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Maternal iron status in early pregnancy and childhood body fat measures and cardio-metabolic risk factors. A population-based prospective cohort.

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Abbreviations

BMI, body mass index; CI, confidence interval; DAG, directed acyclic graph; DXA, Dual-energy X-ray absorptiometry; ECLIA, electrochemiluminescence immunoassay; HFE,

52 hemochromatosis; HDL, high-density lipoprotein; SDS, standard deviation score; TNFα, tumor
53 necrosis factor α.

ABSTRACT

Background Whether maternal iron status during pregnancy is associated with cardio-metabolic health in the offspring is poorly known.

Objectives We aimed to assess the associations of maternal iron status during early pregnancy with body fat measures and cardio-metabolic risk factors in children aged 10 years.

Methods In a population-based cohort study among 3718 mother-child pairs, we measured ferritin, transferrin and transferrin saturation during early pregnancy. We obtained child body mass index (BMI), fat mass index and android/gynoid fat mass ratio by dual-energy X-ray absorptiometry, subcutaneous fat index, visceral fat index, pericardial fat index and liver fat fraction by magnetic resonance imaging and assessed systolic and diastolic blood pressure, serum lipids, glucose, insulin, and C-reactive protein at 10 years.

Results A one-standard deviation score (SDS) higher maternal ferritin was associated with lower fat mass index (difference -0.05 (95% confidence interval (CI) (-0.08, -0.02)) SDS) and subcutaneous fat index (difference -0.06 (95%CI -0.10, -0.02) SDS) in children. One-SDS higher maternal transferrin was associated with higher fat mass index (difference 0.04 (95%CI 0.01, 0.07) SDS), android/gynoid fat mass ratio (difference 0.05 (95%CI 0.02, 0.08) SDS) and subcutaneous fat index (difference 0.06 (95%CI 0.02, 0.10) SDS) in children. Iron status during pregnancy was not consistently associated with organ fat and cardio-metabolic risk factors at 10 years.

Conclusion Maternal lower ferritin and higher transferrin in early pregnancy are associated with body fat accumulation and distribution but are not associated with cardio-metabolic risk factors in childhood. Underlying mechanisms and long-term consequences warrant further study.

Key words: iron, fetal programing, cardiometabolic health, pregnancy, cohort studies

INTRODUCTION

Iron deficiency is the most common micro-nutritional deficiency worldwide affecting around 2 billion of people and is the most common cause of anemia (1, 2). It is estimated that 56% of pregnant females in developing countries and 18% of pregnant females in industrialized countries suffer from anemia (3). Iron supplementation during pregnancy in females with normal iron levels is also becoming a public health concern, especially in countries with a high iron intake and iron status (4). Iron is essential for multiple metabolic and cellular processes, including DNA synthesis and repair, mitochondrial function and is necessary for red blood cell production (5). Iron deficiency leads to reduced enzymatic activity and cellular growth, and produces oxidative stress and mitochondrial damage (5, 6). On the other hand, iron overload generates reactive oxygen species inducing oxidative stress and causing cellular damage, cell death and organ damage (7). In adults, both iron deficiency and iron overload are associated with an adverse cardio-metabolic profile such as an increased risk of cardiovascular diseases and type 2 diabetes mellitus (8-11). Sex related differences can be expected since literature suggested that the link between iron status and cardio-metabolic risk factors might be more relevant in females (12-14).

Deregulated iron homeostasis during early development might be associated with long-term cardio-metabolic risk (7). However, the effects of iron status during pregnancy on the cardio-metabolic risk factors in the offspring are largely unknown (15). In a cohort study among 348 mothers and their offspring, no associations were found for maternal hemochromatosis (HFE) genetic variants with offspring's blood pressure and adiposity at 40-41 years old (16). On the other hand, a Norwegian case-control study in 94 209 mother-child pairs identified an association between maternal HFE genetic variants and a higher risk of type 1 diabetes in children aged 8-17 years old (17).

In this population-based prospective cohort study among 3718 mother-child pairs, we evaluated the association of maternal iron status during early pregnancy with childhood body fat measures and cardio-metabolic risk factors in children aged 10 years old. We

hypothesized that iron status during pregnancy might lead to fetal adaptations with long-term effect in body fat and cardio-metabolic health in the offspring in a sex-specific manner.

METHODS

Study design

The present study is part of the Generation R Study, a prospective population-based cohort (18). The study was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre in Rotterdam. Written informed consent was obtained from the parents or legal representative of the children. Of 8737 prenatally included mothers with singleton children, iron status was measured in 6089 mothers. Mothers whose children had no information on body fat measures and cardio-metabolic risk factors at 10 years were excluded. A total of 3718 mother-child pairs were included in the current study (Supplementary Figure 1).

Maternal iron status

During early pregnancy (median (25^{th}, 75th percentiles) 13.2 (12.2, 14.8) weeks of gestation), non-fasting blood serum and plasma samples were collected from the mothers at different times of the day (19). To characterize maternal iron status, we measured ferritin, iron and transferrin saturation (higher levels indicate higher iron status) and transferrin (higher levels indicate lower iron status). Ferritin was measured on the Cobas e411 analyzer (Roche, Almere, the Netherlands) by electrochemiluminescence immunoassay (ECLIA). Iron was determined by colorimetric assay and transferrin by immunoturbidimetric assay, both measured with C502 on the Cobas 8000 (Roche, Almere, the Netherlands). Quality control samples demonstrated intra- and inter-assay coefficients of variation of 3.2 and 5.9% for ferritin, 0.8 and 1.5% for iron and 1.4 and 2.8% for transferrin, respectively. Transferrin saturation was calculated with formula serum iron*100) / transferrin*25.1 (19). Iron status was categorized into iron deficiency (ferritin <15 µg/L), normal levels (ferritin ≥15 µg/L and ferritin ≤150 µg/L) and iron overload (>150 µg/L) (20).

Feriritin reflects the amount of iron in body stores and transferrin and transferrin saturation are indicators of the adequacy of iron supply (21). Ferritin and transferrin saturation are directly associated with iron status while transferrin is inversely associated with iron status (21).

Childhood body fat measures

At the age of 10 years, all children were invited to participate in detailed body fat follow-up measurements. Weight and height were obtained without shoes and heavy clothing and body mass index (BMI, kg/m^2) was calculated. Age- adjusted and sex-specific SD scores (SDS) of BMI were calculated based on Dutch reference charts (Growth Analyzer 4.0, Dutch Growth Research Foundation) (22). As previously described, we measured total, android and gynoid fat mass by Dual-energy X-ray absorptiometry (DXA) scan (iDXA; General Electrics-Lunar, 2008, Madison, WI, USA, enCORE software v.12.6) (22). We calculated android/gynoid fat mass ratio, which reflects the relation between fat mass in the abdomen (android) and hip (gynoid) regions (23). Measures of abdominal and organ fat, i.e., subcutaneous fat mass, visceral fat mass, pericardial fat mass, and liver fat fraction, were obtained from MRI scans (22). General, abdominal and organ fat indexes independent of height were constructed using an optimal adjustment estimated by log-log regression analyses. Specifically, total fat mass, subcutaneous fat mass, visceral fat mass and pericardial fat mass and height were log-transformed, using natural logarithms. Then, log-adiposity measures were regressed on log-height, and the regression slope was considered as the power by which height should be raised in order to calculate an index uncorrelated with height (22). Therefore, total fat mass and subcutaneous fat mass were divided by height^4 to estimate fat mass index and subcutaneous fat index, respectively and the visceral and pericardial fat mass were divided by height^3 to estimate visceral and pericardial fat indexes, respectively (22).

Childhood cardio-metabolic risk factors

Systolic and diastolic blood pressure were measured 4 times at 1-minute intervals at the right brachial artery with a validated automatic sphygmanometer Datascope Accutor Plus™ (Paramus, NJ, USA). A cuff with a width of approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference was selected. We calculated the mean value for systolic and diastolic blood pressure using the last three measurements of each participant. Thirty minutes fasting blood samples were collected by ante-cubital venipuncture. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and glucose were measured on c702 Cobas 8000 analyzer (23). Insulin was measured with electrochemiluminescence immunoassay (ECLIA) on the E411 module (Roche, Almere, the Netherlands) and C-reactive protein was measured using an immunoturdimetric assay (23, 24). Quality control samples demonstrated intra- and inter-assay coefficients of variation below 2.6% (25-28).

Covariates

Maternal information such as age, education, ethnicity, parity, pre-pregnancy BMI, smoking habits, daily energy intake, psychological distress and folic acid supplement use was obtained from multiple questionnaires during pregnancy (29). Information on gestational age at iron blood sampling was collected during the iron status assessment (19). Since ferritin is an acute phase protein and might be upregulated during inflammation, we included maternal C-reactive protein concentrations which were measured using an immunoturbidimetic assay (27). Information on maternal iron supplement use during pregnancy, gestational age at birth, birthweight and child's sex were obtained from midwife or hospital registries. Information on child's daily dairy, meat, fish and energy intake was obtained from food frequency questionnaires at 8 years old (30).

Statistical analysis

First, we used independent samples T-test, Mann-Whitney U test and Chi-square test to compare characteristics between participants and non-participants in the study. Second, we

assessed the associations of maternal iron status during pregnancy (continuously, and in categories) with body fat measures and cardio-metabolic risk factors in children using linear and logistic regression models. To avoid violation of the assumption of normality of the residuals in the regression models, ferritin, insulin, triglycerides and DXA and MRI body fat measures were natural log-transformed. We constructed SDS [(observed value − mean)/SD] of the sample distribution for all continuous iron biomarkers and child outcomes to enable comparisons of effect sizes. Non-linearity in the associations of iron status on body fat measures and cardio-metabolic risk factors was evaluated with natural cubic splines (three degrees of freedom); and no evidence of non-linearity was observed. We constructed a basic and main model. In the basic model, we adjusted for gestational age at iron blood sampling, child's age and sex. In the main model, we additionally adjusted for the aforementioned covariates since they were related to maternal iron status and one of the child outcomes in previous literature, fulfilled the graphical criteria for confounding by visualizing a directed acyclic graph (DAG, obtained with DAGitty version 3.0) (Supplementary Figure 2) and changed the effect size in ≥ 10% for at least one of the outcomes. For a better visualization of our findings, we plotted the significant associations of iron status in a continuous scale with child outcomes. We additionally adjusted any significant associations in the main model for birthweight, gestational age at birth, and child´s daily dairy, meat, fish and energy intake at 8 years to explore whether these associations were explained by these covariates (31-33). Since females with low hemoglobin and iron status in early pregnancy might have used iron supplement during pregnancy, we performed a sensitivity analysis by additionally adjusting the main models for maternal iron supplement use during pregnancy. As C-reactive protein distribution was still skewed after any transformation, we categorized C-reactive protein into normal (<3 mg/L) and high (≥3 mg/L) concentrations, as previously described (23). Third, we tested for statistical interaction between maternal iron status during pregnancy and child's sex but no statistically significant interactions was observed (p-values>0.05). Fourth, missing data on covariates (up to 28.1%) followed a non-monotonic pattern and was imputed with multiple imputation (m=10)

according to the Markov Chain Monte Carlo using MICE package in R. Software built-in imputation methods were used including predictive mean matching for continuous variables, and polytomous or binary logistic regression for categorical variables. We evaluated the performance of our imputation by inspecting convergence and comparing the distribution of the imputed versus the original values and corroborated the plausibility of our imputed values. To correct for multiple hypothesis testing, we divided 0.05 by the effective number of independent tests based on the correlation structure between outcomes since the exposures were assumed to be representing the same condition and part of the same hypothesis (p-value threshold of 0.006) (34). To illustrate this, we created a correlation matrix between all exposures and outcomes using Spearman correlation analysis. All statistical analyses were performed in R software version 4.0.2 (packages mice, corrplot and visreg; R foundation, Vienna, Austria, https://www.r-project.org/).

RESULTS

Maternal and child characteristics

Table 1 shows the subjects characteristics. The median (25, 75th percentile) maternal ferritin was 55.6ug/L (32.3, 89.8), the mean (SD) transferrin was 2.8g/L (0.4) and the mean (SD) transferrin saturation was 24.9% (10.5). The prevalence of iron deficiency and iron overload was 6.0% and 7.5%, respectively. The mean (SD) child's BMI was 17.5kg/m2 (2.8) and the prevalence of obesity was 3.7%. Non-response analysis showed that mothers of included children were older, had higher educational level, daily energy intake, ferritin, and iron, were more likely to be European, nullipara, non-smokers, users of folic acid supplement, had less psychological distress and their children were more likely female (p-values<0.05). (Supplementary Table 1). Supplementary Figure 3 showed that the correlations between exposures and outcomes were generally weak to moderate, with some strong correlations between childhood body fat measures.

Maternal iron status and childhood body fat measures

Results of the basic models are in Supplementary Table 2. In the main models, after adjustment for potential confounders, one SDS increase of ferritin was associated with lower fat mass index (difference (95% CI) -0.05 (-0.08, -0.02) SDS), and subcutaneous fat index (difference (95% CI) -0.06 (-0.10, -0.02) SDS), while one SDS increase of transferrin was associated with higher fat mass index (difference (95% CI) 0.04 (0.01, 0.07) SDS), android/gynoid fat mass ratio (difference (95% CI) 0.05 (0.02, 0.08) SDS), subcutaneous fat index (difference (95% CI) 0.06 (0.02, 0.10) SDS) and liver fat fraction (difference (95% CI) 0.04 (0.00, 0.09) SDS) (p-values<0.05) (Table 2). The associations of ferritin with fat mass index and subcutaneous fat index and of transferrin with fat mass index, android/gynoid fat mass ratio and subcutaneous fat index remained statistically significant after correction for multiple testing (p-values<0.006) (Table 2). Our effect plots showed these associations across the full range of ferritin and transferrin (Supplementary Figure 4). These associations

did not substantially change after additional adjustment for birthweight, gestational age at birth, and child´s daily dairy, meat, fish and energy intake at 8 years (Supplementary Table 4). Sensitivity analysis by additionally adjusting the main models for iron supplement use did not change the effect sizes or direction of the associations (Supplementary Tables 5). However, only the associations of transferrin with fat mass index, android/gynoid fat mass ratio and subcutaneous fat index remained statistically significant (p-values<0.006). No significant associations were observed for any of the iron status markers with BMI, and visceral and pericardial fat indices.

Maternal iron status and childhood cardio-metabolic risk factors

Results of the basic models are in Supplementary Table 3. In the main models, after adjustment for potential confounders, one SDS increase of ferritin was associated with higher total cholesterol (difference (95% CI) 0.04 (0.00, 0.09) SDS) (p-value<0.05) (Table 3). However, this association did not survive multiple testing correction. No significant associations were observed for any of the iron status markers with blood pressure, HDL cholesterol, triglycerides, glucose, insulin and C-reactive protein. Sensitivity analysis by additionally adjusting for maternal iron supplement use during pregnancy did not change the results (Supplementary Table 6).

DISCUSSION

In this population-based cohort study, we observed that higher maternal ferritin was associated with lower fat mass index and subcutaneous fat index in children aged 10 years while higher maternal transferrin was associated with higher fat mass index, android/gynoid fat mass ratio and subcutaneous fat index. These associations were not explained by birthweight, gestational age at birth, and child´s daily dairy, meat, fish and energy intake at 8 years. Our sensitivity analysis also suggests that these associations are independent of iron supplement use during pregnancy. No consistent associations were found between iron status and organ fat and cardio-metabolic risk factors at 10 years.

Interpretation of main findings

Children with alterations in body fat measures and cardio-metabolic risk factors are at a higher risk of a range of diseases in adulthood (23, 35). As proposed in animal studies, iron status during early pregnancy might be associated with body fat measures and cardio-metabolic risk factors already during childhood (15, 36-38). To the best of our knowledge, no previous population-based studies assessed the association of iron status during early pregnancy with childhood body fat measures (15). We have previously found no associations between maternal hemoglobin during pregnancy and childhood BMI and body fat distribution using data from the current cohort (39). Hemoglobin is not considered a good proxy of the iron status. We found a significant but small positive association between hemoglobin and ferritin in the current study (data not shown). Since iron is incorporated into hemoglobin in a highly prioritized process, hemoglobin lacks sensitivity and specificity to detect early stages of iron deficiency (40). Moreover, other conditions such as hemoglobinopathies, inflammation and chronic diseases can produce anemia (40). A Mendelian randomization study among 348 United Kingdom (UK) adults did not find associations between maternal hemochromatosis (HFE) genetic variants, which are related with iron overload, and BMI and waist circumference; however, the study may have been underpowered (16). In line with this study, we did not find an association of maternal iron

status with BMI in children aged 10 years. However, we found that higher maternal ferritin, representing higher iron status, was associated with lower fat mass index, while higher maternal transferrin, representing lower iron status, was associated with higher fat mass index. Waist circumference is used as a proxy for abdominal fat but it does not distinguish between subcutaneous and visceral abdominal fat (41). In our study, we used more refined body fat distribution measures obtained by DXA and MRI scans. We observed that higher maternal ferritin was associated with lower subcutaneous fat index and higher maternal transferrin was associated with higher android/gynoid fat mass ratio and subcutaneous fat index. No associations were observed between maternal iron status and visceral and pericardial fat indices. Higher maternal transferrin was associated with higher liver fat fraction at 10 years of age but this association did not remain significant after multiple testing correction. We did not observe any association of ferritin categories and transferrin saturation with body fat measures at 10 years. No specific cut-offs for iron deficiency and overload during pregnancy are established and although transferrin saturation is often used to define iron status, this biomarker has limitations as it is more influenced by the rate of iron absorption in the small bowel as well as the iron stores (42). This might partly explain the significant association of ferritin and transferrin but not of ferritin categories and transferrin saturation with child body fat measures and highlight the importance of using multiple iron biomarkers. Our results suggest that lower iron status, as indicated by higher maternal transferrin during early pregnancy, might be adversely associated with body fat accumulation and distribution in childhood. On contrary, higher iron status, as indicated by higher maternal ferritin during early pregnancy, might be favorably associated with body fat development.

Few previous studies have assessed the association of maternal iron status during pregnancy with cardio-metabolic risk factors in the offspring (15). The aforementioned UK Mendelian randomization study did not find an association between maternal HFE genetic variants and blood pressure in the adulthood (16). Moreover, in a cohort study in UK among 362 newborns at 2-6 weeks of age no evidence of an association between maternal iron status and infant brachio-femoral pulse wave velocity was observed (43). On the other hand,

a case-control study among 94 209 Norwegian children aged 8-17 years old observed an association between maternal HFE genetic variants and a higher risk of type 1 diabetes (17). In our study, no associations were observed between maternal iron status and cardio-metabolic risk factors at 10 years. The use of non-fasting blood samples of childhood cardio-metabolic profile might have limited our ability to find associations.

The mechanisms by which maternal iron status during pregnancy might influence offspring body fat accumulation are not fully understood yet. Alterations in placental structure, endocrine and transport functions have been reported during iron deficiency and have also been associated with childhood obesity (44, 45). Placental increased weight (46) and decreased capillarity length and surface were found in iron deficient rats (47). Alteration in regulators of fetal growth and development such as tumor necrosis factor α (TNFα) and leptin have been found increased in placentas of iron deficient rats (48). Moreover, animal experiments showed that maternal iron deficiency during pregnancy leads to disproportionate growth of fetus and offspring obesity (16, 49).

Although the effect estimates of the observed associations are small and might be difficult to translate to clinical settings, the results are relevant since iron status during pregnancy could be modifiable and alterations in childhood body fat measures might persist into adulthood (35). To what extent these small differences become magnified over the course of the life and might increase the risk of disease remains unclear (50). In this study, no data were available on iron status in later stages of pregnancy and during childhood which might help to clarify underlying pathways. Thus, further studies evaluating maternal iron status from pre-conception, at multiple time points during pregnancy and after birth and during childhood with childhood body fat measures are needed to get a better understanding of our findings. Causality and potential underlying mechanisms also need further study. We do not have information on maternal HFE genetic variants in the cohort used in the current study. Further studies with these data should look into the causality of the associations between maternal iron status and childhood body fat measures (51).

Methodological considerations

Strengths of this study are the population-based prospective design, the large sample size with detailed data available on different iron measurements including ferritin, transferrin and iron during early pregnancy and information on childhood MRI and DXA scan adiposity measures. Our research focuses on early pregnancy, which is a critical period of development when tissues and organs are created (52). Dysregulation of iron status during this period migth result in permanent alterations of structural and physiological functions of the fetus (52). The non-response could lead to biased effect estimates if the associations of maternal iron status during pregnancy with childhood body fat measures and cardio-metabolic risk factors differ between mother and children included and not included in the analysis. The non-response analysis showed a selection towards a healthy and wealthy population with low prevalence of iron deficiency, which might have limited the statistical power to detect statistically significant results. Further studies in populations with a higher prevalence of iron deficiency are needed to corroborate our findings. Thus far, no specific cut-offs have been established for iron biomarkers during pregnancy. Therefore, we categorized iron biomarkers based on guidelines for the general population to allow comparison and clinical interpretation with other studies. The non-fasting blood samples of childhood cardio-metabolic risk factors might have resulted in misclassification causing underestimation of the associations. However, non-fasting blood lipids were found to be associated with a higher risk of cardiovascular events (23). Similarly, non-fasting blood samples of maternal iron biomarkers were collected, which might have also resulted in non-differential misclassification (23) Depending on the time of the study visit, iron blood samples were collected at different times of the day. We do not have information on what and when the mothers ate before sampling. However, we believe the influence of this potential misclassification will be minor, as previous studies reported minor or no significant seasonal and diurnal variations (53-55). Moreover, non-fasting iron biomarkers were found to be associated with a higher risk of cardiovascular and cardio-metabolic disease (56-59). Nonetheless, our results should be interpreted with caution and further studies using fasting

samples are warranted to corroborate our findings. Although we adjusted for multiple confounders, residual confounding due to unmeasured characteristics such as genetic characteristics cannot be ruled out.

CONCLUSION

Our study suggests that maternal lower ferritin and higher transferrin in early pregnancy are associated with body fat accumulation and distribution but are not associated with cardio-metabolic risk factors in childhood.

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Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethical Standards disclosure: The study was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre in Rotterdam (MEC-2012-165-NL40020.078.12). Written informed consent was obtained from the parents or legal representative of the children.

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Table 1. Maternal and child characteristics.

Characteristics	Total group (n= 3718)
Maternal characteristics	
Age, mean (SD), years	30.7 (4.7)
Educational level (higher), n (%)	1845 (51.8)
Ethnic background (European), n (%)	2183 (59.6)
Parity (nullipara), n (%)	2216 (59.9)
Body mass index, mean (SD), kg/m^2	23.5 (4.1)
Smoking during pregnancy (yes), n (%)	849 (25.3)
Daily energy intake, mean (SD), kcal	2052 (546)
Psychological distress (yes), n (%)	265 (8.4)
Folic acid supplement use (yes), n (%)	2352 (80.8)
Iron supplement use, yes (%)	471 (17.5)
C-reactive protein, median (25th, 75th percentile), mg/L	4.4 (2.3, 7.9)
Gestational age at iron blood sampling, median (25th, 75th percentile), weeks	13.2 (12.2, 14.8)
Ferritin, median (25th, 75th percentile), ug/L	55.6 (32.3, 89.8)
Iron deficient, n (%)	224 (6.0)
Normal, n (%)	3215 (86.5)
Iron overload, n (%)	279 (7.5)
Iron, mean (SD), (µmol/L)	17.3 (6.6)
Transferrin, mean (SD), (g/L)	2.8 (0.4)
Transferrin saturation, n (%)	24.9 (10.5)
Child characteristics	
Birthweight, mean (SD), kg	3437 (550)
Gestational age at birth, mean (SD), weeks	40.0 (1.7)
Age at follow-up, mean (SD), years	9.8 (0.3)
Sex (female), n (%)	1882 (50.6)
Daily energy intake at 8 years, mean (SD), kcal	1497 (380)
Child's daily dairy intake at 8 years, mean (SD), g	354.9 (217.2)
Child's daily meat intake at 8 years, mean (SD), g	76.2 (39.8)
Child's daily fish intake at 8 years, median (25th, 75th percentile), g	8.6 (4.3, 17.2)

Body mass index, mean (SD), kg/m^2	17.5 (2.8)
Underweight, n (%)	260 (7.0)
Normal weight, n (%)	2803 (75.7)
Overweight, n (%)	503 (13.6)
Obesity, n (%)	137 (3.7)
Total fat mass, median (25th, 75th percentile), g	8422 (6642, 11 740)
Fat mass index, mean (SD), g/cm^4	2.4x10^-5 (1.0x10^-5)
Android/Gynoid fat mass ratio, mean (SD)	0.3 (0.1)
Age at magnetic resonance imaging, mean (SD), years	10.2 (0.6)
Subcutaneous fat mass, median (25th, 75th percentile), g	1294 (944, 2137)
Subcutaneous fat index, mean (SD), g/cm^4	4.3x10^-6 (2.9 x10^-6)
Visceral fat mass, median (25th, 75th percentile), g	369 (271, 507)
Visceral fat index, mean (SD) g/cm^3	1.5x10^-4 (0.7x10^-4)
Pericardial fat mass, median (25th, 75th percentile), g	10.6 (8.0, 14.0)
Pericardial fat index, mean (SD), g/cm^3	4.0x10^-6 (1.6x10^-6)
Liver fat fraction, median (25th, 75th percentile), %	2.0 (1.7, 2.5)
Systolic blood pressure, mean (SD), mmHg	103 (7.9)
Diastolic blood pressure, mean (SD), mmHg	58.5 (6.4)
Total cholesterol, mean (SD), mmol/L	4.3 (0.7)
HDL cholesterol, mean (SD), mmol/L	1.5 (0.3)
Triglycerides, mean (SD), mmol/L	1.1 (0.6)
Glucose, mean (SD), mmol/L	5.2 (0.9)
Insulin, median (25th, 75th percentile), pmol/L	175 (104, 284)
C-reactive protein, median (25th, 75th percentile), mg/L	0.3 (0.3, 0.6)

Values are means (SD), medians (25th, 75th percentile), or absolute numbers (valid percentages) based on observed data. Sample sizes: maternal characteristics: age (n= 3718), educational level (n= 3559), ethnic background (n= 3664), parity (n= 3699), ,body mass index (n= 3123), smoking during pregnancy (n= 3361), daily energy intake (n= 3066), psychological distress (n= 3170), folic acid supplement use (n= 2912), iron supplement use (n= 2696), C-reactive protein (n= 3620), gestational age at iron blood sampling (n= 3718), child characteristics: birthweight (n= 3717), gestational age at birth (n= 3718), daily energy intake at 8 years (n= 2672), daily dairy intake at 8 years (n= 2696), daily meat intake at 8 years (n= 2696), daily fish intake at 8 years (n= 2696), age at follow-up (n= 3714), sex (n= 3718), body mass index (n= 3713), total fat mass (n= 3668), age at magnetic resonance imaging (n= 2297).

Table 2. Associations of maternal ferritin, transferrin and transferrin saturation during pregnancy with childhood body fat measures at 10 years.

Iron status	Difference (95% CI) in standard deviation scores						
	Body mass index n=3711	**Fat mass index n=3667**	**Android/Gynoid fat mass ratio n=3668**	**Subcutaneous fat index n=1907**	**Visceral fat index n=1907**	**Pericardial fat index n=1973**	**Liver fat fraction n=2158**
Ferritin							
Continuous (SDS)	-0.03 (-0.06, 0.00)	**-0.05 (-0.08, -0.02)****	-0.03 (-0.06, 0.00)	**-0.06 (-0.10, -0.02)****	-0.02 (-0.06, 0.03)	0.01 (-0.03, 0.06)	-0.03 (-0.07, 0.02)
Categorical							
Iron deficiency (n=224)	0.05 (-0.08, 0.19)	0.08 (-0.04, 0.20)	0.05 (-0.08, 0.18)	0.15 (-0.01, 0.31)	0.09 (-0.09, 0.26)	0.03 (-0.14, 0.21)	-0.02 (-0.19, 0.15)
Normal (n=3215)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Iron overload (n= 279)	-0.02 (-0.14, 0.11)	-0.03 (-0.14, 0.07)	-0.02 (-0.14, 0.10)	-0.07 (-0.22, 0.07)	-0.02 (-0.17, 0.14)	0.10 (-0.06, 0.26)	-0.10 (-0.26, 0.05)
Transferrin							
Continuous (SDS)	0.01 (-0.02, 0.04)	**0.04 (0.01, 0.07)****	**0.05 (0.02, 0.08)****	**0.06 (0.02, 0.10)****	0.03 (-0.02, 0.07)	0.01 (-0.03, 0.06)	**0.04 (0.00, 0.09)***
Transferrin saturation							
Continuous (SDS)	0.00 (-0.03, 0.04)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)	-0.03 (-0.07, 0.01)	-0.01 (-0.06, 0.03)	0.00 (-0.04, 0.05)	0.00 (-0.05, 0.04)

Values are linear regression coefficients (95% Confidence Intervals) and reflect the change in childhood outcomes in SDS per SDS change in maternal iron status or for maternal iron deficiency (ferritin <15 µg/L) and iron overload (ferritin >150 µg/L) compared with the reference group (ferritin ≥ 15 µg/L and ferritin ≤ 150 µg/L). Models were adjusted for gestational age at iron blood sampling, child's age and sex (except for sex-and age-adjusted BMI SDS), maternal age, daily energy intake, folic acid intake, educational level, parity, ethnicity, body mass index, smoking habits, psychological distress and C-reactive protein. Estimates are based on multiple imputed data. *P-value<0.05. **P-value<0.006.

Table 3. Associations of maternal ferritin, transferrin and transferrin saturation during pregnancy with childhood cardio-metabolic risk factors at 10 years.

Iron status	Difference (95% CI) in standard deviation scores[1]							OR (95% CI)[2]
	Systolic blood pressure n=3580	Diastolic blood pressure n=3580	Total cholesterol n=2572	HDL cholesterol n=2572	Triglycerides n=2568	Glucose n=2573	Insulin n=2567	C-reactive protein (≥3 mg/L) n= 2574
Ferritin								
Continuous (SDS)	0.02 (-0.01, 0.06)	0.02 (-0.02, 0.05)	**0.04 (0.00, 0.09)***	0.02 (-0.03, 0.06)	0.00 (-0.04, 0.04)	-0.04 (-0.08, 0.00)	-0.02 (-0.07, 0.02)	1.06 (0.89, 1.27)
Categorical								
Iron deficiency (n=224)	-0.09 (-0.23, 0.05)	-0.08 (-0.22, 0.06)	-0.05 (-0.22, 0.11)	0.05 (-0.11, 0.21)	-0.08 (-0.24, 0.09)	0.04 (-0.13, 0.20)	0.04 (-0.12, 0.21)	1.08 (0.55, 2.09)
Normal (n=3215)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Iron overload (n= 279)	0.00 (-0.12, 0.13)	-0.05 (-0.18, 0.08)	0.07 (-0.09, 0.22)	-0.04 (-0.19, 0.11)	-0.04 (-0.19, 0.12)	-0.14 (-0.29, 0.02)	-0.12 (-0.27, 0.04)	0.85 (0.43, 1.67)
Transferrin								
Continuous (SDS)	0.00 (-0.03, 0.04)	-0.01 (-0.04, 0.03)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)	0.00 (-0.04, 0.04)	-0.01 (-0.05, 0.03)	0.01 (-0.03, 0.05)	0.92 (0.78, 1.08)
Transferrin saturation								
Continuous (SDS)	0.01 (-0.02, 0.05)	-0.01 (-0.04, 0.03)	0.01 (-0.03, 0.05)	0.00 (-0.04, 0.04)	0.00 (-0.04, 0.05)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.03)	1.08 (0.90, 1.31)

[1]Values are linear regression coefficients (95% Confidence Intervals) and reflect the change in childhood outcomes in SDS per SDS change in maternal iron status or for maternal iron deficiency (ferritin <15 µg/L) and iron overload (ferritin >150 µg/L) compared with the reference group (ferritin ≥ 15 µg/L and ferritin ≤ 150 µg/L).
[2]Values are logistic regression Odds Ratio (OR) with 95% confidence interval (95% CI) and reflect the risk of having C-reactive protein ≥3 mg/L per SDS change in maternal iron status or for maternal iron deficiency (ferritin <15 µg/L) and iron overload (ferritin >150 µg/L) compared with the reference group (ferritin ≥ 15 µg/L and ferritin ≤ 150 µg/L). .
Models were adjusted for gestational age at iron blood sampling, child's age and sex, maternal age, daily energy intake, folic acid intake, educational level, parity, ethnicity, body mass index, smoking habits, psychological distress and C-reactive protein. Estimates are based on multiple imputed data.
*P-value<0.05.