



Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study

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Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study

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Keywords: gestational diabetes, iron intake, iron supplementation, pregnancy, haemoglobin

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Abstract

Objective To investigate the possible association between total daily iron intake from both food and supplements during pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes mellitus (GDM) in women at increased risk of GDM.

Design A prospective cohort study (based on a cluster-randomised controlled trial, where the intervention and the usual care groups were combined).

Setting Primary health care maternity clinics in 14 municipalities in south-western Finland.

Participants 399 pregnant women who were at increased risk of GDM participated in a GDM prevention trial and were followed throughout pregnancy.

Main outcome measurements The main outcome was GDM diagnosed with oral glucose tolerance test at 26-28 weeks' gestation or based on a diagnosis recorded in the Finnish Medical Birth registry. Data on iron intake was collected using a 181-item food frequency questionnaire and separate questions for supplement use at 26-28 weeks' gestation.

Results GDM was diagnosed in 72 women (18.1%) in the study population. The odds ratio (OR) for total iron intake as a continuous variable was 1.005 (95% confidence interval 1.000 to 1.011; $P=0.041$) after adjustment for BMI, age, diabetes in first- or second-degree relatives and GDM or macrosomia in earlier pregnancy. Women in the highest fifth of total daily iron intake had an OR of 1.55 (95% confidence interval 0.81 to 2.96; $P=0.19$) for GDM. After excluding participants with low haemoglobin levels (≤ 120 g/l) already in early pregnancy the OR was 2.21 (95% confidence interval 1.11 to 4.41; $P=0.025$).

Conclusions Our results suggest that high iron intake during pregnancy increases the risk of GDM especially in women who are not anaemic in the beginning of the pregnancy and who are at increased risk of GDM. These findings suggest that routine iron supplementation should be reconsidered in this risk group of women.

Trial registration: Current Controlled Trials ISRCTN33885819

INTRODUCTION

Gestational diabetes mellitus (GDM) is a disturbance in glucose metabolism, which is diagnosed during pregnancy and affects 1-14% of pregnancies in different populations¹. The incidence of gestational diabetes has been increasing the last 20 years². Risk factors for GDM include higher maternal body mass index (BMI), higher age and family history of diabetes³. Approximately half of the GDM cases can be explained by overweight⁴. GDM causes short and long term risks to both the mother and the child. The most common result of GDM is newborn macrosomia, which increases several adverse outcomes during delivery such as shoulder dystocia, perineal lacerations, blood loss and increased caesarean birth⁵. GDM also increases the risk of glucose metabolism disorders and type 2 diabetes in later life of the mother and the newborn^{6,7}.

High iron load and disorders of iron metabolism have been associated with an increased risk of disturbances in glucose metabolism⁸⁻¹⁰. In hereditary hemochromatosis iron accumulation in the body leads to diabetes in 30-60% of patients¹¹. Also in animal models iron has been shown to induce diabetes¹². Iron binding medication is effective in preventing diabetes in iron overload conditions¹³. Frequent blood donation has been shown to result in improvement in glucose metabolism even in healthy people¹⁴. Higher iron stores have been found in gestational diabetes patients measured most commonly by serum ferritin¹⁵⁻¹⁷. High haemoglobin level in early pregnancy has been reported to be an independent risk factor for GDM¹⁸ and lower haemoglobin levels and anaemia during pregnancy have been shown to result in lower risk of GDM¹⁹. A large proportion of women, up to 78% in Finland²⁰, use supplemental iron during pregnancy even with good haemoglobin levels and thus it is an important matter to investigate the possible adverse effects of iron. High iron intake could be especially risky for women with already good iron stores.

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3 Studies of total iron intake and the risk of GDM are scarce: only Bowers et al.²¹ have investigated
4 pre-pregnancy total iron intake in relation to the risk of GDM. Other studies have investigated iron
5 intake either from food or supplements only and have thus failed to report the effect of total iron
6 intake^{22 23}. Only one placebo controlled clinical trial has been conducted on supplemental iron and
7 the risk of GDM²⁴. To our knowledge there are no published studies of total iron intake during
8 pregnancy, haemoglobin and the risk of GDM. The objective of this study was to investigate the
9 possible association between total daily iron intake from both food and supplements during
10 pregnancy, haemoglobin levels in early pregnancy and the risk of gestational diabetes in women at
11 increased risk of GDM.
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25 MATERIAL AND METHDOS

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29 The data of this study was originally collected as a part of a larger gestational diabetes prevention
30 trial, which is described in more detail elsewhere²⁵. The effects of the intervention on GDM and
31 newborns birthweight have been reported elsewhere²⁶. This cluster-randomised trial was carried out
32 in 14 municipalities in Pirkanmaa region in south-western Finland in 2007-2009. The municipalities
33 were randomised in pairs into intervention and usual care municipalities.
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43 The inclusion criteria for the trial was to have at least one of the following risk factors: BMI ≥ 25
44 kg/m²; age ≥ 40 years; GDM, glucose intolerance or newborn's macrosomia (≥ 4500 g) in any earlier
45 pregnancy or type 1 or 2 diabetes in first- or second-degree relatives. Women were excluded from
46 the trial if they had an abnormal measurement in the baseline (8-12 weeks' gestation) glucose
47 tolerance test (fasting blood glucose ≥ 5.3 mmol/l, >10.0 mmol/l at 1 h or >8.6 mmol/l at 2 h); pre-
48 pregnancy diabetes (type 1 or 2); age <18 years; no Finnish language skills; multiple pregnancy;
49 restrictions from physical activity; substance abuse or psychiatric illness. The trial was approved by
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3 the ethical committee of Pirkanmaa Hospital District and written informed consent was provided by
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5 the participants.
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10 A total of 2271 women were screened for the study and of them 726 were preliminary eligible to
11 participate in the study²⁶. Of these women 640 (88.2%) gave an informed consent to participate in
12 the trial. At the baseline (8-12 weeks' gestation) oral glucose tolerance test 174 women had an
13 abnormal result and were thus excluded. Furthermore 38 women had a miscarriage and 29 were lost
14 to follow-up. Finally, 399 participants were included in the analyses (219 in the intervention group
15 and 180 in the usual care group).
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25 The intervention group participated in individual counselling on weight gain, physical activity and
26 diet on five antenatal visits in primary health care maternity clinics. The objectives of the
27 counselling were to guide the participants in monitoring their weight gain, increasing or
28 maintaining their leisure time physical activity and achieving a healthy diet fulfilling the national
29 recommendations. The effects of intensified dietary counselling on food habits and the intake of
30 energy, energy-yielding nutrients, fiber, selected fatty acids and cholesterol have been reported
31 elsewhere²⁷. The counselling was not aiming to influence iron intake. In this study the
32 intervention and usual care groups were combined and these groups had no differences in the
33 incidence of GDM or in iron intake.
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48 Background information of the participants was gathered with a baseline questionnaire at the first
49 prenatal visit (8-12 weeks' gestation). Information of pre-pregnancy BMI (weight self reported and
50 height measured at 8-12 weeks' visit) and haemoglobin were abstracted from maternity cards. In
51 this study we used information of haemoglobin measurements from early pregnancy (weeks 8-12,
52 or 16-18 for those with missing values for 8-12 weeks) to determine the status of body iron stores in
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3 the beginning of pregnancy and before the physiological decrease in haemoglobin levels. The
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5 participants were considered to have good haemoglobin levels if they had a haemoglobin
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7 measurement of over 120 g/l according to the lower limit for normal haemoglobin in non-pregnant
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9 women²⁸. The limit for normal haemoglobin in pregnant women (110 g/l) was not used because of
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11 low frequency of cases with haemoglobin level under 110 g/l in early pregnancy (n=7). All women
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13 underwent a 75 g oral glucose tolerance test at 26-28 weeks' gestation. The criterion for gestational
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15 diabetes diagnosis was to have at least one abnormal value: fasting glucose after an overnight fast
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17 ≥ 5.3 mmol/l, blood glucose > 10.0 mmol/l one hour after or > 8.6 mmol/l two hours after consuming
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19 75 g of glucose. To cover all possible cases information of gestational diabetes was abstracted from
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21 the Finnish Medical Birth Register.
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27 Information of diet and supplement use was obtained by a validated 181-item food frequency
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29 questionnaire²⁹. The participants completed the questionnaire at 8-12 weeks' gestation and again at
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31 26-28 and 36-37 weeks' gestation. In the 8-12 weeks' questionnaire the participants were asked
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33 about their dietary habits during one month before beginning of the pregnancy and in 26-28 and 36-
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35 37 weeks' questionnaires the participants were asked about their dietary habits during the previous
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37 month. The completed questionnaires were checked by a nutritionist and those with more than ten
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39 missing values in the frequency data were completed after consulting the participant on the phone.
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41 In the food frequency questionnaire the dietary habits were assessed by detailed questions of
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43 frequency of use (per day, week, month or not at all) and the portion sizes of specific food items.
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45 The participants were also asked to report their supplement use: brand name of the supplement,
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47 dosage and frequency of use (per day, week or month). The gathered data was then entered into a
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49 food database using a software program of the National Institute for Health and Welfare, Helsinki,
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51 Finland, and coded to daily food record form. Nutrient intakes were calculated by using the 10th
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53 release (updated in 2009) of the Finnish Food Composition Database Fineli (www.fineli.fi) and in-
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3 house software of the National Institute for Health and Welfare, Helsinki. In this study information
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5 of dietary habits and supplement use at 26-28 weeks of gestation was used to get the best estimation
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7 of iron intake during pregnancy and before the onset of GDM.
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10 11 **Statistical methods**

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13 The study population was categorised into five equal groups according to their total daily iron
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15 intake. To better see the differences between women in the highest fifth of total iron intake and the
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17 other women, we combined the three groups in the middle (20, 60 and 20 percent). The groups were
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19 tested for possible differences in risk factors and other background characteristics using Chi-square
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21 test, Kruskal-Wallis test or One-way ANOVA. The incidence of GDM was then investigated
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23 between the iron intake groups and the differences were tested statistically using Chi-square test or
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25 Fisher exact test. Haemoglobin was considered as a potential effect modifier. Women were divided
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27 into two groups according to their haemoglobin level in early pregnancy: >120 g/l or ≤ 120 g/l.
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34 Logistic regression was used to assess the odds ratio (OR) for gestational diabetes. The dependent
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36 variable in the regression model was GDM (yes/no). In the first model iron intake was assessed as a
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38 continuous variable. In the second model the highest fifth of total iron intake (intake ≥ 110 mg) was
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40 compared to the rest of the study population (iron intake under 110 mg). The models were adjusted
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42 for BMI, age (both as continuous), diabetes in first- or second-degree relatives and GDM or
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44 macrosomia in earlier pregnancy (both as categorical). Both models were executed separately for all
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46 women and for women with haemoglobin levels over 120 g/l in early pregnancy (8-12 or 16-18
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48 weeks' gestation).
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54 We also added interaction terms for iron intake and haemoglobin and for iron intake and BMI to the
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56 model but they were not statistically significant and therefore are not presented here. In sensitivity
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3 analyses, we examined the association between the total iron intake and the risk of GDM using
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5 alternative cut-off points for categorising participants based on their iron intake (15, 70 and 15
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7 percent; 25, 50 and 25 percent and 30, 40 and 30 percent) and the same statistical tests as described
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9 above. All analyses were performed with SPSS statistics (version 19).
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11 12 13 14 **RESULTS**

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18 Based on the questionnaire completed at 26-28 weeks' gestation, the mean daily iron intake from
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20 food was 14.4 mg (SD 4.3; median 13.9 mg) among the study population. In the study group 65.7%
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22 of women used supplemental iron and the median of supplemental intake was 27.0 mg. The most
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24 used dosages for iron supplementation were 10.0 mg, 20.0 mg and 100.0 mg of elemental iron
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26 (Fe^{2+}). The median for total iron intake was 27.1 mg (interquartile range 15.5-89.3 mg) (Figure 1).
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28 Iron intake from food was similar in nonusers and users of supplemental iron: users of supplemental
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30 iron had a mean daily intake of 14.5 mg (SD 4.5) and nonusers 14.3 mg (SD 3.9). The mean
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32 haemoglobin level of the study population in the beginning of pregnancy was 134 g/l (SD 11; min
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34 107 g/l; max 169 g/l).
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41 The oral glucose tolerance test at 26-28 weeks' gestation was abnormal in 14.0% of the study
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43 population (n=56, missing value for three women). Furthermore 16 women received a gestational
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45 diabetes diagnosis during their pregnancy. Figure 2 shows the incidence of GDM in fifths of total
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47 iron intake. There was a tendency for a higher incidence of GDM in the highest fifth of total iron
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49 intake.
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54 Between the iron intake groups (20%, 60% and 20%) there were no significant differences in terms
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56 of proportion of primigravida, age, diabetes in first- or second-degree relatives or GDM or
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3 macrosomia in earlier pregnancy (Table 1). Women in the highest iron intake group had a slightly
4
5 lower mean BMI as compared to other women (P=0.05). Haemoglobin levels in early pregnancy
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7 differed between the groups as expected (P<0.001). Table 2 shows the incidence of GDM in the
8
9 three iron intake groups. The incidence was higher in the highest iron intake group (20.8%)
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11 compared to the other two groups (16.7% in both) but the difference was not statistically significant
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13 (P=0.70). After excluding those who had low haemoglobin levels (≤ 120 g/l) already in early
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15 pregnancy, the difference between the groups was even larger but still not statistically significant
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17 (P=0.11).
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23 The logistic regression model shows that after adjustment for risk factors and background
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25 characteristics total iron intake was significantly and positively associated with gestational diabetes
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27 mellitus (Table 3). When iron intake was used as a continuous variable the OR was 1.005 (P=0.041)
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29 for all women and 1.008 (P=0.005) for those with haemoglobin levels over 120 g/l. In the second
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31 model the OR for GDM was 1.55 (95% confidence interval 0.81 to 2.96, P=0.19) for the highest
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33 fifth of iron intake among all women. After excluding women with low haemoglobin levels (≤ 120
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35 g/l) in early pregnancy the OR was 2.21 (95% confidence interval 1.11 to 4.41, P=0.025).
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41 In sensitivity analyses the odds ratios were higher when 15% of women with the highest iron intake
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43 were compared to the rest 85% of women. The odds ratios were lower when 25% or 30% of women
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45 with highest intake were compared to the rest 75% and 70% of women respectively (results not
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47 shown).
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50 51 52 **DISCUSSION** 53 54 55 56 57 58 59 60

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3 In this study we investigated the possible association between total daily iron intake during
4 pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes. To our knowledge
5 there are no similar studies published previously. We discovered that there was a tendency for a
6 higher incidence of GDM in the highest fifth of total iron intake. High iron intake increased the risk
7 of GDM especially for those women who had good haemoglobin levels in early pregnancy: we
8 discovered a two-fold OR for GDM for women in the highest fifth of iron intake with a
9 haemoglobin level of over 120 g/l in early pregnancy.
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20 Our study has several strengths. Firstly unlike most of the previous studies we had detailed
21 information of iron intake from both food and supplements. Secondly we had information of
22 baseline OGTT and could therefore exclude women with undiagnosed prepregnancy diabetes.
23 Additionally Finnish Medical Birth Registry could be utilized to cover those GDM cases that were
24 undiagnosed in the 26-28 weeks' OGTT. We also had information of haemoglobin measurements
25 of the study population during pregnancy although measured by nurses. All of our data was
26 collected prospectively. The participation rate in the original trial was high (88% in both the
27 intervention and the usual care groups) improving the generalisability of the results to other women
28 with risk factors for GDM.
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43 A limitation of this kind of study is that estimating iron intake during pregnancy can be challenging
44 because dietary habits and use of supplements can vary a lot during pregnancy due to nausea and
45 other changes in well being. Iron intake was assessed at a certain point in time, thus it does not
46 cover the intake during the whole pregnancy. In our study we decided to use information of iron
47 intake in mid-pregnancy to get the best estimation on iron intake during pregnancy and before the
48 onset of GDM. We did not use information of iron intake abstracted in the beginning of pregnancy
49 because it covered only dietary habits and supplement use during one month before the pregnancy
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3 when use of supplemental iron is rare. However our information of iron intake covers only one
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5 month in the mid-pregnancy. Additionally we could not assess iron absorption, for example with
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7 blood measurements, which also can vary a lot depending on body iron status and the contents of
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9 meals.
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14 There are also limitations concerning the haemoglobin measurements used in our study. In the
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16 maternity clinics haemoglobin levels are usually screened with a capillary haemoglobin
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18 measurement using finger-stick samples. This method has been demonstrated to be reliable in
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20 determining haemoglobin values but it is however susceptible to handling errors³⁰ and therefore
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22 there can be variation in the test results. Additionally we had a lot of missing values in haemoglobin
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24 measurements and thus we decided to use the haemoglobin measurement at 16-18 weeks' gestation
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26 for those with missing values at weeks 8-12 (n= 28). Although measurements at weeks 16-18 do not
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28 present the haemoglobin levels at early pregnancy our objective was to analyse separately women
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30 who were not anaemic and thus including women who had a haemoglobin level of over 120 g/l still
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32 at weeks 16-18 would help us not to underestimate the proportion of women who were not anaemic
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34 in the beginning of the pregnancy. However for these reasons our results in respect of haemoglobin
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36 measurements should be considered with caution and further studies with reliable and
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38 comprehensive haemoglobin measurements are warranted to confirm our results.
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45 In a somewhat similar study Bo et al.²² discovered that women who used supplemental iron during
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47 mid-pregnancy had a 2- to 3-fold risk of GDM. They however had no information of dietary intake
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49 of iron or haemoglobin levels or other measurements of iron stores. Their findings are however in
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51 line with our results which suggest that dietary intake could play a relatively small part in the
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53 association whereas high supplemental iron intake could be responsible for most of the increase in
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55 GDM risk. Somewhat different results have been reported recently by Bowers et al.²¹ and Qiu et
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3 al.²³. Bowers et al.²¹ investigated dietary and supplemental iron intake during one year before
4 pregnancy utilising the material from the Nurses Health study. They observed no significant effect
5 of total, nonheme or supplemental iron intake on the risk of GDM. It can be argued though, that
6 usually iron supplementation outside of pregnancy is more rare and concerns mainly those who are
7 anaemic. However they did find a significant and positive association between heme iron intake and
8 GDM. Similarly Qiu et al.²³ demonstrated an association between heme iron intake during the time
9 before conception and in early pregnancy and the risk of GDM. To our knowledge only one placebo
10 controlled clinical trial has been conducted to investigate the association between iron
11 supplementation and GDM²⁴. This study did not observe any association. However it can be argued
12 that the intake of supplemental iron was quite low in this study because of only about 50%
13 compliance to a daily supplement of 60 mg of elemental iron.
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29 Iron is a highly reactive component with a possibility to participate in harmful reactions³¹. The
30 human body can excrete iron with very limited mechanisms and thus iron intake is highly regulated
31 according to body iron needs³². Iron could interfere with glucose metabolism in several ways. For
32 example following mechanisms have been proposed: Iron decreases insulin extraction and
33 metabolism in the liver, which leads to peripheral hyperinsulinemia³³. Iron overload results in
34 oxidative stress in pancreatic β -cells, which leads to destruction of the pancreatic islets and thus
35 decreases insulin secretion³⁴. The exact mechanisms, which link iron to diabetes are still unsolved.
36 However the association can be argued to be biologically plausible.
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49 Accumulating body of evidence supports the hypothesis of excess iron as a risk factor for glucose
50 metabolism disorders such as gestational diabetes. Iron deficiency anaemia has been shown to
51 reduce the incidence of GDM¹⁹. Respectively high haemoglobin level (>130 g/l) in early pregnancy
52 has been demonstrated to be an independent risk factor for GDM¹⁸. It seems that iron can affect
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3 glucose metabolism even with no overt iron overload. Women diagnosed with GDM have been
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5 observed to have increased iron stores compared to women without GDM^{15 17}. However many of
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7 these studies have been cross-sectional and it has been criticised that higher serum ferritin levels
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9 could in fact reflect inflammation in the body and could be rather a result than a cause for
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11 diabetes¹⁶.
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16 In summary it seems that high iron intake might be a factor, which increases the risk of GDM
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18 especially in women with already good iron stores. Use of iron supplements during pregnancy is
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20 common even in women with good haemoglobin levels. Our results suggest that routine use of iron
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22 supplements should be reconsidered in non-anaemic women with risk factors for GDM. To confirm
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24 the hypothesis there is need for a large prospective study – ideally a randomised controlled trial –
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26 with reliable and comprehensive information on iron intake from food and supplements during
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28 pregnancy accompanied with serum measurements to determine the level of body iron stores.
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32 33 34 **Author contributions**

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36 AH, TIK and RL designed and conducted research; AH and JR performed statistical analyses; AH
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38 drafted the paper and revised it based on the comments from the other authors. TIK and RL have
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40 the primary responsibility for final content. All authors read, commented and approved the final
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42 manuscript.
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50
51 for dietary calculations. Jatta Puhkala is acknowledged for checking and coding the dietary data.
52
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55 in implementing the study protocol and the pregnant women who participated in the study.
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A competing interest declaration

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that they have no financial or non-financial interests that may be relevant to the submitted work.

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What is already known on this subject

- High iron stores and high iron intake have been associated with the risk of GDM in several studies.
- Anaemia is known to result in lower incidence of GDM.
- There are no previous studies available in which total daily iron intake during pregnancy and haemoglobin levels have been studied in relation to GDM risk.

What this study adds

- Our results suggest that high iron intake during pregnancy increases the risk of GDM especially in women who are not anaemic in the beginning of the pregnancy and who are at increased risk of GDM.
- Our findings suggest that routine iron supplementation should be reconsidered in this risk group of women.

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3 **Figure legends**

4
5 **Figure 1.** Total iron intake in mid pregnancy (26-28 weeks' gestation)

6
7 **Figure 2.** Incidence of GDM (%) in fifths of total iron intake

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Table 1. Background characteristics of iron intake groups (20%, 60% and 20%), means (SD) or frequencies (%)

	20%	60%	20%	P-value
	<14.2 mg/day	14.2 – 109.9 mg/day	110.0+ mg/day	
	(n=78)	(n=233)	(n=78)	
Total Fe intake (mg/day)	11.6 (2.0)	36.0 (25.1)	136.2 (36.5)	-
Primigravida	34 (43.6%)	96 (41.2%)	43 (55.1%)	0.099 ²
BMI (kg/m ²)	26.7 (4.4)	26.5 (4.7)	25.2 (4.3)	0.050 ³
Age (years)	29.3 (5.1)	29.8 (4.4)	29.6 (5.4)	0.70 ³
Diabetes in first- or second-degree relatives	44 (57.1%)	127 (54.5%)	48 (61.5%)	0.55 ²
GDM or macrosomia in previous pregnancy	14 (18.2%)	33 (14.2%)	10 (12.8%)	0.60 ²
Hb (8-12 weeks ¹) (g/l)	135.4 (9.6)	134.0 (10.9)	127.0 (10.0)	<0.001 ⁴

¹ 16-18 for those with missing values at 8-12 weeks (n=28), missing: Hb n=5, 20 and 6, respectively

² Chi-square test, ³ Kruskal-Wallis test, ⁴ One-way ANOVA

Table 2. GDM frequency in iron intake groups (20%, 60% and 20%)

	N	GDM, frequency (%)	P-value
All women			
(20%) <14.2 mg/day	78	13 (16.7)	0.70 ²
(60%) 14.2-109.9 mg/day	233	39 (16.7)	
(20%) 110.0+ mg/day	78	16 (20.8)	
Hb (8-12 weeks¹) >120 g/l			
(20%) <14.2 mg	69	13 (18.8)	0.11 ²
(60%) 14.2-109.9 mg	194	30 (15.5)	
(20%) 110.0+ mg	58	16 (27.6)	
Hb (8-12 weeks¹) ≤120 g/l			
(20%) <14.2 mg	3	0	0.45 ³
(60%) 14.2-109.9 mg	21	3 (14.3)	
(20%) 110.0+ mg	12	0	

¹ 16-18 for those with missing values at 8-12 weeks (n=28)

² Chi-square test

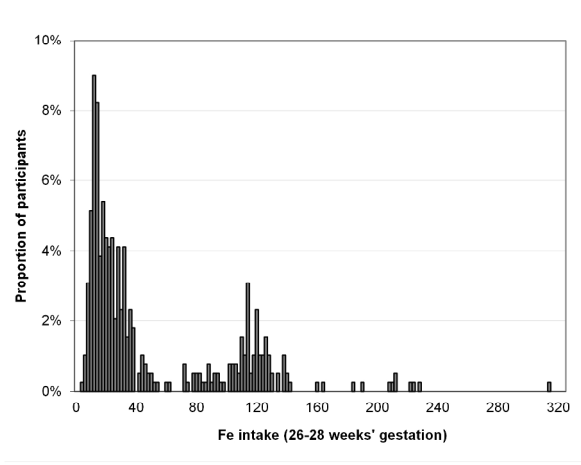
³ Fisher's exact test

Table 3. Incidence of GDM, unadjusted and adjusted ORs (95% CI) for iron intake at 26-28 weeks' gestation as a continuous and a categorical variable separately for all women and for women with haemoglobin >120 g/l

	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI) ²	P-value
Fe intake as a continuous variable				
<i>All women</i>				
Total Fe intake (mg/day)	1.004 (0.999 – 1.008)	0.16	1.005 (1.000 – 1.011)	0.041
BMI			1.11 (1.05 – 1.18)	<0.001
Age			1.03 (0.97 – 1.09)	0.32
Diabetes in first- or second-degree relatives			0.91 (0.52 – 1.59)	0.75
GDM or macrosomia in a previous pregnancy			1.95 (0.97 – 3.89)	0.059
<i>Women with Hb (8-12 weeks' gestation¹) >120 g/l</i>				
Total Fe intake (mg/day)	1.007 (1.001 – 1.012)	0.013	1.008 (1.003 – 1.014)	0.005
BMI			1.11 (1.04 – 1.18)	0.001
Age			1.03 (0.97 – 1.10)	0.28
Diabetes in first- or second-degree relatives			0.80 (0.44 – 1.46)	0.46
GDM or macrosomia in a previous pregnancy			2.03 (0.96 – 4.29)	0.065
Fe intake as a categorical variable				
<i>All women</i>				
Total Fe intake (the highest 20% vs. the lowest 80%)	1.31 (0.70 – 2.44)	0.40	1.55 (0.81 – 2.96)	0.19
BMI			1.11 (1.05 – 1.17)	<0.001
Age			1.03 (0.97 – 1.09)	0.29
Diabetes in first- or second-degree relatives			0.89 (0.51 – 1.55)	0.68
GDM or macrosomia in a previous pregnancy			1.84 (0.92 – 3.64)	0.083
<i>Women with Hb (8-12 weeks' gestation¹) >120 g/l</i>				
Total Fe intake (the highest 20% vs. the lowest 80%)	1.95 (1.005 – 3.78)	0.048	2.21 (1.11 – 4.41)	0.025
BMI			1.10 (1.04 – 1.17)	0.002
Age			1.04 (0.98 – 1.10)	0.24
Diabetes in first- or second-degree relatives			0.78 (0.43 – 1.41)	0.41
GDM or macrosomia in a previous pregnancy			1.87 (0.89 – 3.93)	0.10

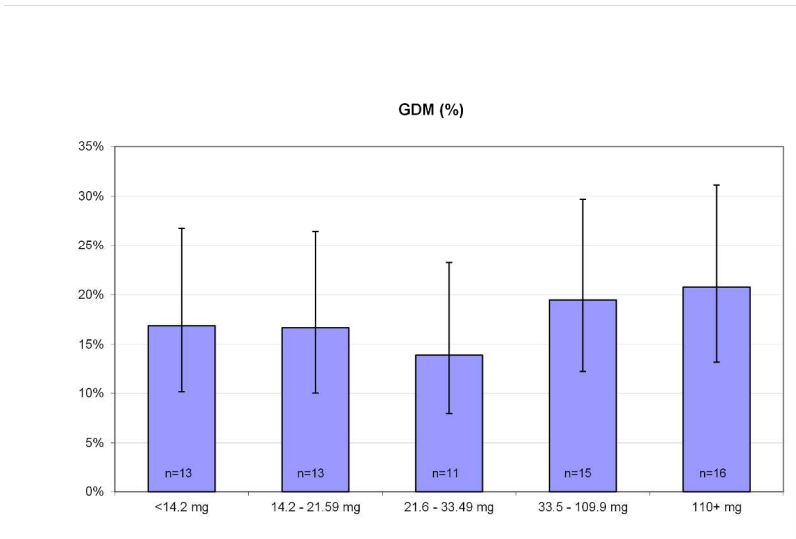
¹ 16-18 weeks' gestation for those with missing values at 8-12 weeks' gestation (n=28) ² Adjusted for other variables in the model (total Fe intake, BMI, age, diabetes in first- or second-degree relatives and GDM or macrosomia in a previous pregnancy)

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Total iron intake in mid pregnancy (26-28 weeks' gestation)
209x297mm (300 x 300 DPI)

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Incidence of GDM (%) in fifths of total iron intake
209x297mm (300 x 300 DPI)

Annika Helin, Tarja I Kinnunen, Jani Raitanen, Riitta Luoto: **Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study**

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Reported on page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4,5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4,5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(b) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	Luoto et al. 2010 (see list of references)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	7,8
		(c) Explain how missing data were addressed	5
		(d) If applicable, explain how loss to follow-up was addressed	
		(e) Describe any sensitivity analyses	7,8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	

1	Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, Figure 1, Table 1
2			(b) Indicate number of participants with missing data for each variable of interest	8, Table 1, Table 2
3			(c) Summarise follow-up time (eg, average and total amount)	
4	Outcome data	15*	Report numbers of outcome events or summary measures over time	8
5	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9, Table 3
6			(b) Report category boundaries when continuous variables were categorized	Table 1
7			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
8	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
9	Discussion			
10	Key results	18	Summarise key results with reference to study objectives	10
11	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10,11
12	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-13
13	Generalisability	21	Discuss the generalisability (external validity) of the study results	10,13
14	Other information			
15	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.



Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study

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Secondary Subject Heading:	Epidemiology, Diabetes and endocrinology, Nutrition and metabolism
Keywords:	gestational diabetes , iron intake, iron supplementation, pregnancy, haemoglobin

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Manuscripts

Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study

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Abstract

Objective To investigate the possible association between total daily iron intake during pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes mellitus (GDM) in women at increased risk of GDM.

Design A prospective cohort study (based on a cluster-randomised controlled trial, where the intervention and the usual care groups were combined).

Setting Primary health care maternity clinics in 14 municipalities in south-western Finland.

Participants 399 pregnant women who were at increased risk of GDM participated in a GDM prevention trial and were followed throughout pregnancy.

Main outcome measurements The main outcome was GDM diagnosed with oral glucose tolerance test at 26-28 weeks' gestation or based on a diagnosis recorded in the Finnish Medical Birth registry. Data on iron intake was collected using a 181-item food frequency questionnaire and separate questions for supplement use at 26-28 weeks' gestation.

Results GDM was diagnosed in 72 women (18.1%) in the study population. The odds ratio (OR) for total iron intake as a continuous variable was 1.006 (95% confidence interval 1.000 to 1.011; P=0.038) after adjustment for BMI, age, diabetes in first- or second-degree relatives, GDM or macrosomia in earlier pregnancy, total energy intake, dietary fibre, saturated fatty acids and total gestational weight gain. Women in the highest fifth of total daily iron intake had an adjusted OR of 1.66 (95% confidence interval 0.84 to 3.30; P=0.15) for GDM. After excluding participants with low haemoglobin levels (≤ 120 g/l) already in early pregnancy the adjusted OR was 2.35 (95% confidence interval 1.13 to 4.92; P=0.023). **Conclusions** Our results suggest that high iron intake during pregnancy increases the risk of GDM especially in women who are not anaemic in early pregnancy and who are at increased risk of GDM. These findings suggest that routine iron supplementation should be reconsidered in this risk group of women.

Trial registration: Current Controlled Trials ISRCTN33885819

ARTICLE SUMMARY

Article focus

- The objective of this study was to investigate the possible association between total daily iron intake from both food and supplements during pregnancy, haemoglobin levels in early pregnancy and the risk of gestational diabetes in women at increased risk of GDM.

Key messages

- Our results suggest that high iron intake during pregnancy increases the risk of GDM especially in women who are not anaemic in the beginning of the pregnancy and who are at increased risk of GDM.
- Our findings suggest that routine iron supplementation should be reconsidered in this risk group of women.

Strengths and limitations of this study

Strengths:

- Unlike most of the previous studies we had detailed information of iron intake from both food and supplements.
- We had information of baseline OGTT and could therefore exclude women with undiagnosed prepregnancy diabetes. Additionally Finnish Medical Birth Registry could be utilized to cover those GDM cases that were undiagnosed in the 26-28 weeks' OGTT.
- We also had information of haemoglobin measurements of the study population during pregnancy although measured by nurses.
- All of our data was collected prospectively.
- The participation rate in the original trial was high (88% in both the intervention and the usual care groups) improving the generalisability of the results to other women with risk

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3 factors for GDM.
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7 Limitations:
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10 - Iron intake was assessed at a certain point in time, thus it does not cover the intake during
11 the whole pregnancy.
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14 - We could not assess iron absorption.
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17 - The haemoglobin levels were screened at the maternity clinics using finger-stick samples,
18 which may not be an optimal measurement tool.
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21 Haemoglobin measurements were missing for 28 women at 8-12 weeks' gestation and we
22 used the measurement at 16-18 weeks' gestation for those women.
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INTRODUCTION

Gestational diabetes mellitus (GDM) is a disturbance in glucose metabolism, which is diagnosed during pregnancy and affects 1-14% of pregnancies in different populations¹. The incidence of gestational diabetes has been increasing the last 20 years². Risk factors for GDM include higher maternal body mass index (BMI), higher age and family history of diabetes³. Approximately half of the GDM cases can be explained by overweight⁴. GDM causes short and long term risks to both the mother and the child. The most common result of GDM is newborn macrosomia, which increases several adverse outcomes during delivery such as shoulder dystocia, perineal lacerations, blood loss and increased caesarean birth⁵. GDM also increases the risk of glucose metabolism disorders and type 2 diabetes in later life of the mother and the newborn^{6,7}.

High iron load and disorders of iron metabolism have been associated with an increased risk of disturbances in glucose metabolism⁸⁻¹⁰. In hereditary hemochromatosis iron accumulation in the body leads to diabetes in 30-60% of patients¹¹. Also in animal models iron has been shown to induce diabetes¹². Iron binding medication is effective in preventing diabetes in iron overload conditions¹³. Frequent blood donation has been shown to result in improvement in glucose metabolism even in healthy people¹⁴. Higher iron stores have been found in gestational diabetes patients measured most commonly by serum ferritin¹⁵⁻¹⁷. High haemoglobin level in early pregnancy has been reported to be an independent risk factor for GDM¹⁸ and lower haemoglobin levels and anaemia during pregnancy have been shown to result in lower risk of GDM¹⁹. A large proportion of women, up to 78% in Finland²⁰, use supplemental iron during pregnancy even with good haemoglobin levels and thus it is an important matter to investigate the possible adverse effects of iron. High iron intake could be especially risky for women with already good iron stores.

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3 Studies of total iron intake and the risk of GDM are scarce: only Bowers et al.²¹ have investigated
4 pre-pregnancy total iron intake in relation to the risk of GDM. Other studies have investigated iron
5 intake either from food or supplements only and have thus failed to report the effect of total iron
6 intake^{22 23}. Only one placebo controlled clinical trial has been conducted on supplemental iron and
7 the risk of GDM²⁴. To our knowledge there are no published studies of total iron intake during
8 pregnancy, haemoglobin and the risk of GDM. The objective of this study was to investigate the
9 possible association between total daily iron intake from both food and supplements during
10 pregnancy, haemoglobin levels in early pregnancy and the risk of gestational diabetes in women at
11 increased risk of GDM.
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25 MATERIAL AND METHDOS

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29 The data of this study was originally collected as a part of a larger gestational diabetes prevention
30 trial, which is described in more detail elsewhere²⁵. The effects of the intervention on GDM and
31 newborns birthweight have been reported elsewhere²⁶. This cluster-randomised trial was carried out
32 in 14 municipalities in Pirkanmaa region in south-western Finland in 2007-2009. The municipalities
33 were randomised in pairs into intervention and usual care municipalities.
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43 The inclusion criteria for the trial were to have at least one of the following risk factors: BMI ≥ 25
44 kg/m²; age ≥ 40 years; GDM, glucose intolerance or newborn's macrosomia (≥ 4500 g) in any earlier
45 pregnancy or type 1 or 2 diabetes in first- or second-degree relatives. Women were excluded from
46 the trial if they had an abnormal measurement in the baseline (8-12 weeks' gestation) glucose
47 tolerance test (fasting blood glucose ≥ 5.3 mmol/l, >10.0 mmol/l at 1 h or >8.6 mmol/l at 2 h); pre-
48 pregnancy diabetes (type 1 or 2); age <18 years; no Finnish language skills; multiple pregnancy;
49 restrictions from physical activity; substance abuse or psychiatric illness. The trial was approved by
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3 the ethical committee of Pirkanmaa Hospital District and written informed consent was provided by
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5 the participants.
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10 A total of 2271 women were screened for the study and of them 726 were preliminary eligible to
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12 participate in the study²⁶. Of these women 640 (88.2%) gave an informed consent to participate in
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14 the trial. At the baseline (8-12 weeks' gestation) oral glucose tolerance test 174 women had an
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16 abnormal result and were thus excluded. Furthermore 38 women had a miscarriage and 29 were lost
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18 to follow-up. Finally, 399 participants were included in the analyses (219 in the intervention group
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20 and 180 in the usual care group).
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25 The intervention group participated in individual counselling on weight gain, physical activity and
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27 diet on five antenatal visits in primary health care maternity clinics. The objectives of the
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29 counselling were to guide the participants in monitoring their weight gain, increasing or
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31 maintaining their leisure time physical activity and achieving a healthy diet fulfilling the national
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33 recommendations. The effects of intensified dietary counselling on food habits and the intake of
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35 energy, energy-yielding nutrients, fiber, selected fatty acids and cholesterol have been reported
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37 elsewhere²⁷. The counselling was not aiming to influence in iron intake. In this study the
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39 intervention and usual care groups were combined and these groups had no differences in the
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41 incidence of GDM or in iron intake.
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47 Background information of the participants was gathered with a baseline questionnaire at the first
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49 prenatal visit (8-12 weeks' gestation). Information of pre-pregnancy BMI (weight self reported and
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51 height measured at 8-12 weeks' visit) and haemoglobin were abstracted from maternity cards. In
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53 this study we used information of haemoglobin measurements from early pregnancy (weeks 8-12,
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55 or 16-18 for those with missing values for 8-12 weeks) to determine the status of body iron stores in
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3 the beginning of pregnancy and before the physiological decrease in haemoglobin levels. The
4 participants were considered to have good haemoglobin levels if they had a haemoglobin
5 measurement of over 120 g/l according to the lower limit for normal haemoglobin in non-pregnant
6 women²⁸. The limit for normal haemoglobin in pregnant women (110 g/l) was not used because of
7 low frequency of cases with haemoglobin level under 110 g/l in early pregnancy (n=7). All women
8 underwent a 75 g oral glucose tolerance test at 26-28 weeks' gestation. The criterion for gestational
9 diabetes diagnosis was to have at least one abnormal value: fasting glucose after an overnight fast
10 ≥ 5.3 mmol/l, blood glucose >10.0 mmol/l one hour after or >8.6 mmol/l two hours after consuming
11 75 g of glucose. To cover all possible cases information of gestational diabetes was abstracted from
12 the Finnish Medical Birth Register.
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27 Information on diet and supplement use was obtained by a validated 181-item food frequency
28 questionnaire²⁹ although the validity of the supplement data was not assessed in the study by
29 Erkkola et al. The participants completed the questionnaire at 8-12 weeks' gestation and again at
30 26-28 and 36-37 weeks' gestation. In the 8-12 weeks' questionnaire the participants were asked
31 about their dietary habits during one month before beginning of the pregnancy and in 26-28 and 36-
32 37 weeks' questionnaires the participants were asked about their dietary habits during the previous
33 month. The completed questionnaires were checked by a nutritionist and those with more than ten
34 missing values in the frequency data were completed after consulting the participant on the phone.
35 In the food frequency questionnaire the dietary habits were assessed by detailed questions of
36 frequency of use (per day, week, month or not at all) and the portion sizes of specific food items.
37 The participants were also asked to report their supplement use: brand name of the supplement,
38 dosage and frequency of use (per day, week or month). The gathered data was then entered into a
39 food database using a software program of the National Institute for Health and Welfare, Helsinki,
40 Finland, and coded to daily food record form. Nutrient intakes were calculated by using the 10th
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3 release (updated in 2009) of the Finnish Food Composition Database Fineli (www.fineli.fi) and in-
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5 house software of the National Institute for Health and Welfare, Helsinki. In this study information
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7 of dietary habits and supplement use at 26-28 weeks of gestation was used to get the best estimation
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9 of iron intake and the intake of energy, macronutrients and dietary fibre during pregnancy and
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11 before the onset of GDM.
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13 14 15 16 **Statistical methods**

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18 The study population was categorised into five equal groups according to their total daily iron
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20 intake. To better see the differences between women in the highest fifth of total iron intake and the
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22 other women, we combined the three groups in the middle (20, 60 and 20 percent). The groups were
23
24 tested for possible differences in risk factors, dietary intake and other background characteristics
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26 using Chi-square test, Kruskal-Wallis test or One-way ANOVA. The incidence of GDM was then
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28 investigated between the iron intake groups and the differences were tested statistically using Chi-
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30 square test or Fisher exact test. Haemoglobin was considered as a potential effect modifier. Women
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32 were divided into two groups according to their haemoglobin level in early pregnancy: >120 g/l or
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34 ≤120 g/l.
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41 Logistic regression was used to assess the odds ratio (OR) for gestational diabetes. The dependent
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43 variable in the regression model was GDM (yes/no). In the first model iron intake was assessed as a
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45 continuous variable. In the second model the highest fifth of total iron intake (intake ≥110 mg) was
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47 compared to the rest of the study population (iron intake under 110 mg). The models were adjusted
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49 for BMI, age, intake of energy, saturated fatty acids and dietary fibre and total gestational weight
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51 gain (all as continuous) as well as diabetes in first- or second-degree relatives and GDM or
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53 macrosomia in earlier pregnancy (both as categorical). Both models were executed separately for all
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3 women and for women with haemoglobin levels over 120 g/l in early pregnancy (8-12 or 16-18
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5 weeks' gestation).
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10 We also added interaction terms for iron intake and haemoglobin and for iron intake and BMI to the
11 model but they were not statistically significant and therefore are not presented here. In sensitivity
12 analyses, we examined the association between the total iron intake and the risk of GDM using
13 alternative cut-off points for categorising participants based on their iron intake (15, 70 and 15
14 percent; 25, 50 and 25 percent and 30, 40 and 30 percent) and the same statistical tests as described
15 above. All analyses were performed with SPSS statistics (version 19).
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24 25 **RESULTS**

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29 Based on the questionnaire completed at 26-28 weeks' gestation, the mean daily iron intake from
30 food was 14.4 mg (SD 4.3; median 13.9 mg) among the study population. In the study group 65.7%
31 of women used supplemental iron and the median of supplemental intake was 27.0 mg. The most
32 used dosages for iron supplementation were 10.0 mg, 20.0 mg and 100.0 mg of elemental iron
33 (Fe^{2+}). The median for total iron intake was 27.1 mg (interquartile range 15.5-89.3 mg) (Figure 1).
34 Iron intake from food was similar in nonusers and users of supplemental iron: users of supplemental
35 iron had a mean daily intake of 14.5 mg (SD 4.5) and nonusers 14.3 mg (SD 3.9). The mean
36 haemoglobin level of the study population in the beginning of pregnancy was 134 g/l (SD 11; min
37 107 g/l; max 169 g/l).
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51 The oral glucose tolerance test at 26-28 weeks' gestation was abnormal in 14.0% of the study
52 population (n=56, missing value for three women). Furthermore 16 women received a gestational
53 diabetes diagnosis during their pregnancy. Figure 2 shows the incidence of GDM in fifths of total
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3 iron intake. There was a tendency for a higher incidence of GDM in the highest fifth of total iron
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5 intake.

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10 Between the iron intake groups (20%, 60% and 20%) there were no significant differences in terms
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12 of proportion of primigravida, age, diabetes in first- or second-degree relatives or GDM or
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14 macrosomia in earlier pregnancy (Table 1). Women in the highest iron intake group had a slightly
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16 lower mean BMI as compared to other women ($P=0.05$). Haemoglobin levels in early pregnancy
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18 differed between the groups as expected ($P<0.001$). With regard to differences in dietary intake,
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20 women in the lowest iron intake group had lower total energy and dietary fibre intakes than the
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22 other women whereas women in the highest iron intake group had higher intake of saturated fatty
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24 acids than the other women. Total gestational weight gain did not differ between the iron intake
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26 groups. Table 2 shows the incidence of GDM in the three iron intake groups. The incidence was
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28 higher in the highest iron intake group (20.8%) compared to the other two groups (16.7% in both)
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30 but the difference was not statistically significant ($P=0.70$). When including women with
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32 haemoglobin levels >120 g/l in early pregnancy ($n=321$, mean Hb 135, SD 9), the difference
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34 between the groups was even larger but still not statistically significant ($P=0.11$).
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41 The logistic regression model shows that after adjustment for potential risk factors and background
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43 characteristics total iron intake was significantly and positively associated with gestational diabetes
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45 mellitus (Table 3). When iron intake was used as a continuous variable the OR was 1.006 ($P=0.038$)
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47 for all women and 1.009 ($P=0.006$) for those with haemoglobin levels over 120 g/l. In the second
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49 model the OR for GDM was 1.66 (95% confidence interval 0.84 to 3.30, $P=0.15$) for the highest
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51 fifth of iron intake among all women. After excluding women with low haemoglobin levels (≤ 120
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53 g/l) in early pregnancy the OR was 2.35 (95% confidence interval 1.13 to 4.92, $P=0.023$).
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57 In sensitivity analyses the odds ratios were higher when 15% of women with the highest iron intake
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3 were compared to the rest 85% of women. The odds ratios were lower when 25% or 30% of women
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5 with highest intake were compared to the rest 75% and 70% of women respectively (results not
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7 shown).

11 DISCUSSION

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16 In this study we investigated the possible association between total daily iron intake during
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18 pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes. To our knowledge
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20 there are no similar studies published previously. We discovered that there was a tendency for a
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22 higher incidence of GDM in the highest fifth of total iron intake. High iron intake increased the risk
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24 of GDM especially for those women who had good haemoglobin levels in early pregnancy: we
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26 discovered a two-fold OR for GDM for women in the highest fifth of iron intake with a
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28 haemoglobin level of over 120 g/l in early pregnancy.
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34 Our study has several strengths. Firstly unlike most of the previous studies we had detailed
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36 information of iron intake from both food and supplements. Secondly we had information on
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38 baseline OGTT and could therefore exclude women with undiagnosed prepregnancy diabetes.
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40 Additionally Finnish Medical Birth Registry could be utilized to cover those GDM cases that were
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42 undiagnosed in the 26-28 weeks' OGTT. We also had information of haemoglobin measurements
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44 of the study population during pregnancy although measured by nurses. All of our data was
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46 collected prospectively. The participation rate in the original trial was high (88% in both the
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48 intervention and the usual care groups) improving the generalisability of the results to other women
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50 with risk factors for GDM.
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3 A limitation of this kind of study is that estimating iron intake during pregnancy can be challenging
4 because dietary habits and use of supplements can vary a lot during pregnancy due to nausea and
5 other changes in wellbeing. Iron intake was assessed at a certain point in time (at 26-28 weeks'
6 gestation covering the previous month), thus it does not cover the intake during the whole
7 pregnancy. In our study we decided to use information of iron intake in mid-pregnancy to get the
8 best estimation on iron intake during pregnancy and before the onset of GDM. We did not use
9 information of iron intake abstracted in the beginning of pregnancy because it covered only dietary
10 habits and supplement use during one month before the pregnancy when use of supplemental iron is
11 rare. However our information on iron intake covers only one month in the mid-pregnancy.
12 Additionally we could not assess iron absorption, for example with blood measurements, which also
13 can vary a lot depending on body iron status and the contents of meals.
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29 There are also limitations concerning the haemoglobin measurements used in our study. In the
30 maternity clinics haemoglobin levels are usually screened with a capillary haemoglobin
31 measurement using finger-stick samples. This method has been demonstrated to be reliable in
32 determining haemoglobin values but it is however susceptible to handling errors³⁰ and therefore
33 there can be variation in the test results. Additionally we had a lot of missing values in haemoglobin
34 measurements and thus we decided to use the haemoglobin measurement at 16-18 weeks' gestation
35 for those with missing values at weeks 8-12 (n= 28). Although measurements at weeks 16-18 do not
36 present the haemoglobin levels at early pregnancy our objective was to analyse separately women
37 who were not anaemic and thus including women who had a haemoglobin level of over 120 g/l still
38 at weeks 16-18 would help us not to underestimate the proportion of women who were not anaemic
39 in the beginning of the pregnancy. However for these reasons our results in respect of haemoglobin
40 measurements should be considered with caution and further studies with reliable and
41 comprehensive haemoglobin measurements are warranted to confirm our results.
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5 In a somewhat similar study Bo et al.²² discovered that women who used supplemental iron during
6 mid-pregnancy had a 2- to 3-fold risk of GDM. They however had no information of dietary intake
7 of iron or haemoglobin levels or other measurements of iron stores. Their findings are however in
8 line with our results which suggest that dietary intake could play a relatively small part in the
9 association whereas high supplemental iron intake could be responsible for most of the increase in
10 GDM risk. Somewhat different results have been reported recently by Bowers et al.²¹ and Qiu et
11 al.²³. Bowers et al.²¹ investigated dietary and supplemental iron intake during one year before
12 pregnancy utilising the material from the Nurses Health study. They observed no significant effect
13 of total, nonheme or supplemental iron intake on the risk of GDM. It can be argued though, that
14 usually iron supplementation outside of pregnancy is more rare and concerns mainly those who are
15 anaemic. However they did find a significant and positive association between heme iron intake and
16 GDM. Similarly Qiu et al.²³ demonstrated an association between heme iron intake during the time
17 before conception and in early pregnancy and the risk of GDM. To our knowledge only one placebo
18 controlled clinical trial has been conducted to investigate the association between iron
19 supplementation and GDM²⁴. This study did not observe any association. However it can be argued
20 that the intake of supplemental iron was quite low in this study because of only about 50%
21 compliance to a daily supplement of 60 mg of elemental iron.
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45 Iron is a highly reactive component with a possibility to participate in harmful reactions³¹. The
46 human body can excrete iron with very limited mechanisms and thus iron intake is highly regulated
47 according to body iron needs³². Iron could interfere with glucose metabolism in several ways. For
48 example following mechanisms have been proposed: Iron decreases insulin extraction and
49 metabolism in the liver, which leads to peripheral hyperinsulinemia³³. Iron overload results in
50 oxidative stress in pancreatic β -cells, which leads to destruction of the pancreatic islets and thus
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3 decreases insulin secretion³⁴. The exact mechanisms, which link iron to diabetes are still unsolved.
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5 However the association can be argued to be biologically plausible.
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10 Accumulating body of evidence supports the hypothesis of excess iron as a risk factor for glucose
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12 metabolism disorders such as gestational diabetes. Iron deficiency anaemia has been shown to
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14 reduce the incidence of GDM¹⁹. Respectively high haemoglobin level (>130 g/l) in early pregnancy
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16 has been demonstrated to be an independent risk factor for GDM¹⁸. It seems that iron can affect
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18 glucose metabolism even with no overt iron overload. Women diagnosed with GDM have been
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20 observed to have increased iron stores compared to women without GDM^{15 17}. However many of
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22 these studies have been cross-sectional and it has been criticised that higher serum ferritin levels
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24 could in fact reflect inflammation in the body and could be rather a result than a cause for
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26 diabetes¹⁶.
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31 In summary it seems that high iron intake might be a factor, which increases the risk of GDM
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33 especially in women with already good iron stores. Use of iron supplements during pregnancy is
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35 common even in women with good haemoglobin levels. Our results suggest that routine use of iron
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37 supplements should be reconsidered in non-anaemic women with risk factors for GDM. To confirm
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39 the hypothesis there is need for a large prospective study – ideally a randomised controlled trial –
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41 with reliable and comprehensive information on iron intake from food and supplements during
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43 pregnancy accompanied with serum measurements to determine the level of body iron stores.
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48 49 **Author contributions**

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51 AH, TIK and RL designed and conducted research; AH and JR performed statistical analyses; AH
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53 drafted the paper and revised it based on the comments from the other authors. SMV and SA were
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3 responsible for the FFQ method and for dietary calculations. TIK and RL have the primary
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5 responsibility for final content. All authors read, commented and approved the final manuscript.
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7

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10
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12
13 the nurses of the participating maternity clinics for their invaluable work in implementing the study
14
15 protocol and the pregnant women who participated in the study.
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18 19 20 **A competing interest declaration**

21
22 All authors have completed the Unified Competing Interest form at
23
24 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare
25
26 that they have no financial or non-financial interests that may be relevant to the submitted work.
27
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29

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36
37 The views expressed in this paper are those of the authors and not necessarily those of the funders
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41 42 **Data Sharing Statement**

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44 Additional unpublished data from the study may be available and it can be enquired of the
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46 authors.
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What is already known on this subject

- High iron stores and high iron intake have been associated with the risk of GDM in several studies.
- Anaemia is known to result in lower incidence of GDM.
- There are no previous studies available in which total daily iron intake during pregnancy and haemoglobin levels have been studied in relation to GDM risk.

What this study adds

- Our results suggest that high iron intake during pregnancy increases the risk of GDM especially in women who are not anaemic in the beginning of the pregnancy and who are at increased risk of GDM.
- Our findings suggest that routine iron supplementation should be reconsidered in this risk group of women.

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3 **Figure legends**

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5 **Figure 1.** Total iron intake in mid pregnancy (26-28 weeks' gestation)

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7 **Figure 2.** Incidence of GDM (%) in fifths of total iron intake

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Table 1. Background characteristics of iron intake groups (20%, 60% and 20%), means (SD) or frequencies (%)

	20%	60%	20%	P-value
	<14.2 mg/day	14.2 – 109.9 mg/day	110.0+ mg/day	
	(n=78)	(n=233)	(n=78)	
Total Fe intake (mg/day)	11.6 (2.0)	36.0 (25.1)	136.2 (36.5)	-
Primigravida	34 (43.6%)	96 (41.2%)	43 (55.1%)	0.099 ²
BMI (kg/m ²)	26.7 (4.4)	26.5 (4.7)	25.2 (4.3)	0.050 ³
Age (years)	29.3 (5.1)	29.8 (4.4)	29.6 (5.4)	0.70 ³
Diabetes in first- or second-degree relatives	44 (57.1%)	127 (54.5%)	48 (61.5%)	0.55 ²
GDM or macrosomia in previous pregnancy	14 (18.2%)	33 (14.2%)	10 (12.8%)	0.60 ²
Hb (8-12 weeks ¹) (g/l)	135.4 (9.6)	134.0 (10.9)	127.0 (10.0)	<0.001 ⁴
Total energy intake (MJ/day)	8.3 (1.5)	10.1 (2.6)	9.9 (2.7)	<0.001 ³
Protein (E%) ⁵	18.0 (2.3)	18.0 (2.2)	18.1 (2.2)	0.81 ⁴
Carbohydrates (E%)	48.0 (4.4)	48.6 (4.8)	47.3 (4.9)	0.071 ⁴
Saccharose (E%)	10.7 (3.4)	10.6 (3.4)	10.8 (2.6)	0.54 ³
Dietary fibre	20.7 (6.0)	26.8 (9.2)	25.8 (8.6)	<0.001 ⁴

(g/day)

Total fat (E%)	32.9 (4.0)	32.3 (4.4)	33.4 (4.1)	0.099 ⁴
Saturated fatty acids (E%)	12.7 (2.0)	12.5 (2.7)	13.3 (2.5)	0.015 ³
Monounsaturated fatty acids (E%)	12.1 (1.9)	11.9 (1.8)	12.2 (1.7)	0.57 ⁴
Polyunsaturated fatty acids (E%)	5.2 (1.0)	5.0 (0.9)	5.0 (0.9)	0.85 ⁴
Total gestational weight gain (kg)	14.1 (5.5)	13.9 (5.3)	14.5 (5.4)	0.81 ⁴

¹ 16-18 for those with missing values at 8-12 weeks (n=28), missing: Hb n=5, 20 and 6, respectively

² Chi-square test, ³ Kruskal-Wallis test, ⁴ One-way ANOVA, ⁵ Percentage of total energy intake. Data on dietary intake was collected by the food frequency questionnaire completed at 26-28 weeks' gestation. **Table 2.** GDM frequency in iron intake groups (20%, 60% and 20%)

	N	GDM, frequency (%)	P-value
All women			
(20%) <14.2 mg/day	78	13 (16.7)	0.70 ²
(60%) 14.2-109.9 mg/day	233	39 (16.7)	
(20%) 110.0+ mg/day	78	16 (20.8)	
Hb (8-12 weeks¹) >120 g/l			
(20%) <14.2 mg	69	13 (18.8)	0.11 ²
(60%) 14.2-109.9 mg	194	30 (15.5)	
(20%) 110.0+ mg	58	16 (27.6)	
Hb (8-12 weeks¹) ≤120 g/l			
(20%) <14.2 mg	3	0	0.45 ³

(60%) 14.2-109.9 mg	21	3 (14.3)
(20%) 110.0+ mg	12	0

¹ 16-18 for those with missing values at 8-12 weeks (n=28)

² Chi-square test

³ Fisher's exact test

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Table 3. Incidence of GDM, unadjusted and adjusted ORs (95% CI) for iron intake at 26-28 weeks' gestation as a continuous and a categorical variable separately for all women and for women with haemoglobin >120 g/l

	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI) ²	P-value
Fe intake as a continuous variable				
<i>All women</i>				
Total Fe intake (mg/day)	1.004 (0.999 – 1.008)	0.16	1.006 (1.000 – 1.011)	0.038
BMI			1.09 (1.02 – 1.16)	0.006
Age			1.01 (0.95 – 1.07)	0.78
Diabetes in first- or second-degree relatives			0.87 (0.49 – 1.55)	0.63
GDM or macrosomia in a previous pregnancy			1.77 (0.86 – 3.64)	0.12
Total energy intake (MJ/day)			0.89 (0.74 – 1.06)	0.19
Dietary fibre (g/day)			1.02 (0.97 – 1.08)	0.43
Saturated fatty acids (E%) ¹			0.96 (0.84 – 1.09)	0.48
Total gestational weight gain			0.94 (0.89 – 1.00)	0.039
<i>Women with Hb (8-12 weeks' gestation²) >120 g/l</i>				
Total Fe intake (mg/day)	1.007 (1.001 – 1.012)	0.013	1.009 (1.003 – 1.015)	0.006
BMI			1.07 (1.00 – 1.15)	0.042
Age			1.01 (0.94 – 1.08)	0.78
Diabetes in first- or second-degree relatives			0.73 (0.39 – 1.36)	0.32
GDM or macrosomia in a previous pregnancy			1.78 (0.80 – 3.95)	0.16
Total energy intake (MJ/day)			0.83 (0.68 – 1.01)	0.066
Dietary fibre (g/day)			1.04 (0.99 – 1.10)	0.14
Saturated fatty acids (E%) ¹			0.98 (0.84 – 1.13)	0.75
Total gestational weight gain			0.93 (0.88 – 0.99)	0.028
Fe intake as a categorical variable				
<i>All women</i>				
Total Fe intake (the highest 20% vs. the lowest 80%)	1.31 (0.70 – 2.44)	0.40	1.66 (0.84 – 3.30)	0.15
BMI			1.09 (1.02 – 1.15)	0.010
Age			1.01 (0.95 – 1.07)	0.77
Diabetes in first- or second-degree relatives			0.84 (0.47 – 1.50)	0.55
GDM or macrosomia in a previous pregnancy			1.65 (0.93 – 3.37)	0.17

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4	Total energy intake (MJ/day)			0.89 (0.80 – 1.06)	0.19
5	Dietary fibre (g/day)			1.02 (0.97 – 1.08)	0.37
6	Saturated fatty acids (E%) ¹			0.97 (0.85 – 1.10)	0.59
7	Total gestational weight gain			0.94 (0.89 – 0.99)	0.029
8					
9	<i>Women with Hb (8-12 weeks' gestation²) >120 g/l</i>				
10	Total Fe intake (the highest 20% vs. the lowest 80%)	1.95 (1.005 – 3.78)	0.048	2.35 (1.13 – 4.92)	0.023
11	BMI			1.07 (1.00 – 1.14)	0.054
12	Age			1.01 (0.95 – 1.08)	0.76
13	Diabetes in first- or second-degree relatives			0.70 (0.38 – 1.31)	0.27
14	GDM or macrosomia in a previous pregnancy			1.60 (0.73 – 3.53)	0.24
15					
16	Total energy intake (MJ/day)			0.83 (0.69 – 1.02)	0.071
17	Dietary fibre (g/day)			1.05 (0.99 – 1.11)	0.10
18	Saturated fatty acids (E%) ¹			0.99 (0.86 – 1.15)	0.92
19	Total gestational weight gain			0.93 (0.87 – 0.99)	0.017
20					

¹ percentage of total energy intake

² 16-18 weeks' gestation for those with missing values at 8-12 weeks' gestation (n=28) ² Adjusted for other variables in the model (total Fe intake, BMI, age, diabetes in first- or second-degree relatives, GDM or macrosomia in a previous pregnancy, total energy intake, dietary fibre, saturated fatty acids and total gestational weight gain)

Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study

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Abstract

Objective To investigate the possible association between total daily iron intake ~~from both food and supplements~~ during pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes mellitus (GDM) in women at increased risk of GDM.

Design A prospective cohort study (based on a cluster-randomised controlled trial, where the intervention and the usual care groups were combined).

Setting Primary health care maternity clinics in 14 municipalities in south-western Finland.

Participants 399 pregnant women who were at increased risk of GDM participated in a GDM prevention trial and were followed throughout pregnancy.

Main outcome measurements The main outcome was GDM diagnosed with oral glucose tolerance test at 26-28 weeks' gestation or based on a diagnosis recorded in the Finnish Medical Birth registry. Data on iron intake was collected using a 181-item food frequency questionnaire and separate questions for supplement use at 26-28 weeks' gestation.

Results GDM was diagnosed in 72 women (18.1%) in the study population. The odds ratio (OR) for total iron intake as a continuous variable was 1.0065 (95% confidence interval 1.000 to 1.011; P=0.0384) after adjustment for BMI, age, diabetes in first- or second-degree relatives ~~and~~, GDM or macrosomia in earlier pregnancy, total energy intake, dietary fibre, saturated fatty acids and total gestational weight gain. Women in the highest fifth of total daily iron intake had an adjusted OR of 1.6655 (95% confidence interval 0.841 to 3.30296; P=0.159) for GDM. After excluding participants with low haemoglobin levels (≤ 120 g/l) already in early pregnancy the adjusted OR was 2.3524 (95% confidence interval 1.134 to 4.9244; P=0.0235).

Conclusions Our results suggest that high iron intake during pregnancy increases the risk of GDM especially in women who are not anaemic in early the beginning of the pregnancy and who are at increased risk of GDM. These findings suggest that routine iron supplementation should be reconsidered in this risk group of women.

Trial registration: Current Controlled Trials ISRCTN33885819

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a disturbance in glucose metabolism, which is diagnosed during pregnancy and affects 1-14% of pregnancies in different populations¹. The incidence of gestational diabetes has been increasing the last 20 years². Risk factors for GDM include higher maternal body mass index (BMI), higher age and family history of diabetes³. Approximately half of the GDM cases can be explained by overweight⁴. GDM causes short and long term risks to both the mother and the child. The most common result of GDM is newborn macrosomia, which increases several adverse outcomes during delivery such as shoulder dystocia, perineal lacerations, blood loss and increased caesarean birth⁵. GDM also increases the risk of glucose metabolism disorders and type 2 diabetes in later life of the mother and the newborn^{6,7}.

High iron load and disorders of iron metabolism have been associated with an increased risk of disturbances in glucose metabolism⁸⁻¹⁰. In hereditary hemochromatosis iron accumulation in the body leads to diabetes in 30-60% of patients¹¹. Also in animal models iron has been shown to induce diabetes¹². Iron binding medication is effective in preventing diabetes in iron overload conditions¹³. Frequent blood donation has been shown to result in improvement in glucose metabolism even in healthy people¹⁴. Higher iron stores have been found in gestational diabetes patients measured most commonly by serum ferritin¹⁵⁻¹⁷. High haemoglobin level in early pregnancy has been reported to be an independent risk factor for GDM¹⁸ and lower haemoglobin levels and anaemia during pregnancy have been shown to result in lower risk of GDM¹⁹. A large proportion of women, up to 78% in Finland²⁰, use supplemental iron during pregnancy even with good haemoglobin levels and thus it is an important matter to investigate the possible adverse effects of iron. High iron intake could be especially risky for women with already good iron stores.

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3 Studies of total iron intake and the risk of GDM are scarce: only Bowers et al.²¹ have investigated
4 pre-pregnancy total iron intake in relation to the risk of GDM. Other studies have investigated iron
5 intake either from food or supplements only and have thus failed to report the effect of total iron
6 intake^{22 23}. Only one placebo controlled clinical trial has been conducted on supplemental iron and
7 the risk of GDM²⁴. To our knowledge there are no published studies of total iron intake during
8 pregnancy, haemoglobin and the risk of GDM. The objective of this study was to investigate the
9 possible association between total daily iron intake from both food and supplements during
10 pregnancy, haemoglobin levels in early pregnancy and the risk of gestational diabetes in women at
11 increased risk of GDM.
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24 MATERIAL AND METHDOS

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29 The data of this study was originally collected as a part of a larger gestational diabetes prevention
30 trial, which is described in more detail elsewhere²⁵. The effects of the intervention on GDM and
31 newborns birthweight have been reported elsewhere²⁶. This cluster-randomised trial was carried out
32 in 14 municipalities in Pirkanmaa region in south-western Finland in 2007-2009. The municipalities
33 were randomised in pairs into intervention and usual care municipalities.
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43 The inclusion criteria for the trial ~~was~~were to have at least one of the following risk factors: BMI
44 ≥ 25 kg/m²; age ≥ 40 years; GDM, glucose intolerance or newborn's macrosomia (≥ 4500 g) in any
45 earlier pregnancy or type 1 or 2 diabetes in first- or second-degree relatives. Women were excluded
46 from the trial if they had an abnormal measurement in the baseline (8-12 weeks' gestation) glucose
47 tolerance test (fasting blood glucose ≥ 5.3 mmol/l, >10.0 mmol/l at 1 h or >8.6 mmol/l at 2 h); pre-
48 pregnancy diabetes (type 1 or 2); age <18 years; no Finnish language skills; multiple pregnancy;
49 restrictions from physical activity; substance abuse or psychiatric illness. The trial was approved by
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3 the ethical committee of Pirkanmaa Hospital District and written informed consent was provided by
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5 the participants.
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9 A total of 2271 women were screened for the study and of them 726 were preliminary eligible to
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11 participate in the study²⁶. Of these women 640 (88.2%) gave an informed consent to participate in
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13 the trial. At the baseline (8-12 weeks' gestation) oral glucose tolerance test 174 women had an
14
15 abnormal result and were thus excluded. Furthermore 38 women had a miscarriage and 29 were lost
16
17 to follow-up. Finally, 399 participants were included in the analyses (219 in the intervention group
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19 and 180 in the usual care group).
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24 The intervention group participated in individual counselling on weight gain, physical activity and
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26 diet on five antenatal visits in primary health care maternity clinics. The objectives of the
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28 counselling were to guide the participants in monitoring their weight gain, increasing or
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30 maintaining their leisure time physical activity and achieving a healthy diet fulfilling the national
31
32 recommendations. The effects of intensified dietary counselling on food habits and the intake of
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34 energy, energy-yielding nutrients, fiber, selected fatty acids and cholesterol have been reported
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36 elsewhere²⁷. The counselling was not aiming to influence iron intake. In this study the
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38 intervention and usual care groups were combined and these groups had no differences in the
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40 incidence of GDM or in iron intake.
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47 Background information of the participants was gathered with a baseline questionnaire at the first
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49 prenatal visit (8-12 weeks' gestation). Information of pre-pregnancy BMI (weight self reported and
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51 height measured at 8-12 weeks' visit) and haemoglobin were abstracted from maternity cards. In
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53 this study we used information of haemoglobin measurements from early pregnancy (weeks 8-12,
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55 or 16-18 for those with missing values for 8-12 weeks) to determine the status of body iron stores in
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3 the beginning of pregnancy and before the physiological decrease in haemoglobin levels. The
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5 participants were considered to have good haemoglobin levels if they had a haemoglobin
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7 measurement of over 120 g/l according to the lower limit for normal haemoglobin in non-pregnant
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9 women²⁸. The limit for normal haemoglobin in pregnant women (110 g/l) was not used because of
10
11 low frequency of cases with haemoglobin level under 110 g/l in early pregnancy (n=7). All women
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13 underwent a 75 g oral glucose tolerance test at 26-28 weeks' gestation. The criterion for gestational
14
15 diabetes diagnosis was to have at least one abnormal value: fasting glucose after an overnight fast
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17 ≥ 5.3 mmol/l, blood glucose > 10.0 mmol/l one hour after or > 8.6 mmol/l two hours after consuming
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19 75 g of glucose. To cover all possible cases information of gestational diabetes was abstracted from
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21 the Finnish Medical Birth Register.
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27 Information on nf diet and supplement use was obtained by a validated 181-item food frequency
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29 questionnaire²⁹ although the validity of the supplement data was not assessed in the study by
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31 Erkkola et al. The participants completed the questionnaire at 8-12 weeks' gestation and again at
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33 26-28 and 36-37 weeks' gestation. In the 8-12 weeks' questionnaire the participants were asked
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35 about their dietary habits during one month before beginning of the pregnancy and in 26-28 and 36-
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37 37 weeks' questionnaires the participants were asked about their dietary habits during the previous
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39 month. The completed questionnaires were checked by a nutritionist and those with more than ten
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41 missing values in the frequency data were completed after consulting the participant on the phone.
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43 In the food frequency questionnaire the dietary habits were assessed by detailed questions of
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45 frequency of use (per day, week, month or not at all) and the portion sizes of specific food items.
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47 The participants were also asked to report their supplement use: brand name of the supplement,
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49 dosage and frequency of use (per day, week or month). The gathered data was then entered into a
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51 food database using a software program of the National Institute for Health and Welfare, Helsinki,
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53 Finland, and coded to daily food record form. Nutrient intakes were calculated by using the 10th
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3 release (updated in 2009) of the Finnish Food Composition Database Fineli (www.fineli.fi) and in-
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5 house software of the National Institute for Health and Welfare, Helsinki. In this study information
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7 of dietary habits and supplement use at 26-28 weeks of gestation was used to get the best estimation
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9 of iron intake and the intake of energy, macronutrients and dietary fibre during pregnancy and
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11 before the onset of GDM.
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14 15 16 **Statistical methods**

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18 The study population was categorised into five equal groups according to their total daily iron
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20 intake. To better see the differences between women in the highest fifth of total iron intake and the
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22 other women, we combined the three groups in the middle (20, 60 and 20 percent). The groups were
23
24 tested for possible differences in risk factors, dietary intake and other background characteristics
25
26 using Chi-square test, Kruskal-Wallis test or One-way ANOVA. The incidence of GDM was then
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28 investigated between the iron intake groups and the differences were tested statistically using Chi-
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30 square test or Fisher exact test. Haemoglobin was considered as a potential effect modifier. Women
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32 were divided into two groups according to their haemoglobin level in early pregnancy: >120 g/l or
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34 ≤120 g/l.
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41 Logistic regression was used to assess the odds ratio (OR) for gestational diabetes. The dependent
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43 variable in the regression model was GDM (yes/no). In the first model iron intake was assessed as a
44
45 continuous variable. In the second model the highest fifth of total iron intake (intake ≥110 mg) was
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47 compared to the rest of the study population (iron intake under 110 mg). The models were adjusted
48
49 for BMI, age ~~(both as continuous)~~, intake of energy, saturated fatty acids and dietary fibre and total
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51 gestational weight gain (all as continuous) as well as diabetes in first- or second-degree relatives
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53 and GDM or macrosomia in earlier pregnancy (both as categorical). Both models were executed
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3 separately for all women and for women with haemoglobin levels over 120 g/l in early pregnancy
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5 (8-12 or 16-18 weeks' gestation).
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9 We also added interaction terms for iron intake and haemoglobin and for iron intake and BMI to the
10 model but they were not statistically significant and therefore are not presented here. In sensitivity
11 analyses, we examined the association between the total iron intake and the risk of GDM using
12 alternative cut-off points for categorising participants based on their iron intake (15, 70 and 15
13 percent; 25, 50 and 25 percent and 30, 40 and 30 percent) and the same statistical tests as described
14 above. All analyses were performed with SPSS statistics (version 19).
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24 25 **RESULTS**

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29 Based on the questionnaire completed at 26-28 weeks' gestation, the mean daily iron intake from
30 food was 14.4 mg (SD 4.3; median 13.9 mg) among the study population. In the study group 65.7%
31 of women used supplemental iron and the median of supplemental intake was 27.0 mg. The most
32 used dosages for iron supplementation were 10.0 mg, 20.0 mg and 100.0 mg of elemental iron
33 (Fe^{2+}). The median for total iron intake was 27.1 mg (interquartile range 15.5-89.3 mg) (Figure 1).
34 Iron intake from food was similar in nonusers and users of supplemental iron: users of supplemental
35 iron had a mean daily intake of 14.5 mg (SD 4.5) and nonusers 14.3 mg (SD 3.9). The mean
36 haemoglobin level of the study population in the beginning of pregnancy was 134 g/l (SD 11; min
37 107 g/l; max 169 g/l).
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51 The oral glucose tolerance test at 26-28 weeks' gestation was abnormal in 14.0% of the study
52 population (n=56, missing value for three women). Furthermore 16 women received a gestational
53 diabetes diagnosis during their pregnancy. Figure 2 shows the incidence of GDM in fifths of total
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3 iron intake. There was a tendency for a higher incidence of GDM in the highest fifth of total iron
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5 intake.

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10 Between the iron intake groups (20%, 60% and 20%) there were no significant differences in terms
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12 of proportion of primigravida, age, diabetes in first- or second-degree relatives or GDM or
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14 macrosomia in earlier pregnancy (Table 1). Women in the highest iron intake group had a slightly
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16 lower mean BMI as compared to other women (P=0.05). Haemoglobin levels in early pregnancy
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18 differed between the groups as expected (P<0.001). With regard to differences in dietary intake,
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20 women in the lowest iron intake group had lower total energy and dietary fibre intakes than the
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22 other women whereas women in the highest iron intake group had higher intake of saturated fatty
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24 acids than the other women. Total gestational weight gain did not differ between the iron intake
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26 groups. Table 2 shows the incidence of GDM in the three iron intake groups. The incidence was
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28 higher in the highest iron intake group (20.8%) compared to the other two groups (16.7% in both)
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30 but the difference was not statistically significant (P=0.70). When including~~After excluding those~~
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32 women with ~~who had low~~ haemoglobin levels (≤ 120 g/l) already in early pregnancy (n=321, mean
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34 Hb 135, SD 9), the difference between the groups was even larger but still not statistically
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36 significant (P=0.11).
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43 The logistic regression model shows that after adjustment for potential risk factors and background
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45 characteristics total iron intake was significantly and positively associated with gestational diabetes
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47 mellitus (Table 3). When iron intake was used as a continuous variable the OR was 1.00~~65~~
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49 (P=0.0~~3844~~) for all women and 1.00~~98~~ (P=0.00~~65~~) for those with haemoglobin levels over 120 g/l.
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51 In the second model the OR for GDM was 1.~~6655~~ (95% confidence interval 0.8~~44~~ to ~~3.302~~.~~96~~,
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53 P=0.1~~59~~) for the highest fifth of iron intake among all women. After excluding women with low
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3 haemoglobin levels (≤ 120 g/l) in early pregnancy the OR was 2.~~3521~~ (95% confidence interval
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5 1.1~~31~~ to 4.~~9241~~, $P=0.0235$).
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10 In sensitivity analyses the odds ratios were higher when 15% of women with the highest iron intake
11 were compared to the rest 85% of women. The odds ratios were lower when 25% or 30% of women
12 with highest intake were compared to the rest 75% and 70% of women respectively (results not
13 shown).
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20 DISCUSSION

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25 In this study we investigated the possible association between total daily iron intake during
26 pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes. To our knowledge
27 there are no similar studies published previously. We discovered that there was a tendency for a
28 higher incidence of GDM in the highest fifth of total iron intake. High iron intake increased the risk
29 of GDM especially for those women who had good haemoglobin levels in early pregnancy: we
30 discovered a two-fold OR for GDM for women in the highest fifth of iron intake with a
31 haemoglobin level of over 120 g/l in early pregnancy.
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43 Our study has several strengths. Firstly unlike most of the previous studies we had detailed
44 information of iron intake from both food and supplements. Secondly we had information on
45 baseline OGTT and could therefore exclude women with undiagnosed prepregnancy diabetes.
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48 Additionally Finnish Medical Birth Registry could be utilized to cover those GDM cases that were
49 undiagnosed in the 26-28 weeks' OGTT. We also had information of haemoglobin measurements
50 of the study population during pregnancy although measured by nurses. All of our data was
51 collected prospectively. The participation rate in the original trial was high (88% in both the
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3 intervention and the usual care groups) improving the generalisability of the results to other women
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5 with risk factors for GDM.
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9 A limitation of this kind of study is that estimating iron intake during pregnancy can be challenging
10 because dietary habits and use of supplements can vary a lot during pregnancy due to nausea and
11 other changes in well-being. Iron intake was assessed at a certain point in time (at 26-28 weeks'
12 gestation covering the previous month), thus it does not cover the intake during the whole
13 pregnancy. In our study we decided to use information of iron intake in mid-pregnancy to get the
14 best estimation on iron intake during pregnancy and before the onset of GDM. We did not use
15 information of iron intake abstracted in the beginning of pregnancy because it covered only dietary
16 habits and supplement use during one month before the pregnancy when use of supplemental iron is
17 rare. However our information on iron intake covers only one month in the mid-pregnancy.
18 Additionally we could not assess iron absorption, for example with blood measurements, which also
19 can vary a lot depending on body iron status and the contents of meals.
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36 There are also limitations concerning the haemoglobin measurements used in our study. In the
37 maternity clinics haemoglobin levels are usually screened with a capillary haemoglobin
38 measurement using finger-stick samples. This method has been demonstrated to be reliable in
39 determining haemoglobin values but it is however susceptible to handling errors³⁰ and therefore
40 there can be variation in the test results. Additionally we had a lot of missing values in haemoglobin
41 measurements and thus we decided to use the haemoglobin measurement at 16-18 weeks' gestation
42 for those with missing values at weeks 8-12 (n= 28). Although measurements at weeks 16-18 do not
43 present the haemoglobin levels at early pregnancy our objective was to analyse separately women
44 who were not anaemic and thus including women who had a haemoglobin level of over 120 g/l still
45 at weeks 16-18 would help us not to underestimate the proportion of women who were not anaemic
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3 in the beginning of the pregnancy. However for these reasons our results in respect of haemoglobin
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5 measurements should be considered with caution and further studies with reliable and
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7 comprehensive haemoglobin measurements are warranted to confirm our results.
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11 In a somewhat similar study Bo et al.²² discovered that women who used supplemental iron during
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13 mid-pregnancy had a 2- to 3-fold risk of GDM. They however had no information of dietary intake
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15 of iron or haemoglobin levels or other measurements of iron stores. Their findings are however in
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17 line with our results which suggest that dietary intake could play a relatively small part in the
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19 association whereas high supplemental iron intake could be responsible for most of the increase in
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21 GDM risk. Somewhat different results have been reported recently by Bowers et al.²¹ and Qiu et
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23 al.²³. Bowers et al.²¹ investigated dietary and supplemental iron intake during one year before
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25 pregnancy utilising the material from the Nurses Health study. They observed no significant effect
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27 of total, nonheme or supplemental iron intake on the risk of GDM. It can be argued though, that
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29 usually iron supplementation outside of pregnancy is more rare and concerns mainly those who are
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31 anaemic. However they did find a significant and positive association between heme iron intake and
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33 GDM. Similarly Qiu et al.²³ demonstrated an association between heme iron intake during the time
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35 before conception and in early pregnancy and the risk of GDM. To our knowledge only one placebo
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37 controlled clinical trial has been conducted to investigate the association between iron
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39 supplementation and GDM²⁴. This study did not observe any association. However it can be argued
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41 that the intake of supplemental iron was quite low in this study because of only about 50%
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43 compliance to a daily supplement of 60 mg of elemental iron.
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51 Iron is a highly reactive component with a possibility to participate in harmful reactions³¹. The
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53 human body can excrete iron with very limited mechanisms and thus iron intake is highly regulated
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55 according to body iron needs³². Iron could interfere with glucose metabolism in several ways. For
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3 example following mechanisms have been proposed: Iron decreases insulin extraction and
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5 metabolism in the liver, which leads to peripheral hyperinsulinemia³³. Iron overload results in
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7 oxidative stress in pancreatic β -cells, which leads to destruction of the pancreatic islets and thus
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9 decreases insulin secretion³⁴. The exact mechanisms, which link iron to diabetes are still unsolved.
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11 However the association can be argued to be biologically plausible.
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16 Accumulating body of evidence supports the hypothesis of excess iron as a risk factor for glucose
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18 metabolism disorders such as gestational diabetes. Iron deficiency anaemia has been shown to
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20 reduce the incidence of GDM¹⁹. Respectively high haemoglobin level (>130 g/l) in early pregnancy
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22 has been demonstrated to be an independent risk factor for GDM¹⁸. It seems that iron can affect
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24 glucose metabolism even with no overt iron overload. Women diagnosed with GDM have been
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26 observed to have increased iron stores compared to women without GDM^{15 17}. However many of
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28 these studies have been cross-sectional and it has been criticised that higher serum ferritin levels
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30 could in fact reflect inflammation in the body and could be rather a result than a cause for
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32 diabetes¹⁶.
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39 In summary it seems that high iron intake might be a factor, which increases the risk of GDM
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41 especially in women with already good iron stores. Use of iron supplements during pregnancy is
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43 common even in women with good haemoglobin levels. Our results suggest that routine use of iron
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45 supplements should be reconsidered in non-anaemic women with risk factors for GDM. To confirm
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47 the hypothesis there is need for a large prospective study – ideally a randomised controlled trial –
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49 with reliable and comprehensive information on iron intake from food and supplements during
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51 pregnancy accompanied with serum measurements to determine the level of body iron stores.
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55 56 | **Author contributions** 57 58 59 60

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3 AH, TIK and RL designed and conducted research; AH and JR performed statistical analyses; AH
4 drafted the paper and revised it based on the comments from the other authors. SMV and SA were
5 responsible for the FFQ method and for dietary calculations. TIK and RL have the primary
6
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9 responsibility for final content. All authors read, commented and approved the final manuscript.
10
11

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17 ~~for dietary calculations.~~ Jatta Puhkala is acknowledged for checking and coding the dietary data.
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21
22 in implementing the study protocol and the pregnant women who participated in the study.
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25 26 27 **A competing interest declaration**

28
29 All authors have completed the Unified Competing Interest form at
30
31 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare
32
33 that they have no financial or non-financial interests that may be relevant to the submitted work.
34
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43
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What is already known on this subject

- High iron stores and high iron intake have been associated with the risk of GDM in several studies.
- Anaemia is known to result in lower incidence of GDM.
- There are no previous studies available in which total daily iron intake during pregnancy and haemoglobin levels have been studied in relation to GDM risk.

What this study adds

- Our results suggest that high iron intake during pregnancy increases the risk of GDM especially in women who are not anaemic in the beginning of the pregnancy and who are at increased risk of GDM.
- Our findings suggest that routine iron supplementation should be reconsidered in this risk group of women.

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3 **Figure legends**
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5 **Figure 1.** Total iron intake in mid pregnancy (26-28 weeks' gestation)
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7 **Figure 2.** Incidence of GDM (%) in fifths of total iron intake
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Table 1. Background characteristics of iron intake groups (20%, 60% and 20%), means (SD) or frequencies (%)

	20%	60%	20%	P-value
	<14.2 mg/day	14.2 – 109.9 mg/day	110.0+ mg/day	
	(n=78)	(n=233)	(n=78)	
Total Fe intake (mg/day)	11.6 (2.0)	36.0 (25.1)	136.2 (36.5)	-
Primigravida	34 (43.6%)	96 (41.2%)	43 (55.1%)	0.099 ²
BMI (kg/m ²)	26.7 (4.4)	26.5 (4.7)	25.2 (4.3)	0.050 ³
Age (years)	29.3 (5.1)	29.8 (4.4)	29.6 (5.4)	0.70 ³
Diabetes in first- or second-degree relatives	44 (57.1%)	127 (54.5%)	48 (61.5%)	0.55 ²
GDM or macrosomia in previous pregnancy	14 (18.2%)	33 (14.2%)	10 (12.8%)	0.60 ²
Hb (8-12 weeks ¹) (g/l)	135.4 (9.6)	134.0 (10.9)	127.0 (10.0)	<0.001 ⁴
<u>Total energy intake</u> <u>(MJ/day)</u>	<u>8.3 (1.5)</u>	<u>10.1 (2.6)</u>	<u>9.9 (2.7)</u>	<u><0.001³</u>
<u>Protein (E%)⁵</u>	<u>18.0 (2.3)</u>	<u>18.0 (2.2)</u>	<u>18.1 (2.2)</u>	<u>0.81⁴</u>
<u>Carbohydrates</u> <u>(E%)</u>	<u>48.0 (4.4)</u>	<u>48.6 (4.8)</u>	<u>47.3 (4.9)</u>	<u>0.071⁴</u>
<u>Saccharose (E%)</u>	<u>10.7 (3.4)</u>	<u>10.6 (3.4)</u>	<u>10.8 (2.6)</u>	<u>0.54³</u>
<u>Dietary fibre</u>	<u>20.7 (6.0)</u>	<u>26.8 (9.2)</u>	<u>25.8 (8.6)</u>	<u><0.001⁴</u>

(g/day)

Total fat (E%) 32.9 (4.0) 32.3 (4.4) 33.4 (4.1) 0.099⁴

Saturated fatty acids (E%) 12.7 (2.0) 12.5 (2.7) 13.3 (2.5) 0.015³

Monounsaturated fatty acids (E%) 12.1 (1.9) 11.9 (1.8) 12.2 (1.7) 0.57⁴

Polyunsaturated fatty acids (E%) 5.2 (1.0) 5.0 (0.9) 5.0 (0.9) 0.85⁴

Total gestational weight gain (kg) 14.1 (5.5) 13.9 (5.3) 14.5 (5.4) 0.81⁴

¹ 16-18 for those with missing values at 8-12 weeks (n=28), missing: Hb n=5, 20 and 6, respectively

² Chi-square test, ³ Kruskal-Wallis test, ⁴ One-way ANOVA, ⁵ Percentage of total energy intake.

Data on dietary intake was collected by the food frequency questionnaire completed at 26-28 weeks' gestation.

Table 2. GDM frequency in iron intake groups (20%, 60% and 20%)

	N	GDM, frequency (%)	P-value
All women			
(20%) <14.2 mg/day	78	13 (16.7)	0.70 ²
(60%) 14.2-109.9 mg/day	233	39 (16.7)	
(20%) 110.0+ mg/day	78	16 (20.8)	
Hb (8-12 weeks¹) >120 g/l			
(20%) <14.2 mg	69	13 (18.8)	0.11 ²
(60%) 14.2-109.9 mg	194	30 (15.5)	
(20%) 110.0+ mg	58	16 (27.6)	
Hb (8-12 weeks¹) ≤120 g/l			
(20%) <14.2 mg	3	0	0.45 ³
(60%) 14.2-109.9 mg	21	3 (14.3)	
(20%) 110.0+ mg	12	0	

¹ 16-18 for those with missing values at 8-12 weeks (n=28)

² Chi-square test

³ Fisher's exact test

Table 3. Incidence of GDM, unadjusted and adjusted ORs (95% CI) for iron intake at 26-28 weeks' gestation as a continuous and a categorical variable separately for all women and for women with haemoglobin >120 g/l

	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI) ²	P-value
Fe intake as a continuous variable				
<i>All women</i>				
Total Fe intake (mg/day)	1.004 (0.999 – 1.008)	0.16	1.006 (1.000 – 1.011) 1.005	0.038 0.041
BMI			1.09 (1.02 – 1.16) 1.11	0.006 <
Age			1.01 (0.95 – 1.07) 1.03	0.78 0.3
Diabetes in first- or second-degree relatives			0.87 (0.49 – 1.55) 0.91	0.63 0.7
GDM or macrosomia in a previous pregnancy			1.77 (0.86 – 3.64) 1.95	0.12 0.0
<u>Total energy intake (MJ/day)</u>			<u>0.89 (0.74 – 1.06)</u>	<u>0.19</u>
<u>Dietary fibre (g/day)</u>			<u>1.02 (0.97 – 1.08)</u>	<u>0.43</u>
<u>Saturated fatty acids (E%)¹</u>			<u>0.96 (0.84 – 1.09)</u>	<u>0.48</u>
<u>Total gestational weight gain</u>			<u>0.94 (0.89 – 1.00)</u>	<u>0.039</u>
<i>Women with Hb (8-12 weeks' gestation^{1,2}) >120 g/l</i>				
Total Fe intake (mg/day)	1.007 (1.001 – 1.012)	0.013	1.009 (1.003 – 1.015) 1.008	0.006 0.005
BMI			1.07 (1.00 – 1.15) 1.11	0.042 0.001
Age			1.01 (0.94 – 1.08) 1.03	0.78 0.2
Diabetes in first- or second-degree relatives			0.73 (0.39 – 1.36) 0.80	0.32 0.4
GDM or macrosomia in a previous pregnancy			1.78 (0.80 – 3.95) 2.03	0.16 0.0
<u>Total energy intake (MJ/day)</u>			<u>0.83 (0.68 – 1.01)</u>	<u>0.066</u>
<u>Dietary fibre (g/day)</u>			<u>1.04 (0.99 – 1.10)</u>	<u>0.14</u>
<u>Saturated fatty acids (E%)¹</u>			<u>0.98 (0.84 – 1.13)</u>	<u>0.75</u>

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<u>Total gestational weight gain</u>			<u>0.93 (0.88 – 0.99)</u>	<u>0.028</u>
Fe intake as a categorical variable				
<i>All women</i>				
Total Fe intake (the highest 20% vs. the lowest 80%)	1.31 (0.70 – 2.44)	0.40	1.66 (0.84 – 3.30) (0.81 – 2.96)	0.150 9
BMI			1.09 (1.02 – 1.15) (1.05 – 1.17)	0.010 0.004
Age			1.01 (0.95 – 1.07) (0.97 – 1.09)	0.770 9
Diabetes in first- or second-degree relatives			0.84 (0.47 – 1.50) (0.51 – 1.55)	0.550 8
GDM or macrosomia in a previous pregnancy			1.65 (0.93 – 3.37) (0.92 – 3.64)	0.170 83
Total energy intake (MJ/day)			0.89 (0.80 – 1.06) (0.97 – 1.08)	0.190 7
Dietary fibre (g/day)			1.02 (0.97 – 1.08) (0.85 – 1.10)	0.370 9
Saturated fatty acids (E%) [†]			0.97 (0.85 – 1.10) (0.89 – 0.99)	0.590 29
<i>Women with Hb (8-12 weeks' gestation²⁴) >120 g/l</i>				
Total Fe intake (the highest 20% vs. the lowest 80%)	1.95 (1.005 – 3.78)	0.048	2.35 (1.13 – 4.92) (1.11 – 4.41)	0.0230 025
BMI			1.07 (1.00 – 1.14) (1.04 – 1.17)	0.0540 002
Age			1.01 (0.95 – 1.08) (0.98 – 1.10)	0.760 4
Diabetes in first- or second-degree relatives			0.70 (0.38 – 1.31) (0.43 – 1.41)	0.270 4
GDM or macrosomia in a previous pregnancy			1.60 (0.73 – 3.53) (0.89 – 3.93)	0.240 0
<u>Total energy intake (MJ/day)</u>			<u>0.83 (0.69 – 1.02)</u>	<u>0.071</u>
<u>Dietary fibre (g/day)</u>			<u>1.05 (0.99 – 1.11)</u>	<u>0.10</u>
<u>Saturated fatty acids (E%)[†]</u>			<u>0.99 (0.86 – 1.15)</u>	<u>0.92</u>

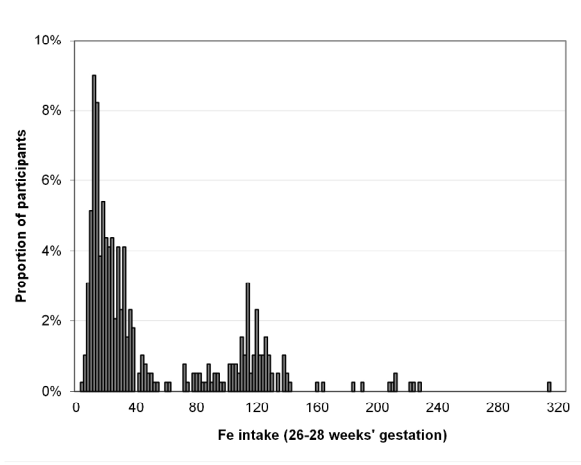
Total gestational weight gain 0.93 (0.87 – 0.99) 0.017

¹ percentage of total energy intake

² 16-18 weeks' gestation for those with missing values at 8-12 weeks' gestation (n=28) ² Adjusted for other variables in the model (total Fe intake, BMI, age, diabetes in first- or second-degree relatives ~~and~~, GDM or macrosomia in a previous pregnancy, total energy intake, dietary fibre, saturated fatty acids and total gestational weight gain)

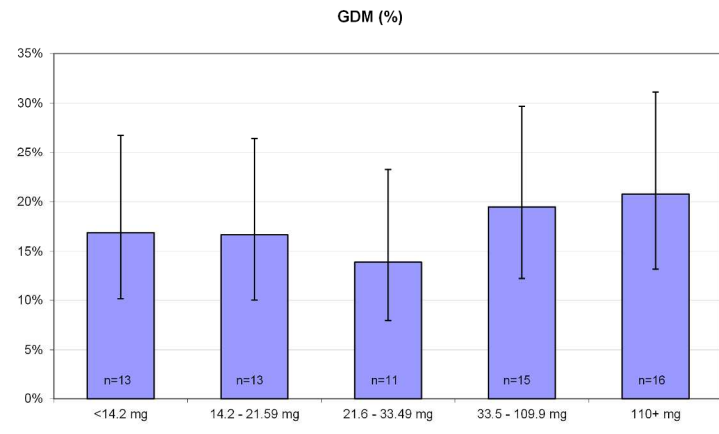
For peer review only

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Total iron intake in mid pregnancy (26-28 weeks' gestation)
209x297mm (300 x 300 DPI)

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Incidence of GDM (%) in fifths of total iron intake
209x297mm (300 x 300 DPI)

Annika Helin, Tarja I Kinnunen, Jani Raitanen, Riitta Luoto: **Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study**

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Reported on page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4,5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4,5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(b) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	Luoto et al. 2010 (see list of references)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	7,8
		(c) Explain how missing data were addressed	5
		(d) If applicable, explain how loss to follow-up was addressed	
		(e) Describe any sensitivity analyses	7,8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	

1	Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, Figure 1, Table 1
2			(b) Indicate number of participants with missing data for each variable of interest	8, Table 1, Table 2
3			(c) Summarise follow-up time (eg, average and total amount)	
4	Outcome data	15*	Report numbers of outcome events or summary measures over time	8
5	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9, Table 3
6			(b) Report category boundaries when continuous variables were categorized	Table 1
7			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
8	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
9	Discussion			
10	Key results	18	Summarise key results with reference to study objectives	10
11	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10,11
12	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-13
13	Generalisability	21	Discuss the generalisability (external validity) of the study results	10,13
14	Other information			
15	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.