

Liver Iron Contents in Rats after Administration of Certain Iron Compounds

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SUMMARY

The effect of consumption of certain iron compounds on liver iron deposition was studied in rats, in each case at 4 dietary iron levels ranging from 70 to 206 mg/100 g diet. In one of two series the basic diet was maize porridge meal, and in the other, a semisynthetic 'balanced' diet was fed.

The siderogenicity of the iron compounds, when fed with maize porridge meal, was found to be in the following order: ferrous chloride > pulverised steel > pulverised cast iron > ferric chloride > ferric citrate = ferric lactate > ferric oxide. The relatively high siderotic effect of cast iron was reduced considerably when fed together with a semisynthetic 'balanced' diet.

It was concluded that the presence of free iron particles in food, as well as the conditions prevailing in the gut as a result of consumption of a predominantly maize diet, are important contributing factors in the development of siderosis in the rat and most probably also in Blacks.

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Iron overload occurs in varying degrees in the majority of adult Blacks in South Africa.¹ Two views have been put forward with regard to the cause of this condition. One group of workers postulated that iron overload occurs as a result of a widespread metabolic defect induced by chronic malnutrition,^{2,3} while others regarded a habitual high iron intake as the primary aetiological factor.^{4,5}

The diet of Blacks contains excessive amounts of iron, most of which is derived from utensils used in cooking and in the brewing of alcoholic beverages.^{4,6} The nature of this extra iron probably depends on the type of food prepared, e.g. home-brewed beer may contain iron lactate. We have also been able to demonstrate the presence of metallic iron particles in certain foods. The use of steel rollers for the grinding of cereals and other foods was found to cause contamination with iron particles.⁷ In a previous investigation⁸ it was found that these particles, when incorporated into a maize diet, increased the deposition of iron in the livers of rats to a much greater extent than did iron citrate. This finding led us to suggest that contamination of foods with free metallic iron might be more important than the presence of organic iron salts in the

aetiology of siderosis in Blacks. However, the possibility cannot be excluded that we have overrated the siderogenic effect of metallic iron particles, since iron in the citrate form might be particularly unavailable for absorption under the relevant experimental conditions, and therefore not an appropriate reference for the evaluation of the relative siderogenicity of iron sources. It was therefore decided to re-evaluate the siderotic effect of metallic iron in a study involving metallic iron as well as several other iron sources.

As maize is the staple diet of the Blacks, it was considered pertinent to study the effect of the iron sources in a predominantly maize dietary context. However, it was also of interest to examine the possible effect on liver iron deposition of other dietary ingredients not well represented in the maize diet. For this reason the degree of siderosis induced by one of the most important iron compounds, viz. cast iron, was also examined while the semisynthetic 'balance' diet was being fed.

MATERIALS AND METHODS

The study entailed feeding rats certain iron compounds at specific levels in a diet for a specific period, measurement of total food intake during the experimental period, and subsequent determination of the non-haemoglobin iron content of the livers of the rats.

Experimental Diets

The predominantly maize diet consisted of maize porridge meal plus 0.3% sodium chloride. The semisynthetic 'balanced' diet contained 5% whole egg protein plus carbohydrates, fats, and a vitamin and mineral mixture at the levels employed in this laboratory on a routine basis whenever semisynthetic diets are used.⁹ In view of the nature of the study the mineral mixture did not include ferric citrate. Details regarding the preparation of maize porridge meal as well as the exact composition of the semisynthetic diet have been published.⁵

The following iron compounds were investigated: (a) ferrous chloride, generally considered to be an iron source of high availability and therefore useful as a reference; (b) ferric chloride, to study the effect of the valence state of the iron; (c) ferric citrate, which has been used as an iron source in several investigations on experimental siderosis; (d) ferric lactate, a salt usually present in Blacks' foods (e.g. *mahewu* and home-made beer) under conditions causing production of lactic acid; (e) metallic iron, present in foods as a contaminant from milling equipment;

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(f) cast-iron powder, which may be introduced into foods from the traditional iron cooking pots used by Blacks; and (g) iron oxide (Fe_2O_3), which may be present in foods through the use of rusted iron utensils. In each case maize porridge meal was used as the basic diet. As mentioned, the semisynthetic 'balanced' diet was used in an additional study involving cast-iron powder. The effect of each iron source was investigated at 4 different levels: approximately 70, 116, 161 and 206 mg/100 g diet.

The metallic iron consisted of an iron powder prepared by grinding a steel rod of the type En 26 (British Standard grading) in a Siebtechnik Vibratory Disc Mill (Model T. 250). The vessel, rings and stone of the mill are made of tungsten carbide, yielding a ground product which is comparatively free from products of wear. Cast-iron powder was similarly prepared from pieces of an iron pot of the type traditionally used by Blacks in the cooking of maize porridge.

Prior to being mixed into the experimental diet, ferrous chloride was coated, by addition of glycerol tripalmitate to a level of 10 (w/w), to prevent oxidation to the ferric state. The glycerol tripalmitate was dissolved in an appropriate volume of chloroform, the solution mixed with the iron salt and the solvent allowed to evaporate under vacuum.

Iron lactate was prepared according to the following method: a quantity of 50 g of $\text{Fe}_2(\text{SO}_4)_3$ was dissolved in 100 ml water and 100 g of 88% lactic acid. To this mixture 110 g $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, dissolved in 500 ml water, was added to precipitate excess sulphate ions. After filtration through a double layer of Whatman No. 1 filter paper, the filtrate was evaporated to a volume of approximately 100 ml. To this concentrated solution about 1 litre of acetone was added very rapidly with continuous stirring. The iron lactate formed was immediately collected on a filter paper in a Buchner funnel and then washed several times with acetone. The final product was dried overnight in a vacuum desiccator.

Rats and Experimental Procedure

The experimental animals were weanling male rats of the F2 generation of a cross between two inbred strains, namely BD V and BD IX. The rats were divided into 8 main groups. Each main group consisted of 4 subgroups (8 rats per subgroup) which received the same basic diet but differing amounts of iron of a specific type.

Each animal was housed individually in a perspex metabolism cage, in an air-conditioned room at $26 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity. In an attempt to equalise food consumption, the amount of diet fed was restricted to a level of 8% of the body mass per day. De-ionised water was offered *ad lib*.

At the end of a 21-day experimental period the rats were killed with ether and the livers excised for determination of the non-haemoglobin iron content.

Chemical Methods

Iron contents of diets were determined according to the L,L'-dipyridyl method¹⁰ after wet ashing of appropriate

samples with 50:50 (v/v) sulphuric acid-perchloric acid mixture.

Livers were homogenised in 9 volumes (w/v) of de-ionised water in a Potter-Elvehjem-type homogeniser with a Teflon pestle. Suitable aliquots of these homogenates were treated according to Kaldor's method¹¹ to extract the non-haemoglobin iron. The L,L'-dipyridyl method was employed in the determination of the iron content of the extract.

RESULTS AND DISCUSSION

The average values obtained for each subgroup for iron intake and liver non-haemoglobin iron content, as well as the source of iron fed when the diet was predominantly maize, are listed in Table I.

TABLE I. DATA ON IRON INTAKE AND NON-HAEMOGLOBIN LIVER IRON IN RATS FED CERTAIN IRON COMPOUNDS IN MAIZE PORRIDGE MEAL

Iron source	Subgroup No.	Iron intake (mg/100 g average body mass)*	Non-haemoglobin (mg/g wet liver)
No iron added	—	5,1 ± 0,04†	0,11 ± 0,01†
Ferrous chloride	1	113,8 ± 0,73	0,53 ± 0,07
	2	187,7 ± 1,47	0,63 ± 0,04
	3	268,4 ± 0,92	0,80 ± 0,05
	4	342,0 ± 0,64	0,93 ± 0,07
Pulverised steel	1	118,4 ± 0,65	0,44 ± 0,04
	2	191,7 ± 1,51	0,52 ± 0,03
	3	262,4 ± 2,38	0,70 ± 0,06
	4	337,2 ± 3,40	0,88 ± 0,06
Pulverised cast iron	1	117,7 ± 0,14	0,36 ± 0,02
	2	196,6 ± 1,19	0,50 ± 0,04
	3	266,1 ± 3,39	0,58 ± 0,05
	4	344,5 ± 2,10	0,75 ± 0,05
Ferric chloride	1	108,4 ± 0,39	0,40 ± 0,03
	2	180,7 ± 0,86	0,43 ± 0,03
	3	255,1 ± 2,17	0,53 ± 0,06
	4	332,7 ± 0,95	0,56 ± 0,03
Ferric citrate	1	115,4 ± 1,05	0,41 ± 0,03
	2	187,1 ± 2,11	0,40 ± 0,02
	3	267,1 ± 3,26	0,45 ± 0,04
	4	342,8 ± 2,64	0,52 ± 0,04
Ferric lactate	1	105,3 ± 2,95	0,31 ± 0,01
	2	144,0 ± 1,22	0,33 ± 0,02
	3	266,8 ± 4,43	0,46 ± 0,02
	4	323,0 ± 3,06	0,50 ± 0,01
Iron oxide	1	112,3 ± 0,48	0,11 ± 0,01
	2	186,3 ± 0,42	0,11 ± 0,01
	3	265,5 ± 0,61	0,15 ± 0,02
	4	330,1 ± 1,69	0,12 ± 0,01

* Average body mass during experimental period.

† Standard error of mean of 8 observations.

The relationships observed between iron intake and non-haemoglobin iron content in the liver are shown in Fig. 1.

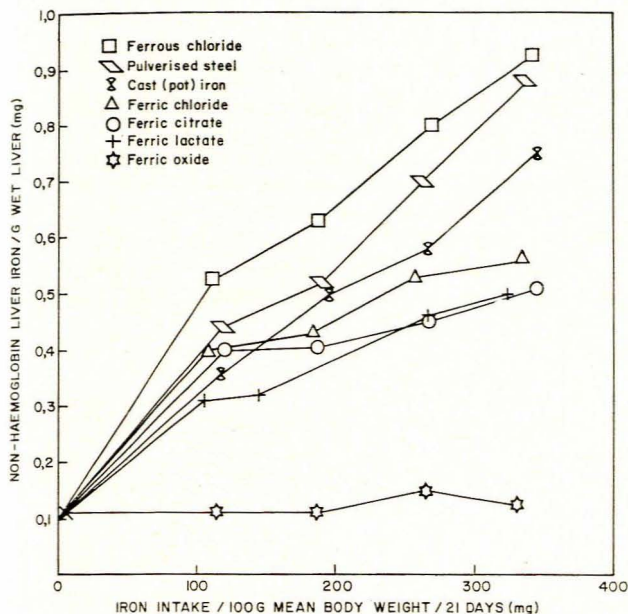


Fig. 1. The effect of type of iron fed on liver non-haemoglobin iron content relative to iron intake in rats when maize porridge meal was the basic diet. Each point represents the average of 8 observations.

Data pertaining to the significance or otherwise of differences among the liver iron contents of rat groups at a particular dietary iron level are given in Table II.

The relevant statistical analyses entailed analyses of variance with the aid of an IBM computer in which use was made of programme BMDPIV as described by Dixon,¹⁵ and application of the multiple comparison technique of Scheffé.¹³

From the results it appeared that liver iron content generally tended to increase with an increase in iron intake. It was also clear, however, that there were considerable differences as regards the quantitative effects of the various iron compounds on liver iron deposition; there were, moreover, differences in the nature of the response of liver iron relative to iron intake.

Generally the different iron compounds appear to fall into 3 distinct classes with regard to effect on liver iron content. Forming one group were ferrous chloride, pulverised steel and pulverised cast iron. These substances yielded the highest liver iron values, while the response curves suggested that the livers had not reached saturation with iron even at the highest iron intake level. Iron oxide falls into a class of its own; in this case the liver iron values remained practically constant at all levels of iron intake. Ferric chloride, ferric citrate and ferric lactate comprised the third group. With respect to the rate of increase of liver iron relative to iron intake, these compounds occupied an intermediate position.

It appears that the valence state of the iron in the different compounds plays a role in the above classification. More iron was deposited in the liver when ferrous chloride was administered than when ferric compounds were fed, the difference becoming more marked as iron

TABLE II. SIGNIFICANCE (+) OR NON-SIGNIFICANCE (-) AT 5% PROBABILITY LEVEL OF DIFFERENCES IN LIVER IRON CONTENT OF RATS FED CERTAIN IRON SOURCES AT CERTAIN DIETARY LEVELS IN A PREDOMINANTLY MAIZE DIET

Iron Source	Level 1 (c. 70 mg/Fe/100 g diet)			Level 2 (c. 116 mg Fe/100 g diet)			Level 3 (c. 161 mg Fe/100 g diet)			Level 4 (c. 206 mg Fe/100 g diet)		
	Ferrous chloride	Pot Iron	Steel	Ferrous chloride	Pot Iron	Steel	Ferrous chloride	Pot Iron	Steel	Ferrous chloride	Pot Iron	Steel
Ferrous chloride	+	+	+	+	+	+	+	+	+	+	+	+
Steel	+	+	+	+	+	+	+	+	+	+	+	+
Pot Iron	+	+	+	+	+	+	+	+	+	+	+	+
Ferric chloride	+	+	+	+	+	+	+	+	+	+	+	+
Ferric citrate	+	+	+	+	+	+	+	+	+	+	+	+
Ferric lactate	+	+	+	+	+	+	+	+	+	+	+	+
Ferric oxide	+	+	+	+	+	+	+	+	+	+	+	+
Ferrous chloride	+	+	+	+	+	+	+	+	+	+	+	+
Pot Iron	+	+	+	+	+	+	+	+	+	+	+	+
Steel	+	+	+	+	+	+	+	+	+	+	+	+
Ferric chloride	+	+	+	+	+	+	+	+	+	+	+	+
Ferric citrate	+	+	+	+	+	+	+	+	+	+	+	+
Ferric lactate	+	+	+	+	+	+	+	+	+	+	+	+
Ferric oxide	+	+	+	+	+	+	+	+	+	+	+	+

* Iron intakes not sufficiently similar to permit a valid comparison.

intake increased. Opinions differ with regard to the relative availability of ferrous and ferric compounds. In several investigations on rats,¹⁴⁻¹⁶ it was found that these compounds were equally well absorbed, while in a very early study (1927), superiority was claimed for ferric salts.¹⁷ A possible reason for this inconsistency in results is that these workers studied the problem while feeding the iron at very low levels. Our results are in concordance with the finding of Brise and Hallberg¹⁸ who observed that when high therapeutic dosages of iron were administered to humans, much more iron was absorbed from a ferrous salt than from the corresponding ferric salt.

Both the metallic iron powders (pulverised steel and cast iron) investigated in the present study would probably, when dissolved in gastric juice, form ferrous chloride, and it was therefore not surprising to find a close agreement between the relevant response curves (Fig. 1). Since ferrous chloride is one of the most readily utilisable iron sources¹⁹ the metallic iron powders must, in view of this agreement, be regarded as physiologically highly available iron sources.

This relatively high availability of the metallic iron powders warrants further consideration from a practical viewpoint. It has been shown^{4,6} that the iron content of foodstuffs can be raised several times through use of the cooking utensils commonly employed by Blacks. As mentioned previously, we were able to extract (with the aid of a magnet) demonstrable quantities of metallic iron particles from certain foods (maize porridge and beer) prepared by Blacks in the traditional iron pots and tins. It is to be expected that the amounts present in foods will depend on a variety of factors, such as the condition and size of the utensil, the cooking time and the type of food prepared. Iron particles may also be introduced into foods as a contaminant from the steel rollers of food-filling machines. Obviously a variety of iron compounds, differing in their siderotic potential, can be expected to form during the preparation of various foods. In view of our data the

presence in food of any free iron particles is obviously an important contributing factor in the development of siderosis in the rat and most probably also in the Blacks.

The poor availability observed in the case of the iron oxide is in concordance with the results of other workers.²⁰ This being so, it seems justifiable to suggest that a biological assay rather than total iron content would be the better index of the siderogenicity of a diet.

The results of the study on the effect of the semisynthetic 'balanced' diet on the siderogenicity of cast iron are listed in Table III and are illustrated in Fig. 2, while the statistical data are given in Table IV.

TABLE IV. SIGNIFICANCE (+) OR NON-SIGNIFICANCE—AT 5% PROBABILITY LEVEL OF DIFFERENCES IN LIVER IRON CONTENT OF RATS FED PULVERISED CAST IRON IN EITHER MAIZE PORRIDGE MEAL OR A SEMISYNTHETIC 'BALANCED' DIET

	Maize porridge meal	Semi-synthetic 'balanced' diet
Level 1 (c. 70 mg Fe/100 g diet)		
Maize porridge meal	—	—
Semisynthetic 'balanced' diet	—	—
Levels 2, 3, 4 (c. 116; 161; 206 mg Fe/100 g diet)		
Maize porridge meal	—	+
Semisynthetic 'balanced' diet	+	—

From Fig. 2 it is clear that the non-iron constituents of the diet played a prominent role in the deposition of iron in the liver. It is evident that the increase in liver iron content which occurred as a result of increased iron intake was much greater in the case of the maize diet than in that of the semisynthetic 'balanced' diet. Although the reason for this difference is by no means clear, there are several factors which might have a bearing on the question. Firstly, we need to consider the possibility that the differences in liver iron content could have been a manifestation of the protein content of the two diets fed; the protein content of the maize diet was approximately 1.9 times that of the 'balanced' diet. It has been shown by other workers that dietary proteins²⁰ as well as free amino acids²¹ have a promotive effect on iron absorption. However, results previously obtained by us⁵ suggest that this difference in the protein contents of the two diets alone cannot explain the great differences in the extent of liver iron deposition observed under the dietary conditions imposed in the present study. In fact, some workers²² have found no differences at all in iron retention from diets of different protein content.

Another factor which might have a bearing on the results under discussion is the possible difference in the motility of the gut under the prevailing dietary conditions. Higginson *et al.*²³ found that the speed of passage of a maize diet through the small intestine was slower than that of other diets, and in view of this they suggested that the increased iron absorption observed in the case of the

TABLE III. DATA ON IRON INTAKE AND NON-HAEMOGLOBIN LIVER IRON IN RATS FED PULVERISED CAST IRON IN EITHER MAIZE PORRIDGE MEAL OR SEMISYNTHETIC 'BALANCED' DIET CONTAINING WHOLE EGG PROTEIN AT 5%

Diet	Subgroup No.	Iron intake (mg/100 g average body mass)*	Non-haemoglobin iron (mg/g wet liver)
Maize porridge meal	0	5,1 ± 0,04†	0,11 ± 0,01†
	1	117,7 ± 0,14	0,36 ± 0,02
	2	196,6 ± 1,19	0,50 ± 0,04
	3	266,1 ± 3,39	0,58 ± 0,05
	4	344,5 ± 2,10	0,75 ± 0,05
Semisynthetic 'balanced' diet	0	5,8 ± 0,02	0,08 ± 0,01
	1	113,6 ± 0,60	0,17 ± 0,01
	2	184,6 ± 0,81	0,20 ± 0,02
	3	267,1 ± 2,10	0,25 ± 0,02
	4	340,6 ± 1,42	0,25 ± 0,02

* Average body mass during experimental period.

† Standard error of mean of 8 observations.

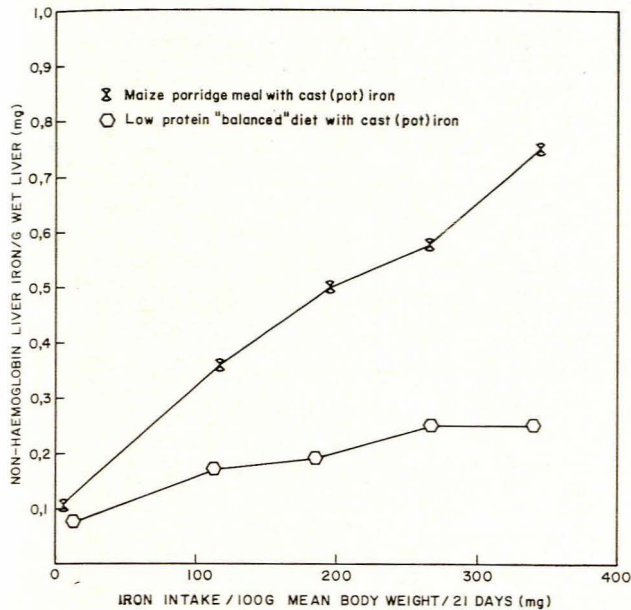


Fig. 2. The effect of dietary iron (pulverised cast iron) on liver non-haemoglobin iron content when the iron was fed at graded levels together with either maize porridge meal or a semisynthetic 'balanced' diet containing whole egg protein at 5%. Each point represents the average of 8 observations.

maize diet, compared with the other diets, was due to the greater time exposure to the intestinal mucosa and the consequent increase in efficiency of absorption. During the course of our experiment we observed that the maize diet was consumed much more slowly than the 'balanced' diet. It seems reasonable to expect that this could be a factor contributing to better iron absorption and the consequent higher liver iron values which were observed when the maize diet was fed. Iron absorption is also influenced by the composition and the level of the mineral mixture incorporated into the diet, the amounts of iron absorbed being reduced as the level of the mineral mixture is raised.²² Hegsted *et al.*²⁴ showed that an excessive iron absorption from an iron citrate-supplemented, predominantly maize

ration could be reduced by the addition of phosphates to the diet. It seems possible that the balanced mineral mixture used in the semisynthetic diet on the one hand, and the unfavourable mineral composition of the maize diet on the other hand, might be additional factors responsible for the differences in the relevant response curves (Fig. 2).

Higginson *et al.*²³ found that when one diet was fed for a period of time and then replaced abruptly with another, the change to a predominantly maize diet always resulted in an increased iron absorption. In view of this phenomenon the authors concluded that the increased absorption observed when the maize diet was fed was due mainly to factors operating in the lumen of the intestine and was not, as has been suggested by others,³ the result of effects on intracellular metabolism of the predominantly maize diet.

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