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

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Complete List of Authors:	Helin, Annika; University of Tampere, School of Health Sciences Kinnunen, Tarja; University of Tampere, School of Health Sciences Raitanen, Jani; University of Tampere, School of Health Sciences Luoto, Riitta; UKK Institute for Health Promotion,
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Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study

Annika Helin, MSc student[1], Tarja I Kinnunen, university teacher[1], Jani Raitanen, statistician[1,2], Riitta Luoto, research director[2,3]

Affiliations:

[1] University of Tampere, School of Health Sciences, FI-33014 University of Tampere, Finland;

[2] The UKK Institute for Health Promotion Research, P. O. Box 30, FI-33501 Tampere, Finland;

[3] The National Institute for Health and Welfare, The Unit of Sexual and Reproductive Health, P.O. Box 30, FI-00271, Helsinki, Finland

Correspondence to: Tarja Kinnunen Tarja.I.Kinnunen@uta.fi

The guarantor: Riitta Luoto

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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 populations[1]. The incidence of gestational diabetes has been increasing the last 20 years[2]. Risk factors for GDM include higher maternal body mass index (BMI), higher age and family history of diabetes[3]. Approximately half of the GDM cases can be explained by overweight[4]. 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 birth[5]. 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 metabolism[8-10]. In hereditary hemochromatosis iron accumulation in the body leads to diabetes in 30-60% of patients[11]. Also in animal models iron has been shown to induce diabetes[12]. Iron binding medication is effective in preventing diabetes in iron overload conditions[13]. Frequent blood donation has been shown to result in improvement in glucose metabolism even in healthy people[14]. Higher iron stores have been found in gestational diabetes patients measured most commonly by serum ferritin[15-17]. High haemoglobin level in early pregnancy has been reported to be an independent risk factor for GDM[18] and lower haemoglobin levels and anaemia during pregnancy have been shown to result in lower risk of GDM[19]. A large proportion of women, up to 78% in Finland[20], 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.

Studies of total iron intake and the risk of GDM are scarce: only Bowers et al.[21] have investigated pre-pregnancy total iron intake in relation to the risk of GDM. Other studies have investigated iron intake either from food or supplements only and have thus failed to report the effect of total iron intake[22 23]. Only one placebo controlled clinical trial has been conducted on supplemental iron and the risk of GDM[24]. To our knowledge there are no published studies of total iron intake during pregnancy, haemoglobin and the risk of GDM. 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.

MATERIAL AND METHDOS

The data of this study was originally collected as a part of a larger gestational diabetes prevention trial, which is described in more detail elsewhere[25]. The effects of the intervention on GDM and newborns birthweight have been reported elsewhere[26]. This cluster-randomised trial was carried out in 14 municipalities in Pirkanmaa region in south-western Finland in 2007-2009. The municipalities were randomised in pairs into intervention and usual care municipalities.

The inclusion criteria for the trial was to have at least one of the following risk factors: BMI ≥25 kg/m^2; age ≥40 years; GDM, glucose intolerance or newborn's macrosomia (≥4500g) in any earlier pregnancy or type 1 or 2 diabetes in first- or second-degree relatives. Women were excluded from the trial if they had an abnormal measurement in the baseline (8-12 weeks' gestation) glucose 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-pregnancy diabetes (type 1 or 2); age <18 years; no Finnish language skills; multiple pregnancy; restrictions from physical activity; substance abuse or psychiatric illness. The trial was approved by

the ethical committee of Pirkanmaa Hospital District and written informed consent was provided by the participants.

A total of 2271 women were screened for the study and of them 726 were preliminary eligible to participate in the study[26]. Of these women 640 (88.2%) gave an informed consent to participate in the trial. At the baseline (8-12 weeks' gestation) oral glucose tolerance test 174 women had an abnormal result and were thus excluded. Furthermore 38 women had a miscarriage and 29 were lost to follow-up. Finally, 399 participants were included in the analyses (219 in the intervention group and 180 in the usual care group).

The intervention group participated in individual counselling on weight gain, physical activity and diet on five antenatal visits in primary health care maternity clinics. The objectives of the counselling were to guide the participants in monitoring their weight gain, increasing or maintaining their leisure time physical activity and achieving a healthy diet fulfilling the national recommendations. The effects of intensified dietary counselling on food habits and the intake of energy, energy-yielding nutrients, fiber, selected fatty acids and cholesterol have been reported elsewhere[27]. The counselling was not aiming to influence in iron intake. In this study the intervention and usual care groups were combined and these groups had no differences in the incidence of GDM or in iron intake.

Background information of the participants was gathered with a baseline questionnaire at the first prenatal visit (8-12 weeks' gestation). Information of pre-pregnancy BMI (weight self reported and height measured at 8-12 weeks' visit) and haemoglobin were abstracted from maternity cards. In this study we used information of haemoglobin measurements from early pregnancy (weeks 8-12, or 16-18 for those with missing values for 8-12 weeks) to determine the status of body iron stores in

the beginning of pregnancy and before the physiological decrease in haemoglobin levels. The participants were considered to have good haemoglobin levels if they had a haemoglobin measurement of over 120 g/l according to the lower limit for normal haemoglobin in non-pregnant women[28]. The limit for normal haemoglobin in pregnant women (110 g/l) was not used because of low frequency of cases with haemoglobin level under 110 g/l in early pregnancy (n=7). All women underwent a 75 g oral glucose tolerance test at 26-28 weeks' gestation. The criterion for gestational diabetes diagnosis was to have at least one abnormal value: fasting glucose after an overnight fast ≥5.3 mmol/l, blood glucose >10.0 mmol/l one hour after or >8.6 mmol/l two hours after consuming 75 g of glucose. To cover all possible cases information of gestational diabetes was abstracted from the Finnish Medical Birth Register.

Information of diet and supplement use was obtained by a validated 181-item food frequency questionnaire[29]. The participants completed the questionnaire at 8-12 weeks' gestation and again at 26-28 and 36-37 weeks' gestation. In the 8-12 weeks' questionnaire the participants were asked about their dietary habits during one month before beginning of the pregnancy and in 26-28 and 36-37 weeks' questionnaires the participants were asked about their dietary habits during the previous month. The completed questionnaires were checked by a nutritionist and those with more than ten missing values in the frequency data were completed after consulting the participant on the phone. In the food frequency questionnaire the dietary habits were assessed by detailed questions of frequency of use (per day, week, month or not at all) and the portion sizes of specific food items. The participants were also asked to report their supplement use: brand name of the supplement, dosage and frequency of use (per day, week or month). The gathered data was then entered into a food database using a software program of the National Institute for Health and Welfare, Helsinki, Finland, and coded to daily food record form. Nutrient intakes were calculated by using the 10th release (updated in 2009) of the Finnish Food Composition Database Fineli (www.fineli.fi) and in-

house software of the National Institute for Health and Welfare, Helsinki. In this study information of dietary habits and supplement use at 26-28 weeks of gestation was used to get the best estimation of iron intake during pregnancy and before the onset of GDM.

Statistical methods

The study population was categorised into five equal groups according to their total daily iron intake. To better see the differences between women in the highest fifth of total iron intake and the other women, we combined the three groups in the middle (20, 60 and 20 percent). The groups were tested for possible differences in risk factors and other background characteristics using Chi-square test, Kruskal-Wallis test or One-way ANOVA. The incidence of GDM was then investigated between the iron intake groups and the differences were tested statistically using Chi-square test or Fisher exact test. Haemoglobin was considered as a potential effect modifier. Women were divided into two groups according to their haemoglobin level in early pregnancy: >120 g/l or ≤120 g/l.

Logistic regression was used to assess the odds ratio (OR) for gestational diabetes. The dependent variable in the regression model was GDM (yes/no). In the first model iron intake was assessed as a continuous variable. In the second model the highest fifth of total iron intake (intake ≥110 mg) was compared to the rest of the study population (iron intake under 110 mg). The models were adjusted for BMI, age (both as continuous), diabetes in first- or second-degree relatives and GDM or macrosomia in earlier pregnancy (both as categorical). Both models were executed separately for all women and for women with haemoglobin levels over 120 g/l in early pregnancy (8-12 or 16-18 weeks' gestation).

We also added interaction terms for iron intake and haemoglobin and for iron intake and BMI to the model but they were not statistically significant and therefore are not presented here. In sensitivity

analyses, we examined the association between the total iron intake and the risk of GDM using alternative cut-off points for categorising participants based on their iron intake (15, 70 and 15 percent; 25, 50 and 25 percent and 30, 40 and 30 percent) and the same statistical tests as described above. All analyses were performed with SPSS statistics (version 19).

RESULTS

Based on the questionnaire completed at 26-28 weeks' gestation, the mean daily iron intake from food was 14.4 mg (SD 4.3; median 13.9 mg) among the study population. In the study group 65.7% of women used supplemental iron and the median of supplemental intake was 27.0 mg. The most used dosages for iron supplementation were 10.0 mg, 20.0 mg and 100.0 mg of elemental iron (Fe^{2+}). The median for total iron intake was 27.1 mg (interquartile range 15.5-89.3 mg) (Figure 1). Iron intake from food was similar in nonusers and users of supplemental iron: users of supplemental iron had a mean daily intake of 14.5 mg (SD 4.5) and nonusers 14.3 mg (SD 3.9). The mean haemoglobin level of the study population in the beginning of pregnancy was 134 g/l (SD 11; min 107 g/l; max 169 g/l).

The oral glucose tolerance test at 26-28 weeks' gestation was abnormal in 14.0% of the study population (n=56, missing value for three women). Furthermore 16 women received a gestational diabetes diagnosis during their pregnancy. Figure 2 shows the incidence of GDM in fifths of total iron intake. There was a tendency for a higher incidence of GDM in the highest fifth of total iron intake.

Between the iron intake groups (20%, 60% and 20%) there were no significant differences in terms of proportion of primigravida, age, diabetes in first- or second-degree relatives or GDM or

macrosomia in earlier pregnancy (Table 1). Women in the highest iron intake group had a slightly lower mean BMI as compared to other women (P=0.05). Haemoglobin levels in early pregnancy differed between the groups as excepted (P<0.001). Table 2 shows the incidence of GDM in the three iron intake groups. The incidence was higher in the highest iron intake group (20.8%) compared to the other two groups (16.7% in both) but the difference was not statistically significant (P=0.70). After excluding those who had low haemoglobin levels (≤120 g/l) already in early pregnancy, the difference between the groups was even larger but still not statistically significant (P=0.11).

The logistic regression model shows that after adjustment for risk factors and background characteristics total iron intake was significantly and positively associated with gestational diabetes mellitus (Table 3). When iron intake was used as a continuous variable the OR was 1.005 (P=0.041) for all women and 1.008 (P=0.005) for those with haemoglobin levels over 120 g/l. In the second model the OR for GDM was 1.55 (95% confidence interval 0.81 to 2.96, P=0.19) for the highest fifth of iron intake among all women. After excluding women with low haemoglobin levels (≤120 g/l) in early pregnancy the OR was 2.21 (95% confidence interval 1.11 to 4.41, P=0.025).

In sensitivity analyses the odds ratios were higher when 15% of women with the highest iron intake were compared to the rest 85% of women. The odds ratios were lower when 25% or 30% of women with highest intake were compared to the rest 75% and 70% of women respectively (results not shown).

DISCUSSION

In this study we investigated the possible association between total daily iron intake during pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes. To our knowledge there are no similar studies published previously. We discovered that there was a tendency for a higher incidence of GDM in the highest fifth of total iron intake. High iron intake increased the risk of GDM especially for those women who had good haemoglobin levels in early pregnancy: we discovered a two-fold OR for GDM for women in the highest fifth of iron intake with a haemoglobin level of over 120 g/l in early pregnancy.

Our study has several strengths. Firstly unlike most of the previous studies we had detailed information of iron intake from both food and supplements. Secondly 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 factors for GDM.

A limitation of this kind of study is that estimating iron intake during pregnancy can be challenging because dietary habits and use of supplements can vary a lot during pregnancy due to nausea and other changes in well being. Iron intake was assessed at a certain point in time, thus it does not cover the intake during the whole pregnancy. In our study we decided to use information of iron intake in mid-pregnancy to get the best estimation on iron intake during pregnancy and before the onset of GDM. We did not use information of iron intake abstracted in the beginning of pregnancy because it covered only dietary habits and supplement use during one month before the pregnancy

when use of supplemental iron is rare. However our information of iron intake covers only one month in the mid-pregnancy. Additionally we could not assess iron absorption, for example with blood measurements, which also can vary a lot depending on body iron status and the contents of meals.

There are also limitations concerning the haemoglobin measurements used in our study. In the maternity clinics haemoglobin levels are usually screened with a capillary haemoglobin measurement using finger-stick samples. This method has been demonstrated to be reliable in determining haemoglobin values but it is however susceptible to handling errors[30] and therefore there can be variation in the test results. Additionally we had a lot of missing values in haemoglobin measurements and thus we decided to use the haemoglobin measurement at 16-18 weeks' gestation for those with missing values at weeks 8-12 (n= 28). Although measurements at weeks 16-18 do not present the haemoglobin levels at early pregnancy our objective was to analyse separately women who were not anaemic and thus including women who had a haemoglobin level of over 120 g/l still at weeks 16-18 would help us not to underestimate the proportion of women who were not anaemic in the beginning of the pregnancy. However for these reasons our results in respect of haemoglobin measurements should be considered with caution and further studies with reliable and comprehensive haemoglobin measurements are warranted to confirm our results.

In a somewhat similar study Bo et al.[22] discovered that women who used supplemental iron during mid-pregnancy had a 2- to 3-fold risk of GDM. They however had no information of dietary intake of iron or haemoglobin levels or other measurements of iron stores. Their findings are however in line with our results which suggest that dietary intake could play a relatively small part in the association whereas high supplemental iron intake could be responsible for most of the increase in GDM risk. Somewhat different results have been reported recently by Bowers et al.[21] and Qiu et

al.[23]. Bowers et al.[21] investigated dietary and supplemental iron intake during one year before pregnancy utilising the material from the Nurses Health study. They observed no significant effect of total, nonheme or supplemental iron intake on the risk of GDM. It can be argued though, that usually iron supplementation outside of pregnancy is more rare and concerns mainly those who are anaemic. However they did find a significant and positive association between heme iron intake and GDM. Similarly Qiu et al.[23] demonstrated an association between heme iron intake during the time before conception and in early pregnancy and the risk of GDM. To our knowledge only one placebo controlled clinical trial has been conducted to investigate the association between iron supplementation and GDM[24]. This study did not observe any association. However it can be argued that the intake of supplemental iron was quite low in this study because of only about 50% compliance to a daily supplement of 60 mg of elemental iron.

Iron is a highly reactive component with a possibility to participate in harmful reactions[31]. The human body can excrete iron with very limited mechanisms and thus iron intake is highly regulated according to body iron needs[32]. Iron could interfere with glucose metabolism in several ways. For example following mechanisms have been proposed: Iron decreases insulin extraction and metabolism in the liver, which leads to peripheral hyperinsulinemia[33]. Iron overload results in oxidative stress in pancreatic β-cells, which leads to destruction of the pancreatic islets and thus decreases insulin secretion[34]. The exact mechanisms, which link iron to diabetes are still unsolved. However the association can be argued to be biologically plausible.

Accumulating body of evidence supports the hypothesis of excess iron as a risk factor for glucose metabolism disorders such as gestational diabetes. Iron deficiency anaemia has been shown to reduce the incidence of GDM[19]. Respectively high haemoglobin level (>130 g/l) in early pregnancy has been demonstrated to be an independent risk factor for GDM[18]. It seems that iron can affect

glucose metabolism even with no overt iron overload. Women diagnosed with GDM have been observed to have increased iron stores compared to women without GDM[15 17]. However many of these studies have been cross-sectional and it has been criticised that higher serum ferritin levels could in fact reflect inflammation in the body and could be rather a result than a cause for diabetes[16].

In summary it seems that high iron intake might be a factor, which increases the risk of GDM especially in women with already good iron stores. Use of iron supplements during pregnancy is common even in women with good haemoglobin levels. Our results suggest that routine use of iron supplements should be reconsidered in non-anaemic women with risk factors for GDM. To confirm the hypothesis there is need for a large prospective study – ideally a randomised controlled trial – with reliable and comprehensive information on iron intake from food and supplements during pregnancy accompanied with serum measurements to determine the level of body iron stores.

Author contributions

AH, TIK and RL designed and conducted research; AH and JR performed statistical analyses; AH drafted the paper and revised it based on the comments from the other authors. TIK and RL have the primary responsibility for final content. All authors read, commented and approved the final manuscript.

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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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Figure legends

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

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

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

	20% <14.2 mg/day (n=78)	60% 14.2 – 109.9 mg/day (n=233)	20% 110.0+ mg/day (n=78)	P-value
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 weeks[1]) >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 weeks[1]) ≤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)

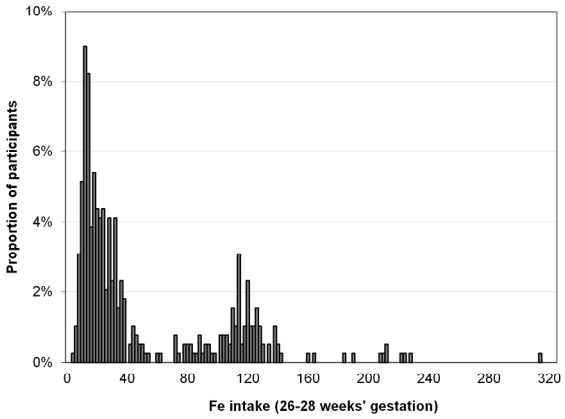
[2] Chi-square test

[3] Fisher's exact test

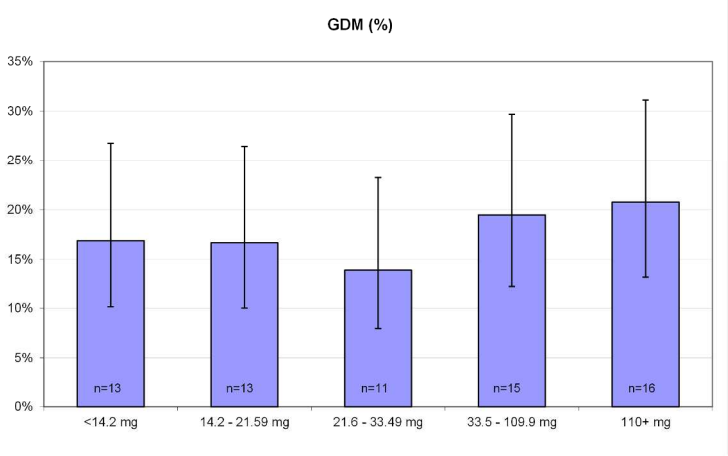
Table 3. Incidence of GDM, unadjusted and adjusted ORs (95% CI) for iron intake at 26-28 weeks' gestation as a continuous and a categorical variable separately for all women and for women with haemoglobin >120 g/l

	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI) [2]	P-value
Fe intake as a continuous variable				
All women				
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
Women with Hb (8-12 weeks' gestation[1]) >120 g/l				
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				
All women				
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
Women with Hb (8-12 weeks' gestation[1]) >120 g/l				
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)



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



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	

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
		(b) Indicate number of participants with missing data for each variable of interest	8, Table 1, Table 2
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
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
		(*b*) Report category boundaries when continuous variables were categorized	Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
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
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
Generalisability	21	Discuss the generalisability (external validity) of the study results	10,13
Other information			
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.

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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

Annika Helin, MSc student[1], Tarja I Kinnunen, university teacher[1], Jani Raitanen, statistician[1,2], Suvi Ahonen, researcher[1,4], Suvi M Virtanen, research professor[1,3,4], Riitta Luoto, research director[2,5]

Affiliations:

[1] University of Tampere, School of Health Sciences, FI-33014 University of Tampere, Finland;

[2] The UKK Institute for Health Promotion Research, P. O. Box 30, FI-33501 Tampere, Finland;

[3] The National Institute for Health and Welfare, The Unit of Nutrition, P.O. Box 30, FI-00271 Helsinki, Finland;

[4] Science Center of Pirkanmaa Hospital District, Tampere University Hospital, P. O. Box 2000, FI-33521 Tampere, Finland;

[5] The National Institute for Health and Welfare, The Unit of Sexual and Reproductive Health, P.O. Box 30, FI-00271, Helsinki, Finland

Correspondence to: Tarja Kinnunen Tarja.I.Kinnunen@uta.fi

The guarantor: Riitta Luoto

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 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

Strenghts:

- 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

factors for GDM.

Limitations:

- Iron intake was assessed at a certain point in time, thus it does not cover the intake during the whole pregnancy.

- We could not assess iron absorption.

- The haemoglobin levels were screened at the maternity clinics using finger-stick samples, which may not be an optimal measurement tool.

Haemoglobin measurements were missing for 28 women at 8-12 weeks' gestation and we used the measurement at 16-18 weeks' gestation for those women.

INTRODUCTION

Gestational diabetes mellitus (GDM) is a disturbance in glucose metabolism, which is diagnosed during pregnancy and affects 1-14% of pregnancies in different populations[1]. The incidence of gestational diabetes has been increasing the last 20 years[2]. Risk factors for GDM include higher maternal body mass index (BMI), higher age and family history of diabetes[3]. Approximately half of the GDM cases can be explained by overweight[4]. 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 birth[5]. 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 metabolism[8-10]. In hereditary hemochromatosis iron accumulation in the body leads to diabetes in 30-60% of patients[11]. Also in animal models iron has been shown to induce diabetes[12]. Iron binding medication is effective in preventing diabetes in iron overload conditions[13]. Frequent blood donation has been shown to result in improvement in glucose metabolism even in healthy people[14]. Higher iron stores have been found in gestational diabetes patients measured most commonly by serum ferritin[15-17]. High haemoglobin level in early pregnancy has been reported to be an independent risk factor for GDM[18] and lower haemoglobin levels and anaemia during pregnancy have been shown to result in lower risk of GDM[19]. A large proportion of women, up to 78% in Finland[20], 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.

Studies of total iron intake and the risk of GDM are scarce: only Bowers et al.[21] have investigated pre-pregnancy total iron intake in relation to the risk of GDM. Other studies have investigated iron intake either from food or supplements only and have thus failed to report the effect of total iron intake[22 23]. Only one placebo controlled clinical trial has been conducted on supplemental iron and the risk of GDM[24]. To our knowledge there are no published studies of total iron intake during pregnancy, haemoglobin and the risk of GDM. 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.

MATERIAL AND METHDOS

The data of this study was originally collected as a part of a larger gestational diabetes prevention trial, which is described in more detail elsewhere[25]. The effects of the intervention on GDM and newborns birthweight have been reported elsewhere[26]. This cluster-randomised trial was carried out in 14 municipalities in Pirkanmaa region in south-western Finland in 2007-2009. The municipalities were randomised in pairs into intervention and usual care municipalities.

The inclusion criteria for the trial were to have at least one of the following risk factors: BMI ≥25 kg/m^2; age ≥40 years; GDM, glucose intolerance or newborn's macrosomia (≥4500g) in any earlier pregnancy or type 1 or 2 diabetes in first- or second-degree relatives. Women were excluded from the trial if they had an abnormal measurement in the baseline (8-12 weeks' gestation) glucose 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-pregnancy diabetes (type 1 or 2); age <18 years; no Finnish language skills; multiple pregnancy; restrictions from physical activity; substance abuse or psychiatric illness. The trial was approved by

the ethical committee of Pirkanmaa Hospital District and written informed consent was provided by the participants.

A total of 2271 women were screened for the study and of them 726 were preliminary eligible to participate in the study[26]. Of these women 640 (88.2%) gave an informed consent to participate in the trial. At the baseline (8-12 weeks' gestation) oral glucose tolerance test 174 women had an abnormal result and were thus excluded. Furthermore 38 women had a miscarriage and 29 were lost to follow-up. Finally, 399 participants were included in the analyses (219 in the intervention group and 180 in the usual care group).

The intervention group participated in individual counselling on weight gain, physical activity and diet on five antenatal visits in primary health care maternity clinics. The objectives of the counselling were to guide the participants in monitoring their weight gain, increasing or maintaining their leisure time physical activity and achieving a healthy diet fulfilling the national recommendations. The effects of intensified dietary counselling on food habits and the intake of energy, energy-yielding nutrients, fiber, selected fatty acids and cholesterol have been reported elsewhere[27]. The counselling was not aiming to influence in iron intake. In this study the intervention and usual care groups were combined and these groups had no differences in the incidence of GDM or in iron intake.

Background information of the participants was gathered with a baseline questionnaire at the first prenatal visit (8-12 weeks' gestation). Information of pre-pregnancy BMI (weight self reported and height measured at 8-12 weeks' visit) and haemoglobin were abstracted from maternity cards. In this study we used information of haemoglobin measurements from early pregnancy (weeks 8-12, or 16-18 for those with missing values for 8-12 weeks) to determine the status of body iron stores in

the beginning of pregnancy and before the physiological decrease in haemoglobin levels. The participants were considered to have good haemoglobin levels if they had a haemoglobin measurement of over 120 g/l according to the lower limit for normal haemoglobin in non-pregnant women[28]. The limit for normal haemoglobin in pregnant women (110 g/l) was not used because of low frequency of cases with haemoglobin level under 110 g/l in early pregnancy (n=7). All women underwent a 75 g oral glucose tolerance test at 26-28 weeks' gestation. The criterion for gestational diabetes diagnosis was to have at least one abnormal value: fasting glucose after an overnight fast ≥5.3 mmol/l, blood glucose >10.0 mmol/l one hour after or >8.6 mmol/l two hours after consuming 75 g of glucose. To cover all possible cases information of gestational diabetes was abstracted from the Finnish Medical Birth Register.

Information on diet and supplement use was obtained by a validated 181-item food frequency questionnaire[29] although the validity of the supplement data was not assessed in the study by Erkkola et al. The participants completed the questionnaire at 8-12 weeks' gestation and again at 26-28 and 36-37 weeks' gestation. In the 8-12 weeks' questionnaire the participants were asked about their dietary habits during one month before beginning of the pregnancy and in 26-28 and 36-37 weeks' questionnaires the participants were asked about their dietary habits during the previous month. The completed questionnaires were checked by a nutritionist and those with more than ten missing values in the frequency data were completed after consulting the participant on the phone. In the food frequency questionnaire the dietary habits were assessed by detailed questions of frequency of use (per day, week, month or not at all) and the portion sizes of specific food items. The participants were also asked to report their supplement use: brand name of the supplement, dosage and frequency of use (per day, week or month). The gathered data was then entered into a food database using a software program of the National Institute for Health and Welfare, Helsinki, Finland, and coded to daily food record form. Nutrient intakes were calculated by using the 10th

release (updated in 2009) of the Finnish Food Composition Database Fineli (www.fineli.fi) and in-house software of the National Institute for Health and Welfare, Helsinki. In this study information of dietary habits and supplement use at 26-28 weeks of gestation was used to get the best estimation of iron intake and the intake of energy, macronutrients and dietary fibre during pregnancy and before the onset of GDM.

Statistical methods

The study population was categorised into five equal groups according to their total daily iron intake. To better see the differences between women in the highest fifth of total iron intake and the other women, we combined the three groups in the middle (20, 60 and 20 percent). The groups were tested for possible differences in risk factors, dietary intake and other background characteristics using Chi-square test, Kruskal-Wallis test or One-way ANOVA. The incidence of GDM was then investigated between the iron intake groups and the differences were tested statistically using Chi-square test or Fisher exact test. Haemoglobin was considered as a potential effect modifier. Women were divided into two groups according to their haemoglobin level in early pregnancy: >120 g/l or ≤120 g/l.

Logistic regression was used to assess the odds ratio (OR) for gestational diabetes. The dependent variable in the regression model was GDM (yes/no). In the first model iron intake was assessed as a continuous variable. In the second model the highest fifth of total iron intake (intake ≥110 mg) was compared to the rest of the study population (iron intake under 110 mg). The models were adjusted for BMI, age, intake of energy, saturated fatty acids and dietary fibre and total gestational weight gain (all as continuous) as well as diabetes in first- or second-degree relatives and GDM or macrosomia in earlier pregnancy (both as categorical). Both models were executed separately for all

women and for women with haemoglobin levels over 120 g/l in early pregnancy (8-12 or 16-18 weeks' gestation).

We also added interaction terms for iron intake and haemoglobin and for iron intake and BMI to the model but they were not statistically significant and therefore are not presented here. In sensitivity analyses, we examined the association between the total iron intake and the risk of GDM using alternative cut-off points for categorising participants based on their iron intake (15, 70 and 15 percent; 25, 50 and 25 percent and 30, 40 and 30 percent) and the same statistical tests as described above. All analyses were performed with SPSS statistics (version 19).

RESULTS

Based on the questionnaire completed at 26-28 weeks' gestation, the mean daily iron intake from food was 14.4 mg (SD 4.3; median 13.9 mg) among the study population. In the study group 65.7% of women used supplemental iron and the median of supplemental intake was 27.0 mg. The most used dosages for iron supplementation were 10.0 mg, 20.0 mg and 100.0 mg of elemental iron (Fe^{2+}). The median for total iron intake was 27.1 mg (interquartile range 15.5-89.3 mg) (Figure 1). Iron intake from food was similar in nonusers and users of supplemental iron: users of supplemental iron had a mean daily intake of 14.5 mg (SD 4.5) and nonusers 14.3 mg (SD 3.9). The mean haemoglobin level of the study population in the beginning of pregnancy was 134 g/l (SD 11; min 107 g/l; max 169 g/l).

The oral glucose tolerance test at 26-28 weeks' gestation was abnormal in 14.0% of the study population (n=56, missing value for three women). Furthermore 16 women received a gestational diabetes diagnosis during their pregnancy. Figure 2 shows the incidence of GDM in fifths of total

iron intake. There was a tendency for a higher incidence of GDM in the highest fifth of total iron intake.

Between the iron intake groups (20%, 60% and 20%) there were no significant differences in terms of proportion of primigravida, age, diabetes in first- or second-degree relatives or GDM or macrosomia in earlier pregnancy (Table 1). Women in the highest iron intake group had a slightly lower mean BMI as compared to other women (P=0.05). Haemoglobin levels in early pregnancy differed between the groups as excepted (P<0.001). With regard to differences in dietary intake, women in the lowest iron intake group had lower total energy and dietary fibre intakes than the other women whereas women in the highest iron intake group had higher intake of saturated fatty acids than the other women. Total gestational weight gain did not differ between the iron intake groups. Table 2 shows the incidence of GDM in the three iron intake groups. The incidence was higher in the highest iron intake group (20.8%) compared to the other two groups (16.7% in both) but the difference was not statistically significant (P=0.70). When including women with haemoglobin levels >120 g/l in early pregnancy (n=321, mean Hb 135, SD 9), the difference between the groups was even larger but still not statistically significant (P=0.11).

The logistic regression model shows that after adjustment for potential risk factors and background characteristics total iron intake was significantly and positively associated with gestational diabetes mellitus (Table 3). When iron intake was used as a continuous variable the OR was 1.006 (P=0.038) for all women and 1.009 (P=0.006) for those with haemoglobin levels over 120 g/l. In the second model the OR for GDM was 1.66 (95% confidence interval 0.84 to 3.30, P=0.15) for the highest fifth of iron intake among all women. After excluding women with low haemoglobin levels (≤120 g/l) in early pregnancy the OR was 2.35 (95% confidence interval 1.13 to 4.92, P=0.023).

In sensitivity analyses the odds ratios were higher when 15% of women with the highest iron intake

were compared to the rest 85% of women. The odds ratios were lower when 25% or 30% of women with highest intake were compared to the rest 75% and 70% of women respectively (results not shown).

DISCUSSION

In this study we investigated the possible association between total daily iron intake during pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes. To our knowledge there are no similar studies published previously. We discovered that there was a tendency for a higher incidence of GDM in the highest fifth of total iron intake. High iron intake increased the risk of GDM especially for those women who had good haemoglobin levels in early pregnancy: we discovered a two-fold OR for GDM for women in the highest fifth of iron intake with a haemoglobin level of over 120 g/l in early pregnancy.

Our study has several strengths. Firstly unlike most of the previous studies we had detailed information of iron intake from both food and supplements. Secondly we had information on 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 factors for GDM.

A limitation of this kind of study is that estimating iron intake during pregnancy can be challenging because dietary habits and use of supplements can vary a lot during pregnancy due to nausea and other changes in wellbeing. Iron intake was assessed at a certain point in time (at 26-28 weeks' gestation covering the previous month), thus it does not cover the intake during the whole pregnancy. In our study we decided to use information of iron intake in mid-pregnancy to get the best estimation on iron intake during pregnancy and before the onset of GDM. We did not use information of iron intake abstracted in the beginning of pregnancy because it covered only dietary habits and supplement use during one month before the pregnancy when use of supplemental iron is rare. However our information on iron intake covers only one month in the mid-pregnancy. Additionally we could not assess iron absorption, for example with blood measurements, which also can vary a lot depending on body iron status and the contents of meals.

There are also limitations concerning the haemoglobin measurements used in our study. In the maternity clinics haemoglobin levels are usually screened with a capillary haemoglobin measurement using finger-stick samples. This method has been demonstrated to be reliable in determining haemoglobin values but it is however susceptible to handling errors[30] and therefore there can be variation in the test results. Additionally we had a lot of missing values in haemoglobin measurements and thus we decided to use the haemoglobin measurement at 16-18 weeks' gestation for those with missing values at weeks 8-12 (n= 28). Although measurements at weeks 16-18 do not present the haemoglobin levels at early pregnancy our objective was to analyse separately women who were not anaemic and thus including women who had a haemoglobin level of over 120 g/l still at weeks 16-18 would help us not to underestimate the proportion of women who were not anaemic in the beginning of the pregnancy. However for these reasons our results in respect of haemoglobin measurements should be considered with caution and further studies with reliable and comprehensive haemoglobin measurements are warranted to confirm our results.

In a somewhat similar study Bo et al.[22] discovered that women who used supplemental iron during mid-pregnancy had a 2- to 3-fold risk of GDM. They however had no information of dietary intake of iron or haemoglobin levels or other measurements of iron stores. Their findings are however in line with our results which suggest that dietary intake could play a relatively small part in the association whereas high supplemental iron intake could be responsible for most of the increase in GDM risk. Somewhat different results have been reported recently by Bowers et al.[21] and Qiu et al.[23]. Bowers et al.[21] investigated dietary and supplemental iron intake during one year before pregnancy utilising the material from the Nurses Health study. They observed no significant effect of total, nonheme or supplemental iron intake on the risk of GDM. It can be argued though, that usually iron supplementation outside of pregnancy is more rare and concerns mainly those who are anaemic. However they did find a significant and positive association between heme iron intake and GDM. Similarly Qiu et al.[23] demonstrated an association between heme iron intake during the time before conception and in early pregnancy and the risk of GDM. To our knowledge only one placebo controlled clinical trial has been conducted to investigate the association between iron supplementation and GDM[24]. This study did not observe any association. However it can be argued that the intake of supplemental iron was quite low in this study because of only about 50% compliance to a daily supplement of 60 mg of elemental iron.

Iron is a highly reactive component with a possibility to participate in harmful reactions[31]. The human body can excrete iron with very limited mechanisms and thus iron intake is highly regulated according to body iron needs[32]. Iron could interfere with glucose metabolism in several ways. For example following mechanisms have been proposed: Iron decreases insulin extraction and metabolism in the liver, which leads to peripheral hyperinsulinemia[33]. Iron overload results in oxidative stress in pancreatic β-cells, which leads to destruction of the pancreatic islets and thus

decreases insulin secretion[34]. The exact mechanisms, which link iron to diabetes are still unsolved. However the association can be argued to be biologically plausible.

Accumulating body of evidence supports the hypothesis of excess iron as a risk factor for glucose metabolism disorders such as gestational diabetes. Iron deficiency anaemia has been shown to reduce the incidence of GDM[19]. Respectively high haemoglobin level (>130 g/l) in early pregnancy has been demonstrated to be an independent risk factor for GDM[18]. It seems that iron can affect glucose metabolism even with no overt iron overload. Women diagnosed with GDM have been observed to have increased iron stores compared to women without GDM[15 17]. However many of these studies have been cross-sectional and it has been criticised that higher serum ferritin levels could in fact reflect inflammation in the body and could be rather a result than a cause for diabetes[16].

In summary it seems that high iron intake might be a factor, which increases the risk of GDM especially in women with already good iron stores. Use of iron supplements during pregnancy is common even in women with good haemoglobin levels. Our results suggest that routine use of iron supplements should be reconsidered in non-anaemic women with risk factors for GDM. To confirm the hypothesis there is need for a large prospective study – ideally a randomised controlled trial – with reliable and comprehensive information on iron intake from food and supplements during pregnancy accompanied with serum measurements to determine the level of body iron stores.

Author contributions

AH, TIK and RL designed and conducted research; AH and JR performed statistical analyses; AH drafted the paper and revised it based on the comments from the other authors. SMV and SA were

responsible for the FFQ method and for dietary calculations. TIK and RL have the primary responsibility for final content. All authors read, commented and approved the final manuscript.

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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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Data Sharing Statement

Additional unpublished data from the study may be available and it can be enquired of the authors.

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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Figure legends

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

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

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

	20% <14.2 mg/day (n=78)	60% 14.2 – 109.9 mg/day (n=233)	20% 110.0+ mg/day (n=78)	P-value
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 weeks[1]) >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 weeks[1]) ≤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				
All women				
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
Women with Hb (8-12 weeks' gestation[2]) >120 g/l				
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				
All women				
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

Total energy intake (MJ/day)			0.89 (0.80 – 1.06)	0.19
Dietary fibre (g/day)			1.02 (0.97 – 1.08)	0.37
Saturated fatty acids (E%)[1]			0.97 (0.85 – 1.10)	0.59
Total gestational weight gain			0.94 (0.89 – 0.99)	0.029
Women with Hb (8-12 weeks' gestation[2]) >120 g/l				
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
BMI			1.07 (1.00 – 1.14)	0.054
Age			1.01 (0.95 – 1.08)	0.76
Diabetes in first- or second-degree relatives			0.70 (0.38 – 1.31)	0.27
GDM or macrosomia in a previous pregnancy			1.60 (0.73 – 3.53)	0.24
Total energy intake (MJ/day)			0.83 (0.69 – 1.02)	0.071
Dietary fibre (g/day)			1.05 (0.99 – 1.11)	0.10
Saturated fatty acids (E%)[1]			0.99 (0.86 – 1.15)	0.92
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, 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

Annika Helin, MSc student[1], Tarja I Kinnunen, university teacher[1], Jani Raitanen, statistician[1,2], Suvi Ahonen, researcher[1,4], Suvi M Virtanen, research professor[1,3,4], Riitta Luoto, research director[2,5~~3~~]

Affiliations:

[1] University of Tampere, School of Health Sciences, FI-33014 University of Tampere, Finland;

[2] The UKK Institute for Health Promotion Research, P. O. Box 30, FI-33501 Tampere, Finland;

[3] The National Institute for Health and Welfare, The Unit of Nutrition, P.O. Box 30, FI-00271 Helsinki, Finland;

[4] Science Center of Pirkanmaa Hospital District, Tampere University Hospital, P. O. Box 2000, FI-33521 Tampere, Finland;

[~~3~~5] The National Institute for Health and Welfare, The Unit of Sexual and Reproductive Health, P.O. Box 30, FI-00271, Helsinki, Finland

Correspondence to: Tarja Kinnunen Tarja.I.Kinnunen@uta.fi

The guarantor: Riitta Luoto

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

Word count ~~3311~~3424

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 1.006~~5~~ (95% confidence interval 1.000 to 1.011; P=0.038~~41~~) 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 1.66~~55~~ (95% confidence interval 0.84~~1~~ to 3.30~~2.96~~; P=0.15~~9~~) for GDM. After excluding participants with low haemoglobin levels (≤120 g/l) already in early pregnancy the adjusted OR was 2.35~~21~~ (95% confidence interval 1.13~~1~~ to 4.92~~41~~; P=0.023~~5~~).

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

INTRODUCTION

Gestational diabetes mellitus (GDM) is a disturbance in glucose metabolism, which is diagnosed during pregnancy and affects 1-14% of pregnancies in different populations[1]. The incidence of gestational diabetes has been increasing the last 20 years[2]. Risk factors for GDM include higher maternal body mass index (BMI), higher age and family history of diabetes[3]. Approximately half of the GDM cases can be explained by overweight[4]. 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 birth[5]. 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 metabolism[8-10]. In hereditary hemochromatosis iron accumulation in the body leads to diabetes in 30-60% of patients[11]. Also in animal models iron has been shown to induce diabetes[12]. Iron binding medication is effective in preventing diabetes in iron overload conditions[13]. Frequent blood donation has been shown to result in improvement in glucose metabolism even in healthy people[14]. Higher iron stores have been found in gestational diabetes patients measured most commonly by serum ferritin[15-17]. High haemoglobin level in early pregnancy has been reported to be an independent risk factor for GDM[18] and lower haemoglobin levels and anaemia during pregnancy have been shown to result in lower risk of GDM[19]. A large proportion of women, up to 78% in Finland[20], 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.

Studies of total iron intake and the risk of GDM are scarce: only Bowers et al.[21] have investigated pre-pregnancy total iron intake in relation to the risk of GDM. Other studies have investigated iron intake either from food or supplements only and have thus failed to report the effect of total iron intake[22 23]. Only one placebo controlled clinical trial has been conducted on supplemental iron and the risk of GDM[24]. To our knowledge there are no published studies of total iron intake during pregnancy, haemoglobin and the risk of GDM. 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.

MATERIAL AND METHDOS

The data of this study was originally collected as a part of a larger gestational diabetes prevention trial, which is described in more detail elsewhere[25]. The effects of the intervention on GDM and newborns birthweight have been reported elsewhere[26]. This cluster-randomised trial was carried out in 14 municipalities in Pirkanmaa region in south-western Finland in 2007-2009. The municipalities were randomised in pairs into intervention and usual care municipalities.

The inclusion criteria for the trial ~~was~~ were to have at least one of the following risk factors: BMI ≥25 kg/m^2; age ≥40 years; GDM, glucose intolerance or newborn's macrosomia (≥4500g) in any earlier pregnancy or type 1 or 2 diabetes in first- or second-degree relatives. Women were excluded from the trial if they had an abnormal measurement in the baseline (8-12 weeks' gestation) glucose 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-pregnancy diabetes (type 1 or 2); age <18 years; no Finnish language skills; multiple pregnancy; restrictions from physical activity; substance abuse or psychiatric illness. The trial was approved by

the ethical committee of Pirkanmaa Hospital District and written informed consent was provided by the participants.

A total of 2271 women were screened for the study and of them 726 were preliminary eligible to participate in the study[26]. Of these women 640 (88.2%) gave an informed consent to participate in the trial. At the baseline (8-12 weeks' gestation) oral glucose tolerance test 174 women had an abnormal result and were thus excluded. Furthermore 38 women had a miscarriage and 29 were lost to follow-up. Finally, 399 participants were included in the analyses (219 in the intervention group and 180 in the usual care group).

The intervention group participated in individual counselling on weight gain, physical activity and diet on five antenatal visits in primary health care maternity clinics. The objectives of the counselling were to guide the participants in monitoring their weight gain, increasing or maintaining their leisure time physical activity and achieving a healthy diet fulfilling the national recommendations. The effects of intensified dietary counselling on food habits and the intake of energy, energy-yielding nutrients, fiber, selected fatty acids and cholesterol have been reported elsewhere[27]. The counselling was not aiming to influence in iron intake. In this study the intervention and usual care groups were combined and these groups had no differences in the incidence of GDM or in iron intake.

Background information of the participants was gathered with a baseline questionnaire at the first prenatal visit (8-12 weeks' gestation). Information of pre-pregnancy BMI (weight self reported and height measured at 8-12 weeks' visit) and haemoglobin were abstracted from maternity cards. In this study we used information of haemoglobin measurements from early pregnancy (weeks 8-12, or 16-18 for those with missing values for 8-12 weeks) to determine the status of body iron stores in

the beginning of pregnancy and before the physiological decrease in haemoglobin levels. The participants were considered to have good haemoglobin levels if they had a haemoglobin measurement of over 120 g/l according to the lower limit for normal haemoglobin in non-pregnant women[28]. The limit for normal haemoglobin in pregnant women (110 g/l) was not used because of low frequency of cases with haemoglobin level under 110 g/l in early pregnancy (n=7). All women underwent a 75 g oral glucose tolerance test at 26-28 weeks' gestation. The criterion for gestational diabetes diagnosis was to have at least one abnormal value: fasting glucose after an overnight fast ≥5.3 mmol/l, blood glucose >10.0 mmol/l one hour after or >8.6 mmol/l two hours after consuming 75 g of glucose. To cover all possible cases information of gestational diabetes was abstracted from the Finnish Medical Birth Register.

Information on~~f~~ diet and supplement use was obtained by a validated 181-item food frequency questionnaire[29] although the validity of the supplement data was not assessed in the study by Erkkola et al. The participants completed the questionnaire at 8-12 weeks' gestation and again at 26-28 and 36-37 weeks' gestation. In the 8-12 weeks' questionnaire the participants were asked about their dietary habits during one month before beginning of the pregnancy and in 26-28 and 36-37 weeks' questionnaires the participants were asked about their dietary habits during the previous month. The completed questionnaires were checked by a nutritionist and those with more than ten missing values in the frequency data were completed after consulting the participant on the phone. In the food frequency questionnaire the dietary habits were assessed by detailed questions of frequency of use (per day, week, month or not at all) and the portion sizes of specific food items. The participants were also asked to report their supplement use: brand name of the supplement, dosage and frequency of use (per day, week or month). The gathered data was then entered into a food database using a software program of the National Institute for Health and Welfare, Helsinki, Finland, and coded to daily food record form. Nutrient intakes were calculated by using the 10th

release (updated in 2009) of the Finnish Food Composition Database Fineli (www.fineli.fi) and in-house software of the National Institute for Health and Welfare, Helsinki. In this study information of dietary habits and supplement use at 26-28 weeks of gestation was used to get the best estimation of iron intake and the intake of energy, macronutrients and dietary fibre during pregnancy and before the onset of GDM.

Statistical methods

The study population was categorised into five equal groups according to their total daily iron intake. To better see the differences between women in the highest fifth of total iron intake and the other women, we combined the three groups in the middle (20, 60 and 20 percent). The groups were tested for possible differences in risk factors, dietary intake and other background characteristics using Chi-square test, Kruskal-Wallis test or One-way ANOVA. The incidence of GDM was then investigated between the iron intake groups and the differences were tested statistically using Chi-square test or Fisher exact test. Haemoglobin was considered as a potential effect modifier. Women were divided into two groups according to their haemoglobin level in early pregnancy: >120 g/l or ≤120 g/l.

Logistic regression was used to assess the odds ratio (OR) for gestational diabetes. The dependent variable in the regression model was GDM (yes/no). In the first model iron intake was assessed as a continuous variable. In the second model the highest fifth of total iron intake (intake ≥110 mg) was compared to the rest of the study population (iron intake under 110 mg). The models were adjusted for BMI, age ~~(both as continuous)~~, intake of energy, saturated fatty acids and dietary fibre and total gestational weight gain (all as continuous) as well as diabetes in first- or second-degree relatives and GDM or macrosomia in earlier pregnancy (both as categorical). Both models were executed

separately for all women and for women with haemoglobin levels over 120 g/l in early pregnancy (8-12 or 16-18 weeks' gestation).

We also added interaction terms for iron intake and haemoglobin and for iron intake and BMI to the model but they were not statistically significant and therefore are not presented here. In sensitivity analyses, we examined the association between the total iron intake and the risk of GDM using alternative cut-off points for categorising participants based on their iron intake (15, 70 and 15 percent; 25, 50 and 25 percent and 30, 40 and 30 percent) and the same statistical tests as described above. All analyses were performed with SPSS statistics (version 19).

RESULTS

Based on the questionnaire completed at 26-28 weeks' gestation, the mean daily iron intake from food was 14.4 mg (SD 4.3; median 13.9 mg) among the study population. In the study group 65.7% of women used supplemental iron and the median of supplemental intake was 27.0 mg. The most used dosages for iron supplementation were 10.0 mg, 20.0 mg and 100.0 mg of elemental iron (Fe^{2+}). The median for total iron intake was 27.1 mg (interquartile range 15.5-89.3 mg) (Figure 1). Iron intake from food was similar in nonusers and users of supplemental iron: users of supplemental iron had a mean daily intake of 14.5 mg (SD 4.5) and nonusers 14.3 mg (SD 3.9). The mean haemoglobin level of the study population in the beginning of pregnancy was 134 g/l (SD 11; min 107 g/l; max 169 g/l).

The oral glucose tolerance test at 26-28 weeks' gestation was abnormal in 14.0% of the study population (n=56, missing value for three women). Furthermore 16 women received a gestational diabetes diagnosis during their pregnancy. Figure 2 shows the incidence of GDM in fifths of total

iron intake. There was a tendency for a higher incidence of GDM in the highest fifth of total iron intake.

Between the iron intake groups (20%, 60% and 20%) there were no significant differences in terms of proportion of primigravida, age, diabetes in first- or second-degree relatives or GDM or macrosomia in earlier pregnancy (Table 1). Women in the highest iron intake group had a slightly lower mean BMI as compared to other women (P=0.05). Haemoglobin levels in early pregnancy differed between the groups as excepted (P<0.001). With regard to differences in dietary intake, women in the lowest iron intake group had lower total energy and dietary fibre intakes than the other women whereas women in the highest iron intake group had higher intake of saturated fatty acids than the other women. Total gestational weight gain did not differ between the iron intake groups. Table 2 shows the incidence of GDM in the three iron intake groups. The incidence was higher in the highest iron intake group (20.8%) compared to the other two groups (16.7% in both) but the difference was not statistically significant (P=0.70). When including~~After excluding~~ ~~those~~ women with ~~who had low~~ haemoglobin levels ~~(≤~~>120 g/l~~)~~ ~~already~~ in early pregnancy (n=321, mean Hb 135, SD 9), the difference between the groups was even larger but still not statistically significant (P=0.11).

The logistic regression model shows that after adjustment for potential risk factors and background characteristics total iron intake was significantly and positively associated with gestational diabetes mellitus (Table 3). When iron intake was used as a continuous variable the OR was 1.006~~5~~ (P=0.038~~41~~) for all women and 1.009~~8~~ (P=0.006~~5~~) for those with haemoglobin levels over 120 g/l. In the second model the OR for GDM was 1.66~~55~~ (95% confidence interval 0.8~~41~~ to 3.30~~2.96~~, P=0.15~~9~~) for the highest fifth of iron intake among all women. After excluding women with low

haemoglobin levels (≤120 g/l) in early pregnancy the OR was 2.3521 (95% confidence interval 1.131 to 4.9241, P=0.0235).

In sensitivity analyses the odds ratios were higher when 15% of women with the highest iron intake were compared to the rest 85% of women. The odds ratios were lower when 25% or 30% of women with highest intake were compared to the rest 75% and 70% of women respectively (results not shown).

DISCUSSION

In this study we investigated the possible association between total daily iron intake during pregnancy, haemoglobin in early pregnancy and the risk of gestational diabetes. To our knowledge there are no similar studies published previously. We discovered that there was a tendency for a higher incidence of GDM in the highest fifth of total iron intake. High iron intake increased the risk of GDM especially for those women who had good haemoglobin levels in early pregnancy: we discovered a two-fold OR for GDM for women in the highest fifth of iron intake with a haemoglobin level of over 120 g/l in early pregnancy.

Our study has several strengths. Firstly unlike most of the previous studies we had detailed information of iron intake from both food and supplements. Secondly we had information onf 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 factors for GDM.

A limitation of this kind of study is that estimating iron intake during pregnancy can be challenging because dietary habits and use of supplements can vary a lot during pregnancy due to nausea and other changes in well-being. Iron intake was assessed at a certain point in time (at 26-28 weeks' gestation covering the previous month), thus it does not cover the intake during the whole pregnancy. In our study we decided to use information of iron intake in mid-pregnancy to get the best estimation on iron intake during pregnancy and before the onset of GDM. We did not use information of iron intake abstracted in the beginning of pregnancy because it covered only dietary habits and supplement use during one month before the pregnancy when use of supplemental iron is rare. However our information on~~f~~ iron intake covers only one month in the mid-pregnancy. Additionally we could not assess iron absorption, for example with blood measurements, which also can vary a lot depending on body iron status and the contents of meals.

There are also limitations concerning the haemoglobin measurements used in our study. In the maternity clinics haemoglobin levels are usually screened with a capillary haemoglobin measurement using finger-stick samples. This method has been demonstrated to be reliable in determining haemoglobin values but it is however susceptible to handling errors[30] and therefore there can be variation in the test results. Additionally we had a lot of missing values in haemoglobin measurements and thus we decided to use the haemoglobin measurement at 16-18 weeks' gestation for those with missing values at weeks 8-12 (n= 28). Although measurements at weeks 16-18 do not present the haemoglobin levels at early pregnancy our objective was to analyse separately women who were not anaemic and thus including women who had a haemoglobin level of over 120 g/l still at weeks 16-18 would help us not to underestimate the proportion of women who were not anaemic

in the beginning of the pregnancy. However for these reasons our results in respect of haemoglobin measurements should be considered with caution and further studies with reliable and comprehensive haemoglobin measurements are warranted to confirm our results.

In a somewhat similar study Bo et al.[22] discovered that women who used supplemental iron during mid-pregnancy had a 2- to 3-fold risk of GDM. They however had no information of dietary intake of iron or haemoglobin levels or other measurements of iron stores. Their findings are however in line with our results which suggest that dietary intake could play a relatively small part in the association whereas high supplemental iron intake could be responsible for most of the increase in GDM risk. Somewhat different results have been reported recently by Bowers et al.[21] and Qiu et al.[23]. Bowers et al.[21] investigated dietary and supplemental iron intake during one year before pregnancy utilising the material from the Nurses Health study. They observed no significant effect of total, nonheme or supplemental iron intake on the risk of GDM. It can be argued though, that usually iron supplementation outside of pregnancy is more rare and concerns mainly those who are anaemic. However they did find a significant and positive association between heme iron intake and GDM. Similarly Qiu et al.[23] demonstrated an association between heme iron intake during the time before conception and in early pregnancy and the risk of GDM. To our knowledge only one placebo controlled clinical trial has been conducted to investigate the association between iron supplementation and GDM[24]. This study did not observe any association. However it can be argued that the intake of supplemental iron was quite low in this study because of only about 50% compliance to a daily supplement of 60 mg of elemental iron.

Iron is a highly reactive component with a possibility to participate in harmful reactions[31]. The human body can excrete iron with very limited mechanisms and thus iron intake is highly regulated according to body iron needs[32]. Iron could interfere with glucose metabolism in several ways. For

example following mechanisms have been proposed: Iron decreases insulin extraction and metabolism in the liver, which leads to peripheral hyperinsulinemia[33]. Iron overload results in oxidative stress in pancreatic β-cells, which leads to destruction of the pancreatic islets and thus decreases insulin secretion[34]. The exact mechanisms, which link iron to diabetes are still unsolved. However the association can be argued to be biologically plausible.

Accumulating body of evidence supports the hypothesis of excess iron as a risk factor for glucose metabolism disorders such as gestational diabetes. Iron deficiency anaemia has been shown to reduce the incidence of GDM[19]. Respectively high haemoglobin level (>130 g/l) in early pregnancy has been demonstrated to be an independent risk factor for GDM[18]. It seems that iron can affect glucose metabolism even with no overt iron overload. Women diagnosed with GDM have been observed to have increased iron stores compared to women without GDM[15 17]. However many of these studies have been cross-sectional and it has been criticised that higher serum ferritin levels could in fact reflect inflammation in the body and could be rather a result than a cause for diabetes[16].

In summary it seems that high iron intake might be a factor, which increases the risk of GDM especially in women with already good iron stores. Use of iron supplements during pregnancy is common even in women with good haemoglobin levels. Our results suggest that routine use of iron supplements should be reconsidered in non-anaemic women with risk factors for GDM. To confirm the hypothesis there is need for a large prospective study – ideally a randomised controlled trial – with reliable and comprehensive information on iron intake from food and supplements during pregnancy accompanied with serum measurements to determine the level of body iron stores.

Author contributions

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

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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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Figure legends

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

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

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

	20% <14.2 mg/day (n=78)	60% 14.2 – 109.9 mg/day (n=233)	20% 110.0+ mg/day (n=78)	P-value
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 weeks[1]) >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 weeks[1]) ≤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				
All women				
Total Fe intake (mg/day)	1.004 (0.999 – 1.008)	0.16	1.006 (1.000 – 1.011)~~1.005 (1.000 – 1.011)~~	0.038~~0.041~~
BMI			1.09 (1.02 – 1.16)~~1.11 (1.05 – 1.18)~~	0.006~~< 0.001~~
Age			1.01 (0.95 – 1.07)~~1.03 (0.97 – 1.09)~~	0.78~~0.32~~
Diabetes in first- or second-degree relatives			0.87 (0.49 – 1.55)~~0.91 (0.52 – 1.59)~~	0.63~~0.75~~
GDM or macrosomia in a previous pregnancy			1.77 (0.86 – 3.64)~~1.95 (0.97 – 3.89)~~	0.12~~0.059~~
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
Women with Hb (8-12 weeks' gestation[12]) >120 g/l				
Total Fe intake (mg/day)	1.007 (1.001 – 1.012)	0.013	1.009 (1.003 – 1.015)~~1.008 (1.003 – 1.014)~~	0.006~~0.005~~
BMI			1.07 (1.00 – 1.15)~~1.11 (1.04 – 1.18)~~	0.042~~0.001~~
Age			1.01 (0.94 – 1.08)~~1.03 (0.97 – 1.10)~~	0.78~~0.28~~
Diabetes in first- or second-degree relatives			0.73 (0.39 – 1.36)~~0.80 (0.44 – 1.46)~~	0.32~~0.46~~
GDM or macrosomia in a previous pregnancy			1.78 (0.80 – 3.95)~~2.03 (0.96 – 4.29)~~	0.16~~0.065~~
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

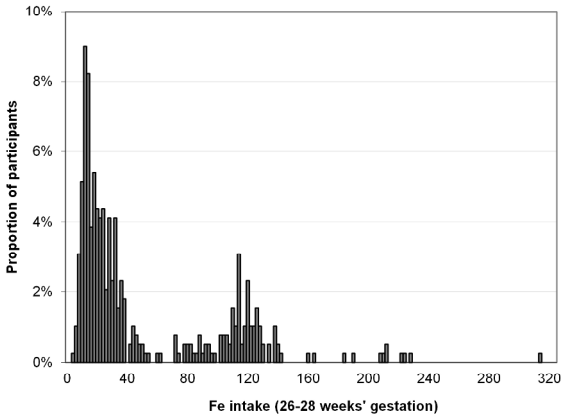
Total gestational weight gain			0.93 (0.88 – 0.99)	0.028
Fe intake as a categorical variable				
All women				
Total Fe intake (the highest 20% vs. the lowest 80%)	1.31 (0.70 – 2.44)	0.40	1.66 (0.84 – 3.30)~~1.55 (0.81 – 2.96)~~	0.15~~0.19~~
BMI			1.09 (1.02 – 1.15)~~1.11 (1.05 – 1.17)~~	0.010~~< 0.001~~
Age			1.01 (0.95 – 1.07)~~1.03 (0.97 – 1.09)~~	0.77~~0.29~~
Diabetes in first- or second-degree relatives			0.84 (0.47 – 1.50)~~0.89 (0.51 – 1.55)~~	0.55~~0.68~~
GDM or macrosomia in a previous pregnancy			1.65 (0.93 – 3.37)~~1.84 (0.92 – 3.64)~~	0.17~~0.083~~
Total energy intake (MJ/day)			0.89 (0.80 – 1.06)~~1.02 (0.97 – 1.08)~~	0.19~~0.37~~
Dietary fibre (g/day)			1.02 (0.97 – 1.08)~~0.97 (0.85 – 1.10)~~	0.37~~0.59~~
Saturated fatty acids (E%)[1]			0.97 (0.85 – 1.10)~~0.94 (0.89 – 0.99)~~	0.59~~0.029~~
Total gestational weight gain			0.94 (0.89 – 0.99)	0.029
Women with Hb (8-12 weeks' gestation[21]) >120 g/l				
Total Fe intake (the highest 20% vs. the lowest 80%)	1.95 (1.005 – 3.78)	0.048	2.35 (1.13 – 4.92)~~2.21 (1.11 – 4.41)~~	0.023~~0.025~~
BMI			1.07 (1.00 – 1.14)~~1.10 (1.04 – 1.17)~~	0.054~~0.002~~
Age			1.01 (0.95 – 1.08)~~1.04 (0.98 – 1.10)~~	0.76~~0.24~~
Diabetes in first- or second-degree relatives			0.70 (0.38 – 1.31)~~0.78 (0.43 – 1.41)~~	0.27~~0.41~~
GDM or macrosomia in a previous pregnancy			1.60 (0.73 – 3.53)~~1.87 (0.89 – 3.93)~~	0.24~~0.10~~
Total energy intake (MJ/day)			0.83 (0.69 – 1.02)	0.071
Dietary fibre (g/day)			1.05 (0.99 – 1.11)	0.10
Saturated fatty acids (E%)[1]			0.99 (0.86 – 1.15)	0.92

28

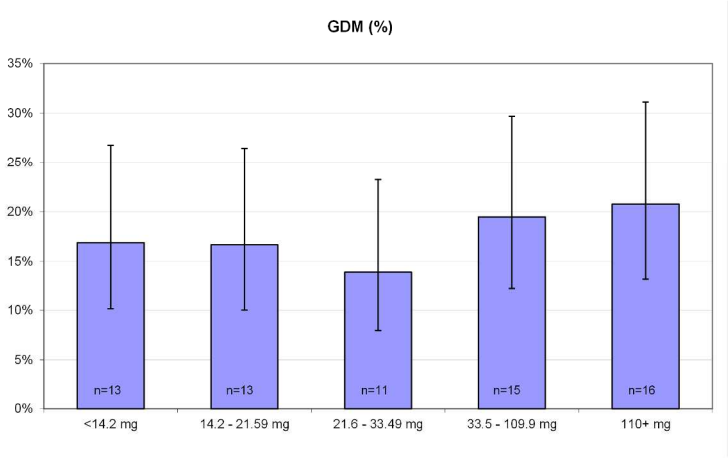
Total gestational weight gain	0.93 (0.87 – 0.99)	0.017

[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)



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



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	

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
		(b) Indicate number of participants with missing data for each variable of interest	8, Table 1, Table 2
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
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
		(*b*) Report category boundaries when continuous variables were categorized	Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
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
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
Generalisability	21	Discuss the generalisability (external validity) of the study results	10,13
Other information			
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.