

# **Iron Therapy in Inflammatory Bowel Disease**

## **Effects on Oxidative Stress and Disease Activity**

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## List of publications

This thesis is based on the following papers, referred to in the text by their roman numerals:

- I Erichsen K, Hausken T, Ulvik RJ, Svardal A, Berstad A, Berge RK. Ferrous fumarate deteriorated plasma antioxidant status in patients with Crohn disease. *Scand J Gastroenterol* 2003;38:543-548.  
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- II Erichsen K, Milde AM, Arslan G, Helgeland L, Gudbrandsen OA, Ulvik RJ, Berge RK, Hausken T, Berstad A. Low-dose oral ferrous fumarate aggravated intestinal inflammation in rats with DSS-induced colitis. *Inflamm Bowel Dis* 2005;11:744-748.  
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- III Erichsen K, Ulvik RJ, Nysaeter G, Johansen J, Ostborg J, Berstad A, Berge RK, Hausken T. Oral ferrous fumarate or intravenous iron sucrose for patients with inflammatory bowel disease. *Scand J Gastroenterol* 2005;40:1058-1065.  
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- IV Erichsen K, Ulvik RJ, Grimstad T, Berge RK, Berstad A, Hausken T. Effects of ferrous sulphate and non-ionic iron-polymaltose complex on markers of oxidative tissue damage in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2005;22:831-838.  
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# **1. Introduction**

## **1.1 Purpose of the introduction**

The purpose of the introduction is to bring the reader into the concept of inflammatory bowel disease associated anaemia and its management, with emphasis on problems related to deterioration of oxidative stress during iron therapy.

## **1.2 Inflammatory bowel disease**

Inflammatory bowel disease (IBD) is a group of disorders of the gastrointestinal tract characterised by intestinal inflammation and a chronic relapsing course. IBD has traditionally been categorised as either ulcerative colitis (UC) or Crohn's disease (CD) on the basis of clinical, radiological, endoscopic and histological criteria. About 10 % of colitis cases show overlapping features of the two major forms and are called intermediate colitis.

Although the aetiology of IBD remains to be defined, recent experimental and clinical studies suggest that the initiation and pathogenesis of these diseases are multi-factorial, involving interactions among genetic, environmental and immune factors (1).

### **1.2.1 Epidemiology**

Inflammatory bowel disease is not evenly distributed world-wide. There is a clear tendency to a higher incidence in developed countries compared with less developed countries (2). North America, the United Kingdom and Scandinavia have the highest rates (2). In Norway, the incidence of UC and CD was reported to be 13.6 per 100 000 and 5.8 per 100 000, respectively (3; 4). UC and CD are most commonly

diagnosed in late adolescence and early adulthood, but the diagnosis may occur at all ages.

### **1.2.2 Pathology and clinical presentation**

UC is a mucosal disease that usually involves the rectum and extends proximally to involve all or part of the colon. Proximal spread occurs in continuity without areas of uninvolved mucosa. The major symptoms of UC are urgent diarrhoea, rectal bleeding, passage of mucus, and crampy abdominal pain. The severity of symptoms correlates with the extent of the disease.

CD can affect any part of the gastrointestinal tract from the mouth to the anus, but most commonly affects the small intestine and/or the colon. The inflammation is transmural and segmental, with normal areas between patches of diseased intestine. Consequences of the inflammation include fistula formation to other loops of bowel, the urinary bladder, vagina, or perianal skin, abdominal or perianal abscesses, and intestinal strictures. The site and behaviour of the disease influences the clinical manifestations. The most common symptoms are diarrhoea, crampy abdominal pain, fever, anorexia, and weight loss.

Extraintestinal manifestations of UC and CD can affect multiple organ systems, such as the eyes, skin, and joints, as well as gastrointestinal organs, including the liver and gall bladder.

## **1.3 Anaemia in inflammatory bowel disease**

### **1.3.1 Definition of anaemia**

Anaemia is defined as a lowered concentration of blood haemoglobin (Hb). The degree of anaemia is often scaled as; mild, haemoglobin 10 g/dL to normal limits; moderate, haemoglobin 8 to 10 g/dL; and severe, haemoglobin less than 8 g/dL (5).

### 1.3.2 Prevalence

One third of patients with UC and CD suffer from anaemia defined as blood haemoglobin < 12 g/dL (6; 7). While the understanding of IBD has grown over the past decades, the prevalence of IBD associated anaemia has changed only little (8).

### 1.3.3 Clinical consequences

In chronic disease, symptoms of anaemia are often insidious in onset and may seem to be less than expected from the haemoglobin concentration. It has been argued that patients adapt to low haemoglobin if anaemia develops slowly. However, in the recent past it has been suggested that the process of adaptation to chronic anaemia is in fact adaptation to a lower quality of life (9).

Chronic fatigue is a frequent symptom of IBD, and is commonly caused by anaemia (8). Dyspnoea and tachycardia are due to peripheral hypoxia, while central hypoxia may lead to symptoms such as headache, dizziness, vertigo or tinnitus. Nausea, anorexia and weight loss are also associated with anaemia. A common finding is menorrhagia and amenorrhoea among women (5). Men may suffer from impotence (5).

### 1.3.4 Aetiology

IBD associated anaemia is commonly a combination of iron deficiency anaemia (IDA) and anaemia of chronic diseases (ACD). Other mechanisms of anaemia may occasionally be relevant.

#### *Iron deficiency*

Iron deficiency is defined as a reduction in total body iron to an extent that iron stores are completely exhausted. *Absolute iron deficiency* is accompanied by IDA. In *latent iron deficiency* iron stores are empty but anaemia has not yet developed.

In man the normal diet should contain 13-18 mg of iron per day of which only 1-2 mg are absorbed (10; 11). Balance is achieved through daily iron losses from the gut, skin and sweat of 1-2 mg (12). In iron-deficient states, absorption may increase to a maximum of 2-4 mg (10; 11). Therefore, ongoing losses of  $\geq 10$  ml of blood (1 ml of blood contains 0.5 mg of iron) daily will result in iron deficiency (10).

Chronic intestinal bleeding in IBD may exceed the amount of iron that can be absorbed from the diet, resulting in a negative iron balance (13). Although iron absorption in IBD is generally normal, it can occasionally be impaired in extensive CD of the duodenum and upper jejunum (14).

Food aversion and self-reported intolerance are common in IBD and this will affect the amount of iron available in the diet (15; 16). Various reports have pointed to diminished iron intake in IBD predominantly due to avoidance of iron fortified cereals which may be perceived to exacerbate abdominal symptoms (17; 18).

### *Anaemia of chronic disease*

Anaemia of chronic disease (ACD) occurs in patients with activation of cell mediated immunity, such as infections, immune mediated inflammatory disorders or malignancy (19). The cytokines most often implicated in the pathogenesis are tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin-1, interleukin 6 and interferons (19). Several factors contribute to the development of ACD.

A hallmark of ACD is disturbances in iron homeostasis, with increased uptake and retention of iron within cells of the reticuloendothelial system, and down regulation of duodenal iron absorption. This leads to hypoferraemia, limiting iron availability in erythroid marrow and subsequent iron deficient erythropoiesis. The term *functional iron deficiency* is applied for situations where there is an inadequate iron supply for erythropoiesis despite the fact that the iron stores are sufficiently filled with iron.

Cytokines directly affect erythropoiesis by inhibiting the proliferation and differentiation of erythroid progenitor cells, and the life span of red cells are shortened (19).

Erythropoietin, a hormone secreted by the kidney, regulates erythroid proliferation centrally. Under normal physiological conditions, erythropoietin concentration is inversely related to tissue oxygenation and haemoglobin levels. Serum erythropoietin levels in patients with ACD appear to be inadequately low for the degree of anaemia (8). However, this does not hold true for all diseases underlying ACD (20), but has been shown for UC and CD (6; 21).

### *Vitamin B12 and folate deficiency*

Vitamin B12 (cobalamin) and folic acid are vitamins involved in a series of complex biochemical reactions.

Clinical evidence of vitamin B12 deficiency occurs late as body stores have to be depleted to less than 10% (8). Vitamin B12 deficiency is most often a consequence of terminal ileal disease or resection. Of those patients with ileal resections of > 60 cm, almost all malabsorb vitamin B12, and if < 60 cm, about half have vitamin B12 malabsorption (22). An association with gastric Crohn's disease has also been recognised (23).

Clinical evidence of folic acid deficiency occurs early as folate stores last only 1-2 months. Folic acid is absorbed in the duodenum and jejunum and deficiency may arise from inadequate intake, malabsorption, or drug interactions (sulfasalazine, methotrexate).

### *Drug induced anaemia*

Azathioprine and 6-mercaptopurine have a direct myelosuppressive effect.

Sulfasalazine and 5-aminosalicylic acid have been related to a minor degree of hemolysis or aplasia (24-26).

Less common causes of anaemia in IBD are autoimmune haemolytic anaemia (27) and myelodysplastic syndromes (28).

### **1.3.5 Laboratory diagnosis**

Monitoring of IBD patients with complete blood counts is a routine measure and iron parameters, vitamin B12 and folic acid must be checked regularly.

The lower haemoglobin cut off level is about 12.0 g/dL in women and 13.5 g/dL in men. One must keep in mind that patients may have habitual haemoglobin levels considerably higher than the cut off level, and anaemia may therefore be present even with haemoglobin within the normal range.

Mean cell volume (MCV) and mean cell haemoglobin (MCH) characterise the anaemia. Microcytosis and hypochromia indicate red cell iron deficiency with or without concomitant ACD. Macrocytosis points to vitamin B12 or folic acid deficiency. An increase in MCV without anaemia is seen during azathioprine and 6-mercaptopurine therapy. MVC and MCH are late markers and abnormalities in these parameters suggest longstanding deficiencies.

The serum ferritin reflects the iron stores. However, ferritin is an acute phase reactant, complicating the interpretation of test results. When serum ferritin is less than 15 µg/L, iron stores are definitely depleted. A serum ferritin concentration below 30 µg/L is diagnostic of IDA in a patient with anaemia (29; 30). Serum ferritin concentrations above 50 µg/L usually exclude iron deficiency in patients with ACD (30). The C-reactive protein (CRP) is generally considered to be the best laboratory marker of inflammation, but the degree of elevation that invalidates the serum ferritin criteria of iron deficiency has not been accurately defined. A reasonable interim guideline to eliminate an influence of inflammation on serum ferritin is a CRP below 30 mg/L (31; 32).

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In pure IDA, the serum transferrin concentration, or total iron binding capacity (TIBC), is increased, while the transferrin saturation is decreased (<16%). In ACD, both serum transferrin and transferrin saturation are decreased.

Increased serum transferrin receptor (sTfR) is also a useful indicator of iron deficiency. The sTfR is usually normal in ACD, and only increased within the reference range in the combined state of ACD and iron deficiency (33). The ratio of sTfR/log ferritin has been proposed for the identification of patients with ACD and iron deficiency (30), but such ratios are often impractical in clinical practise.

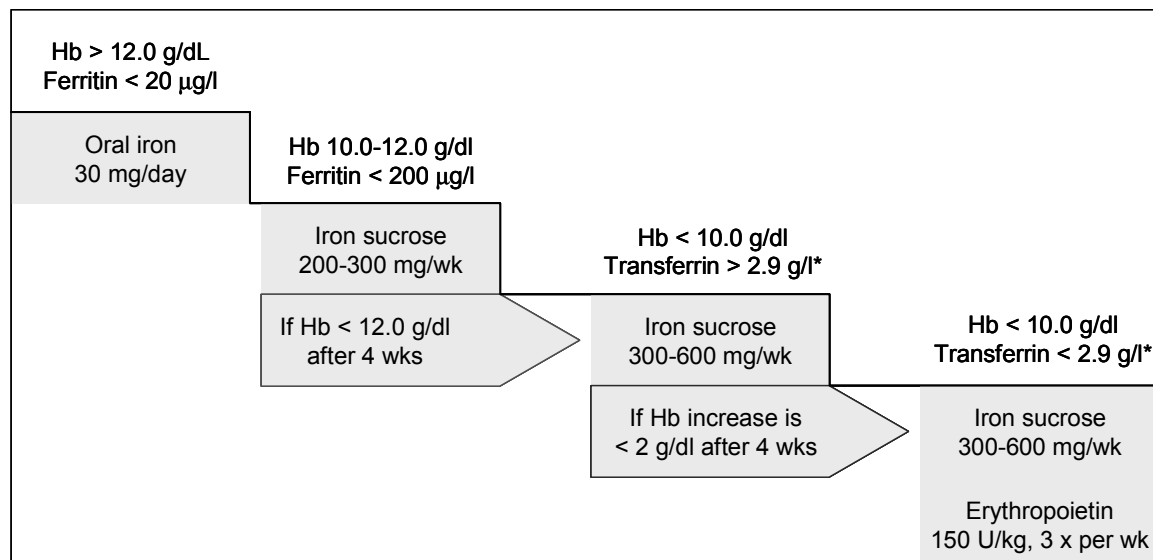
Two new erythroid indices of iron deficient erythropoiesis have been introduced recently. The proportion of hypochromic red cells (HYPO) permits earlier detection of iron deficient erythropoiesis than the MCV, but it still takes a few weeks to become abnormal (31; 34). A more rapid change occurs with the reticulocyte haemoglobin content (CHr) that falls within a couple of days (35). These parameters can be assayed by only a few models of haematology analysers and availability is therefore limited.

### **1.3.6 Treatment**

The therapeutic goal is normalisation of haemoglobin levels (8). In anaemic cancer patients, improvements in quality of life were detected at haemoglobin levels of up to 14 g/dL (36).

Intestinal inflammation must be treated adequately to reduce blood loss and immune activation. The treatment for iron deficiency is iron supplementation. Patients who do not respond to iron supplementation may benefit of erythropoietin administration. The therapeutic algorithm for IBD associated anaemia developed by Gasche et al. is shown in Figure 1. In cases of vitamin B12 or folic acid deficiency, appropriate substitution is needed.

This paragraph focuses on efficacy and tolerability of oral and intravenous iron administration in IBD patients. Iron therapy in general and the relation between iron and oxidative stress are outlined in paragraphs 1.5 and 1.6 respectively.



**Figure 1** Adjusting therapy to the degree of anaemia. The place of oral iron therapy is mainly prevention of iron deficiency anaemia. In patients with normocytic or microcytic anaemia, iron sucrose demonstrates the best efficacy and tolerability. The amount of iron needed relates to the degree of anaemia and can be estimated using the approximation that an increase of 1 g/dl haemoglobin (Hb) requires about 200 mg of intravenous iron. It is recommended that iron therapy is suspended in cases of acute infection (for example, abscess) or at transferrin saturation > 50%. \*Instead of transferrin, the soluble transferrin receptor (below 50 nmol/l) or erythropoietin concentration (below 100U/l) can be applied. *Gut* 2004;53:1190-1197. Reproduced with permission from the BMJ Publishing Group.

### *Oral iron preparations*

Oral iron supplements commonly contain iron in the form of ferrous salts (ferrous sulphate, ferrous fumarate and ferrous gluconate). Nausea, abdominal pain, bloating and diarrhoea are frequent side-effects in the IBD population leading to poor compliance (37). Iron absorption is limited, and treatment for several months is necessary to correct IDA. Chronic inflammatory disease may reduce iron absorption. Also, oral iron may not be sufficient to compensate ongoing blood loss in IBD patients with severe anaemia (7). Because of these limitations oral iron preparations are recommended only for the prevention of anaemia (8). However, until recently



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oral iron supplementation was also recommended for the treatment of anaemia with haemoglobin > 10.5 g/dL (37).

Ferric iron-polymaltose complex and haeme iron polypeptide are available in some countries, but experience and evidence for the use of these formulations in the IBD population are lacking.

### *Intravenous iron preparations*

Parenteral iron formulations are ferric hydroxide carbohydrate complexes (iron dextran, iron sucrose, iron gluconate). During the last few years, experience of using intravenous (i.v.) iron sucrose in various forms of iron deficiency has evolved (38). The efficacy and tolerability of iron sucrose in IBD patients has been tested in several studies.

Using single doses of 200 mg iron sucrose, twice weekly during the first two weeks and once weekly thereafter, approximately 75% of patients with UC and CD responded to the treatment after eight weeks (21; 39). Response was defined as an increase in haemoglobin of  $\geq 2.0$  g/dL. In another study, 91% of IBD patients responded after 12 weeks of iron sucrose treatment (40). No serious adverse events were observed when using individual doses of 200 mg iron sucrose (21; 39; 40). Transient burning at the site of venipuncture, bitter taste, transient fever and transient hypotension were experienced in a few patients (21; 39; 40).

To date, no data is available on the efficacy and tolerance of other i.v. iron formulations in IBD patients.

### *Recombinant human erythropoietin*

Supraphysiological doses of erythropoietin can overcome the inhibition of erythropoiesis in ACD (41). IBD patients not responding to iron sucrose alone benefit from combined erythropoietin treatment (21; 39). For the reason of costs, erythropoietin should be reserved for patients who are resistant to i.v. iron therapy (37). In patients with severe anaemia, low plasma erythropoietin (below 100 U/L),

sTfR (below 50 nmol/L or 2.7 mg/L) or transferrin levels (below 2.9 g/L) indicated resistance to iron sucrose (42). Transferrin of 2.9 g/L is equivalent to TIBC of 73  $\mu\text{mol/L}$ .

## 1.4 Basic aspects of iron metabolism

All mammalian cells require iron for a wide range of vital biologic processes. Iron is centrally involved in oxygen transport by haemoglobin and myoglobin, in electron transport during mitochondrial respiration, and in DNA synthesis. However, iron is also a potentially toxic substance. The shift back and forth between its two oxidation states – ferrous ( $\text{Fe}^{2+}$ ) and ferric ( $\text{Fe}^{3+}$ ) – via single electron transfer reactions is the property that makes iron such an essential component of the cytochromes in the respiratory chain. This redox property also contributes to its potential toxicity. Redox cycling between ferrous and ferric iron can promote the generation of highly reactive oxygen species, which can damage lipids, proteins and nucleic acids. Normal iron homeostasis allows cells to benefit from the usefulness of iron while avoiding its deleterious effects.

There is no physiologic pathway for excretion of iron. Iron losses occur primarily through bleeding and sloughing of mucosal and skin cells. To maintain iron balance, intestinal iron absorption is meticulously controlled. Normal adults have approximately 4000 mg of total body iron, of which about 3000 mg is found in haemoglobin in erythrocytes and their precursors, and 800-1200 mg as storage iron. Small amounts of iron also occur in myoglobin and cytochrome enzymes. Only 1-2 mg of iron enters and leaves the body on a daily basis.

### 1.4.1 Iron absorption, transport and storage

Dietary iron consists of two components, haeme iron (present in meat, fish and poultry) and non-haeme iron or inorganic iron (in vegetables, cereals etc.). Haeme iron and non-haeme iron are absorbed through distinct pathways (43).

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Non-haeme iron is transported into the enterocytes in the ferrous ( $\text{Fe}^{2+}$ ) form, and most iron absorption takes place in the proximal duodenum, near the gastric outlet. Luminal factors strongly influence iron absorption. The gastric acid helps to keep iron in the more soluble ferrous form ( $\text{Fe}^{2+}$ ). At the neutral pH of the duodenum, ferrous iron is rapidly oxidised to ferric iron ( $\text{Fe}^{3+}$ ), which precipitates into poorly absorbed iron hydroxide and iron oxide polymers. Ascorbic acid potentiates iron absorption by stabilising the reduced ion (44). In contrast, compounds that bind iron make it less available for the absorptive mechanism. Examples of such inhibitors are polyphenols (e.g. as in tea and coffee) and phytates (e.g. as in wheat bran, oat, rice and maize).

The first step in iron absorption is transfer across the apical (luminal) membrane of the enterocyte. First, ferric iron is reduced to ferrous iron by a brush border ferrireductase (DCYTB). Transfer of ferrous iron is then carried out by divalent metal transporter 1 (DMT1), a transmembrane protein that functions as a general transporter of divalent metals. Once inside the enterocyte, iron has two possible fates. Some remains stored within the cell; that iron is ultimately lost from the body at the end of the enterocyte lifespan. The remainder is transferred across the basolateral surface through ferroportin, a transmembrane protein similar to DMT1. A ferroxidase, hephaestin, acts in some way to facilitate basolateral iron transport (45). Basolateral plasma transfer is increased in iron deficiency and decreased when body iron is plentiful.

Proteolytic digestion of myoglobin and haemoglobin results in the release of haeme ( $\text{Fe}^{2+}$ -protoporphyrin IX), which is maintained in a soluble form by globin degradation products so that it remains available for absorption. Intact haeme is transported across the brush border membrane by an unknown mechanism. Inside the enterocyte iron is released from the porphyrin ring, and then further processed as inorganic iron. Haeme iron from meat is readily absorbed, regardless of other dietary components. Up to 30% may be available for absorption (46). On the other hand, purified haeme forms large insoluble polymers and are poorly absorbed (47).

Iron circulates bound to transferrin, a very abundant plasma protein that binds two iron atoms avidly with a dissociation constant of approximately  $10^{22}$  at physiologic pH (48). Transferrin keeps ferric iron soluble and prevents iron from reacting with other molecules by attenuating its redox activity. It aids in delivery of iron to cells by binding to a specific cell-surface transferrin receptor (TFR1). TFR1 is present in low abundance in most if not all mammalian cell types, but is expressed at high levels by cell types with large needs for iron, such as developing erythroid precursors.

In general, two cell types are considered important for storage of iron that is not needed for immediate use. Macrophages recover iron from effete erythrocytes. They also internalise iron from intravenously administered iron sucrose and iron dextran. Hepatocytes acquire both transferrin bound and non-transferrin bound iron from plasma. Both the macrophages and the hepatocytes have large capacity for iron storage.

At a molecular level, iron storage occurs primarily in the protein ferritin. It consists of 24 subunits that sequester iron hydroxide phosphates in a central core. Iron release occurs when intracellular pools become depleted. Degradation of ferritin leads to the formation of hemosiderin, a non-homogeneous conglomerate of iron, protein and membrane breakdown products.

Measurable changes in intestinal iron absorption and tissue iron distribution are found in situations of abnormal iron availability (iron overload or deficiency), accelerated erythropoiesis, hypoxia and inflammation. Situations that require decreased iron availability are associated with interruption of intestinal absorption and retention of iron by macrophages. In contrast, situations requiring increased iron availability are associated with increased intestinal absorption and enhanced macrophage iron release. Hepcidin, a 25-amino-acid peptide secreted into the plasma from the hepatocytes, is probably a key regulator of intestinal iron absorption and also of recycling of iron via macrophages (11). Increased hepcidin expression results in reduced iron absorption and iron recycling whereas reduced plasma hepcidin acts to increase iron absorption and release of iron through macrophages (11).

## 1.4.2 Iron and erythropoiesis

Erythropoiesis dominates iron metabolism. About 20-25 mg of iron per day is required to support the haemoglobinisation of new erythrocytes (49). Most of it comes from recycling of iron already in the body. The primary source is macrophages, which phagocytose damaged erythrocytes to scavenge their iron from haemoglobin and return it to the circulation. The amount of iron passing through this macrophage recycling system every day approximates the amount needed for erythropoiesis.

## 1.5 Iron therapy

### 1.5.1 Oral iron preparations

In otherwise healthy patients, oral iron should be given preference over parenteral iron as the initial approach to treatment of iron deficiency.

Oral iron supplements are commonly in the form of ferrous salts. These are ionic compounds composed of the metal iron (cation) and an acid (anion), so that the product is neutral (without a net charge). The most common are ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ), ferrous fumarate ( $\text{FeC}_4\text{H}_2\text{O}_4$ ) and ferrous gluconate ( $\text{Fe}(\text{C}_6\text{H}_{11}\text{O}_7)_2$ ).

Ingested ferrous salts are solubilised and ionised by the gastric acid, and the iron is absorbed by the same mechanisms as dietary inorganic iron (outlined in paragraph 1.4.1). However, the tight control of dietary iron absorption seems to be partly out of control after pharmacological doses of ferrous salts. With increasing doses, the absolute amounts of iron absorbed increase (50; 51).

Standard iron replacement therapy for adults is 2-3 mg elemental iron/kg body weight in three divided doses. Reticulocytosis may be observed as early as 4 days after start of treatment and will reach a maximum at 7-10 days. An increase in the haemoglobin concentration will follow. Therapy needs to continue for 2-3 months after correction

of anaemia in order to replenish the body iron stores (52). The usual cause of failure to respond is non-compliance. There is no significant difference in efficacy (53) or side-effects (54) between different ferrous compounds when the same amount of elemental iron is administered.

The major difficulty with oral iron is the nausea, vomiting and epigastric discomfort that occurs within an hour or two of ingestion. These symptoms vary in proportion to the concentration of ionisable iron in the upper gastrointestinal tract and can be reduced by taking the iron supplement with food. However, administration with food decreases bioavailability. Many patients also complain of bloating, constipation or diarrhoea, but these lower gastrointestinal side-effects appear not to be dose related (31). Enteric coated ferrous formulations are promoted on the basis of reduced side-effects, but any reduction in symptoms is invariably due to a parallel decrease in iron absorption (8; 31).

Ferric iron-polymaltose complex and haeme iron polypeptide are also available in some countries. Ferric iron-polymaltose complex is a ferric ( $\text{Fe}^{3+}$ ) hydroxide carbohydrate complex similar to the parenteral iron formulations. Two smaller studies demonstrated that iron-polymaltose complex was as efficient as ferrous sulphate in correcting blood haemoglobin levels in iron deficiency anaemia, but iron-polymaltose complex did not increase serum ferritin to the same extent as ferrous sulphate (55; 56). Haeme iron polypeptide is produced by hydrolysis of bovine haemoglobin. The extraction technique leaves peptides from the globin subunits covalently bound to the haeme ring, improving solubility (57). The product is thought to have improved bioavailability and promises lower side-effects (57). Larger series have not yet been treated with any of these compounds.

### **1.5.2 Intravenous iron preparations**

Indications for intravenous iron therapy are given in Table 1.

**Table 1.** Indications for intravenous iron therapy

High iron needs	Significant gastrointestinal bleeding Erythropoietin therapy Menorrhagia Pregnancy
Need for fast correction of anaemia	Post-partum anaemia Post-operative anaemia
Failure of oral iron therapy	Intolerance Poor compliance Malabsorption

Direct administration of iron into the circulation requires formulations that prevent the cellular toxicity of iron salts (58). I.v. iron agents consist of a core of non-ionic ferric hydroxide molecules surrounded by a shell of carbohydrate. They differ from each other by the size of the core and the identity and the density of the surrounding carbohydrate.

After i.v. administration, ferric hydroxide carbohydrate complexes are taken up by the reticuloendothelial system. Within macrophages, iron is released from the iron-carbohydrate compound and either stored as ferritin or released into the circulation where it combines with transferrin for transport to the bone marrow. A small fraction, however, likely bypasses the intracellular steps and donates iron directly to transferrin in plasma (59). Three different products are currently available.

### *Iron dextran*

Iron dextran is a stable parenteral iron product with molecular weight of 100-300 kD. The plasma half-life is 30 to 60 hours (60). Stability allows administration of high single doses of 500-1000 mg iron. The reticulocyte count can increase within 7 days and haemoglobin responses occur within 1-2 weeks of iron dextran administration (61).

Anaphylactic reactions to the dextran moiety are seen in up to 1.7% of infusions (62). The risk of a life-threatening/serious acute reaction is on the order of 0.7% (62). The reaction typically occurs during the first several minutes of administration. A test dose should be given to all new patients started on iron dextran, and in patients receiving repeat doses after an interval of non-treatment (61; 63). In addition, special caution should be exercised in patients with multiple drug allergies/intolerance (63).

Delayed reactions of hypotension, arthralgias, myalgias, malaise, nausea, and vomiting occurred in about 10% of patients after high doses (61; 64; 65).

Iron dextran may be administered intravenously by slow injection or infusion. It may also be administered by the intramuscular route. However, pain on injection, staining of the skin, unpredictable delivery, and absorption of iron make the intramuscular route undesirable (61).

One form of iron dextran is currently available in some European countries, and marketed under the name of Cosmofer<sup>®</sup>. Iron dextran Cosmofer<sup>®</sup> is identical to iron dextran InFed<sup>®</sup> available in the United States.

### *Iron sucrose*

Iron sucrose has a molecular weight of 43 kD. The plasma half life is 5-6 hours (66). Iron sucrose, like iron gluconate, is more readily available for erythropoiesis than iron dextran (38). Increase in haemoglobin concentration is noted after one week of iron sucrose administration (67).

There is no risk of anaphylactic reactions, and a test dose is not required. Iron sucrose must be administered intravenously by slow injection or infusion. Single doses of up to 300 mg iron sucrose are safe (68). The maximal recommended dosage is 600 mg/week, but this amount exceeds the physiological needs of the erythropoiesis. If the infusion speed is too fast or the single total iron dose is too high transient hypotension, tachycardia, dyspnoea, nausea and lower back pain can occur (8; 68).



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Iron sucrose is available in Europe, North America, and most countries world-wide as Venofer<sup>®</sup>.

### *Iron gluconate*

Ferric gluconate has a molecular weight of 38 kD, and a plasma half-life of 1 h (58; 61; 69).

Iron gluconate has not been associated with anaphylactic reactions, and a test dose is not required. A standard dose of 125 mg may be administered intravenously by slow injection or infusion. Doses of 250 mg i.v. have been reported to be well tolerated (70). High infusion speed or high single iron doses may cause nausea, hypotension, tachycardia and dyspnoea (8). The use of iron gluconate for iron deficiency in patients on dialysis has been found to be safe and superior to iron dextran (71; 72).

Ferric gluconate is available in several European countries and the United States as Ferrlecit<sup>®</sup>.

## 1.6 Oxidative stress

Oxidative stress is defined as an absolute or relative increase in reactive oxygen species (ROS), and may be caused by increased production of ROS, or diminished antioxidant defences, or often a combination of these. Oxidative stress may lead to oxidative damage of biological molecules and is believed to constitute a major tissue-destructive mechanism (73).

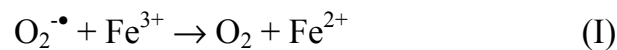
Under normal conditions, about 2% of the oxygen we breathe is used to make ROS, a production approximately balanced by the antioxidant system (74). During the inflammatory response, activated neutrophils and macrophages release large amounts of ROS. Although ROS generation by phagocytes is essential for an effective defence against invading microbes, its sustained overproduction during chronic inflammation may cause extensive tissue destruction (75).

### 1.6.1 Reactive oxygen species

Reduction-oxidation (or redox) reactions involve loss of one or more electrons by one molecule (oxidation) and simultaneous gain by another (reduction). A free radical (denoted  $\bullet$ ) is any atom or molecule that contains one or more unpaired electrons, and is typically unstable and reactive.

ROS are intermediates that appear in the stepwise reduction of oxygen to water. These include superoxide anion ( $\text{O}_2^{\bullet-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radical ( $\text{OH}^\bullet$ ). Occasionally, the term is expanded to include hypochlorous acid ( $\text{HOCl}$ ), peroxynitrite anion ( $\text{ONOO}^-$ ), and nitric oxide ( $\text{NO}$ ).

$\text{O}_2^{\bullet-}$  and  $\text{H}_2\text{O}_2$  are not considered as particular reactive intermediates (76). However, in the presence of “free” iron they may react to form the extremely reactive  $\text{OH}^\bullet$  in the so-called Haber-Weiss reaction (reaction I + II):



and



$\text{OH}^\bullet$  is considered to be the most reactive ROS (76). It can attack and damage almost every molecule in living cells, including lipids, proteins, and DNA.

To promote the generation of ROS, iron must be either freely water-soluble or weakly bound to small organic ions (77). In plasma and tissues, the concentration of such low molecular chelated iron is low due to the tight control of iron homeostasis.

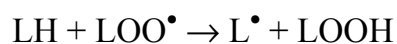
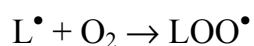
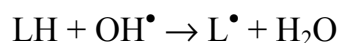
### 1.6.2 Oxidative damage

The overall high reactivity and short half-life of ROS implies that the tissue damage they inflict is generally close to the site of generation.

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### *Membrane lipids*

The polyunsaturated fatty acids located within the cell membrane lipid bilayer are major targets for ROS attack (78). They are particularly effectively attacked by OH<sup>•</sup>, thereby initiating the process of lipid (LH) peroxidation. Once initiated, lipid peroxidation propagates as a chain reaction, and a single oxidative event can thus affect many lipid molecules.



The end products of the chain reaction are a variety of lipid hydroperoxides (LOOH), which decompose to aldehydes.

Once initiated, lipid peroxidation is most successfully terminated by lipid soluble chain-breaking antioxidants such as vitamin E (79).

Extensive lipid peroxidation in the cell membrane has a profound effect on its fluidity and, as such, on the activity of transmembrane enzymes, transporters, receptors, and other membrane proteins (80; 81). As a result, lipid peroxidation causes changes in membrane permeability and selectivity, and ultimately leads to alterations in cell volume homeostasis and cellular metabolism (82). Some products of peroxide decomposition are also cytotoxic (83).

Frequently used markers of lipid peroxidation are malondialdehyde (MDA), thiobarbituric acid-reacting substances (TBARS), lipid hydroperoxides and F<sub>2</sub>-isoprostanes.

### *Proteins and DNA*

Proteins are the most abundant cell constituents, which make them important ROS targets (84). Moreover, a relative minor oxidative modification of a single protein can lead to a marked change (in most cases lowering) in its biological activity. Similar to

lipid peroxidation,  $\text{OH}^\bullet$  seems to be most effective in inducing oxidative protein damage (84). The process of protein oxidation frequently introduces new functional groups, such as hydroxyls and carbonyls, which contribute to altered function, turnover, and degradation (85).

Both nuclear and mitochondrial DNA are known targets of ROS attack (86). Many types of DNA modification can occur, and may in turn lead to malignant transformation or cell death (76).

### **1.6.3 Antioxidant defences**

An antioxidant is any substance that considerably delays or inhibits oxidation. Roughly, the components of the antioxidant defence system can be categorised into non-enzymatic and enzymatic antioxidants.

#### *Non-enzymatic antioxidants*

Non-enzymatic antioxidant defences are largely extracellular. The binding of free metal ions, in particular iron and copper, is an important defence mechanism. Iron bound to transferrin and lactoferrin, and copper bound to ceruloplasmin, can not catalyse redox reactions (78). Haptoglobin and haemopexin bind free haeme and haeme proteins to minimise their ability to participate in redox reactions (78).

Several dietary compounds with antioxidant properties, which normally originate from natural sources, such as fruits and vegetables are considered to be of importance. Vitamin E ( $\alpha$ -tocopherol) is a lipid soluble chain breaking antioxidant,  $\beta$ -Carotene is a lipid soluble radical scavenger, while vitamin C (ascorbic acid) is a water soluble radical scavenger (78).

Reduced glutathione, a tripeptide with a reactive sulphhydryl group, is an important intracellular antioxidant. Apart from being a substrate for the enzyme glutathione peroxidase (see below), glutathione serves as a scavenger of several ROS (87). During

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its antioxidant function, reduced glutathione is converted to its oxidised state, upon which it is reduced back again by the enzyme glutathione reductase.

### *Antioxidant enzymes*

The most important component of the endogenous defence system against ROS is the enzymatic machinery that is found in all cells. The antioxidant enzymes remove key ROS in a two-step pathway. Superoxide dismutases (SOD) convert  $O_2^{\bullet-}$  to  $H_2O_2$ , which is subsequently neutralised to water by catalase or glutathione peroxidase (GPO). The enzymes are not consumed by the reaction (78).

#### **1.6.4 Oxidative stress in anaemia**

The prooxidant-antioxidant balance may be altered in anaemia. Tissue hypoxia is accompanied by an absolute increase in ROS production due to changes in cellular metabolism, higher flux rates in catecholamine metabolism, and activation of leukocytes (88). Also, the antioxidant system is markedly weakened by anaemia. The erythrocytes represent a major component of the antioxidant capacity of the blood, comprising the antioxidant enzymes SOD and catalase, and the glutathione system.

#### **1.6.5 Oxidative stress in inflammatory bowel disease**

##### *Pathogenesis*

Oxidative tissue damage constitutes an important pathogenic factor in IBD (89). ROS are produced in large amounts by infiltrating leukocytes in the inflamed mucosa.

Using direct and indirect measurements of lipid peroxidation, increased levels of lipid peroxidation products have been consistently found in IBD and are mostly correlated with either inflammation or disease activity (90-92). With regard to protein damage, the protein carbonyl content was increased in colonic biopsies from both UC and CD patients (93). Also, the levels of and the balance between the most important

antioxidants were seriously impaired within inflamed intestinal mucosa of IBD patients compared with normal mucosa (94; 95).

### *Role of iron*

When iron meets the inflamed intestinal mucosa it may promote the production of  $\text{OH}^\bullet$  and thereby aggravate tissue damage. Both haeme from chronic mucosal bleeding and dietary iron are sources of redox active iron (96). Most of ingested iron is not taken up, but passed on with the faecal stream. The bulk of inorganic iron is stabilised in iron hydroxide polymers (97; 98). However, a small but significant fraction of the total iron concentration in faeces exists as low molecular chelated iron (99). Bile pigments and amino acids are potential chelators within the intestinal tract (77). Furthermore, low dose oral ferrous sulphate supplementation markedly increased the concentration of weakly chelated iron in faeces (99).

The oxidative potential of i.v. iron compounds is considerably less than that of ferrous salts. Also, i.v. administration does not favour intestinal iron accumulation.

## 2. Aims of the study

The overall objective of the present study was to investigate the effects of iron therapy on markers of oxidative stress and clinical disease activity in IBD.

The specific aims of the four papers included in the thesis were:

- I To compare plasma antioxidant status in patients with CD with that of healthy controls, and to see if oral intake of ferrous fumarate changed plasma antioxidant status. In addition, to evaluate the effects of ferrous iron therapy on disease activity, and to evaluate iron absorption in patients with CD and iron deficiency.
- II To evaluate the effects of low-dose oral ferrous fumarate given by gavage on intestinal inflammation and plasma redox status in DSS-induced colitis in Wistar rats.
- III To evaluate the effects of oral ferrous fumarate and intravenous iron sucrose on clinical disease activity and plasma redox status, and to compare the two treatment modalities in patients with CD or UC, and iron deficiency anaemia.
- IV To compare the effects of oral ferrous sulphate and oral iron-polymaltose complex on markers of oxidative tissue damage and clinical disease activity in patients with CD or UC, and iron deficiency.

### 3. Materials and methods

An overview of study designs, experimental groups, inclusion criteria, interventions and measurements are given in Table II and Table III. Details are outlined or referred in the papers. Only a few issues need to be commented here.

**Table II.** Overview of study designs, experimental groups, inclusion criteria, and interventions.

	Study design	Experimental groups	Inclusion criteria	Interventions
Study I	Prospective, case-control	10 CD patients 10 healthy controls	Iron deficiency	Ferrous fumarate tabs 120 mg/day for 7 days
Study II	Factorial design	40 Wistar rats: Control (8) Sham gavage (8) Iron (8) DSS (8) Iron + DSS (8)		Iron: Ferrous fumarate 0.60 mgFe <sup>2+</sup> /kg/day days 1-14 DSS: DSS 5% in drinking water days 8-14
Study III	Prospective, randomised, non-blinded, crossover	11 CD patients 8 UC patients	Iron deficiency anaemia: Hb < 12 g/dL in females Hb < 13 g/dL in males and Ferritin < 50 µg/L	Ferrous fumarate tabs 120 mg/day for 14 days and Iron sucrose 200 mg i.v. 3 times during 14 days
Study IV	Prospective, randomised, non-blinded, with 2 parallel treatment groups	24 CD patients 17 UC patients	Iron deficiency: MCV < 80 fL or Ferritin < 15 µg/L or Soluble transferrin receptor > 1.54 mg/L	Ferrous sulphate tabs 100 mg twice daily for 14 days or Iron polymaltose complex tabs 200 mg once daily for 14 days

Abbreviations: DSS = dextran sulfate sodium; Hb = haemoglobin; MCV = mean corpuscular volume



**Table III.** Overview of measurements. In study I, III, and IV measurements were carried out before and after intervention. In study III, measurements were carried out at sacrifice of the rats.

	Clinical disease activity	Blood samples	Urine samples	Faecal samples	Histology
Study I	CDAI for CD CDAI diary for all	Routine lab. inv. Iron absorption Aminothiols Vitamin C, E Beta-carotene			
Study II		Plasma iron MDA Aminothiols Vitamin A, C, E		GMP	Crypt and inflammation scores
Study III	HBSI for CD SCCAI for UC CDAI diary for all	Routine lab. inv. MDA Aminothiols Vitamin A, C, E Beta-carotene		Calprotectin	
Study IV	HBSI for CD SCCAI for UC CDAI diary for all	Routine lab. inv. MDA Aminothiols Vitamin A, C, E Beta-carotene	8-iso PGF <sub>2α</sub>	Calprotectin	

Abbreviations: CDAI = Crohn's Disease Activity Index; HBSI = Harvey-Bradshaw Simple Index of Crohn's Disease Activity; SCCAI = Simple Clinical Colitis Activity Index; Routine lab. inv. = routine laboratory investigations; MDA = malondialdehyde; 8-iso PGF<sub>2α</sub> = Urine 8-isoprostaglandin F<sub>2α</sub>; GMP = granulocyte marker protein

### 3.1 Experimental groups

Patients were recruited from the outpatient clinics at Haukeland University Hospital (study I, III, and IV), Førde County Hospital (study III), Haugesund Hospital (study III), Stord Hospital (study III), and Stavanger University Hospital (study IV). In study I, healthy controls were recruited from medical students and staff at Haukeland University Hospital.

## 3.2 Clinical disease activity

In paper I, clinical disease activity was evaluated according to Crohn's Disease Activity Index (CDAI) (100). Scores of  $> 150$  indicate active CD. CDAI has no upper limit.

In paper III and IV, the Harvey-Bradshaw Simple Index of Crohn's Disease Activity (101) was applied for patients with CD, and the Simple Clinical Colitis Activity Index (102) was applied for patients with UC. These indices were used because they are similar regarding design and clinical significance of a given change in score. For the Harvey-Bradshaw Simple Index, maximum score is 25 and scores of  $\geq 5$  indicate active CD. For the Simple Clinical Colitis Activity Index maximum score is 20 and scores of  $\geq 4$  indicate active UC. To allow pooling of results from patients with CD and UC, disease activity scores were calculated as actual score divided by maximum score.

## 3.3 Laboratory investigations

In paper I, plasma levels of the total, reduced and oxidised forms of aminothiols (glutathione, cysteine, cysteinyl-glycine and homocysteine) were measured. To determine the reduced and oxidised forms, a derivatising agent was added to the vacutainer tubes before blood sampling. This was done with an insulin syringe with a very thin needle.

In paper III and IV, plasma levels of the reduced and oxidised forms were not given. When conducting these studies, the insulin syringes were no longer available, and we had to use somewhat thicker needles to inject the derivatising agents into the vacutainer tubes. This often resulted in loss of vacuum in the tubes, and hence the tubes were filled with a variable volume of blood during sampling. Also, between study I and III/IV, the Laboratory of Clinical Biochemistry at Haukeland University Hospital changed from tubes with liquid additives to tubes with dry additives. We often observed that the tubes with dry additives were incompletely filled during

sampling. Since the volume of derivatising agents added was constant, but the volume of blood drawn was highly variable, the dilution of the blood varied. As a consequence, the results of the analyses of the reduced and oxidised forms of aminothiols in study III and IV were incoherent.

## 4. Summary of results

### 4.1 Paper I

#### Ferrous fumarate deteriorated plasma antioxidant status in patients with Crohn disease

At inclusion, plasma reduced cysteine was lower ( $p=0.038$ ) in patients compared with controls. One week of ferrous iron supplementation further decreased reduced cysteine ( $p<0.001$ ) and decreased plasma reduced glutathione ( $p=0.004$ ) in the patients. Following one week of ferrous fumarate supplementation the CDAI tended to increase ( $p=0.071$ ). Patients, but not controls, experienced more diarrhoea, abdominal pain and nausea. Serum iron increased significantly in patients (from  $5.8\pm 3.2$  to  $30.9\pm 13.1$   $\mu\text{mol/L}$ ) and controls (from  $18.4\pm 8.0$  to  $34.5\pm 12.5$   $\mu\text{mol/L}$ ) after an oral iron load test. Variation in iron absorption was large among patients.

### 4.2 Paper II

#### Low-dose oral ferrous fumarate aggravated intestinal inflammation in rats with DSS-induced colitis

DSS significantly increased histological colitis scores ( $p<0.001$ ) and faecal GMP ( $p<0.01$ ). Ferrous fumarate further increased histological colitis scores ( $p<0.01$ ) in DSS induced colitis. DSS + ferrous fumarate decreased plasma vitamin A as compared with controls ( $p<0.01$ ). Otherwise, no changes were seen in plasma MDA, plasma antioxidant vitamins or plasma amino thiols.

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### 4.3 Paper III

Oral ferrous fumarate or intravenous iron sucrose for patients with inflammatory bowel disease

Following oral ferrous fumarate clinical disease activity ( $p=0.037$ ), general well being score (i.e. patients felt worse) ( $p=0.027$ ) and abdominal pain score ( $p=0.027$ ) increased, while no changes were seen following iron sucrose. CRP and faecal calprotectin were unchanged after both treatments. As compared with iron sucrose, ferrous fumarate increased CDAI scores of general well being ( $p=0.049$ ), while changes in clinical disease activity ( $p=0.14$ ) and abdominal pain score ( $p=0.20$ ) did not differ. Ferrous fumarate did not significantly alter plasma MDA or plasma antioxidants. Iron sucrose increased plasma MDA ( $p=0.004$ ) and decreased plasma vitamin C ( $p=0.017$ ) and beta-carotene ( $p=0.008$ ). Changes in plasma parameters did not differ significantly between treatments.

### 4.4 Paper IV

Effects of ferrous sulphate and non-ionic iron-polymaltose complex on markers of oxidative tissue damage in patients with inflammatory bowel disease

Following ferrous sulphate plasma malondialdehyde (MDA) increased ( $p=0.02$ ), while urine 8-isoprostaglandin  $F_{2\alpha}$  (8-iso-PGF $_{2\alpha}$ ) and plasma antioxidants did not change significantly. Iron-polymaltose complex did not change plasma MDA, urine 8-iso-PGF $_{2\alpha}$  or plasma antioxidants. Comparing the two treatments, changes in plasma MDA tended to differ ( $p=0.08$ ), while urine 8-iso-PGF $_{2\alpha}$  and plasma antioxidants did not differ. Neither ferrous sulphate nor iron-polymaltose complex

altered clinical disease activity indices and alterations in stool frequency, abdominal pain and nausea did not differ between treatments.

## 5. General discussion

### 5.1 Oxidative stress

Tissue damage due to oxidative stress is considered important in the pathogenesis of IBD (89). It was hypothesised that oral ferrous iron therapy would enhance intestinal inflammation by catalysing production of ROS. In our first study we found significantly lower plasma levels of reduced cysteine and beta-carotene in patients compared with controls. Following one week of ferrous fumarate therapy plasma levels of reduced cysteine and reduced glutathione decreased in patients, but not in controls. These findings were discussed in an editorial by C. Gasche (103), and commented in a letter to the Editor by P. Nielsen (104) which again was commented by the author (105). These publications are enclosed after paper I. In study III, we found no significant change in plasma redox status of IBD patients following ferrous fumarate 120 mg/day for two weeks. However, in study IV, plasma MDA increased and urine 8-iso PGF<sub>2α</sub> tended to increase after two weeks of ferrous sulphate 200 mg/day for two weeks, indicating increased lipid peroxidation. Significant changes in plasma MDA in study IV but not in study III may be due to higher ferrous iron dosage in study IV. No changes in plasma redox status were found following iron-polymaltose complex treatment. To our knowledge, there are no other publications regarding oral iron therapy and oxidative stress in patients with IBD.

In study II, low-dose oral ferrous fumarate was given by gavage to Wistar rats with DSS-induced colitis. Ferrous fumarate increased histologic colitis scores in rats with colitis. Plasma antioxidant status, however, remained largely unchanged.

Administration by gavage allowed exact iron dosage. The ferrous fumarate dose given was 0.60 mg Fe<sup>2+</sup>/kg/day. In humans, normal iron content in the diet is 0.15-0.20 mg Fe<sup>2+</sup>/kg/day, and standard iron replacement therapy is 2-3 mg Fe<sup>2+</sup>/kg/day.

Lately, several studies of experimental colitis in rats have demonstrated increased intestinal inflammation as assessed by histology following iron fortification of the diet (106-109). In these studies, the diet was enriched with iron salts in the order of 10 to 100 times the normal iron content. In DSS-induced colitis in mice, twofold, fivefold, and 10-fold iron enriched diets increased the severity of colitis in a nearly dose-dependent manner (110). Increased intestinal inflammation was associated with increased colonic (106; 108; 109) and plasma lipid peroxidation products (107) and decreased plasma antioxidant vitamins (106), consistent with increased oxidative stress. Furthermore, in a long term carcinogenesis experiment in DSS-induced colitis in mice, a two-fold iron-enriched diet significantly increased colorectal tumor incidence as compared with animals fed on a control diet (110).

Control animals on iron supplementation had normal colonic histology in our study, and in all the cited studies. It seems therefore that pre-existing inflammation is necessary for visible damage to occur within these dose ranges.

Taken together, present evidence suggests that caution should be exercised in the use of oral ferrous iron therapy to IBD patients. Haeme iron formulations are probably not safer alternatives, because haeme bound iron is redox active (111). Iron-polymaltose may be an alternative for oral iron administration. Iron-polymaltose complex has a high complex stability, and a significant increase in the concentration of weakly bound iron in faeces is therefore not likely to occur following oral intake. However, evidence regarding safety and efficacy of iron-polymaltose complex in IBD patients is still inadequate.

In study III, treatments with i.v. iron sucrose and oral ferrous fumarate to patients with CD and UC were compared with regard to plasma redox status. Following iron sucrose therapy plasma MDA increased, and plasma vitamin C and plasma beta-carotene decreased, indicating increase in oxidative stress. After ferrous fumarate therapy changes were not significant. Clinically, this study is relevant because these are the ways we treat the patients. Scientifically, however, it is quite unfair. Iron sucrose in a rather high dose was administered i.v., which is in the same compartment



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as redox status was measured. It is likely that i.v. administered iron exerts its catalytical properties mainly in the intravascular compartment. Following oral iron administration it is the intestinal mucosa that is heavily exposed to the iron load. Apparently, changes in redox status in the gastrointestinal tract have to be substantial to be recognised in plasma, while intravascular changes are more readily recognised.

No i.v. iron compounds generate detectable free iron after infusion (112). However, all i.v. iron agents show evidence of a labile, biologically active iron fraction (112). The rate of labile iron release is estimated to 2-6% of total iron and follows the sequence iron dextran < iron sucrose < iron gluconate (59; 113; 114). Transferrin scavenges most of this labile iron (114), but some is bound to serum albumin, citrate, and other negatively charged ligands (115). This non-transferrin-bound iron (NTBI) has the potency to become redox active (98). The extent of NTBI following iron infusion is likely to be dose dependent (113; 116). Labile iron release may be responsible for the reactions seen when giving too much of any i.v. iron agent too fast (112).

In patients with chronic renal failure, increased plasma levels of MDA was found following infusions of 100 mg iron sucrose (116-118) and 125 mg iron gluconate (119). Increase in MDA was significantly related to NTBI (116). Also, infusion of 100 mg iron sucrose to healthy volunteers increased oxygen radical formation assessed by whole-blood electron spin resonance (120). The acute effects of i.v. iron are believed to be modest and transient, but chronic, repeated administration may produce longer lasting pro-oxidant effects (121).

The long term significance of increased oxidative stress after i.v. iron is currently unknown. In IBD, concerns are mainly related to the possibility of i.v. iron to influence intestinal inflammation. However, it was recently shown that systemic (intraperitoneal) administration of iron dextran in a moderately high dose did not exacerbate intestinal inflammation or increase colorectal tumor incidence in experimental colitis in mice (122). In chronic renal failure, oxidative stress is proposed to contribute to the increased risk of cardiovascular disease (118; 123).

Still, there is little evidence that i.v. iron adversely affects survival in patients with dialysis dependent chronic renal failure (121). Besides, anaemia itself increases oxidative stress, whereas increasing haemoglobin levels to  $> 11$  g/dL reduces oxidative stress (124). To date, the benefits of improving anaemia with intravenous iron are thought to outweigh the potential for long term iron toxicity (124).

Most studies conclude that higher doses of i.v. iron yield better results in term of efficacy (63). However, even if unproven, the potential for exacerbation of oxidative stress-related disease give rise to safety concerns regarding the administration of high doses. Until we have more information, it therefore seems prudent to avoid frequent high-dose iron infusions.

The goal of i.v. iron therapy in IBD associated anaemia is to provide sufficient iron to support maximal erythropoiesis, but to avoid iron excess. In normal circumstances, about 20-25 mg iron per day is required to support the haemoglobinisation of new erythrocytes. In anaemia and erythropoietin therapy, the erythropoietic activity is increased. A large portion of the required iron comes from red cell destruction even in anaemia, and the amount of supplemented i.v. iron required is probably between 100 and 200 mg per week. Depending of the degree of anaemia, single infusions of 200 mg iron sucrose can be given once weekly or every second week until target haemoglobin is reached.

We measured plasma (study I-IV) and urine (study IV) parameters as markers of oxidative stress. These are not ideal for estimation of oxidative stress in the gastrointestinal tract of IBD patients. Measurements in colonic biopsies would possibly be more sensitive. However, multiple biopsies are needed. In the animal models of IBD, 8-16 biopsies per colon were necessary to reveal the deleterious effects of iron on histologic colitis changes (125; 126), while 100 mg of colonic tissue was used to measure lipid peroxidation products (109). Furthermore, rectal biopsies may be inadequate for evaluation of oxidative tissue damage following oral iron therapy. The faeces passes through rectum during defecation, otherwise the rectum is practically empty. Consequently, contact time between faecal iron and the

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rectal mucosa is short. Faecal transit time is longer in the colon, increasing the probability of faecal iron to catalyse production of ROS. Therefore, flexible sigmoidoscopy or full colonoscopy with multiple biopsies, before and after iron therapy, is necessary for an optimal evaluation. In clinical practise this would take a lot of resources and make recruitment of patients more difficult.

Faecal bacteria may also be involved in oral iron toxicity. The intestinal flora is thought to play a pathogenic role in human IBD (1) as well as in experimental colitis (127). Oral iron supplementation could exacerbate intestinal inflammation by altering the microbial balance in an unfavourable manner. In line with this, ferrous sulphate supplementation in rats led to strong alterations in faecal flora such as to favour the growth of microorganisms with a pathogenic potential (128). Besides, the respiratory activity of faecal bacteria is a ready biologic source of ROS (77).

## 5.2 Clinical disease activity

In study I and III, IBD patients given oral ferrous fumarate therapy experienced increase in clinical disease activity and symptoms like abdominal pain, nausea and diarrhoea. Side-effects were more pronounced in CD patients compared with healthy controls. In study IV, an enteric coated ferrous sulphate compound had less impact on clinical disease activity and abdominal pain and nausea. Better tolerance may be related to the slow-release formulation or to low disease activity in the patients studied. There was no difference in tolerance when comparing ferrous sulphate and iron-polymaltose complex. However, three of 21 patients discontinued ferrous sulphate, while one of 19 patients discontinued iron-polymaltose complex, due to gastrointestinal side-effects.

It is believed that at least the upper the gastrointestinal side-effects seen with ferrous salts are related to oxidation with formation of ROS within the lumen of the gut (129). Furthermore, iron induced alterations in the microbial balance may possibly

cause gastrointestinal discomfort, especially in patients with small intestinal bacterial overgrowth.

In study I and IV, IBD patients taking ferrous iron tablets experienced increased stool frequency. Healthy controls experienced decreased stool frequency. The reason for changes in stool frequency is unknown.

In study III, iron sucrose infusions were well tolerated, and had no influence on clinical disease activity or specific gastrointestinal symptoms in IBD patients. When comparing iron sucrose and ferrous fumarate, alterations (from before to after treatment) in clinical disease activity did not reach statistical significance. However, all the patients completed iron sucrose treatment, while two patients discontinued ferrous fumarate treatment due to side-effects. Incorrectly, this distinction was not taken into account in the statistical calculations, as the two non-compliant patients were excluded from the analyses. Including these patients in the statistics by assigning post-treatment disease activity and symptom scores as described in paper IV, alterations in clinical disease activity ( $p=0.04$ ) and general well-being ( $p=0.03$ ) differed significantly, and alterations in abdominal pain ( $p=0.05$ ) tended to differ.

### 5.3 Iron absorption

In study I, iron absorption was evaluated by an oral iron load test. CD patients with iron deficiency anaemia had increased iron absorption compared with healthy controls with normal iron status. However, there was larger variation in iron absorption among patients than controls, especially after 1 week of ferrous fumarate therapy. A few patients with CD located in the ileum and colon had little serum iron increase 2 hours after iron administration. These patients had clinically active disease with CDAI values between 200 and 300 at inclusion, and decreased intestinal iron absorption may therefore reflect anaemia of chronic disease. Iron absorption can occasionally be impaired in extensive CD of the duodenum and upper jejunum (14).

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However, anaemia of chronic disease is probably a more common reason for decreased iron absorption in IBD.

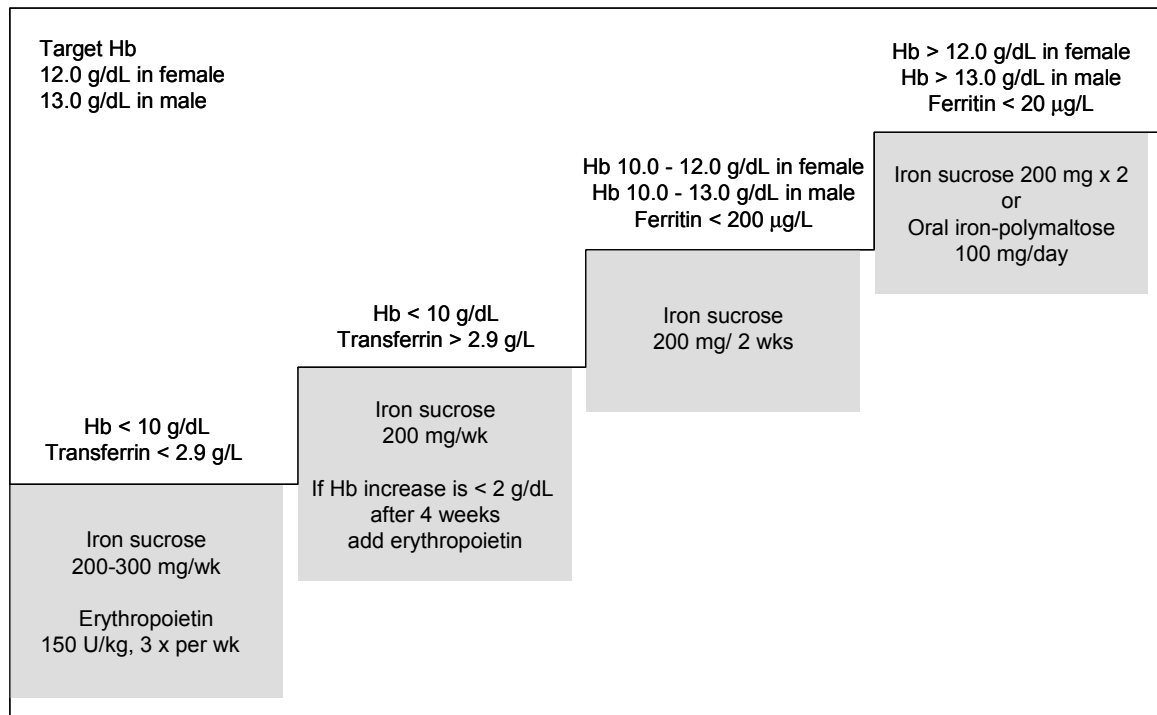
## 6. Conclusions

The main conclusions of the present study are as follows:

1. Oral ferrous iron therapy increased oxidative stress in patients with IBD. Oxidative changes are believed to occur primarily in areas of inflamed intestinal mucosa and may aggravate intestinal inflammation.
2. Oral iron-polymaltose complex therapy did not affect plasma redox status in patients with IBD.
3. Low-dose oral ferrous fumarate therapy increased intestinal inflammation in DSS-induced colitis in rats.
4. I.v. iron sucrose therapy increased oxidative stress in patients with IBD. This oxidative stress is believed to occur in the intravascular department, to be dose dependent and transient. Its long term significance is currently unknown.
5. Side-effects of oral ferrous fumarate therapy were more pronounced in CD patients compared with healthy controls. IBD patients given ferrous fumarate experienced increase in clinical disease activity and gastrointestinal symptoms. Enteric coated ferrous sulphate tablets and iron-polymaltose complex tablets had less impact on disease activity and symptoms. There was no difference in tolerability of ferrous sulphate and iron-polymaltose complex.
6. I.v. iron sucrose therapy was well tolerated, and had no influence on clinical disease activity scores or gastrointestinal symptoms in IBD patients. Iron sucrose showed better tolerance as compared with oral ferrous fumarate.
7. CD patients absorb iron. However, iron absorption may be decreased due to anaemia of chronic disease.

## 7. Clinical implications

In consequence of the evidences recently discussed we propose a modified algorithm for the treatment of IBD associated anaemia (Figure 2). The main differences from the algorithm given by Gasche et al. (Figure 1) are the lower i.v. iron sucrose dose, and the omission of oral ferrous iron in the treatment of latent iron deficiency.



**Figure 2** Algorithm for the treatment of IBD associated anaemia.

Complete blood count, iron status, vitamin B12 and folic acid must be checked before start of iron therapy. Intestinal inflammation should be actively treated to reduce blood loss and to alleviate anaemia of chronic disease. It is recommended that iron therapy is stopped in patients with ongoing infection (e.g. bacteraemia or abscess) or at transferrin saturation > 50% (8; 63).

Depending on the degree of anaemia, 200 mg iron can be given every week or every second week. Iron sucrose can be given as single doses of 100 or 200 mg, diluted in 100 mL saline 0.9%. Recommended speed of infusion is 30 min/100 mg iron sucrose. The total amount of iron needed to correct anaemia can be estimated using the

approximation that 200 mg of i.v. iron is required for a haemoglobin increase of 1 g/dL. Moreover, another 500 mg of iron should be given to replenish the iron stores. Alternatively, the total iron dose to be administered can be calculated from the formula: Total iron deficit (mg) = body weight (kg) \* 2.4 \* (target haemoglobin (g/dL) – actual haemoglobin (g/dL)) + 500 mg (40). In cases of ongoing blood loss the total amount of iron needed may be increased.

Transferrin levels below 2.9 g/L (equivalent to TIBC of 73 µmol/L), soluble transferrin receptor below 50 nmol/L (equivalent to 2.7 mg/L), or erythropoietin below 100 U/L indicated resistance to iron sucrose in IBD patients with haemoglobin ≤ 10.5 g/dL (42). These parameters may help to identify patients who benefit from additional erythropoietin treatment. The number of weeks proposed for iron sucrose therapy alone before adding erythropoietin should be complied with judgement. In patients with ongoing blood loss, treatment with iron sucrose alone should be extended. Patients with active IBD, anaemia with haemoglobin > 10 g/dL and a moderate response to i.v. iron therapy may also gain profit by combination therapy with erythropoietin.

Treatment of latent iron deficiency, iron stores are empty but anaemia has not yet developed, is a matter of discussion. If the patient does not have any complaints attributed to iron deficiency the situation can be awaited. Alternatively, i.v. iron sucrose may be administered twice, or oral iron-polymaltose may be given for several weeks. Iron-polymaltose is currently not available in Scandinavia, but may be imported with exemption from registration given by national medicines authorities. However, data regarding safety and efficacy of iron-polymaltose in IBD patients is still inadequate.

Haemoglobin and iron status (serum iron, TIBC, ferritin) should be assessed regularly during after iron therapy. Percent transferrin saturation is derived from dividing the serum iron into the TIBC and multiplying by 100. Because i.v. iron compounds interfere with clinical laboratory determination of serum iron, blood samples should be taken before or 2 days after an iron sucrose infusion (60). Serum ferritin levels



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may not accurately reflect iron stores for a period of up to 1 week after i.v. iron therapy (63).



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