (VCP 1998)

Age group:	Males			Females		
	VCP 1 1988	VCP 2 1992	VCP 3 1998	VCP 1 1988	VCP 2 1992	VCP3 1998
1-4	6.1	5.5	6.1	5.8	5.3	5.8
4-7	7.8	7.5	7.0	7.0	6.6	6.6
7-10	8.9	8.6	8.4	8.2	7.7	7.8
10-13	10.3	9.9	9.9	9.6	8.7	9.1
13-16	11.7	11.0	10.9	10.4	9.2	9.0
16-19	12.9	11.9	11.5	9.9	9.7	9.9
19-22	13.5	12.8	12.2	10.1	10.0	9.5
22-50	13.1	13.0	13.0	10.6	10.5	10.7
50-65	12.3	12.9	12.9	10.2	10.5	10.7
65+	11.7	11.7	11.4	9.7	9.9	10.1

6. IRON SUPPLEMENTATION AND FORTIFICATION: CURRENT POLICY

About 10% of the Dutch population use multi-vitamin/mineral preparations. The iron contents of these supplements were not determined (VCP 1998) but usually range between 9 and 15 mg per daily dose. Daily intake of substantial amounts of iron for longer periods may result in a iron overload situation, depending on the absorption efficiency of the iron complex in the preparation.

Iron fortification at the level of the recommended daily allowance (up to 14 mg) per daily intake in foods is presently allowed (Warenwetbesluit Toevoeging micro-voedingsstoffen aan levensmiddelen 1996). In infant food, iron supplementation is also allowed, even without giving the amount added on packaging (Warenwetbesluit Produkten voor bijzondere voeding 1992).

Functional foods such as iron-fortified cereal products are generally used in substitution for bread, which is an important iron source. This may implicate that iron-fortified foods have little adverse effects for iron-overloaded individuals, although this obviously depends on the amount added. In the U.S., Kellogg's and General Mills cornflakes contain 31 mg and 60 mg iron per 100 g product, respectively. Supplementation of 7 mg iron per 100 g breakfast product would already result in twice the mean daily intake (Johansson 2000).

In the Netherlands, many iron-fortified products, particularly cereal products consumed by both children and adults, have already been introduced (Compendium Dieetproducten en Voedingsmiddelen 1999/2000). Iron contents in these products vary from 7.5 to 100 mg per 100 g (e.g. Cereal Bloedwijn 7.5mg/100ml, Liga Brunchy 8mg/100g, Liga Switch 8mg/100g, Cereal Fruity Bar 8.1mg/100g, Liga Glutenex 20mg/100g, De Ruijter Roosvicee Ferro 100mg/100ml). For comparison, iron contents of bread is 1.0 (white) to 2.8 (whole-wheat) mg per 100 g (NEVO 1993).

Recently, an iron-fortified milk (Campina/Melkunie Junior Plus, 1.75 mg per 100 ml) was introduced in the Netherlands. Consumption during 8 weeks was shown to result in a small but significant increase of ferritin (Scholz-Ahrens et al. 2000). The authors only put emphasis on a possible iron deficiency and omitted to mention possible adverse effects of iron overload.

Further introduction of new iron-enriched food products can cause a general exceeding of the recommended daily intake in the population, at least in some age groups. As the health effects of high iron intake are at least very suspected from public health point of view, this development must be questioned until further scientific evidence becomes available.

7. CONCLUSIONS AND RECOMMENDATIONS

The mean daily intake of iron in the Netherlands seems to be adequate for the majority of the population.

The present prevalence of iron deficiency in the Netherlands is probably much lower than generally assumed.

In certain subgroups (children and adolescents in rapid growth periods and lactating women) iron fortification may possibly be needed. However, more research is required to estimate the real prevalence of iron deficiency in these subgroups.

A low hemoglobin concentration does not necessarily reflect a low iron status or iron deficiency. Therefore, the diagnosis 'iron deficiency' based on only a hemoglobin test may not be correct. The determination of the transferrin receptor - in combination with red cell parameters and ferritin - is currently the best method to diagnose real iron deficiency.

The prescription of iron medication or supplementation is only recommended after exclusion of anemia due to chronic diseases, general infections and gastrointestinal disorders, which are common causes of low hemoglobin levels.

Iron accumulation due to genetic disorders in the Netherlands is more common than generally assumed. The prevalence of homozygote carriers of the C282Y gene of hemochromatosis is estimated at 0.5% and heterozygote carriers at 10% of the general population.

Iron overload is an established risk factor for cardiovascular disease. Particularly men (>20 years) and postmenopausal women may be at risk. The hemochromatosis heterozygotes have an increased risk for cardiovascular disease as well, particularly in combination with other risk factors.

A high unabsorbed iron concentration in the intestinal tract is possibly associated with an increased risk of colon cancer. The increased risk of liver cancer in hemochromatosis homozygotes is evident.

At present, the currently available data do not support radical changes in dietary recommendations with respect to iron intake. Further studies are advised to investigate whether a decrease in the daily intake of iron, particularly in men aged 20-50 years, must be aimed for.

Presently, iron supplementation of food products should be avoided because of the low prevalence of iron deficiency and the associations between iron overload and several chronic diseases. Further studies are required to investigate these relationships. Especially the hemochromatosis homozygotes, but also the heterozygotes (which constitute about 10% of the general population in the Netherlands) should be protected from excess iron intake.

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REFERENCES

Alanen A et al
Abstract Book of BioIron '99, Sorrento, Italy 1999, Abstract 240

Anderson GJ and Powell LW
Haemochromatosis and control of intestinal iron absorption
Lancet 353(9170) 1999

Andrews NC
Disorders of iron metabolism
N Engl J Med 341: 1986-1995 1999

Asperen IA van, et al
Body iron stores and mortality due to cancer and ischemic heart disease: a 17-year follow-up study of elderly men and women
Int J Epidemiol 24(4): 665-670 1995

Barton JC et al
Genetic and clinical description of hemochromatosis probands and heterozygotes: evidence that multiple genes linked to the major histocompatibility complex are responsible for hemochromatosis
Blood Cells Mol Dis 23(1): 135-145 1997

Beaton GH, Corey PN and Steele C
Conceptual and methodological issues regarding the epidemiology of iron deficiency and their implications for studies of the functional consequences of iron deficiency
Am J Clin Nutr 50: 575-588 1989

Beutler E et al
Mutation analysis in hereditary haemochromatosis
Blood Cells Mol Dis 22: 187-194 1996

Beutler E and Gelbart T
HLA-H mutations in the Ashkenazi Jewish population
Blood Cells Mol Dis 23(1): 95-98 1997

Borch-Iohnsen B
Risk evaluation of iron
Institute for Nutrition Research – University of Oslo – Norway 2000: 16 pp

Borch-Iohnsen B and Trygg K
Past and present: iron status and iron intake among adults in Norway
Scand J Nutr 41: 125-128 1997

Borot N
Mutations in the MHC class I-like candidate gene for hemochromatosis in French patients
Immunogenetics 45(5): 320-324 1997

Borrello S, DeLeo M and Galeotti T
Defective gene expression of MnSOD in cancer cells
Molec Aspects Med 14: 253-258 1993

Bothwell TH.
Overview and mechanisms of iron regulation
Nutr Rev 53: 237-245 1995

Brussaard JH et al
Iron intake and iron status among adults in the Netherlands
Eur J Clin Nutr 51: S51-S58 1997

Bulaj ZJ et al
Clinical and biochemical abnormalities in people heterozygous for hemochromatosis
N Engl J Med 335: 1799-1805 1996

Cabantchik ZI et al
Physiological and pathological properties of labile forms of iron inside and outside mammalian cells
Abstract Book of BioIron '99, Sorrento, Italy 1999, 17

Cassanelli S et al
Abstract Book of BioIron '99, Sorrento, Italy 1999, Abstract 249

Cogswell ME et al
Iron overload, public health and genetics: evaluating the evidence for hemochromatosis screening
Ann Intern Med 129: 971-979 1998

Cohen AR
Choosing the best strategy to prevent childhood iron deficiency
JAMA 281: 2247-2248 1999

Compendium Dieetproducten en Voedingsmiddelen 1999/2000
27e editie 1999/2000
Bohn Stafleu Van Loghum Houten/Diegem

Corti MC, Gaziano M and Hennekens CH
Iron status and risk of cardiovascular disease
Ann Epidemiol 7: 62-68 1997

Crawford R
New perspectives on iron deficiency
Abstract Book of BioIron '99, Sorrento, Italy 1999

Crichton RR
'Inorganic biochemistry of iron metabolism'
Ellis Horwood 1991

Datz C et al
Predominance of the HLA-H Cys282Tyr mutation in Austrian patients with genetic haemochromatosis
J Hepatol 27(5): 773-779 1997

Distante S et al
High prevalence of the hemochromatosis-associated Cys282Tyr HFE gene mutation in a healthy Norwegian population in the city of Oslo, and its phenotypic expression
Scand J Gastroenterol 34(5): 529-534 1999

Distante S et al
High prevalence of C282Y HFE gene mutation in a healthy population in Norway
Abstract Book of BioIron '99, Sorrento, Italy 1999

McDonnell SM et al
Population-based screening for hemochromatosis using phenotypic and DNA testing among employees of health maintenance organizations in Springfield, Missouri
Am J Med 107(1): 30-37 1999

Edwards CQ and Kushner JP
Screening for hemochromatosis
N Engl J Med 328: 1616-1620 1993

FDA
Press Release: "Warnings Required on Iron-Containing Drugs and Supplements"
Jan. 15, 1997
http://www.fda.gov/opacom/campaigns/3iron.html August 2000

Feder JN et al
A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis
Nat Genet 13: 399-408 1996

Ferguson B et al
Serum transferrin receptor distuinguishes the anemia of chronic disease from iron deficiency anemia
J Lab Clin Med 119: 385-390 1992

Fontecave M
'4th Winter Research Conference on Iron Metabolism and Free Radicals'
Valloire 1999

Frey GH and Krider DW
Serum ferritin and myocardial infarct
W V Med J 90: 13-15 1994

Gezondheidsraad
Vroege opsporing van ijzerstapelingsziekte
Publication no. 1999/21

Gillum RF et al
Serum transferrin saturation, stroke incidence and mortality in women and men
Am J Epidemiol 144: 59-68 1996

Griffiths WJH, Kelly AL and Cox TM
Inherited disorders of iron storage and transport
Molec Med Today 5: 431-438 1999

Harrison et al
The role of dietary factors in the intestinal and diffuse histologic subtypes of gastric adenocarcinoma: a case-control study in the U.S.
Cancer 80: 1021-1028 1997

Iron Disorders Institute U.S.
www.irondisorders.org August 2000

Ironpanel Australia
www.ironpanel.org.au August 2000

Izaks GJ, Westendorp RGJ and Knook DL
The definition of anemia in older persons
JAMA 281: 1714-1717 1999

Jackson HA et al
Br J Haematol 108(S1): 47 2000

Jezequel P et al
Allele frequencies of hereditary hemochromatosis gene mutations in a local population of West Brittany
Hum Genet 102: 332-333 1998

Johansson L
Norwegian food and nutrition policy - Enrichment
National Council on Nutrition and Physical Activity - Norway - 2000

Jonsson JJ et al
Prevalence of iron deficiency and iron overload in the adult Icelandic population
J Clin Epidemiol 44(12): 1289-1297 1991

Jouanolle AM et al
Prevalence of the C282Y mutation in Brittany: penetrance of genetic hemochromatosis?
Ann Genet 41(4): 195-198 1998

Jouanolle AM et al
Abstract 254 BioIron 1999

Kato I et al
Iron intake, body iron stores and colorectal cancer risk in women: a nested case control study
Int J Cancer 80: 693-698 1999

Kepczyk MT et al
A prospective multidisciplinary evaluation of premenopausal women with iron-deficiency anemia
Am J Gastroenterol 94(1): 109-115 1999

Kiechl S et al
Body iron stores and the risk of carotid atherosclerosis
Circulation 96: 3300-3307 1997

Klipstein-Grobusch K et al
Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam study
Am J Clin Nutr 69(6): 1231-1236 1999

Kuratko CN
Decrease of manganese superoxide dismutase activity in rats fed high levels of iron during colon carcinogenesis
Food Chem Toxicol 36: 819-824 1998

Lai C et al
Non-promoting effects of iron from beef in the rat colon carcinogenesis model
Cancer Lett 112: 87-91 1997

Liao Y, Cooper RS and McGee DL
Iron status and coronary heart disease: negative findings from the NHANES I epidemiologic follow-up study
Am J Epidemiol 139: 704-712 1994

Looker AC et al
Prevalence of iron deficiency in the U.S.
JAMA 277: 973-976 1997

Lowik MRH et al
Changes in the diet in the Netherlands: 1987-88 to 1992
Int J Food Sci Nutr 49 Suppl 1: S5-S68 1998

Lozoff B et al
Long-term developmental outcome of infants with iron deficiency
N Engl J Med 325: 687-6694 1991

Lucotte G
Celtic origin of the C282Y mutation of hemochromatosis
Blood Cells Mol Dis 24(4): 433-438 1998

Lund EK et al
Oral ferrous sulfate supplements increase the free radical-generating capacity of feces from healthy volunteers
Am J Clin Nutr 69: 250-255 1999

Magnusson MK et al
Low iron-binding capacity as a risk factor for myocardial infarction
Circulation 89: 102-108 1994

Meltzer HM and Alexander J
The scientific basis for the Norwegian regulation of fortification of foods with vitamins and minerals
National Institute of Public Health - Norway - 2000

Merryweather-Clarke AT et al
A retrospective anomynous pilot study in screening newborns for HFE mutations in scandinavian populations
Human Mutation 13: 154-159 1999

Merryweather-Clarke AT et al
The effect of HFE mutations on serum ferritin and transferrin saturation in the Jersey population
Br J Haematol 101(2): 369-373 1998

Meyers DG et al
Possible association of a reduction in cardiovascular events with blood donation
Heart 78: 188-193 1997

Milman N et al
Iron status in Danes updated 1994
Ann Hematol 78(9): 393-400 1999

Milman N et al
Iron status in 268 Danish women aged 18-30 years
Ann Hematol 77 (1-2): 13-19 1998

Morrison HI et al
Serum iron and risk of fatal acute myocardial infarction
Epidemiology 5(2): 243-246 1994

Nassar BA et al
Relation of HFE gene mutations, high iron stores and early onset coronary artery disease
Can J Cardiol 14(2): 215-220 1998

Nederlandse Voedingsnormen
Voedingscentrum 1989 and 1999 (1st and 2nd edition, respectively)

Nelson M, White J and Rhodes C
Haemoglobin, ferritin and iron intakes in British chidren aged 12-14 years: a preliminary investigation
Br J Nutr 70(1): 147-155 1993

Nelson M, Bakaliou F and Trivedi A
Iron deficiency anemia and physical performance in adolescent girls from different ethnic backgrounds
Br J Nutr 72(3): 427-433 1994

Voorlichtingsbureau voor de Voeding
NEVO Tabel: Nederlands Voedingsstoffenbestand 1993

Niederau C et al
Screening for hemochromatosis and iron deficiency in employees and primary care patients in Western Germany
Ann Intern Med 128: 337-345 1998

Niederau C
Diabetes mellitus in hemochromatosis
Z Gastroenterol suppl !: 22-32 1999

Nielsen P et al
Prevalence of the C282Y and H63D mutations in the HFE gene in patients with hereditary haemochromatosis and in control subjects from Northern Germany
Br J Haematol 103(3): 842-845 1998

North M et al
Serum transferrin receptor levels in patients undergoing evaluation of iron stores: correlation with other parameters, and observed versus predicted results
Clin Lab Haematol 19: 93-97 1997

Olynyk JK et al
A population-based study of the clinical expression of the hemochromatosis gene
N Engl J Med 341: 718-724 1999

Van Ooik A et al
Duplicaat 24-uurs voedingen 1994 - ijzerinname -
RIVM rapport nr. 515004001 1996

Oski FA
Iron deficiency in infancy and childhood
N Engl J Med 329(3): 190-193 1993

Pratico D et al
Iron-dependent human platelet activation and hydroxyl radical formation
Circulation 99: 3118-3124 1999

Provan D
Mechanisms and management of iron deficiency anaemia
Br J Haematol 105 Suppl 1: 19-26 1999

Punnonen K et al
Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency
Blood 89: 1052-1057 1997

Reddy MB, Hurrell RF and Cook JD
Estimation of nonheme-iron bioavailability from meal composition
Am J Clin Nutr 71: 937-943 2000

Rezazadeh H and Athar M
Evidence that iron-overload promotes DMBA-induced skin tumorigenesis in mice
Redox Rep 3: 303-309 1997

Roeser HP et al
The use of serum transferrin receptor measurements to assess iron status during pregnancy
Abstract Book of BioIron '99, Sorrento, Italy 1999

Roest M et al
Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular death in women
Circulation 100: 1268-1273 1999

Rossi E et al
Compound heterozygous hemochromatosis genotype predicts increased iron and erythrocyte indices in women
Clin Chem 46(2): 162-166 2000

Rossi E et al
Abstract Book of BioIron '99, Sorrento, Italy 1999, Abstract 257

Ryan E, O'keane C and Crowe J
Hemochromatosis in Ireland and HFE
Blood Cells Mol Dis 24(4): 428-432 1998

Salonen JT et al
High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men
Circulation 86: 803-811 1992

Salonen JT, Tuomainen TP and Kontula K
Role of C282Y mutation in haemochromatosis gene in development of type 2 diabetes in healthy men: prospective cohort study
Brit Med J 320: 1706-1707 2000

Sanchez M et al
Prevalence of the Cys282Tyr and His63Asp HFE gene mutations in Spanish patients with hereditary hemochromatosis and in controls
J Hepatol 29(5): 725-728 1998

Sandstead HH
Causes of iron and zinc deficiencies and their effects on brain
J Nutr 130: 447S-449S 2000

Scholl TO and Reilly T
Anemia, iron and pregnancy outcome
J Nutr 130: 443S-447S 2000

Scholz-Ahrens K et al
IJzer toegevoegd aan melk is biologisch beschikbaar
Voeding Nu 6: 16-17 2000

Sempos CT et al
Body iron stores and the risk of coronary heart disease
N Engl J Med 330(16): 1119-1124 1994

Sempos CT, Looker AC and Gillum RF
Iron and heart disease: the epidemiologic data
Nutr Rev 54: 3 1996

Sempos CT and Looker AC
Iron status and the risk of coronary heart disease
Nutr Metab Cardiovasc Dis 9: 294-303 1999

Siemelink M, Jansen EHJM, Piersma AH and Opperhuizen A
Active components in food supplements
RIVM report 650250 001, April 2000.

Slingerland RJ, Van der Graaf F and Vader HL
Een ijzersterk verhaal?! Een niewe test op hemochromatose
Medisch Journaal St Joseph Ziekenhuis Veldhoven 26(4): 136-138 1997

Sterke PHL de
Hemochromatose
In press 1999

Swinkels DW and Marx JJM
Diagnostiek en behandeling van primaire hemochromatose
Nederlands Tijdschrift voor Geneeskunde 143(27): 1404-1408 1999

Symons MCR and Gutteridge JMC
Free radicals and iron: Chemistry, biology and medicine
Oxford Science Publications 1998

Tuomainen TP et al
Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene C282Y mutation: a prospective cohort study in men in eastern Finland
Circulation 100: 1274-1279 1999

Tuomainen TP et al
Association between body iron stores and the risk of acute myocardial infarction in men
Circulation 97: 1461-1466 1998

Valk B de and Marx JJM
Iron, atherosclerosis and ischemic heart disease
Arch Intern Med 159: 1542-1548 1999

Valk B et al
Non-transferrin-bound iron is present in plasma of subjects with heterozygous hereditary hemochromatosis and normal plasma transferrin saturation
Abstract Book of BioIron '99, Sorrento, Italy 1999, Abstract 262

Voedingscentrum, Den Haag
Zo eet Nederland. Resultaten van de Voedselconsumptiepeiling (VCP) 1997-1998 (1998)

Uchida T
Overview of iron metabolism
Int J Hematol 62: 193-202 1995

Weinberg ED
Iron loading and disease surveillance
Emerging Infectious Diseases 5(3): 346-352 1999

Weinberg ED
Association of iron with colorectal cancer
Biometals 7(3): 211-216 1994

Weiss G
Iron and anemia of chronic disease
Kidney Int Suppl 69: S12-17 1999

APPENDIX
Review table of iron status and cardiovascular disease

Study:	+/-	Case study:	Measures:	Results:	Comments
Roest et al. 1999 Circ 100: 1268-1273	+	-Cardiovascular death and presence of wt-heterozygote-homozygote -Cohort study, women, N=12239	DNA-screening (C282Y)	-Risk significantly increased in heterozygotes compared to wt (RR=1.6, adjusted for age, smoking, obesity and hypertension) [1.6: 95% CI, 1.1-2.4] -Risk particularly increased (18.9 fold) for smoker/hypertensive/heterozygous compared to non sm/non-hy/wt	
Klipstein et al. 1999 Am J Clin Nutr 69: 1231-1236	+	-Myocardial infarction and serum ferritin -Nested C-C, N=172 (7983)	Ferritin, iron, transferrin, ceruloplasmin, C-reactive protein, dietary (total and heme) iron	-Serum ferritin >200 µg/l risk 1.81 compared to <200 µg/l (adjusted for age, sex, smoking, alcohol,body mass index) [1.82: 95% CI, 0.90-3.69, p=0.096] -No association: serum iron, serum transferrin, total dietary iron -Dietary heme iron OR=4.0 (adjusted for energy, fat and cholesterol) [4.0: 95% CI, 1.17-15.87, p=0.031]	-Fasting bloodsamples?
Tuomainen et al. 1999 Circ 100: 1274-1279	+	-C282Y occurrence and first myocardial infarction -Prospective cohort, men, N=1150	DNA-screening (C282Y)	-Increased risk of first acute myocardial infarction (2.3-fold) in C282Y heterozygous men compared to noncarriers (adjusted for the major risk factors) [2.3: 95% CI, 1.1-4.8, p=0.03]	
Tuomainen et al. 1998 Circ 97: 1461-1466	+	-1 Acute myocardial infarction and TfR/ferritin -2 Acute myocardial infarction and regular blood donation -Nested C-C, men, N=197	1 TfR, serum ferritin, C-reactive protein (fasting blood samples)	-1 Mean serum TfR/ferritin 28.6% lower among cases than controls (15.1, 21.3 resp.) -Low serum TfR/ferritin 2.0-2.9 fold increased risk compared to high TfR/ferritin (adjusted for the strong risk factors and inflammation) [2.0: 95% CI, 0.9-4.2, p=0.081; 2.9: 95% CI, 1.3-6.6, p=0.011] -2 Blood donors RR=0.14 compared to nondonors	
Nassar et al. 1998 Can J Cardiol 14: 215-220	±	-C282Y and H63D mutations (and plasma ferritin) and coronary artery disease -Study population: patients, N=300	C282Y, H63D, plasma ferritin	-Significant higher plasma ferritin levels in men with early CVD (onset <50 y) compared to men with CVD onset >65 y (234 vs 136 µg/l) -In women no differences in plasma ferritin levels -No difference in prevalence of heterozygoous-homozygous-compound hetrozygous state between CVD onset <50 y and >65 y	-Inflammation/ infection markers not determined -Fasting bloodsamples? -Small number of patients (150 vs 150)
Kiechl et al. 1997 Circulation 96: 3300-3307	+	-Carotid atherosclerosis and serum ferritin -Prospective survey, N=826	Ultrasonographic measurements, serum ferritin, serum iron, TS, HDL and LDL cholesterol, inflammation/ infection marker (fasting blood samples)	-Strong association between serum ferritin and atherosclerosis progression (through modification of the atherogenic potential of LDL cholesterol (OR=1.55, 1.77 and 2.05 respectively for a 1-SD unit increase in ferritin at LDL levels of 2.5, 3.6 and 4.9 mmol/l) [1.55: 95% CI, 1.30-1.85; 1.77: 95% CI, 1.40-2.24; 2.05: 95% CI, 1.50-2.80]	
Meyers et al. 1997 Heart 78: 188-193	+	-Cardiovascular events and blood donation -Prospective cohort, N=2966	Occurrence of cardiovascular events and level of blood donation (telephone survey follow up)	-Current blood donation in non-smoking men was associated with a reduced risk of cardiovascular events (OR=0.67) [0.67: 95% CI, 0.45-0.99] -No association between blood donation and cardiovascular events in women	
Gillum et al. 1996 Am J Epidemiol 144: 59-68	±	-Stroke incidence and serum TS -Cohort follow up (NHANES I), N=5033	-TS, serum iron, TIBC, hemoglobin	-significant 'U-shaped' association between stroke death and TS (RR=1.58 and 1.81 respectively for TS=20-29% and >44% compared to TS=30-36, adjusted for age, smoking, diabetes, history of heart disease, education, blood pressure, race and sex) [1.58: 95% CI, 1.04-2.39, p<0.05 and 1.81: 95% CI, 1.01-3.26, p<0.05] -No significant association between stroke incidence and TS	-Ferritin not measured -No fasting blood samples -Inflammation/ infection not determined and/or excluded

Van Asperen et al. 1995 Int J Epidemiol 24: 665-670	-	-Ischemic heart disease and 'iron status' -Follow-up study, N=260	Hemoglobin, TS, TIBC (fasting blood samples)	-No clear association -Among men: trend of borderline significantce between TS and risk	-Small number of participants -Inflammation/ infection at baseline unknown -Ferritin not measured
Kiechl et al. 1994 Arterioscler Thromb 14: 1625-1630	+	-Carotid atherosclerosis and serum ferritin -Prospective survey, N=847	Ultrasonographic measurements, serum ferritin, serum iron, TS, HDL and LDL cholesterol, inflammation/ Infection marker (fasting blood samples)	-Strong association (OR=1.54 per 100 µg/l) between ferritin and carotid artery disease in men and women (adjusted for all age, sex and all major risk factors) -Effect more prominent when associated with hypercholesterolemia	
Morrison et al. 1994 Epidemiology 5: 243-246	+	-Fatal acute myocardial infarction and serum iron, dietary iron and use of iron supplements -Cohort, N=9920	-TS, hemoglobin, serum iron, UIBC, serum total cholesterol, food consumption questionnaire	-Risk of fatal acute myocardial infarction significantly increased in both men and women in the serum iron >175 µg/dl group compared to <120 µg/dl group (RR=2.18 and 5.53 respectively) [2.18: 95% CI, 1.01-4.74 and 5.53: 95% CI, 1.69-18.12] -Risk higher (RR=4.60) in men with high serum cholesterol levels (>240 mg/dl) and high serum iron (>175 µg/dl) compared to men with serum cholesterol <240 mg/dl and serum iron >175 µg/dl (RR=1.46) -No association between either dietary iron intake or use of iron supplements and fatal acute myocardial infarction risk	-Serum ferritin not measured -No inflammation/ infection markers determined -Fasting bloodsamples?
Solymoss et al. 1994 Coron Artery Dis 5: 231-235	-	-Coronary artery disease and serum ferritin -Retrospective patient study, N=299	Elective coronary arteriography, serum ferritin	-No association between serum ferritin and presence or severity of angiographically determined coronary artery disease	-Determinations were performed after CVD events: serum ferritin levels tend to decrease to normal values -All participants were patients -Fasting blood samples? -Inflammation/ infection markers not determined
Frey and Krider 1994 W V Med J 90: 13-15	-	-Myocardial infarction and serum ferritin -Retrospective study, N=298	Serum ferritin, lipid profile, cholesterol (fasting blood samples(baseline))	-No association between serum ferritin and the occurrence of myocardial infarction	-Small study population -Participants were former mine workers and outpatients -Uncommon method used for serum ferritin determination
Magnusson et al. 1994 Circulation 89: 102-108	±	-Myocardial infarction and low iron binding capacity, N=2036	Iron, ferritin, TIBC, lipid profile, cholesterol (fasting blood samples)	-Serum ferritin higher in cases than in controls and TIBC lower in cases than in controls (both not significant) -TIBC negative risk factor in men (RR=0.95) [0.95: 95% CI, 0.92-0.98] -Each increase in TIBC of 1 µmol/l associated with a 5.1% decrease in myocardial infarction risk	
Liao et al. 1994 Am J Epidemiol 139: 704-712	-	-Coronary heart disease and serum iron / TS -Cohort follow up (NHANES I), N=4237	TS, serum iron, TIBC	-No association	-Ferritin not measured -No fasting blood samples -Inflammation/ infection not determined and/or excluded

Sempos et al. 1994 N Engl J Med 330: 1119-1124	-	-Coronary heart disease and TS -Cohort follow up (NHANES I), N=4518	TS, serum iron, TIBC, hemoglobin, hematocrit, erythrocyte sedimentation (as a measure for inflammation)	-No association	-Erythrocyte sedimentation reliable measure? -Possible bias because of missing data (coronary heart disease incidence) -Ferritin not measured -No fasting blood samples
Aschero et al. 1994 Circulation 89: 969-974	+	-Coronary disease and dietary (heme and total) iron -Cohort, men, N=44933	Food frequency questionnaire at baseline	-No association between total dietary iron intake and coronary disease -Incidence of fatal coronary disease or nonfatal myocardial infarction higher among men with high heme intake compared to low heme intake (RR=1.42, adjusted for the established risk factors and fat and cholesterol)	-No clinical determinations performed, heme iron is indeed correlated to serum ferritin, but this method is not completely reliable
Salonen et al. 1992 Circulation 86: 803-811	+	-Myocardial infarction and serum ferritin -Population study, men, N=1931	Serum ferritin, hemoglobin, LDL and HDL cholesterol, dietary iron intake questionnaire (fasting blood samples)	-Men with serum ferritin >200 µg/l had a 2.2 fold risk factor for acute myocardial infarction compared to men with serum ferritin <200 µg/l (adjusted for all major risk factors) [2.2: 95% CI, 1.2-4.0, p<0.01] -Association was stronger in men with serum LDL cholesterol >5.0 mmol/l compared to <5.0 mmol/l	-Inflammation/ infection not determined and/or excluded

(+ = Results in favour of the iron hypothesis; - = Results weakening the iron hypothesis; ± = Indistinct results)

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