

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

3
4 Hugo G. Quezada-Pinedo, MD^{1,2}, Vincent Jaddoe, MD PhD^{1,3}, Liesbeth Duijts, MD PhD^{2,4}
5 Taulant Muka, MD PhD⁵, Marijn J. Vermeulen, MD PhD², Irwin K.M. Reiss, MD, PhD², Susana
6 Santos, PhD^{1,3,6,7}

7
8 ¹The Generation R Study Group, Erasmus MC, University Medical Center Rotterdam,
9 Rotterdam, the Netherlands

10 ²Department of Pediatrics, Division of Neonatology, Erasmus MC – Sophia Children's Hospital,
11 University Medical Center Rotterdam, Rotterdam, the Netherlands

12 ³Department of Pediatrics; Erasmus MC – Sophia Children's Hospital, University Medical Center
13 Rotterdam, Rotterdam, the Netherlands

14 ⁴Department of Pediatrics, Division of Respiratory Medicine and Allergology, Sophia Children's
15 Hospital, University Medical Center Rotterdam, Rotterdam, the Netherlands

16 ⁵Institute of Social and Preventive Medicine (ISPM), University of Bern, Mittelstrasse 43, 3012,
17 Bern, Switzerland

18 ⁶EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600
19 Porto, Portugal

20 ⁷Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR),
21 Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal

22
23 **Word count manuscript: 3695**

24 **Word count abstract: 248**

25 **Number of tables: 3**

26 **Number of figures: 0**

27 **Financial Support:** The Generation R Study is made possible by financial support from the
28 Erasmus Medical Centre, Rotterdam, the Erasmus University Rotterdam and the Netherlands
29 Organization for Health Research and Development. Hugo G. Quezada-Pinedo received
30 funding from Academy Ter Meulen grant of the Academy Medical Sciences Fund of the Royal
31 Netherlands Academy of Arts & Sciences (KNAWWF/1327/TMB202116). Vincent Jaddoe
32 received funding from the European Research Council (grant number ERC-2014CoG-648916).
33 Liesbeth Duijts received funding from the European Union's Horizon 2020 research and
34 innovation programme (LIFECYCLE, grant agreement No 733206, 2016; EUCAN-Connect grant
35 agreement No 824989; ATHLETE, grant agreement No 874583). The researchers are
36 independent from the funders. The study sponsors had no role in the study design, data
37 analysis, interpretation of data, or writing of this report.

38
39 **Conflict of interest:** The authors declare no competing interests.

40 41 **Corresponding author**

42 Susana Santos, PhD.

43 Generation R Study Group, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the
44 Netherlands, Na-2907; PO Box 2040, 3000 CA Rotterdam, the Netherlands.

45 Tel: *31 10 7043405, Fax: *31 10 7036811, E-mail: s.dasilvasantos@erasmusmc.nl

46
47 **Running title:** iron status and cardio-metabolic health

48 49 **Abbreviations**

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

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

54 **ABSTRACT**

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

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

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

66 **Results** A one-standard deviation score (SDS) higher maternal ferritin was associated with
67 lower fat mass index (difference -0.05 (95% confidence interval (CI) (-0.08, -0.02)) SDS) and
68 subcutaneous fat index (difference -0.06 (95%CI -0.10, -0.02) SDS) in children. One-SDS
69 higher maternal transferrin was associated with higher fat mass index (difference 0.04
70 (95%CI 0.01, 0.07) SDS), android/gynoid fat mass ratio (difference 0.05 (95%CI 0.02, 0.08)
71 SDS) and subcutaneous fat index (difference 0.06 (95%CI 0.02, 0.10) SDS) in children. Iron
72 status during pregnancy was not consistently associated with organ fat and cardio-metabolic
73 risk factors at 10 years.

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

78

79 **Key words:** iron, fetal programming, cardiometabolic health, pregnancy, cohort studies

80 **INTRODUCTION**

81 Iron deficiency is the most common micro-nutritional deficiency worldwide affecting around 2
82 billion of people and is the most common cause of anemia (1, 2). It is estimated that 56% of
83 pregnant females in developing countries and 18% of pregnant females in industrialized
84 countries suffer from anemia (3). Iron supplementation during pregnancy in females with
85 normal iron levels is also becoming a public health concern, especially in countries with a
86 high iron intake and iron status (4). Iron is essential for multiple metabolic and cellular
87 processes, including DNA synthesis and repair, mitochondrial function and is necessary for
88 red blood cell production (5). Iron deficiency leads to reduced enzymatic activity and cellular
89 growth, and produces oxidative stress and mitochondrial damage (5, 6). On the other hand,
90 iron overload generates reactive oxygen species inducing oxidative stress and causing
91 cellular damage, cell death and organ damage (7). In adults, both iron deficiency and iron
92 overload are associated with an adverse cardio-metabolic profile such as an increased risk
93 of cardiovascular diseases and type 2 diabetes mellitus (8-11). Sex related differences can
94 be expected since literature suggested that the link between iron status and cardio-metabolic
95 risk factors might be more relevant in females (12-14).
96 Deregulated iron homeostasis during early development might be associated with long-term
97 cardio-metabolic risk (7). However, the effects of iron status during pregnancy on the cardio-
98 metabolic risk factors in the offspring are largely unknown (15). In a cohort study among 348
99 mothers and their offspring, no associations were found for maternal hemochromatosis
100 (HFE) genetic variants with offspring's blood pressure and adiposity at 40-41 years old (16).
101 On the other hand, a Norwegian case-control study in 94 209 mother-child pairs identified an
102 association between maternal HFE genetic variants and a higher risk of type 1 diabetes in
103 children aged 8-17 years old (17).

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

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

109

110 **METHODS**

111 **Study design**

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

120 **Maternal iron status**

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

134

135 Feritin reflects the amount of iron in body stores and transferrin and transferrin saturation
136 are indicators of the adequacy of iron supply (21). Ferritin and transferrin saturation are
137 directly associated with iron status while transferrin is inversely associated with iron status
138 (21).

139 **Childhood body fat measures**

140 At the age of 10 years, all children were invited to participate in detailed body fat follow-up
141 measurements. Weight and height were obtained without shoes and heavy clothing and
142 body mass index (BMI, kg/m²) was calculated. Age- adjusted and sex-specific SD scores
143 (SDS) of BMI were calculated based on Dutch reference charts (Growth Analyzer 4.0, Dutch
144 Growth Research Foundation) (22). As previously described, we measured total, android
145 and gynoid fat mass by Dual-energy X-ray absorptiometry (DXA) scan (iDXA; General
146 Electrics-Lunar, 2008, Madison, WI, USA, enCORE software v.12.6) (22). We calculated
147 android/gynoid fat mass ratio, which reflects the relation between fat mass in the abdomen
148 (android) and hip (gynoid) regions (23). Measures of abdominal and organ fat, i.e.,
149 subcutaneous fat mass, visceral fat mass, pericardial fat mass, and liver fat fraction, were
150 obtained from MRI scans (22). General, abdominal and organ fat indexes independent of
151 height were constructed using an optimal adjustment estimated by log-log regression
152 analyses. Specifically, total fat mass, subcutaneous fat mass, visceral fat mass and
153 pericardial fat mass and height were log-transformed, using natural logarithms. Then, log-
154 adiposity measures were regressed on log-height, and the regression slope was considered
155 as the power by which height should be raised in order to calculate an index uncorrelated
156 with height (22). Therefore, total fat mass and subcutaneous fat mass were divided by
157 height⁴ to estimate fat mass index and subcutaneous fat index, respectively and the
158 visceral and pericardial fat mass were divided by height³ to estimate visceral and
159 pericardial fat indexes, respectively (22).

160

161 **Childhood cardio-metabolic risk factors**

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

174 **Covariates**

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

185 **Statistical analysis**

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

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

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

228

229

230

231 **RESULTS**

232 **Maternal and child characteristics**

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

244

245 **Maternal iron status and childhood body fat measures**

246 Results of the basic models are in Supplementary Table 2. In the main models, after
247 adjustment for potential confounders, one SDS increase of ferritin was associated with lower
248 fat mass index (difference (95% CI) -0.05 (-0.08, -0.02) SDS), and subcutaneous fat index
249 (difference (95% CI) -0.06 (-0.10, -0.02) SDS), while one SDS increase of transferrin was
250 associated with higher fat mass index (difference (95% CI) 0.04 (0.01, 0.07) SDS),
251 android/gynoid fat mass ratio (difference (95% CI) 0.05 (0.02, 0.08) SDS), subcutaneous fat
252 index (difference (95% CI) 0.06 (0.02, 0.10) SDS) and liver fat fraction (difference (95% CI)
253 0.04 (0.00, 0.09) SDS) (p-values<0.05) (Table 2). The associations of ferritin with fat mass
254 index and subcutaneous fat index and of transferrin with fat mass index, android/gynoid fat
255 mass ratio and subcutaneous fat index remained statistically significant after correction for
256 multiple testing (p-values<0.006) (Table 2). Our effect plots showed these associations
257 across the full range of ferritin and transferrin (Supplementary Figure 4). These associations

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

266

267 **Maternal iron status and childhood cardio-metabolic risk factors**

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

276

277

278

279

280

281 **DISCUSSION**

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

290

291 **Interpretation of main findings**

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

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

331 Few previous studies have assessed the association of maternal iron status during
332 pregnancy with cardio-metabolic risk factors in the offspring (15). The aforementioned UK
333 Mendelian randomization study did not find an association between maternal HFE genetic
334 variants and blood pressure in the adulthood (16). Moreover, in a cohort study in UK among
335 362 newborns at 2-6 weeks of age no evidence of an association between maternal iron
336 status and infant brachio-femoral pulse wave velocity was observed (43). On the other hand,

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

342

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

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

365 **Methodological considerations**

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

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

396

397 **CONCLUSION**

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

401

402 **Acknowledgments:** The Generation R Study is managed by the Erasmus Medical Centre in
403 close collaboration with the School of Law and the Faculty of Social Sciences at Erasmus
404 University, Rotterdam, the Municipal Health Service, Rotterdam area, and the Stichting
405 Trombosedienst and Artsenlaboratorium Rijnmond (Star-MDC), Rotterdam. We
406 acknowledge the contribution of children and their parents, general practitioners, hospitals,
407 midwives and pharmacies in Rotterdam. The authors thank Dr. Luis Alberto Antonio
408 Sanchez Ramirez from the Blood Bank of the National Institute of Neoplastic Diseases
409 (*Instituto Nacional de Enfermedades Neoplasicas* in Spanish) in Peru for valuable
410 assistance in the interpretation of the laboratory technical documents.

411

412 **Author contributions:** HQ, SS contributed to the conception and design, acquisition of
413 data, analyses and interpretation of the data, drafted the article, revised it critically for
414 important intellectual content, and gave final approval of the version to be published. VJ, LD,
415 TM, MV, IR contributed to the conception and design, acquisition of data, revised the drafted
416 manuscript critically for important intellectual content, and gave final approval of the version
417 to be published.

418

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

422

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

REFERENCES

1. Teichman J, Nisenbaum R, Lausman A, Sholzberg M. Suboptimal iron deficiency screening in pregnancy and the impact of socioeconomic status in a high-resource setting. *Blood Advances*. 2021.
2. Raffaelli G, Manzoni F, Cortesi V, Cavallaro G, Mosca F, Ghirardello S. Iron Homeostasis Disruption and Oxidative Stress in Preterm Newborns. *Nutrients*. 2020;12(6).
3. Sekhavat L, Davar R, Hosseinidezoki S. Relationship between maternal hemoglobin concentration and neonatal birth weight. *Hematology*. 2011;16(6):373-6.
4. Brannon PM, Taylor CL. Iron Supplementation during Pregnancy and Infancy: Uncertainties and Implications for Research and Policy. *Nutrients*. 2017;9(12).
5. Muckenthaler MU, Rivella S, Hentze MW, Galy B. A Red Carpet for Iron Metabolism. *Cell*. 2017;168(3):344-61.
6. Walter PB, Knutson MD, Paler-Martinez A, Lee S, Xu Y, Viteri FE, et al. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;99(4):2264-9.
7. Roemhild K, von Maltzahn F, Weiskirchen R, Knüchel R, von Stillfried S, Lammers T. Iron metabolism: pathophysiology and pharmacology. *Trends in Pharmacological Sciences*. 2021;42(8):640-56.
8. Hsu HS, Li CI, Liu CS, Lin CC, Huang KC, Li TC, et al. Iron deficiency is associated with increased risk for cardiovascular disease and all-cause mortality in the elderly living in long-term care facilities. *Nutrition*. 2013;29(5):737-43.
9. Salonen JT, Nyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation*. 1992;86(3):803-11.

10. Klip IT, Voors AA, Swinkels DW, Bakker SJ, Kootstra-Ros JE, Lam CS, et al. Serum ferritin and risk for new-onset heart failure and cardiovascular events in the community. *Eur J Heart Fail.* 2017;19(3):348-56.
11. Kunutsor SK, Apekey TA, Walley J, Kain K. Ferritin levels and risk of type 2 diabetes mellitus: an updated systematic review and meta-analysis of prospective evidence. *Diabetes/Metabolism Research and Reviews.* 2013;29(4):308-18.
12. Sheu WH, Chen YT, Lee WJ, Wang CW, Lin LY. A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. *Clin Endocrinol (Oxf).* 2003;58(3):380-5.
13. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *Jama.* 2004;291(6):711-7.
14. Brewer CJ, Wood RI, Wood JC. mRNA regulation of cardiac iron transporters and ferritin subunits in a mouse model of iron overload. *Exp Hematol.* 2014;42(12):1059-67.
15. Quezada-Pinedo HG, Cassel F, Duijts L, Muckenthaler MU, Gassmann M, Jaddoe VVW, et al. Maternal Iron Status in Pregnancy and Child Health Outcomes after Birth: A Systematic Review and Meta-Analysis. *Nutrients.* 2021;13(7):2221.
16. Alwan NA, Lawlor DA, McArdle HJ, Greenwood DC, Cade JE. Exploring the relationship between maternal iron status and offspring's blood pressure and adiposity: A Mendelian randomization Study. *Clin Epidemiol.* 2012;4(1):193-200.
17. Stordal K, McArdle HJ, Hayes H, Tapia G, Viken MK, Lund-Blix NA, et al. Prenatal iron exposure and childhood type 1 diabetes. *Sci rep.* 2018;8(1):9067.
18. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. *Eur J Epidemiol.* 2016;31(12):1243-64.
19. Quezada-Pinedo HG, Mensink-Bout SM, Reiss IK, Jaddoe VVW, Vermeulen MJ, Duijts L. Maternal iron status during early pregnancy and school-age, lung function, asthma, and allergy: The Generation R Study. *Pediatric Pulmonology.* 2021;56(6):1771-8.

20. World Health Organization. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations 2011. Available from: https://www.who.int/vmnis/indicators/serum_ferritin.pdf.
21. Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF, et al. Biomarkers of Nutrition for Development (BOND)—Iron Review. *The Journal of Nutrition*. 2018;148(suppl_1):1001S-67S.
22. Santos S, Monnereau C, Felix JF, Duijts L, Gaillard R, Jaddoe VWV. Maternal body mass index, gestational weight gain, and childhood abdominal, pericardial, and liver fat assessed by magnetic resonance imaging. *International Journal of Obesity*. 2019;43(3):581-93.
23. Silva CCV, Vehmeijer FOL, El Marroun H, Felix JF, Jaddoe VWV, Santos S. Maternal psychological distress during pregnancy and childhood cardio-metabolic risk factors. *Nutr Metab Cardiovasc Dis*. 2019;29(6):572-9.
24. Qureshi F, Derks IPM, Jaddoe VWV, Williams MA, Koenen KC, Tiemeier H, et al. Mental Health in Early Childhood and Changes in Cardiometabolic Dysregulation by Preadolescence. *Psychosomatic Medicine*. 2021;83(3).
25. Santos S, Gaillard R, Oliveira A, Barros H, Hofman A, Franco OH, et al. Subcutaneous fat mass in infancy and cardiovascular risk factors at school-age: The generation R study. *Obesity (Silver Spring)*. 2016;24(2):424-9.
26. Geurtsen ML, Wahab RJ, Felix JF, Gaillard R, Jaddoe VWV. Maternal Early-Pregnancy Glucose Concentrations and Liver Fat Among School-Age Children. *Hepatology*. 2021;74(4):1902-13.
27. Ernst GDS, de Jonge LL, Hofman A, Lindemans J, Russcher H, Steegers EAP, et al. C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: the Generation R Study. *American Journal of Obstetrics and Gynecology*. 2011;205(2):132.e1-.e12.
28. van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PY, et al. Chronic air pollution exposure during pregnancy and maternal and fetal

C-reactive protein levels: the Generation R Study. *Environ Health Perspect.* 2012;120(5):746-51.

29. Cajachagua-Torres KN, Jaddoe VWV, de Rijke YB, van den Akker ELT, Reiss IKM, van Rossum EFC, et al. Parental cannabis and tobacco use during pregnancy and childhood hair cortisol concentrations. *Drug and Alcohol Dependence.* 2021;225:108751.

30. Mou Y, Jansen PW, Raat H, Nguyen AN, Voortman T. Associations of family feeding and mealtime practices with children's overall diet quality: Results from a prospective population-based cohort. *Appetite.* 2021;160:105083.

31. Thane CW, Walmsley CM, Bates CJ, Prentice A, Cole TJ. Risk factors for poor iron status in British toddlers: further analysis of data from the National Diet and Nutrition Survey of children aged 1.5-4.5 years. *Public Health Nutr.* 2000;3(4):433-40.

32. Holmlund-Suila EM, Hauta-alus HH, Enlund-Cerullo M, Rosendahl J, Valkama SM, Andersson S, et al. Iron status in early childhood is modified by diet, sex and growth: Secondary analysis of a randomized controlled vitamin D trial. *Clinical Nutrition.* 2022;41(2):279-87.

33. Bouglé D, Brouard J. Iron in Child Obesity. Relationships with Inflammation and Metabolic Risk Factors. *Nutrients.* 2013;5(6):2222-30.

34. Sol CM, Santos S, Asimakopoulos AG, Martinez-Moral MP, Duijts L, Kannan K, et al. Associations of maternal phthalate and bisphenol urine concentrations during pregnancy with childhood blood pressure in a population-based prospective cohort study. *Environ Int.* 2020;138:105677.

35. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. *Obes Rev.* 2016;17(2):95-107.

36. Gambling L, Dunford S, Wallace DI, Zuur G, Solanky N, Srai SKS, et al. Iron deficiency during pregnancy affects postnatal blood pressure in the rat. *The Journal of physiology.* 2003;552(Pt 2):603-10.

37. Crowe C, Dandekar P, Fox M, Dhingra K, Bennet L, Hanson MA. The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. *The Journal of physiology*. 1995;488 (Pt 2)(Pt 2):515-9.
38. Gambling L, McArdle HJ. Iron, copper and fetal development. *Proc Nutr Soc*. 2004;63(4):553-62.
39. Walter PB, Knutson MD, Paler-Martinez A, Lee S, Xu Y, Viteri FE, et al. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. *Proceedings of the National Academy of Sciences*. 2002;99(4):2264-9.
40. Georgieff MK. Iron deficiency in pregnancy. *Am J Obstet Gynecol*. 2020;223(4):516-24.
41. Wahab RJ, Voerman E, Jansen PW, Oei EHG, Steegers EAP, Jaddoe VWV, et al. Maternal Glucose Concentrations in Early Pregnancy and Cardiometabolic Risk Factors in Childhood. *Obesity (Silver Spring, Md)*. 2020;28(5):985-93.
42. Li J, Lange LA, Duan Q, Lu Y, Singleton AB, Zonderman AB, et al. Genome-wide admixture and association study of serum iron, ferritin, transferrin saturation and total iron binding capacity in African Americans. *Human molecular genetics*. 2015;24(2):572-81.
43. Alwan NA, Cade JE, McArdle HJ, Greenwood DC, Hayes HE, Ciantar E, et al. Infant arterial stiffness and maternal iron status in pregnancy: A UK birth cohort (Baby VIP Study). *Neonatology*. 2015;107(4):297-303.
44. Ouyang F, Parker MG, Luo Z-C, Wang X, Zhang H-J, Jiang F, et al. Maternal BMI, gestational diabetes, and weight gain in relation to childhood obesity: The mediation effect of placental weight. *Obesity*. 2016;24(4):938-46.
45. Peng S, Deysenroth MA, Di Narzo AF, Cheng H, Zhang Z, Lambertini L, et al. Genetic regulation of the placental transcriptome underlies birth weight and risk of childhood obesity. *PLOS Genetics*. 2019;14(12):e1007799.
46. Roberts H, Woodman AG, Baines KJ, Jeyarajah MJ, Bourque SL, Renaud SJ. Maternal Iron Deficiency Alters Trophoblast Differentiation and Placental Development in Rat Pregnancy. *Endocrinology*. 2021;162(12).

47. Lewis RM, Doherty CB, James LA, Burton GJ, Hales CN. Effects of Maternal Iron Restriction on Placental Vascularization in the Rat. *Placenta*. 2001;22(6):534-9.
48. Gambling L, Charania Z, Hannah L, Antipatis C, Lea RG, McArdle HJ. Effect of Iron Deficiency on Placental Cytokine Expression and Fetal Growth in the Pregnant Rat¹. *Biology of Reproduction*. 2002;66(2):516-23.
49. Gambling L, McArdle HJ. Iron, copper and fetal development. *Proceedings of the Nutrition Society*. 2004;63(4):553-62.
50. Breton CV, Marsit CJ, Faustman E, Nadeau K, Goodrich JM, Dolinoy DC, et al. Small-Magnitude Effect Sizes in Epigenetic End Points are Important in Children's Environmental Health Studies: The Children's Environmental Health and Disease Prevention Research Center's Epigenetics Working Group. *Environ Health Perspect*. 2017;125(4):511-26.
51. Bédard A, Lewis SJ, Burgess S, John Henderson A, Shaheen SO. Maternal iron status during pregnancy and respiratory and atopic outcomes in the offspring: A Mendelian randomisation study. *BMJ Open Respir Res*. 2018;5(1).
52. Kwon EJ, Kim YJ. What is fetal programming?: a lifetime health is under the control of in utero health. *Obstet Gynecol Sci*. 2017;60(6):506-19.
53. Sennels HP, Jørgensen HL, Hansen A-LS, Goetze JP, Fahrenkrug J. Diurnal variation of hematology parameters in healthy young males: The Bispebjerg study of diurnal variations. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2011;71(7):532-41.
54. Haus E, Lakatua DJ, Swoyer J, Sackett-Lundeen L. Chronobiology in hematology and immunology. *Am J Anat*. 1983;168(4):467-517.
55. Touitou Y, Touitou C, Bogdan A, Reinberg A, Auzeby A, Beck H, et al. Differences between young and elderly subjects in seasonal and circadian variations of total plasma proteins and blood volume as reflected by hemoglobin, hematocrit, and erythrocyte counts. *Clin Chem*. 1986;32(5):801-4.
56. Bowers KA, Olsen SF, Bao W, Halldorsson TI, Strøm M, Zhang C. Plasma Concentrations of Ferritin in Early Pregnancy Are Associated with Risk of Gestational

Diabetes Mellitus in Women in the Danish National Birth Cohort. *The Journal of Nutrition*. 2016;146(9):1756-61.

57. Daphne Lvd, Diederick EG, Mark R, Joannes JMM, Hieronymus AV, Yvonne TvdS. Serum Ferritin Is a Risk Factor for Stroke in Postmenopausal Women. *Stroke*. 2005;36(8):1637-41.

58. Nguyen LT, Buse JD, Baskin L, Sadrzadeh SMH, Naugler C. Influence of diurnal variation and fasting on serum iron concentrations in a community-based population. *Clinical Biochemistry*. 2017;50(18):1237-42.

59. Suárez-Ortegón M-F, McLachlan S, Fernandez-Real J-M, Tuomainen T-P, Aregbesola A, Wild SH. Serum ferritin and incident cardiometabolic diseases in Scottish adults. *Cardiovascular Diabetology*. 2022;21(1):26.

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)

¹Values 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).

²Values 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.