Ferritin levels throughout childhood and metabolic syndrome in adolescence

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Abbreviations:
MetS : Metabolic syndrome
HDL-C: High density lipoprotein cholesterol
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
TG: Triglyceride
BMI: Body mass index
WC: Waist circumference
CRP: C-reactive protein
HOMA-IR: homeostasis model assessment-Insulin Resistance

Abstract

Background and aim:
Increased ferritin levels have been widely associated with cardiovascular risk in adults. Whether ferritin levels and their changes during childhood are related to metabolic syndrome (MetS) at adolescence is unknown. We aimed to evaluate these associations using levels of ferritin at 5, 10 and 16 years and their linear increases and patterns of sustained increased levels across childhood.

Methods
There were four samples evaluated according to non-missing values for study variables at each stage (5 years: 562; 10 years: 381; and 16 years: 567 children; non-missing values at any stage: 379). MetS risk was evaluated as a continuous Z score. Patterns of sustained increased ferritin (highest tertile) and slope of the change of ferritin per year across the follow-up were calculated.

Results
Ferritin levels in the highest versus lowest tertile at five and 16 years were significantly positively associated with MetS risk Z score at adolescence in boys and these associations were unaffected by adjustment for covariates. Having high, compared to low/moderate ferritin level at 2 or more time periods between 5 and 16 years was related to higher Mets Z-score in boys only[e.g. 5-10 years adjusted-beta (95 %CI):0.26(0.05-0.48),P<0.05]. In girls, ferritin Z score at 10 and 16 years was positively and independently associated with HOMA-IR Z score. In girls, the slope of ferritin per year in the highest tertile was positively associated with MetS risk Z-score[adjusted-beta (95 %CI):0.21(0.05-0.38),P<0.05].

Conclusions
Ferritin levels throughout childhood are positively related to cardiometabolic risk in adolescence, with associations varying by sex.

Introduction

Increased iron stores, defined by ferritin levels, have been associated with metabolic syndrome[1] and risk of diabetes in general adult populations in many studies.[2, 3] Iron is an active cofactor of oxidative biological reactions whose products have deleterious effects on insulin sensitivity and endothelial function.[4] Interestingly, not only iron overload but also iron deficiency is related to worsening of metabolic profile trough mechanisms still unclear.[5, 6]

To date, little is understood about whether the iron-cardiometabolic risk link exists in early life. Children have a lower capacity for iron storage than adults since this physiological function becomes fully developed in adulthood. It is unknown if changes in ferritin levels across infancy and adolescence are associated with metabolic syndrome, and if these changes could have an additive effect on cardiometabolic risk. Whether increased ferritin levels, low ferritin levels or both are involved in higher cardiometabolic risk during childhood is also unknown. Therefore, we conducted a study to evaluate longitudinal and cross-sectional associations between ferritin levels measured at 5, 10 and 16 years with metabolic syndrome and its components at 16-17 years in a cohort of Chilean children. In addition, we evaluated if a linear increase and sustained patterns of increased ferritin across childhood were related to MetS risk in adolescence.

Methods

Subjects

The cohort included Chilean infants of low/middle socio-economic status from urban Santiago recruited between 1991 and 1996 for a trial of iron supplementation. The infants, recruited at 4 months, were healthy, full-term singleton infants weighing 3 kg or more at birth. At 6 months, those who did not have iron deficiency anaemia were randomized to receive iron supplementation or usual nutrition between 6 and 12 months. The cohort was assessed for developmental outcomes, including ferritin levels, in infancy and at 5, 10 and 16 years.[7] At 16-

17 years, participants were also assessed for obesity and cardiovascular risk.[8] Three samples from the cohort were evaluated: 1) participants with no missing values for ferritin and covariates at 5 years and for cardiometabolic risk outcomes at 16 years (n=565); 2) participants with no missing values for ferritin and covariates at 10 years and for cardiometabolic risk outcomes at 16 years (n=381); 3) participants with no missing values for ferritin, covariates and cardiometabolic risk outcomes at 16 years (n=567). A sample with no missing values for the study variables at any stage of the follow-up was also evaluated (n=379). The study was approved by the institutional review boards of the University of Michigan, the Institute of Nutrition and Food Technology (University of Chile), and the University of California, San Diego. Participants and their primary caregiver provided informed and written consent.

Methods for clinical measurements of blood pressure, body mass index and waist circumference and biochemical markers of fasting glucose, triglycerides, and HDL cholesterol has been described previously[7, 8].

Metabolic syndrome and insulin resistance

It was decided to evaluate a continuous MetS score instead of using some paediatric MetS definitions. The use of the latter approach had supposed lower statistical power on the basis of few cases meeting categorical criteria from MetS defnitions (e.g high glucose as glucose > 100 mg/dL). Similarly, there is no consensus about cut-off values in the different components of MetS in children and adolescents. A continuous variable of Z score or scale of SD units for MetS was created from an average of Z scores of blood pressure, glucose, triglycerides, HDL cholesterol and waist circumference.[9] Distribution of these MetS components was normalised if required, before calculating the Z scores. The Z scores of diastolic and systolic blood pressures were averaged to get a single Z score for blood pressure, and the HDL-C Z score was multiplied by -1 before obtaining the overall MetS score, to ensure that all of the components

of MetS had the same positive association with regard to cardiometabolic risk. Insulin resistance was estimated by using the formula of the homeostatic model assessment (HOMA-IR) as: (glucose[mg/dL] x insulin [mU/mL])/ 405.[10] We estimated proportions of non-overweight, overweight and obesity using both the CDC and WHO reference populations. Cut-points used were < 1 BMI z score for non-overweight, ≥ 1 BMI Z score and <2 Z BMI z score overweight, and ≥ 2 BMI z score for obesity.

Data analysis

The analyses were conducted in female and male subjects separately given differences by sex in cardiometabolic outcomes. Study variables were described as median (and interquartile range) and proportions, and differences were estimated by Mann-Whitney U test and Chi Square test, respectively. Wilcoxon and sign tests were used to detect significant differences or changes in values of ferritin across the different time points during the follow-up.

We defined two kinds of variable of change over time for ferritin levels as exposure variable. One was on the basis of ferritin levels defined in tertiles at 5, 10 and 16 years for participants with data available at those time points. Patterns of ferritin levels were then identified from different combinations of high ferritin (highest tertile) and low/moderate ferritin (lowest and middle tertile). The second approach was the calculation of the slope of ferritin concentration per year and use this parameter as continuous and categorical variable (tertiles). The slope provides information on variation of ferritin concentration by each unit of time, in this case years (11 years of follow-up), which were extrapolated from the trend line of the three time points: 5, 10 and 16 years. Multiple linear regression was used to evaluate associations of ferritin tertiles (with lowest tertile as reference) at each stage of follow up and patterns and slope of ferritin across the follow-up with the variation of MetS Z score. For the patterns of repeated ferritin measurements, low/moderate ferritin at two stages of an interval or in all of

the stages of follow-up was used as reference. We calculated sex-specific z-scores for ferritin in our cohort at each age (5, 10, and 16 years) and used them to model associations between ferritin at each age and Z scores or SD units of MetS components and insulin resistance using linear regression. The adjustment consisted of covariates at the respective stage (5, 10, and 16-17 years): age, BMI Z score, Tanner stage, and haemoglobin levels. At adolescent stage (16-17 years) C reactive protein (CRP) levels were also available and cross-sectional associations were furtherly adjusted for this inflammatory marker. Associations between patterns of repeated ferritin measurements and cardiometabolic risk variables in adolescence were adjusted for covariates at the end of a determined interval (e.g. if ferritin pattern during the interval 5-10 years, covariates used were those at 10 years). We also adjusted this kind of associations by using baseline values of covariates along with changes of these across the interval (if ferritin pattern during the interval 5-10 years, for instance BMI Z score at 5 years and also its change between 5 and 10 years were covariates).

To approximate to normal distribution ferritin, SBP, DBP and CRP values were log-transformed in girls and boys, WC and TG in girls and HDL-C in boys. In boys WC was transformed as (1/square)*-1, TG as (1/square root)*-1, and HDL-C as its logarithm.

All analyses were performed using Stata 14.0.

Results

The study variables are described by sex and age at follow-up in Table 1. No differences by sex were found at 5 years of age for ferritin, haemoglobin, and BMI Z score. At 10 years of age, girls had higher sexual development than boys (Table 1). At the adolescent stage, boys had higher levels of ferritin, haemoglobin, systolic and diastolic blood pressure, glucose compared to girls, and girls had higher sexual development, HDL-C and insulin levels compared to boys. In boys there was a significant increase in ferritin levels showed in Table 1

from 5 to 10 years and from 10 to 16-17 years (Wilcoxon and Sign tests P<0.05). In girls, changes in ferritin levels were also significant, but with increase from 5 to 10 years, and decrease from 10 to 16-17 years (Wilcoxon and Sign tests P<0.05). The slope for average annual change in ferritin across the follow-up was higher in boys than girls [mean(SD) 0.67 ± 1.82 v. -0.50(1.38), P <0.001]. Specific slopes of change in ferritin levels between 5 and 10 years were [mean(SD)] 0.89 ± 3.23 and 0.99 ± 2.55 in boys and girls respectively. Between 10 and 16-17 years the slope for boys was 0.49 ± 3.01 and for girls -1.68 ± 2.31.

With regard overweight and obesity, this Chilean cohort presented a considerable proportion of overweight and obesity (~44% at 5 and 10 years, and 34% at 16-17 years) (Supplementary table 1). Serum ferritin levels increased across categories of non-overweight, overweight, and obesity in each age group analysed (Supplementary table 2).

Table 2 shows linear regression analyses for relationships between sex-specific Z score of ferritin at the three ages and sex-specific Z scores for MetS components and insulin resistance at adolescent stage. In girls, Z scores for ferritin levels at 10 and 16-17 years of age were significantly (P=0.046 and P=0.014) and independently associated with the Z score of insulin resistance but not with any of the MetS components at the adolescent stage. In boys, ferritin level Z score at 5 years old was inversely associated with the Z score of HDL-C in adolescence in unadjusted and adjusted models, whereas significant unadjusted associations with Z scores of waist circumference, HDL-C and insulin resistance did not remain significant after adjustment for covariates. In adolescence, there were significant cross-sectional associations between Z scores of ferritin and fasting glucose and triglycerides independent of adjustment for covariates in boys and with insulin resistance (measured by HOMA-IR) in both sexes.

Ferritin levels in the highest tertile at five years (compared to the lowest tertile) were positively associated with MetS risk Z score in adolescence in boys (Figure 1), and this association was unaffected by adjustment for baseline BMI Z score and haemoglobin levels. Also in boys, there

was a significant cross-sectional association between ferritin levels measured in adolescence in the highest tertile and increasing MetS risk Z score, independently of adjustment for BMI Z score, CRP levels, haemoglobin levels and Tanner stage. No significant associations were found between ferritin at 10 years and MetS Z score at adolescent stage. No significant associations were found in girls (Figure 1).

Having high, compared to low/moderate ferritin level at 2 or more time periods between 5 and 16 years was related to higher Mets risk z-score in boys only ($p<0.05$) (Figure 2). A sensitivity analysis by adjusting the associations in figure 2 for baseline values of covariates along with changes of these across the interval, showed very similar beta coefficients to those in figure 2 (Supplementary table 3).

The slope values for ferritin levels as a continuous variable were marginally associated ($P=0.06$) with MetS risk z-score only in girls and significantly associated with MetS risk z-score when comparing highest to lowest tertile of ferritin slope (Supplementary table 4). High ferritin levels at the three time points was associated with MetS rik Z score in boys only (Supplementary table 5). More details on these associations are provided in the supplementary material as "additional results" along with a sensitivity analysis and an extra-adjustment.

In the different age stages, the correlation between ferritin and MetS Z scores was stronger in overweight (including obese) children in comparison with non-overweight children (Supplementary figures 1, 2 and 3).

Discussion

This study showed that ferritin levels in infancy and childhood are positively and longitudinally associated with cardiometabolic risk at adolescent stage. Moreover, patterns of sustained high levels of ferritin throughout childhood were associated with worse metabolic profile in

adolescence. In general, in male but not in female subjects the associations of ferritin at different ages with MetS were independent of covariates. However, only in girls, a linear increase in terms of the slope of ferritin from measurements at 5, 10 and 16 years, was independently associated with MetS risk. The above findings highlight serum ferritin as a factor associated with cardiometabolic risk from early life and indicate sex differences in the association.

Contrast with previous studies

Only five studies conducted to date on the association of ferritin and other iron status markers with cardiometabolic risk variables in children were identified from a systematic literature search performed in September 2016. All studies are cross-sectional and four are from Asian paediatric populations (Table 3). All these studies included children and/or adolescents from general populations, except the study by Bougle and Brouard in Canadian children, who studied overweight and obese children specifically.[11] This latter study is the only one, along with the present studies, reporting associations adjusted for inflammatory markers. Lee et al. , like our study, evaluated outcomes of MetS and its components,[12] while the others evaluated MetS components (categorical or continuous variables). In general, the studies showed no significant association between ferritin and most of the cardiometabolic risk outcomes they evaluated. Zhu et al. did not find association between ferritin and any MetS component in 1126 Chinese children.[13] Ferritin levels were significantly and positively associated with waist circumference in the studies by Lee et al. and Jeon et al. in Korean children,[12, 14] with triglycerides in the studies by Bougle and Brouard and Kim et al.,[11, 15] and inversely associated with HDL-C in the studies by Bougle and Brouard, Kim et al., and Lee et al.[11, 12, 15] None of the studies reported associations between ferritin and fasting glucose or blood pressure.

The contrasting and significant cross-sectional findings in Chilean boys with regard to the above studies could be explained in terms of differences in age range and study design. In the Chilean cohort, participants were evaluated at the same age at each evaluation and cardiometabolic risk outcomes were at the adolescent stage (16-17 years), a higher and narrower age range than in the above studies.

The longitudinal and cross-sectional positive associations between ferritin at each stage of childhood (except at 10 years) and MetS described in the present study were significant in boys but not in girls. This sex difference appears to be explained by a threshold effect derived from higher levels of cardiometabolic risk factors in boys at the adolescent stage, rather than by higher iron stores in boys since at 5 years there was no significant sex difference in ferritin levels. Two recent prospective studies in adults which evaluated both sexes have also shown significant associations between ferritin and development of MetS with stronger associations for men than for premenopausal women,[16, 17] although one study in a Swiss population did not find difference by sex.[18] However, some cross-sectional studies have described non-significant ferritin-MetS associations in men.[19-21] The association could be stronger in men given higher iron storage capacity in comparison with women.

The relationship between the pattern of repeated measurements of ferritin and MetS appeared to differ by sex. In boys, a pattern of sustained high ferritin levels throughout the follow-up was significantly associated with MetS risk, whereas in girls a linear change in ferritin levels across follow-up was associated with MetS risk. It is of notice that the slope of change in ferritin levels in girls tended to be negative due to puberty-related menstrual iron losses. Therefore, an association between the slope of change in ferritin levels and HOMA-IR should be interpreted as the less decline in ferritin concentration across the follow-up the higher the values of the insulin resistance index. In boys the association appear markedly influenced by threshold

effects of ferritin and/or cardiometabolic risk outcomes. There are no studies on trajectories of ferritin and cardiometabolic risk in adults, although one study evaluated change in ferritin regarding development of MetS after 6.5 years follow-up.[22] In this study conducted in a Finnish population, men or women who had incident MetS presented higher changes in ferritin values between baseline and the end of the follow-up.[22] Future studies should test patterns of sustained high ferritin and slopes from repeated measurements of ferritin over time regarding cardiometabolic outcomes in adults and other paediatric populations to contrast our findings by sex.

Only in girls, there was a longitudinal association between ferritin at 10 years and insulin resistance at adolescence, and later at adolescence, ferritin levels were cross-sectionally associated with the index of insulin resistance (HOMA-IR) in both sexes. This could be explained in terms of sexual development differences. At 10 years, 60% of the girls were in adolescent stage v. 29% in boys, and at 16-17 years both sexes reached adolescent stage. None of the three studies that evaluated relationship between ferritin and HOMA-IR in Table 3 found those variables significantly associated.[11, 12, 15]. In line with our findings, Aigner et al. found in Caucasian adolescents an isolated inverse association between the soluble transferrin receptor (sTfR)/ ferritin index (lower values represent higher body iron content) and HOMA-IR in girls, and consistent inverse associations with systolic and diastolic blood pressure, and triglyceride levels in boys[23]. However, in that study no associations with ferritin and sTfR were separately evaluated. The finding in Chilean girls contrasts with the lack of association between ferritin at 10 and 16 years with MetS score in the same girls, as higher insulin resistance is strongly correlated with higher MetS scores.Given that the relationship between iron and glucose/insulin metabolism is bi-directional,[5] insulin levels could modulate iron stores throughout the growth process in girls. However, the difference by sex in the ferritin-MetS association must be mainly explained by menstrual blood loses in girls.

Serum ferritin levels were increased in overweight and obese children, and the correlation between serum ferritin at the different age stages and MetS Z score at adolescence was stronger in overweight children v. non-overweight children. A higher body mass implies a higher capacity for iron storage. Higher ferritin in people with obesity could also be explained by insulin resistance rather than by obesity itself [24,25]. Since ferritin is an acute phase reactant, their circulating levels might be influenced by conditions in which chronic low grade inflammation is prominent, with parallel iron retention in several tissues, as an evolutionary mechanism to avoid iron use by potential pathogens [26]. Another known source of increased serum ferritin levels is liver injury, which results in the release of ferritin into the bloodstream [27]. In this sense, hepatic steatosis, a condition characterized by lipid accumulation in the liver in terms of triglycerides and fatty acids, appears to lead to an environment of increased oxidative stress, insulin resistance and necrotic signals in obese subjects, ending up in hepatic damage [28]. In addition, adipocytes secrete inflammatory interleukins which contribute to increased inflammatory activity of obese subjects [29].Thus, liver injury, hypertrophied adipocytes and inflammation concur during the clinical course of overweight and obesity, to alter the circulating levels of ferritin. However, despite the influence of weight status, the ferritin-MetS association found in boys remained significant after adjustment for BMI Z score.

Ethnicity is an important aspect to take into account when it comes to cardiometbolic risk. The vast majority of the Chilean population is mestizo: mixed Spanish and Indigenous ancestry. Although, Indigenous ethnicities are different according to geographic location, several studies have shown higher risk of diabetes and cardiovascular disease in Indigenous groups such as PIMA (Arizona, U.S), Australian and Canadian Aboriginal communities [30,31,32]. Although genetic susceptibility may be implied, as observed for the PIMA community[29], social inequalities are more likely to explain why Indigenous ethnic groups are more prone to cardiometabolic risk[31,32]. Since these groups are ethnic minorities, poverty, lower educational level and higher rates of unemployment might be associated with higher cardiometabolic risk.

In addition to the partial Indigenous ancestry, the Chilean children of this cohort belong to low and middle socioeconomic status, and thus higher cardiometabolic risk is expected. However, the above aspects would support an inverse ferritin-MetS association rather than a positive association as it was found. In fact, because of poor nutrition socioeconomically deprived or marginal communities would tend to have lower iron levels concomitantly with hypercaloric diets, unfavourable environments for physical activity and higher rates of cardiovascular risk factors[33] . However, further studies in children from diverse ethnic groups are needed to confirm the longitudinal and cross-sectional associations we found.

Several limitations have to be mentioned. First, because of lack of availability of variables, there was no evaluation of cross-sectional associations between ferritin cardiometabolic risk at 5 and 10 years since cardiometabolic risk outcomes were measured only at the adolescent stage. Second, there were no available measures of subclinical/clinical inflammation at 5 and 10 years, meaning that it is not possible to account for ferritin acting as an acute phase reactant. Adjustment for BMI Z score might partly have corrected the associations for the potential effect of subclinical inflammation derived from adiposity on ferritin levels. It is also important to bear in mind that CRP levels in adolescence were not associated with cardiometabolic risk and insulin resistance and did not affect the associations in the multivariable models (data not shown). Third, hepatic injury increases ferritin levels and the associations reported in this study were not adjusted for hepatic injury markers. However, in general children were in good health status. Our findings in girls might need further characterisation in large samples at different tanner stages to properly evaluate puberty-related changes in iron status and cardiometabolic risk.

On the other hand, our study has some relevant strengths. To the best of our knowledge this is the first study evaluating longitudinal association between ferritin levels and cardiometabolic risk in paediatric populations. Moreover, the Chilean cohort analysis is the first study analysing patterns of three repeated measurements of ferritin over time regarding cardiometabolic risk. The study is also the first to adjust the cross-sectional ferritin-cardiometabolic risk association in children for a systemic inflammation marker such as CRP levels. The evaluation of the children at specific ages in the Chilean cohort allowed a more precise characterisation of the association in terms of a more homogeneous group of subjects in comparison with previous studies.

Conclusion

Both serum ferritin at different time points in childhood and its change or patterns of sustained high concentration throughout childhood are associated with MetS and insulin resistance but with differences by sex in those relationships. Patterns of sustained high ferritin and slopes from repeated measurements of ferritin over time should be tested regarding cardiometabolic outcomes in adults and other paediatric populations to establish whether the difference by sex of the present study is replicable. It is uncertain if the association between high ferritin and higher cardiometabolic risk reported in the Chilean cohort, commonly described in adults, would remain significant after adjustments for hepatic function markers.

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