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Citation for published version:
Suárez-Ortegón, MF, Prats-Puig, A, Bassols, J, Carreras-Badosa, G, McLachlan, S, Wild, SH, López-Bermejo, A & Fernández-Real, JM 2023, 'Iron status and cardiometabolic risk in children', *Diabetes Research and Clinical Practice*, vol. 202, 110795. https://doi.org/10.1016/j.diabres.2023.110795

Digital Object Identifier (DOI):
10.1016/j.diabres.2023.110795

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Diabetes Research and Clinical Practice

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Download date: 30. Jul. 2023

IRON STATUS AND CARDIOMETABOLIC RISK IN CHILDREN

Abbreviated title: Iron status and cardiometabolic risk in children.

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Disclosure Statement: No potential conflicts of interest relevant to this article exist.

ABSTRACT

Aim: We aimed to evaluate associations between serum ferritin and transferrin and variables related to the metabolic syndrome (MetS) in children.

Methods: Cross-sectional and longitudinal study in prepubertal children(n=832) aged 3-14 years. A subset(n=203) were re-examined after a mean follow-up of 3.7±0.8 years[range 2-6]. Outcomes were MetS and MetS components scores, glycosylated haemoglobin (HbA1c), and their follow-up change.

Results: Children with low ferritin had increased HbA1c Z scores (ANCOVA,P=0.003). Ferritin was inversely associated with glycaemia [fully adjusted β (95% confidence interval): -2.35(-4.36 to -0.34)]. Transferrin was associated with diastolic blood pressure[β: 0.02(0.01-0.04)] and log-HOMA-IR [β:0.001(0.0005-0.002)]. MetS risk score worsened during follow-up in children with the lowest baseline ferritin levels. In contrast, at baseline ferritin was positively associated with all (except glycaemia) the MetS-related variables but adjustments for inflammatory, hepatic function, and body mass markers attenuated those associations(P>0.05).

Conclusions: Lower iron status was independently associated with glycaemic markers and MetS in children, whereas higher ferritin levels were related to other cardiometabolic risk markers under the influence of inflammation, hepatic injury and body mass. Research is required to study whether this mixed pattern is part of an early risk or would be explained by a normal transition during growth and development.

Keywords: transferrin, ferritin, metabolic syndrome, insulin resistance, glycosylated haemoglobin, children

INTRODUCTION

Iron is an essential micronutrient since it is a functional or structural factor for enzymes and proteins involved in large set of physiological processes such as growth, immune response, tissue oxygenation, genetic expression (1). Iron deficiency is a common disorder in children due to high iron requirements on the basis of an accelerated metabolism during growth (2). Therefore, children have lower capacity to store iron than adults which is reflected in lower ranges of serum ferritin concentration, the iron store protein (3).

Increased serum ferritin levels have been widely associated with metabolic syndrome (MetS) and risk of cardiometabolic disease (CMD) in adults populations (4,5). Alteration of insulin signalling by increased oxidative stress derived from pro-oxidant properties of iron could be a possible mechanism for relationships of iron metabolism with type 2 diabetes (T2D) and cardiovascular disease (CVD) (6,7). Although iron excess is the most common derangement of iron status reported in relation to the risk of T2D, a literature review of iron status and cardiovascular disease showed that associations exist with both iron excess and iron deficiency. Theoretical biological mechanisms underlying the relationship between iron deficiency and cardiometabolic risk are still unclear and nutritional status could confound the association (8).

Cardiometabolic risk seems to be programmed in the early stages of life as a result of interaction of genetic and environmental factors (9). In fact, children and adolescents are a current target population to detect individuals at risk in order to prevent burden of CMD in adulthood (10). Definitions of metabolic syndrome (MetS) in adolescents in terms of obesity, and abnormalities of blood pressure, lipids and glucose levels have been suggested by

different authors(11,12,13) and prevalence of this syndrome and its components have been estimated in several studies (14).

In the light of the relevance of early detection of cardiometabolic risk, it is also important to investigate the effects of potential new risk factors identified in adults, such as iron metabolism, on CMD risk in children. So far there is scarce information available in children on association patterns between iron metabolism and metabolic profile. The aim of this study was to describe the cross-sectional relationship between iron metabolism markers (ferritin and transferrin), carbohydrates metabolism, Mets and insulin resistance in prepubertal children, and also prospective relationship in a subsample after 3.7.7 ± 0.8 [2-6 years] years of follow-up.

METHODS

Subjects

The study population consisted of 832 school-aged non-consanguineous children of European ancestry (398 boys and 434 girls aged 3.4 - 14 years old, 8.2 ± 2.0 years) included in a cross-sectional study of cardiovascular risk factors in prepubertal children. The sample size was calculated to have an 80% power to detect a significant correlation with a Pearson correlation coefficient of at least 0.30. Subjects were consecutively recruited among those seen in a primary care setting in the Alt Empordà and Girona localities in north-eastern Spain between 2009 and 2014. Both localities have similar socio-cultural background since they belong to the same region in Spain, Girona province, in the autonomous community of Catalonia. The sample was obtained in a small area of 100,000 inhabitants approximately, and the inclusion was restricted to local population and immigrant population did not participate in the study because the original project was also focused on genetic polymorphisms of European-Spanish

ancestry. Thus, little population variability is expected. Participation ranged from 50 to 70% among the different centres. Of the 832 participants whose data were analysed at baseline, 207 children [94 boys and 109 girls] were longitudinally after a mean follow-up of 3.7 ± 0.8 years [range 2-6]. A flow diagram summarising the inclusion of the subjects is shown in supplementary figure S1.

Inclusion criteria at baseline were: 1) European ancestry; 2) age between 3.4 and 14 years; 3) no pubertal development, as judged by a specifically trained nurse using Tanner criteria (breast stage I; testicular volume < 4 mL) (15,16). In order to avoid findings biased by acute or chronic illness the exclusion criteria were: 1) major congenital anomalies; 2) abnormal blood count, liver or kidney or thyroid functions 3) evidence of chronic illness or prolonged use of medication; 4) acute illness or use of medications in the month preceding potential enrolment.

The study protocol was approved by the Institutional Review Board of Dr. JosepTrueta Hospital. Signed consent for participation in the study was obtained from parents.

Clinical assessments

Clinical examination and fasting venous blood sampling were performed in the morning. A local anaesthetic cream was used to minimize the discomfort of venepuncture. Weight was measured wearing light clothes with a calibrated scale and height was measured with a Harpenden stadiometer. Age- and sex-adjusted Z-score values for current weight, height and body mass index (BMI) were calculated using regional normative data (17). Waist circumference (WC) was measured in the supine position at the umbilical level.

Blood pressure (BP) was measured in the supine position on the right arm after 10 min rest; an electronic sphygmomanometer (Dinamap Pro 100, GE Healthcare, Chalfont St. Giles,

United Kingdom) with cuff size appropriate for arm circumference was used. Averages of three readings taken at 5-min intervals were recorded in each subject.

Nutritional status was by self-administered questionnaires. A 16-item Mediterranean Diet Quality Index was used to assess dietary intakes. A higher score is indicative of a better nutritional status; the questionnaire has been validated in children (18).

Laboratory variables

All serum samples were obtained between 8:00 and 9:00 AM under fasting conditions. Serum glucose was analysed by the hexokinase method. Insulin was measured by immunochemiluminiscence (IMMULITE 2000, Diagnostic Products, Los Angeles, CA). Lower detection limit was 0.4 mIU/L and intra- and inter-assay CVs were less than 10%. HDL cholesterol was quantified by homogenous method of selective detergent with accelerator. Total serum triglycerides were measured by monitoring the reaction of glycerol-phosphate-oxidase and peroxidase.

Serum alanine transaminase (ALT) and gamma glutamyltranspeptidase (GGT) were measured by colorimetry using automated tests. Intra- and inter-assay coefficients of variation were <4% for these tests. Serum levels of high-sensitivity C-reactive protein (CRP) were measured using the ultrasensitive latex immunoassay CRP Vario (Sentinel Diagnostics, Abbott Diagnostics Europe, Milan, Italy). Lower detection limit was 0.2 mg/L and intra- and inter-assay CVs were less than 3%. Children with hs-CRP values above 10.0 mg/L were excluded from the study as they indicate the presence of significant acute inflammation (19). Serum ferritin was measured by microparticle enzyme immunoassay (AxSYMTM; Abbot Laboratories, USA) with intra- and inter-assay CVs <6%. Serum transferrin, was determined by routine laboratory tests (Beckman, Fullerton, CA).Whole-blood haemoglobin concentrations (EDTA sample) were determined by routine laboratory tests (Coulter

Electronics) and glycosylated haemoglobin (HbA1C) was measured by the high-performance liquid chromatography method (Bio-Rad, Munich, Germany, and autoanalyserJokoh HS- 10). Intra-assay and inter-assay coefficients of variation were less than 4%. Serum albumin concentrations were determined using the bromcresol green procedure (Abbott Laboratories, USA) with intra- and inter-assay CVs <5%.

Insulin resistance, weight status, anaemia and iron deficiency

Fasting insulin resistance was estimated from fasting insulin and glucose levels using the homeostasis model assessment [HOMA-IR = (fasting insulin in mU/L) x (fasting glucose in mg/dL)/405] (20). Categories of weight status were defined according to BMI Z score's cut-off points as follows: underweight<-1, normal weight -1 to 1, overweight>1 and< 2, and obesity>2 (21). Anaemia was defined as haemoglobin <11g/dL for children <5 years, < 11.5 g/dL for children 5 to 11 years, and < 12 g/dL for children aged 12 years and older (22). Serum ferritin < 7 µg/L was categorised as iron deficiency (23).

Metabolic syndrome risk score

Metabolic syndrome is the cluster of abnormalities of blood pressure, lipids, glucose levels and abdominal obesity. Since there is no consensus on definition and cut-points for MetS and its components for young children (< 10 years old) we calculated a continuous risk score for MetS according to the method described previously by Brage et al. in prepubertal children (24). Z scores for each one of the five markers related to MetS (blood pressure, HDL-C, triglycerides, glucose and waist circumference) were calculated by gender and quintiles of age. For blood pressure, the average of Z scores for systolic (SBP) and diastolic blood pressures (DBP) was used, and Z scores for HDL-C were multiplied by -1 to indicate higher risk with increasing values such as the other components. Triglycerides (baseline and after

follow-up) and waist circumference (baseline) were log-transformed before calculation of Z scores. The MetS risk score was calculated from the average of the Z scores from each component.

Data analysis

Study variables were described in each gender as medians and their interquartile range. Differences between sexes were estimated by Mann–Whitney U test. Multivariate linear regression analyses were conducted by sex and in the whole sample to evaluate and adjust cross-sectional associations of ferritin and transferrin (exposure variables) with MetS-related variables (SBP, DBP, WC, glucose, triglycerides and HDL-C) and HOMA-IR at baseline (outcome variables). Subclinical inflammation and/or infection, hepatic disorders, and overweight/obesity use to increase ferritin levels and are associated with raising of cardiometabolic risk markers. Therefore the regression coefficients were adjusted for age and the above-mentioned potential confounders in terms of CRP levels, ALT and GGT levels and categories of weight according to BMIZ score (underweight/normal weight/overweight/obesity), and also sex (if whole sample). Relationships with SBP and DBP were additionally adjusted for height since this variable has influence on blood pressure during growth. Changes per year of follow up in MetS-related markers, MetS and HOMA-IR were calculated as: (measurement 2- measurement 1)/ years of follow-up. Longitudinal associations were evaluated on the basis of iron markers at baseline regarding the change per year in MetS-related markers and HOMA-IR, adjusting for covariates at baseline. The relationship of glycaemia and HbA1C Z scores, and MetS risk score with the iron markers at baseline was evaluated by estimating differences in these variables values across sex-specific tertiles of ferritin and transferrin, and adjustments were the same as mentioned above. Before conducting the analyses skewed variables were log- transformed [ferritin, CRP, ALT, GGT,

WC(baseline) and triglycerides] to approximate to normal distribution, except CRP levels which were inverse log-transformed. There were missing data for some variables and therefore the number of valid cases in the adjustment models are defined by the variable with less cases. Valid cases for each variable are described in results section. Significance level was set at $p<0.05$. Statistical analyses were performed using SPSS version 12.0 (SPSS Inc, Chicago, IL

RESULTS

In the whole group, median and its interquartile range for ferritin was 35.3 (25-51) µg/L, and for transferrin was 273(250-297) mg/dL. Characteristics of the study population are presented by sex in Table 1. At baseline boys were significantly older and had higher levels of glucose, GGT and HDL-C than girls. Girls had higher values of diastolic blood pressure, triglycerides values, C reactive protein and insulin than boys. In the subgroup who were followed up, again boys had higher levels of HDL-C, and girls had higher values of insulin, HOMA-IR and triglycerides values. There were no significant cross-sectional differences by sex in anthropometric variables, iron markers and MetS score at baseline and after follow-up.

Prevalence of underweight and overweight/obesity were 11.1% and 38%, respectively. Anaemia was found in 29 cases (3.4%) of the children. Only 4 cases had iron deficiency according to ferritin levels< 7 µg/L, and 18 had ferritin levels < 10 µg/L. In 530 children with measurement of serum albumin, none of them had low values of albumin (<3.2 g/dL), an indicator of undernutrition. The median (interquartile range) for overall nutritional score was 8.0(6.0-9.0) and no significant difference was found by gender (P=0.143). At baseline all the children were pre-pubertal.

Ferritin: cross-sectional analyses

Children in the lowest tertile (vs. higher tertiles) of serum ferritin had increased glycaemia and HbA1c Z scores (Figure 1). In the fully adjusted model, MetS risk score at baseline was not significantly different across tertiles of ferritin at baseline (Figure 1).

Serum ferritin was also linearly and inversely associated with fasting glucose (Table 2). There were initial age and sex-adjusted positive associations between ferritin and blood pressures, triglycerides, HDL-C levels and HOMA-IR that did not remain statistically significant after adjustments for inflammatory, hepatic function, and body mass markers (Table 2). Analyses by sex (Table 3) showed that in boys ferritin was associated with fasting glucose.

Ferritin: prospective analyses

Children with the lowest baseline serum ferritin levels were those in whom Mets risk score worsened during follow-up (Figure 1). The linear correlation of this relationship is shown in supplementary figure S2. There were no significant differences in changes per year for fasting glycaemia, HbA1C Z scores by tertiles of ferritin at baseline (Figure 1).

After full adjustment for covariates, ferritin at baseline was statistically significantly negatively associated with the change per year in waist circumference values (Table 2).

Transferrin: cross-sectional analyses

In age and sex adjusted models, values of HbA1C Z score and MetS risk score at baseline were increased in children within highest tertile of transferrin (the higher the transferrin, the lower the iron status), although further adjustments attenuated these associations (Figure 2). Transferrin levels were significantly and positively associated with diastolic blood pressure and HOMA-IR, and with HDL-C after full adjustment for covariates (Table 2). In boys transferrin was associated with HDL-C, whereas in girls transferrin was associated with diastolic blood pressure and HOMA-IR (Table 3).

Transferrin: longitudinal analyses

There were no differences in changes per year for fasting glycaemia, HbA1C or MetS Z scores by tertiles of transferrin at baseline in the whole population (Figure 2).

Transferrin was inversely associated with the change in HDL-C levels after full adjustment (Table 2). In the girls, transferrin was also associated with HDL-C levels in the prospective component of the study (Table 3).

Nutritional status variables and iron markers

There were no statistically significant differences in nutritional score across tertiles of ferritin or transferrin (Krusskall-Wallis U test, P>0.05). No significant difference was found for prevalence of anaemia or underweight by tertiles of ferritin (χ^2 test, P>0.05). On the other hand anaemia was significantly more prevalent in the lowest tertile of transferrin (6.1% vs 2.2% in middle and highest tertiles) (χ^2 test, P=0.012), and underweight was more prevalent in the highest tertile of transferrin (15.2 %) compared to the middle tertile (7.3%) (P=0.004). We additionally adjusted the significant cross-sectional and longitudinal associations of ferritin and transferrin for anaemia and the associations remained unaffected.

Sub-analyses by age and sex

Supplemental tables S1-S4 describe cross-sectional and prospective associations between Z scores iron and glucose metabolism markers by groups of age (< 8, ≥ 8 years) and sex. The previously described inverse cross-sectional associations between ferritin and glucose levels Z scores, and between transferrin and glucose metabolism markers was stronger in older children (≥ 8 years) (Table S1). In boys, ferritin and glucose Z scores were cross-sectionally inversely related as describedfor the whole group (Table S3). The prospective association

between ferritin and MetS Z scores was specifically observed in younger children (Table S2). In older children, transferrin and MetS, HbA1C, and HOMA-IR Z scores were significantly associated (Table S1), and in younger children an inverse association between ferritin and change in glucose Z score was observed (Table S2)."

DISCUSSION

In this study we evaluated associations between two iron status markers and cardiometabolic risk in a population of children. We found associations of MetS and MetS-related variables with low and high iron status but in a different way. At baseline lower body iron stores were independently associated with glycaemic markers, and longitudinally with increased cardiometabolic risk (Mets risk score). Second, transferrin was positively and independently associated with insulin resistance (at baseline) and inversely with the change in HDL-C levels during follow-up. These independent associations for both iron markers would suggest an association between low iron status and cardiometabolic risk. On the other hand, at baseline serum ferritin was linearly and positively associated with all (except glycaemia) the MetS-related variables and HOMA-IR but adjustments for inflammatory, hepatic function, and body mass markers completely attenuated those associations.

A limited number of studies have evaluated the associations of MetS-related variables and insulin resistance with ferritin or transferrin in children. Bougle and Brouard evaluated associations of ferritin with cardiometabolic risk markers exclusively in 502 obese children (11 ± 3.0 years old), finding significant associations with triglycerides and HDL-C (negative correlation) with adjustments for age, gender, fibrinogen levels and BMI Z score (25). On the other hand they did not find significant relationships with blood pressure, fasting glucose and HOMA-IR. Lee et al. reported significant non-adjusted correlations of ferritin with waist

circumference and HDL-C (negative) but not with blood pressure, triglycerides, fasting glucose and HOMA-IR in 1350 children (9.19 ± 1.3 years old) (26). Both studies did not conduct analyses by sex. In 1493 schoolchildren aged 9–13 years, Moschonis et al. did not find differences in ferritin levels by categories of normal/high waist circumference but found a positive association between the highest quartile of visceral fat (estimated by bioelectrical impedance analysis) and iron deficiency (defined as transferrin saturation < 16%) adjusting for nutritional intakes and Tanner stage (27). Meanwhile Zhang et al. found inconsistent cross-sectional associations (adjusted for age, sex and CRP levels) between iron status and components of metabolic syndrome in children age 6-12 years of the Chinese National Nutrition and Health Survey (22). While whole blood iron, serum ferritin and total body iron were inversely related to low HDL-C, whole blood iron was positively associated with high glucose levels, and serum ferritin lacked of inverse or positive relationship with glucose levels (28). The children from Lee et al., and Moshonis et al. studies had comparable ferritin concentrations to those from children in our study (26, 27), but those from Bougle and Brouard's and Zhang et al.`s studies had higher ferritin (45.3 ± 26.7 µg/L and 87.3±49.3 µg/L, respectively) (25,28). Our inverse association between ferritin and fasting glucose is in contrast with Bougle and Brouard and Lee et al.'s studies which found no association for these markers (25, 26). Marked differences in ferritin levels (Zhang et al.), population specifically obese (Bougle and Brouard) or lack of adjustments (Lee et al.) could explain this discrepancy (25, 26, 28). We previously described a positive prospective association between serum ferritin and MetS in a cohort of Chilean children. However, unlike this Spanish cohort, in that study the associations could not be adjusted for inflammatory and liver injury markers and the outcome, MetS, was estimated in the adolescent stage while the baseline of the exposure was at pre-pubertal stage (29).

In the cross-sectional component of the study associations between ferritin and transferrin and MetS-related markers and insulin resistance differed. The significant inverse relationship between ferritin and fasting glucose and glycosylated haemoglobin persisted across all of the adjustment models and transferrin was consistently associated with diastolic blood pressure and insulin resistance. This suggests that these markers, in addition to reflecting iron metabolism may also be involved in other pathophysiological processes related to glucose homeostasis in childhood. Our finding of positive relationships between transferrin and DBP or insulin resistance is in line with a study in adults by Vari et al. (4). Transferrin levels has also been inversely associated with arterial stiffness parameters referring to rigidity of the arterial wall (such as pulse wave velocity) in kidney-transplant patients (30).The longitudinal associations of ferritin with MetS, and ferritin and transferrin with HDL-C are in line with the cross-sectional study, in terms of the lower the iron status, the higher the cardiometabolic risk. Low iron status is characterized by lower ferritin levels regarding low iron stores, and by higher transferrin levels as signal of high demand for the metal.

Some previous epidemiological studies have found increased cardiometabolic risk in subjects with low iron status. In fact, the associations found between iron markers (iron intake, transferrin saturation and serum ferritin concentration) and risk of coronary heart disease have been described to be positive, negative or U-shaped in longitudinal studies in adults (8). Importantly, nutritional status is an important confounding factor. There is increasing evidence describing the double burden of malnutrition and cardiometabolic risk, particularly in developing countries. Coexistence of at least one nutritional deficiency (iron or vitamin A) and one cardiometabolic risk factor (among overweight/obesity, abdominal obesity, hypertension, hyperglycaemia, diabetes or dyslipidaemia) was observed in 23.5% of a West-

African population, and this phenomenon was significantly higher in the low income group (31).

The children in our study did not have evidence of malnutrition on the basis of serum albumin (measured in 530 subjects) and no differences in weight or nutritional score were identified between children with low and high ferritin levels. Additional adjustment for anaemia did not affect the associations between iron status and cardio-metabolic risk. Current findings could be related to life-course physiological transitions and/or subclinical iron deficiency. In early stages of life, metabolic processes related to glucose homeostasis may be more iron-dependent than in adulthood. Changes in cardiometabolic risk markers could be sensitive to modest reductions in iron status within the narrower normal range suggested for children than for adults. Children do not accumulate as much iron as adults since body compartments are still developing, and negative balance of iron storage is a more common finding than iron excess, and has physiological implications. Our hypothesis of subclinical iron deficiency is supported by a recent recommendation for higher cut-off points to establish iron deficiency in adults (serum ferritin <30 µg/L instead of <12µg/L) (32), and normal ranges for children may also need to be reviewed.

Interestingly, iron deficiency has been associated with elevation in fasting blood glucose despite increased insulin sensitivity in animal models (33). A dose-response relationship has been described between anaemia and hyperglycaemia in rats (34). However mechanisms underlying this association are still unknown.

HbA1c is known to be spuriously increased in iron deficiency due to an elongation of the erythrocyte lifespan. However, this spuriously increased HbA1c with iron deficiency is not

accompanied by concomitant rise in glucose indices, as recently noted in a systematic review (35). The association here found between low iron status and increased HbA1c seems not spurioussince fasting glucose had the same association pattern with iron status.

Increased or higher iron status was also related to cardiometabolic risk, in terms of positive associations between ferritin levels and all the MetS-related variables (except glycaemia), but this relationship was completely influenced by inflammation, hepatic injury and body mass. Ferritin might appear increased in serum via ferritin released by damaged hepatocytes, by synthesis stimulated during acute phase since ferritin is also an acute phase reactant, and as result of increased mass of adipocytes which contain ferritin or by increased synthesis due to low grade-inflammation effects of excess adiposity (36, 37). This non-independent relationship seems to agree with findings from a meta-analysis on increased iron and MetS in adults we previously conducted. We found that the high ferritin-MetS association, although globally significant and independent, appear to be partially influenced by hepatic injury and body mass since adjustment for markers of these variables weakened the pooled association (38). Moreover, higher cut-off points used to define high ferritin concentrations were more strongly associated with high triglycerides component, which in turn was the component with the strongest association with high serum ferritin (38). Therefore, children might tend to replicate the adult pattern on iron excess-cardiometabolic risk but would not reach the threshold effect needed for significant independent associations since their serum ferritin range is narrower and lower.

Our subgroup analyses suggested few differences by age and sex. Cross-sectional significant associations in the whole sample were replicated mainly in the older age group. This is coherent with the fact that cardiometabolic risk variables increase with age and associations

in the older group might be more evident. The prospective association between ferritin Z score and worsening of MetS Z score seen in the whole group was only observed in children younger than 8 years. This finding is likely related to lower statistical power since among the 203 children with follow-up, only 57 were 8 years old or older and 34 had overweight/obesity at baseline. Associations of ferritin with glucose, and between transferrin and HOMA-IR, appear to differ by sex but confirmation of this finding is required.

A 38% (around third) of the children from our study had overweight or obesity. This prevalence is in agreement with official data from Spanish general paediatric and adult populations in 2011 and 2012, respectively (39, 40). In Spanish children, the overweight/obesity trends have been stable during the last two decades (32). During this same period the prevalence of overweight has also been stable in Spanish adults (35.8%) but the prevalence of obesity has increased from 8.0% in 1987 to 16.5% in 2012 (39). The prevalence of underweight reported in our study (11.1%) is also in line with the official data in Spanish children (39).

Some limitations have to be mentioned. Follow-up was not achieved for the whole sample. In addition, this analysis lacked a socioeconomic status variable since the information on this background was not aggregated in the primary care data system. This information would have been useful as covariate to test the independence of the inverse associations found between iron markers and cardiometabolic risk. The findings of this analysis may not be generalised before replication to other populations, in which iron status and distribution of cardiometabolic risk factors could be different. Therefore, further studies in other countries and worldwide regions should be conducted to consolidate an association pattern. The findings should also be seen within a more systemic perspective. The microbiome is

increasingly recognized to interact with iron metabolism: Not only dietary iron influences the composition and function of the microbiome, but also the latter significantly affects iron absorption. Further studies might characterize the impact of microbiome on the iron status-cardiometabolic risk relationship in children and adolescents (41).

On the other hand, the strength of this study is the simultaneous evaluation of markers of iron storage and transport regarding insulin resistance, MetS and its markers (anthropometric and biochemical) with a broad set of covariates for multivariate cross-sectional and longitudinal analyses. To the best of our knowledge, this is the first article with these evaluations in young children.

In conclusion, lower iron status was independently associated with glycaemic markers and MetS in children, whereas higher ferritin levels were related to other cardiometabolic risk markers under the influence of inflammation, hepatic injury and body mass. Research is required to study whether this mixed pattern is part of an early risk or would be explained by a normal transition during growth and development.

Acknowledgments: G.C.-B. is an Investigator of the pFIS Fund for Scientific Research from Carlos III National Institute of Health, Madrid, Spain. J.B. is an Investigator of the Miguel Servet Fund from the National Institute of Health Carlos III, Madrid, Spain. A.L.-B. is an Investigator of the I3 Fund for Scientific Research, Ministry of Science and Innovation, Madrid, Spain. This work was supported by grant no. 10/00553 (to A.L.-B.) from the National Institute of Health Carlos III (Fund FIS for Health Research, Spain) a project funded in part by FEDER (Fondo Europeo de Desarrollo Regional).

Funding: This work was supported by grant no. 10/00553 (to A.L.-B.) from the National Institute of Health Carlos III (Fund FIS for Health Research, Spain) a project funded in part by FEDER (Fondo Europeo de Desarrollo Regional).

Author contributions: M.F.S.O conceived the study design, analysed data, and wrote the manuscript. A.P.P coordinated field work, researched and analysed data and wrote the manuscript. J.B and G.C.B coordinated field work and researched data. S.H.W, S.M and J.M.F.R supervised the analysis, reviewed/edited the manuscript and contributed to the discussion. A.L.B coordinated the project, researched data and edited the manuscript. M.F.S.O and A.P.P are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Baynes RD, Stipanuk MH (2000) Iron. In :Stipanuk M (ed) Biochemical and physiological aspects of human nutrition. Saunders, Philadelphia.

2. Stoltzfus RJ. Iron interventions for women and children in low-income countries. J Nutr. 2011;141(4):756S-762S.

3. Worwood M. "Indicators of the iron status of populations: ferritin." WHO, CDC. Assessing the iron status of populations, 2nd ed. Geneva, World Health Organization (2007): 35-74.

4.Vari IS, Balkau B, Kettaneh A, Andre P, Tichet J, Fumeron F, et al. Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population: Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes care.2007 ;30(7):1795-801.

5.Forouhi NG, Harding AH, Allison M, Sandhu MS, Welch A, Luben R, et al. Elevated serum ferritin levels predict new-onset T2D: Results from the EPIC-Norfolk prospective study. Diabetologia. 2007;50(5):949-56.

6. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes. 2002;51(8):2348-54.

7.Basuli D, Stevens RG, Torti FM, Torti SV. Epidemiological associations between iron and cardiovascular disease and diabetes.Frontiers in pharmacology. 2014;5:117.

8.Lapice E, Masulli M, Vaccaro O. Iron deficiency and cardiovascular disease: an updated review of the evidence. Current Atherosclerosis Reports. 2013;15(10):358.

9.Vaag A, Brøns C, Gillberg L, Hansen NS, Hjort L, Arora GP, Thomas N, Broholm C, Ribel-Madsen R, GrunnetLG.Genetic, nongenetic and epigenetic risk determinants in developmental programming of type 2 diabetes.ActaObstetGynecol Scand. 2014;93(11):1099-108.

10. Nguyen QM, Srinivasan SR, Xu JH, Chen W, Kieltyka L, Berenson GS.Utility of childhood glucose homeostasis variables in predicting adult diabetes and related cardiometabolic risk factors: the Bogalusa Heart Study. Diabetes Care 2010;33(3):670-5.

11. Cook S, Weitzman M, Auinger P, et al. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. Arch PediatrAdolesc Med 2003;157:821-7.

12.de Ferranti SD, Gauvreau K, Ludwig DS, et al. Prevalence of the metabolic syndrome in American adolescents: findings from the third national health and nutrition examination survey. Circulation 2004;110:2494-7.

13.Zimmet P, Alberti KG, Kaufman F, et al. IDF Consensus Group. The metabolic syndrome in children and adolescents - an IDF consensus report.Pediatr Diabetes 2007;8:299-306.

14.Poyrazoglu S, Bas F, Darendeliler F. Metabolic syndrome in young people. CurrOpinEndocrinol Diabetes Obes. 2014 Feb;21(1):56-63.

15.Marshall WA, Tanner JM.Variations in pattern of pubertal changes in girls. Arch. Dis. Child. 1969;44(235):291-303.

16.Marshall WA, Tanner JM.Variations in the pattern of pubertal changes in boys. Arch. Dis. Child. 1970;45(239):13-23.

17. Carrascosa Lezcano A, Fernández García JM, Fernández Ramos C, FerrándezLongás A, López-Siguero JP, Sánchez González E, Sobradillo Ruiz B, Yeste Fernández D; Grupo Colaborador Español. Spanish cross-sectional growth study 2008. Part II. Height, weight and body mass index values from birth to adulthood. AnPediatr (Barc) 2008; 68(6): 552-69.

18. Serra-Majem L, Ribas L, Ngo J, Ortega RM, Garcia A, Perez-Rodrigo C et al. Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public health nutrition 2004; 7(7): 931-5.

19. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003:111:1805-1812.

20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia ;28:412–419.

21. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee.World Health OrganTech Rep Ser. 1995;854:1-452

22. World Health Organization.Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. World Health Organization.

23. Siimes MA, Addiego JE Jr, DallmanPR.Blood. Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children.1974;43(4):581-90.

24.Brage S, Wedderkopp N, Ekelund U, Franks PW, Wareham NJ, Andersen LB, Froberg K; European Youth Heart Study (EYHS). Features of the metabolic syndrome are associated with objectively measured physical activity and fitness in Danish children: the European Youth Heart Study (EYHS). Diabetes Care. 2004;27(9):2141-8.

25.Bouglé D, Brouard J. Iron in child obesity. Relationships with inflammation and metabolic risk factors.Nutrients. 2013;5(6):2222-30.

26. Lee HJ, Jang HB, Park JE, Park KH, Kang JH, Park SI, Song J. Relationship between Serum Levels of Body Iron Parameters and Insulin Resistance and Metabolic Syndrome in Korean Children.Osong Public Health Res Perspect. 2014;5(4):204-10.

27.Moschonis G, Chrousos GP, Lionis C, Mougios V, Manios Y; Healthy Growth Study group. Association of total body and visceral fat mass with iron deficiency in preadolescents: the Healthy Growth Study. Br J Nutr. 2012;108(4):710-9.

28. Zhang H, Wang L, Li S, Liu X, Li Y, He Y, Man Q, Yang L. Association of iron storage markers with metabolic syndrome and its components in Chinese rural 6–12 years old children: the 2010–2012 China National Nutrition and Health Survey. *Nutrients*. 2020;12(5):1486.

29. Suárez-Ortegón MF, Blanco E, McLachlan S, Fernandez-Real JM, Burrows R, Wild SH, Lozoff B, Gahagan S. Ferritin levels throughout childhood and metabolic syndrome in adolescent stage. *Nutr Metab Cardiovasc Dis*. 2019;29(3):268-78.

30. Kovács D, Lőcsey L, Laczik R, Szabó L, Zsom L, Kabai K, Fedor R, Nemes B, Asztalos L, Soltész P. Three-year longitudinal clinical trial of arterial function assessed by aoscillometric non-invasive method in comparison with carotid sclerosis and transferrin kidney-transplanted patients.Transplant Proc. 2014;46(6):2168-70.

31. Zeba AN, Delisle HF, Renier G, Savadogo B, Baya B. The double burden of malnutrition and cardiometabolic risk widens the gender and socio-economic health gap: a study among adults in Burkina Faso (West Africa). *Public Health Nutr*. 2012; 15:2210-2219.

32.CamaschellaC.Iron-deficiency anemia.NEngl J Med. 2015;372(19):1832-43.

33.Davies KJ, Donovan CM, Refino CJ, Brooks GA, Packer L, Dallman PR. Distinguishing effects of anemia and muscle iron deficiency on exercise bioenergetics in the rat. *Am J Physiol.* 1984;246:E535–E543

34. Yamagishi H, Komabayashi T. Alteration of glucose metabolism and increased fructosamine in iron-deficiency anemic rats. *Nutr Res.* 2003;23:1547–1553

35. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG.The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. Diabetologia. 2015.

36. Zhao L, Zhang X, Shen Y, Fang X, Wang Y, Wang F. Obesity and iron deficiency: a quantitative meta-analysis. Obes Rev. 2015;16(12):1081-93.

37. Adams PC, Barton JC. A diagnostic approach to hyperferritinemia with a non-elevated transferrin saturation. J Hepatol. 2011;55(2):453-8.

38. Suárez-Ortegón MF, Ensaldo-Carrasco E, Shi T, McLachlan S, Fernández-Real JM, Wild SH. Ferritin, metabolic syndrome and its components: A systematic review and meta-analysis. Atherosclerosis. 2018; 275:97-106.

39. de Ruiter I, Olmedo-Requena R, Sánchez-Cruz JJ, Jiménez-Moleón JJ. Trends in child obesity and underweight in Spain by birth year and age, 1983 to 2011. Revista Española de Cardiología (English Edition). 2017;70(8):646-55.

40. Basterra-Gortari FJ, Bes-Rastrollo M, Ruiz-Canela M, Gea A, Martinez-Gonzalez MÁ. Prevalence of obesity and diabetes in Spanish adults 1987–2012. Medicina Clínica (English Edition). 2017 Mar 22;148(6):250-6.

41. Mayneris-Perxachs J, Moreno-Navarrete JM, Fernández-Real JM. The role of iron in host-microbiota crosstalk and its effects on systemic glucose metabolism. Nat Rev Endocrinol. 2022; 18:683-698.

FIGURE LEGENDS

Figure 1. Fasting glucose, glycosylated haemoglobin and metabolic syndrome Z scores at baseline (Left) and their changes per year (right) across tertiles of ferritin at baseline. Data are mean (95% confidence interval). Z scores are age- and sex-specific. Tertiles of ferritin: tertile 1 ≤ 28.2 µL; tertile 2 28.3-44.9 µL; tertile 3 > 44.9 µL. * Adjusted for age and gender. † adjusted for age, gender, CRP, ALT, GGT levels and weight status (underweight/normal weight/ overweight/obesity). Trend or differences across tertiles were estimated by using ANCOVA. Skewed variables were log- transformed [CRP, ALT, GGT,] except CRP levels which were inverse log-transformed. MetS, metabolic syndrome. CRP, C reactive protein. ALT, Alanine aminotransferase. GGT, gamma-glutamyl transferase.

Figure 2. Fasting glucose, glycosylated haemoglobin and metabolic syndrome Z scores at baseline (Left) and their changes per year (right) across tertiles of transferrin at baseline. Data are mean (95% confidence interval). Z scores are age- and sex-specific. Tertiles of transferrin: tertile 1 ≤ 257 µg/dL ; tertile 2 258-289 µL; tertile 3 > 289 µL. * Adjusted for age and sex. † adjusted for age, sex, CRP, ALT, GGT levels and weight status (underweight/normal weight/ overweight/obesity). Trend or differences across tertiles were estimated by using ANCOVA. Skewed variables were log- transformed [CRP, ALT, GGT,] except CRP levels which were inverse log-transformed. MetS, metabolic syndrome. CRP, C reactive protein. ALT, Alanine aminotransferase. GGT, gamma-glutamyl transferase.