Factors associated with serum ferritin levels and iron excess: results from the EPIC-EurGast study

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ABSTRACT

Purpose: Excess iron is involved in the development of non-communicable diseases such as cancer, type 2 diabetes and cardiovascular conditions. We aimed to describe the prevalence of excess iron and its determinants in healthy European adults.

Methods: Sociodemographic, lifestyle, iron status, dietary information, and *HFE* genotyping were obtained from controls in the EPIC-EurGast study, encompassing 7 European countries. High sensitivity C-reactive protein (hsCRP) was measured to address possible systemic inflammation. Descriptive and multivariate analyses were used to assess iron status and its determinants.

Results: Out of the 828 participants (median age: 58.7 years), 43% were females. Median serum ferritin and prevalence of excess iron were 143.7µg/L and 35.2% in males, respectively, and 77µg/L and 20% in females, both increasing with latitude across Europe. Prevalence of *HFE* C282Y mutation was significantly higher in Northern and Central Europe (~11%) than in the South (5%). Overweight/obesity, age, and daily alcohol and heme iron intake were independent determinants for iron status, with sex differences, even after excluding participants with hsCRP>5mg/L. Obese males showed a greater consumption of alcohol, total and red meat, and heme iron, compared with those normal weight.

Conclusion: Obesity and higher alcohol and heme iron intake were the main risk factors for excess iron in males while only age was associated with iron overload in females. Weight control and promoting healthy lifestyle may help prevent iron overload, especially in obese people. Further research is needed to clarify determinants of excess iron in healthy adult population, helping to reduce the associated comorbidities.

Keywords: serum ferritin, iron status, iron overload, excess iron, EPIC

INTRODUCTION

Iron absorption and metabolism are regulated through a complex homestasis, in order to prevent both deficiency and excess. The harmful effects of iron deficiency have been extensively investigated, while interest in excess iron has been on the rise for the past decades. However, there are no well-established data on the prevalence of iron overload for the general population, and the only estimates in Europe concern pregnant women (8.7%-42%) [1, 2]. The high reactivity of iron makes it a highly oxidative metal, its excess being toxic to several organs. While iron overload is associated with some non-communicable diseases, such as type 2 diabetes [3–6], cardiovascular conditions [7, 8], cancer [9, 10] and chronic respiratory pathologies [11, 12], some other studies reported controversial findings [13, 14].

Mutations in the *HFE* gene, involved in hepcidin expression and intestinal iron absorption, have been proposed as one of the main causes of iron overload [15, 16]. The geographical distribution of *HFE* genotypes across Europe has been previously reported, with higher prevalence of *HFE* mutations in Central and Northern Europe than in Southern areas [17, 18]. The homozygous C282Y genotype is especially associated with hereditary hemochromatosis (HH), which results in very high (>1,000 µg/L) levels of serum ferritin (SF) [19]. However, mild iron overload (SF >200 µg/L in males and >150 µg/L in females) can also be harmful and should be treated [20]. Scientific evidence supports that mild excess iron could be caused by less frequent *HFE* genotypes [21] and also non-genetic factors, including many individual, environmental and lifestyle characteristics that might influence iron status beyond genetic polymorphisms. It is well known that average SF concentrations are higher in males than females and also that iron stores increase with age [22]. Unhealthy lifestyle habits such as smoking and alcohol consumption also lead to increased SF levels [23, 24]. Other determinants of iron stores include body mass index (BMI) [25, 26], habitual blood donation [27], and physical activity (PA) –with high SF levels for both sedentary and intense exercise behaviors– [22]. Some of these conditions have been previously found to contribute to systemic inflammation, which could underlie certain associations with iron overload [28–30].

Moreover, diet has a great influence over iron status; suboptimal iron intake mostly leads to iron deficiency and anemia. In contrast, excessive consumption of certain food groups and nutrients (e.g. heme iron and meat) [4, 31–33] contributes to increases in iron levels, especially in individuals with the aforementioned genetic predisposition. Furthermore, dietary fiber affects intestinal absorption of many nutrients, including iron [34], although evidence is conflicting to date.

Considering that non-communicable diseases constitute a major health issue and their association with excess iron remains unclear, this study aimed to describe the frequency of excess iron and its dietary, sociodemographic, and lifestyle determinants, for the healthy, European, adult population.

METHODOLOGY

Study participants

The subjects in this study were participants from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Detailed information about EPIC study has been described elsewhere [35]. Briefly, they were control subjects selected from a nested case-control design (EurGast study), who were age- and sex-matched with the cases at each participating center.

Analyses comprised 828 individuals from 7 of the 10 countries involved in the EPIC cohort (Germany, Spain, France, Italy, the United Kingdom [UK], Sweden and the Netherlands). Approximately 60% of Spanish participants, distributed throughout all centers, in addition to some participants from two Italian centers (Ragusa and Turin) were blood donors. According to Italian law regulating blood donation, hemoglobin level must be >13.5g/dl in males and >12.5g/dl in females [36]; at the local blood transfusion center, the “alert” level of SF for periodic donors are 15µg/L in males and 12µg/L in females, ensuring that subjects from Ragusa and Turin had SF levels higher than these thresholds by the time of recruitment.

Data collection and laboratory procedures

Dietary assessment was performed using validated center-specific dietary questionnaires [35, 37]. To record diet history, most centers used an extensive self-administered food frequency questionnaire (FFQ) while in Spain and Italy it was completed during an interview. Malmö (Sweden) combined a FFQ, a 7-day dietary recall and an interview. Total energy intake, some food groups (total, red and processed meat, fruit, vegetables and legumes, and dairy products), and some nutrients that modulate iron absorption (fiber, calcium, vitamin C and heme iron) were assessed. Daily consumption of each food and nutrient was calculated from information collected by validated dietary questionnaires and, then, dietary variables were expressed as nutrient density per 2000 Kcal ([g/Kcal]*2000).

Anthropometric measurements were collected at recruitment, except in France and part of the UK where they were self-reported [35]. Information on education level and lifestyle including smoking status, alcohol intake and PA [38] was recorded. PA index was created from the Cross-Classification of Occupational and Combined Recreational and Household Activity using Metabolic Equivalents-hours/week [39].

Based on previous evidence about geographical differences across Europe in regards with lifestyle habits and *HFE* genotype distribution [17, 18, 40, 41], the countries were grouped according to the following geographic region: “Southern Europe” (Italy, Spain and France); “Central Europe” (Germany, the Netherlands and the UK); “Northern Europe” (Sweden).

Biochemical analyses of iron biomarkers were performed for all participants at the “Laboratori de Referència Sud de Catalunya” (Tarragona, Spain). SF was measured by electrochemiluminescence immunoassay (Elecsys analyser, Roche Diagnostics, Mannheim, Germany) and serum iron by immune chemiluminescence. Serum transferrin and high-sensitive C-reactive protein (hsCRP) were measured by immunoturbidimetry (Modular Analytics P800 chemistry analyser, Roche Diagnostics, Mannheim, Germany). Total iron binding capacity (TIBC, µg/dL) was calculated as follows: [serum transferrin (mg/dL)*1.43].

Iron sufficiency was established at SF levels 15-200µg/L for males and 15-150µg/L for females, while excess iron was set at SF>200µg/L and SF>150µg/L, respectively [20].

Genotyping of *HFE* polymorphisms (rs1800562, C282Y and rs1799945, H63D) was performed on 403 participants, using the Illumina BeadStation Platform and GoldenGate technology (San Diego, CA) at the laboratory of the Spanish National Genotyping Center (Barcelona, Spain).

Statistical Analysis

Median and interquartile range were used to describe iron biomarkers (SF, serum transferrin, serum iron and TIBC) given their non-normal distribution. The remaining continuous variables were expressed as mean and standard deviation, and categorical data as percentages. Natural logarithm transformation was applied to normalize the distribution of SF. Continuous variables were compared using Student’s t-test and ANOVA while chi-square was used for frequencies. Information on dietary intake, *HFE* genotype, iron status, and sociodemographic and lifestyle characteristics was described by country and geographical region. A multivariate linear regression and logistic regression were performed to assess the association between possible determining factors and SF levels and excess iron, respectively, in the overall sample and by sex. Based on descriptive analyses, multivariate models included the following a priori variables: center, sex, age, BMI (<18.5, 18.5-24.9, 25-29.9, ≥30 Kg/m^2), educational level (uncompleted primary school, primary/secondary school, technical/professional education, and higher/vocational education), frequency and daily amount of alcohol consumption (never/former, <1 serving (s)/d, >1-2 s/d, >2-3 s/d, >3 s/d; 1 serving is 14g), smoking (non-smoker, ex-smoker and smoker), PA (inactive, moderate, active), *HFE* genotype (wild-type [WT], carriers of C282Y mutation, carriers of H63D mutation), hsCRP, and dietary intake. Regarding dietary intake, regression models were performed separately adjusting for nutrients and food groups to avoid overfitting.

To explore the effect of blood donation on SF levels, sensitivity analyses were performed excluding only the Spanish subjects, since the Italian law ensures that blood donors had SF levels higher than the recommended thresholds and, therefore, comparable to those of non-blood donors. In addition, we did not observe statistically significant differences in median SF levels between Italian donors and non-donors, which reinforced our decision.

In order to assess the effect of systemic inflammation in iron stastus we performed a sensitivity analysis by excluding participants with hsCRP>5 mg/L.

Statistical analyses were performed using SPSS v 25.0 (Windows; Chicago, IL, USA) and significance was set at $p<0.05$.

RESULTS

Out of the 828 participants (43% females), 28.6% had excess iron. Their characteristics are presented in **Table 1**. The median age at recruitment was 58.7 (35-77) years with no statistically significant differences by sex. Males reported statistically significant greater percentages of excess iron, overweight, inactive behaviour, alcohol consumption, higher education levels and smoking habit than females. *HFE* genotyping was performed on 403 participants, out of which 29.1% had some mutation (21.3% H63D/WT, 6.2% C282Y/WT, 1.6% C282Y/H63D); given the few individuals with both heterozygous mutations (n=7), they were considered together with those heterozygous carriers of C282Y mutation. Results showed that H63D mutation was more abundant than C282Y (22.8% and 8.7%, respectively) and more prevalent in females than males (30.9% and 15.8%, respectively). These two mutations were always in heterozygous form. Participants from France (n=8) and those with iron deficiency (SF<15µg/L, n=35) were excluded from further analyses given their low representation.

The median SF level was 107.2µg/L for the overall study population, with a highly significant difference between males (143.7µg/L) and females (77.0µg/L). The difference on SF levels by sex was strong enough to remain after excluding Spanish participants, whose concentrations were markedly lower than the others; thus, the overall SF median in the sensitivity analyses rose up to 118.6µg/L, being 163.5µg/L for males and 84.3µg/L for females (**data not shown**). We also found a significant difference between males and females in the prevalence of iron deficiency (2.8% and 6.2%, respectively) and iron excess (35.2% and 20.0%, respectively). The difference in the prevalence of iron deficiency by sex was no longer statistically significant in sensitivity analyses (2.3% and 4.8%, respectively) (**data not shown**). Just 10.1% of participants showed hsCRP>5 (**Table 1**).

Additionally, SF levels were significantly higher in participants from Central and Northern Europe, smokers and alcohol consumers as well as in obese males and those with technical/professional and high/vocational education (**Table S1**). Values for serum transferrin, serum iron and TIBC were in the optimal ranges for both sexes (**Table 1**).

Table 2 compares participants' characteristics and dietary intake across countries and geographic regions. A statistically significant pattern of increasing SF levels was observed from Southern (79.6µg/L) towards Central (132.8µg/L) and Northern Europe (127.6µg/L), which remained in the sensitivity analyses after excluding Spain (Southern Europe: 90.4µg/L) (**data not shown**). Likewise, the prevalence of iron excess was significantly higher in Central and Northern Europe than in the Southern regions for males (43.5%, 40.4% and 23.8%, respectively), although not for females (22.3%, 21.9% an 19.4%, respectively).

Statistically significant differences by geographic region in regards to *HFE* genotype were only found for the C282Y mutation (p=0.031), it being less frequent in Southern Europe (5.0%) than in Central (11.3%) and Northern areas (11.1%). That difference remained evident when Spanish participants were left out of the analyses, since the frequency of C282Y mutation in the Southern region dropped to 3% (p=0.004) (**data not shown**).

As for dietary intake across Europe, higher energy intake and greater consumption of total and red meat, fruits, vegetables and legumes, and heme iron were observed for participants in the South of Europe. Highest consumption of processed meat, dairy products and fiber was described for Northern European countries. The consumption of vitamin C was very similar across geographical regions with no significant differences among them. Regarding alcohol intake, participants in Southern Europe reported the highest daily amount consumed followed by those from Central and Northern regions (**Table 2**).

The sex-specific predictors of iron status are detailed in **Table S2**. Compared with males with iron sufficiency, those with excess iron showed a greater prevalence of obesity (11.6% and 21.1%, respectively), and reported a higher daily consumption of total and processed meat, and alcohol. In females, only fruits intake differed significantly between those with iron sufficiency, who reported a greater amount, and excess iron. Participants with excess iron were older than those with iron sufficiency, but the difference was statistically significant only in females (61 and 57.5 years, respectively).

Being male, obesity, increasing age, and consuming higher amounts of heme iron, calcium and alcohol were positively associated with SF in the overall study population (**Table 3**). In contrast, higher consumption of vitamin C tended to be associated with lower SF levels. The following characteristics were also positively associated with excess iron: increasing age (OR:1.04, 95%CI:1.01,1.09), overweight (OR:1.81, 95%CI:1.16,2.83), obesity (OR:3.02, 95%CI:1.70,5.38), and consuming a higher daily amount of heme iron (OR:1.65, 95%CI:1.22,2.24). When stratified by sex, associations of excess iron with overweight, obesity, and dietary intake of heme iron, calcium and alcohol turned more strength in males than overall sample, while age became a stronger determining factor only for females. No statistically significant effect of genetics was found on iron biomarkers or iron excess. When adjusted for food groups' consumption instead of nutrient intake as well as following the sensitivity analyses, regression models rendered similar results (**data not shown**).

Sensitivity analyses excluding participants with hsCRP>5 mg/L yielded similar results to those performed in total sample **(Table S3)**. The main predictive factors remained unchanged, including the daily intake of heme iron and alcohol. Regarding BMI, the observed effect of being overweight on SF levels and the risk of excess iron disappeared while the association with obesity remained only for males.

Obese males in our study showed worse dietary habits than their counterparts; it is, compared with those with normal weight, they consumed a greater daily amount of alcohol (14.35g vs 28.74g) and animal-origin products such as total meat (89.02g vs 117.01g), red meat (33.52g vs 49.39g), and heme iron (1.04g vs 1.54g). Indeed, the daily alcohol consumption and heme iron intake, together with calcium intake, showed a statistically significant effect on iron status in the adjusted multivariate analyses, suggesting that having several factors at the same time would additively increase the risk of excess iron.

DISCUSSION

This study presents, for the first time and jointly, the median SF levels and prevalence of excess iron in 7 European countries, representing Northern, Central, and Southern Europe. We found an association between several factors including dietary, sociodemographic, and lifestyle and SF levels and excess iron in a sample of healthy adults.

The median SF concentration observed in our study was within the optimal range [20] and similar to those reported in previous studies for the European population [4, 5]. We found that median SF for Spain (64.5µg/L) was lower than in the other countries, although agreed with recent studies in the Spanish population [42, 43], and the overall median increased when Spain was excluded from the analyses. An interesting finding in this study was that a substantial number of participants (28.6%) had excess iron with significant differences among population groups and geographic regions. Given the scarcity of studies providing data on prevalence of excess iron in different countries, we could not appropriately compare our data with other studies.

This study also identified sex, age, BMI, and some nutritional and lifestyle aspects as relevant factors for SF concentrations and excess iron.

Sex, age and BMI

The higher SF levels observed in males could be explained by differences reported in iron metabolism as females, especially pre-menopausal females, have lower iron levels than males due to menstrual losses and childbearing [22, 44, 45]. In addition to sex, age also plays an important role in iron status. We observed a progressive increase of SF concentrations with age in both sexes, reinforcing the existing evidence [44, 46]. Age-related iron accumulation and dyshomeostasis could explain that, as extensively reported in the litterature [47, 48]; the expression of ferroportin, the only known cellular iron exporter in mammals, is downregulated in aging, which could partially explain poor iron recycling and iron accumulation in various tissues [49]. Consequently, a strong association between increasing age and excess iron in females was also found in this study, surely due to SF concentrations and iron stores continuing to increase after menopause, as has been pointed to above [22].

Our findings of a positive association between SF levels and overweight/obesity indicates that obese males, but not females, were more than five times more likely to develop excess iron than those with normal weight. These results would confirm previous findings that increasing BMI may lead to hyperferritinemia [26, 44], usually related with high CRP concentration [26]. Systemic inflammation and insulin resistance, typical of obesity, are considered possible causes of the excess iron [26, 44, 49, 50]. However, the observed effects in our sample remained unchanged after excluding the individuals with high hsCRP. This is of special interest given that obese subjects had a diet rich in proinflammatory foods, including animal products (meat and heme iron), and a higher consumption of alcohol, similar to what has been widely describe in former studies [51–53]. This leads us to believe that SF should not be used as the only indicator of iron status in obese people and that factors associated with obesity should also be screened in clinical practice to address the multicausal origin of iron overload in these patients.

Geographic region

We found an increase in iron excess from the South to Central and Northern European countries in males, although not in females. Significant variations in diet and *HFE* genotype according to country and geographic region were also found. Regarding the geographic distribution of *HFE* mutations, the greater prevalence of C282Y mutation observed in Northern (11.1%) and Central (11.3%) Europe than in Southern region (5.0%) was in line with previous reports describing its geographic gradient across Europe [17, 18]. As for the H63D mutation, its prevalence was greater than that of C282Y in the total sample and each country, which also matches previous reports [17] despite not finding a statistically significant geographic gradient across Europe in this case.

All this makes us consider that the impact observed on biomarkers and iron status could be due to the population's characteristics and lifestyle, and not strictly to their geographic location.

***HFE* genotype**

Mutations in *HFE* gene have been established as one the main causes of iron overload due to the characteristic increased iron absorption associated with this condition [15, 16]. However, contrary to expectations, no significant differences were found in our study in SF levels and excess iron according to the *HFE* genotype. Although *HFE* mutations are common in the European population and their association with SF concentrations has been previously reported [4, 54, 55], it is also true that their clinical penetrance is low [56], which could have prevented us from observing a substantial effect on iron status in our sample. This was in line with previous findings in a Spanish population, in which no effect of *HFE* mutations on SF was found although they observed an effect on hemoglobin concentration [57].

Diet and lifestyle

As expected, and in accordance with published studies [5, 58, 59], each additional daily mg of heme iron intake increased

SF concentration, and showed a strong positive association with excess iron although only in males. Similar results obtained for meat consumption, once the regression models were adjusted for food groups instead of nutrients reinforced this finding. As for dietary vitamin C, the interplay with the fiber content in fruits and vegetables could lead to the striking conclusion of vitamin C reducing SF levels. We speculate that the inhibitory effect of fiber on intestinal iron absorption could have possibly counteracted the enhancer effect of vitamin C [60].

Statistically significant differences were observed for consumption of most food groups and nutrients, based on country and geographic region. People in Southern Europe reported higher total and red meat as well as heme iron intake, which promote iron absorption [60]. On the other hand, they reported the lowest fiber intake, lessening its inhibitory effect on nutrient bioavailability [34, 60]. If only diet was considered, it could be thought that SF levels and, consequently, the prevalence of iron excess, should be higher in Southern Europe; contrary to that, the values were higher in Central and Northern countries. We believe that factors other than diet, such as the higher prevalence of the C282Y mutation, could be behind this finding.

Several studies have observed that some lifestyle, especially toxic habits, may alter iron status [23, 59]. We noted that SF concentration was higher in participants who reported frequent alcohol consumption, which is consistent with previous results [61], although this association was not significant after adjusting for potential confounders. In addition, we found a positive association between each daily 10-gram increase of alcohol and SF levels and the risk of excess iron, though only in males; this association has been reported before [62, 63] and recently reinforced [23, 24, 64]. Studies suggest that alcohol deregulates the synthesis and expression of hepcidin in the liver, leading to increased intestinal iron absorption and, consequently, to iron overload [61, 62, 64, 65]. Also smoking has repetitively been identified as a determining factor for iron overload [23, 66]. Smokers tend to have higher SF concentrations than non-smokers/ex-smokers [66, 67], which was observed in this study (127.7µg/L and 97.7µg/L, respectively). Extensive literature suggests that cigarette smoking disrupts iron homeostasis, leading to a systemic iron overload and excessive deposits [66, 68]. Despite this being widely accepted [69, 70], we were not able to observe the effect of smoking status on iron overload after multivariate adjusted analyses. This suggests that other lifestyle habits could be related to iron levels to a greater extent than smoking in our study population.

Regarding PA, our results agree with a prior study [69], as we found no association with SF levels or excess iron. Few researches have explored the role PA plays in iron status in the general population, as studies generally focus on athletes or consider high-intensity exercise, in which case significant variations in hepcidin and SF levels were observed [71, 72]. Researchers argue that SF concentrations may increase in response to exercise-induced acute inflammation and not actually reflect iron storage [71, 72]. We believe, therefore, that the lack of effect of PA on iron status observed in our study could be due to the fact that the intensity of exercise usually performed by the general population is low-medium rather than high, as in the aforementioned investigations.

Strengths and limitations

This is the first time that determinant factors of SF levels and excess iron have been jointly evaluated in a population from different European countries as part of the same study. Data used covered a wide variety of variables from a well-established cohort. The hsCRP assay provides an estimate of systemic inflammation, and including it as an adjustment variable reinforced our findings by allowing us to rule out that SF levels were due to an infection or inflammation process. Moreover, *HFE* genotyping constitutes valuable information in relation to iron status. However, some limitations should be considered. First, the study population used controls selected in a nested case-control study, so that it may not be representative of the general European population. The sample size may limit the interpretation of some of our findings, especially in stratified analyses. Also, around 60% of Spanish participants were blood donors that could lead to SF levels being skewed; however, similar studies in the general population in Europe [4, 5] and Spain [42, 43] obtained comparable results. Furthermore, we reached similar findings from sensitivity analyses excluding Spanish subjects. The high median age of the participants imply that results should be extrapolated with caution. In addition, biochemical analyses were performed using samples collected at baseline, which prevented us from monitoring iron status over time. Finally, hemoglobin data could have been useful for further verification of the association with *HFE* genotypes but they were not available.

CONCLUSIONS

We found a moderate-high prevalence of excess iron, which increased from the Southern to Northern Europe and was higher in males than females. Geographical differences between European regions were only found for C282Y mutation in *HFE* gen, whose prevalence was higher in the Northern and Central countries than in the South. This could explain the increase in SF levels and the prevalence of excess iron towards Northern Europe, although genetics ended up not showing a powerful enough effect by itself to constitute a determining factor for iron overload. Predictive factors of excess iron were different by sex; while only age was related to iron overload in females, the main risk factors in males were obesity, and a higher intake of alcohol and heme iron. Thus, an integrative clinical approach including strategies for weight control as well as the promotion of lifestyle changes towards healthier habits could help control iron status and prevent iron overload, especially in obese people. Finally, more research is needed to further clarify the determinants of SF and excess iron in the healthy adult population. A better understanding of the associated factors may help reduce the incidence of associated comorbidities.

DECLARATIONS

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Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval This study was approved by the Ethical Committees at the International Agency for Research on Cancer (IARC) and in each of the EPIC centers. It has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Consent to participate All participants gave their informed consent prior to their inclusion in the study.

Consent for publication Not applicable

Availability of data and material Not applicable

Code availability Not applicable

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