Evaluation of iron status in European adolescents through biochemical iron indicators: the HELENA Study

M Ferrari¹, L Mistura¹, E Patterson², M Sjöström², LE Díaz³, P Stehle⁴, M Gonzalez-Gross⁴, M Kersting⁶, K Widhalm⁷, D Molnár⁸, F Gottrand⁹, S De Henauw¹⁰, Y Manios¹¹, A Kafatos¹², LA Moreno¹³ and C Leclercq¹ on behalf of the HELENA Study Group

Background/Objectives: To assess the iron status among European adolescents through selected biochemical parameters in a cross-sectional study performed in 10 European cities.

Subjects/Methods: Iron status was defined utilising biochemical indicators. Iron depletion was defined as low serum ferritin (SF <15 µg/l). Iron deficiency (ID) was defined as high-soluble transferrin receptor (sTfR >8.5 mg/l) plus iron depletion. Iron deficiency anaemia (IDA) was defined as ID with haemoglobin (Hb) below the WHO cutoff for age and sex: 12.0 g/dl for girls and for boys aged 12.5–14.99 years and 13.0 g/dl for boys aged ≥15 years. Enzyme linked immunosorbent assay was used as analytical method for SF, sTfR and C-reactive protein (CRP). Subjects with indication of inflammation (CRP >5 mg/l) were excluded from the analyses. A total of 940 adolescents aged 12.5–17.49 years (438 boys and 502 girls) were involved.

Results: The percentage of iron depletion was 17.6%, significantly higher in girls (21.0%) compared with boys (13.8%). The overall percentage of ID and IDA was 4.7 and 1.3%, respectively, with no significant differences between boys and girls. A correlation was observed between log (SF) and Hb (r = 0.36, P < 0.01), and between log (sTfR) and mean corpuscular haemoglobin (r = −0.30, P < 0.01). Iron body stores were estimated on the basis of log (sTfR/SF). A higher percentage of negative values of body iron was recorded in girls (16.5%) with respect to boys (8.3%), and body iron values tended to increase with age in boys, whereas the values remained stable in girls.

Conclusions: To ensure adequate iron stores, specific attention should be given to girls at European level to ensure that their dietary intake of iron is adequate.

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Introduction

Iron Deficiency (ID) continues to be one of the most prevalent nutritional deficiencies in the world, particularly in developing countries (World Health Organization (WHO), 2003). WHO estimated that ID occurs in about 66–80% of...
the world’s population (WHO, 2003). ID has many negative effects on health, including changes in immune function, cognitive development, temperature regulation, energy metabolism and work performance (Dallman, 1986). On the other hand, iron is a pro-oxidant and thus a possible risk factor for coronary heart disease. However, a recent review suggests that there is no strong association between body iron stores and coronary heart disease in adults (Zegrean, 2009).

The diagnosis of ID is based primarily on laboratory measurements of biochemical iron indicators. There is no full international consensus on the indicators to be used for assessing iron status, as each indicator has its own limitations because of either poor sensitivity or specificity, or because it is modified by conditions other than ID (Dallman et al., 1993). There are three general approaches to assess ID in populations. In the first one, used in studies conducted by Hallberg et al. (Hallberg et al., 1993) and Armstrong (Armstrong, 1989), ID is defined as a low level of serum ferritin (SF). In the second one, used in the US in the National Health and Nutrition Examination Survey (NHANES) II and III, the multiple-criteria model is used to classify ID; it consists of two or three abnormal values for transferrin saturation, SF and erythrocyte protoporphyrin (Cogswell et al., 2009). The main limitation of these two approaches is that inflammation leads to high ferritin values, even though iron stores are low. The third approach proposed by WHO (2004) and adopted by Zimmermann (2008) is based on the measurements of SF and soluble transferrin receptor (sTfR) in combination with C-reactive protein (CRP) to eliminate false negative cases in presence of inflammation. The third approach was considered more appropriate and was therefore used in the present study. It is well known that SF concentrations reflect the size of the iron stores (Cook, 1999) and that low SF levels identify ‘iron depletion’ (WHO, 2001). On the other hand, the measurement of sTfR has been reported to be useful in the diagnosis of ‘tissue ID’ (Skikne et al., 1990) and its values are not influenced by acute or chronic inflammatory conditions (Ferguson et al., 1992; Olivares et al., 1995). An approach to estimate body iron was developed by Cook et al. (Cook et al., 2003) using the formula: body iron (mg/kg) = -(log(sTfR/SF)–2.8229)/0.1207 to allow the full range of the iron status of a population to be evaluated, with negative values representing a deficit of iron with respect to the quantity required to maintain a normal haemoglobin (Hb) concentration.

Adolescents are at risk of ID because of their high iron requirements, especially during the growth spurt period (Moreno, 2008). In boys, increased muscular growth leads to increased demand for iron. In girls, in addition to the fast growth, the onset of menstruation leads to iron losses (Wharton and Wharton, 1987). As a consequence, ID occurs in adolescents also in developed countries, if their additional iron needs are not met (Dallman et al., 1996). Some studies suggested that ID can have detrimental effects on memory and learning processes in preadolescents and adolescents (Beard and Connor, 2003).

The data available on iron status in European adolescents, are derived from studies utilising different methodologies, different indicators of iron status and varying criteria to define ID (Herberg et al., 2001). There was, therefore, a need to investigate the situation of ID among European adolescents with a harmonised methodology.

Within the framework of the HELENA Study (Healthy Lifestyle in Europe by Nutrition in Adolescents), funded within the 6th EU framework programme, a biochemical evaluation of iron status was performed with a cross-sectional approach. The objective of the present paper is to report the iron status of European adolescents.

Materials and methods

Study design and sample selection

A detailed description of HELENA-cross-sectional study (HELENA-CSS) in terms of sampling and recruitment approaches, standardisation and harmonisation processes, data collection, analysis strategies and quality-control activities has been published elsewhere (Moreno et al., 2008). The study was approved by the Research Ethics Committees of each country involved according to the Declaration of Helsinki and International Conferences on Harmonization for Good Clinical Practice. Written informed consent was obtained from the parents of the adolescents and the adolescents themselves (Béghin et al., 2008). Briefly, subjects with an age range of 12.5–17.49 years were recruited in 10 cities across Europe. Selection of cities was based on two criteria: regional distribution and presence of an active research group assuring sufficient expertise and resources to successfully perform epidemiological studies. Within the study, Stockholm (Sweden) represented Northern Europe. Athens, Heraklion (Greece), Rome (Italy) and Zaragoza (Spain) represented Southern Europe. Pecs (Hungary) represented Eastern Europe. Ghent (Belgium) and Lille (France) represented Western Europe, whereas Dortmund (Germany) and Vienna (Austria) represented Central Europe. As the overall aim of the HELENA Study was to assess the nutritional status of the adolescent population in Europe, during the design phase, the variability of a large set of nutritional status indices obtained in previous studies was checked. Body mass index was the indicator that showed the largest variability. Therefore, the sample size was estimated according to the mean body mass index and variance values for each sex and age strata. This procedure ensured to adequately describe all the variables included in the study. For the sample size calculation, a confidence level of 95% and an error of ± 0.3 kg/m² were assumed, leading to a sample size of 3000 adolescents. More details on the procedure are provided by Moreno et al. (2008).

Blood sampling was performed in one-third of the adolescents recruited. Therefore, the total expected subsample was 1000 adolescents (100 subjects for each city). The size of subsample was chosen, so as to assess the
imunological parameters with sufficient precision. Such parameters were those with the highest variability within the blood measurements that were included in the study as discussed by Moreno et al. (2008). The selection procedure for schools and classes including the selection of a subset of classes for blood sampling has been applied centrally by one partner (Ghent) for all study centres.

Blood sampling and laboratory measures

The blood sampling procedure and sample logistics have been described in detail elsewhere (González-Gross et al., 2008). Briefly, fasting blood samples were collected by venipuncture at school between 0800 and 1000 hours in the morning. Whole-blood samples for the red blood parameters (Hb, red blood cell, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration and red cell distribution width) were sent directly to the local laboratory of each country to be analysed. Samples for iron status analyses were clotted at room temperature for at least 30 min, followed by centrifuged at 3500 r.p.m. for 10 min. Serum samples were shipped to the central laboratory in Bonn University (IEL, Germany), Department of Nutrition and Food Science, to be stored at −80 °C before being shipped to Italy in May 2007, within 6 months of the collection. The aliquots of serum samples were analysed in the Human Nutrition Laboratory of the National Research Institute on Food and Nutrition (INRAN). SF, sTfR and CRP were measured by using an enzyme linked immunosorbent assay (Erhardt et al., 2004). A commercially available control sample from Bio-Rad Liquichek Immunology Control Level 3 (Bio-Rad, Milan, Italy) was used to obtain a calibration curve on each plate.

A quality-control serum was produced in house by pooling human serum from four healthy volunteers to monitor accuracy and precision of the determinations. The coefficient of variation from 10 replicates of the quality-control serum pool was 5.3, 6.4 and 12.4% for SF, sTfR and CRP, respectively. Additionally, the interassay coefficient of variation of the pool samples analysed in a total of 32 different analytical runs (four replicates) was 10.8, 14.8 and 23.4% for SF, sTfR and CRP, respectively.

INRAN laboratory is involved in an external quality assurance program of CDC Vital-Eqa for SF, sTfR and CRP analyses (Haynes et al., 2008). In Spring 2007, the measures of iron indicators of this study were within the acceptable range, except for CRP that was just below the inferior limit (2.42 mg/l versus an acceptable range of 2.70–5.38 mg/l) and sTfR that was at the superior limit (7.3 mg/l versus an acceptable range of 1.7 and 7.3 mg/l).

Anaemia and iron classification

Anaemia was defined as Hb concentration below the cutoff set by WHO (WHO, 2004), 12.0 g/dl for girls aged 12 years and above, and for boys aged 12–14; and 13.0 g/dl for boys aged 15 years or above. Abnormal values for iron indicators were defined as concentration <15 μg/l for low SF (WHO, 2004), >200 μg/l and >150 μg/l (boys and girls, respectively) for high SF (WHO, 2004; severe risk of iron overload) and >8.5 mg/l for high sTfR (Cook et al., 1993). A participant was considered to have active inflammation, if the CRP concentration exceeded 5 mg/l (Woodruff et al., 2006). The methodology to classify the iron status is described in Table 1. The classification of iron status is considered in presence of normal CRP concentration.

Total body iron was estimated with the use of the formula: body iron (mg/kg) = − (log sTfR/SF) −2.8229)/0.1207 (Cook et al., 2003).

Statistical analyses

The Kruskal–Wallis test was used for comparing hematological parameters among iron sufficiency, iron depletion and ID. The Student’s t-test was used to evaluate the significance of differences between mean values of iron indicators between genders. Pearson correlation was used to test the linear relation between hematological variables. The χ2-test was used for comparing proportions. Iron status indicators, such as sTfR and SF, were transformed to logarithms before performing statistical analyses. The results in the tables are shown using the original units without any log transformation.

As already performed by Ortega et al. (Ortega et al., 2010), all the analyses conducted on the HELENA data were adjusted by a weighing factor to balance the sample according to the age and sex distribution of the theoretical sample to guarantee representation of each of the stratified groups. The counts of subjects (n) are reported in the tables as unweighted values. All the statistical analyses were performed using the SPSS statistical software package for Microsoft Windows (version 12.00, SPSS Inc., Chicago, IL, USA).

Results

Iron indicators and iron status

The HELENA-CSS consists of 1089 adolescent subjects with blood measurements. A total of 102 subjects were excluded

| Classification | Values of iron indicators |
|----------------|---------------------------|
| SF (μg/l)      | sTfR (mg/l)               |
| Iron sufficiency | ≥ 15                    | ≤ 8.5                |
| Iron depletion  | < 15                     | < 8.5                |
| ID             | < 15                     | > 8.5                |
| IDA            | < 15                     | > 8.5                |

Abbreviations: CRP, C-reactive protein; ID, iron deficiency; IDA, iron deficiency with anaemia; SF, serum ferritin; sTfR, soluble transferrin receptor.

aIron status is considered in the presence of CRP <5 mg/l.
bWithout anaemia.
cWithout ID and with or without anaemia.
because at least one of the iron indicators (SF, n = 80; sTfR, n = 84) or Hb (n = 16) was missing. The final sample of 987 adolescents included 465 boys and 522 girls, that is, 90.7% of the adolescents who participated in the blood sampling.

A total of 47 subjects had high levels of CRP (27 boys and 20 girls) and were excluded. Therefore, the present study of iron status was performed on a total of 940 adolescents (438 boys and 502 girls), with no indication of the presence of inflammation.

The mean age (standard deviation) of adolescents was 14.7 (1.2) years both in boys and in girls, and the mean body mass index was 21.2 (3.8) and 21.1 (3.3) kg/m², respectively, for boys and girls. The reported age of menarche in the present study ranged from 9 to 16 years of age. A total of 58 (12%) girls reported not to have experienced menarche at the time of the study. In all, 39 and 43% of the boys and girls, respectively, declared to have smoked cigarettes at least once. Mean, median and percentiles (25–75th) values of red blood parameters, SF and sTfR are summarised in Table 2. Percentage of iron sufficiency, abnormal values of iron indicators, anaemia, iron depletion, ID and iron deficiency anaemia (IDA) are presented in Table 3.

In boys, the mean (standard deviation) of Hb was 14.7 (1.2) g/dl (14.3 (1.0) g/dl aged 12.5–14.99 years and 15.2 (1.2) g/dl aged 15 years or above). In girls it was 13.8 (1.2) g/dl. The mean values of SF suggest a gender-related significant difference (P<0.05) with a lower mean SF value in girls (27.9 µg/l) than in boys (36.6 µg/l). The percentage of low SF values was also higher in girls (26.3%) compared with boys (17.9%) (P<0.01) (Table 3). No single case of high level of SF was identified (SF >200 µg/l for boys and >150 µg/l for girls), indicating no risk of iron overload (WHO, 2004) in the study sample.

A total of 23 subjects had high values (>8.5 mg/l) of sTfR (that could have indicated ID) but with normal values of SF (indicating no iron depletion), and could therefore not be categorised in one of the iron status classifications (Table 1). They were excluded from the analyses in terms of the classification of ID.

The percentage of iron depletion was 17.6%, significantly higher in girls (21.0%) than in boys (13.8%) (P<0.05) (Table 3).

The overall prevalence of anaemia was 4.4% and it ranged from 1.1% (Stockholm) to 10.1% (Heraklion) among cities. Intermediate prevalences were 1.4% for Zaragoza, 3.2% for Pecs, 3.6% for Dortmund, 4.0% for Rome, 4.6% for Ghent, 5.5% for Lille, 5.6% for Vienna and 5.7% for Athens. The overall prevalence of anaemia without ID was 3.1% (4.6% in girls and 1.4% in boys) and ranged from 0% (Stockholm and Zaragoza) to 7.6% (Heraklion). The second highest prevalence was observed in Athens (5.7%). Intermediate

### Table 2 Haemoglobin, red blood and iron status parameters in European adolescents

| Parameter | Boys (n = 438) | Girls (n = 502) |
|-----------|---------------|----------------|
| **M**     | s.d. | Me | 25–75th percentiles | **M** | s.d. | Me | 25–75th percentiles |
| Hb g/dl   | 14.7 | 1.2 | 14.7 | 14.0–15.6 | 13.8 | 1.2 | 13.4 | 12.8–14.1 |
| RBC 10^{12}/l | 5.2 | 0.4 | 5.1 | 4.9–5.4 | 4.7 | 0.4 | 4.7 | 4.5–4.9 |
| MCV fl    | 83.6 | 4.6 | 84.0 | 81.5–86.2 | 84.9 | 5.1 | 85.5 | 82.4–88.2 |
| MCH µg/l  | 28.6 | 1.9 | 28.8 | 27.7–29.8 | 28.6 | 2.1 | 29.0 | 29.0–30.0 |
| MCHC g/dl | 34.3 | 1.0 | 34.2 | 33.6–34.9 | 33.7 | 1.0 | 33.7 | 33.7–34.3 |
| RDW %     | 12.9 | 0.9 | 12.9 | 12.5–13.3 | 12.9 | 1.1 | 12.8 | 12.8–13.4 |
| SF µg/l   | 36.6  | 23.9 | 32.0 | 17.9–49.3 | 37.9 | 19.7 | 24.1 | 24.1–38.0 |
| sTfR mg/l | 5.9  | 1.9 | 5.8 | 4.6–7.0 | 6.0  | 3.3 | 5.7 | 5.7–7.0 |

**Abbreviations:** Hb, haemoglobin; M, mean; Me, median; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; RDW, red cell distribution width; SF, serum ferritin; sTfR, soluble transferrin receptor.

**n=12** boys and **n=10** girls were excluded because of very high values of sTfR and normal values of SF.

**Abbreviations:** ID, iron deficiency; IDA, iron deficiency with anaemia; SF, serum ferritin; sTfR, soluble transferrin receptor.

**The count of subjects (n) are reported in the table as unweighted values.**

**Indicators and cutoff values used to identify abnormal values of iron indicators and iron status are reported in Table 1.**

**All data were adjusted by a weighing factor to balance the sample according to the theoretical age distribution of the European adolescent population.**

**The mean age (standard deviation) of adolescents was 14.7 (1.2) years both in boys and in girls.**

**The percentage of low SF values was also higher in girls (26.3%) compared with boys (17.9%) (P<0.01).**

**The overall prevalence of anaemia was 4.4% and it ranged from 1.1% (Stockholm) to 10.1% (Heraklion) among cities.**

**Intermediate prevalences were 1.4% for Zaragoza, 3.2% for Pecs, 3.6% for Dortmund, 4.0% for Rome, 4.6% for Ghent, 5.5% for Lille, 5.6% for Vienna and 5.7% for Athens.**

**The overall prevalence of anaemia without ID was 3.1% (4.6% in girls and 1.4% in boys) and ranged from 0% (Stockholm and Zaragoza) to 7.6% (Heraklion).**

**The second highest prevalence was observed in Athens (5.7%).**

**Intermediate overall prevalence of anaemia without ID was 3.1% (4.6% in girls and 1.4% in boys) and ranged from 0% (Stockholm and Zaragoza) to 10.1% (Heraklion).**

**The overall prevalence of anaemia was 4.4% and it ranged from 1.1% (Stockholm) to 10.1% (Heraklion) among cities.**

**Intermediate prevalences were 1.4% for Zaragoza, 3.2% for Pecs, 3.6% for Dortmund, 4.0% for Rome, 4.6% for Ghent, 5.5% for Lille, 5.6% for Vienna and 5.7% for Athens.**

**The overall prevalence of anaemia without ID was 3.1% (4.6% in girls and 1.4% in boys) and ranged from 0% (Stockholm and Zaragoza) to 7.6% (Heraklion).**

**The second highest prevalence was observed in Athens (5.7%).**

Intermediate
prevalences were 1.8% for Dortmund, 1.9% for Vienna, 3.0% for Rome, 3.2% for Pecs, 4.1% for Lille and 4.6% for Ghent. Percentage of iron depletion ranged from 9.6% (Zaragoza) to 23.0% (Pecs). Percentage of ID ranged from 0% (Lille) to 11.3% (Athens). Percentage of IDA ranged from 0% (Athens, Gent, and Pecs) to 2.8% (Vienna).

Among adolescents with anaemia (n = 40), 27.5% (n = 11) were classified as IDA, with abnormal SF and sTfR values, whereas 24.3% (n = 10) had abnormal SF values but with normal sTfR values (iron depletion without ID) and 47.5% (n = 19) had anaemia without ID, with normal SF and sTfR values.

Log(SF) and log(sTfR) were significantly correlated with the red cell parameters (MCV, MCH, mean corpuscular haemoglobin concentration and red cell distribution width) (Table 4). In the total sample, a significant correlation was observed between log(SF) and Hb concentration (r = 0.36, P < 0.01), and between log(SF) and log(sTfR) (r = 0.33, P < 0.01). For log(sTfR), a significant correlation was observed with log(SF), MCH (r = 0.30, P < 0.01) and MCV (r = -0.26, P < 0.01) No significant correlation was observed between red blood cell and iron indicators (log(SF) and log(sTfR); r = 0.13 and r = 0.07, respectively).

The mean of red cell parameters in the different classification of iron status is reported in Table 5. Statistically significant differences between iron sufficiency, iron depletion and ID were observed for red cell parameters for boys and girls. There were significant differences (P < 0.001) for Hb, MCV, MCH, mean corpuscular haemoglobin concentration and red cell distribution width between iron sufficiency group and the iron depletion and ID groups. No significant difference was observed between iron sufficiency group and ID group for any of the red cell parameters considered.

Estimation of body iron
Body iron was calculated according to the formula of Cook et al. (Cook et al., 2003), and the cumulative percentage distributions by gender are shown in Figure 1. Mean

### Table 4 Pearson correlation coefficients and significance of correlations (P) between iron parametersa (n = 940)b

|          | Hb   | RBC  | MCV  | MCH  | MCHC | RDW  | Log(SF) | Log(sTfR) |
|----------|------|------|------|------|------|------|---------|-----------|
| Boys     |      |      |      |      |      |      |         |           |
| Log(SF)  | 0.25**| 0.11*| 0.17**| 0.19**| 0.10**| -0.14**| -         | -0.25**   |
| Log(sTfR)| 0.16**| 0.07 | -0.16**| -0.22**| -0.20**| 0.09**| 0.004*  | -         |
| Girls    |      |      |      |      |      |      |         |           |
| Log(SF)  | 0.35**| 0.004*| 0.34**| 0.41**| 0.40**| -0.42**| -        | -0.39**   |
| Log(sTfR)| 0.21**| 0.11 | -0.32**| -0.35**| -0.29**| 0.35**| 0.35**  | -         |
| Total    |      |      |      |      |      |      |         |           |
| Log(SF)  | 0.36**| 0.13 | 0.24**| 0.32**| 0.32**| -0.31**| -        | -0.33**   |
| Log(sTfR)| 0.16**| 0.07 | -0.26**| -0.30**| -0.24**| 0.26**| 0.26**  | -         |

**Abbreviations:** Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; RDW, red cell distribution width; SF, serum ferritin; sTfR, soluble transferrin receptor.

**a**All data were adjusted by a weighing factor to balance the sample according to the theoretical age distribution of the European adolescent population.

**b**The count of subjects (n) are reported in the table as unweighted values.

**c**P < 0.05; **d**P < 0.01 boys versus girls.

### Table 5 Haematological characteristics according to iron sufficiency, iron depletion and IDa

|                      | Iron sufficiency | Iron depletion | ID |
|----------------------|-----------------|----------------|----|
|                      | Boys (n = 344)b | Girls (n = 361)b | Total (n = 705)b | Boys (n = 63)b | Girls (n = 104)b | Total (n = 167)b | Boys (n = 18)b | Girls (n = 27)b | Total (n = 45)b |
| Mean Hb, g/dl (s.d.)| 14.9 (1.2)     | 13.6 (0.9)     | 14.2 (1.2)*    | 14.4 (1.0)     | 13.3 (1.6)     | 13.7 (1.4)     | 13.7 (0.9)     | 12.0 (1.4)     | 12.7 (1.4)     |
| Mean RBC, 1012/l (s.d.)| 5.2 (0.4) | 4.7 (0.3) | 4.9 (0.4)* | 5.2 (0.4) | 4.7 (0.5) | 4.8 (0.5) | 5.0 (0.4) | 4.8 (0.3) | 4.8 (0.4) |
| Mean MCV, fl (s.d.) | 84.1 (4.4) | 85.7 (4.6) | 84.8 (4.5)* | 82.1 (4.6) | 84.5 (4.5) | 83.4 (4.7) | 82.0 (4.2) | 78.2 (8.0) | 80.1 (6.4) |
| Mean MCH, pg (s.d.) | 28.8 (1.8) | 29.0 (1.8) | 28.8 (1.8)* | 28.0 (1.9) | 28.3 (1.8) | 28.3 (1.9) | 27.6 (1.7) | 25.1 (3.5) | 26.3 (2.9) |
| Mean MCHC, g/dl (s.d.) | 34.3 (1.0) | 33.8 (0.9) | 34.1 (0.9)* | 34.1 (1.0) | 33.5 (0.9) | 33.7 (1.0) | 33.6 (1.0) | 32.0 (1.8) | 32.8 (1.6) |
| Mean RDW, % (s.d.)  | 12.9 (0.9)  | 12.7 (0.9)  | 12.8 (0.9)*  | 13.2 (1.0)  | 13.2 (1.0)  | 13.2 (1.0)  | 13.6 (0.9)  | 14.6 (2.0)  | 14.1 (1.6)  |

**Abbreviations:** Hb, haemoglobin; ID, iron deficiency; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; RDW, red cell distribution width.

**a**All data were adjusted by a weighing factor to balance the sample according to the theoretical age distribution of the European adolescent population.

**b**The count of subjects (n) are reported in the table as unweighted values.

**c**P < 0.001 statistically different between iron sufficiency, iron depletion and ID.
(standard deviation) body iron was significantly higher ($P<0.001$; Student’s t-test) in boys (4.4 (3.2) mg/kg) than in girls (3.3 (4.1) mg/kg). Girls showed a slight shift of the body iron distribution curve to the left in comparison with boys in which higher positive values were observed. The reference line indicates the cutoff point for tissue ID (body iron <0 mg/kg). The percentage of negative values was significantly higher ($P<0.001$; $\chi^2$) in girls (16.5%) with respect to boys (8.3%). The distribution of body iron stores by age was also examined. Figure 2 shows that the linear relationship between the body iron and the age of the adolescents is not very strong. The body iron increased slightly with age ($r=0.28$) but this trend did not reach statistical significance. On the other hand, it tended to be stable ($r=0.025$) in girls.

The present study is the first report evaluating iron status in adolescents across multiple European countries. As discussed by Moreno et al. (2008), the selected cities were equivalent and comparable between countries but the samples were representative for the cities and not for the countries. Therefore, the HELENA Study does not allow for statistical comparison of differences in iron status between regions. However, the prevalence of iron status stages according to geographical location of the cities adopted for this study are provided underneath for descriptive purpose.

In the present study, the overall proportion of iron depletion was 17.6%; it was significantly higher in girls (21.0%) than in boys (13.8%). The prevalence of iron depletion by geographical location was: 23% in Eastern Europe (Pecs), 19% in Northern Europe (Stockholm), 18% in Western Europe (17% in Ghent and 19% in Lille), 17% in Central Europe (16% in Dortmund and 19% in Vienna) and 15% in Southern Europe (14% in Athens, 17% in Heraklion, 19% in Rome and 10% in Zaragoza). These data are in the range of findings from a recent review on iron status in European adolescents (Hercberg et al., 2001) where the highest prevalence of iron depletion was observed in girls in Ireland (43%) and in boys in Denmark (16%), whereas the lowest prevalence was observed in girls in Finland (4.7%) and in boys in France where no cases were detected.

For ID, in the present study, there was a tendency for a higher percentage in girls (5.4%) with respect to boys (3.9%) but the difference was not significant. The percentage of ID in girls in our study was slightly lower than the 8.7% prevalence observed in girls aged 12–16 years in the US (Halterman et al., 2001) but in line with the 6% prevalence observed in girls aged 13–19 years in Canada (Deegan et al., 2005). Both the prevalences observed in our study and in the US are below the cutoff established by WHO to determine ‘no prevalence’ of ID, that is, 10% (WHO, 2004). Both are

### Discussion

The present study is the first report evaluating iron status in adolescents across multiple European countries. As discussed by Moreno et al. (2008), the selected cities were equivalent and comparable between countries but the samples were representative for the cities and not for the countries. Therefore, the HELENA Study does not allow for statistical comparison of differences in iron status between regions. However, the prevalence of iron status stages according to geographical location of the cities adopted for this study are provided underneath for descriptive purpose.

In the present study, the overall proportion of iron depletion was 17.6%; it was significantly higher in girls (21.0%) than in boys (13.8%). The prevalence of iron depletion by geographical location was: 23% in Eastern Europe (Pecs), 19% in Northern Europe (Stockholm), 18% in Western Europe (17% in Ghent and 19% in Lille), 17% in Central Europe (16% in Dortmund and 19% in Vienna) and 15% in Southern Europe (14% in Athens, 17% in Heraklion, 19% in Rome and 10% in Zaragoza). These data are in the range of findings from a recent review on iron status in European adolescents (Hercberg et al., 2001) where the highest prevalence of iron depletion was observed in girls in Ireland (43%) and in boys in Denmark (16%), whereas the lowest prevalence was observed in girls in Finland (4.7%) and in boys in France where no cases were detected.

For ID, in the present study, there was a tendency for a higher percentage in girls (5.4%) with respect to boys (3.9%) but the difference was not significant. The percentage of ID in girls in our study was slightly lower than the 8.7% prevalence observed in girls aged 12–16 years in the US (Halterman et al., 2001) but in line with the 6% prevalence observed in girls aged 13–19 years in Canada (Deegan et al., 2005). Both the prevalences observed in our study and in the US are below the cutoff established by WHO to determine ‘no prevalence’ of ID, that is, 10% (WHO, 2004). Both are
also far lower than that observed in other parts of the world as in Nepal, with an ID prevalence of 56% observed in adolescents girls (Woodruff et al., 2006). The slightly lower prevalence in our study may, in part, result from a different approach to define ID. The impact of ID on the cognitive functioning of adolescent girls who are at the highest risk of ID requires major clarification. In fact, there is only limited evidence that iron supplementation may improve attention, concentration and intelligent quotient (Falkingham et al., 2010). The prevalence of ID by geographical location was: 6% in both Northern Europe (Stockholm) and Southern Europe (11% in Athens, 6% in Heraklion, 3% in Rome and Zaragoza) plus Eastern Europe (Pecs), 5% in Central Europe (3% in Dortmund and 7% in Vienna) and 2% in Western Europe (no cases in Ghent and 4% in Lille). No comparable data are available in the literature in relation to ID in adolescents in the European context for comparison with the results of the present study.

The percentage of IDA was 1.3% (0.5% in boys and 2.0% in girls), with a (non-significantly) higher value in girls. The prevalence of IDA by geographical location was: 2% in Central Europe (2% in Dortmund and 3% in Vienna), 1% in both Northern Europe (Stockholm) and Southern Europe (no cases in Athens, 2% in Heraklion, 1% in both Rome and Zaragoza), 0.6% in Western Europe (no cases in Ghent and 1% in Lille) and no cases in Eastern Europe (Pecs). These data are similar to the estimates of prevalence in adolescents in Canada (Deegan et al., 2005), the United States (Looker et al., 2002) and Sweden (Samulson et al., 1996), ranging from 0 to 3%. In the present study, the proportion of anaemia was 4.4%, consistent with the estimates of the worldwide prevalence of anaemia during adolescence: 27% in developing countries and 6% in developed countries (Kara et al., 2006). The observed prevalence of anaemia not due to ID (3.1%) is probably overestimated because of unavoidable misclassifications that are inherent to the definition of limits. In fact, the limits set to define anaemia are based on 95% reference ranges, and therefore it is assumed that 2.5% of healthy individuals would be classified as anaemic. On the other hand, a proportion of anaemia without ID may be originated from genetic Hb disorders, as subjects with such disorders were not excluded. In our study, the highest prevalence of anaemia without ID among cities was observed in two cities of Greece (7.6% in Heraklion and 6.3% in Athens). Greece is known to be heavily affected by haemoglobinopathies (thalassaemia and sickle-cell disease). In fact, according to Angastiniotis et al. (1995), the highest percentage of carriers of thalassaemia and sickle-cell disorders is observed in Greece (9.0%).

In our study, no single case of iron overload was identified through SF, a stored body iron marker.

Results of the present study on correlations between iron status parameters are all plausible. SF and sTfR that were chosen in this study as iron indicators showed a correlation with red cell parameters. The observed correlation coefficients between log(sTfR) and red cell parameters (MCV and MCH) are not surprising, as sTfR is a measure of ID erythropoiesis (Metzgeroth et al., 2005) as MCV and MCH. The present data are in general agreement with the result of a previous study showing a correlation between sTfR and red cell parameters in iron-deficient adolescents (Choi, 2003). These findings are confirmed, as the concentrations of the mean of red cell parameters decreased gradually (except for red cell distribution width that increased) with increasingly negative iron balance, as ID appeared (sTfR > 8.5 mg/l) (Table 5). The results of the present study document a positive correlation between log(SF) level and Hb concentration ($r = 0.39$, $P < 0.01$; Table 3) comparable to those observed in other studies among children: $r = 0.24$ (Jeremiah et al., 2007) and $r = 0.26$ (Ayoya et al., 2010). The low correlation between these two indicators is because of the fact that they are related to differing stages of ID. The correlation between the indicators of iron status and red cell parameters may be considered as a confirmation of the reliability of these indicators (WHO, 2004; Zimmermann, 2008) in the assessment of iron status in adolescents.

The correlation of SF with Hb and the higher percentage of iron depletion in girls with respect to boys suggest that the risk of developing ID anaemia is higher in girls. If iron depletion is not corrected, it may indeed progress to ID. In addition, the calculated measure of body iron was lower in girls than in boys. The distribution of body iron stores by age (Figure 2) suggests that in boys, body iron stores may have slightly increased during and after their rapid growth period, whereas in girls, iron stores remain low during the whole pubertal period. One possible explanation for the lower iron status in girls, besides growth, is the loss of iron with menstruation. In previous studies, it was observed that the growth spurt associated with menstrual status, blood loss and a low iron intake may have adverse effects on iron stores in adolescents girls (Ilich-Ernst et al., 1998).

Conclusions

Our study confirms SF and sTfR as biochemical indicators that could be used in the assessment of iron status in adolescents, a problematic group of population, for the period of rapid growth. On the basis of WHO guidelines (2004) for the interpretation of the iron status data at population level, the results indicate that, in the European context, ID is not prevalent neither in boys nor in girls, and that iron depletion is prevalent in girls but not in boys. Adolescent girls therefore constitute a group at risk of ID, and specific attention should be given to them during adolescence to ensure that their dietary intake of iron is adequate to their requirements.

Conflict of interest

The authors declare no conflict of interests.
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Appendix

Helena Study group

Co-ordinator: Luis A Moreno

Core group members: Luis A Moreno, Frédéric Gottrand, Stefaan De Henauw, Marcela González-Gross, Chantal Gilbert.

Steering committee: Anthony Kafatos (President), Luis A Moreno, Christian Libersa, Stefaan De Henauw, Jackie Sáchez, Frédéric Gottrand, Mathilde Kesting, Michael Sjöström, Dènes Molnár, Marcela González-Gross, Jean Dollongeville, Chantal Gilbert, Gunnar Hall, Lea Maes, Luca Scalfi.

Project manager: Pilar MeleÁndez

1. Universidad de Zaragoza (Spain): Luis A Moreno, Jesús Fleta, José A Casajús, Gerardo Rodríguez, Concepción Tomás, María I Mesana, Germán Vicente-Rodríguez, Adoración Villarroya, Carlos M Gil, Ignacio Ara, Juan Revenga, Carmen Lachen, Juan Fernández Alvira, Gloria Bueno, Aurora Lázaro, Olga Bueno, Juan F León, Jesús Mª Garagorri, Manuel Bueno, Juan Pablo Rey López, Iris Iglesia, Paula Velasco, Silvia Bel.
2. Consejo Superior de Investigaciones Científicas (Spain): Ascensión Marcos, Julia Warna¨berg, Esther Nova, Sonia Gómez, Esperanza Ligia Díaz, Javier Romeo, Ana Veses, Mari Ángeles Puertollano, Belén Zapatera, Tamara Pozo.
3. Université de Lille 2 (France): Laurent Béghin, Christian Libersa, Frédéric Gottrand, Catalina Iliescu, Juliana Von Berlepsch.
4. Research Institute of Child Nutrition Dortmund, Rheinische Friedrich-Wilhelms-Universität Bonn (Germany): Mathilde Kersting, Wolfgang Sichert-Hellert, Ellen Koeppen.
5. Pécsi Tudományegyetem (University of Pécs) (Hungary): Dénès Molnár, Eva Erhardt, Katalin Csernus, Katalin Török, Szilvia Bokor, Mrs Angeliki Papadaki, Katerina Sarri, Anna Viskadourou, Christos Hatzis, Michael Kiriakakis, George Tsibinos, Constantine Vardavas Manolis Sbokos, Eva Protoyeraki, Maria Fasoulaki.
6. Instituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (Italy): Davide Arcella, Elena Azzini, Emma Barrison, Noemi Bevilacqua, Pasquale Buonocore, Giovanna Catasta, Laura Censi, Donatella Ciarpica, Paola D’Acapito, Marika Ferrari, Myriam Gallo, Cinzia Le Donne, Catherine Leclercq, Giuseppe Malani, Beatrice Motrenzo, Lorenza Mistura, Antoinette Pasquali, Rafaele Piccinelli, Angela Polito, Raffaella Spada, Stefania Sette, Maria Zaccaria.
7. University of Napoli ‘Federico II’ Dept of Food Science (Italy): Luca Scalfi, Paola Vitaglione, Concetta Montagnese.
8. Ghent University (Belgium): Ilse De Bourdeaudhuij, Stefaan De Henauw, Tineke De Vriendt, Lea Maes, Christophe Matthys, Carine Vereecken, Mieke de Maeyer, Charlene Ottevaere.
9. Medical University of Vienna (Austria): Kurt Widhalm, Katharina Schiffl, Sabine Dietrich.
10. Harokopio University (Greece): Yannis Manios, Eva Grammatikaki, Zoi Bouloubasi, Tina Louisa Cook, Sofia Eleutheriou, Orsalia Consta, George Moschos, Ioanna Katsaroli, George Kranou, Stalo Papoutsou, Despoina Keke, Ioanna Petraki, Elena Bellou, Sofia Tanagra, Kostadina Kallianoti, Dionysia Argyropoulou, Katerina Kondaki, Stamatoula Tsikrika, Christos Kairisogos.
11. Institut Pasteur de Lille (France): Jean Dollongeville, Aline Meirhaeghe.
12. Karolinska Institutet (Sweden): Michael Sjöström, Patrick Bergman, Maria Hagström, Lena Hallström, Mårten Hallberg, Eric Poortvliet, Julia Wärnberg, Nico Zimmermann AM (2008). Methods to assess iron and iodine status. Br J Nutr 99, S2-S9.
Rizzo, Linda Beckman, Anita Hurtig Wennlöf, Emma Patterson, Lydia Kwak, Lars Cernerud, Per Tillgren, Stefaan Sörensen.

16. Asociación de Investigación de la Industria Agroalimentaria (Spain): Jackie Sánchez-Molero, Elena Picó, Maite Navarro, Blanca Vladel, José Enrique Carreres, Gema Merino, Rosa Sanjuán, María Lorente, María José Sánchez, Sara Castelló.

17. Campden and Chorleywood Food Research Association (United Kingdom): Chantal Gilbert, Sarah Thomas, Elaine Allchurch, Peter Burguess.

18. SIK—Institutet för Livsmedel och Bioteknik (Sweden): Gunnar Hall, Annika Astrom, Anna Sverkén, Agneta Broberg.

19. Meurice Recherche and Development asbl (Belgium): Annick Masson, Claire Lehoux, Pascal Brabant, Philippe Pate, Laurence Fontaine.

20. Campden and Chorleywood Food Development Institute (Hungary): Andras Sebok, Tunde Kuti, Adrienn Hegyi.

21. Productos Aditivos SA (Spain): Cristina Maldonado, Ana Llorente.

22. Cárnicas Serrano SL (Spain): Emilio García.

23. Cederroth International AB (Sweden): Holger von Fircks, Marianne Lilja Hallberg, Maria Messerer.

24. Lantmännen Food R and D (Sweden): Mats Larsson, Helen Fredriksson, Viola Adamsson, Ingmar Börjesson.

25. European Food Information Council (Belgium): Laura Fernández, Laura Smillie, Josephine Wills.

26. Universidad Politécnica de Madrid (Spain): Marcela González-Gross, Agustín Meléndez, Pedro J. Benito, Javier Calderón, David Jiménez-Pavón, Jara Valtueña, Paloma Navarro, Alejandro Urzaqui, Ulrike Albers, Raquel Pedrero, Juan José Gómez Lorente.