# Factors associated with serum ferritin levels and iron status: 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. The aim was to describe the prevalence of excess iron and its determinants in the healthy European adult population.

**Methods:** Sociodemographic, lifestyle, iron status, and dietary information, as well as *HFE* genotyping, were obtained from controls participating in the nested case-control EPIC-EurGast study, encompassing 7 European countries. Statistical analyses were performed using SPSS software.

**Results:** Out of the 828 participants, 80.7% age 50 or older and 43% were women. Median serum ferritin (SF) levels and the prevalence of excess iron were 143.70 µg/L and 19.7% in males, respectively, and 76.95 µg/L and 10.7% in females, both of them increasing with latitude. The prevalence of *HFE* C282Y mutation was significantly greater in Northern (11.1%) and Central Europe (11.3%) than in the South (5%). Excess iron was more prevalent among obese individuals and those aged 50 or above. Body mass index, age ≥50 years, and daily alcohol and heme iron intake constituted independent determinant factors for SF levels and excess iron, with variations based on sex.

**Conclusion**: The prevalence of excess iron was moderate-high and greater in males. Genetics did not constitute a determining factor for iron overload, in favor of other individual, sociodemographic and lifestyle factors. Further research is needed to clarify the determinants of SF and excess iron in the healthy adult population, which would help reduce the incidence of associated comorbidities.

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

## INTRODUCTION

Iron is an essential micronutrient for life and it is strictly regulated by the human metabolism, in order to prevent both deficiency and excess. The harmful effects of different states of iron deficiency have been extensively investigated, while interest in excess iron has been in 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, with some studies reporting prevalences between 8.7% and 42% [1, 2]. The high reactivity of iron causes oxidative stress so that its excessive deposition becomes toxic for several organs. Iron overload has been associated with an increased risk of developing non-communicable diseases, such as type 2 diabetes [3–6], cardiovascular conditions [7, 8], cancer [9, 10] and chronic respiratory pathologies [11, 12]. However, the evidence in this regard remains controversial, with some studies showing little or no significant positive association [13, 14].

Mutations in the *HFE* gene, a gene associated with hepcidin expression and involved in intestinal iron absorption, have been proposed as one of the main causes of iron overload [15, 16]. A geographical distribution of *HFE* genotypes across Europe has been previously widely reported, with higher prevalence of *HFE* mutations in Central and Northern Europe, than in more Southern areas [17, 18]. The homozygous C282Y genotype has been especially associated with the development of hereditary hemochromatosis (HH), which results in very high (>1,000 µg/L) levels of serum ferritin (SF) [19]. However, mild iron overload with SF concentrations below those reached in HH (>200 µg/L in males and >150 µg/L in females) can also be harmful and should be treated [20]. In addition, non-HH excess iron could be underpinned by other less frequent *HFE* genotypes [21] along with other non-genetic factors. Indeed, scientific evidence supports the idea that diverse individual, environmental and lifestyle characteristics might influence iron status beyond genetic polymorphisms. It has been well described that average SF concentrations are higher in males than in females and also that iron stores increase with age [22]. A number of studies have shown that unhealthy 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) −with obesity increasing SF levels [25, 26], habitual blood donation [27], and physical activity −with no changes found for moderate exercise but increased SF levels for both sedentary and intense exercise behaviors [22]. Moreover, dietary habits have great influence over iron status; also, 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) [28–30] contributes to iron levels increases, especially in individuals with the aforementioned genetic predisposition. Furthermore, the binding capacity and viscosity of dietary fiber affect intestinal absorption of many nutrients, including that of iron and other minerals [31]. To date, evidence for fiber is conflicting, with some data showing reduced indicators of iron status [32] and others showing no association between SF levels or iron status and higher consumption of fiber or fiber-rich foods [33].

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, and were selected according to a nested case-control design. More specifically, the data included in the present study belonged to subjects in the control group, who were age and sex matched with the cases at each participating center. The EPIC study is a large multicenter prospective cohort including more than 500,000 subjects recruited between 1992 and 2000 from 23 centers in 10 European countries. Detailed information has been described elsewhere [34].

The analyses for the present work were based on the controls from the EurGast study, comprising 828 individuals from 7 out of the 10 countries involved in the EPIC cohort (Germany, Spain, France, Italy, the United Kingdom [UK], Sweden and the Netherlands). Around the 60% of participants from Spain, distributed throughout all centers, in addition to the participants from two Italian centers (Ragusa and Turin) were blood donors. In regards to the later, according to the Italian law that regulates the blood donation, the Hb level must be higher than 13.5 g/dl in males and 12.5 g/dl in females; at the local blood transfusion center, the "alert" level of SF for periodic (high frequent) donors are 15 µg/L in males and, commonly, 12 µg/L in females. It means that by the time of recruitment the majority of the subjects from EPIC Ragusa and EPIC Turin had a level of SF higher than the above thresholds.

### Data collection and laboratory procedures

Dietary assessment was performed by using validated country/center-specific dietary questionnaires [34, 35]. To record diet history, most centers used an extensive self-administered food frequency questionnaire (FFQ) while in Spain and Italy they completed a FFQ during an interview. Malmö (Sweden) used a combined method which included a FFQ, a 7-day dietary recall and an interview. Dietary variables were expressed as nutrient density per 2,000 kcal ([g/Kcal] x 2,000). Total energy intake, several food groups (including total, red and processed meat, fruit, vegetables and legumes, and dairy products), as well as some nutrients that exert a modulation of iron absorption (fiber, vitamin C and heme iron) were assessed.

Anthropometric measurements were collected at recruitment, except in France and part of the UK, where they were selfreported [34]. Height and weight were used to calculate BMI (Kg/m$^2$). Information on education level and lifestyle including smoking status, alcohol intake and physical activity [36] was also recorded. Physical activity index was created from the Cross-Classification of Occupational and Combined Recreational and Household Activity using Metabolic Equivalents (MET)-hours/week [37].

The participating countries were grouped according to their geographic region: "Southern Europe" included Italy, Spain and France; "Central Europe" included Germany, the Netherlands and the UK; "Northern Europe" was represented by Sweden.

Biochemical determination of iron biomarkers was done for all participants. SF levels were measured by electrochemiluminescence immunoassay using an Elecsys analyzer (Roche Diagnostics, Mannheim, Germany). Serum iron was measured by immune chemiluminescence. Serum transferrin and high sensitive C-reactive protein (hsCRP) were measured by immunoturbidimetry using a Modular Analytics P800 chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Total iron binding capacity (TIBC, µg/dL) was calculated by using the following equation: [serum transferrin (mg/dL)*1.43]. All of these procedures were performed at "Laboratori de Referència Sud de Catalunya" (Tarragona, Spain).

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

Genotyping of the two main functional polymorphisms (rs1800562, C282Y and rs1799945, H63D) of the *HFE* gene was carried out on 403 participants, by 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 for the description of iron biomarkers (SF, serum transferrin, serum iron and TIBC) while the remaining continuous variables were expressed as the mean and standard deviation (SD) values and categorical data were presented as percentages. Natural logarithm transformation was applied to normalize the distribution of SF. Student's t-test and ANOVA were used to compare continuous variables and chi-squared testing was applied for frequencies. Information on dietary intake, *HFE* genotype, and iron status as well as sociodemographic and lifestyle characteristics was described by country and geographical region. Multivariate linear regressions and logistic regressions were performed to assess the association between possible determining factors and SF levels and excess iron, respectively, in the overall sample and stratified by sex. Based on previous knowledge and descriptive analyses, multivariate models included the following a priori variables: study center, sex, age (<50 and ≥50 years), BMI (<18.5, 18.5-24.9, 25-29.9, and ≥30 Kg/m$^2$), educational level (uncompleted primary school, primary/secondary school, technical/professional education, and higher/vocational education), frequency of alcohol consumption (never/former, < 1 serving/d, >1-2 servings/d, >2-3 servings/d, >3 servings/d; 1 serving is 14 g) ) and daily amount, smoking (never/former and current), physical activity (inactive, moderate, and active), *HFE* genotype (wild type, carriers of C282Y mutation, and carriers of H63D mutation), hsCRP, and dietary intake. Regarding dietary intake, regression models were performed separately adjusting for nutrients (fiber, heme iron, calcium, vitamin C) and food groups (meat, red meat, processed meat, fruits, vegetables and legumes, dairy products) in order to avoid overfitting.

In order 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.

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

## RESULTS

A total of 828 subjects were included, whose characteristics are presented in **Table 1** for the overall sample and stratified by sex. The population was 43% females and the mean age at recruitment was 57.7 (28-78) years, with no statistically significant differences by sex. We found that 28.6% of participants had excess iron. Males reported statistically significant higher percentages of excess iron, overweight, inactive behaviour, alcohol consumption and smoking habit than females. Male participants also had higher education levels compared to females. The HFE genotyping was performed on 403 participants, out of which 29.1% had some mutation (21.3% H63D/wild type, 6.2% C282Y/wild type, and 1.6% C282Y/H63D); given the low number of individuals (n=7) with both mutations in heterozygous form, they were jointly considered with those heterozygous carriers of C282Y mutation. Results showed that H63D mutation was more abundant than that of C282Y (22.8% and 8.7%, respectively) and more prevalent in females than in males (30.9% and 15.8%, respectively). The presence of these two mutations was always in heterozygous form. Participants from France (n=8) and those with iron deficiency (SF<15 μg/L, n=35) were excluded from further analyses given their low representation in the overall sample.

The median SF level was 107.20 μg/L for the study population overall, with a highly significant ($p<0.001$) difference between males (143.70 µg/L) and females (76.95 µg/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 118.60 µg/L, being 163.50 µg/L for males and 84.32 µg/L for females (data not shown). We also found a significant difference by sex in the prevalence of both iron deficiency (2.8% and 6.2% for males and females, respectively) and iron excess (35.2% and 20% for males and females, respectively). In this case, the difference in regards with prevalence of iron deficiency between males and females was no longer statistically significant in sensitivity (2.3% and 4.8%, respectively) (data not shown).

Additionally, SF levels were significantly higher in participants over 50 years of age, those from Central and Northern Europe, smokers and alcohol consumers as well as in obese males and those with technical/professional and high/vocational education. As expected, blood donation resulted in a significantly decrease of SF levels of the entire sample in both sexes (**Table S1**). Values for serum transferrin, serum iron and TIBC were in the optimal ranges both in males and females.

**Table 2** shows the differences in age, BMI, *HFE* genotype, biochemical iron parameters and iron status, as well as dietary intake across countries and geographic regions. A statistically significant pattern of increasing SF levels was observed from Southern (79.57 µg/L) towards Central (132.80 µg/L) and Northern Europe (127.60 µg/L), which remained in the sensitivity analyses after excluding Spain (Southern Europe: 90.42 µg/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 no significant differences were found 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%) than in Central (11.3%) and Northern areas (11.1%). That difference between South and Central-Northern Europe was even notorious 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, higher energy intake and greater consumption of total and red meat, fruits, vegetables and legumes, and heme iron were reported by 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 different geographical regions with no statistically significant differences among them. Regarding alcohol intake, participants in Southern Europe reported the highest daily amount consumed followed by those from Central Europe and, further afield, by Northern regions.

The sex-specific predictors of iron status are detailed in **Table 3**. Excess iron was more prevalent in obese males and

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females 50 years of age or older. Males with excess iron (SF>200 µg/L) reported a higher consumption of total and processed meat as well as a greater daily alcohol intake. In females, only fruits intake differed significantly between those with iron sufficiency, who reported a greater amount, and excess iron.

**Table 4** shows that male participants, those obese, aged 50 years or older, and a higher reported heme iron, calcium and alcohol intake were positively associated with SF in the overall study population. On the contrary, increasing consumption of vitamin C tended to be associated with lower SF levels. Furthermore, the following characteristics were also positively associated with excess iron: age 50 or older (OR=2.03, 95%CI=1.15, 3.59), 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, the associations of excess iron with overweight, obesity, and dietary intake of heme iron, calcium and alcohol remained significant only for males, while age remained a determining factor solely for females, with a greater magnitude for them than for the total sample (ORs 3.16 and 2.03, respectively). No statistically significant effect of genetics was found on iron biomarkers or iron excess in the total sample or in either sex. When adjusted for food groups' consumption instead of for nutrient intake, regression models rendered similar results (data not shown). In addition, sensitivity analyses excluding Spain yielded similar results (data not shown).

## DISCUSSION

This study presents, for the first time and altogether, median levels of SF and the prevalence of excess iron in 7 European countries, representing the Northern, Central, and Southern geographic regions of Europe. The study also shows the association of several factors including dietary, sociodemographic, lifestyle, and genetic characteristics, with SF levels and excess iron in a sample of healthy adults.

The median SF concentration observed in our study (107.20 µg/L; 143.70 µg/L in males and 76.95 µg/L in females) were within the optimal range (SF 15-200 µg/L and 15-150 µg/L, respectively) [20] and similar to those reported in previous studies for European population [4, 5]. We found that median SF for Spain (64.45 µg/L) was quite lower than the other countries, although agreed with recent studies in Spanish population [38, 39], and the global median increased to 118.60 µg/L when Spain was excluded from the analyses. On the other hand, the SF levels found in our study were slightly higher than those reported by other authors [40, 41]. A possible explanation could be that they evaluated populations younger than ours: in addition, these studies were conducted in Korea, a country with great differences in terms of dietary habits, as well as social and genetic backgrounds compared to Europe. Also, an interesting finding in this study was that a substantial number of participants (28.6%) showed excess iron (SF>150 µg/L in females and SF>200 µg/L in males) with significant differences among population groups and geographic regions. We must highlight here the difficulty of comparing our results with others, given the limited availability of studies providing updated data on prevalence of excess iron in other countries.

Regarding the *HFE* gene, the prevalence of C282Y mutation was higher in Northern (11.1%) and Central (11.3%) Europe than in the Southern region (5%), which coincided 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 (22.8% and 8.7%, respectively) and each country. This matches previously existing knowledge [17], although we did not find a statistically significant geographic gradient across Europe in this case.

Yet another main finding of this study was the identification of sex, age, BMI, and some nutritional and lifestyle aspects as relevant factors for SF concentrations and excess iron.

**Sex, age and BMI**

We found a significant difference in iron status by sex, with SF concentration and the percentage of excess iron greater in males (143.70 µg/L and 35.2%, respectively) than in females (76.95 µg/L and 20%, respectively). To this regard, it is well known that SF levels are lower in females than males, especially before the menopause due to menstrual losses and childbirth [22, 42, 43]. However, not only sex but also age plays an important role in iron status. We observed that SF increased after the age of 50 in both sexes, reinforcing the existing evidence about a progressive increase of SF concentrations with age [42, 44]. Age-related iron accumulation and dyshomeostasis could underlie these findings [45, 46]; in this regards, the expression of ferroportin, the only known cellular iron exporter in mammals, has been found to be downregulated in aging, which could partially explain poor iron recycling and iron accumulation in various tissues [47]. Consequently, a strong association between age and excess iron in females after 50 was also found, surely due to SF concentrations continuing to increase after menopause, increasing in turn the iron stores, as has been pointed to above [22].

Moreover, a positive association between overweight/obesity and SF was found in this study, especially in males. Our results indicate that obese males were more than four times more likely to develop excess iron than those with normal weight, while no significant association was found in females. These results would confirm previous findings that increasing BMI may lead to hyperferritinemia [26, 42], sometimes related with high CRP concentration [26]. Systemic inflammation and insulin resistance, typical comorbidities of obesity, have been considered as possible causes of the excess iron [26, 42, 47]. This leads us to thinking that SF should not be used as the only indicator of iron status in obese people, and that our results must be interpreted with caution in clinical practice.

**Geographic region**

We found an increase in SF concentrations from the South to the Center and, consequently, a greater percentage of excess iron in Central European countries, than in the Southern regions for males, although no statistically significant difference was found for females. The impact observed on biomarkers and iron status could be due to the population's lifestyle and not strictly to their geographic location. In line with this, our results showed significant variations in diet and *HFE* genotype according to country and geographic region, which would actually lie behind the differences in iron status.

***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]. In contrast to our hypothesis, no significant differences were found in our study in SF levels and excess iron according to the *HFE* genotype, neither in the crude nor in the adjusted analyses. This was in line with previous findings in Spanish population, in which no effect of *HFE* mutations on SF but on hemoglobin concentration was observed [48]. In addition, the geographic distribution of *HFE* mutations has been well-described as more prevalent in Central and Northern Europe than in Mediterranean countries [17, 18]. Our results are in agreement with this, having found a significantly higher percentage of the C282Y mutation in participants from countries in the upper half of the continent (10-13%) than in Southern ones (3-8%).

**Diet and lifestyle**

As expected, and in accordance with published studies [49, 50], each additional daily mg of heme iron intake increased the SF concentration, and showed a strong positive association with excess iron in males, while no effect was found in females. This finding was reinforced by similar results obtained for meat consumption, once the regression models were adjusted for food groups instead of nutrients. 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 [51].

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 [51]; the consumption of red meat was also higher in Southern and Central Europe than in the Northern region. On the other hand, countries of Southern Europe reported the lowest fiber intake, lessening its inhibitory effect on nutrient bioavailability [31, 32, 51]. If only diet was considered, it could be thought that SF levels and,

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consequently, the prevalence of iron overload, should be higher in Southern Europe; contrary to that, the values were higher in Central and Northern countries. We believe that other factors besides diet, as the highest prevalence of the C282Y mutation, could be the cause of this finding.

Several studies have observed that some lifestyle, especially toxic habits, may alter iron status [23, 50]. We noted that SF concentration was higher when participants reported increasing alcohol consumption. Although this association was lost after adjusting for other possible determinant factors, it was in line with results reported by Harrison-Findik et al. [52]. As for the amount of alcohol intake, 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 [53–55] and recently reinforced [23, 24, 56]. 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 [52–57]. Also smoking has also repetitively been identified as a determining factor for iron overload [23, 58]. Smokers tend to have higher SF concentrations than non-smokers [58–60], which was also initially observed in this study (127.65 µg/L and 97.72 µg/L, respectively). Extensive literature suggests that cigarette smoking disrupts iron homeostasis, which leads to a systemic iron overload and excessive deposits [58, 59, 61]. Although this is widely accepted, some former controversial findings [62–64] agree with our results, which showed a lack of effect of smoking on SF levels and no association with iron overload, after having controlled for related factors.

Regarding physical activity, our results were in accordance with a prior study [62], as we found no association with SF levels or excess iron. We also notice that only a few pieces of research have analyzed the role that physical activity plays in iron status in the general population. Conversely, studies generally focus on athletes or consider high-intensity exercise, in which case significant variations in hepcidin and SF levels were observed [65–67]. In relation to this finding, researchers have argued that SF concentrations may increase as a response to exercise-induced acute inflammation and not actually as a reflection of iron storage [66, 67]. We believe, therefore, that the lack of effect of physical activity on iron status observed in our study could be due to the fact that the intensity of exercise that the general population usually does is low-medium instead of high, as in the aforementioned investigations.

**Strengths and limitations**

This is the first time that a population from different European countries has been jointly evaluated as part of the same study assessing the determinant factors of SF levels and excess iron. In addition, the data used covered a wide variety of variables from a well-established cohort. In addition, 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 was selected according to a nested case-control design, so that it is not representative of the general population. Second, the sample size may limit the interpretation of some stratified analyses. Third, that around 60% of Spanish participants were blood donors could lead to think that results on SF levels may be skewed; in this regards, however, similar studies in general population in Europe [4, 5] and Spain [38, 39] obtained comparable results  Furthermore, we performed sensitivity analyses excluding Spanish subjects from which we reached similar findings. Another possible limitation was the high mean age of the participants, which imply that the results should be extrapolated with caution. In addition biochemical determinations were performed only once, which prevented us from monitoring iron status over time. And finally, hemoglobin measurements were not available, which could have been useful for further verification of the association with *HFE* genotypes.

## CONCLUSIONS

There was a moderate-high prevalence of excess iron, which increased from the South to the North of Europe and was higher in males than in females. H63D mutation in the *HFE* gene was more prevalent than C282Y, in both sexes. Geographical differences between European regions were only found for C282Y, 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 sufficient effect by itself to constitute a determining factor for iron overload. Other factors associated with increased SF concentrations and excess iron have, indeed, been found. These include, obesity, age > 50 years, increasing alcohol and heme iron intake as the main ones. 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 would 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.

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**Table 1. Characteristics of the participants in total sample and by gender.**

| | Total (n=828) | Males (n=472) | Females (n=356) |
|---|---|---|---|
| **Iron biomarkers** | | | |
| Serum ferritin (µg/L) | 107.20 [140.67] | **143.70 [179.06]** | **76.95 [92.43]** |
| Serum transferrin (mg/dL) | 260.00 [46] | **257.50 [44]** | **263.00 [52]** |
| Serum iron (µg/dL) | 110.00 [50.00] | **113.00 [54.00]** | **106.00 [42.00]** |
| Total iron binding capacity (µg/dL) | 371.80 [67.21] | **368.23 [62.56]** | **376.09 [74.36]** |
| **Sociodemographic characteristics** | | | |
| Age (years) | | | |
| <50 | 160 (19.3) | 91 (19.3) | 69 (19.4) |
| ≥50 | 668 (80.7) | 381 (80.7) | 287 (80.6) |
| Education level | | | |
| Uncompleted primary school | 58 (7.2) | **20 (4.4)** | **38 (10.9)** |
| Primary and secondary school | 403 (50.2) | **214 (47.1)** | **189 (54.3)** |
| Technical or professional education | 184 (22.9) | **119 (26.2)** | **65 (18.7)** |
| High or vocational education | 157 (19.6) | **101 (22.2)** | **56 (16.1)** |
| Body mass index ($Kg/m^2$) | | | |
| Normal weight, 18.5-25 | 293 (35.4) | **145 (30.7)** | **148 (41.6)** |
| Overweight, >25-30 | 401 (48.4) | **257 (54.4)** | **144 (40.4)** |
| Obesity, >30 | 134 (16.2) | 70 (14.8) | 64 (18.0) |
| ***HFE* genotype¶** | | | |
| Wild-type genotype | 276 (68.5) | **157 (73.0)** | **119 (63.3)** |
| Carrier of C282Y mutation | 35 (8.7) | **24 (11.2)** | **11 (5.9)** |
| Carrier of H63D mutation | 92 (22.8) | **34 (15.8)** | **58 (30.9)** |
| **Lifestyle** | | | |
| Physical activity | | | |
| Inactive | 103 (13.9) | **92 (22.0)** | **11 (3.4)** |
| Moderate | 572 (77.4) | **297 (70.9)** | **275 (85.9)** |
| Active | 64 (8.7) | 30 (7.2) | 34 (10.6) |
| Smoking | | | |
| No smoker/Ex-smoker | 554 (66.9) | **269 (57.0)** | **285 (80.1)** |
| Smoker | 274 (33.1) | **203 (43.0)** | **71 (19.9)** |
| Frequency of alcohol intake | | | |
| Never/Former | 131 (15.8) | **44 (9.3)** | **87 (24.4)** |
| < 1 serving/d (<14 g/d) | 449 (54.2) | **231 (48.9)** | **218 (61.2)** |
| >1-2 servings/d (>14-28 g/d) | 114 (13.8) | **80 (16.9)** | **34 (9.6)** |
| >2-3 servings/d (>28-42 g/d) | 57 (6.9) | **46 (9.7)** | **11 (3.1)** |
| >3 servings/d (>42 g/d) | 77 (9.3) | **71 (15.1)** | **6 (1.7)** |
| **Country** | | | |
| France | 8 (1.0) | **0 (0)** | **8 (2.2)** |
| Italy | 186 (22.5) | **81 (17.2)** | **105 (29.5)** |
| Spain | 155 (18.7) | 89 (18.9) | 66 (18.5) |
| United Kingdom | 156 (18.8) | **118 (25.0)** | **38 (10.7)** |
| The Netherlands | 88 (10.6) | **24 (5.1)** | **64 (18.0)** |
| Germany | 155 (18.7) | **113 (23.9)** | **42 (11.8)** |
| Sweden | 80 (9.7) | 47 (10.0) | 33 (9.3) |
| **Geographic region** | | | |
| Southern Europe[1] | 341 (41.2) | **170 (36.0)** | **171 (48.0)** |
| Central Europe[2] | 407 (49.2) | **255 (54.0)** | **152 (42.7)** |
| Northern Europe[3] | 80 (8.9) | 47 (10.0) | 33 (9.3) |
| **Iron status*** | | | |
| Iron deficiency | 35 (4.2) | **13 (2.8)** | **22 (6.2)** |
| Iron sufficiency | 556 (67.0) | **293 (62.0)** | **263 (73.8)** |
| Iron excess | 237 (28.6) | **166 (35.2)** | **71 (20.0)** |

The results are expressed in median [interquartile range] and n (%). Significant differences ($p<0.05$) by gender were highlighted in bold.
¶ The sample size of *HFE* genotype was 403 subjects. All participants with mutations in *HFE* gene were heterozygous carriers.
[1] Southern Europe (Italy and Spain); [2] Central Europe (United Kingdom, The Netherlands and Germany); [3] Northern Europe (Sweden).

**Table 2. Description of the HFE genotype, iron status and dietary intake of the participants, by country and geographic region.**

| | Country | | | | | | Geographic region | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spain‡ | Italy‡ | The Netherlands | United Kingdom | Germany | Sweden | Southern Europe[1] | Central Europe[2] | Northern Europe[3] | p southern vs central Europe | p southern vs northern Europe | p central vs northern Europe |
| Sex (male) | 89 (57.4) | 81 (43.5) | 24 (27.3) | 118 (75.6) | 113 (72.9) | 47 (58.8) | 170 (49.9) | 255 (63.9) | 47 (58.8) | **<0.001** | 0.152 | 0.383 |
| Age (years) | 53.48 (7.77) | 56.56 (8.24) | 58.06 (5.81) | 64.08 (8.00) | 56.14 (7.56) | 54.65 (6.88) | 55.16 (8.16) | 59.67 (8.21) | 54.65 (6.88) | **<0.001** | 1.000 | **<0.001** |
| Body mass index (Kg/m$^2$) | 28.28 (3.93) | 26.69 (4.09) | 25.72 (3.53) | 26.02 (3.00) | 26.92 (3.53) | 24.78 (3.07) | 27.41 (4.09) | 26.30 (3.36) | 24.78 (3.07) | **<0.001** | **<0.001** | **0.002** |
| **Iron biomarkers** | | | | | | | | | | | | |
| Serum ferritin (μg/L) | 64.45 [102.08] | 90.42 [114.67] | 102.15 [114.32] | 106.75 [120.22] | 186.40 [220.90] | 127.60 [160.38] | 79.57 [109.01] | 132.80 [153.26] | 127.60 [160.38] | **<0.001** | **0.007** | 0.885 |
| Serum transferrin (mg/dL) | 268.00 [43] | 263.00 [41] | 273.00 [49] | 259.00 [47] | 253.00 [47] | 245.00 [39] | 264.50 [42] | 259.00 [50] | 245.00 [39] | 1.000 | **<0.001** | **<0.001** |
| Serum iron (μg/dL) | 105.50 [44.50] | 104.00 [51.25] | 108.00 [48.75] | 117.00 [54.75] | 113.00 [45.00] | 114.00 [37.75] | 104.00 [49.00] | 114.00 [51.00] | 114.00 [37.75] | **0.006** | 0.986 | 1.000 |
| Total iron binding capacity (μg/dL) | 383.24 [61.85] | 376.09 [58.99] | 390.39 [69.71] | 370.37 [66.85] | 361.79 [67.21] | 349.64 [56.13] | 378.24 [60.06] | 370.37 [71.50] | 349.64 [56.13] | 1.000 | **<0.001** | **<0.001** |
| **Iron status*** | | | | | | | | | | | | |
| Males | | | | | | | | | | **<0.001** | **0.024** | 0.692 |
| Iron sufficiency | 66 (77.6) | 59 (74.7) | 12 (54.5) | 78 (69.0) | 50 (44.2) | 28 (59.6) | 125 (76.2) | 140 (56.5) | 28 (59.6) | | | |
| Iron excess | 19 (22.4) | 20 (25.3) | 10 (45.5) | 35 (31.0) | 63 (55.8) | 19 (40.4) | 39 (23.8) | 108 (43.5) | 19 (40.4) | | | |
| Females | | | | | | | | | | 0.534 | 0.745 | 0.958 |
| Iron sufficiency | (89.7) | 73 (75.3) | 49 (79.0) | 30 (85.7) | 29 (69.0) | 25 (78.1) | 125 (80.6) | 108 (77.7) | 25 (78.1) | | | |
| Iron excess | 6 (10.3) | 24 (24.7) | 13 (21.0) | 5 (14.3) | 13 (31.0) | 7 (21.9) | 30 (19.4) | 31 (22.3) | 7 (21.9) | | | |
| ***HFE* genotype¶** | | | | | | | | | | | | |
| Wild-type genotype | 38 (61.3) | 72 (72.7) | 29 (56.9) | 53 (71.6) | 56 (71.8) | 26 (72.2) | 110 (68.3) | 138 (68.0) | 26 (72.2) | 0.944 | 0.647 | 0.613 |
| Carrier of C282Y mutation | 5 (8.1) | 3 (3.1) | 5 (9.8) | 8 (10.8) | 10 (12.8) | 4 (11.1) | 8 (5.0) | 23 (11.3) | 4 (11.1) | **0.031** | 0.164 | 0.969 |
| Carrier of H63D mutation | 19 (30.6) | 24 (24.2) | 17 (33.3) | 13 (17.6) | 12 (15.4) | 6 (16.7) | 43 (26.7) | 42 (20.7) | 6 (16.7) | 0.178 | 0.208 | 0.579 |
| **Daily alcohol intake (g)** | 17.86 (26.22) | 15.71 (19.61) | 11.36 (14.77) | 8.63 (11.94) | 20.99 (38.45) | 4.21 (5.60) | 16.68 (22.84) | 14.00 (26.59) | 4.21 (5.60) | 0.379 | **<0.001** | **0.002** |
| **Daily dietary intake†** | | | | | | | | | | | | |
| Energy (Kcal) | 2268.87 (711.37) | 2241.97 (692.06) | 2010.00 (579.89) | 2139.84 (602.27) | 2083.06 (770.82) | 1745.57 (653.90) | 2254.20 (700.00) | 2088.89 (668.95) | 1745.57 (653.90) | **0.003** | **<0.001** | **<0.001** |
| Foods | | | | | | | | | | | | |
| Total meat (g) | 119.68 (44.18) | 87.42 (39.14) | 101.17 (51.49) | 86.54 (53.43) | 104.34 (44.78) | 80.11 (31.65) | 102.08 (44.46) | 96.73 (50.32) | 80.11 (31.65) | 0.356 | **<0.001** | **0.011** |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Red meat (g) | 41.36 (28.72) | 41.94 (26.60) | 57.32 (32.55) | 38.78 (31.51) | 31.14 (21.48) | 14.73 (7.75) | 41.67 (27.54) | 39.91 (29.90) | 14.73 (7.75) | 1.000 | **<0.001** | **<0.001** |
| Processed meat (g) | 35.72 (30.11) | 19.98 (15.61) | 27.60 (22.37) | 21.69 (17.50) | 59.09 (32.72) | 39.04 (18.36) | 27.13 (24.59) | 37.60 (30.76) | 39.04 (18.36) | **<0.001** | **0.001** | 1.000 |
| Fruit (g) | 309.90 (247.31) | 338.00 (197.69) | 257.58 (180.30) | 229.81 (172.80) | 152.96 (111.58) | 211.36 (143.42) | 325.23 (221.73) | 205.96 (159.48) | 211.36 (143.42) | **<0.001** | **<0.001** | 1.000 |
| Vegetables and legumes (g) | 282.13 (136.38) | 154.78 (95.14) | 145.76 (66.37) | 275.41 (156.65) | 135.57 (80.80) | 121.33 (120.85) | 212.67 (131.84) | 192.07 (131.97) | 121.33 (120.85) | 0.100 | **<0.001** | **<0.001** |
| Dairy products (g) | 261.90 (203.65) | 216.66 (167.77) | 477.43 (261.29) | 412.55 (187.83) | 233.18 (190.16) | 466.93 (271.15) | 237.22 (186.04) | 356.90 (230.47) | 466.93 (271.15) | **<0.001** | **<0.001** | **<0.001** |
| Nutrients | | | | | | | | | | | | |
| Fiber (g) | 23.43 (6.55) | 19.94 (5.17) | 24.44 (5.81) | 22.83 (7.28) | 21.71 (6.16) | 23.11 (5.34) | 21.53 (6.08) | 22.75 (6.61) | 23.11 (5.34) | **0.025** | 0.128 | 1.000 |
| Vitamin C (mg) | 134.86 (73.97) | 110.03 (54.22) | 123.88 (61.58) | 135.22 (62.99) | 111.76 (52.79) | 106.93 (61.04) | 121.32 (65.05) | 123.55 (59.64) | 106.93 (61.04) | 1.000 | 0.188 | 0.088 |
| Heme iron (mg) | 1.76 (0.88) | 1.13 (0.61) | 1.10 (0.59) | 0.79 (0.46) | 1.20 (0.60) | 0.32 (0.77) | 1.41 (0.80) | 1.02 (0.58) | 0.32 (0.77) | **<0.001** | 0.786 | **0.002** |

The results are expressed in n (%), mean (standard deviation), and median [interquartile range].

‡ Spain: the 60% of participants were blood donors. Italy: the participants from Ragusa and Turin were blood donors.

[1] Southern Europe (Italy and Spain); [2] Central Europe (United Kingdom, The Netherlands and Germany); [3] Northern Europe (Sweden).

* For males, iron deficiency: SF<15 µg/L; iron sufficiency: SF 15-200 µg/L; iron excess as SF>200 µg/L.

For females, iron deficiency: SF<15 µg/L; iron sufficiency: SF 15-150 µg/L; iron excess as SF>150 µg/L.

¶ The sample size of *HFE* genotype was 403 subjects. All participants with mutations in *HFE* gene were heterozygous carriers.

† Dietary intake was expressed as nutrient density per 2,000 kcal ([g/Kcal] x 2,000).

All differences between countries were statistically significant (p<0.05), except for iron status in females.

**Table 3. Characteristics of participants and dietary intake by iron status.**

| | Males | | Females | |
|---|---|---|---|---|
| | Sufficiency SF≥15-200 μg/L (n=293) | Excess SF>200 μg/L | Sufficiency (n=263) | Excess SF>150 μg/L (n=71) |
| **Sociodemographic characteristics** | | | | |
| Age (years) | | | | |
| <50 | 63 (21.5) | 25 (15.1) | **52 (20.2)** | **4 (5.9)** |
| ≥50 | 230 (78.5) | 141 (84.9) | **206 (79.8)** | **64 (94.1)** |
| Educational level | | | | |
| Uncompleted primary school | 13 (4.6) | 5 (3.2) | 31 (12.3) | 6 (9.1) |
| Primary or secondary school | **147 (51.8)** | **60 (38.2)** | 132 (52.2) | 40 (60.6) |
| Technical or professional education | 72 (25.4) | 45 (28.7) | 51 (20.2) | 11 (16.7) |
| High or vocational education | **52 (18.3)** | **47 (29.9)** | 39 (15.4) | 9 (13.6) |
| Body mass index (BMI, Kg/m$^2$) | | | | |
| Normal weight, 18.5-25 | **103 (35.2)** | **34 (20.5)** | 111 (43.0) | 22 (32.4) |
| Overweight, >25-30 | 156 (53.2) | 97 (58.4) | 100 (38.8) | 30 (44.1) |
| Obesity, >30 | **34 (11.6)** | **35 (21.1)** | 47 (18.2) | 16 (23.5) |
| ***HFE* genotype*** | | | | |
| Wild-type genotype | 98 (73.7) | 57 (74.0) | 84 (61.8) | 24 (66.7) |
| Carrier of C282Y mutation | 13 (9.8) | 9 (11.7) | 10 (7.4) | 1 (2.8) |
| Carrier of H63D mutation | 22 (16.5) | 11 (14.3) | 42 (30.9) | 11 (30.6) |
| **Lifestyle** | | | | |
| Physical activity | | | | |
| Inactive | 57 (21.8) | 33 (22.8) | 7 (3.0) | 2 (3.3) |
| Moderate | 187 (71.6) | 99 (68.3) | 199 (86.1) | 52 (85.2) |
| Active | 17 (6.5) | 13 (9.0) | 25 (10.9) | 7 (11.5) |
| Smoking | | | | |
| No smoker/Ex-smoker | 171 (58.4) | 91 (54.8) | 207 (80.2) | 55 (80.9) |
| Smoker | 122 (41.6) | 75 (45.2) | 51 (19.8) | 13 (19.1) |
| Frequency of alcohol intake | | | | |
| Never/Former | **22 (9.7)** | **5 (3.4)** | 36 (17.5) | 8 (12.9) |
| < 1 serving/d (<14 g/d) | 127 (55.9) | 72 (49.0) | 143 (69.4) | 46 (74.2) |
| >1-2 servings/d (>14-28 g/d) | 35 (15.4) | 31 (21.1) | 17 (8.3) | 6 (9.7) |
| >2-3 servings/d (>28-42 g/d) | 18 (7.9) | 17 (11.6) | 6 (2.9) | 1 (1.6) |
| >3 servings/d (>42 g/d) | 25 (11.0) | 22 (15.0) | 4 (1.9) | 1 (1.6) |
| **Daily alcohol intake (g)** | **17.86 (21.38)** | **24.27 (39.62)** | 6.32 (10.03) | 6.64 (10.68) |
| **Daily nutritional intake†** | | | | |
| Energy (Kcal) | 2325.23 (629.48) | 2334.98 (810.54) | 1840.22 (601.26) | 1802.80 (495.25) |
| Foods | | | | |
| Total meat (g) | **96.51 (46.84)** | **108.34 (45.46)** | 92.21 (48.27) | 95.45 (42.85) |
| Red meat (g) | 38.72 (27.61) | 41.12 (30.25) | 35.90 (27.59) | 38.93 (31.30) |
| Processed meat (g) | **32.55 (25.48)** | **44.55 (32.51)** | 28.85 (26.88) | 32.81 (24.50) |
| Fruits (g) | 219.37 (178.72) | 191.51 (155.15) | **328.32 (209.68)** | **271.26 (193.73)** |
| Vegetables and legumes (g) | 188.97 (132.04) | 168.61 (118.95) | 216.11 (140.67) | 184.47 (115.47) |
| Dairy products (g) | 275.98 (207.22) | 305.44 (224.02) | 374.45 (250.28) | 345.30 (220.19) |
| Nutrients | | | | |
| Fiber (g) | 21.07 (5.91) | 20.99 (5.70) | 24.23 (6.52) | 22.85 (5.87) |
| Proteins (g) | 80.98 (14.92) | 81.99 (15.63) | 86.00 (16.39) | 83.87 (14.47) |
| Total iron (mg) | 12.46 (2.84) | 12.70 (2.74) | 12.79 (2.64) | 12.77 (2.04) |
| Heme iron (mg) | 1.21 (0.78) | 1.34 (0.70) | 1.15 (0.71) | 1.22 (0.63) |
| Calcium (mg) | 846.30 (280.85) | 893.00 (288.45) | 1056.54 (359.09) | 994.91 (288.30) |
| Vitamin C (mg) | 110.38 (55.38) | 104.08 (44.22) | 141.32 (69.75) | 129.73 (77.17) |

The results are expressed in n (%) and mean (standard deviation). Significant differences ($p<0.05$) were highlighted in bold.

* The sample size of *HFE* genotype was 403 subjects. All participants with mutations in *HFE* gene were heterozygous carriers.

† Dietary intake was expressed as nutrient density per 2,000 kcal ([g/Kcal] x 2,000).

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**Table 4. Factors associated with levels of serum iron biomarkers and excess iron.**

| Serum ferritin levels (µg/L) | | | | | | |
|---|---|---|---|---|---|---|
| | Total | | Male | | Female | |
| | $R^2c$=0.254; $F_{675,13}$=18.65 p<0.001 | | $R^2c$=0.181, $F_{388,13}$=7.62 p<0.001 | | $R^2c$=0.179, $F_{286,11}$=6.67 p<0.001 | |
| **Variable** | **β** | **95%CI** | **β** | **95%CI** | **β** | **95%CI** |
| [1] Sex (female) | **-0.42** | **-0.55, -0.30** | | | | |
| Age (≥50 years) | **0.44** | **0.30, 0.59** | **0.38** | **0.19, 0.58** | **0.54** | **0.33, 0.76** |
| Body mass index (overweight) | 0.04 | -0.09, 0.17 | 0.08 | -0.10, 0.26 | -0.07 | -0.25, 0.12 |
| Body mass index (obesity) | **0.19** | **0.02, 0.36** | **0.27** | **0.03, 0.51** | 0.08 | -0.16, 0.32 |
| Vitamin C intake (100 mg/d) | **-0.23** | **-0.32, -0.14** | **-0.20** | **-0.35, -0.05** | **-0.24** | **-0.35, -0.13** |
| Heme iron intake (mg/d) | **0.10** | **0.02, 0.18** | **0.10** | **0.00, 0.21** | 0.12 | -0.01, 0.24 |
| Calcium intake (mg/d) | **0.02** | **0.00, 0.04** | **0.05** | **0.02, 0.08** | 0.01 | -0.02, 0.03 |
| Alcohol intake (10 g/d) | **0.04** | **0.01, 0.06** | **0.03** | **0.00, 0.06** | 0.08 | -0.01, 0.15 |
| hsCRP (mg/L) | **0.02** | **0.00, 0.04** | 0.02 | -0.04, 0.04 | **0.04** | **0.01, 0.08** |
| *HFE* genotype (carriers of C282Y) | 0.19 | -0.48, 0.10 | 0.02 | -0.34, 0.38 | 0.55 | -1.06, 0.05 |
| *HFE* genotype (carriers of H63D) | 0.06 | -0.26, 0.13 | 0.00 | -0.31, 0.30 | 0.04 | -0.30, 0.22 |

| Excess iron | | | | | | |
|---|---|---|---|---|---|---|
| | Total | | Male | | Female | |
| | p<0.001 | | p<0.001 | | p=0.020 | |
| **Variable** | **OR** | **95%CI** | **OR** | **95%CI** | **OR** | **95%CI** |
| [1] Sex (female) | 0.62 | 0.36, 1.08 | | | | |
| Age (≥50 years) | **2.03** | **1.15, 3.59** | 1.47 | 0.72, 3.00 | **3.16** | **1.01, 10.90** |
| Body mass index (overweight) | **1.81** | **1.16, 2.83** | **2.15** | **1.16, 3.99** | 1.29 | 0.61, 2.72 |
| Body mass index (obesity) | **3.02** | **1.70, 5.38** | **5.18** | **2.30, 11.67** | 1.65 | 0.62, 4.43 |
| Vitamin C intake (100 mg/d) | 0.74 | 0.49, 1.12 | 0.71 | 0.38, 1.32 | 0.85 | 0.45, 1.59 |
| Heme iron intake (mg/d) | **1.65** | **1.22, 2.24** | **2.37** | **1.53, 3.66** | 1.19 | 0.69, 2.06 |
| Calcium intake (mg/d) | 1.04 | 0.98, 1.12 | **1.18** | **1.05, 1.31** | 0.90 | 0.79, 1.01 |
| Alcohol intake (10 g/d) | 1.07 | 0.96, 1.19 | **1.12** | **1.01, 1.27** | 0.88 | 0.63, 1.25 |
| hsCRP (mg/L) | 1.02 | 0.96, 1.08 | 1.01 | 0.94, 1.08 | 1.09 | 0.98, 1.23 |
| *HFE* genotype (carriers of C282Y) | 1.31 | 0.51, 3.39 | 1.76 | 0.49, 6.30 | 0.17 | 0.01, 2.80 |
| *HFE* genotype (carriers of H63D) | 0.89 | 0.43, 1.81 | 0.90 | 0.27, 2.89 | 0.42 | 0.11, 1.57 |

[1]Adjusted for: center EPIC, sex, age, body mass index, educational level, physical activity, alcohol intake, smoking, high-sensitivity C-reactive protein (hsCRP), *HFE* genotype, and daily nutritional intake (including energy, fiber, heme iron, calcium and vitamin C).

[2]Adjusted for: model 1 – hsCRP.

Reference categories: Sex (female), age (<50 years), body mass index (normal weight), *HFE* genotype (wild type).

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**Table S1. Serum ferritin levels (μg/L) of participants according to sociodemographic and lifestyle characteristics.**

| | Total sample (n=828) | | Males (n=472) | Females (n=356) |
|---|---|---|---|---|
| | **N (%)** | **Median [IQR]** | **Median [IQR]** | **Median [IQR]** |
| All | 828 (100) | 107.20 [140.67] | 143.70 [179.06] | 76.95 [92.43] |
| Subset CRP≤10 mg/L | 803 (97.0) | 104.60 [137.25] | 139.40 [176.78] | 75.10 [88.96] |
| Subset CRP≤5 mg/L | 744 (89.9) | 103.55 [137.46] | 138.95 [176.11] | 74.41 [85.46] |
| **Sociodemographic characteristics** | | | | |
| Age (years) | | | | |
| <50 | 160 (19.3) | **65.17 [105.81]** | **103.70 [171.07]** | **40.75 [42.50]** |
| ≥50 | 668 (80.7) | **117.65 [138.14]** | **157.50 [172.82]** | **85.27 [97.23]** |
| Educational level | | | | |
| Uncompleted primary school | 58 (7.2) | **68.24 [83.26]** | **81.12 [156.20]** | 59.74 [70.23] |
| Primary or secondary school | 403 (50.3) | **102.30 [129.51]** | **133.85 [159.43]** | 80.91 [93.80] |
| Technical or professional education | 184 (22.9) | **127.35 [141.25]** | **167.20 [166.29]** | 96.24 [95.06] |
| High or vocational education | 157 (19.6) | **115.30 [190.68]** | **185.80 [214.37]** | 59.72 [89.14] |
| Body mass index (BMI, $Kg/m^2$) | | | | |
| Normal weight, 18.5-25 | 293 (35.4) | **91.10 [114.26]** | **124.70 [129.78]** | 72.36 [76.26] |
| Overweight, >25-30 | 401 (48.4) | **115.10 [149.54]** | **147.80 [170.59]** | 74.10 [103.72] |
| Obesity, >30 | 134 (16.2) | **129.15 [216.51]** | **208.45 [270.83]** | 98.87 [107.89] |
| ***HFE* genotype*** | | | | |
| Wild-type genotype | 276 (68.5) | 110.45 [146.78] | 153.80 [187.29] | 75.90 [93.65] |
| Carrier of C282Y mutation | 35 (8.7) | 81.27 [148.70] | 173.15 [207.52] | 68.77 [54.28] |
| Carrier of H63D mutation | 92 (22.8) | 100.04 [114.41] | 146.55 [151.69] | 86.63 [98.35] |
| **Lifestyle** | | | | |
| Physical activity | | | | |
| Inactive | 103 (13.9) | 125.00 [200.41] | 135.05 [211.27] | 63.20 [60.79] |
| Moderate | 572 (77.4) | 101.35 [132.97] | 139.40 [169.44] | 77.45 [89.41] |
| Active | 64 (8.7) | 117.65 [146.01] | 189.50 [162.63] | 95.73 [100.18] |
| Smoking | | | | |
| No smoker/Ex-smoker | 554 (66.9) | **97.72 [130.19]** | 137.80 [187.17] | 74.34 [88.98] |
| Smoker | 274 (33.1) | **127.65 [151.82]** | 162.80 [168.23] | 84.98 [104.77] |
| Frequency of alcohol intake | | | | |
| Never/Former | 131 (15.8) | **70.47 [76.94]** | **83.61 [85.32]** | 62.91 [81.44] |
| < 1 serving/d (<14 g/d) | 449 (54.2) | **104.70 [133.18]** | **149.40 [173.13]** | 83.21 [100.55] |
| >1-2 servings/d (>14-28 g/d) | 114 (13.8) | **137.50 [168.05]** | **177.40 [196.47]** | 76.16 [108.83] |
| >2-3 servings/d (>28-42 g/d) | 57 (6.9) | **129.90 [169.64]** | **143.00 [165.49]** | 58.43 [57.90] |
| >3 servings/d (>42 g/d) | 77 (9.3) | **161.00 [237.85]** | **181.10 [237.87]** | 105.40 [166.96] |
| **Geographic region** | | | | |
| Southern Europe[1] | 341 (41.5) | **79.03 [108.97]** | **103.00 [144.17]** | **62.13 [91.24]** |
| Central Europe[2] | 407 (49.2) | **132.80 [151.27]** | **181.20 [183.67]** | **92.91 [88.60]** |
| Northern Europe[3] | 80 (8.9) | **127.60 [160.38]** | **152.10 [220.85]** | **70.61 [106.75]** |