The effect of dietary iron intake on the development of iron overload among homozygotes for haemochromatosis

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Abstract

Objective: To quantify the role of dietary Fe in total body Fe (TBI) accumulation among homozygotes for the *HFE* gene associated with haemochromatosis.

Design: A Monte Carlo model was built to simulate Fe accumulation based on findings from human feeding experiments and national dietary surveys. A hypothetical cohort of 1000 homozygotes with starting age 25 years was used in 39-year simulations. The impact of reducing dietary Fe intake on Fe accumulation was tested.

Results: In the baseline model without any dietary intervention, by age 64, the percentage of males with TBI > 10 g, >15 g and >20 g was 93·2%, 49·6% and 14·7%, respectively. When the Fe intake of individuals in the cohort was reduced to ≤200% of the recommended dietary allowance (RDA), the corresponding percentages were 92·0%, 40·5% and 10·2%, respectively. The corresponding figures were 91·0%, 40·0% and 9·3% for Fe defortification and 70·3%, 21·3% and 4·1% when Fe intake was capped at 100% RDA. Similar trends were seen with sexes combined, although the impact of interventions was less. Sensitivity analysis revealed that the rate of Fe accumulation and the impact of dietary interventions are highly dependent on assumptions concerning Fe absorption rates.

Conclusions: Variation in Fe intake as currently observed in the USA contributes to variation in Fe accumulation among homozygotes, when continued over an extended period. Lifelong dietary habits and national fortification policy can affect the rate of Fe accumulation, although the magnitude of the effect varies by gender, the TBI level of interest and factors affecting the Fe absorption rate.

Since the discovery of a candidate gene for hereditary haemochromatosis (HH) in 1996, HH has been found to be one of the most common autosomal recessive disorders in the US population, estimated to affect about one million people[1]. Among the mutations that have been reported, the prevalence of C282Y/C282Y genotype is 0·30% among non-Hispanic whites, 0·06% among non-Hispanic blacks and 0·03% among Mexican-Americans, with no gender differences[2]. The C282Y homozygote genotype is most closely associated with HH, presenting in 67–95% of clinically diagnosed HH cases[1–6].

Clinically, Fe overload in individuals with *HFE* gene mutations can lead to fatal complications such as liver cirrhosis and diabetes[7–11]. However, the severity of the disease varies greatly. It is well established that the majority of homozygotes will have increased transferrin saturation and serum ferritin (SF)[3,12–16], but the percentage of homozygotes who will develop clinical symptoms is still unclear. Factors influencing the progress of the disease remain poorly understood. A complex gene–gene interaction and gene–environment interaction is proposed[17,18].

Some environmental factors are found to influence the manifestation of HH, for example gender, age and blood donation[18]. Dietary Fe may be another factor. A few feeding studies on Fe absorption among individuals with genetic mutations showed that the feedback control of Fe absorption was impaired in patients with HH[19–21]. Their absorption of both haem and non-haem Fe was higher than in normal individuals. Consistently, two studies suggested that higher dietary Fe intake may accelerate Fe accumulation in patients with HH[22,23]. Current recommendations on the management of HH recommend avoiding vitamin C and Fe supplements, but do not suggest control of dietary Fe intake[24].

The purpose of the current paper is to present a Monte Carlo model that simulates the Fe accumulation process in homozygotes for HH and to use the model to estimate

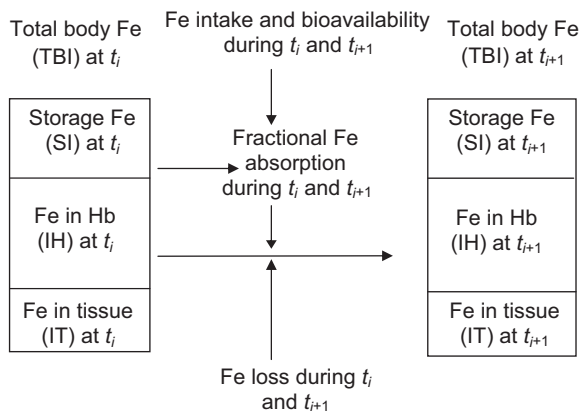† Correspondence address: Tower 400, 27000 W 11 Mile Road, Southfield, MI 48034, USA.

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whether dietary modification can be used as an effective prevention and treatment for HH.

Research design and methods

Model overview

We developed a Monte Carlo simulation model using @Risk 4·5 for Excel (Palisades Inc., Newfield, NY, USA). The model generated a cohort of 1000 homozygotes with *HFE* mutations. Each individual in the cohort is followed up from 25 to 64 years of age, unless death occurs. The process of Fe accumulation in the cohort was simulated. The simulation outcome was total body Fe (TBI) because, according to clinical observations, this is an indicator closely related to the development of specific clinical symptoms and fatal complications.

Figure 1 illustrates the biological framework of the simulation model. TBI in the model was composed of three components: (i) Fe in Hb; (ii) non-storage Fe in tissues; and (iii) storage Fe. The small amount of transport Fe in the plasma and extracellular fluid was neglected in the model. During an individual's life, TBI is a changing variable. As shown in Fig. 1, TBI at a certain time point (t_{i+1}) in the model is a function of previous Fe status (t_i) and Fe loss and Fe absorption during the time period between t_i and t_{i+1}. Data sources used to define TBI at t_0 and parameters used to simulate Fe absorption and Fe loss during various time periods are listed in Table 1[25–32]. Model structure is described in detail below. The impact of reducing dietary Fe on Fe accumulation in the cohort was tested using the model.

Fig. 1 Framework for simulating the dynamic change of iron status

Model structure

Baseline status

The simulation cohort started at age 25 years. To estimate each individual's TBI at t_0, their gender, height, weight, Hb and SF levels must be defined. The gender ratio and the distributions of height, weight and Hb for each gender group were calculated using data from the third National Health and Nutrition Examination Survey (NHANES III), since there is no evidence that homozygotes are different from non-homozygotes in values for these variables. However, SF levels differ between individuals with wild genotype and those with *HFE* mutations. Distributions of SF for males and females were based on SF levels of homozygotes between the ages of 20 and 29 years reported in a large cross-sectional survey[16]. Monte Carlo sampling was used to assign specific values for these variables to each individual in the model according to these distributions.

Fe in Hb was calculated based on Hb level and total blood volume estimated from weight and height, and non-storage Fe in tissues estimated from body weight[33–35]. Storage Fe in tissues was calculated from SF level based on the linear relationship between SF and storage Fe among homozygotes. Data from three phlebotomy studies were used to quantify the linear relationship between SF and storage Fe in homozygotes[36–38]. This procedure generated the starting TBI level for each homozygote in the cohort.

Two time units were used to facilitate the simulation of changes in Fe status once individuals left baseline (t_0) and were projected into the future: monthly and annual.

Monthly updating

Events simulated at monthly intervals include Fe absorption from food and supplements, daily Fe loss and menstruation. Within one simulation month, each individual was assumed to follow the same dietary intake. Since no evidence suggests the diet of homozygotes is different from that of non-homozygotes, data from the Continuing Survey of Food Intakes by Individuals (CSFII) 1994–96 were used to estimate meat, Fe, Ca and ascorbic acid intakes for each gender and age group. Data were normalized using Cox–Box transformations, then ANOVA was used to estimate average dietary intake levels, intra- and inter-individual variations[39]. Using Monte Carlo sampling, specific values were assigned to each individual according to these distributions. An individual's

Table 1 Sources of data used in the simulation model

Variable	Data source
Age, gender, height, weight, Hb level	NHANES III
Daily Fe, vitamin C, Ca and meat intake, Fe supplement usage during pregnancy	NHANES III, CSFII 1994–96, reference 25
Daily Fe loss, Fe loss from menses, Fe requirement from pregnancy, blood donations	References 26–30, NHANES III
Fe absorption	References 19 and 21
Mortality and fertility	References 31 and 32

NHANES III, Third National Health and Nutrition Examination Survey; CSFII 1994–96, Continuing Survey of Food Intakes by Individuals.

mean intake level of a nutrient for the month was assigned based on the average intake and inter-individual variance for his/her subgroup, then daily intake values were assigned based on each person's mean intake level and intra-individual variance. Linear equations were used to estimate the percentage of dietary Fe from fortification based on Fe intake in previous work[25].

Fe absorption was determined by meal composition and the individual's Fe status. Monsen's model and the regression equation by Hallberg *et al.* were used to calculate bioavailable Fe based on the amount of Fe, Fe absorption enhancers (ascorbic acid and meat) and Fe absorption inhibitors (Ca) contained in the diet[40,41]. In normal individuals, the feedback control of Fe absorption by Fe status is a critical part of Fe homeostasis. Feeding studies using homozygotes as subjects showed that although this process is greatly impaired, there is still a weak feedback control of Fe absorption by Fe status. After Fe bioavailability was calculated from meal composition, linear regression equations were used to predict the absorption rates of haem and non-haem Fe adjusted to each individual's Fe store. Data reported by Bezwoda *et al.* and Lynch *et al.* were used to build the regression model[19,21]. The amount of Fe absorbed was calculated using Fe intake and Fe absorption rates and was assumed to be the amount of Fe that can be utilized in the body.

Loss of gastrointestinal epithelium is a major cause of basal Fe loss. Because intestinal epithelial cells are actually Fe-deficient in HH, basal loss among homozygotes may be lower than in normal individuals. However, because there are no data available, basal loss of homozygotes was still assumed to be 14 μg/kg per d[30]. Average daily loss was calculated according to body weight and Fe loss per unit body weight. For women of reproductive age in the cohort, the amount of menstrual blood loss per menstrual cycle was assigned based on an empirical distribution[29]. The amount of Fe lost each month during menstruation was calculated based on the amount of blood loss and Hb level. By the end of each month, storage Fe was updated, taking into account Fe absorption and Fe loss during the month. Consequently, the SF level was updated corresponding to the new amount of storage Fe.

Annual updating

Events simulated on an annual interval include blood donations and pregnancy. Pregnancy was simulated as an event happening within a given simulation year. Fe loss during pregnancy was based on experts' judgement[26–28,42]. The most commonly used distribution to quantify experts' opinions, triangle distribution, was applied. The small loss of Fe from breast milk was neglected. The probabilities of giving birth were estimated from national vital statistics and varied according to mother's age[31]. Fe supplement usage during pregnancy, the length of taking supplements and the amount of Fe

taken were simulated based on data from NHANES III. NHANES III data were also used to calculate the probability of being a blood donor for each age and gender group and the frequency of blood donation each year. For blood donors in the simulation model, Fe loss through blood donation was calculated using frequency of blood donation per year, units of blood donated each time and Hb level.

At the end of each simulation year, TBI, Hb and SF levels were updated as the result of these events. Distributions of total body Fe were recalculated following the rule that Fe in the body is used to build tissue and maintain normal Hb level first, and then extra Fe will be stored[43]. Fe in the body was first allocated to body tissues, then to maintain a normal Hb level. Only after meeting these two needs was the remaining Fe apportioned to storage. If the amount of TBI was insufficient to maintain a normal Hb level, the highest Hb level possible was calculated.

Mortality rates were obtained from national vital statistics reports. The mortality rates for different gender, age and ethnic groups in 1994 were used in the model[32]. Based on these rates, mortality in various subgroups was randomly assigned.

Dietary modification

The simulation outcomes were the percentage of the cohort with TBI > 10 g, TBI > 15 g and TBI > 20 g. Among normal individuals, TBI is less than 5 g[28]. Twenty grams of TBI is usually thought to be a threshold related to specific clinical manifestation among homozygotes for *HFE* gene mutation[44]; however, the implications of mild or moderate TBI levels are still in debate. The percentages of the cohort with TBI > 10 g, TBI > 15 g and TBI > 20 g were used to illustrate the number of individuals in the cohort with various degrees of Fe overload.

Dietary modification was designed based on the recommended dietary allowance (RDA) for each age and gender group[30]. In the first intervention, if an individual's food Fe intake was >200 % of the RDA for his/her gender and age group, his/her Fe intake was reduced to 200 % RDA by decreasing non-haem Fe intake; if an individual's food Fe intake was already ≤200 % RDA, no dietary modification was made. Similarly, in another intervention tested, each individual's Fe intake from food was capped to 100 % RDA. To test the efficacy of the interventions, 100 % compliance with dietary modification was assumed. Fe defortification was also tested. The percentages of individuals with various degrees of Fe overload under different dietary interventions were compared.

Sensitivity analysis

Because absorption of non-haem Fe is a critical assumption in the model, sensitivity analysis was performed to determine its impact on the simulation outcome.

When analysing data from feeding studies among homozygotes of HH, a linear relationship was used to describe the relationship between non-haem Fe absorption rate and SF level[19,21]. The equation was:

$$\log(\text{non-haem Fe absorption rate}) = \alpha - \beta \times \text{SF} + \varepsilon.$$

Slope β reflects the average feedback control of Fe absorption by Fe status. When data from feeding studies were analysed[19,21], the estimated slope was $\beta_1 = 0.0004$, which was used to build the main simulation model. Since the subjects used in these feeding studies may be a selective subgroup of homozygotes whose feedback control of Fe absorption was likely to be more severely damaged than those who had not developed HH, three steeper slopes were arbitrarily chosen ($\beta_2 = 0.0005$, $\beta_3 = 0.0008$ and $\beta_4 = 0.002$) and used for sensitivity analysis. If, for a woman with wild genotype and 500 mg Fe storage, the non-haem Fe absorption rate is 5 % for a certain meal, Fig. 2 shows the average non-haem Fe absorption rate for homozygotes by SF level for the same meal under different slope assumptions.
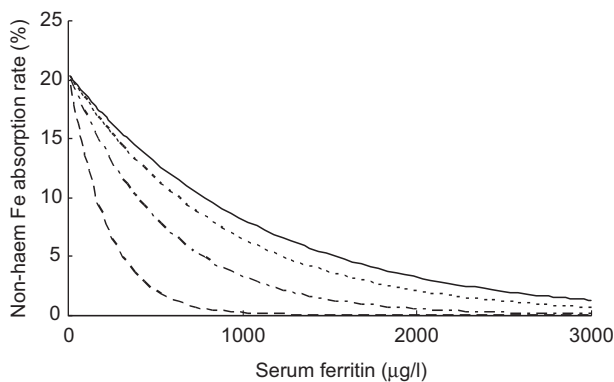
Fig. 2 Average non-haem iron absorption rates under different slope assumptions (——, $\beta_1 = 0.0004$; - - -, $\beta_2 = 0.0005$; – · – ·, $\beta_3 = 0.0008$; – – –, $\beta_4 = 0.002$)
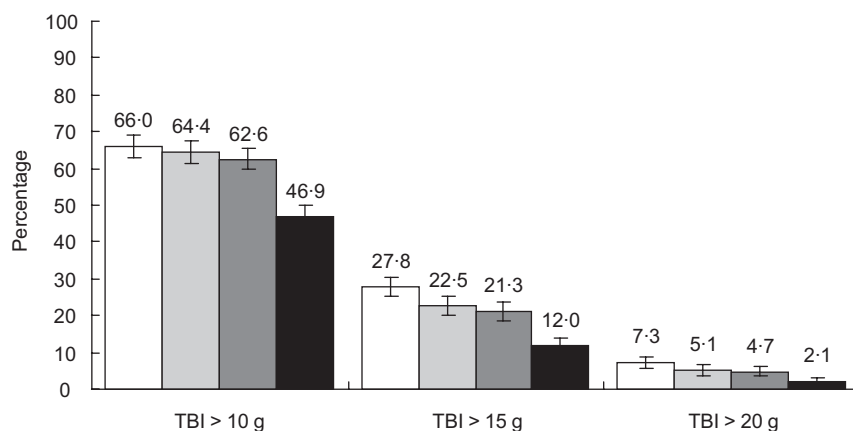
Simulations were run under different slope assumptions and the percentages of the cohort with TBI > 10 g, TBI > 15 g and TBI > 20 g were calculated and compared. The effect of capped Fe intake at 100 % RDA on Fe accumulation was tested under different slope assumptions.

Results

When Fe absorption data from feeding studies were used to build the model ($\beta_1 = 0.0004$), without dietary change, 66.0 % of the simulation cohort reached TBI > 10 g, 27.8 % of the cohort accumulated TBI > 15 g and only 7.3 % of the cohort reached TBI > 20 g (Fig. 3). Fe defortification or reducing Fe intake to 200 % RDA slightly reduced the speed of Fe accumulation and their sizes of impact were similar. With stricter dietary change, when Fe intake was capped at 100 % RDA, Fe accumulation was substantially slower and the percentage of the cohort reaching high TBI levels was lower.

Since a majority of those with increased TBI were males, the rate of Fe accumulation and the impact of different dietary interventions were more obvious when men were analysed separately (Fig. 4).

Sensitivity analysis

When steeper slopes (β) for the regression equation on non-haem Fe absorption and SF were applied, individuals with the same body Fe stores would absorb less Fe from the same meal (Fig. 2). The percentage of individuals who reached >10 g, >15 g and >20 g TBI also decreased gradually as the slope changed from β_1 to β_4 (Table 2). As the percentage of the cohort with TBI > 10 g, >15 g and >20 g decreased under different slope assumptions, the impact of diet on the development of Fe overload also reduced. With slope $\beta_2 = 0.0005$, 4.2 % of the cohort reached TBI > 20 g in the no intervention model; this

Fig. 3 Percentage of the cohort reaching total body iron (TBI) >10 g, >15 g and >20 g under different simulation scenarios (□, no dietary change; ▨, dietary iron intake capped at 200 % of the recommended dietary allowance (RDA); ▨, iron defortification; ■, dietary iron intake capped at 100 % RDA), with 95 % confidence interval represented by vertical bars, in homozygotes for the *HFE* gene associated with hereditary haemochromatosis

number decreased to 1·1 % when Fe intake was capped at 100 % RDA. When slope $\beta_3 = 0.0008$ was used in the simulation model, the corresponding estimates were 2·0 % and 1·0 %.

Discussion

Overall, the present simulation results demonstrate that variation in Fe intake and variation in the damage to the control of Fe absorption among homozygotes contribute to variation in the rate of Fe accumulation among homozygotes. This suggests that lifelong dietary habits and national fortification policy can affect the rate of Fe accumulation among homozygotes for HH.

Two knowledge gaps currently prevent us from fully quantifying the exact impact of dietary modification on the health outcomes of homozygotes for HH. First, as sensitivity analysis reveals, the rate of Fe accumulation and the impact of dietary interventions are dependent on assumptions concerning Fe absorption rate, which reflects the severity of damage on the Fe absorption control system. The effect of dietary intervention is strongest among individuals whose Fe absorption regulation has been impaired significantly, which is very likely to be true among patients who are diagnosed with HH or individuals with a family history of HH. As the assumed Fe absorption rate (and degree of regulatory impairment) decreases, so does the percentage of adults with Fe overload and the effect of dietary modification. In particular, dietary modification has a smaller effect in reducing severe Fe overload (TBI > 20 g) but still a modest effect in reducing mild and moderate Fe overload.

These sensitivity analyses are important because our current understanding of the degree of impairment on Fe absorption control is based on feeding studies using patients who were already diagnosed with HH. Such patients may have a higher degree of regulatory impairment than the average for all homozygotes. Feeding studies using more randomly selected homozygotes are needed, including those diagnosed with HH as well as asymptomatic homozygotes. Such studies would provide a more accurate understanding of the variation in impairment of the Fe absorption control system among homozygotes for HH and thus more accurate simulation results.

The finding that, under lesser degree of Fe absorption regulatory impairment, dietary modification is more
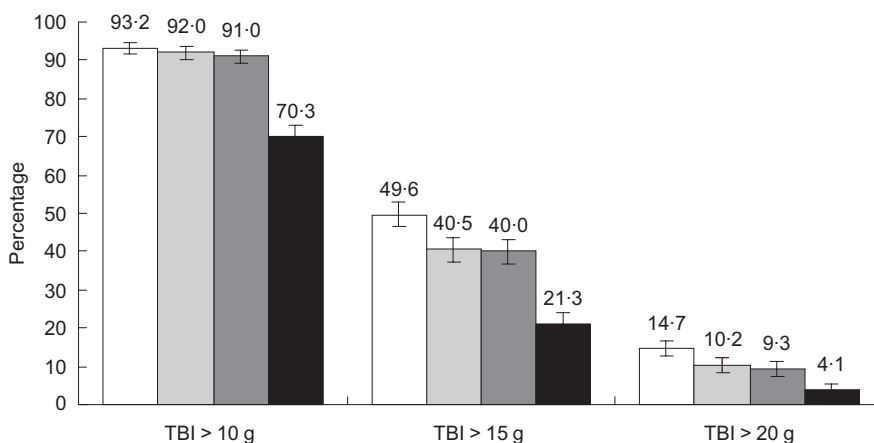
Fig. 4 Percentage of males in the cohort reaching total body iron (TBI) >10 g, >15 g and >20 g under different simulation scenarios (□, no dietary change; ▨, dietary iron intake capped at 200 % of the recommended dietary allowance (RDA); ▦, iron defortification; ■, dietary iron intake capped at 100 % RDA), with 95 % confidence interval represented by vertical bars, in homozygotes for the *HFE* gene associated with hereditary haemochromatosis

Table 2 Percentages of the cohort reaching certain TBI levels under different iron absorption assumptions in homozygotes for the *HFE* gene associated with hereditary haemochromatosis

	No dietary change						Fe intake ≤100 % RDA					
	TBI > 10 g		TBI > 15 g		TBI > 20 g		TBI > 10 g		TBI > 15 g		TBI > 20 g	
	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
β_1	66·0	63·1, 68·9	27·8	25·0, 30·6	7·3	5·7, 8·9	46·9	43·9, 49·9	12·0	10·0, 14·0	2·1	1·2, 2·9
β_2	58·0	54·9, 61·1	18·0	15·6, 20·4	4·2	3·0, 5·4	37·9	37·3, 38·5	7·7	4·7, 10·7	1·1	0·4, 1·7
β_3	49·7	46·6, 52·8	12·5	10·5, 14·6	2·0	1·1, 2·9	31·5	30·9, 32·1	5·7	2·8, 8·6	1·0	0·4, 1·6
β_4	10·0	8·1, 11·9	2·7	1·7, 3·7	0·6	0·1, 1·1	8·3	7·8, 8·8	2·2	0·5, 3·9	0·6	0·1, 1·1

TBI, total body Fe; RDA, recommended dietary allowance.

efficacious in reducing mild and moderate Fe overload (compared with severe overload) is potentially significant. This is because mild and moderate Fe overload is far more common than is severe overload. However, the public health implications of this remain unclear because studies of the relationship between mild to moderate Fe overload and the risk of chronic diseases have not produced consistent findings. Some studies found no increased risk for chronic diseases among adults with mild to moderate Fe overload[45–48], but others reached the opposite conclusion[49–51]. More prospective studies looking at the risk of chronic diseases caused by mild to moderate Fe overload are needed.

In principle, dietary modification might be achieved by changing diet habits among homozygotes for HH. This intervention is targeted so individuals without such genetic mutation will not be impacted. To estimate the efficacy of this intervention, we assumed 100 % compliance in our simulation study. In reality, the effectiveness of the intervention also relies on the compliance to both genetic screening test and dietary change. Fe defortification operates at the population level, so the issue of compliance is not involved. However, defortification may have an unwanted impact on other segments of the population who are at risk of Fe deficiency. For instance, the prevalence of Fe deficiency increased among school girls in Sweden after Fe fortification of flour was halted[52]. Similarly, in our simulations of the general US population we estimated that defortification may increase Fe-deficiency anaemia among 3–5-year-olds and women aged 20–49 years[53].

In summary, the present study provides the first available evidence that variation in dietary Fe intake by homozygotes for HH does affect the rate of total body Fe accumulation over the course of several decades and that dietary interventions can slow this accumulation. None the less, the public health implications of this finding remain unclear because of generally poor compliance with dietary modifications and continued uncertainty concerning the health effects of mild or moderate Fe overload. Microsimulations of the type employed here can play an increasingly important role in guiding policy choices for conditions like HH that require decades to develop and may be too difficult or expensive to study through primary intervention trials.

Acknowledgements

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