

Original Article

Supplemental iron intake and the risk of glucose intolerance in pregnancy: re-analysis of a randomised controlled trial in Finland

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Abstract

Observational studies suggest that high iron intake during pregnancy is associated with the risk of gestational diabetes. As such studies are prone to bias, we re-analysed data from a randomised controlled trial of iron supplementation to see whether it supports the risk found in observational studies. The trial was conducted in primary health care setting in five municipalities in Finland in 1985–1986. The participants were 2944 women (95% of pregnant women in the area) who were randomly allocated either to (1) the selective iron group (elemental iron 50 mg twice a day only if diagnosed as anaemic, continuing until their haemoglobin increased to 110 g L⁻¹) or (2) the routine iron group (elemental iron 100 mg day⁻¹ throughout the pregnancy regardless of haemoglobin level). The numbers of women in the analyses were 1358 and 1336, respectively. The main outcome measure was a composite variable including any glucose intolerance-related outcome (e.g. glucosuria, gestational diabetes, large-for-gestational-age child) in mothers' or children's patient records during pregnancy and post-partum. There were no statistically significant differences in the incidence of the primary outcome between the selective iron and the routine iron groups (13.0 vs. 11.0%, $P = 0.12$). The most common outcome was large-for-gestational-age calculated from children's hospital data (8.3 vs. 8.2%, $P = 0.95$). The results were mainly similar when stratified by the mothers' baseline haemoglobin level, body mass index or gestational weight gain. Routine iron supplementation throughout pregnancy did not increase the risk of glucose intolerance during pregnancy. The results need to be confirmed in future trials.

Keywords: iron supplementation, randomised controlled trial, pregnancy, glucose intolerance, gestational diabetes.

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Trial registration: Not done (recruitment in 1985–1986)

Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that is recognised during pregnancy for the first time (American Diabetes Association 2013). GDM and milder glucose intolerance increase the risk of macrosomia and other adverse pregnancy outcomes (Stotland *et al.* 2004;

HAPO Study Cooperative Research Group *et al.* 2008; Ju *et al.* 2008). In the long term, GDM predicts the risk of overweight, type 2 diabetes and metabolic syndrome in the child (Dabelea 2007; Clausen *et al.* 2008, 2009; Pirkola *et al.* 2010) and type 2 diabetes in the mother (Kim *et al.* 2002; Lee *et al.* 2007). The main risk factors for GDM are being overweight in pre-pregnancy, advanced maternal age and family history

of diabetes (American Diabetes Association 2003). As the incidence of GDM has been increasing over the last 20 years (Ferrara 2007; Galtier 2010) and it has been estimated that only about half of the cases are explained by people being overweight (Kim *et al.* 2010), it is important to search for other modifiable risk factors.

It has been hypothesised that iron may have a role in the development of GDM. High haemoglobin (Hb) level in early pregnancy has been positively (Lao *et al.* 2002) and low Hb level negatively (Lao & Ho 2004) associated with the risk of GDM, although not in all studies (Chen *et al.* 2006). Higher levels of serum ferritin, transferrin and some other markers of iron stores have been found among women with GDM compared with women without GDM (Lao *et al.* 2001; Chen *et al.* 2006; Afkhami-Ardekani & Rashidi 2009; Soubasi *et al.* 2010; Derbent *et al.* 2013). Serum ferritin level is also elevated in inflammatory states and therefore may not necessarily indicate increased body iron stores (Williams *et al.* 2002; Mainous *et al.* 2004). Higher levels of hepcidin, a hormone-regulating iron metabolism, have also been associated with GDM (Derbent *et al.* 2013).

Regarding iron intake from food and/or supplements, the results of the previous observational studies are partly contradictory (Bo *et al.* 2009; Bowers *et al.* 2011; Qiu *et al.* 2011; Helin *et al.* 2012). As observational studies are prone to confounding, the effect of iron supplementation on the risk of GDM should be examined in randomised controlled trials. One such study from Hong Kong has been reported to date (Chan *et al.* 2009). It found no difference in the incidence of GDM between women

randomised to take 60 mg elemental iron or a placebo daily, but the overall compliance to the intervention as well as participation in the follow-up were relatively low. More evidence is needed on this topic because iron supplementation and GDM are common in pregnancy (e.g. 78% and 13% of pregnant women in Finland, respectively; Arkkola *et al.* 2006; National Institute for Health and Welfare 2012). We examined the effects of a trial comparing routine and selective iron supplementation on the risk of glucose intolerance in pregnant women.

Material and methods

Study design and participants

We used the opportunity to analyse data from an old trial conducted in Finland in the mid-1980s (Hemminki *et al.* 1989). The original trial was a population based multi-centre randomised controlled trial aiming to study the health effects of routine iron supplementation on mothers' and infants' health. The methods and the main results have been described previously (Hemminki *et al.* 1989, 1991; Hemminki & Rimpela 1991a,b; Hemminki & Merilainen 1995). Pregnant women were recruited in municipal maternity clinics in Tampere and five neighbouring municipalities in Southern Finland between April 1985 and May 1986. Briefly, all 51 municipal midwives (or public health nurses) working at 27 different clinics recruited the participants and carried out the study. All women attending the maternity clinics for the first time during their pregnancy ($n = 3098$) were screened for eligibility (Fig. 1). The exclusion criteria for

Key messages

- Previous observational studies have suggested that high iron intake during pregnancy might increase the risk of gestational diabetes, but more evidence is needed from randomised controlled trials.
- We used the opportunity to analyse data from a well-implemented randomised controlled trial of iron supplementation from the 1980s.
- There were no differences in the composite outcome variable (including any glucose intolerance-related outcome) between women in the routine iron supplementation group and women who received iron supplementation only if they were diagnosed as anaemic.
- The results suggest that routine iron supplementation may not increase the risk of glucose intolerance in pregnancy.

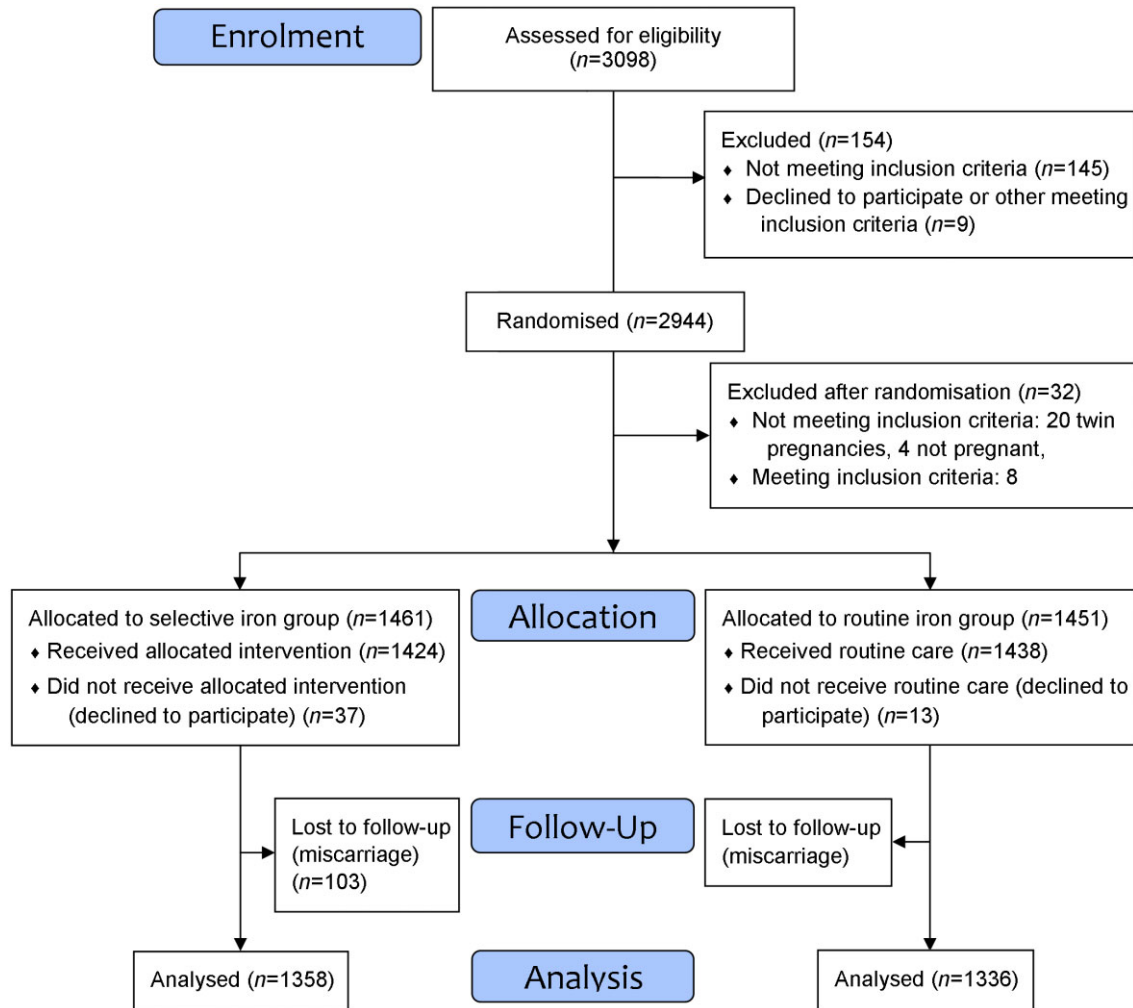


Fig. 1. Flow diagram of the randomised controlled trial.

participation were a serious chronic disease, $\text{Hb} \leq 110 \text{ g L}^{-1}$, high probability of moving from the area before the birth of the child or enrolling later than at 16 weeks gestation. A total of 154 (5.0%) women were excluded before randomisation, nine of them would have been eligible.

In total, 2944 women were randomised either to the selective iron group or the routine iron group by using random codes created by computer in blocks of 10 per clinic. The codes were kept in sealed envelopes stored in containers and the midwives were told to take them in order. The midwives gave the participants oral and written information on the study only after the

randomisation and asked them to participate and to sign the informed consent form. Thirty-two women were excluded after randomisation (Fig. 1). In addition, 103 (7.0%) participants in the selective iron group and 115 (7.9%) participants in the routine iron group had a miscarriage and were not included in the analyses. The final study population was therefore 1358 (93.0%) and 1336 (92.1%) participants in the two groups, respectively. This included also 37 and 13 women, respectively, who declined participation in the intervention, but were willing for data collection. They were asked to take iron as they wished. The study was conducted in accordance with Helsinki

Declaration and the study protocol was accepted in seven ethics committees in the area.

Intervention

The participants in the routine iron group were advised to take elemental iron 100 mg day⁻¹ throughout the pregnancy regardless of their Hb level (Hemminki *et al.* 1989). This advice was very similar to that in usual care practices in the 1980s. The participants in the selective iron group were advised to take elemental iron (50 mg twice a day) only if their Hb fell below 100 g L⁻¹ after 14 weeks gestation on two consecutive visits and anaemia was confirmed in the laboratory. They were asked to continue supplementation for 2 months or until their Hb increased to 110 g L⁻¹. As the midwives requested, the protocol was slightly changed in the middle of the study so that the participants in the selective iron group were also allowed to start iron supplementation if their Hb was <105 g L⁻¹ after 33 weeks gestation.

The participants' compliance to their assigned treatment was assessed by questionnaires filled in during the routine prenatal visits at 28 and 36 weeks gestation. The participants were asked if they had used any iron containing supplements during the preceding 2 weeks and what the dosage had been. In the routine iron group of those answering this question (86–92% of all), 82% at 28 weeks and 73% at 36 weeks gestation had used iron containing supplements daily or almost daily (Hemminki *et al.* 1989). Among participants using iron supplements, the reported mean dosage of elemental iron was 124 mg day⁻¹ at both time points. In the selective iron group of those answering this question (84–89% of all), 92% at 28 weeks and 84% at 36 weeks gestation had not used any iron containing supplements. Among participants using iron supplements, the reported mean dosage of elemental iron was 107 mg day⁻¹ at 28 weeks and 121 mg day⁻¹ at 36 weeks gestation.

Outcomes and data collection

GDM was not originally an outcome in the trial and it was not assessed systematically among all partici-

pants, but was abstracted from patient records as recognised in the usual care. In the 1980s, GDM was usually screened among women with risk factors (e.g. diabetes in close relatives, overweight, glucosuria, birthweight ≥ 4500 g in any previous pregnancy; Hyvönen 1991). All outcome data were based on children's hospital records or on routinely and prospectively recorded midwives' notes during prenatal and post-natal visits (Hemminki *et al.* 1989). In total, the percentage of missing forms was 0.5% for children's records and 0.4% for the midwives forms. All births occurred in a hospital. Midwives themselves abstracted the data into study forms. The hospital data were abstracted by a trained research assistant who was not aware of the intervention assignment.

The primary outcome of the present study was a composite variable including any glucose intolerance-related outcome described in detail in Table 1. Large for gestational age (LGA) was considered one of the indicators of glucose intolerance as women with GDM are more likely to have a LGA child as compared with other pregnant women (Wendland *et al.* 2012).

The following data were obtained from the midwives' forms: mothers' age, parity, height (all at the first visit), and gestation weeks, body weight and Hb at the four data collection visits (around 12, 20, 28 and 36 weeks gestation). Data on pre-pregnancy weight (self-reported at 12 weeks gestation) and socio-economic status and smoking during pregnancy (at 28 weeks gestation) were obtained from the mothers' questionnaires. The definition of socio-economic status was based on the classification of Statistics Finland for the year 1980 (Central Statistical Office of Finland 1981). The eight occupational categories were recoded into five by combining categories 'employers' and 'entrepreneurs' into 'self-employed persons' and categories 'retired', 'students' and 'others, e.g. unemployed' into 'others' (Table 2). Gestation weeks at delivery were categorised into four categories (<37, 37.0–38.9, 39.0–41.9 and ≥ 42.0 weeks).

In sub-analyses, the participants were categorised by their pre-pregnancy body mass index (BMI) to underweight (BMI < 18.5 kg m⁻²), normal weight (BMI 18.5–24.9 kg m⁻²), overweight (BMI 25.0–29.9 kg m⁻²) and obese (BMI ≥ 30 kg m⁻²) women.

Table 1. Definitions of the study outcomes

Outcome	Description of the outcome*	The primary data source
1. The primary reason for referring to a specialist in maternity hospital	Abnormal blood glucose values in daily follow-up measurements, elevated blood sugar level or glucosuria	Midwives' notes
2. The primary reason for inpatient care before delivery	Abnormal blood glucose values in daily follow-up measurements, elevated blood sugar level or glucosuria	Midwives' notes
3. Discharge diagnosis from birth hospital	Abnormal glucose tolerance or gestational diabetes	Midwives' notes
4. Discharge diagnosis from birth hospital	Grouped using ICD 8 codes, including glucosuria, abnormal result in the oral glucose tolerance test, pre-diabetes (impaired glucose tolerance) or diabetes mellitus during pregnancy	Children's hospital records
5. Discharge diagnosis from birth hospital	ICD 8 code: A large for date child	Children's hospital records
6. Large for gestational age	Birthweight above the 90th percentile of birthweight, defined separately for both sexes and each week of gestational age [†]	Children's hospital records
7. Composite outcome (the primary outcome)	Any of the above outcomes	Midwives' notes, children's hospital records

*Most common terms used in the primary data source or in the eighth revision of the International Classification of Diseases (ICD 8). [†]The researchers calculated the variable based on birthweight, gestational age at birth and sex. The reference data for the 90th percentile of birthweight were obtained from the Finnish Medical Birth Register (year 1987).

They were also categorised based on whether their gestational weight gain was below, within or above the recommended weight gain, using the recommendations of the Institute of Medicine (IOM): 12.5–18.0 kg for underweight, 11.5–16.0 kg for normal weight, 7.0–11.5 kg for overweight and 5.0–9.0 kg for obese women (IOM 2009).

Statistical methods

The data were analysed by the intention to treat. Additionally, per-protocol analyses were performed for the outcomes after excluding the 50 women who refused participation in the intervention but agreed to data collection. Post-hoc power calculations showed that our sample size was adequate to detect a statistically significant between-group difference in the composite outcome if the incidence of the outcome was 11% among the routine iron group and <7.8% or >14.7% in the selective iron group (assuming 80% power and two-tailed *P*-value 0.05).

Descriptive data are given as numbers (%) and means (SD). A chi-square test was used to compare percentages and a *t*-test to compare means between the groups. When comparing the incidence of the composite outcome between the groups, the analyses

were also stratified by baseline Hb (<130, 130–139.9, ≥140 g L⁻¹), BMI and gestational weight gain groups. All tests were two-sided and *P*-values < 0.05 were regarded as statistically significant. The data were analysed using the SPSS statistical software package version 20 (SPSS Inc., Chicago, IL, USA).

Results

Participants' background characteristics and pregnancy data

The participants' background characteristics were very similar in the two groups (Table 2). The participants were 28 years old and had a BMI of 22 kg m⁻², on average, in both groups. The percentages of overweight or obese participants were relatively low in both groups. The groups were also very similar regarding the number of previous births, socioeconomic status and smoking.

The timing of the first visit was similar in both groups (Table 2). Regardless of randomisation the mean Hb level was slightly higher in the selective iron group than in the routine iron group at the first visit (137 vs. 136 g L⁻¹, *P* = 0.031). As expected, from 28 weeks gestation onwards, the routine iron group had higher mean Hb level than the selective iron group

Table 2. Background characteristics and pregnancy data, by the iron supplementation groups, means (SD) or numbers (%)

	Missing values (%) [*]	Selective iron (n = 1358)	Routine iron (n = 1336)	<i>t/χ</i> ²	df	<i>P</i> -value [†]
Background characteristics						
Age, years, mean (SD)	0, 0	27.8 (5.6)	27.7 (4.9)			
Pre-pregnancy body mass index (BMI), kg m ⁻² , mean (SD)	5.2, 3.6	22.4 (3.4)	22.3 (3.5)			
Categorised pre-pregnancy BMI, <i>n</i> (%)	5.2, 3.6					
Underweight, BMI < 18.5 kg m ⁻²		80 (6.2)	108 (8.4)			
Normal weight, BMI 18.5–24.9 kg m ⁻²		1004 (78.0)	961 (74.6)			
Overweight, BMI 25.0–29.9 kg m ⁻²		153 (11.9)	174 (13.5)			
Obese, BMI ≥ 30 kg m ⁻²		50 (3.9)	45 (3.5)			
Previous births, <i>n</i> (%)	4.5, 4.6					
0		559 (43.1)	557 (43.7)			
1		506 (39.0)	499 (39.1)			
≥2		232 (17.9)	219 (17.2)			
Socio-economic status, <i>n</i> (%)	4.2, 2.4					
Upper level employees		179 (13.8)	186 (14.3)			
Lower level employees		659 (50.7)	635 (48.7)			
Workers		355 (27.3)	363 (27.8)			
Self-employed persons		27 (2.1)	29 (2.2)			
Others (e.g. unemployed, pensioners, students, unknown status)		81 (6.2)	91 (7.0)			
Smokers, <i>n</i> (%) [‡]	8.7, 7.5					
Non-smoker		966 (77.9)	939 (76.0)			
Quitted smoking during pregnancy		79 (6.4)	90 (7.3)			
Occasional smoker		73 (5.9)	71 (5.7)			
Smoker		122 (9.8)	136 (11.0)			
Pregnancy data						
Weeks gestation at the first prenatal visit, weeks, mean (SD)	8.0, 8.0	11.5 (2.0)	11.6 (2.1)	-0.322	2476	0.75
Haemoglobin, g L ⁻¹ , mean (SD)						
At 12 weeks gestation (baseline)	2.3, 2.1	136.5 (9.4)	135.7 (9.3)	2.162	2633	0.031
At 20 weeks gestation	2.1, 2.4	126.4 (8.9)	127.0 (8.6)	-1.622	2632	0.11
At 28 weeks gestation	2.9, 2.9	123.9 (9.5)	127.0 (8.6)	-8.718	2614	<0.001
At 36 weeks gestation	7.9, 7.0	124.8 (9.6)	131.2 (9.3)	-17.117	2491	<0.001
Body weight, kg, mean (SD)						
Pre-pregnancy	4.8, 3.1	60.9 (10.0)	60.8 (10.8)	0.315	2586	0.75
At 12 weeks gestation	1.9, 2.0	63.1 (10.3)	62.9 (10.2)	0.443	2639	0.66
At 20 weeks gestation	1.5, 1.6	66.1 (10.2)	66.0 (10.2)	0.336	2650	0.74
At 28 weeks gestation	2.1, 1.9	70.0 (10.3)	69.8 (10.5)	0.329	2639	0.74
At 36 weeks gestation	5.5, 5.6	73.6 (10.6)	73.5 (10.6)	0.420	2542	0.68
Gestational weight gain [§] , kg, mean (SD)	9.6, 8.2	12.6 (4.1)	12.6 (4.1)	-0.131	2453	0.90
Categorised gestational weight gain, <i>n</i> (%) [¶]	10.0, 8.5			1.408	2	0.50
Below the recommendations		404 (33.1)	431 (35.3)			
Within the recommendations		569 (46.6)	545 (44.6)			
Above the recommendations		249 (20.4)	246 (20.1)			
Weeks gestation at delivery, weeks, mean (SD)	0.7, 1.0	39.8 (1.9)	39.9 (1.8)	-1.766	2669	0.08
Categorised weeks gestation at delivery, <i>n</i> (%)	0.7, 1.0			4.285	3	0.23
<37 weeks		52 (3.8)	36 (2.7)			
37.0–38.9 weeks		139 (10.2)	150 (11.2)			
39.0–41.9 weeks		995 (73.3)	964 (72.2)			
≥42.0 weeks		171 (12.6)	186 (13.9)			

*Percentage of women with missing data in the selective iron and the routine iron groups, respectively. †Two-sided independent samples *t*-test for continuous variables, two-sided χ^2 -test for categorised variables. Differences in the baseline values were not tested statistically. ‡At 28 weeks gestation on average in both groups. §From pre-pregnancy to 36 weeks gestation. ¶Compared with the recommendations of the Institute of Medicine 2009.

(131 vs. 125 g L⁻¹ at 36 weeks gestation, $P < 0.001$). There were no differences between the groups in body weight measurement at any of the visits, in the average gestational weight gain from pre-pregnancy to 36 weeks gestation or in the percentages of participants gaining weight below, within or above the IOM's recommendations. The average duration of the pregnancy and the percentages of participants giving birth pre- or post-term were also similar between the groups.

The incidence of glucose intolerance-related outcomes

The incidence of the composite outcome was 13.0% in the selective iron group and 11.0% in the routine iron group ($P = 0.12$; Table 3). When the six outcome variables were compared separately, some of the outcomes were slightly, but non-significantly, more common in the selective iron group. 'Large for gestational age' (outcome 6) was the most common outcome (8% in both groups). The length and birthweight of the child and the proportion of children with birthweight ≥ 4000 g or ≥ 4500 g were similar in the two groups (Table 3). The results of the per-protocol analyses were very similar.

When stratifying the composite outcome by Hb level at the first study visit, the results were fairly

similar in the two higher strata (Table 4). Among women with Hb < 130 g L⁻¹, the selective iron group had a higher incidence of the outcome than the routine iron group. Among participants with Hb < 120 g L⁻¹, the incidence of the outcome was 29.4% ($n = 15$) in the selective iron group and 21.8% ($n = 12$) in the routine iron group ($P = 0.37$). Among participants with Hb ≥ 150 g L⁻¹, the respective numbers were 12.9% ($n = 9$) and 9.4% ($n = 6$; $P = 0.52$). Regardless of the iron group, the incidence of the outcome was not associated with baseline Hb level.

When stratifying the composite outcome by the pre-pregnancy BMI, no significant differences were observed between the groups within any of the strata (Table 4). Regardless of the iron group, the incidence of the outcome increased remarkably with increasing BMI, being 4- to 5-fold in obese women compared with underweight women.

When stratifying the composite outcome by gestational weight gain, the selective iron group had a higher incidence of the outcome than the routine iron group among women who gained weight within the recommendations (Table 4). No differences were observed between the groups among women gaining weight either below or above the recommendations. Regardless of the iron group, women with lower than recommended weight gain had lower incidence of the

Table 3. Incidence of glucose intolerance-related outcomes*, and child birthweight and length by the iron supplementation groups, numbers (%) or means (SD)

	Selective iron ($n = 1358$)	Routine iron ($n = 1336$)	t/χ^2	df	P -value [†]
Glucose intolerance-related outcomes, n (%)					
1. The primary reason for referring to a specialist in maternity hospital	11 (0.8)	6 (0.4)	1.399	1	0.24
2. The primary reason for inpatient care before delivery	11 (0.8)	11 (0.8)	0.001	1	0.97
3. Discharge diagnosis from birth hospital	3 (0.2)	3 (0.2)	0.000	1	0.98
4. Discharge diagnosis from birth hospital	62 (4.6)	47 (3.5)	1.904	1	0.17
5. Discharge diagnosis from birth hospital	12 (0.9)	8 (0.6)	0.742	1	0.39
6. Large for gestational age	112 (8.3)	109 (8.2)	0.004	1	0.95
7. Composite outcome	176 (13.0)	147 (11.0)	2.445	1	0.12
Child birthweight and length [‡]					
Length of the child, cm, mean (SD)	50.1 (2.5)	50.2 (2.8)	-1.585	2687	0.11
Birthweight, g, mean (SD)	3566 (565)	3580 (570)	-0.646	2688	0.52
Birthweight ≥ 4000 g, n (%)	288 (21.3)	295 (22.1)	0.281	1	0.60
Birthweight ≥ 4500 g, n (%)	44 (3.2)	53 (4.0)	1.011	1	0.32

*The outcomes are defined in Table 1. [†]Two-sided χ^2 -test for categorised variables and two-sided independent samples t -test for continuous variables. [‡]Missing values were 0.2% in the selective iron and 0.1% in the routine iron groups.

Table 4. Incidence of the composite outcome* by the iron supplementation groups, stratified by haemoglobin level, pre-pregnancy body mass index (BMI) and gestational weight gain, n (%)

	<i>n</i> [†]	Selective iron (<i>n</i> = 1358)	Routine iron (<i>n</i> = 1336)	χ^2	df	<i>P</i> -value [‡]
Haemoglobin level at the first visit						
<130 g L ⁻¹	337, 375	53 (15.7)	39 (10.4)	4.476	1	0.034
130–139.9 g L ⁻¹	598, 595	71 (11.9)	69 (11.6)	0.022	1	0.88
≥140 g L ⁻¹	392, 338	48 (12.2)	37 (10.9)	0.297	1	0.59
		0.18 [§]	0.80 [§]			
Pre-pregnancy BMI						
Underweight, <18.5 kg m ⁻²	80, 108	4 (5.0)	8 (7.4)	0.446	1	0.50
Normal weight, 18.5–24.9 kg m ⁻²	1004, 961	116 (11.6)	96 (10.0)	1.248	1	0.26
Overweight, 25.0–29.9 kg m ⁻²	153, 174	34 (22.2)	28 (16.1)	1.991	1	0.16
Obese, ≥30 kg m ⁻²	50, 45	13 (26.0)	12 (26.7)	0.005	1	0.94
		<0.001 [§]	<0.001 [§]			
Gestational weight gain, <i>n</i> (%)[¶]						
Below the recommendations	404, 431	31 (7.7)	36 (8.4)	0.130	1	0.72
Within the recommendations	569, 545	93 (16.3)	60 (11.0)	6.688	1	0.010
Above the recommendations	249, 246	39 (15.7)	45 (18.3)	0.607	1	0.44
		0.001 [§]	<0.001 [§]			

*The composite outcome is defined in Table 1. [†]Numbers in the selective iron and the routine iron groups, respectively. [‡]Two-sided χ^2 -test.

[§]*P*-value for trend between the strata within the selective iron and the routine iron groups. [¶]Compared with the recommendations of the Institute of Medicine 2009.

outcome than those with recommended or above recommended weight gain.

Discussion

As opposite to the hypothesis, this randomised controlled trial found no statistically significant differences in the incidence of the glucose intolerance-related outcomes during pregnancy between women who were prescribed iron supplements only if they were diagnosed as anaemic and women who were prescribed elemental iron (100 mg day⁻¹) routinely regardless their Hb level. The results were mainly the same when stratified by the baseline BMI. Thus, our trial does not support the hypothesis that iron supplements during pregnancy increase the risk of GDM.

The present study has several strengths. A randomised controlled trial is the best design to explore the effects of iron supplementation. Compliance with the selective or the routine iron group was good based on both self-reported supplement use and the differences observed in Hb measurements at different stages of pregnancy. The follow-up was almost complete (Fig. 1), the proportion of missing data was

low and the data were analysed according to the intention-to-treat principle. The study population was large and representative. The results are applicable to healthy European pregnant women who are mostly normal weight.

The main limitation of the present study is that the oral glucose tolerance test had not been performed systematically to all participants. Consequently, the composite outcome covers also milder forms of glucose intolerance and may include some women who did not have glucose intolerance. However, the finding of a good correlation between pre-pregnancy BMI and GDM supports the validity of our outcome. Furthermore, the risk of information bias regarding the outcome should be low because the same criteria were applied to both groups and the risk of GDM was not generally suspected to be related to iron intake in the 1980s. However, the results may be biased towards a null finding. Another important limitation is that data on iron stores or dietary intake of iron was not available. The association between high iron intake and glucose intolerance may be related to iron metabolism and iron stores rather than iron intake per se. We did not have data on some potential

confounders such as family history of diabetes mellitus or GDM in any previous pregnancy. The large number of women randomised suggests that these factors were equally distributed between the groups, but we could not confirm it. Although our data were collected nearly 30 years ago, the biological mechanism of the potential association between iron supplementation and glucose intolerance should have remained the same.

Our findings are in concordance with those of the only previous randomised controlled trial conducted in Hong Kong ($n = 1164$; Chan *et al.* 2009). In that study, the incidence of GDM was similar (11%) in the iron supplementation group (60 mg day⁻¹ elemental iron) and the placebo group. A strength of the study was that GDM was defined by oral glucose tolerance test and data on serum ferritin and transferrin were also available. However, 40% of the women were lost to follow-up, compliance with the intervention was low (53–56%) and 43–46% of the women had used other dietary supplements (possibly including iron), which may explain why the Hb levels were similar between the groups at 28 weeks gestation.

Previous observational studies have generally supported the hypothesis that excessive iron intake in pregnancy could be associated with increased risk of GDM. A prospective cohort study ($n = 3158$) in the United States found that the intake of dietary heme iron during pregnancy was directly associated but the intake of dietary non-heme iron tended to be inversely associated with the risk of GDM (Qiu *et al.* 2011). An Italian case-control study ($n = 1000$) reported that the risk of GDM was elevated among women using iron supplements during pregnancy compared with women not using them (Bo *et al.* 2009). In our previous cohort study ($n = 399$; Helin *et al.* 2012), high total iron intake (from both diet and supplements) increased the risk for GDM, but the result was statistically significant only after excluding women with baseline Hb < 120 g L⁻¹. In addition, the results from the US Nurses' Health Study ($n = 13\,475$) suggested that higher pre-pregnancy intake of dietary heme iron increased linearly the risk for GDM, but the intake of total iron, non-heme iron or iron supplements were not related to the risk for GDM (Bowers *et al.* 2011). The measurement

methods of iron intake and GDM were heterogeneous in these observational studies.

In the present study, the risk of glucose intolerance-related outcomes was unexpectedly higher in the selective iron group than in the routine iron group when including women with Hb < 130 g L⁻¹ or women with weight gain within the recommendations only. These observations are difficult to explain, especially because two of the major risk factors of GDM – BMI and age – were similar between the groups within these two subgroups of women (results not shown). Moreover, iron supplement use was not higher than expected in the selective iron group (results not shown). We cannot exclude the possibility that low Hb may have led women in the selective iron group to eat iron-rich food. The observations might also be explained by effect modification or they could be chance findings resulting from multiple testing.

There are some potential explanations for why the results of the observational studies and the randomised controlled trials are different. Firstly, the original hypothesis may be wrong and the findings in observational studies may be due to residual confounding (e.g. iron stores or metabolism as a confounder). Secondly, the phenomenon may be different depending on the main source, type (supplements and diet vs. diet only) and the dosage of iron intake. Thirdly, the findings of the present study may be wrong for example due to limitations of our outcome measure.

There are still many open questions regarding the potential association between dietary or supplementary iron intake and the risk of GDM. Absorption of different forms of iron varies and is affected for example by body iron stores and other dietary factors (Cao & O'Brien 2013). The roles of heme and non-heme iron need to be better understood (Cao & O'Brien 2013). In addition, better understanding of iron metabolism during pregnancy, the markers of body iron stores and the possible effects of iron on glucose regulation at the cell level is warranted.

In conclusion, the study found no effect of iron supplementation on the risk of glucose intolerance in Finnish women pregnant in the 1980s. The findings of the previous observational studies may be

confounded or biased. To confirm our results, future trials should include the glucose tolerance test to define GDM, measures of iron intake from diet and additional markers of iron status.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Contributions

EH was the principal investigator of the original trial conducted in the 1980s; TIK, EH and RL designed the research; TIK performed statistical analyses and wrote the paper. All authors interpreted the findings, commented the drafts and approved the final draft of the manuscript.

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