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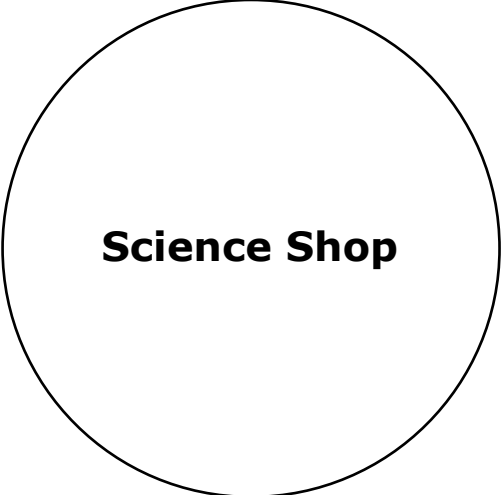
Dietary advice in *HFE*-hemochromatosis

Gerdien (G.M.) van Doorn, MSc, Wageningen University, Division of Human Nutrition
With the cooperation of: Ir. Irene (I.M.G.) Gosselink, Plant Research International

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WAGENINGEN UR
For quality of life



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Client	Hemochromatosis Association Netherlands, in collaboration with: Philip de Sterke Wilma Meerveld Hilly Hegeman Contact: Philip de Sterke, pdesterke@hotmail.com
Project coordinator	<i>Ir.</i> Irene (I.M.G.) Gosselink, Plant Research International, part of Wageningen UR
Project execution	Gerdien (G.M.) van Doorn, MSc Email address: gerdienvandoorn@hotmail.com
Financial support	Wageningen UR Science Shop
Advisory commission	Dr <i>ir.</i> Alida (A) Melse-Boonstra, Wageningen University, part of Wageningen UR, Division of Human Nutrition Dr <i>ir.</i> Hans (J.C.M.) Verhoef, Wageningen University, part of Wageningen UR, Department of Animal Sciences

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Dietary advice in *HFE*-hemochromatosis

Report Number 285

Gerdien van Doorn, Wageningen, May 2012

Hemochromatosis Society Netherlands

With the cooperation of: Philip de Sterke,
Wilma Meerveld and Hilly Hegeman
P.O. Box 252
2260 AG Leidschendam
www.hemochromatose.nl
info@hemochromatose.nl

The Hemochromatosis Society Netherlands (HVN) represents the interests of patients who are faced with hemochromatosis (iron overload disease). The HVN provides information to patients and their therapists. The HVN organizes regional and national meetings for hemochromatosis patients and their families, and provides telephone support as well as oral and written information. The HVN also provides information about hemochromatosis and related disorders to general practitioners, medical specialists, hospitals and health insurers. The HVN promotes and supports scientific research into the detection and treatment of hemochromatosis.

Plant Research International Business Unit Agrosystems

Droevendaalsesteeg 1
6708 AP Wageningen
www.pri.wur.nl/uk

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Wageningen University Division of Human Nutrition

Bomenweg 2
6703 HD Wageningen
<http://humannutrition.wur.nl/uk>

The Division of Human Nutrition provides academic education and conducts scientific research on the importance of diet to the health of human beings. They study human nutrition studies at the cellular level, the level of the individual person in a given population, and in the population as a whole; they also study the interrelationships among these three levels.

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Foreword by the client

Primary hemochromatosis is an inherited disorder. As a result of this condition too much iron is absorbed from the diet causing iron overload.

One of the most frequently asked questions in primary hemochromatosis is: "How does nutrition influence iron overload?"

It is a question that no one could answer well.

In any case, there has been no universally agreed upon answer that could be scientifically substantiated.

This is remarkable, as the condition is not rare. The disease is often hidden. Among diabetics, rheumatic and heart disease patients, as well as various others (mostly with vague complaints), hemochromatosis is hiding.

The medical literature is mostly focused on solving the problem of iron overload through many bloodlettings, so called therapeutic phlebotomies, and is barely focused on preventing iron overload.

Solving the iron overload condition consists mainly of medical interventions. In the eyes of patients, preventing iron overload by limiting the intake of iron is a very nice solution. On the one hand you tackle the problem at the same time it gives you as a patient the opportunity to take action yourself.

When we questioned doctors about the effect of the diet on iron overload, we received answers that were not clear enough for us. Perhaps we asked the wrong question since very few physicians study nutrition in medical school.

The knowledge of the Dutch nutrition center with regards to iron was also limited and dietitians often asked us for advice. We could not answer this question directly, and we certainly could not provide answers that had been scientifically substantiated.

When we heard that the Science Shop Wageningen was researching food on behalf of patients, it was clear to us that we were at the right address.

Because our question was taken seriously by the Science Shop, we as patients asked them to answer our pressing question:

"What influence has food on the absorption of iron?"

Hilly Hegeman
Wilma Meerveld
Philip de Sterke

Foreword by the author

'What to do with nutrition in hemochromatosis?' is a frequently asked question that arises when the subject of diet in hemochromatosis is touched upon. Also when searching for information on the internet this question is often asked. To quote two patients:

"I see many questions but why can't I find any answers? I would like more information on a low iron diet and the genetics that cause increased iron-values";

and

"My husband and children have been diagnosed with hemochromatosis. I read a lot of useful questions but no answers I'm urgently looking for a good list of low-iron food, nutritional tips and recipes. "

Moreover (well intentioned) advice on nutrition in hemochromatosis can be found on the Internet. One example is the site 'human-and-health'. Here is mentioned that "*certain foods (...) proportionally contain more iron than others, such as (...) breakfast cereals like cornflakes (...)*". In addition a 'low-iron diet' is mentioned several times. Specific advice is given not to consume more than the recommended daily amount of iron (18 mg), too much vitamin C, any alcohol and avoid iron-and multi-vitamin preparations. Also 'gezondheidsplein.nl' says that as a hemochromatosis patient you can "*eat two steaks every day, if you only make sure you go often enough to the blood bank to deplete your iron stores.*" You can also find there that one of the hemochromatosis patients "*limits spinach to one time every three weeks,*" a warning is given (and it is criticized) for the (large) amount of iron added to foods. Also it is mentioned that iron can be ingested by cooking in cast iron pots and pans (such as a wok) and that attention is needed for adequate fluid intake (except coffee), especially before and after a phlebotomy. A whole range of advice is available. What is missing is the rationale for nutritional advice in hemochromatosis.

This report aims to provide dietary advice which is based on what is known so far about the effect of a diet, particularly on iron overload in *HFE*-hemochromatosis. The reason that the recommendations in principle apply only to the group of individuals with *HFE*-gene mutations and are focused on the minimization of iron overload is that little research has been done on the effect of food in non-*HFE*-associated iron overload. Also little research is done into other (than iron overload) related health problems in *HFE*-hemochromatosis. Individuals with *HFE*-gene mutations – those with iron overload caused by an abnormality of the *HFE*-gene – are also the largest group of hemochromatosis patients (>90%) and therefore the advice is intended for the majority of hemochromatosis patients. Based on this dietary advice, patients can make an informed choice of whether and how they adjust their diet.

The structure of the report is as follows: As an introduction we start with an outline of the current dietary advice in *HFE*-hemochromatosis and a description of factors other than diet that affect the iron status. Then investigations into the influence of various dietary factors on iron overload are described. Finally the information, with a consideration of the role of nutrition in hemochromatosis, will lead to a (more) concrete advice. The report ends with a conclusion, discussion and subsequent recommendations for further research.

The appendices are diverse in nature but are only available in Dutch. Information on the physiology of iron metabolism in health and in hemochromatosis, and the implementation of nutrition research can be found in Appendices I and II. Furthermore, results of nutrition studies in hemochromatosis are found in tables in Appendix III. Appendix IV provides a closer examination of iron-fortified foods in the Netherlands. A summary with key information for the patient is finally provided in Appendix V.

These appendices can be downloaded from the website of the Scienceshop Wageningen UR (www.wetenschapswinkel.wur.nl)

The text references are made to the relevant Appendix. In addition, references are made to the glossary for clarification of certain terms and abbreviations by the use an asterisk (*'). This glossary is provided at the end of the core document.

Gerdien van Doorn

Summary

The standard treatment for hemochromatosis, a genetic disease in which too much iron is absorbed in the body, is phlebotomy (periodic withdrawal of blood). Usually some dietary advice is given but it is not scientifically substantiated advice. This report discusses current scientific knowledge to create well founded nutritional advice.

There is a rigorous control mechanism for the amount of iron the body absorbs from food. The absorption is therefore dependent on several factors such as iron status and needs and also genetic factors. In *HFE*-hemochromatosis the regulation of iron absorption is disrupted so that more iron is transported from the intestine into the bloodstream. The extent to which more iron is passed through depends on the gene mutation: homozygosity for C282Y (C282Y/C282Y) and compound heterozygosity (C282Y/H63D) increases the potential, H63D homozygosity (H63D/H63D) and single heterozygosity (C282Y/wt or H63D/wt) gives less potential for iron overload.

The total amount of iron in the diet affects the iron overload in hemochromatosis patients, at least for C282Y homozygotes. Besides the total amount of iron in the diet, the type of iron in the diet should be taken into account: heme iron and non-heme iron. Heme iron is only found in meat. Non-heme iron also comes from plant sources and non-cellular animal sources (egg and milk products). The two forms of iron have a different kind of absorption in the intestines. Heme iron has a higher bioavailability than non-heme iron because of a different absorption mechanism and a relatively low influence of enhancers and inhibitors. Enhancers are nutrients that promote iron absorption from food. Alcohol, organic acids (especially vitamin C) and (an unknown factor in) meat are known as enhancers of the non-heme iron absorption. Proven inhibitors of non-heme iron are phytate (phytic acid), polyphenols and calcium. The total effect on iron uptake of the enhancers and inhibitors in the diet is difficult to determine and is not an addition and subtraction of the individual impact.

An important question, when it comes to the role of diet in the treatment of *HFE*-hemochromatosis, is how much bloodletting may be omitted if the diet is adjusted. By choosing foods that contain relatively little iron, the total iron intake can be significantly reduced. Theoretically – taking into account the bioavailability of the different forms of iron – with relatively minor adjustments this could reduce the number of phlebotomies by at least two per year. The completeness of the diet in the sense of providing sufficient other vitamins and minerals can (and should) be safeguarded.

In addition to reducing the number of phlebotomies, nutrition may affect co-morbidities in *HFE*-hemochromatosis like colorectal cancer, cardiovascular and vascular diseases, liver diseases and infection with the bacterium *Vibrio vulnificus*.

For the overall dietary advice in *HFE*-hemochromatosis the rules for a healthy diet prepared by the Dutch Nutrition Center is the starting point. Therefore, the recommended amounts of fruits, vegetables, potatoes, bread, cheese and milk are the same as they are for the general population. In addition to the general recommendations, some specific recommendations can be given: consumption of (red and/or organ) meats is not recommended, because this is a major contributor to the total iron uptake. A great alternative to meat is the inclusion of non iron-fortified meat substitutes including (oily) fish explicitly, twice a week. Fruit and (vitamin C-rich)-drinks are best consumed with non-iron-containing foods (for example between meals). Consumption of alcohol leads to multiple health risks and should therefore be avoided.

While there is no convincing evidence that iron fortification of foods has a detrimental effect on the iron status of *HFE*-hemochromatosis patients, the use is discouraged on rational grounds. Taking iron and vitamin C-containing supplements is also not recommended. Iron-fortified foods and iron-containing supplements are also a 'threat' to carriers of *HFE*-gene mutations. It is expected that consumption of these products will cause their disease to manifest itself sooner than would otherwise be the case.

1 Introduction

Hemochromatosis is a disease in which the metabolism of iron is disturbed. This disturbance is also present in the intestinal cells, where more dietary iron is absorbed and/or transited¹ than necessary. Dietary advice might help hemochromatosis patients. Nutrition is currently not a standard part of hemochromatosis treatment.

Theoretically considered, the accumulation of iron can be prevented if no iron is ingested. A small amount of iron is needed to offset daily losses for example through feces and skin. In this way, iron deficiency, often leading to iron deficiency anemia, is prevented. Moreover, it is unavoidable to take in iron because almost all foods contain the nutrient. Iron is also part of foods that provide the basis for a healthy diet. A diet that provides no iron is thus deficient, it falls short in the supply of energy and/or vitamins and/or other mineral substances needed for good health. However, the amount of iron ingested can be limited by mainly choosing products with a low iron concentration.

The absorption of iron varies by type and form and is dependent on the presence of other nutrients. The forms of iron that can be distinguished in the diet are heme and non-heme iron. Both forms have their own way of 'processing' in the body, resulting in varying degrees of absorption. Besides the iron found naturally in food – the native iron –, iron is added to foods and supplements. This iron also has – depending on the form – a varying degree of absorption. Other nutrients also influence the absorption of iron. They can increase or decrease the absorption. By paying attention to both the quantity, type and form of iron and other substances in the diet it might be possible to influence the iron accumulation in the body.

The nutritional advice that is given to patients during the maintenance phase² is generally fully optional. Phlebotomy is proposed as an easy and natural method of treatment; following a diet is, compared with phlebotomy, considered as less effective [3] or efficient [4]. The working group Hereditary hemochromatosis that drafted "evidence-based guidelines for the diagnosis and treatment of hereditary hemochromatosis according to the method of the Institute for Healthcare Improvement CBO" commented that there is uncertainty about the benefit of dietary advice. They also indicated that less iron is "certainly better", but they were in doubt whether any decrease in the frequency of phlebotomy, outweighs the reduced quality of life resulting from following a diet [5]. The caution with which the existing diet is described is a logical consequence of this dubious position.

Some dietary guidelines are given in varying degrees. A healthy diet is recommended by the European Association of the Study of the Liver rather than restrictions of excessive iron intake through food [6]. Also, the directive of this organization argues that alcohol consumption in hemochromatosis can worsen liver damage and can increase the absorption* of iron. A recommendation is lacking. It is also often lacking in Dutch educational materials for both physicians [7] and patients [8], when it comes to the treatment of hemochromatosis. Nevertheless, alcohol intake is also mentioned in other resources for both groups [3] [5]. Also, the use of food supplements that contain iron [3] and/or a (high) dose of vitamin C [3] [6] is discouraged. Furthermore, drinking (black) tea during meals and the intake of much (red) meat is mentioned [3] [5].

From the question of what effect nutrition has on the accumulation of iron in hemochromatosis patients, in the following section various studies are discussed to arrive at dietary advice.

1 Iron absorption from food in the body take place in two steps. More about this can be found in Appendix II.

2 The treatment of hemochromatosis can be divided into a depletion and maintenance phase. The depletion phase* covers the period of frequent – weekly to monthly – phlebotomies to remove the largest amount of stored iron. During the maintenance phase, the amount of iron removed is that stored between two phlebotomies.

2 Non-nutritional factors that affect iron status

The iron in the diet we ingest is not fully absorbed. On the one hand, this incomplete absorption is due to the bioavailability* of iron. On the other hand it is the body that, in a healthy situation, is very careful in regulating the absorption* of iron to meet the demand. Large fluctuations in iron intake during the day and subsequent days will thus not easily cause health problems. The degree of iron uptake is thus dependent on several factors (Explanation 1, page 15). Animal studies suggest that a complex system – with a close correlation between iron status and iron-demand, genetics and nutrition – exists to regulate the absorption in the gastrointestinal tract [9].

Explanation 1 Absorption of nutrients

The amount of a nutrient which is absorbed depends on factors related to the substance and the body. Professor West of Wageningen University enclosed these factors with the concept of SLAMENGHI. It allows to define the bioavailability* of a nutrient: "the part of the ingested substance that is available to the body for the use of normal physiological functions or for storage" [1]. The letters of the word represent individual concepts Species, Linkages at the molecular level, Amount consumed in a meal, Matrix, Effectors of absorption and bioconversion, Nutrient status of the host, Genetic factors, Host-related factors, Interactions among all variables [1]. Specifically for iron this can be literally (' figuratively ') 'translated' to:

- Species: ferrous, ferric
- Linkages at the molecular level: heme, non-heme iron
- Amount of iron per meal
- Matrix: food structure e.g. the presence of iron in the seeds of cereals
- Effectors of iron absorption* and bioconversion: enhancers* and inhibitors*
- Iron status
- Genetic factors: gene mutation(s) for genes involved in iron absorption
- Host-related factors: e.g. iron demand, influenced by for example pregnancy
- Interaction among all variables: not applicable

It is indisputable that the iron status leads to differences in iron absorption. To maintain the level of iron in the body, it seems that the body is 'equipped' with a feedback mechanism [10]. All in all, the lower the iron status and/or the higher the requirement for iron of a person, the higher the efficiency of absorption* and vice versa [11] [12]. This is related to the hormone hepcidin, a hormone³ that probably plays a key role in maintaining the balance of the iron status of the body⁴ [14]. One component of this is the influence of ferroportin⁵, a transport protein⁶ in the intestine that regulates the throughput of iron into the bloodstream [13] [15] (Figure 1 , page 16). The higher the serum ferritin-value⁷, the higher the serum hepcidin-value⁸, the lower the absorption* of iron [16]. (More information can be found in Appendix I.) It was suspected that the feedback system explicitly would commence from a serum ferritin of 60 µg/L [11]. Since research also pointed to 'set point' values from 14 to 350 µg/l [17] and because the mean serum ferritin concentration of the general population is higher than 60 µg/l [12]⁹, the 'value shift' of 60 to 70 µg/l is under discussion.

3 A substance that is secreted by a gland and causes an effect in the body.

4 This refers to the total amount of iron in the body. In practice it is usually derived from the ferritin values in serum (part of the blood) [13], see footnote 7.

5 The transport protein that iron transports from the inner to the outer of the cell.

6 A protein particle that transports other substances in or out of the cell.

7 The amount of ferritin in the serum (part of the blood), which turns out to be a good reflection of body iron stores [13].

8 The amount of hepcidin in serum (part of blood).

9 The normal values for serum ferritin will vary from site. In general, male and female values of 15-320 mg/l and 6-155 mg/l respectively held [18].

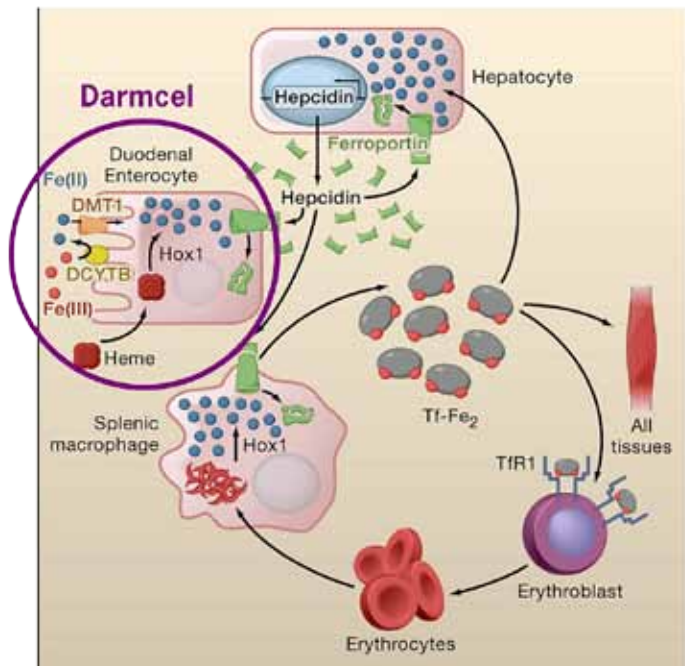


Figure 1: Overview iron metabolism [2].

It is known that genetic abnormalities play a role in iron uptake (Explanation 2 , page 17). When there is a defect in the *HFE*-gene* the feedback system works less well. The gene regulates the production of the HFE-protein*, which in turn is related to the production of hepcidin [19]. Because of the lower production of the HFE-protein* and hence the hepcidin, more iron is transported from the enterocyte into the bloodstream. Presumably, the feedback mechanism still works in hemochromatosis [20], but it has a much higher 'adjustment' [21] [22].

The extent to which the control mechanism is still active, is probably different for each gene mutation [23] [24]. Homozygosity for the C282Y (C282Y/C282Y) and compound heterozygosity (C282Y/H63D) give more potential for iron overload, and H63D-homozygosity (H63D/H63D) and single heterozygosity (C282Y/wt or H63D/wt) reduces the potential for iron overload [25]. (People with the "milder" forms are often not diagnosed with hemochromatosis, because the amount of iron that is accumulated in the course of a lifetime is not high enough to cause complaints.) To what extent the gene mutation S65C and others, in the past suspect, (combinations of) mutations also contribute to iron overload is doubtful [13] [19] [26].

Besides the gene mutation and the iron status other factors affect iron absorption in the body. Women lose blood during menstruation and pregnancy – they lose hemoglobin¹⁰ and hence iron. This makes a difference in iron overload amongst men and women of childbearing age [18] [27]. Also, inflammatory activity, drug use and the metabolic syndrome¹¹ and/or Body Mass Index* have an effect on iron overload [12 , 13]. All of these conditions, therefore, result in differences in the amount of iron that is absorbed in individuals with hemochromatosis. Estimates range from 3 to even 10 mg of iron uptake per day, compared with a regular uptake of 1 mg daily in persons without iron deficiency or surplus [28] [29].

¹⁰ An iron-containing red protein particle in red blood cells that can bind oxygen.

¹¹ A combination of frequent abnormalities of blood pressure (hypertension), blood glucose values (diabetes mellitus, also known as diabetes), cholesterol (hypercholesterolemia), obesity and the often associated proteinuria (increased protein excretion in the urine).

Explanation 2 *HFE*-mutations

Hemochromatosis has, genetically looked at, various forms. The different forms give rise to generally more or less iron overload and hence expression of the disease. The deviations in the *HFE* gene* that are currently known, are C282Y, H63D and S65C. (The letters refer to amino acids that are replaced, the numbers to the place in the *HFE*-protein* where another amino acid is incorporated.) The mutations can occur in different configurations: (The genetic information is stored in chromosomes, they occur in pairs, because both parents provide one *HFE* gene at conception.)

- homozygosity: same genetic defect on both chromosomes
 - C282Y/C282Y
 - H63D/H63D
 - S65C/S65C
- heterozygosity: a genetic defect on any of the chromosomes
 - C282Y/wt
 - H63D/wt
 - S65C/wt
- compound heterozygosity: different genetic abnormalities in both chromosomes
 - C282Y/H63D
 - H63D/S65C
 - S65C/C282Y

If no abnormality occurs on both chromosomes, this is indicated as wt/wt.

3 Diet-related factors that affect iron status

The next section focuses on nutrients. These are associated with foods rich in the substance. First, the uptake of various forms of iron (the origin-related groups: native and non-native) are discussed, then the enhancers* and inhibitors* of iron uptake. Under the heading "mechanism" a description of what is known about the influence of specific dietary factors on iron metabolism is discussed. Subsequently, results of studies with hemochromatosis patients and persons without hemochromatosis are described successively. The studies are usually divided into experimental and observational studies. In appendix II, the differences between these studies are further explained. Furthermore, the methods, results and conclusions of the mentioned studies on dietary research in hemochromatosis can be found in Appendix III.

The studies on the effect of food in hemochromatosis show that in recent years primarily through observational studies * attention has been given to (food) factors that lead to the expression of hemochromatosis. Since 2005, no further experimental studies * have been conducted. (Mid-2011 probably an investigation will be launched [Dr. K. Allen, The Royal Children's Hospital, Australia, personal communication]). The research before this stems mainly from the time before 1996 when the location of the mutation in the *HFE*-gene* (and thus determination of the hemochromatosis type) had not yet been identified.

3.1 Native iron

The iron that is naturally in the diet, is indicated as 'native iron'. There are two distinct forms: heme and non-heme iron. Heme iron is only found in poultry, fish and meat, and their derivatives. Non-heme iron is widely present in animal and vegetable foods.

In the study of iron absorption it is important to separate these two forms. The bioavailability* of the two forms is different which is attributable to a different route of uptake; heme iron is absorbed 15-35% [14] [30] and non-heme -iron is absorbed 5-15% [10] [11]. (More background information on the absorption of iron can be found in appendix I.) Furthermore, it seems that the extent to which iron absorption decreases is dependent on the form or type of iron.

Mechanism

When there is a failure in the system for the uptake of iron, the iron in the diet will contribute to iron overload. If less is taken in, less can be absorbed. This 'logic' does not apply to people without the iron metabolism disorder because they have the strong regulatory effect of hepcidin, related to iron status and -requirement. In addition, the body is equipped with a so-called "mucosal block" effect. This concept shows the influence on uptake at intestine level: by changing the intestinal mucosa, the absorption* of iron a short time after a high iron dose, can be lower than that at a later point in time [31]. Whether this applies only to one form of iron (heme or non-heme iron) and whether – and to what extent – hepcidin is involved in this mechanism – and hence whether the system still works in *HFE*-hemochromatosis – is unknown.

Effect in practice

One of the first research groups that investigated the absorption of iron by hemochromatosis patients was the group of Walters [20]. They indeed found a higher absorption* of iron in individuals with hemochromatosis compared with individuals without hemochromatosis when exposed to higher doses of iron [20]. Although the genetic backgrounds of the people in this survey (in 1975) were unknown¹², it is likely that the hemochromatosis patients mostly were C282Y-homozygous individuals.

¹² Since 1996, it has been possible to determine the gene mutation for *HFE*-hemochromatosis. Before that time, the person with a (too) high serum ferritin value was 'suspected' of hemochromatosis, so called 'idiopathic'* hemochromatosis.

The results in individuals heterozygous for the C282Y-gene mutation are contradictory. One research group found a difference in iron absorption from a meal [32], yet, another research group found no difference [30].

Studies of individuals without a defect in iron metabolism indicate a positive relationship between the serum ferritin value and total iron intake [25] [33] and consumption of iron-rich foods such as liver and paté [34].

Conclusion

The total iron intake affects the total iron overload in hemochromatosis, at least for C282Y-homozygotes.

3.1.1 Heme iron

In general, heme iron comprises quite a large proportion of the supply of iron needs.. Meat, chicken and fish are the only products that deliver heme iron. The contribution to the total iron intake of heme iron for non-vegetarians is between 10 and 15% [14]. Due to the high bioavailability* of heme iron it contributes to (over) 40% of the total iron intake [14]. Especially red meat is rich in heme iron; it contains more than 1.5 mg of heme iron per 100 grams of prepared product [35]. On the other hand, an equally large piece of prepared chicken delivers 0.2 mg of heme iron [35]. Because heme iron is found only in meat, there are also studies in which the relationship between meat intake and iron status can be taken into account when assessing a possible relationship between heme iron intake and the iron status in hemochromatosis.

Mechanism

That heme iron has a high bioavailability*, can thus be explained 1) heme iron in contrast to non-heme iron can pass 'directly' through the intestinal wall and hence is absorbed [10] and 2) heme iron is less 'captured' by other substances [36]. (More background information can be found in appendix I.)

Effect in practice

Several absorption studies* indicate that the absorption of heme iron in humans with *HFE*-gene defects is higher than that in persons without *HFE*-gene*- defects: Both Bezwoda et al. [21], Hunt and Zeng [30] as well as Lynch et al. [32] worked out a higher absorption* percentage, although the difference between the group of hemochromatosis patients and the control group* was not always statistically significant*. Lynch et al. [32] calculated a similar uptake between (idiopathic*) hemochromatosis patients and controls * with a serum ferritin value of 50 µg/l and found no correlation between heme iron intake and serum ferritin values in hemochromatosis patients. Several researchers investigated the possible difference in the uptake of heme iron in an iron-fortified meal and they found no difference between the control group* and the people with C282Y/wt-hemochromatosis [30] [37]. Hunt and Zeng [30] however, found a correlation between heme iron uptake and serum ferritin value. Some of the above mentioned and other [23] researchers also found a positive relationship between heme iron intake and serum ferritin values in hemochromatosis, which indicates a higher uptake.

Observational studies, as well as experimental studies*, show no hard links between the heme iron intake and serum ferritin values: Van der A et al. [25] split the heme iron intake into three categories and also divided the risk of iron overload caused by the *HFE*-mutation (Explanation 2 *HFE*-mutations, page 17) into three groups, namely: none, small and large. In the lowest category of intake (0.00-1.42 mg daily) they found a statistically significant* difference for the effect of the intake of heme iron on the serum ferritin values for the groups with a small (C282Y/wt , H63D/wt and H63D/H63D) and a high (C282Y/H63D and C282Y/C282Y) potential for iron overload compared to the group without potential. Additionally, it seemed that at a high intake of heme iron there was an association between the 'high risk of iron overload group' and serum ferritin levels of persons over the age of 50 years [25]. In the study by Cade et al. [34] and Greenwood et al. [38] (the same research group), the effect of heme iron intake on serum ferritin values were only for C282Y/C282Y-group such that it could be seen as a statistically significant* difference between this group and the group without genetic mutations. Greenwood et al. [38] calculated that the effect of heme iron intake on the serum ferritin value was twice as great for the C282Y-homozygotes than for other groups.

Several observational studies have investigated the effect of meat intake on serum ferritin values. The intake of red meat did not give statistically significant* different serum ferritin levels for groups with and without mutation of the *HFE*-gene* in several studies [9] [39] [40]. Rossi et al. [39] found, however – although the difference was not statistically significant* – higher serum-ferritin-values for men with C282Y/wt who daily eat meat (approximately 15% higher than in subjects without a mutation). Allen et al. [41] described a similar result: in the 'C282Y/C282Y-group' with elevated serum-ferritin there were more men and women with 'meat consumption of more than four days a week' than in the group with normal serum ferritin values.

The absorption studies* a weak to absent relationship between heme iron intake and serum ferritin values indicate for persons without a *HFE*-gene* mutation. Also for hemochromatosis patients it was shown in experimental studies* that the intake of heme iron is not considered statistically associated with iron status [30] [37]. The other way around, an increase in serum ferritin levels in subjects without hemochromatosis using a meal with high bioavailability* of iron, an iron-fortified meal and supplements could not be explained by a higher heme iron absorption [10] [32] [42]; the contribution of the non-heme iron to the absorption increased. Probably, the absorption of both forms of iron stays similar in serum ferritin levels to about 10 µg/l (uptake rate of approximately 50%) [42] and decreases then for both forms of iron, although this reduction is smaller for heme than non-heme iron [43].

Many observational studies in people without hemochromatosis have found relationships between heme iron intake and serum ferritin levels. This correlation was found in women [25] [33] [34] [38] and the elderly with an adequate iron status [44]. Aranda et al. [45] were the only ones that found no effect. Fleming et al. [44] and Greenwood [38] calculated for each intake of 1 mg of heme iron per day an increase of the serum ferritin value of 46% and 41% respectively.

However, for the meat intake of the general population in observational studies, statistically significant* relationships were found between meat intake (both red and white) and serum ferritin levels [9] [33] [34] [39] [46]. Also the intake of fish and chicken may be related to the iron status [33]. Further the lower average serum ferritin value of vegetarians confirms an interrelationship between meat intake and the serum ferritin value [33].

Conclusion and discussion

Although many observations can be placed in the investigations (see Appendix III, column 'Conclusion + comments'), it can be concluded that the heme iron intake is little – and in relation to non-heme iron is less – influenced by iron status. Ideas about the 'transition' of (a part of) the heme iron [36] support this notion.

To what extent a reduced sensitivity to the already relatively high uptake of heme iron (due to the high bioavailability*) with higher serum ferritin levels also applies to hemochromatosis patients cannot be said with much certainty on the basis of the investigations. While it appears that the heme iron absorption is relatively higher than in subjects without genetic mutations of the *HFE*-gene*, it is impossible to substantiate this, because in the studies where the absorption* rate was calculated the serum ferritin values were below 40 µg/l [21] [30]. At relatively low serum ferritin values for hemochromatosis patients, the uptake of heme iron is about 35% [21] [30].

The results for the coherence of the heme iron intake and serum ferritin values in observational studies are fairly obvious. Meat increases the non-heme iron absorption (see 3.3.3 'Factor X' in meat', page 28) and in observational studies* this cannot be measured. (Only the relationship between meat intake and serum ferritin levels can be determined.) In observational studies, it was found that there was a relationship between heme iron intake (and meat) and serum ferritin levels in people who had a healthy iron metabolism. This was not found in hemochromatosis patients and may be related to the study population and the methods used (see Appendix III, table 'Effects of nutrients and food on the iron status, found in observational studies', column 'Conclusion and comments'). Because it is common to ingest heme iron only from meat, it is good to include the 'enhancer' effect of meat and the influence of meat instead of only looking at the direct effect of heme iron on serum ferritin values. Based on the above observation it is expected that the intake of meat (including the heme iron) is a relatively large contributor to the overall iron status in hemochromatosis patients. Advice for reducing meat intake in *HFE*-hemochromatosis in order to reduce the absorption of iron is thus in place. The group of persons who load the most iron – men and non-menstruating women with the C282Y homozygosity and compound heterozygosity of C282Y/H63D – will benefit most from a reduction in meat intake.

3.1.2 Non-heme iron

Non-heme iron is all the iron from vegetable and non-cellular animal sources (such as egg and milk products) and about half the iron in meat, fish and chicken [47].

Mechanism

The uptake of non-heme iron in the enterocyte can be divided into several steps. To our knowledge, only one transport protein* is known. This transport cell can only take up a specific form of iron – a 'ferrous' part [36] [48]. Hence the iron that is not yet 'ferrous' – most of the non-heme iron in the diet – has to be transformed to be 'ferrous'. Certain conditions influence the transformation [49]. Also, the conversion as well as the uptake of non-heme iron increases with iron deficiency, because the production of cells that are involved increases [48]. (Appendix I provides a detailed description of the mechanism.)

Effect in practice

The adaptation of the body to the demand of iron has been proven in hemochromatosis patients. Absorption studies* indicated that there is a relationship between serum ferritin values and the non-heme iron absorption in idiopathic* hemochromatosis patients [32] and C282Y-heterozygotes (with iron-fortified foods) [30] [50]. Also it looks like the uptake of non-heme iron at higher doses is less compared to that of lower doses [22]. The moment of 'feedback' seems – different from those without mutations of the *HFE*-gene* – to start at serum ferritin values >200 µg/l [32]. A higher intake of non-heme iron in idiopathic* (including heterozygous) hemochromatosis patients [21] [32] than in assumed healthy subjects has been found at least. Other researchers found no increased uptake in diagnosed heterozygous hemochromatosis patients, particularly not if large amounts of iron were added for an enriched meal [30] [50].

For non-hemochromatosis patients observational studies also have proven an inverse relationship with serum ferritin values. Experimental studies* [32] [50] showed that the non-heme iron absorption is lower at higher serum ferritin values. Also an observational study by Cade et al. [34] showed that at a higher non-heme iron intake, the serum ferritin value is lower. However, the latter was not found in the Framingham Heart Study [44], a study by Van der A et al. [25] and a French study [33].

Conclusion and discussion

The results of investigations into the relationship between serum ferritin values and the non-heme iron intake and uptake do not point in one direction. However, the absorption studies* seem to indicate that the iron status determines the absorption* of non-heme iron. The non-heme iron intake has, due to the mechanism of uptake, therefore no effect on the iron status in individuals without hemochromatosis with a good iron status [12]. Individuals with abnormalities in the *HFE*-gene* in general take up – especially at low serum ferritin levels – more of the non-heme iron. It is expected that for the mutations C282Y/C282Y C282Y/H63D the non-heme iron absorption will be larger than for the C282Y/wt-mutation at an average non-heme iron intake and serum ferritin value. Hence that hemochromatosis patients would be wise to limit their non-heme iron intake to prevent an increase in the degree of iron overload.

The bioavailability* of non-heme iron is strongly influenced by meal composition. Thus, the absorption* can range from 2-45%, depending on the amount of enhancers* and inhibitors* (see 3.3 'Enhancers and inhibitors', page 25) in the diet [44]. In the study by Hunt and Zeng [30], for compound heterozygosity for C282Y and H63D, the effect of high doses of non-heme iron was only reflected in the serum ferritin value when iron was taken under 'favorable conditions' (taking into account the amount of inhibiting substances and stimulating nutrients and -agents). According to Roe et al. [50] the difference of non-heme iron absorption* in C282Y-heterozygotes is not strongly correlated with those of persons without any *HFE*-gene mutation who have a diet with a relatively large amount of enhancers* (440 mg vitamin C and 364 grams of meat , fish or chicken a day¹³).

13 In comparison, the recommended daily allowances for vitamin C and meat are 70 mg and 100 grams respectively.

3.2 Non-native iron

Iron that is not naturally present in foods is labeled as non-native iron. These include iron added to food, iron that is ingested through food supplements and iron that ends up in food due to 'pollution' ('contaminant iron').

3.2.1 Iron-fortified foods

Iron-fortified foods can be viewed or distinguished from multiple perspectives; based on the type and form used and the product group. When food is fortified, however, usually only the non-heme iron form is used [47]. The non-heme iron compounds added to products can be roughly split into three groups: elemental iron powders, iron salts and iron chelates. Each type has properties that have advantages and disadvantages for a specific application. In the Netherlands, enrichment of iron is mainly used in: cereals, meat substitutes, (between) biscuits, bread and apple syrup. It is also permitted to add iron to black olives for the preservation of the color. This is stipulated in the Commodities¹⁴ [51]: Application of iron to prevent color change is explicitly approved only for black olives. The added content is usually about 15 mg per 100 gram drained weight, the maximum allowed amount (appendix IV).

Also the uptake of the added iron is dependent on the medium with which it is taken. This is because the effectiveness of the added iron is also determined by the presence or absence of enhancers* and inhibitors*. Thus – as with the naturally occurring iron in the diet – the presence of meat or ascorbic acid (naturally present or added [47], see 3.3.2 'Organic acid') and fat [47] will affect the absorption [52] .

Mechanism

It is assumed that the added iron enters the body in the same way as the naturally present non-heme iron in food [11] .

Effect in practice

The results of absorption studies * in hemochromatosis have been mixed: One study found a difference with added iron sulfate [32] and the other study no difference with added iron citrate [53] and iron ascorbate [21] and iron sulfate [50] in the uptake between (idiopathic*) hemochromatosis patients and/or carriers* and people who have a good iron status and/or people with iron deficiency. For hemochromatosis patients, the effects of the use of iron-fortified foods, found in observational studies, are less dubious: Well known is the study of Olsson et al. [54] who studied what effect stopping the enrichment of flour with iron carbonyl in Sweden had on iron overload in people with hemochromatosis. The followed subjects absorbed less iron (-0.65 mg/day; before: 4.27, after: 3.63, approximately 12% and 27% of total absorbed iron) and needed fewer phlebotomies per year for maintenance treatment [54]. Cook [28] also predicted, based on the results of the study, that continued enrichment of flour will lead to an accumulation of 0.1-2.1 mg per year, depending on the serum ferritin value and assuming an intake of enriched iron of 3 mg per day.

In people without hemochromatosis, it is suspected that the effect of iron enrichment is absent or negligible. Experimental studies showed that iron status did not improve when men took 7.5 mg daily for two years as well as 10 mg for 500 days of ferrous sulfate [12]. Another study indicated a linear relationship between the absorption* of iron and a serum ferritin value at a dose of 3 mg Fe²⁺ [55], supposing that the serum ferritin value is the determinant factor in uptake.

In Scandinavian countries and the United Kingdom a debate about the use of iron-fortified flour gave rise to a study on population level for its effect. In the countries mentioned, flour has long been fortified with iron to ensure an adequate intake. A study in the United Kingdom suggested that little or nothing of the added iron would be absorbed [34]. Also in the UK Women's Cohort Study – as well as for the consumption of iron-fortified breakfast cereals – no conclusive link was found between the intake of iron-enriched white or brown bread and serum ferritin values [34]. Similarly in Denmark there was no change in the iron status of elderly people after stopping the enrichment of flour [12].

14 The law that controls the composition and inspection of food.

Conclusion and discussion

Iron fortification of food has, several times, been found to have no effect, or negligible effect. The assumption is that the iron-enrichment will have no adverse effect; a high iron level resulting from intake of iron-fortified foods is not expected [55]. Furthermore, it is thought that added iron will not negatively affect the uptake of other divalent ions¹⁵ such as zinc, manganese and copper [47]. Do iron-fortified foods pose a 'danger' to hemochromatosis patients? Possibly the type of iron used in the enrichment along with the bioavailability* and the iron status of the individual are ultimately the common factors that determine the absorption of the added iron. Unfortunately, the studies made with the hemochromatosis patients are different for the type of iron used and the genetic mutations. Rationally considered, the use of fortified products by hemochromatosis patients is inadvisable. At a relatively high consumption of iron-fortified foods, the iron intake can quickly go above 100% of the recommended daily allowance (nutrient)*. For hemochromatosis patients this can lead to accelerated higher serum ferritin levels, thus reducing the interval between phlebotomies. Cook [28] deduced from studies into the effect of fortification of wheat flour – established as national policy – that a 20% increased intake of iron from fortified wheat flour would require 1-2 phlebotomies more per year. Moreover, the use of iron-fortified products is also a potential 'danger' for carriers*. For them symptoms may occur earlier. Bezwoda et al. [22] estimate that heterozygotes will have an iron storage in the body at most up to 4.3 gram compared to 1.6 gram at the age of 50 with the use of enriched flour (from 13.0 compared to 16.5 mg per 500 gram flour, respectively). In addition, they suspected that the time required for manifestation of iron overload would be shortened by 20% [22]. Appendix IV has a more extensive discussion of the use of iron-enrichment and also provides more information about the types of iron that may be used, the effects of iron enrichment and an overview of a random selection of iron-fortified foods.

3.2.2 Iron-containing supplements

In addition to iron supplements, most multi-vitamin and mineral supplements contain iron. As with the fortified foods, the type of iron that is part of the preparation varies. In the Dutch Commodities decree on supplements [56] it was established that the following forms of iron may be added to supplements: iron (II) carbonate, iron (II) citrate, iron (III) ferric or ferrous ammonia citrate, iron (II) gluconate, iron (II) fumarate, sodium iron (III) diphosphate, iron (II) lactate, iron (II) sulfate, iron (III) diphosphate (iron (III) pyrophosphate), iron (III) saccharate, elemental iron (carbonyl + electrolytic + hydrogen reduced) and ferrous bisglycinate. The iron in most preparations is present in the ferrous form, the more directly absorbable form of non-heme iron [57]. The dose of iron in a preparation is currently (still) unlimited¹⁶.

Effect in practice

While it is unethical to experimentally investigate the effect of the use of iron supplements in hemochromatosis, data is available. This data come from inquiring about the use of dietary supplements containing iron in the time that people were not yet diagnosed with hemochromatosis. A poll found that 27% of (idiopathic*) hemochromatosis patients used iron-containing supplements before the diagnosis [58]. Allen et al. [41] reported in their compliance study¹⁷ that nearly one fifth of newly diagnosed patients with an elevated serum ferritin stopped the use of multi-vitamin tablets. Barton et al. [59] described a case-study¹⁸; before being diagnosed, a C282Y homozygous patient took an iron supplement with 60 mg of ferrous gluconate for seven years. It was calculated that 20.9% of the supplemented iron was absorbed and stored in the body of the person [59]. Consumption of dietary preparations containing iron and/or vitamin C appear to influence the iron status. Large observational studies like the Framingham Heart Study [44] showed (63%) higher

15 Atoms that are electrically charged by a lack or excess of one or more electrons, and that both can take up or cede (one) electron (s).

16 Written response from Mr J. Homma, senior inspector, specialised expert BED at the Dutch Food and Drug Administration dated 26/01/2011 "at present there are (yet) no set limits for the amount of iron for use in dietary supplements. The Directive 2002/46/EC that will provide limits at this point has not yet been completed. National member states may include limit values in their legislation, but these may not hinder trade. The Netherlands has not determined a national standard for the amount of iron and always refers to the safety standards that should be observed under HACCP procedures by manufacturers, importers and sellers."

17 Research into the participation in a treatment (eg. follow-up of given advice).

18 A detailed study of a single research object, such as a specific person or an individual case, with the aim of understanding the research object in its various aspects, complexity and possible development as thoroughly as possible.

serum ferritin values while using iron supplements. Other studies have also suggested a positive relationship between iron supplementation and the serum ferritin value [10]. Hallberg [55] suggested, however, that iron from supplements probably does not influence iron stores as much as native iron. Cade et al. [34] also assumed that the effect of supplements on iron status is low to negligible when food supplies enough iron. They found, like Pedersen and Milman [46] in their observational study, no relationship between iron supplement use and serum ferritin levels in subjects without hemochromatosis.

However, as a result of a screening into iron supplementation doubts are raised that iron supplementation has no effect on iron absorption* when there is an adequate iron status: In Norway, out of 120 people, who were identified as having idiopathic hemochromatosis on the basis of their symptoms and elevated ferritin levels, eleven were found to have no mutation of the C282Y or H63D when diagnostic accuracy was checked by determination of *HFE*-gene mutations [12]. Of these eleven, nine persons used iron supplements for 10 to 50 years [12].

Conclusion and discussion

The use of iron-containing supplements may not be meaningful and perhaps not without risk if the serum ferritin levels are good. Firstly, the question is how high the uptake of the iron in the multi-vitamin and mineral supplements is, because it has to compete with the other metals that are included in the supplements (zinc, copper, manganese) [46]. On the other hand, it seems to be absorbed and cause higher serum ferritin values. The use will therefore be risky, and – according to research – not only for carriers* of genetic defects of the *HFE*-gene*, but also for non-hemochromatosis patients. With the fact that in 2003 more than 20% of the Dutch population used multi-vitamin supplements and thus used 5.0 mg (26%) (women) and 3.9 mg (19%) (men) of extra iron daily [60], this is no unreal 'danger'. The use of supplements with iron, if iron deficiency does not exist, is for that reason, not recommended.

3.2.3 Contaminant Iron

Small amounts of iron are ingested inadvertently by 'pollution'. Examples include iron from materials used, for example when cooking. This iron belongs to the non-heme iron group [47].

Effect in practice

Although the use of equipment for the preparation of food has changed significantly, iron-containing material is still being used. These include cast iron pans, steel woks, frying pans and pancakes griddles. In addition, many kitchen utensils are made of stainless steel, which consist of at least 50% iron [61]. This iron can be released and thus end up in food [11]. This iron is also available for absorption [61] [62]. Especially when food is prepared in iron-containing material and with prolonged contact with acidic products it is likely that the iron ends up in the food [63]. The amount coming from the cooking equipment can all-in-all provide for a substantial increase in the iron intake and status [11]. The ingestion of contaminant iron can be more than 3 mg of iron per day according to Zhou and Brittin [62].

Conclusion

the use of iron cookware will increase the iron content of food. Limiting the intake of iron by this route is recommended.

3.3 Enhancers and inhibitors

All kinds of nutrients in addition to the iron itself affect the absorption of iron. These substances are referred to as 'enhancers'* and 'inhibitors*', substances which increase or decrease iron uptake. The enhancers* and inhibitors* mainly have an impact on the uptake of non-heme iron; heme iron absorption* is hardly affected. For heme iron, thus far, the only 'suspicious' uptake-influencing

compounds are calcium (inhibitory) [10] [44], and meat or meat cysteine or non-dairy proteins (increasing) [44]. Non-heme iron has more enhancers* and inhibitors*.

The enhancers* for non-heme iron exert their influence on iron absorption in several ways. Alcohol, meat and organic acids are identified as having an absorption* enhancing effect on the absorption of iron.

Besides the enhancers* there are many inhibitors* of iron absorption. For certain substances the influence is 'proven' (such as phytates [14] [48]), others are suspected (such as protein from milk, eggs and soybeans [14]).

3.3.1 Alcohol

One of the treatment guidelines in hemochromatosis is to avoid (large volumes of) alcohol. Certain kinds of wine and beer contain a relatively large amount of iron [34] [44]. This can lead to an increase of the serum ferritin levels with the consumption of alcohol, in addition to a greater absorption of iron from what is eaten with alcohol.

Mechanism

It is proven that alcohol has the effect of increasing serum ferritin values. It is not entirely clear via which 'route' alcohol increases the iron status. It is thought that alcohol increases the permeability of the gut for nutrients such as iron [34].

Impact in practice

The studies evaluating the effect of alcohol intake on the iron status of hemochromatosis patients are of course limited to observational studies. These studies showed higher serum ferritin levels when alcohol consumption increased [9] [40] [41] [45]. Alcohol intake seems to have a dose-response relationship: the serum-ferritin-value is proportional to the alcohol intake [40]. McCune et al. [40] calculated a more than twice-as-high chance of having iron overload (transferrin saturation >50% and serum ferritin value >300 µg/l) with more than five units of alcohol per week, compared to (less than) five units of alcohol per week for C282Y homozygotes. Regardless of the effect of alcohol consumption on the serum ferritin values, research showed that alcohol accelerated the expression of the hemochromatosis disease in Danish men [46].

Also in several observational studies in the general population, the relationship between alcohol intake and serum ferritin values was demonstrated, including by Fleming et al. [44], Cade et al. [34], McCune et al. [40], Milward et al. [9], Lee et al. [64], Pedersen and Milman [46] and Aranda et al. [45].

Conclusion and discussion

The use of alcohol is not recommended for hemochromatosis patients. The interrelationship between alcohol intake and an elevated serum ferritin value is obvious.

Consuming iron free alcoholic beverages without consuming other iron-containing foods will in theory not increase the serum ferritin. However there are some indications that in this case also the serum ferritin rises: Alcohol will inhibit ferritin uptake by the liver [44], by setting in motion inflammatory reactions in the liver [39] and suppressing newly formed red blood cells, or the survival of red blood cells and thus less iron is used and thus preserved [46]. Hepcidin is possibly a substance that plays a role in the mechanism of increased iron absorption in alcohol intake [24] [65]. Regardless of the iron overload, alcohol causes a reduction in hepcidin production, which leads to a higher transit of iron in the intestinal cells [65]. Oxidative stress¹⁹ may play an underlying explanatory role [65]. The advice not to consume alcohol is at least given to reduce the chance of further liver diseases [46] [65] [66].

3.3.2 Organic acids

A number of organic acids are evidently enhancers* of iron absorption*. Organic acids that independently of each other increase the absorption of iron are: malic acid, ascorbic acid, lactic acid and tartaric acid [33] [44]. For citric acid the effect is not well known; it could be both stimulating

19 A metabolic condition in which more than a normal amount of reactive oxygen compounds in the cell are formed or are present.

and inhibiting the absorption of iron [33]. It is possible that in combination, the acids increase or diminish the iron uptake-enhancing effect [9]. Also, the difference in concentrations of acids and other inhibitors in products that contain organic acids may have differing effects on the absorption* of iron [9].

Organic acids are found in a wide range of foods: in fruits and vegetables many and various organic acids are found. Especially citrus fruits, strawberries, broccoli, spinach and cabbage are high in ascorbic acid, better known as vitamin C [67]. Malic acid is located in (unripe) fruit. Lactic acid is often used in the preparation of fermented foods such as yogurt, cheese and sauerkraut. Lactic acid also arises in the fermentation of certain products, such as the formation of wine. Tartaric acid occurs in grapes and derived products such as wine.

For ascorbic acid, the effect is most obvious. Ascorbic acid negates the inhibition of iron absorption* for all inhibitors* such as polyphenols [11] [14], phytate, calcium and proteins in dairy products, and increases the absorption of both types of iron found naturally in food as well as supplemental iron [14]. It explains about 8% of the variation in the absorption* of iron in persons without the *HFE*-gene* defect [68]. Ascorbic acid will only increase iron absorption when iron-containing foods are ingested at the same time [44]. Most studies into the effect of organic acids on iron uptake focus on ascorbic acid and/or vegetable consumption.

Storing and processing can negate the uptake-enhancing effect of fruits and vegetables. This is especially true for ascorbic acid because the amount of ascorbic acid rapidly decreases. However, some 'derivatives' of ascorbic acid – for example those used as antioxidants in food – are able to maintain their iron uptake-stimulating effect [14].

Mechanism

Organic acids affect the availability of iron by influencing chemical reactions in the stomach and small intestine [33]. The acids can convert dietary iron into the absorbable 'ferrous-form' [9] [14] [33]. In addition, the iron binds so it cannot be bound to other nutrients that inhibit the uptake [33]. All in all, the organic acids improve solubility and thus they change the availability of iron for absorption by the specific transport cells on the surface of intestinal cells [10]. (In Appendix I more can be read about the aspects of uptake.)

Effect in practice

In two studies, Bezwoda et al. measured [21] [22] the absorption of iron sulfate in combination with 30 mg of ascorbic acid. Compared with the same meal in non-hemochromatosis patients and a non-ascorbic acid enriched meal in hemochromatosis patients, the absorption of iron – although not statistically significant* – was much higher.

Two observational studies reported results of the ascorbic acid intake on serum ferritin values of hemochromatosis patients. McCune et al. [40] found no effect of ascorbic acid. This group however, also calculated a more than three times increased risk of iron overload with an intake of less than or equal to seven pieces of fresh fruit per week for C282Y-homozygous persons. Milward et al. [9] on the other hand found no effect of fruit and vegetable consumption on serum ferritin levels in individuals with *HFE*-gene* mutations.

The iron absorption-enhancing effect of ascorbic acid has been shown in experimental studies* in individuals with a healthy iron metabolism. Ascorbic acid seems to increase the absorption of iron proportionally [11] [33], with the first 50-100 mg having the most uptake enhancing effect on the absorption* of iron [44]. Not all researchers, however, found equally large effects for the long term [69].

Observational studies gave conflicting results for the effect of ascorbic acid or fruit and vegetable consumption on iron absorption: In some studies, ascorbic acid (not supplemented) was [34] [44] associated with the serum ferritin value [11], but in others this association was not found [9] [34]. Also for the consumption of fruit and/or vegetables there is no agreement between the results: Milward et al. [9] found that a higher consumption of non-citrus fruit – the fruit with the least ascorbic acid – the serum ferritin values in men were decreased. They also concluded that any citrus fruit or juice will not lead to increased iron stores [9]. In a study by Cade et al. [34] among women, no relationship between fruit and vegetable consumption and serum ferritin values was noted, while the use of fruit juice was negatively associated with serum ferritin.

Supplemented vitamin C does not appear to lead to higher serum ferritin levels [9] [34] [44].

Conclusion and discussion

The effect of organic acids mainly focuses on ascorbic acid intake or fruit consumption. The effect on serum ferritin levels and thereby iron overload is unclear. In the experimental studies* usually iron is added to a meal and it is therefore not possible to indicate the effect of the naturally present quantity and naturally present dietary iron. Similarly, in observational studies* the time of intake of vitamin C is often not included in the analysis of the results. This may give a bias, because fruits like ascorbic acid-rich product are often consumed in between meals (thus not in combination with other iron-rich foods). Furthermore, it is hardly possible in these studies to include the effect of the total composition of the fruit – thus all the influence of the inhibiting and stimulating factors. This is partly because of the large differences in the presence of inhibitors* and enhancers* per fruit (species), and partly because the content of organic acids varies strongly per fruit (species) (e.g. deterioration of the content during storage). The mismatched results from observational studies, both in studies with and without hemochromatosis patients, can be explained hereby.

Yet it is generally accepted that ascorbic acid has an effect on the non-heme iron absorption*, both as a supplement and in food [44]. It is therefore not possible to exclude that consumption of a specific fruit (species) (like juice) and/or vegetable (also) has an effect on the absorption* of iron in hemochromatosis. However, the use of supplements containing vitamin C is not recommended for hemochromatosis patients: It is discouraged during the depletion phase* because it could cause heart problems (see 'Heart and blood vessel disease', page 37) [6].

3.3.3 Factor 'X' in meat

Meat is not only a source of 'heme iron' that contributes significantly to the absorption of iron; it also increases the absorption of both heme [44] and non-heme iron [10]. Which nutrient (or a combination of several nutrients) is responsible, is still unknown. The contribution of the 'meat factor' to the variation in serum ferritin values is estimated at 4% [68] and appears to be dose-dependent [14]. 30 mg of muscle tissue (for instance in the form of steak), will have the same iron absorption-enhancing effect as 25 mg of ascorbic acid [14].

Mechanism

A reason for the uptake-enhancing effect of meat has not been found. 'Suspect' substances of this 'meat factor' are amongst others proteins (not from milk) [33], cysteine-containing peptides [12] [33] – that may have the same effect as organic acids [14] – and oligosaccharides [33].

Effect in practice

The extent to which the meat factor actually contributes to an increase of the iron status is difficult to define because of the difference in effect of the more available heme iron. There are no studies evaluating the effect specifically for hemochromatosis patients. Surveys of people without hemochromatosis are indicative of the absorption-enhancing effect: Also white meat and fish – less rich sources of heme iron than red meat – are in fact linked to higher serum ferritin values [33] [34].

Conclusion and discussion

Meat contributes to increased iron status, not only because of the presence of the better bioavailability of heme iron, but also for another reason. The still unknown factor could explain the association between serum ferritin levels and the intake of iron-rich foods such as liver and paté that Cade et al. [34] found.

3.3.4 Phytate (Phytic acid)

Phytates (phytic acid) are in the shell of nuts, seeds, legumes and grains. By processing the products – such as milling, soaking, cooking, baking and fermenting – phytic acid is degraded to some degree [10] [14] [33] [57]. If 95% of the phytic acid in grains is broken down, the iron is better absorbed from a meal [10]. In addition, the inhibitory effect of phytate can be partially reversed by vitamin C [33] (80 mg of vitamin C – more than is usual in a meal [55] – per 25 mg phytate – a reasonable amount for a regular meal – [70]) and meat [55].

Mechanism

Phytate (phytic acid) is well known for its inhibitory effect on non-heme iron absorption [10] [11] [14] [48] [69]. Of the substances present in plant foods it is (one of [10]) the [14] strongest inhibitors. The phosphorus content in the phytate is responsible; negatively charged groups form very stable complexes with minerals such as iron and convert them into substances that are not absorbed [57]. It is estimated that the phosphorus part of the phytates explains 19% of the variation in the absorption* of iron [68]. Even low concentrations have an influence and at increasing concentrations, the influence on the absorption* of iron * further increases [14].

Effect in practice

An inhibitory effect of phytic acid may be derived from relationships amongst foods and serum ferritin levels. Thus, the inhibitory effect of legumes is due to the presence of phytate [33]. Legumes were found, as well as nuts and seeds – also phytate-containing foods – in a study by Cade et al. [34] to have a negative correlation with serum ferritin values.

Conclusion and discussion

Studies for the effect of phytate on the absorption* of iron are scarce. A reason for this could be that it is not easy to separate the effect of phytate from the effect of other substances that are present in phytate-containing foods. In addition, it is difficult to investigate the effect since there also needs to be investigation into which types of phytic acid (in what concentration) are present [33]. Also, the effect of phytate may not be studied much in observational studies, because no comprehensive food tables for phytate levels are available [12].

Although it is generally accepted that phytate (phytic acid) inhibits the uptake of non-heme iron, little is known about the ultimate effect in hemochromatosis patients. Physiologically, it is unlikely that the effect in hemochromatosis patients is different from that of persons without hemochromatosis. It can therefore be assumed that phytate has an inhibitory effect, although it cannot explicitly be indicated to which extent it inhibits the absorption of iron.

3.3.5 Polyphenols

Polyphenols comprise a group of substances in plant products with a characteristic chemical compound. They inhibit non-heme iron absorption*. The group of polyphenols can be divided into: tannins, flavonoids, phenylpropanoids and lignans. Of the first two types the occurrence in food is most familiar and most studied. Tannins are known as the most potent inhibitors among the polyphenols [11]. Polyphenols are found in drinks such as tea [10], coffee [10], cocoa [10], red wine [10] and herbal tea [10] [14] and often determine the flavor, color and texture of these beverages. Of these tea varieties Ceylon teas ("Wewesse Ceylon Broken"), have the highest content of polyphenols [71]. Various hemochromatosis treatment guidelines write about the benefit of tea consumption with meals to reduce iron absorption.

Mechanism

Like the phytates, polyphenols have a strong attraction for non-heme iron and bind themselves to the iron, whereby an insoluble and non-absorbable complex is created [14] [33] [69] [71].

Effect in practice

Research into the effects of polyphenols on iron absorption in hemochromatosis patients is limited to the consumption of tea. A study by Kaltwasser et al. [71] is considered as leading in the advice to consume tea with meals to reduce iron absorption in hemochromatosis patients. In two different studies the researchers found an effect of the consumption of black tea with meals. First, they used an isotope study* to measure the absorption of iron from a test meal with labelled iron, the absorption of iron proved to be decreased by 70% (from 22.1% to 6.9%) when consuming tea. In the second study, the effects of drinking black tea on iron absorption were measured after one year, including using the number of phlebotomies (translated into the amount of iron removed). The amount of stored iron was, for the group that drank tea with meals – although not statistically significant* – lower than the group which drank nothing or something else with meals (69 mg before the intervention* - and 105 mg per month for the control group*). The final effect after one year was less than expected from the isotope study* [71].

In the observational study by Milward et al. [9], no association was found in hemochromatosis patients between tea consumption and serum ferritin values.

Research amongst persons without *HFE* -hemochromatosis also gave no hard evidence for an inhibiting effect of iron absorption by polyphenols. Cade et al. [34] and Milward et al. [9] found no (statistically significant*) change of serum ferritin values by the ingestion of tea and polyphenols. Also for the intake of coffee Milward [9] found no effect on iron absorption, based on serum ferritin values. Fleming et al. [44] found, however, statistically significant* lower serum ferritin values with higher coffee-, but not with higher tea-consumption. Experimental studies, however, showed that tea has a higher inhibitory effect of iron absorption* than coffee [44].

Not only the quantity but also the type of polyphenol determines the effect: In comparable amounts of polyphenols the difference in effect between spices such as chili or turmeric, and the effect of black tea was larger than that of herbal teas and wine [14].

Conclusion and discussion

Polyphenols inhibit the absorption* of iron because they bind iron in the gut. This will also be the case with hemochromatosis patients.

With regards to the results of the study by Kaltwasser et al. [71] some reservations can be noted: The main objection to the design of the second study by Kaltwasser et al. [71] is that the people were not involuntarily assigned, but based on habits related to drinking tea. It is therefore possible that they found an association that does not exist; that the polyphenols in tea are not associated with serum ferritin values, but with another substance or intake (of the amount) of another food – related to the intake of tea – and ‘responsible’ for the effect (for example, the tea drinkers ate less meat (whether before or during the trial)).

The research methods may explain that no effect of tea was found among persons without hemochromatosis in several observational studies. Often these studies omitted detailing the specific timings of intake , but it’s quite common to drink tea between meals rather than during the meal. So when the iron intake is usually higher, usually no polyphenols are taken from tea. It is thus a question whether tea consumption affects the absorption of iron.

However hemochromatosis patients can pay attention to the intake of polyphenols – for example by drinking black tea – together with non-heme iron-containing food. It has to be realized, however, that the tea in the study by Kaltwasser et al. [71] had been steeped for a prolonged time (to make the tea extra strong) and that intake of a relatively large amount of polyphenols may also have an inhibitory effect on the absorption of other substances. The advice of tea consumption with meals to prevent iron overload has to be reserved and kept in perspective as to its effects.

3.3.6 Calcium

Calcium is also an inhibitor of the absorption* of iron [10] [14] [47] [48], for both heme and non-heme iron [12]. Especially dairy (products) are rich in calcium.

Mechanism

A physiological explanation for the inhibitory effect of calcium has not been found yet. It seems that iron in the absorption process has competition with calcium and other similar substances [37].

Effect in practice

To our knowledge, only one study analyzed the effect of calcium intake on serum ferritin values in hemochromatosis patients. In this observational study, a negative correlation between serum ferritin levels and calcium intake was found in all men with *HFE*-gene* mutations [45].

Much research has been done on the inhibitory effect of calcium on the absorption* of iron in persons without *HFE*-gene mutations*. Experimental studies point toward a lowering effect in meal-studies with dairy products and added calcium [11]. Ingestion of dairy (products) *between* meals, however, showed an improvement of the absorption* of iron [11]. It may be that the effect of calcium on a meal is dose-dependent. A stronger inhibition seems to occur at a higher calcium intake [14].

However, an intake below 50 mg (approximately 45 ml of dairy) showed no effect [33].

Some observational studies confirm the inhibitory effect of calcium: Pedersen and Milman [46] found lower serum ferritin values when the subjects in the study consumed more milk. Also in French studies, lower values were found at a higher calcium and dairy intake [33]. Cade et al. [34] could

not demonstrate an association between serum ferritin values and calcium intake (through diet and/or supplements). The relationship between calcium and iron uptake is therefore not shown in all studies, as Fleming et al. [44] already have concluded.

Conclusion and discussion

Will calcium intake reduce the absorption* of iron in hemochromatosis patients? This question has not been answered yet. It seems that the effects in the healthy population are visible only in single-meal studies* and that calcium has a limited effect on the absorption of iron* if the effect is examined over several meals [14]. If calcium has an inhibitory effect on the absorption of iron, however, the effect is weak.

3.3.7 Other

Besides the above mentioned substances there are more substances suspected to affect the absorption of iron. Nutrients that are also associated with higher iron uptake include animal protein, and albumin [14]. Vitamin A also seems to be a promoter of non-heme iron absorption, although there is insufficient evidence [14]. In addition, fermented soy products – like soy sauce – sometimes are included in the list of enhancers of iron absorption* [12]. Substances which may also be associated with the inhibition of iron absorption are found in (raw) eggs, such as avidin [12], sulfides [14] [46] and other substances in the yolk [9]. Eggs are also associated with lower serum ferritin levels, when consuming more than four units per week (Pedersen's study) [46]. In addition, several elements other than the above organic elements – such as copper [12], zinc [47] and magnesium [12] [47] – are associated with lower iron absorption. Other substances are tannates, phosphates, oxalates, carbonates [14] [48], dietary fibers [33], milk casein [14] [47] and whey [14]. In addition, certain foods were found to correlate with the absorption of iron and/or serum ferritin levels. This may be related to the above mentioned substances. Thus, it can explain an association that was found between serum ferritin levels and proteins from soybeans [14] and between serum ferritin levels and starch [34]. It may also be the result of interaction among a set of substances.

3.3.8 Overall conclusion

The addition and subtraction of the individual effect of inhibitors* and enhancers* does not reflect what the final result of absorption of iron will be. Thus, the effect of a substance in the food in which it occurs or when taken in combination with other foods can be strengthened or weakened.

Several studies have shown that the influence of enhancers* and inhibitors* on the absorption of iron is less with an intake of a varied diet over a longer time than with ingestion of just one meal [11] [14] [28] [33]. Long-term studies also indicate that the absorption of iron from a diet with a high bioavailability* reduces and vice versa the absorption of iron from a low bioavailable diet increases [69]. The difference in the outcome of the absorption* of iron between so called single- and multiple-meal studies* can be 50 percent [10]. In a single meal study* researchers worked out an influence of enhancers* and inhibitors* up to 13.5 percent, while the influence of a multiple-meal study* was up to 8 percent [28]. Probably an uptake enhancing effect by 'enhancers*', with a low iron status and a meal with a low amount of iron, lasts till the iron status is adequate and the iron values, at the next meal, are increased again [33]. In subjects with a good iron status, the influence of other nutrients on the absorption* of iron is probably limited.

It is possible that one or more enhancer(s) and/or inhibitor(s) have (some degree of) an independent effect. There are indications [69] of this. Also finding relationships between enhancers* and inhibitors* and the serum ferritin values in observational studies is an indication for this. Cook and Reddy [72] calculated, from experimental studies, that the phosphorus derived from phytates has the greatest (19%) contribution to the spread of non-heme iron absorption*, and vitamin C and meat have a relatively smaller (8% and 4% respectively) contribution [68].

Conclusion and discussion

Possibly phytate, dietary fiber, calcium and several polyphenols act as inhibitors, and alcohol, vitamin C and meat are stimulating substances and indicators of serum ferritin values. The effect of inhibitors* and enhancers* is generally much less when analyzing the effect in a non-experimental setting. Presumably, the iron status is, in a typical Western diet, crucial in the use of enhancers* and

inhibitors* to increase or decrease the absorption of iron (probably only or mainly non-heme iron). The results of observational and experimental studies* have limitations as far as the translation into practice is concerned. For example, the experimental studies* almost always used only fortified foods. In addition, the observational studies analyzed effects of nutrients and thus nothing is known about the intake of other nutrients simultaneously. For this reason not much can be said about the duration of a given nutrients effect.

Hemochromatosis patients can probably affect iron absorption partly by paying attention to the intake of enhancers* and inhibitors* with meals. Well conducted studies that have been done on the effects of enhancers* and inhibitors* over a long term in hemochromatosis patients are scarce. In addition, absorption studies* in people who are (compound) heterozygous for hemochromatosis [30] [32] do not show a clear effect. However, it is seen as physiologically unlikely that the effect will be much different from that of persons without a gene defect with regard to iron metabolism.

Hence, ingestion of large amounts of vitamin C together with non-heme iron-rich foods is not recommended and intake of calcium with iron-rich foods – including meat – is recommended. Further, drinking black tea with meals possibly reduces the chances for iron overload.

4 The role of nutrition in the treatment of *HFE*-hemochromatosis

Hemochromatosis and diet are linked. First, in terms of intake of the central substance iron by means of nutrition. In addition, however, also because of concomitant diseases – so called co-morbidities – it has there are points of interest. Hence, the final nutritional advice below, with its potential for reducing that iron overload which is directly related to the genetic defect under discussion, may also reduce the risk of certain diseases also related to that defect.

4.1 Iron Overload

4.1.1 Reduction of phlebotomies

The ultimate question to answer is how many phlebotomies may be omitted when changes are made in the diet. Especially for people who do not tolerate phlebotomy very well, and for whom the treatment is painful and causes disruption of their daily lives, this is a question that we would like to answer. Dietary changes cannot lead to a low iron uptake so that iron overload does not continue. Changes in the diet cannot replace phlebotomies when they are necessary. In the depletion phase* phlebotomy is necessary to remove as much of the excess iron as possible, including that stored in the liver. That's the only way for the body to get rid of the large amount of iron. After the depletion it is not possible to survive without ingesting some iron from food. This is because almost all food contains some iron and when eating only low iron products, shortages of other nutrients will arise.

The central question cannot have an unambiguous or binding answer. This is partly because both the intake and the accumulation of iron varies from person to person and thus many issues need to be sorted out before conclusions can be drawn. As indicated, the accumulation per unit of time depends on the feedback mechanism, guided by the serum ferritin values. Thus, the iron uptake at the time of diagnosis is the lowest and immediately after phlebotomy the highest. It is calculated that at diagnosis the iron uptake is comparable to persons without hemochromatosis, who absorb about 1 mg daily [22]. During the depletion phase* the uptake possibly rises, as is found in individuals with idiopathic* hemochromatosis [73].

In the past, studies on the degree of iron overload were done per unit of time, based on the number of phlebotomies (the amount of iron removed). Investigations into the increase in serum ferritin ranged from 8 to 430 µg/l/year for idiopathic* patients [74]. Adams et al. [74] calculated for C282Y homozygotes, based on the increase in serum ferritin between two phlebotomies, an average serum ferritin increase of 99 µg/l per year (equivalent to 742.5 mg per year [74] = 2.0 mg day²⁰). However, they found an extensive variation from person to person: the iron storage had a range from 1.2 to 241 µg/l/year [74].

The individual differences are not easy to explain. The hypothesis that diet can contribute to the prevention of iron overload is quite plausible since all the iron has to be ingested through the diet. If less is ingested, then less can be absorbed. This is especially in the period just after a phlebotomy; it is 'interesting' to take food relatively low in iron during that period, because the absorption* is the highest at that time.

The relationship between the number of phlebotomies and nutritional intake for people with different *HFE* -gene mutations has not been studied. However, some researchers have focused on the effect of the enrichment of food with iron on the rise (or decrease) of the serum ferritin values in hemochromatosis patients. Cook [28] brought forward an yearly iron storage of 2.1 grams – of which 0.4 grams was greater because of the daily use of enriched flour (basis fortification of flour with 3 mg

20 Data are based on assumptions that 1 µg/l ferritin corresponds to 7.5 mg of body iron and removing 500 ml of blood decreases the ferritin concentration by 33 µg/l, which equates to 250 mg of body iron [74].

iron). This equates to approximately eight phlebotomies per year. Tao [75] predicted the percentage of C282Y homozygous hemochromatosis patients in whose bodies a certain amount of iron will have been stored after 39 years (starting age: 25 years), in response to the use of iron-fortified flour. Here he took into account the different levels of intake for the population (based on the NHANES III study) that could be achieved by fortification of flour. By continuing the policy of the use of iron-enriched flour, 14.7% of the homozygous C282Y-hemochromatosis patients would show clinical symptoms of hemochromatosis at the age of 64 (assuming that this is the case with 20 grams of total body iron). If the fortification were to be reduced to a value where the population average would exactly meet the recommended daily intake (nutrient)*, the mentioned percentage would be 4.1. In the latter case, however, more than 70% of the people with the C282Y/C282Y mutation would have a total body iron content of 10 grams by their 64th year. These calculations show the impact of iron-enrichment for the population using standard products.

4.1.2 Reducing iron intake

The intake of iron in a day can be significantly different according to the food choices made. To illustrate this, two different 'daily diets' are shown in Figure 2, page 35 and Figure 3, page 36. The products chosen and therefore the amount of iron is different. For both 'daily diets' the recommended daily intake (food)* for a man between 50 and 70 years is the starting point²¹. These recommended daily allowances (food)* will provide sufficient variation in the diet to most adequately cover the recommended daily allowance (nutrients)*. By choosing different products for the recommended daily allowances (food)* and foods that additionally can be eaten and drunk, the intake of iron can be quite different.

It is clear that it is not possible to predict the amount of iron that will be absorbed from an imaginary diet. This will depend on the iron status of the person, the contribution of the enhancers* and inhibitors* and the extent to which the heme iron is absorbed more than the non-heme iron. Yet it can be stated with confidence that more iron is absorbed in hemochromatosis from a diet relatively rich in iron than from foods which supply relatively less iron. At minimal, meat at a warm meal will contribute. If the amount will make a difference for the person in question is debatable. This is because the amount of iron absorbed is probably not commensurate with the amount of iron ingested. If a food delivers twice as much iron you cannot automatically say that twice as much iron is absorbed. It is however likely that the uptake of iron from a 'daily diet' that contains much iron will be higher, especially if the serum ferritin levels are not very high.

Theoretically, the difference between the two proposed 'daily diets' is 12.9 mg non-heme iron and 1.3 mg of heme iron. Assuming an average uptake (based on persons without hemochromatosis) not influenced by the serum ferritin, this will result in a difference in absorption of 1.6 mg per day. This represents an amount of 589 mg per year, representing over two phlebotomies per year²². Whether this is reality, is still a question mark. The data on which the calculations are made are based on the iron metabolism in subjects without hemochromatosis. As stated, in hemochromatosis patients the uptake can be greater – especially shortly after phlebotomy – and there is an influence from other factors described in SLAMENGHI. It is clear that relatively small differences in the daily intake can contribute considerably to the iron overload [12].

21 The reason to show this group of people as an example is because most people who are discovered with hemochromatosis are male and belong to this age category.

22 Data are based on a larger absorption* of heme iron compared to non-heme iron (25% and 10% respectively [14] [30] [11]) and the assumptions that 1 µg/l ferritin corresponds to 7.5 mg body iron and that removing 500 ml of blood will lead to a decrease in ferritin concentration of 33 µg/l, equivalent to 250 mg body iron [74].

Figure 2 Detailed food report of a daily diet that provides relative a lot of iron.

		Iron (mg)	
		Heme	Non-heme
Breakfast			
Whole wheat bread	3 slice (105 gr.).		2.7
Spreads	3 portions (18 gr.)		* 0.0
Cheese Leiden 20+	1-2 toppings (30 gr.)		0.0
Chocolate Sprinkles	1 portion (15 gr.)		0.5
Cappuccino coffee prepared	1 standard (150 gr.)		0.5
Mid-morning			
Chocolate medium-fat	1 glass (175 gr.)		0.5
Fruit (average, excl. citrus)	1 dish (125 gr.)		0.4
Lunch			
Whole wheat bread	3 slice (105 g).		2.7
Spreads	3 portions (18 gr.)		* 0.0
Boiled egg	1 piece (50 gr.)		1.0
Apple syrup	1 portion (15 gr.)		2.2
Coconut Bread	1 portion (20 gr.)		0.3
Buttermilk	1 glass (175 gr.)		0.0
Mid-afternoon			
Apple juice	1 glass (150 gr.)		0.3
Muesli Biscuits	1 piece (29 gr.)		0.5
Dinner			
Prepared beef	1 portion (80 gr.)	1.5	0.9
Boiled potatoes	3 medium (210 gr.)		1.3
Cooked spinach	4 vegetable spoons (210 gr.)		2.9
Low fat chocolate pudding	1 dish (150 gr.)		0.6
Late evening			
Espresso Coffee	1 cup (125 gr.)		0.3
Red Wine	1 wine glass (100 gr.)		0.6
Cashew nuts	2 handfuls (30 gr.)		1.5
		Energy (kcal)	Protein (gr.)
			Total iron (mg)
			[heme iron (mg)]
Daily total		2088	93 (17%)
Recommendation		2062	46 (= 9% energy)
51-year-old man			9.0

* = unknown quantity

Figure 3 Detailed food report of a daily diet that provides relative less iron.

		Iron (mg)	
		Heme	Non-heme
Breakfast			
Wheat Bread	3 slice (105 gr.)		1.8
Spreads	3 portions (18 gr.)		* 0.0
Cooked Chicken	1 portion (15 gr.)	0.0	0.1
Gouda Cheese full fat	1 slice (24 gr.)		0.0
Jam	1 portion (15 gr.)		0.1
Semi-skimmed milk	1 glass (175 gr.)		0.0
Mid-morning			
Black Coffee	1 cup (125 gr.)		0.3
Sprits Cookie	1 piece (25 gr.)		0.2
Lunch			
Wheat Bread	3 slices (105 gr.)		1.8
Spreads	3 portions (18 gr.)		* 0.0
Sandwich Spread	1 portion (15 gr.)		0.3
Peanut butter	1 portion (15 gr.)		0.2
Honey	1 portion (15 gr.)		0.1
Semi-skimmed milk	1 glass (175 gr.)		0.0
Mid-afternoon			
Tea	1 large cup (225 gr.)		0.0
Tea Biscuit	2 pieces (10 gr.)		0.1
Apple	1 piece (135 gr.)		0.3
Dinner			
Canned tuna in water	1 small (80 gr.)	0.2	0.6
Margarine	1 tablespoon (13 gr.)		0.0
Cooked spaghetti	Two portions (180 gr.)		0.7
Vegetables cooked average	3 Spoons vegetables (165 gr.)		1.0
Tomato Sauce (ready made)	4 tablespoons (48 gr.)		0.1
Custard-cream	1 dish (150 gr.)		0.0
Late evening			
Black Coffee	1 cup (125 gr.)		0.3
Cracknel	1 piece (12 gr.)		0.1
Pear	1 medium (160 gr.)		0.1
	Energy (kcal)	Protein (gr.)	Total iron (mg) [heme iron (mg)]
Daily total	2094	87 (17%)	8.3 [0.2]
Recommendation 51-year-old man	2062	46 (= 9% energy)	9.0

* = unknown quantity

4.2 Co-morbidities

Besides the risk of iron overload, hemochromatosis has a number of other health issues to which food (substances) consumption is relevant. These are linked to a possible increased risk of colon cancer, heart and blood vessel diseases, liver diseases and a greater susceptibility for infection with *Vibrio vulnificus*.

4.2.1 Colon cancer

A high iron status and/or –ingestion of iron is suspected of increasing the risk of colorectal cancer [47]. Based on this it is thought that hemochromatosis carries an increased risk of colon cancer. There are several proposed mechanisms:

- degradation of the DNA and thus uncontrolled growth of cells by production of free radicals by the excess iron;
- the use of iron as a nutrient by cells, leading to accelerated production or growth of cells;
- an impaired defence mechanism against pathogens [12].

Epidemiological studies gave contradictory results when analyzing iron status and colorectal cancer [12] [76]. In a group with different *HFE*-mutations, Chan et al. [76] found no association between hemochromatosis and colon cancer. This corresponded with other studies on heterozygous *HFE*-mutations. But the studies that were done into the relationship between hemochromatosis, iron intake and the development of colorectal cancer, there were mainly individuals with heterozygous defects involved or were done during a period when no typing of genetic defects was possible [76]. Recent research by Osborne et al. [77] indicated an increased risk of colon cancer in C282Y-homozygotes. It is therefore likely that a (frequent) higher iron status caused by hemochromatosis increases the risk of colon cancer. This must be clarified by further research.

4.2.2 Heart and blood vessel diseases

Explicit advice on diet with regard to heart and blood vessel diseases is mainly restricted mainly to the intake of vitamin C in the period around a phlebotomy. Vitamin C stimulates the release of iron from the cells where it is stored. Transferrin is thus saturated too quickly, allowing in more substances that are harmful to cells in the blood. This can lead to cardiac arrhythmias and heart muscle diseases [78]. The knowledge about this is however based on a single fatal event [6]. It is advised, because of this described risk, to limit the vitamin C intake from supplements to 500 mg per day [6] [58]. Individuals with a defect in the *HFE*-gene* may be at an increased risk of heart and blood vessel diseases. Two studies reported at least a larger number of heart and blood vessel diseases in subject with C282Y/wt [39]. In subjects with homozygous hemochromatosis no greater amount of atherosclerosis – a risk factor for heart and blood vessel diseases – was found [18]. The risk of heart and blood vessel diseases can be reduced by following the recommendations for fruit-, vegetable- and fish intake. It is also desirable to limit the intake of saturated fat and sodium (salt).

4.2.3 Liver diseases

With the discovery of hemochromatosis often fibrosis or cirrhosis can be seen in the liver. This indicates degradation of liver tissues. It is possible that the damage can be reversed over time (fibrosis). Alcohol is not recommended because it can increase liver damage [6].

4.2.4 Infections

High iron levels in the blood and tissues and reduced hepcidin in plasma can increase the risk of infection. There are known cases of hemochromatosis patients who became infected with the bacterium *Vibrio vulnificus* after eating raw oysters and other shellfish [58]. Infection with this bacterium can be fatal [79].

5 Dietary advice in *HFE*-hemochromatosis

All in all, the information (with marginal notes in regards to the desirability and feasibility of following a diet), leads to a dietary advice for those with *HFE*- hemochromatosis, and suggests the desirability and feasibility of following such advice: desirability because of the prevention of other diseases by the accumulation of deficits caused by an imbalanced diet, feasibility because of the availability of diet products and taste aspects. A healthy diet – as drafted by the Dutch Nutrition Center with the Food Pyramid – forms a starting point for describing the overall effect and the backbone for the practical advice.

5.1 Vegetables

Vegetables contribute around 7% to the total iron intake [80]. In addition to the iron, other valuable nutrients such as antioxidants are delivered. It is important to note that the bioavailability* of iron is negatively influenced by the presence of various inhibitors* in the vegetables.

Some vegetables are rich in iron: dark green leafy vegetables like spinach, chard, purslane, clarion and agoumawiri, beans (such as green beans and lentils), and fennel. Examples of other vegetables which deliver much less iron are cucumber, carrot and chicory. The difference when taking the recommended daily allowance (nutrient)* into account can be a change in iron intake of 2 mg per day and a hypothetical iron uptake of 73 mg per year.

For the intake of other nutrients it is especially important to not limit the total vegetable intake and to have plenty of varied and colorful vegetables. It may be better not to eat iron-rich vegetables too often. When eating certain high iron vegetables, it may be wise to combine them with a meat substitute (such as an egg and spinach), because meat also increases the non-heme iron absorption.

5.2 Fruit

Fruit is, because of its vitamin C content, often mentioned as a concern in hemochromatosis. Vitamin C can make iron (from other foods) better available for absorption. Yet it is unnecessary to limit fruit intake. It contains many valuable nutrients. Perhaps fruit consumption, as required for healthy Dutch people (two fruits per day), is specifically important in hemochromatosis because of the greater chance of preventing unbound iron in the blood, which may cause oxidative damage. The antioxidants in the fruit can possibly prevent oxidative damage.

Fruit contains relatively little iron (average 0.3 mg per 100 grams). An exception is dried fruit (including raisins), so hemochromatosis patients are better off limiting their dried fruit intake to small portions. In addition, fruit is best eaten in between meals, so that the influence on the absorption of iron from other foods is not enhanced.

5.3 Potatoes, rice, pasta, legumes

With a warm meal the choice of the starch or carbohydrates requires little attention. There are some comments that can be made concerning the relatively iron-rich legumes and whole grain varieties of grains. The iron content in potatoes, white and brown rice, *non-whole-wheat* pasta and legumes is similar. However, a whole-wheat pasta contains almost three times as much iron as the non-whole-wheat product. Presumably, the presence of more inhibitors, compensates for the higher concentration of iron, making the uptake from whole-wheat varieties probably the same. Whole-wheat products are preferred over more refined products because of the presence of other valuable nutrients and dietary fiber. In addition, legumes are an excellent source of nutrients and inhibitors, making them an excellent food source – for example as a meat substitute – in *HFE*-hemochromatosis is.

5.4 Bread

Bread is responsible for about one fifth of the total amount of iron ingested by Dutch people [81]. For pasta and bread, the whole grain variety continues to be the preferred product, despite the increased contribution of iron; the greater amount of iron will not outweigh the effect of the inhibitors and/or healthy value.

Bread (substitutes) enriched with iron are discouraged. Most basic bread substitutes as well as non-enriched varieties are present in the shops.

5.5 Cheese and milk (products)

Cheese and milk (products) are low in iron. Although there are suggestions for an inhibitory effect of calcium, no clear associations between calcium intake and iron absorption have been found yet. In addition, because the mechanism and thus the impact of the *HFE*-mutation is not clear, no advice can be given that differs from the Dutch Guidelines on Food choice regarding the intake of calcium from dairy products and/or supplements.

5.6 Meat (products), chicken, fish, eggs, meat substitutes

Meat (products), chicken, fish, eggs and meat-substitutes offer a variety of valuable nutrients such as protein, vitamins and minerals, including iron. However in hemochromatosis, it is important to reduce meat intake to a minimum. This is because meat contains a lot of iron and is a (the only) source of heme iron. The better absorbable heme iron is not a nutrient that is you need to stay healthy. As meat is a very large contributor to the absorption of iron, omitting it results in a big gain. Assuming an average serving of 100 grams (size of a meat patty), this piece of beef delivers 1.7 mg heme iron and 1.1 mg of non-heme iron. Consumption of this protein source in theory adds 0.53 mg to the absorption of iron per day²³. Replacement of this piece of meat with one that does not have heme iron and only a small amount of non-heme iron (like a cheese soufflé or meat substitute based on mycoprotein²⁴; 0.1 and 0.3 mg non-heme iron, respectively) leads to a theoretical bioavailable iron content of 0.03 mg. This difference of 0.5 mg, on an annual basis, leads to a profit of more than 180 mg per phlebotomy, a difference of more than two phlebotomies over three years.

Perhaps the bioavailability* of iron from meat by the 'meat factor' is underestimated: If the maximum bioavailability* (mentioned in the literature) for both iron forms (35% for heme iron [14] [30] and 15% for non-heme iron [11]) is calculated, 0.76 mg will be available. All in all, this piece of meat gives – without taking into account the feedback mechanism and the increased uptake in hemochromatosis – an increase in serum ferritin greater than 275 mg per year. Replacement of 100 grams of beef per day through a low-iron product will also theoretically reduce the number therapeutic phlebotomies by one per year²⁵.

To clarify: the above is a theoretical framework for a common portion of beef. However, this estimate is quite reasonable since hemochromatosis patients are better in absorbing iron than non-hemochromatosis patients.

If no meat is eaten, in particular the risk of shortages of some nutrients is higher. This applies especially to vitamin B12. Dagnelie [82] calculated that the recommended daily intake (nutrient)* of 2.8 µg (micrograms) per day is feasible if meat is eaten 2-3 times a week. Also, instead of meat, fish twice a week will suffice for the recommended daily allowance (nutrient)* of vitamin B12. The recommendation will also be met by a large consumption of dairy products: more than 3 glasses of milk and more than two slices of cheese per day [82]. Compared with (other) kinds of meat, fish

23 Assuming a bioavailability* of heme and non-heme iron of respectively 25 [30] [14] and 10% [11].

24 Protein derived from fungi, yeasts or fungi.

25 Data are based on assumptions that 1 µg/l ferritin corresponds to 7.5 mg of body iron and that a reduction of 500 ml of blood leads to a decrease in ferritin concentration of 33 µg/l, which equates to 250 mg body iron [74].

contains little iron and little heme iron [35]. It is therefore a good source of vitamin B12 for hemochromatosis patients.

In addition to vitamin B12, inadequate intake of other B vitamins is a risk in a primarily vegetarian diet. This is because meat is rich in vitamin B3 and especially pork is rich in vitamin B1 [80]. Adequate intake of these substances is most likely achieved when fish is eaten twice a week. Fish twice a week – including oily fish once weekly – is thus also recommended for hemochromatosis patients.

Meat is also a rich source of zinc. The consequences for a hemochromatosis patient who eats no meat (possibly twice a week (oily) fish) with regard to zinc intake is still an outstanding issue. In any case, it seems that iron and zinc compete for the same receptor on the cell [83]. There are also indications that not only the absorption of iron, but also those of other metals are disturbed in hemochromatosis [84]. It is thus possible that a lot more or even less is absorbed in hemochromatosis. Further research should show whether the absorption of zinc in hemochromatosis is less, greater or equal before consequences in terms of dietary intake can be drawn.

HFE-hemochromatosis patients are wise to limit their meat consumption and to eat (oily) fish twice a week, not only from the perspective of iron overload, but also in view of the risk of concomitant diseases (such as those of heart and blood vessels). If hemochromatosis patients want to eat meats, those that contain little or no (heme) iron are preferred. The rule of thumb is: the redder, the more heme iron. An exception to this rule is organ meat, that is rich in (non-heme) iron; 100 grams of liver for example yields an average of the entire recommended daily intake (nutrient)* per day for men and women over 50 years [35] [85].

It is sensible to eat an alternative to meat, so other substances that are present in meat are ingested. This may be a ready-made (non-iron-enriched) meat substitute, but also other protein sources can be used: chicken, fish, eggs, cheese, nuts, legumes (or derivatives such as soy bean products curd/tofu, tempeh), peanuts, nuts and seeds. These products do not contain heme iron, and relatively little non-heme iron. In addition, they provide many vitamins and minerals that are also present in meat and can therefore be safely used as a substitute for meat (products). Also, to add variety protein-rich crops – so called pseudo cereals – may be chosen as a substitute for meat and potatoes, rice or pasta. Readily available variants are quinoa and amaranth. None of the meat substitutes provide all the substances that meat contains, therefore meat is unique as a nutrient. A variety of types of meat substitutes, will guarantee the intake of other nutrients.

If reducing meat intake takes a lot of effort, “in between solutions” can be worked out, such as replacing a part of the meat with a meat substitute, preferably based on dairy, to suppress the non-heme and heme iron uptake with calcium.

Because of the risk of infection with the bacterium *Vibrio vulnificus* and the fatal consequences, it is advised not to eat raw seafood or prepared food that is sprinkled with sea water. Marine animals that are cooked, and to which no fresh seawater has been added later, can be eaten without risk [58] [84]. The bacterium is not common in Europe and therefore it is particularly relevant to be aware of this in America and Asia [79].

5.7 Baking and frying products, oils and spreads

Fat products such as bread spreads and cooking fats such as margarine are free of iron. The use of a liquid variant of baking and frying fat (with a high content of polyunsaturated fat) is recommended to reduce the risk of heart disease.

5.8 Beverages

Drinking beverages can lead to an increased iron intake or absorption if those liquids contain iron, vitamin C, or alcohol. Various types of beverages supply different amounts of iron. Nursing Sister Blood Wine is an extreme example with 10 mg of iron per 100 ml glass. Water, tea, gin, beer (excluding old brown) and most soft drinks (no juices) contain no iron or a negligible amount of iron.

When drinking coffee, iron is ingested, 0.3 mg in a cup of 125 ml. Also, most wines, many types of liquor, some whiskeys and old brown beer contribute to iron intake. Furthermore, liqueur, whiskey, beer and other alcoholic beverages are not recommended because the alcohol promotes the absorption of iron and to avoid (further) damage to the liver.

Depending on what and how much is drunk, the contribution of liquids to iron intake can increase quickly. 1 glass (150 ml) orange juice, 3 cups (125 ml) coffee, one cup (150 ml) of chocolate milk and 100 ml of wine delivers 3.2 mg of iron. Replacing these liquids with tea, milk and soft drinks will lead to a reduction in the absorption of iron by 117 mg (nearly half a therapeutic phlebotomy) on an annual basis²⁶. This estimate may be low because neither the uptake-enhancing effect of vitamin C and alcohol, nor the possible uptake-inhibiting effect of drinking strong black tea with meals was taken into account. Because of the contribution of vitamin C, fruit juice is best drunk without consuming other iron-containing foods.

5.9 Other

Chocolate and black olives contain relatively high amounts of iron. Chocolate is a delicacy and not a necessary food to stay healthy. Dark chocolate sprinkles deliver 1.2 mg of iron per serving (on a piece of bread). When using a portion per day this gives an annual intake of 44 mg of iron by only using the sprinkles. Milk chocolate delivers about half the iron of the pure form and thus is preferred²⁷. The iron content of black olives may be higher than other olives because of iron gluconate, an additive intended to preserve the black color. One serving of 20 grams of black olives provides 3.4 mg of iron.

As described, the use of cooking equipment can contribute to an increased iron intake: Especially when acid products are prepared for a long time in (stainless steel and/or cast iron) pans, this is a concern.

Most food supplements are multi-vitamin and mineral supplements, and they also contain iron. The iron content varies widely. A diet as recommended by the Dutch Nutrition Center will supply, for the majority of the healthy Dutch population, enough vitamins and minerals to stay healthy. Supplementing with preparations will not be necessary for them. Also for hemochromatosis patients, there is still no advice to take extra vitamins and/or minerals. Should the use of a preparation be required, it is wise to choose a tablet that contains no iron and vitamin C, provided there is no shortage of iron (e.g. too many or too deep phlebotomies (ferritin to low)).

Besides concerns for certain iron-rich foods or products that enhance or reduce the absorption, it is important to prevent obesity. In several studies [34] [39] [64] there was found to be a positive correlation between the body mass index (BMI)* and the ferritin values: the higher the body mass index*, the higher the serum ferritin values. Pursuing and maintaining a body mass index* between 20 and 25 is thus recommended.

26 Assuming an absorption* -efficiency of 10% [11] and removal of 250 mg of iron per phlebotomy [74].

27 Written response from Mrs. M. Jansen, NEVO team member at the National Institute for Health and Environment, dated 05/19/2011:

"The iron content of chocolate products has been a discussion point at earlier stage. In 1990, analysis were done on milk chocolate and this resulted in a level of 3.2 mg ijzer/100g. For dark chocolate, these levels were then also used. Code 717 is a recipe of hazelnut- and milk chocolate and roughly comes to the same level.

In 2000 we received information from different kinds of chocolate spreads via manufacturer De Ruijter. They had calculated this level based on the ingredients. Since this level is much higher than 'normal' chocolate, we have corresponded with a producer and this showed that the high levels could be true. We were in doubt whether we, on this basis, had to adjust the other chocolate products. This would then be an estimate by us, because we did not have analyzed data at our disposal. This time we did not do this. In hindsight I think we probably should have done an adjustment for code 432, dark chocolate, as it should always be higher than milk chocolate, because it has more cocoa mass in it."

6 Conclusions

The guidelines for the treatment of hemochromatosis are mainly aimed at “solving” the effects of the genetic abnormality. Removing the excess iron by phlebotomy is proposed as the predominant therapy and attention to the prevention of increased iron levels is subject to caution, based on the ‘burden’ that dieting entails. Even though changes in diet usually will not eliminate the need for therapeutic phlebotomies during the maintenance phase, they can affect the amount of iron that accumulates per time unit. Obviously the degree of iron overload differs from person to person. This also depends on the persons usual diet and it is determined by the difference in the degree to which iron is absorbed. Individuals with the homozygous C282Y (C282Y/C282Y) and the compound heterozygous (C282Y/H63D) form of *HFE*-hemochromatosis, will profit most because they have the greatest potential to absorb excess iron. They would therefore be wise to reduce their iron intake to a minimum.

In reducing the absorption of iron, it should be paramount to ensure the intake of other vitamins and minerals. Hence the recommended daily intake (food) per day* of the Dutch Nutrition Center should be a starting point. An emphatically important additional aspect is meat intake. (Red) Meat is a source of easily absorbed heme iron and is not recommended in *HFE*-hemochromatosis. If one eats (oily) fish twice per week, nutritional deficiencies from the absence of meat in one’s diet, are not expected. The other days of the week can be chosen for a meat substitute such as eggs, cheese, nuts, legumes (including soy products like tofu, tempeh), peanuts, seeds and fish. Obviously the non-iron-fortified types are advised. While it is not well known what the uptake of the added iron from iron-fortified food is in hemochromatosis, these foods are better avoided by hemochromatosis patients.

Alcohol should be avoided by hemochromatosis patients. In many ways it has a detrimental effect on the course of hemochromatosis and concomitant diseases. Not only the persons most at risk of iron overload are advised to consume no alcohol, but also those with the less ‘serious’ variants C282Y/wt, H63D/wt and H63D/H63D are advised to abstain from alcohol.

When a patient wants to use a vitamin- and mineral supplement, an iron-free multi-vitamin supplement can be taken in consultation with the doctor, if there is no iron deficiency. The total amount of vitamin C from (those) preparations ingested, is best kept below 500 mg.

It is impossible to predict the effect of dietary changes on the accumulation of iron in the body. Besides the fact that it depends on the genetic abnormality and dietary habits of the person, the uptake will depend on many factors, such as the times of meals and the iron requirement. Another aspect is the interaction with other nutrients, which may have a stimulating or inhibitory effect on iron absorption. It cannot be clearly indicated what the impact of these substances will be on iron intake, but it is probably limited.

All in all, meats, alcoholic beverages, and iron-fortified foods and preparations are the most adverse factors affecting iron overload. It is not inconceivable that by reasonable changes in the diet two phlebotomies per year can be omitted. Whether the burden of dieting outweighs the reduction in phlebotomies, is at the discretion of the patient.

7 Discussion

The advised diet is pretty much identical to the current nutrition aspects in *HFE*-hemochromatosis. The possible influence of meat, dietary preparations and iron-enriched flour, and the clear contribution of alcohol to the accumulation of iron is mentioned in the Dutch guidelines [5] and in articles [9] [58] on the treatment of hemochromatosis. Drinking black tea with meals is also described several times as a possible beneficial complementary 'therapy' [5] [6] [58]. Although several researchers did not mention all the same nutrients, the above advice 'covers' what was previously cited.

What is different is the idea of the added value of following a diet for hemochromatosis. One therapeutic phlebotomy will remove 250 mg of iron at once [74], while subjects with a healthy iron balance absorb, at most, only 5mg per day, and hemochromatosis patients most probably absorb 8-10 mg per day [29]. In addition, the amount of iron per day that enters the body is only 1/20 of the total amount per day that is circulating through the blood [2]. What information exists, suggests that the dietary variations in the 'treatment' of iron overload have little extra value if plotted against results of therapeutic phlebotomy.

However, there is no evidence undermining the role of nutrition in controlling iron overload. Several researchers [12] [24] [86] indicate a potential role for food or mention that there is no evidence that diet plays no role. Research by McCune et al. [40] demonstrates this, with the fact that 43% of the variability in the transferrin and serum ferritin values of first-degree relatives of C282Y-homozygotes cannot be explained by genetic, psychological and lifestyle factors included in their model.

While it is correct that phlebotomies have a faster effect than diet changes, it is likely that the combination of phlebotomy and diet modifications is the best treatment for hemochromatosis. Changes in nutrition can perhaps reduce the number of required phlebotomies. That this preventive method for iron overload gets virtually no attention is quite remarkable because – although (almost) no research is done – it is expected that bloodletting also has an impact on other health aspects, such as the vitamin status of patients.

The complaints of fatigue after a phlebotomy may be related to the depth of phlebotomy. At present, 50 µg/l is the serum ferritin value, which is considered the "end-point" of phlebotomies. This is not a scientifically based value [5], but based on 1) the theory that it is necessary for lower iron concentrations in body tissue to cause an iron deficiency, and 2) to at least have a guideline [6]. There appears to be much discussion about the cutoff value. This is because for years it has been known that uptake is the highest after a phlebotomy and then decreases until the next phlebotomy [20]. To avoid a (rapid) increase in ferritin it is a good idea – especially shortly after the phlebotomy – to limit iron intake to ensure that a minimum of iron is absorbed. Deep phlebotomies (to a low ferritin level), however, show a greater 'sensitivity' for the transit of iron from the enterocyte into the blood and it is hardly possible to eat 'against' this. Another argument for maintaining a higher 'set point' at/ after the phlebotomy are the serum ferritin values of the population. These were in the Framingham Heart Study 108 µg/l for men and 72 µg/l for women [44], and in a study by Alexander et al., were 36.6 µg/l and 13.6 µg/l for adult male and female vegetarians respectively. By maintaining a higher ferritin value after phlebotomy, it might also be less frustrating for the patient to maintain a diet, as efforts will translate to better (low) serum ferritin levels.²⁸ Recently Ramos et al. [88] argued for adjusting the cut-off value in phlebotomy due to a higher absorption at lower serum ferritin levels.

The number of phlebotomies that ultimately can be reduced by following a diet can not be indicated. The added value of the diet will – related to iron accumulation and dietary habits – differ from person to person. Dietary changes may explain the fact that half of twenty patients, who were followed over

²⁸ Besides a discussion on the cut-off value after phlebotomy it is also realistic to question the normal range, up to 200 and 300 µg/l for women and men respectively. It appears that one fifth of the women and two fifths of the men had iron overload in the liver, while serum ferritin was below the specified maximum values [87].

a period of over four years, did not need any phlebotomies after the depletion phase* [74]. One can see that by conscientiously following the suggested dietary guidelines, theoretically, one can eliminate more than one phlebotomy each year. Hence it is unrealistic to stick to the belief that dietary changes have only a slight benefit compared to phlebotomies.

In addition: Although food has a nearly negligible effect on the total number of phlebotomies per year, for the patient there might be every reason to follow a diet. First, there is presence of personal effort, the need/self-empowerment of doing something to contribute to the prevention of (symptoms and/or severe complications of) iron overload. Second, physicians may underestimate the impact of phlebotomy relative to following a diet so far as the patient is concerned [12]. For many patients, therapeutic phlebotomies are a very unpleasant affair: 95% of patients who underwent phlebotomy, would prefer to swallow a pill with a 5% probability of serious adverse consequences as an alternative to phlebotomy [6]. The importance of food seems to be a message in general still to be recognized by physicians. An active attitude of physicians with regards to nutrition may assist the patient in responding to his illness by making changes where possible.

The doctor will be able to inform the patient well about a number points: First, the patient should know that the condition causing hemochromatosis will not go away, because the genetic defect cannot be undone by nutrition. Phlebotomy will therefore continue to be needed if ferritin levels are too high. However, it is possible to try to limit iron overload so much as possible through diet modifications. The patient must thereby know that it is unrealistic and unhealthy to limit iron intake to zero. A diet that provides all other necessary nutrients is more important than avoiding all iron in the diet. Third, the patients should know that the effect of dietary changes depends on the degree of accumulation that takes place, and on the opportunities that remain in the diet to reduce the intake or absorption of iron. Furthermore, the patient should be aware that dietary changes will not immediately “fix” or prevent symptoms linked to hemochromatosis. The effect is not immediately visible, but – depending on the frequency of phlebotomy – over a long-term. Supporting the patient nutritionally will therefore translate into a concrete evaluation of the increase in serum ferritin over time. Motivating the patient is an issue requiring attention. It is good if the patient knows that he would be well advised to implement changes to his/her diet slowly. In this way, it is best maintained for the long term. A reference to a dietitian when there are questions or motivational problems could be considered.

Besides more attention to nutrition in the treatment of hemochromatosis patients, more attention should be given to preventing the expression of the disease by symptoms. Persons who are diagnosed with an abnormality of the *HFE*-gene*, but do not yet have (too) high a serum ferritin value, can benefit from dietary advice, so that they may try to postpone the beginning of complaints. That the patient is motivated to do this was shown in research by Allen et al. [41]. Although it was stated that it was not compulsory, nearly one fifth of the carriers* decreased their meat intake, although their serum ferritin values were considered ‘normal’. Also, many followed up the advice to reduce their alcohol intake and supplement use [41]. Thus, by changes in diet, the onset of symptoms can be delayed and the prevalence of the number of hemochromatosis patients reduced.

From this perspective, this diet applies to one in two hundred people. Unfortunately most of these people do not know that they accumulate excess iron, because they do not know about their genetic defect. This issue cuts to the discussion of the possibility of screening at the population level. For the time being there is no screening in the Netherlands due to the high false positive results and low prevalence of hemochromatosis [5].

Other specific issues in the prevention of hemochromatosis include the use of iron-fortified foods and dietary supplements containing iron, and blood donations. The use of fortified foods and supplements leads to more cases and an ‘earlier’ discovery of hemochromatosis. From this viewpoint a (further) discussion about the usefulness of adding iron to food, rather than prescribing iron supplements in proven cases of anemia, is in place. Furthermore, subjects with a predisposition for iron overload are strongly advised to become blood donors. In a study by Allen et al. [41], almost half the people, after the discovery of the C282Y homozygosity, became blood-donors.

All in all, it is incorrect to regard nutrition as an ineffective part of the treatment of hemochromatosis. Both patients and practitioner(s) must realize that the effect of food changes are not instantly noticeable, unlike the removal of iron with a phlebotomy.

8 Recommendations

In preparing the nutritional advice, gaps became apparent, indicating the need for further research into the effects of diet in hemochromatosis.

First, more research is needed into the physiological and epidemiological substantiation of the link between diet and iron overload. In the field of physiology* there has been much research into (genetic influences on) the various receptors involved in iron uptake. Experimental and observational human studies have, however, rarely been published in recent years. Much of this kind of research is dated and thus based on unknown genetic defects, without clear understanding of definition(s). In addition, the studies often had very limited numbers of test subjects. Also, the variety of gene defects limited the prediction of the effect of dieting. Furthermore, the duration of the studies was sometimes not optimal to consider a result as "solid". The drafters of the directive *Diagnosis and treatment of hereditary hemochromatosis* [5] came to a similar conclusion: "In general, it can be said that the literature found refers to source articles that often have been written before genetic testing was available, that there is no clear and sometimes no definition of hereditary hemochromatosis given and that the methodological quality often is low (case reports, observational studies* without control group*, low numbers of participants and non-blinded or not the same way of diagnosis applied to all." [5] Also Swinkels et al. [19] concluded that "Especially the last 10 years in the literature there is little evidence at hand regarding the diagnosis and treatment." [19]. There should be more attention to this, certainly when it comes to following the drawn up recommendations after the diagnosis of hemochromatosis.

It is also desirable that more attention be given to other nutritional aspects in hemochromatosis. For example, it would be useful to find out more about the amount of vitamins and minerals that are lost during phlebotomies. Also, the influence of hemochromatosis in the uptake of other metals such as cobalt, zinc and lead and the role of other nutrients such as certain vitamins in (preventing) the absorption of iron may get more attention. It would be good to investigate the possibility of undoing the harmful effects of free iron in the blood. In this way, perhaps some patient complaints such as joint problems may be 'solved'. At the same time specific advice regarding the possible use of dietary supplements can be given.

Furthermore, the discussion regarding iron-fortified foods and the limit of iron in dietary supplements may be further boosted. To avoid more cases of hemochromatosis in the future it might be good to only provide dietary supplements containing iron only by prescription. Furthermore, a group of experts should come together to discuss the pros and cons of enriching foods with iron (as well as with other micronutrients), and come to a considered decision regarding the possible problems of enriched products. In the course of time it can hereby also be evaluated whether the desired effect is achieved. In undeveloped countries this is already common practice; there are 'National Fortification Alliances' established before a product is allowed to be enriched. Politicians, industry, international organizations and civil organizations of the country itself are participating in consultations to enforce the policy of legalization and regulation surrounding food's enrichment²⁹.

Finally, it would be desirable if the disease and the role of nutrition became better known among doctors and nutritionists. Doctors, could refer patients to dietitians for an appropriate dietary advice and an estimate of the possible effects of diet change. Records of the effects should be kept in order to eventually better substantiate the results of dietary advice.

²⁹ See also: <http://www.gainhealth.org/programs/gain-national-fortification-program/national-fortification-alliances> // 2001, access date 07-04-2011.

9 Afterword

Dietary advice for hemochromatosis was drafted. However, the conclusions were less definite than previously hoped. It was not for nothing that Adams and Baron [58] said that "Evidence-based dietary advice for patients with hemochromatosis could diminish the rate of increase in iron stores, and decrease the expressivity of iron overload due to hemochromatosis in populations." Many international hemochromatosis societies provide dietary advice, but without a scientific basis. Floating on the internet there are several recommendations that lead to unnecessary restrictions and/or are irresponsible because they make food intake incomplete. It is the hope that this will eliminate a lot of questions for hemochromatosis patients and that it will also provide an incentive for more research to answer the questions that are still open and questions that have newly arisen.

Further searching and sifting through applied research and the search for the physiology* of specific nutrients that promote or inhibit the uptake of heme or non-heme iron and the influence of iron types which are used in food fortification, will perhaps clarify the effects of dietary changes in hemochromatosis, and will also apply to family members who know they have the genotype, but who do not yet have iron overload so severe that it produces physical complaints.

This report is written especially for that group of patients who are interested and motivated; those who want to know what they can watch and those who are willing to adjust their diet where needed. For example, they can examine the impact of their diet on their iron status, and determine whether or not diet can reduce their need for phlebotomy by at least one phlebotomy per year, like a patient who shared her experiences on the Internet:

"My husband and I both have HH. I'm homozygous C282Y and he's a compound H63D/C282Y. He probably would eat a "whole cow every 3 months", drinks alcohol and never thinks about the iron content of his diet. His recent tests showed Ferritin of 33 and Tsat of 35%. He only phlebs 3 times a year. I rarely eat red meat, take care about dietary iron intake, drink tea with meals and no longer touch alcohol. My latest tests show Ferritin of 77, Tsat of 93%, my serum iron is always high and I haven't had a phleb since February 2009.

HHers still require some dietary iron and we each have different iron loading patterns. Micro-managing your diet could come close to taking over your life and won't necessarily make too big a difference to your phlebbing schedule."

Although it is standard to say "thanks", the acknowledgments below are no less than standard: Thanks go out to the supervisory committee, in particular Irene Gosselink and Alida Melse for their direct supervision of the project. In addition, a 'thank you' is in place for the hemochromatosis patients for their contribution in the broad sense: regular supply of lots of information, asking questions, giving comments and so on. Through their contributions, this report has become one by and for the hemochromatosis patient.

Glossary

Absorption	Uptake
Absorption-study	Research aimed at measuring the absorption of a nutrient in the body
Bioavailability	The degree to which a nutrient can be absorbed into the body
Body Mass Index	Measure of the relationship between body weight and length, usually used to provide an indication of over- or underweight (also referred to as BMI)
Carrier	A person who has one copy of a mutated, pathogenic gene and who (not at an early age) has no symptoms of the disease, but can transmit the mutated gene to his or her children
Control group	Group of individuals in an investigation into the effect of a particular treatment or procedure to investigate the treatment or surgery that does not undergo the treatment or procedure (see Appendix II)
Depletion phase	The first period after the diagnosis hemochromatosis, in which the excess iron in the body is reduced by frequent therapeutic phlebotomy over a relatively short period of time
Enhancer	A substance or circumstance that enhances the absorption* of a nutrient
Experimental study	To investigate a particular aspect by deliberately changing circumstances (see Appendix II)
Hepcidin	A hormone that regulates the iron metabolism throughout the body
HFE-protein	A protein involved in iron uptake (HFE = high ferritin)
<i>HFE</i> -gene	The piece of DNA allowing formation of the HFE-protein* (HFE = high ferritin)
Idiopathic	No known cause (Before it was possible to determine the genetic mutation, hemochromatosis could only be diagnosed based on the symptoms)
Inhibitor	Substance or circumstance that deteriorates the absorption* of a nutrient
Intervention Group	The group of persons in an investigation into the effect of a particular treatment or procedure who undergoes the treatment or intervention that is being tested – e.g. “subject population” (see Appendix II)
Isotope study	Study into the absorption of a nutrient that makes use of certain charged particles
Multiple-meal study	Research where the effect of multiple meals is measured
Observational research	A study where relationships are sought by means of looking at effects
Physiology	The science related to the functioning of living organisms
Recommended daily amount/recommended daily intake (nutrient)	The recommended amount of a specific nutrient to take per day, the amount is based on the average need of the Dutch population, covering most of them
Recommended daily amount/recommended daily intake (food)	A recommended amount of a specific food group to take for a day, to cover the intake of the recommended daily allowance (nutrients) for most of the Dutch population
Single-meal study	Research where the effect of a single meal is measured
Statistically significant	A designation to rule out that a result is or is not based on chance (this is usually a certainty of 95%, indicated by ‘ $p < 0.05$ ’)

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Attachments

Appendix I: Physiological aspects of the (disturbed) iron metabolism (in *HFE*-hemochromatosis)

Appendix II: From research on nutrition to advice on nutrition in *HFE*-hemochromatosis

Appendix III: Overview of dietary intervention and observational studies in *HFE*-hemochromatosis

Appendix IV: Nutrition (food products) with added iron

Appendix V: Summary of key information for patients

Please note: attachments are only available in Dutch.

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Wageningen UR Science Shop
P.O. Box 9101
6700 HB Wageningen
The Netherlands
T + 31 317 48 39 08
E scienceshop@wur.nl

www.wetenschapswinkel.wur.nl/uk

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