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1 **Original article**

2

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

5

6 Authors List: Marika Ferrari, PhD¹, Lorenza Mistura, Msc¹, Emma Patterson, PhD², Michael
7 Sjöström, PhD², Ligia Esperanza Díaz, PhD³, Peter Stehle, PhD⁴, Marcela Gonzalez-
8 Gross, PhD^{4,5}, Mathilde Kersting, PhD⁶, Kurt Widhalm, MD⁷, Dénes Molnár, PhD⁸, Frederic
9 Gottrand, PhD⁹, Stefaan De Henauw, PhD¹⁰, Yannis Manios, PhD¹¹, Anthony Kafatos, PhD¹²,
10 Luis A. Moreno, PhD¹³, Catherine Leclercq, PhD¹, on behalf of the HELENA Study Group*

11 ¹National Research Institute on Food and Nutrition, Rome, Italy; ²Department of Biosciences and
12 Nutrition, Karolinska Institutet, Huddinge, Sweden; ³Immunonutrition Research Group, Department of
13 Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN), Instituto del
14 Frío, Spanish National Research Council (CSIC), Madrid, Spain; ⁴Department of Nutrition and Food
15 Sciences-Nutritional Physiology, University of Bonn, Germany; ⁵Departement of Health and Human
16 Performance. Facultad de Ciencias de la Actividad Fisica y del Deporte-INEF, Universidad
17 Politécnica de Madrid Spain; ⁶Research Institute of Child Nutrition Dortmund, Germany;
18 ⁷Department of Paediatrics and Adolescents Medicine, Div. Clinical Nutrition, Medical University of
19 Vienna, Vienna, Austria; ⁸Department of Paediatrics, University of Pécs, H-7623, Pécs-József A.7,
20 Hungary; ⁹U995, IFR114, University Lille2. Lille France; ¹⁰Department of Public Health, Ghent
21 University, Ghent, Belgium; ¹¹Department of Nutrition and Dietetics, Harakopio University, Athens,
22 Greece; ¹²Preventive medicine and Nutrition Unit, University of Crete, School of Medicine,
23 Heraklion, Crete, Greece; ¹³Growth, Exercise, Nutrition and Development (GENUD) Research Group,
24 Escuela Universitaria de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain

25

26

27

1 Corresponding author:

2 Marika Ferrari, National Research Institute on Food and Nutrition, Via Ardeatina, 546, 00178

3 Rome, Italy. Tel: +39 06 51494571, Fax: +0039 06 51494550, E-mail: ferrari@inran.it

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6

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4

5 ***Helena Study Group**

6 **Co-ordinator:** Luis A. Moreno

7 **Core Group members:** Luis A. Moreno, Frédéric Gottrand, Stefaan De Henauw, Marcela
8 González-Gross, Chantal Gilbert.

9 **Steering Committee:** Anthony Kafatos (President), Luis A. Moreno, Christian Libersa,
10 Stefaan De Henauw, Jackie Sánchez, Frédéric Gottrand, Mathilde Kersting, Michael Sjostrom,
11 Dénes Molnár, Marcela González-Gross, Jean Dallongeville, Chantal Gilbert, Gunnar Hall,
12 Lea Maes, Luca Scalfi.

13 **Project Manager:** Pilar Meléndez

14 **1. Universidad de Zaragoza (Spain):** Luis A. Moreno, Jesús Fleeta, José A. Casajús, Gerardo
15 Rodríguez, Concepción Tomás, María I. Mesana, Germán Vicente-Rodríguez, Adoración
16 Villarroya, Carlos M. Gil, Ignacio Ara, Juan Revenga, Carmen Lachen, Juan Fernández
17 Alvira, Gloria Bueno, Aurora Lázaro, Olga Bueno, Juan F. León, Jesús M^a Garagorri, Manuel
18 Bueno, Juan Pablo Rey López, Iris Iglesia, Paula Velasco, Silvia Bel.

19 **2. Consejo Superior de Investigaciones Científicas (Spain):** Ascensión Marcos, Julia
20 Wörnberg, Esther Nova, Sonia Gómez, Esperanza Ligia Díaz, Javier Romeo, Ana Veses, Mari
21 Angeles Puertollano, Belén Zapatera, Tamara Pozo.

22 **3. Université de Lille 2 (France) :** Laurent Béghin, Christian Libersa, Frédéric Gottrand,
23 Catalina Iliescu, Juliana Von Berlepsch.

24 **4. Research Institute of Child Nutrition Dortmund, Rheinische Friedrich-Wilhelms-**
25 **Universität Bonn (Germany):** Mathilde Kersting, Wolfgang Sichert-Hellert, Ellen Koeppen.

- 1 **5. Pécsi Tudományegyetem (University of Pécs) (Hungary):** Dénes Molnar, Eva Erhardt,
2 Katalin Csernus, Katalin Török, Szilvia Bokor, Mrs. Angster, Enikő Nagy, Orsolya Kovács,
3 Judit Repásy.
- 4 **6. University of Crete School of Medicine (Greece):** Anthony Kafatos, Caroline
5 Codrington, María Plada, Angeliki Papadaki, Katerina Sarri, Anna Viskadourou, Christos
6 Hatzis, Michael Kiriakakis, George Tsibinos, Constantine Vardavas Manolis Sbokos, Eva
7 Protoyeraki, Maria Fasoulaki.
- 8 **7. Institut für Ernährungs- und Lebensmittelwissenschaften –Ernährungsphysiologie.**
9 **Rheinische Friedrich Wilhelms Universität (Germany):** Peter Stehle, Klaus Pietrzik,
10 Marcela González-Gross, Christina Breidenassel, Andre Spinneker, Jasmin Al-Tahan, Miriam
11 Segoviano, Anke Berchtold, Christine Bierschbach, Erika Blatzheim, Adelheid Schuch, Petra
12 Pickert.
- 13 **8. University of Granada (Spain):** Manuel J. Castillo, Ángel Gutiérrez, Francisco B. Ortega,
14 Jonatan R Ruiz, Enrique G. Artero, Vanesa España-Romero, David Jiménez-Pavón, Palma
15 Chillón.
- 16 **9. Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (Italy):** Davide Arcella,
17 Elena Azzini, Emma Barrison, Noemi Bevilacqua, Pasquale Buonocore, Giovina Catasta,
18 Laura Censi, Donatella Ciarapica, Paola D’Acapito, Marika Ferrari, Myriam Galfo, Cinzia Le
19 Donne, Catherine Leclercq, Giuseppe Maiani, Beatrice Mauro, Lorenza Mistura, Antonella
20 Pasquali, Raffaella Piccinelli, Angela Polito, Raffaella Spada, Stefania Sette, Maria Zaccaria.
- 21 **10. University of Napoli "Federico II" Dept of Food Science (Italy):** Luca Scalfi, Paola
22 Vitaglione, Concetta Montagnese.
- 23 **11. Ghent University (Belgium):** Ilse De Bourdeaudhuij, Stefaan De Henauw, Tineke De
24 Vriendt, Lea Maes, Christophe Matthys, Carine Vereecken, Mieke de Maeyer, Charlene
25 Ottevaere.

- 1 **12. Medical University of Vienna (Austria):** Kurt Widhalm, Katharina Phillipp, Sabine
2 Dietrich..
- 3 **13. Harokopio University (Greece):** Yannis Manios, Eva Grammatikaki, Zoi Bouloubasi,
4 Tina Louisa Cook, Sofia Eleutheriou, Orsalia Consta, George Moschonis, Ioanna Katsaroli,
5 George Kraniou, Stalo Papoutsou, Despoina Keke, Ioanna Petraki, Elena Bellou, Sofia
6 Tanagra, Kostalena Kallianoti, Dionysia Argyropoulou, Katerina Kondaki, Stamatoula
7 Tsikrika, Christos Karaiskos.
- 8 **14. Institut Pasteur de Lille (France) :** Jean Dallongeville, Aline Meirhaeghe.
- 9 **15. Karolinska Institutet (Sweden) :** Michael Sjöstrom, Patrick Bergman, María
10 Hagströmer, Lena Hallström, Mårten Hallberg, Eric Poortvliet, Julia Wärnberg, Nico Rizzo,
11 Linda Beckman, Anita Hurtig Wennlöf, Emma Patterson, Lydia Kwak, Lars Cernerud, Per
12 Tillgren, Stefaan Sörensen.
- 13 **16. Asociación de Investigación de la Industria Agroalimentaria (Spain):** Jackie Sánchez-
14 Molero, Elena Picó, Maite Navarro, Blanca Viadel, José Enrique Carreres, Gema Merino,
15 Rosa Sanjuán, María Lorente, María José Sánchez, Sara Castelló.
- 16 **17. Campden & Chorleywood Food Research Association (United Kingdom):** Chantal
17 Gilbert, Sarah Thomas, Elaine Allchurch, Peter Burgess.
- 18 **18. SIK - Institutet foer Livsmedel och Bioteknik (Sweden):** Gunnar Hall, Annika Astrom,
19 Anna Sverkén, Agneta Broberg.
- 20 **19. Meurice Recherche & Development asbl (Belgium) :** Annick Masson, Claire Lehoux,
21 Pascal Brabant, Philippe Pate, Laurence Fontaine.
- 22 **20. Campden & Chorleywood Food Development Institute (Hungary):** Andras Sebok,
23 Tunde Kuti, Adrienn Hegyi.
- 24 **21. Productos Aditivos SA (Spain):** Cristina Maldonado, Ana Llorente.

- 1 **22. Cárnicas Serrano SL (Spain):** Emilio García.
- 2 **23. Cederroth International AB (Sweden):** Holger von Fircks, Marianne Lilja Hallberg,
3 Maria Messerer.
- 4 **24. Lantmännen Food R&D (Sweden):** Mats Larsson, Helena Fredriksson, Viola
5 Adamsson, Ingmar Börjesson.
- 6 **25. European Food Information Council (Belgium):** Laura Fernández, Laura Smillie,
7 Josephine Wills.
- 8 **26. Universidad Politécnica de Madrid (Spain):** Marcela González-Gross, Agustín
9 Meléndez, Pedro J. Benito, Javier Calderón, David Jiménez-Pavón, Jara Valtueña, Paloma
10 Navarro, Alejandro Urzanqui, Ulrike Albers, Raquel Pedrero, Juan José Gómez Lorente.
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12

1 **Abstract**

2 **Objectives:** To assess the iron status among European adolescents through selected
3 biochemical parameters in a cross sectional study performed in 10 European cities.

4 **Methods:** Iron status was defined utilizing biochemical indicators. Iron depletion was defined
5 as low serum ferritin (SF<15µg/l). Iron Deficiency (ID) was defined as high soluble
6 Transferrin Receptor (sTfR >8.5 mg/l) plus iron depletion. Iron Deficiency Anaemia (IDA)
7 was defined as ID with haemoglobin (Hb) below the WHO cut-off for age and sex: 12.0 g/dl
8 for girls and for boys aged 12.5-14.99 years and 13.0 g/dl for boys aged ≥15 years. Enzyme
9 Linked ImmunoSorbent Assay (ELISA) was used as analytical method for SF, sTfR and CRP.
10 Subjects with indication of inflammation (C-Reactive Protein, CRP > 5 mg/l) were excluded
11 from the analyses. 940 adolescents aged 12.5 to 17.49 years (438 boys and 502 girls) were
12 involved.

13 **Results:** The percentage of iron depletion was 17.6%, significantly higher in girls (21.0%)
14 compared to boys (13.8%). The overall percentage of ID and IDA was 4.7% and 1.3%
15 respectively, with no significant differences between boys and girls. A correlation was
16 observed between Log(SF) and Hb ($r=0.36$, $p<0.01$) and between Log(sTfR) and Mean
17 Corpuscular Haemoglobin, MCH ($r=-0.30$, $P<0.01$). Iron body stores were estimated on the
18 basis of Log(sTfR/SF). A higher percentage of negative values of body iron was recorded in
19 girls (16.5%) respect to boys (8.3%) and body iron values tended to increase with age in boys
20 while the values remained stable in girls.

21 **Conclusion:** In order to ensure adequate iron stores, specific attention should be given to girls
22 at European level to ensure that their dietary intake of iron is adequate.

23

24 **Keywords:** Anaemia, iron deficiency, adolescent, Europe

25

26

1 **Introduction**

2 Iron Deficiency (ID) continues to be one of the most prevalent nutritional deficiencies in the
3 world, particularly in developing countries (World Health Organization (WHO), 2003). WHO
4 estimated that ID occurs in about 66-80% of the world's population (WHO, 2003). ID has
5 many negative effects on health, including changes in immune function, cognitive
6 development, temperature regulation, energy metabolism and work performance (Dallman,
7 1986). On the other hand, iron is a pro-oxidant and thus a possible risk factor for coronary
8 heart disease (CHD). However, a recent review suggests that there is no a strong association
9 between body iron stores and coronary heart disease in adults (Zegrean, 2009).

10 The diagnosis of ID is based primarily on laboratory measurements of biochemical iron
11 indicators. There is no full international consensus on the indicators to be used for assessing
12 iron status since each indicator has its own limitations due to either poor sensitivity or
13 specificity, or because it is modified by conditions other than ID (Dallman *et al.*, 1993). There
14 are three general approaches to assessing ID in populations. In the first one, used in studies
15 conducted by Hallberg et al (Hallberg *et al.*, 1993) and Amstrong (Amstrong, 1989) ID is
16 defined as a low level of serum ferritin (SF). In the second one, used in the US in the National
17 Health and Nutrition Examination Survey (NHANES) II and III, the multiple-criteria model is
18 used to classify ID; it consists of two or three abnormal values for transferrin saturation (TS),
19 SF and erythrocyte protoporphyrin (EP) (Cogswell *et al.*, 2009). The main limitation of these
20 two approaches is that inflammation leads to high ferritin values even though iron store are
21 low. The third approach proposed by WHO (2004) and adopted by Zimmermann (2008) is
22 based on the measurements of SF and soluble Transferrin Receptor (sTfR) in combination
23 with C-Reactive Protein (CRP), in order to eliminate false negative cases in presence of
24 inflammation. The third approach was considered more appropriate and was therefore used in
25 the present study. It is well known that SF concentrations reflect the size of the iron stores
26 (Cook, 1999) and that low SF levels identify "iron depletion" (WHO, 2001). On the other

1 hand, the measurement of sTfR has been reported to be useful in the diagnosis of “tissue ID”
2 (Skikne *et al.*, 1990) and its values are not influenced by acute or chronic inflammatory
3 conditions (Ferguson *et al.*, 1992; Olivares *et al.*, 1995). An approach to estimate body iron
4 was developed by Cook *et al.* (Cook *et al.*, 2003) using the formula: body iron (mg/kg) = -
5 $[\text{Log}(\text{sTfR}/\text{SF}) - 2.8229] / 0.1207$ to allow the full range of the iron status of a population to be
6 evaluated with negative values representing a deficit of iron respect to the quantity required to
7 maintain a normal haemoglobin concentration.

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

15 Few data are available on iron status in European adolescents and these data derive from
16 studies utilizing different methodologies, different indicators of iron status and varying
17 criteria to define ID (Hercberg *et al.*, 2001). There was, therefore, a need to investigate the
18 situation of ID among European adolescents with a harmonized methodology.

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

23

24 **Materials and methods**

25 *Study design and sample selection*

1 A detailed description of HELENA-cross sectional study (HELENA-CSS) in terms of
2 sampling and recruitment approaches, standardization and harmonization processes, data
3 collection, analysis strategies and quality control activities has been published elsewhere
4 (Moreno *et al.*,2008). The study was approved by the Research Ethics Committees of each
5 country involved according to the Declaration of Helsinki and International Conferences on
6 Harmonization for Good Clinical Practice. Written informed consent was obtained from the
7 parents of the adolescents and the adolescents themselves (Béghin *et al.*, 2008). Briefly,
8 subjects with an age range of 12.5-17.49 years were recruited in ten cities across Europe.
9 Selection of cities was based on two criteria: regional distribution and presence of an active
10 research group assuring sufficient expertise and resources to successfully perform
11 epidemiological studies. Within the study, Stockholm (Sweden) represented Northern Europe.
12 Athens, Heraklion (Greece), Rome (Italy) and Zaragoza (Spain) represented Southern Europe.
13 Pecs (Hungary) represented Eastern Europe. Ghent (Belgium) and Lille (France) represented
14 Western Europe whereas Dortmund (Germany) and Vienna (Austria) represented Central
15 Europe. As the overall aim of the HELENA study was to assess the nutritional status of the
16 adolescent population in Europe, during the design phase the variability of a large set of
17 nutritional status indices obtained in previous studies was checked. Body Mass Index (BMI)
18 was the indicator that showed the largest variability. Therefore, the sample size was estimated
19 according to the mean BMI and variance values for each sex and age strata. This procedure
20 ensured to adequately describe all the variables included in the study. For the sample size
21 calculation, a confidence level of 95% and an error of $\pm 0.3 \text{ kg/m}^2$ were assumed leading to a
22 sample size of 3000 adolescents. More details on the procedure are provided by Moreno et al.
23 (2008).

24 Blood sampling was performed in one third of the adolescents recruited. Therefore, the total
25 expected sub-sample was 1,000 adolescents (100 subjects for each city). The size of sub-
26 sample was chosen so as to assess the immunological parameters with sufficient precision.

1 Such parameters were those with the highest variability within the blood measurements that
2 were included in the study as discussed by Moreno et al (2008). The selection procedure for
3 schools and classes, including the selection of a subset of classes for blood sampling has been
4 applied centrally by one partner (Ghent) for all study centers.

5

6 *Blood sampling and laboratory measures*

7 The blood sampling procedure and sample logistics have been described in detail elsewhere
8 (González *et al.*, 2008). Briefly, fasting blood samples were collected by venipuncture at
9 school between eight and ten o' clock in the morning. Whole blood samples for the red blood
10 parameters (Haemoglobin, Hb, Red Blood Cell, RBC, Mean Corpuscular Volume, MCV,
11 Mean Corpuscular Haemoglobin, MCH, Mean Corpuscular Haemoglobin Concentration,
12 MCHC and Red Cell Distribution Width, RDW) were sent directly to the local laboratory of
13 each country to be analysed. Samples for iron status analyses were clotted at room
14 temperature for at least 30 minutes and then centrifuged at 3,500 rpm for 10 minutes. Serum
15 samples were shipped to the central laboratory in Bonn University (IEL, Germany)
16 Department of Nutrition and Food Science, to be stored at -80°C before being shipped to Italy
17 in May 2007, within six months to the collection. The aliquots of serum samples were
18 analysed in the Human Nutrition Laboratory of the National Research Institute on Food and
19 Nutrition (INRAN). SF, sTfR and CRP were measured by using an Enzyme Linked
20 ImmunoSorbent assay (ELISA) (Erhardt *et al.*, 2004). A commercially available control
21 sample from Bio-Rad (Liquichek Immunology Control, Level 3) was used to obtain a
22 calibration curve on each plate.

23 A quality-control serum was produced in-house by pooling human serum from four healthy
24 volunteers in order to monitor accuracy and precision of the determinations. The coefficient
25 of variation (CV) from 10 replicates of the quality control serum pool was 5.3%, 6.4% and
26 12.4% for SF, sTfR and CRP respectively. Additionally, the inter-assay CV of the pool

1 samples analysed in a total of 32 different analytical runs (4 replicates) was 10.8%, 14.8% and
2 23.4% for SF, sTfR and CRP respectively.

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

8

9 *Anaemia and iron classification*

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

19 Total body iron was estimated with the use of the formula: body iron (mg/kg) = -
20 [Log(sTfR/SF)-2.8229]/0.1207 (Cook *et al.*, 2003).

21

22 *Statistical analyses*

23 The Kruskal Wallis test was used for comparing haematological parameters among iron
24 sufficiency, iron depletion and ID. The student t test was used to evaluate the significance of
25 differences between mean values of iron indicators between gender. Pearson correlation was
26 used to test the linear relation between haematological variables. The χ^2 test was used for

1 comparing proportions. Iron status indicators such as sTfR and SF were transformed to
2 logarithms before performing statistical analyses. The results in the tables are shown using the
3 original units without any log-transformation.

4 As already performed by Ortega et al (Ortega et al., 2010) all the analyses conducted on the
5 HELENA data were adjusted by a weighing factor to balance the sample according to the age
6 and sex distribution of the theoretical sample, in order to guarantee representation of each of
7 the stratified groups. The counts of subjects (n) are reported in the tables as unweighted
8 values. All the analyses were performed using SPSS 12.00 for Windows.

9

10 **Results**

11 *Iron indicators and iron status*

12 The HELENA-CSS consists of 1,089 adolescent subjects with blood measurements. A total of
13 102 subjects were excluded because at least one of the iron indicators (SF n= 80; sTfR n= 84)
14 or haemoglobin (Hb n =16) was missing. The final sample of 987 adolescents included 465
15 boys and 522 girls i.e. 90.7% of the adolescents who participated in the blood sampling.

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

19 The mean age (standard deviation, SD) of adolescents was 14.7 (1.2) years both in boys and
20 in girls and the mean BMI was 21.2 (3.8) and 21.1 (3.3) kg/m² respectively for boys and girls.

21 The reported age of menarche in the present study ranged from 9 to 16 years of age. Fifty-
22 eight (12%) girls reported not to have experienced menarche at the time of the study. 39% and
23 43% of the boys and girls respectively declared to have smoked cigarettes at least once.

24 Mean, median (Me) and percentiles (25th-75th) values of red blood parameters, SF and sTfR
25 are summarized in Table 2. Percentage of iron sufficiency, abnormal values of iron indicators,
26 anaemia, iron depletion, ID and IDA are presented in Table 3.

1 In boys, the mean (SD) of Hb was 14.7 (1.2) g/dl (14.3 (1.0) g/dl aged 12.5-14.99 years and
2 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
3 suggest a gender-related significant difference ($p<0.05$) with a lower mean SF value in girls
4 (27.9 $\mu\text{g/l}$) than in boys (36.6 $\mu\text{g/l}$). The percentage of low SF values was also higher in girls
5 (26.3%) compared to boys (17.9%) ($p<0.01$) (Table 3). No single case of high level of SF was
6 identified (SF>200 $\mu\text{g/l}$ for boys and >150 $\mu\text{g/l}$ for girls) indicating no risk of iron overload
7 (WHO, 2004) in the study sample.

8 Twenty three subjects had high values (> 8.5 mg/l) of sTfR (that could have indicated iron
9 deficiency) but with normal values of SF (indicating no iron depletion) and could therefore
10 not be categorized in one of the iron status classification (Table 1). They were excluded from
11 the analyses in terms of the classification of ID.

12 The percentage of iron depletion was 17.6%, significantly higher in girls (21.0%) than in boys
13 (13.8%) ($p<0.05$) (Table 3).

14 The overall prevalence of anaemia was 4.4% and it ranged from 1.1% (Stockholm) to 10.1%
15 (Heraklion) among cities. Intermediate prevalences were 1.4% for Zaragoza, 3.2% for Pecs,
16 3.6% for Dortmund, 4.0% for Rome, 4.6% for Ghent, 5.5% for Lille, 5.6% for Vienna and
17 5.7% for Athens. The overall prevalence of anaemia without ID was 3.1% (4.6% in girls and
18 1.4% in boys) and ranged from 0% (Stockholm and Zaragoza) to 7.6% (Heraklion). The
19 second highest prevalence was observed in Athens (5.7%). Intermediate prevalences were
20 1.8% for Dortmund, 1.9% for Vienna, 3.0% for Rome, 3.2% for Pecs, 4.1% for Lille and
21 4.6% for Ghent. Percentage of iron depletion ranged from 9.6% (Zaragoza) to 23.0% (Pecs).
22 Percentage of ID ranged from 0% (Lille) to 11.3% (Athens). Percentage of IDA ranged from
23 0% (Athens, Gent, and Pecs) to 2.8 % (Vienna).

24 Among adolescents with anaemia (n=40), 27.5% (n=11) were classified as IDA, with
25 abnormal SF and sTfR values whereas 24.3% (n=10) had abnormal SF values but with normal

1 sTfR values (iron depletion without ID) and 47.5% (n=19) had anaemia without ID, with
2 normal SF and sTfR values.

3 Log(SF) and Log(sTfR) were significant correlated with the red cell parameters (MCV, MCH,
4 MCHC and RDW) (Table 4). In the total sample, a significant correlation was observed
5 between Log(SF) and haemoglobin concentration ($r=0.36$, $p<0.01$) and between Log (SF) and
6 Log(sTfR) ($r=0.33$, $p<0.01$). For Log(sTfR) a significant correlation was observed with Log
7 (SF), MCH ($r=-0.30$, $p<0.01$) and MCV ($r=-0.26$, $p<0.01$) No significant correlation was
8 observed between RBC and iron indicators [Log(SF) and Log (sTfR; $r =0.13$ and $r=0.07$
9 respectively].

10 The mean of red cell parameters in the different classification of iron status is reported in
11 Table 5. Statistically significant differences between iron sufficiency, iron depletion and ID
12 were observed for red cell parameters for boys and girls. There were significant differences
13 ($p<0.001$) for Hb, MCV, MCH, MCHC and RDW between iron sufficiency group and the
14 iron depletion and ID groups. No significant difference was observed between iron
15 sufficiency group and ID group for any of the red cell parameters considered.

16

17 *Estimation of body iron*

18 Body iron was calculated according to the formula of Cook et al (Cook *et al.*, 2003) and the
19 cumulative percentage distributions by gender is shown in Figure 1. Mean (SD) body iron
20 was significantly higher ($p<0.001$; Student t test) in boys [4.4 (3.2) mg/kg] than in girls [3.3
21 (4.1) mg/kg]. Girls showed a light shift of the body iron distribution curve to the left in
22 comparison to boys in which higher positive values were observed. The reference line
23 indicates the cut-off point for tissue iron deficiency (body iron < 0 mg/kg). The percentage of
24 negative values was significant higher ($p<0.001$; χ^2) in girls (16.5%) respect to boys (8.3%).

25 The distribution of body iron stores by age was also examined. Figure 2 shows that the linear
26 relationship between body iron and the age of the adolescents is not very strong. Body iron

1 increased slightly with age ($r=0.28$) but this trend did not reach statistical significance. On the
2 other hand, it tended to be stable ($r=0.025$) in girls.

3

4 **Discussion**

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

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

22 For ID, in the present study, there was a tendency for a higher percentage in girls (5.4%)
23 respect to boys (3.9%) but the difference was not significant. The percentage of ID in girls in
24 our study was slightly lower than the 8.7% prevalence observed in girls aged 12-16 years in
25 the US (Halterman *et al.*, 2001) but in line with the 6% prevalence observed in girls aged 13-
26 19 years in Canada (Deegan *et al.*, 2005). Both the prevalence observed in our study and in

1 the US are below the cut-off established by WHO to determine “no prevalence” of ID, i.e.
2 10% (WHO, 2004). Both are also far lower than that observed in other parts of the world as in
3 Nepal with an ID prevalence of 56% observed in adolescents girls (Woodruff *et al.*, 2006).
4 The slightly lower prevalence in our study may in part result from a different approach to
5 define ID. The impact of ID on the cognitive functioning of adolescent girls who are at
6 highest risk of ID requires major clarification. In fact there is only limited evidence that iron
7 supplementation may improve attention, concentration and Intelligent Quotient (IQ)
8 (Falkingham *et al.*, 2010). The prevalence of ID by geographical location was: 6% in both
9 Northern Europe (Stockholm) and Southern Europe (11% in Athens, 6% in Heraklion, 3% in
10 Rome and Zaragoza) plus Eastern Europe (Pecs), 5% in Central Europe (3% in Dortmund and
11 7% in Vienna) and 2% in Western Europe (no cases in Ghent and 4% in Lille). No
12 comparable data are available in the literature in relation to ID in adolescents in the European
13 context for comparison with the results of the present study.

14 The percentage of IDA was 1.3% (0.5% in boys and 2.0% in girls), with a (non-significantly)
15 higher value in girls. The prevalence of IDA by geographical location was: 2% in Central
16 Europe (2% in Dortmund and 3% in Vienna), 1% in both Northern Europe (Stockholm) and
17 Southern Europe (no cases in Athens, 2% in Heraklion, 1% in both Rome and Zaragoza),
18 0,6% in Western Europe (no cases in Ghent and 1% in Lille) and no cases in Eastern Europe
19 (Pecs). These data are similar to the estimates of prevalence in adolescents in Canada (Deegan
20 *et al.*, 2005), the United States (Looker *et al.*, 2002) and Sweden (Samulson *et al.*, 1996),
21 ranging from 0% to 3%. In the present study, the proportion of anaemia was 4.4%, consistent
22 with the estimates of the worldwide prevalence of anaemia during adolescence: 27% in
23 developing countries and 6% in developed countries (Kara *et al.*, 2006). The observed
24 prevalence of anaemia not due to ID (3.1%) is probably overestimated due to unavoidable
25 misclassifications which are inherent to the definition of limits. In fact, the limits set to define
26 anaemia are based on 95% reference ranges and therefore it is assumed that 2.5% of healthy

1 individuals would be classified as anaemic. On the other hand, a proportion of anaemia
2 without ID may be originated from genetic haemoglobin disorders since subjects with such
3 disorders were not excluded. In our study the highest prevalence of anaemia without ID
4 among cities was observed in two cities of Greece (7.6% in Heraklion and 6.3% in Athens).
5 Greece is known to be heavily affected by haemoglobinopathies (thalassemia and sickle-cell
6 disease). In fact, according to Angastiniotis *et al* (1995), the highest percentage of carriers of
7 thalassemia and sickle-cell disorders is observed in Greece (9.0%).

8 In our study, no single case of iron overload was identified through serum ferritin, a stored
9 body iron marker.

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

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

11

12 **Conclusion**

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

21

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26

1 **Competing interests**

2 None of the authors had any conflict of interests.

3

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9

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- 14

1 **Table 1** Iron status^a as defined in the present paper

2

Classification	Values of iron indicators	
	SF ($\mu\text{g/l}$)	sTfR (mg/l)
Iron sufficiency ^b	≥ 15	≤ 8.5
Iron depletion ^c	< 15	≤ 8.5
Iron Deficiency (ID) ^b	< 15	> 8.5
Iron Deficiency with Anaemia (IDA)	< 15	> 8.5

3

^a Iron status is considered in presence of CRP >5 mg/l

4

^b With or without anaemia

5

^c Without ID and with or without anaemia

6

1 **Table 2** Haemoglobin, red blood and iron status parameters in European adolescents^a. The
 2 data are presented as means (M), standards deviations (SD), median (Me) and 25th and 75th
 3 centiles
 4

Parameter	Boys (n = 438) ^b					Girls (n =502) ^b					
	M	SD	Me	25 th -75 th centiles		M	SD	Me	25 th -75 th centiles		
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	pg	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		27.9	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	

5 Hb = Haemoglobin; RBC = Red blood cells; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin;
 6 MCHC = Mean Corpuscular Haemoglobin Concentration; RDW = Red Cell Distribution Width; SF = Serum Ferritin; sTfR =
 7 soluble Transferrin Receptor

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

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

11 * (p<0.05) statistically significant difference between mean values in boys and girls
 12
 13

1 **Table 3** Proportion of European adolescents having anaemia, abnormal values of iron
 2 indicators , iron sufficiency, iron depletion, ID and IDA^a
 3

Iron status ^c	Total (n =940) ^b		Boys (n = 438) ^b		Girls (n =502) ^b	
	n	%	n	%	n	%
Low SF	210	22.4	78	17.9 ^{**}	132	26.3
High sTfR	66	7.0	30	6.9	36	7.2
Anaemia	41	4.4	8	1.8 ^{**}	33	6.6
<i>Classification^d</i>						
Iron sufficiency	705	75.2	344	79.3 [*]	361	71.7
Iron depletion	167	17.6	63	13.8 [*]	104	21.0
ID	45	4.7	18	3.9	27	5.4
IDA	11	1.3	2	0.5	9	2.0

4 ^aAll data were adjusted by a weighing factor to balance the sample according to the theoretical age distribution of the
 5 European adolescent population

6 ^bThe count of subjects (n) are reported in the table as unweighted values

7 ^c Indicators and cut-off values used to identify abnormal values of iron indicators and iron status are reported in Table 1

8 ^d 23 subjects (13 boys and 10 girls) were excluded because of with high values of sTfR and normal values of SF .

9 ^{*}P<0.05; ^{**}P<0.01 statistically significant between girls and boys

10

1 **Table 4** Pearson correlation coefficients and significance of correlations (P) between iron
 2 parameters^a (n= 940)^b
 3

	Hb	RBC	MCV	MCH	MCHC	RDW	Log SF	Log sTfR
<i>Boys</i>								
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**	-0.25**	-
<i>Girls</i>								
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**	-0.39**	-
<i>Total</i>								
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**	-0.33**	-

4 RBC = Red blood cells; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin;

5 MCHC = Mean Corpuscular Haemoglobin Concentration; RDW = Red Cell Distribution Width

6 ^aAll data were adjusted by a weighing factor to balance the sample according to the theoretical age distribution of the
 7 European adolescent population

8 ^bThe count of subjects (n) are reported in the table as unweighted values

9 *P<0.05; **P<0.01 boys versus girls

Table 5 Haematological characteristics according to iron sufficiency , iron depletion and ID^a

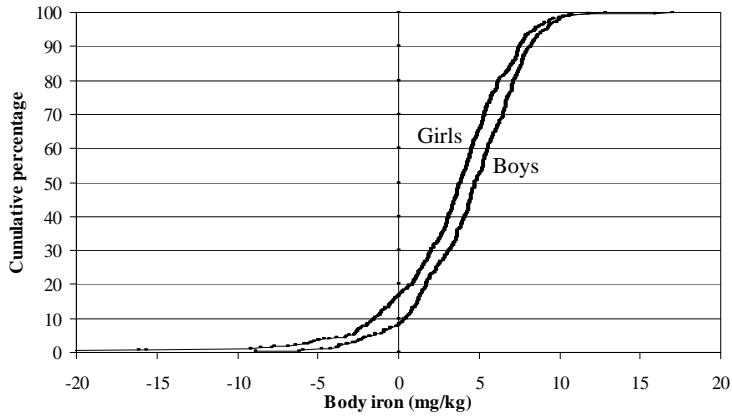
	Iron sufficiency			Iron depletion			ID		
	Boys (n=344) ^b	Girls (n=361) ^b	Total (n=705) ^b	Boys (n=63) ^b	Girls (n=104) ^b	Total (n=167) ^b	Boys (n=18) ^b	Girls (n=27) ^b	Total (n=45) ^b
Mean Hb, g/dl (s.d.)	14.9 (1.2)	13.6 (0.9)	14.2* (1.2)	14.4 (1.0)	13.3 (1.6)	13.7 (1.4)	13.7 (0.9)	12.0 (1.4)	12.7 (1.4)
Mean RBC, 10 ¹² /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)

^aAll data were adjusted by a weighing factor to balance the sample according to the theoretical age distribution of the European adolescent population

^bThe count of subjects (n) are reported in the table as unweighted values

*P<0.001 statistically different between iron sufficiency, iron depletion and ID

Figure 1. Cumulative percentage distributions of estimated body iron¹ in boys and girls from the HELENA Study



¹Total body iron was estimated using the formula: $\text{body iron (mg/kg)} = -[\log(\text{sTfR}:\text{ferritin ratio}) - 2.8229] / 0.1207$ developed by Cook¹⁶. Positive values indicate the presence of storage iron, whereas negative values indicate tissue iron deficiency.

Figure 2. Distribution of body iron stores by age in boys and girls in the HELENA Study.

